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OUTCOME OF HUMAN PAPILLOMAVIRUS INFECTION AMONG MEN IN THE FINNISH FAMILY HPV STUDY

by

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To my Family

ABSTRACT

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Outcome of human papillomavirus infection among men in the Finnish Family HPV Study

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Annales Universitatis Turkuensis, Medica-Odontologica, Turku, Finland, 2014.

Emerging evidence on the role of human papillomavirus (HPV) infections for the pathogenesis of a number of diseases has increased the interest in male HPV infection.

This thesis is part of the Finnish Family HPV Study on the dynamics of HPV infection in 329 families. The present study focuses on the male cohort (n=131). Oral scrapings were taken at seven and genital scrapings at two follow-up visits. HPV-genotyping was performed by nested PCR and a Luminex®-based Multimetrix Assay. Demographic data were collected with structured questionnaires at two follow-up-points.

At baseline, asymptomatic HPV infections were common in both spouses (men oral 18.3%; genital 35.9%; women oral 17.2%, cervical 18.8%) but HPV genotype-specific concordance among the spouses was low. Risky sexual behavior of women but not of men was associated with HPV concordance. Changing the partner and marital status increased the risk of incident genital HPV infections in men. Seventeen HPV-genotypes were detected in the oral mucosa of the males; the point-prevalence varied from 15% to 31%. The incidence-time of oral HPV infection fluctuated between 3.9 and 25.7 months. Most of the HPV-positive men cleared their oral HPV infection and genotype-specific oral HPV persistence was detected in only 14% of the men. The time of HPV persistence ranged from 6.0 to 30.7 months. Smoking increased the risk while a history of genital warts protected against oral high risk (HR)-HPV persistence.

These results suggest that asymptomatic HPV infections in men are common. A stable marital relationship protects against incident genital HPV infections. Smoking increases the risk of oral HR-HPV persistence and smoking seems to be an important cofactor for HPV infection.

Key words: Human papillomavirus, man, oral HPV infection, genital HPV infection, HPV genotype, high-risk, low-risk, prevalence, incidence, clearance, persistence, risk factors, sexual behavior, smoking

TIIVISTELMÄ

Katja Kero

Ihmisen papilloomaviruksen taudinkuva suomalaisperheiden seurantatutkimuksen mieskohortissa

Suupatologia ja naistentaudit ja synnytysoppi, Turun yliopisto; Turun yliopistollinen keskussairaala
Annales Universitatis Turkuensis, Medica-Odontologica, Turku, Suomi, 2014.

Tieto ihmisen papilloomaviruksen (HPV) yhteydestä eri anatomisten alueiden sairauksien syntyyn on lisännyt mielenkiintoa miehen papilloomavirustulehduksen taudinkulun selvittämiseksi.

Tämä väitöskirjatyö on osa suomalaista seurantatutkimusta, jossa tutkitaan HPV-infektioiden tartuntareittejä 329 perheessä. Väitöstyössä keskitytään tutkimukseen osallistuneiden 131 miehen aineistoon. Suun limakalvonäytteet otettiin seitsemässä aikapisteessä. Lisäksi otettiin sukuelinalueen näytteet kahdella seurantakäynnillä. Riskitekijöitä kartoittava kyselytutkimus teetettiin tutkimuksen alkutilanteessa sekä viimeisellä seurantakäynnillä.

Oireettomat HPV infektiot olivat alkutilanteessa yleisiä molemmilla sukupuolilla (miesten suu 18,3 % ja sukuelinalue 35,9 %, naisten suu 17,2 % ja kohdunsuu 18,8 %), mutta HPV:n genotyyppien vastaavuus partnerien välillä oli vähäinen. Naisen, mutta ei miehen, seksuaalinen riskikäyttäytyminen oli yhteydessä pariskunnan HPV tyyppien vastaavuuteen. Partnerin ja siviilisäädyn vaihtaminen lisäsivät miehen riskiä saada uusia HPV infektioita. Miehen suun limakalvonäytteistä löytyi kaikkiaan 17 eri HPV tyyppiä. Suun HPV-tulehduksen esiintyvyys vaihteli eri aikapisteissä 15 %:sta 31 %:iin. Uusien HPV tulehdusten ilmaantumisaika vaihteli 3,9 ja 25,7 kuukauden välillä. Suun HPV infektio parani valtaosalla miehistä. Suun krooninen HPV-infektio todettiin 14 %:lla miehistä. Näiden infektioiden keskimääräinen kesto vaihteli 6.0:sta 30.7:ään kuukauteen. Tupakointi lisäsi korkean riskin HPV tyyppien aiheuttamien suun kroonisten infektioiden riskiä, kun taas aikaisemmin sairastetut sukuelinten kondyloomat suojasivat siltä.

Tuloksemme osoittavat, että miehen oireeton HPV tulehdus on yleinen suussa ja sukuelinten alueella. Vakaa parisuhde suojaa uusilta HPV tulehduksilta. Tupakoinnilla on keskeinen merkitys suun HPV-infektion kroonistumisessa.

Avainsanat: ihmisen papilloomavirus, mies, suun HPV-infektio, sukuelinalueen HPV-infektio, HPV genotyyppi, esiintyvyys, ilmaantuvuus, parantuminen, persistointi, taudin eteneminen, korkean riskin HPV, matalan riskin HPV, vaaratekijät, seksuaalikäyttäytyminen, tupakointi

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ABBREVIATIONS

DNA	deoxyribonucleic acid
E	early protein
GEE	generalized estimating equation
HIM	The Human Papillomavirus Infection in Men -Study
HIV	human immunodeficiency virus
HNSCC	head and neck squamous cell carcinoma
HPV	human papillomavirus
HR	high-risk type
IARC	International Agency for Research on Cancer
ICC	intra-class correlation coefficient
ISH	in situ hybridization
L	late protein
LCR	long control region
LR	low-risk type
MMR	male months at risk
mRNA	messenger ribonucleic acid
MSM	men who have sex with men
MSW	men who have sex with women
NCR	noncoding region
ORF	open reading frame
OSCC	oral squamous cell carcinoma
PCR	polymerase chain reaction
RNA	ribonucleic acid
SNR	short noncoding region
STD	sexually transmitted disease
TZ	transformation zone
VLP	virus-like particle
CIN	cervical intraepithelial lesion
AIN	anal intraepithelial lesion

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by the Roman numerals I–IV

I 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.

II Kero K, Rautava J, Syrjänen K, Kortekangas-Savolainen O, Grenman S, Syrjänen S. Stable marital relationship protects men from oral and genital HPV infections. Eur J Clin Microbiol Infect Dis 2014 Feb 7. [Epub ahead of print].

III Kero K, Rautava J, Syrjänen K, Grenman S, Syrjänen S. Oral mucosa as a reservoir of human papillomavirus: Point prevalence, genotype distribution, and incident infections among males in a 7-year prospective study. Eur Urol 2012;62:1063-70.

IV Kero K, Rautava J, Syrjänen K, Willberg J, Grenman S, Syrjänen S. Smoking increases oral HPV persistence among men: 7-year follow-up study. Eur J Clin Microbiol Infect Dis 2014;33(1):123-33.

1 INTRODUCTION

Persistent human papillomavirus (HPV) infection is associated with malignancies in the anogenital and head and neck regions, but the natural history of HPV infections and the predictors of HPV persistence are not fully known. Previously, HPV-studies have largely focused on females, since HPV infection is the main cause for cervical cancer. There is, however, increased interest on male HPV-infections, since evidence is mounting that such infections are related to important diseases also among males.

Anogenital HPV-infections are common among men. Most of the infections clear spontaneously within a year. Sexual behavior of the partners contributes to HPV transmission. Risk factors relating to sexual history, e.g., life-time number of sexual partners, sexual frequency and sexual orientation especially among men having sex with men (MSM), increase the acquisition of anogenital HPV infection. On the other hand, circumcision reduces the incidence of anogenital HPV infections (Albero et al., 2012). Unlike women whose risk of acquiring cervical infection decreases with age (Munoz et al., 2004; Castle et al., 2005), men seem to have a similar risk for incident genital HPV infection throughout their lives (Giuliano et al., 2011, Lenzi et al., 2013).

The natural course of oral HPV infections among men is unknown. Factors associated with oral HPV infection include smoking, older age, immunodeficiency, MSM and open mouth kissing, but the role of oral sex as a risk factor is controversial (Rintala et al., 2006; Kreimer et al., 2011; Pickard et al., 2012; Edelstein et al., 2012; Gillison et al., 2012).

In addition to sexual transmission, there are other routes of transmission of the HPV. Vertical transmission from mother to neonate and transmission through everyday babycare routines make acquisition of a HPV infection early in life possible. Since it is difficult to define the time of the first infection, the natural history of HPV infection is not very well known.

The focus of this study was on assessing the natural history of oral and genital HPV infections in men participating to the Finnish Family HPV study. Persistent HPV infection is the key event in malignant transformation in head and neck and cervical cancer. Therefore, we evaluated factors related to persistence of oral and genital HPV infections.

2 REVIEW OF LITERATURE

2.1 Human papillomaviruses

2.1.1 Structure of the human papillomavirus

Human papillomaviruses (HPV) are a group of small DNA-viruses approximately 55 nm in diameter. The HPV genome contains 8,000 base pairs in a double-stranded DNA molecule with 72 capsomeres, which are enveloped in an icosahedral protein capsid. Although the genome is small, the temporal and spatial pattern of gene expression is complex and is dependent on a fully differentiating epithelium (Doorbar, 2005) described in detail in Chapter 2.2.1. The genome of HPV involves eight open reading frames (ORF) that are further divided into three functional regions (**Figure 1**).

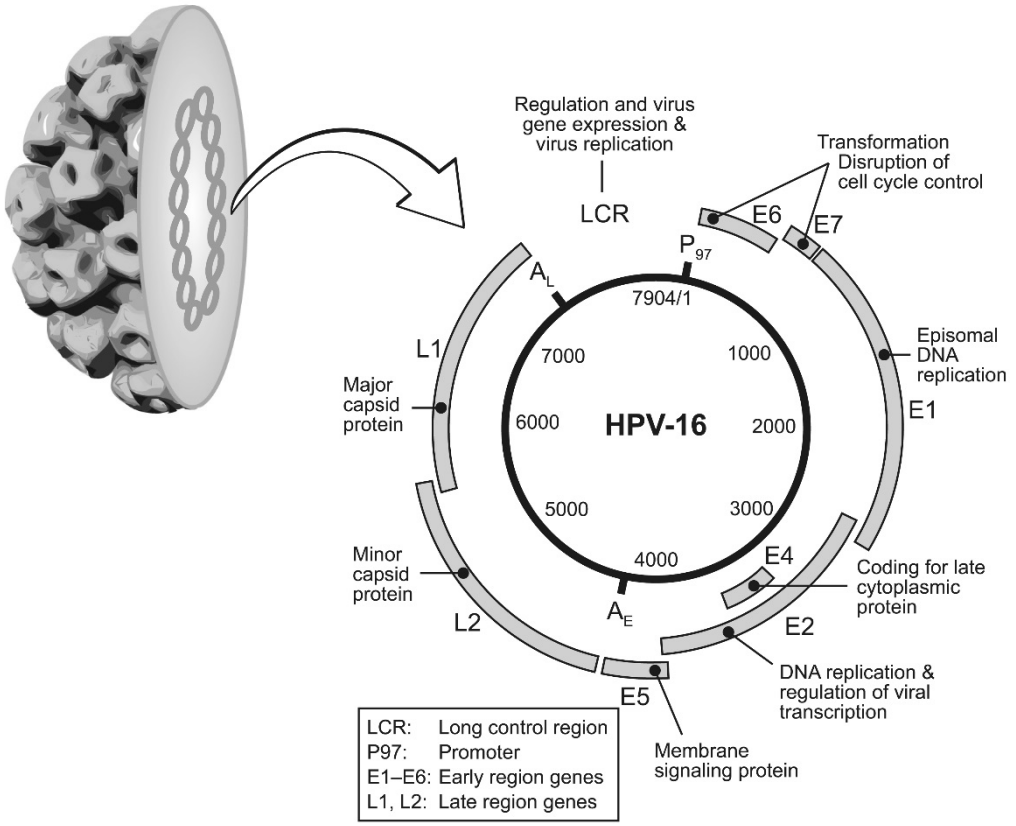

Figure 1. HPV genome.

2.1.2 Classification of HPVs

Classification according to clinical manifestations

HPVs can be divided into different subcategories by their predilection for either cutaneous or mucosal surfaces (de Villiers, 2004; Bzhalava et al., 2013). To date, forty HPVs are known to infect the mucosal sites of the body, typically the anogenital tract and the oral mucosa. HPVs can be grouped also with regard to their malignant potential into high-risk (HR), low-risk (LR) and probable low-risk types (zur Hausen, 2002).

Taxonomy

HPVs are classified into different genotypes according to differences in nucleotide sequences and homology in L1 open reading frames (ORF). Until now, over 170 HPV genotypes have been described (de Villiers, 2013). The number of the HPV genotype is assigned in chronological order by the time of characterization. L1 is the most conserved region within the genome and is used for identification of new genotypes. Thus, if the DNA sequence of the L1 gene differs by more than 10% compared to a HPV genotype already classified, this HPV is entered as a new genotype. A difference of 2 -10% in the DNA sequence is defined as a subtype and a less than 2% difference is defined as an intratype variant (de Villiers et al., 2004).

The different HPV genotypes comprise the Papillomavirus family, which is further divided into genera, Species, types and subtypes. The genus alpha-papillomaviruses (**Figure 2**) contain most of the human HPV types infecting the mucosa (de Villiers, 2013).

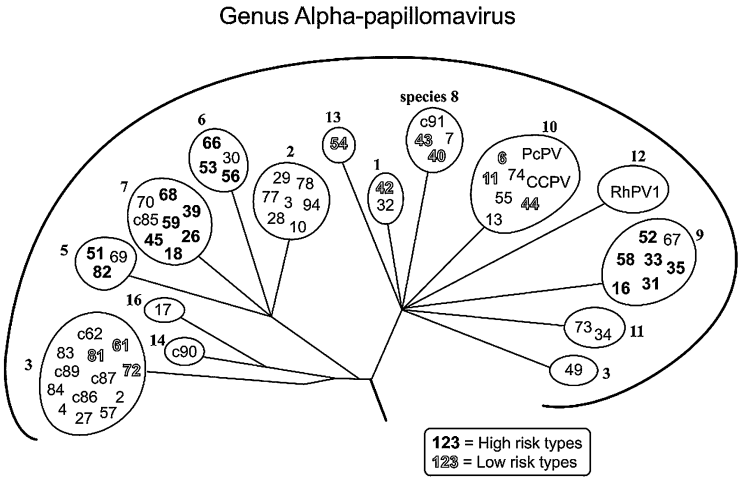

Figure 2. HPV genotypes of the genus alpha-papillomavirus (modified from De Villiers et al., Virology 2004:324;17-24).

2.2 HPV infection

2.2.1 Host cells and host epithelia

Papillomaviruses are strictly Species-specific: HPVs infect only humans. HPV targets squamous cell epithelia and cells with the potential for squamous cell maturation. HPVs are epitheliotropic and affect the skin or mucosal sites of the human body. Cells capable of differentiating are required for the infection, since cell cycle progression through the early stages of mitosis is essential for HPV infection (Pyeon et al., 2009). Stratified squamous cell epithelium provides favorable surroundings for HPV infection since this tissue undergoes constant renewal. The squamous epithelium of the genital mucosa consists of 20 to 30 layers of cells, while the oral mucosa contains fewer cell layers. The basal cells divide actively; one of the two cells resulting from mitosis remains as a basal cell and the other one enters the suprabasal layer to be pushed upwards by continuously dividing cells beneath. **Figure 3** shows the layers of the squamous cell epithelium of the oral mucosa, while **Figures 4** and **5** show the structure of the male genital organs and oral cavity (modified from Kapit W, Elson LM, Anatomy Colouring Book, publisher Benjamin Cummings, 1993, ISBN 0-06-455016-8, s. 115).

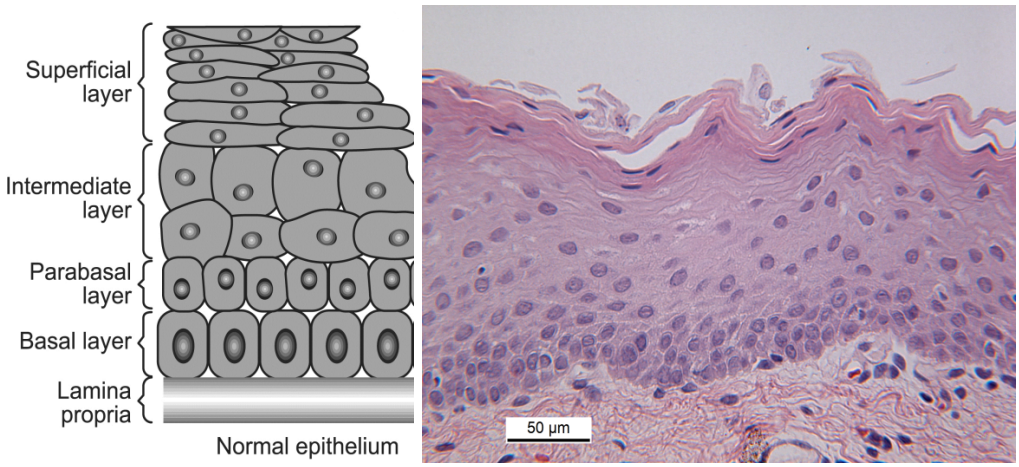

Figure 3. Stratified squamous cell epithelia of the oral mucosa.

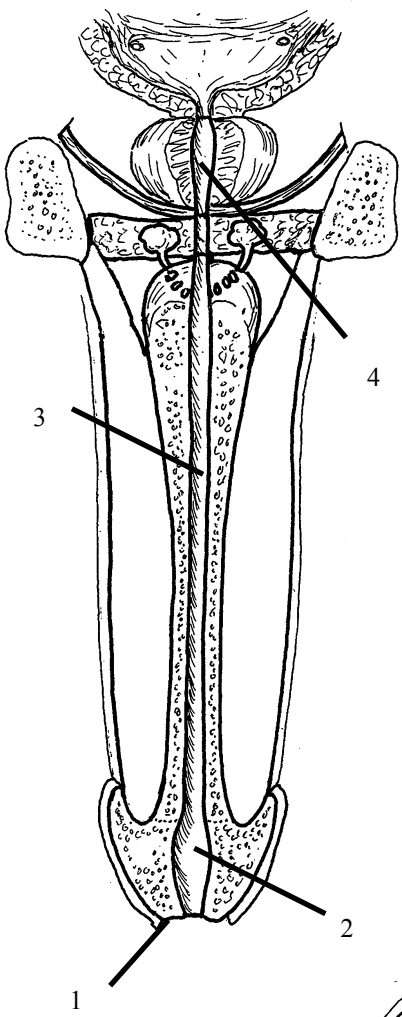

Figure 4. Anatomy of male genital tract

Types of epithelia in external genitalia of the male

Stratified squamous cell epithelium with a varying degree of keratinization covers the outer genitals. Keratinization tends to disappear in the area between the outer and inner parts of the prepuce (1). The distal part of the male urethra is the fossa navicularis (2). It is covered by stratified squamous cell epithelium, similar to the one in the glans penis. The proximal part of the urethra (3) is lined with pseudostratified columnar epithelium and there is a junction zone between the two different types of epithelia (stratified squamous and stratified columnar epithelia). A bladder-like epithelium covers the prostatic part of the urethra (4).

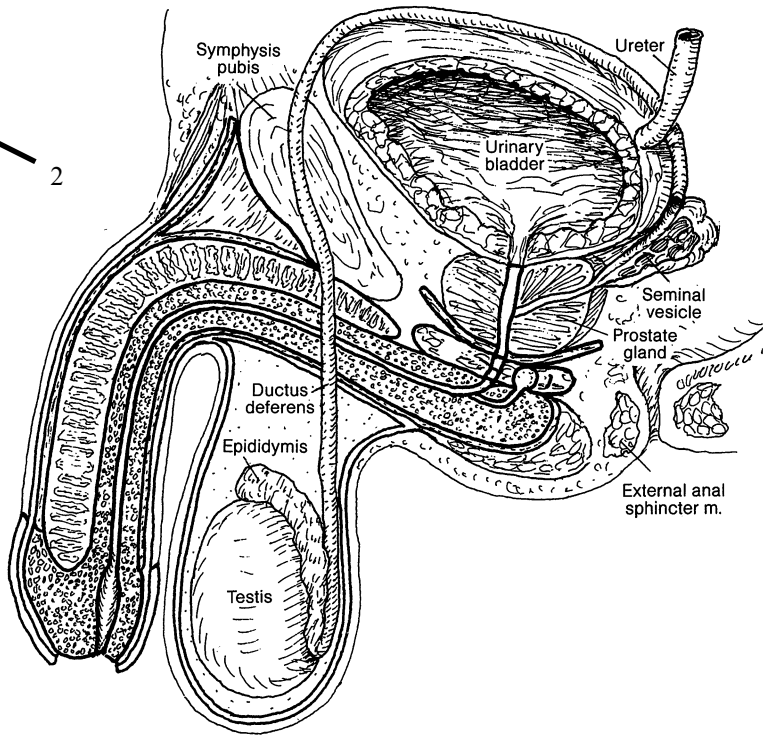

Figure 5. Anatomy of the oral cavity (**a**) and oropharynx in the head and neck region (**b**).

Types of epithelia in the oral site

The oral cavity includes the mobile part of the tongue (the base of the tongue is situated in the oropharynx), gums, floor of the mouth, buccal mucosa, and hard palate.

The oral mucosa consists of stratified squamous epithelium. There are three different types of epithelia in the oral cavity: 1) *masticatory mucosa* with keratinized epithelium covering the hard palate and the attached gingiva; 2) *lining mucosa* with non-keratinized epithelium covering the buccal mucosa and the inner mucosa of the lips and the floor of the mouth; 3) *specialized mucosa* covering the dorsal part of the tongue where there is partly keratinized epithelium, taste buds and lingual papillae.

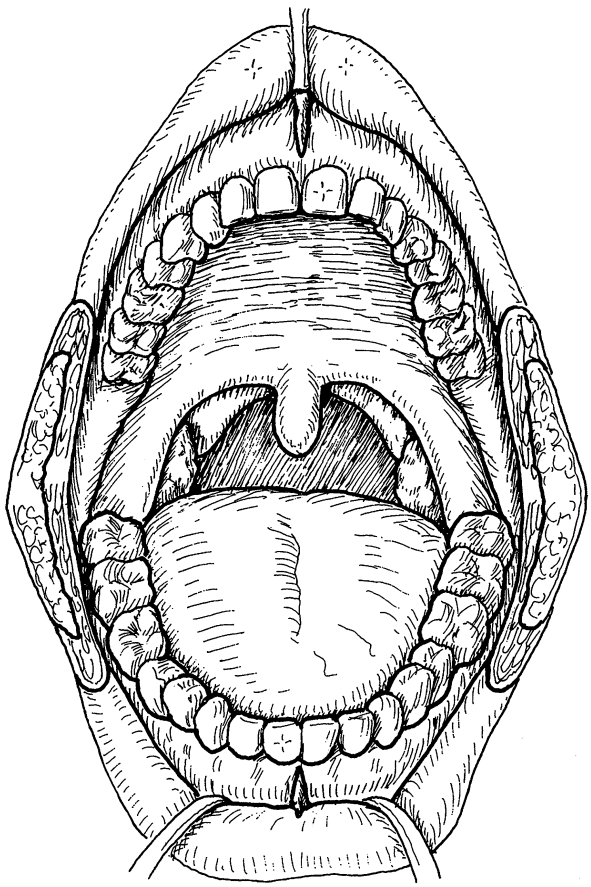

Figure 5 a. The oral cavity.

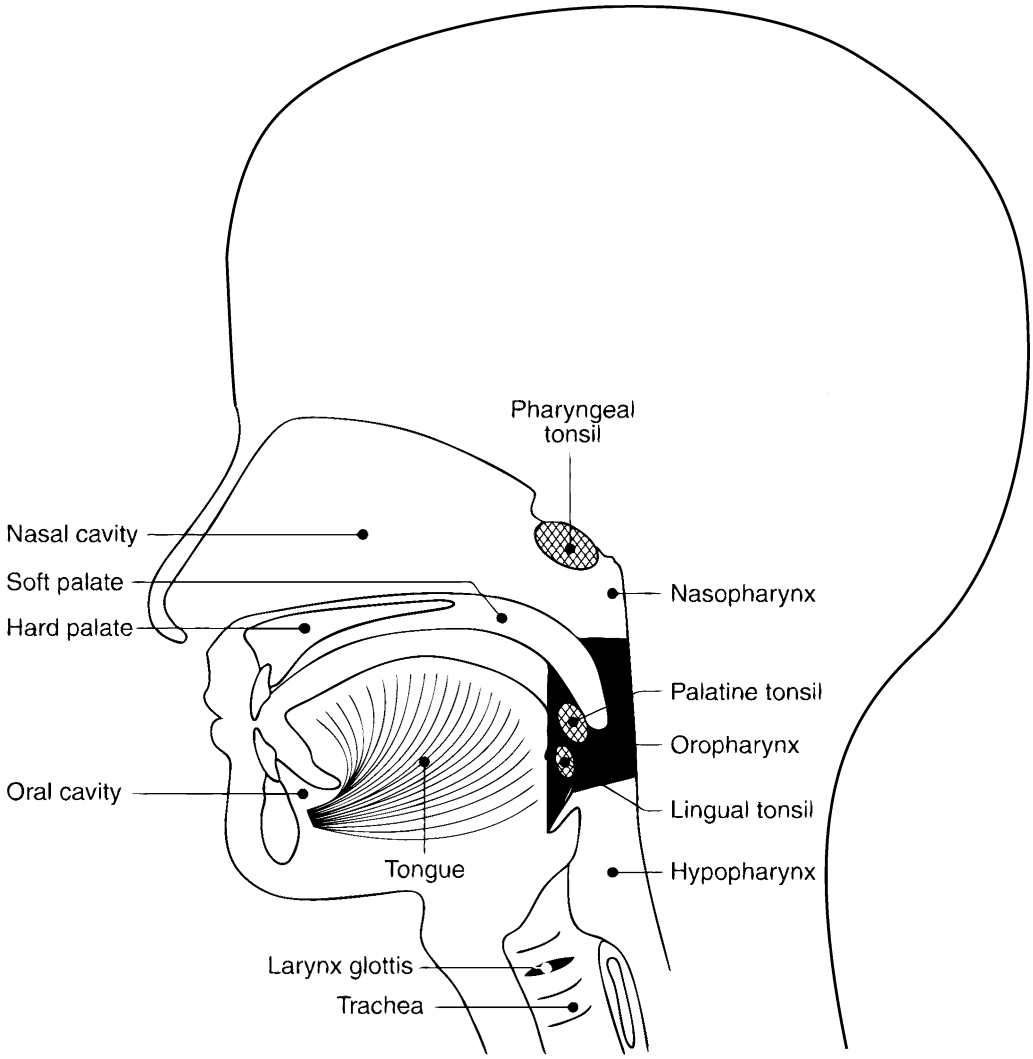

Figure 5 b. The oropharynx in the head and neck region.

2.2.2 Life cycle of papillomavirus infection

The HPV infection cycle begins with viral entry into the primitive basal cells, where the virus establishes itself in the nucleus as an episome with a low copy number (around 50-100 copies per cell). At this stage, the viral early proteins E1, E2, E6 and E7 are expressed at a low level (Doorbar, 2005). After cell division, the infected daughter cells arise from the primitive stem-cell-like compartment to the proliferating layer of the epithelium called the suprabasal region. At this stage, viral expression is still minimal (Stanley et al., 2007; Doorbar et al., 2012). When the infected

keratinocyte enters the differentiating compartment of the epithelium and exits the cell cycle, there is massive up-regulation of the expression of all early viral genes, also the early genes E6 and E7. These genes stimulate cellular proliferation and DNA synthesis by interfering with and ultimately inhibiting several cell-cycle regulators. This allows high level amplification of the viral genome (Doorbar, 2005). Now the infected cells contain at least 1,000 viral copies. Finally, in the terminally differentiated cells, the surface late proteins L1 and L2 are expressed and the viral DNA is packaged and shed from the surface of the epithelium. The duration of the infectious cycle is 2-3 weeks and harmonizes with the maturation time of the basal keratinocyte (Doorbar, 2005; Stanley, 2010). Part of the infectious cycle takes place in epithelial cells that have already exited the cell cycle and are differentiating. This poses a challenge to the HPV, since it requires cellular DNA polymerases and replication factors unavailable in non-dividing cells. Consequently, the virus encodes proteins that reactivate cellular DNA synthesis in noncoding cells, inhibit apoptosis and delay differentiation of the infected keratinocytes. This permits the viral replication necessary for HPV. An important adverse event may occur in when there is interference with the normal cell cycle of the epithelium: HR-HPV replication may deregulate the growth control in the infected cell and further develop into cancer (zur Hausen, 2002). The infectious cycle of papillomavirus is presented in **Figure 6.**

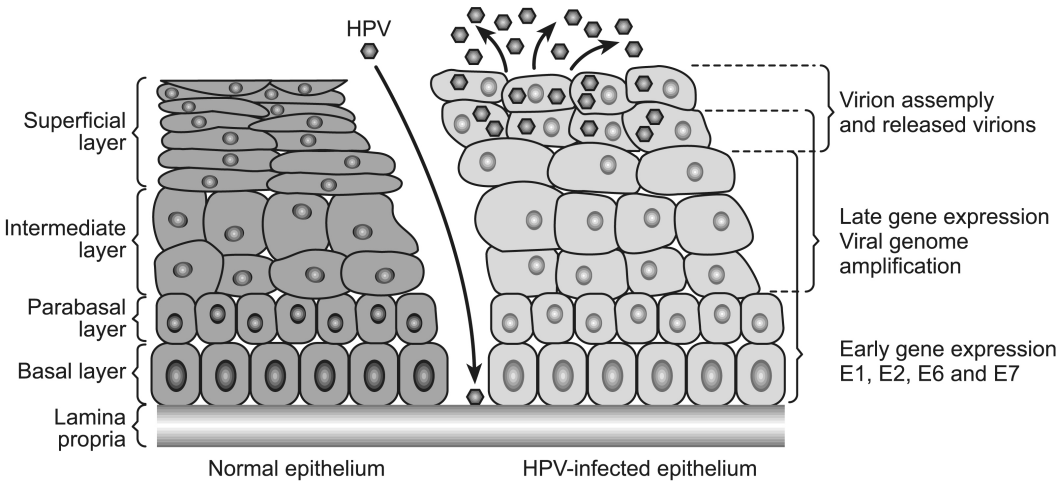

Figure 6. Infectious cycle of the papillomavirus (modified from Moody and Laimins. Human papillomavirus oncoproteins: pathways to transformation. Nat Rev Cancer 2010;10(8):550-60).

2.3 Natural course of the HPV infection

The natural history of genital HPV infections in women is well-characterized, although the key question of why a HPV infection becomes persistent still remains unanswered. Very little is known about the role of the first HPV infection as a modulator of a subsequent HPV infection occurring at another anatomical site.

Thus far, the literature on the natural history of HPV infections has focused on infections of the female genital tract, and data on male infections have been scanty. There is, nevertheless, an obvious need for information on the natural course of male HPV infections, and this has led to some large male cohort follow up-studies (Giuliano et al., 2011; Kreimer et al., 2011). Differences between the characteristics of infections of distinct anatomical regions have not been defined, and thus sampling has been targeted at multiple anatomical sites in the head and neck region and in anogenital site. A simplified concept of the natural course of cervical HPV infections is presented in **Figure 7.**

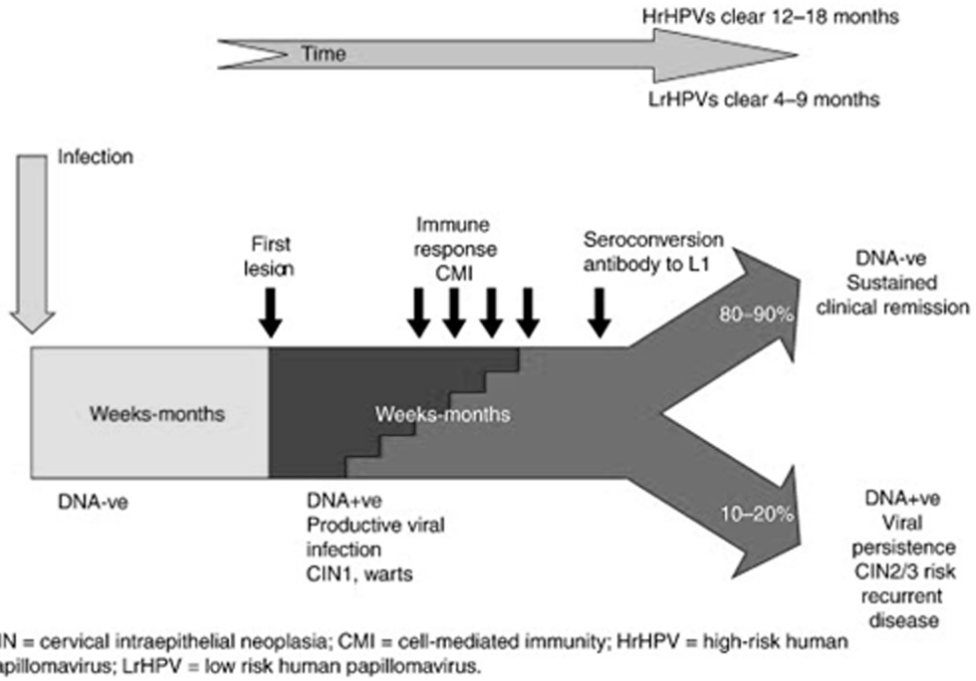

Figure 7. Natural course of cervical HPV infection. Re-publication permission from Professor Margaret Stanley, Director of Research, Department of Pathology, University of Cambridge (**Figure** from Gynecol Oncol. 2010:117(2Suppl):S5-10).

2.3.1 Transmission

A fundamental challenge presented by HPV transmission is that there are no means to trace the exact time of the first HPV infection. Most HPV infections are asymptomatic and both sexes can be carriers (Syrjänen and Syrjänen, 2000; Reiter et al., 2010; Gillison et al., 2012). Sexual intercourse has been regarded as the primary route for transmission of HPV infection requiring skin-skin, skin-mucosa, or mucosa-mucosa contact (Burchell et al., 2006). Semen has also been suggested as a vector for HPV transmission from male to female partners, yet data is controversial (Rintala et al., 2004; Wang et al., 2010). Transmission model studies imply that the median probability of transmission of HPV

per sex act is 40% (range 5 – 100%) (Burchell et al., 2006). At present, the scanty data on genotype-specific concordance among sexual partners report concordance rates varying from 2% to 87% (Hippeläinen et al., 1994b; Castellsague et al., 1997; Bleeker et al., 2005; Reiter et al., 2010). Bleeker and co-workers (2005) reported a cross-sectional concordance of female and male sexual partners with the same genotype in 37% of the cases. Higher concordance rates have been related to short abstinence times before sampling (Moscicki et al., 2012; Widdice et al., 2013).

HPV may also be transmitted through autoinoculation, i.e., transmission of the virus between anatomic sites of the same individual (Moscicki et al., 2012). In females, HPV residing in the anal region might serve as a reservoir for the cervix and vice versa (Moscicki et al., 2012). This assumption gains substantial support by the fact that there is often vaginal discharge on the perineum. Consequently, non-penetrative sex and fingers may also contribute to transmission (Koliopoulos et al., 2013). In a study on 212 sexually active university students aged 18-24 years, current infection with the same HPV genotype in the genitals and hyponychium of the fingers was associated with incident oral infections (Edelstein et al., 2012). The association was higher than reported between the oral cavity and the uterine cervix in women. Interestingly, hyponychial infection was detected with the same HPV genotype as in the oral acvity in 2/3 of males with an oral HPV infection. However, factors possibly related to autoinoculation, e.g. masturbation, fingernail biting, current genital warts and circumcision, were not risk factors in that study (Edelstein et al., 2012).

HPV infection can also be transmitted via manual-to-genital or oral-to-genital routes (heteroinoculation) (Kellokoski et al., 1990; Hippeläinen et al., 1994a; Hippeläinen et al., 1994b; Syrjänen, 2010). Also vertical transmission from mother to neonate during and after pregnancy occurs (Syrjänen and Puranen, 2000; Rintala et al., 2005a; Sarkola et al., 2008a; Sarkola et al., 2008b; Syrjänen, 2010; Merckx et al., 2013). A recent meta-analysis indicated that children of HPV-positive mothers had a 33% higher risk of becoming infected than children of HPV-negative mothers. The risk was 45% when only HR-HPV infection was considered (Merckx et al., 2013). Thus, the literature implies that the mother is playing a significant role as the main transmitter of HPV infection to her child (Syrjänen, 2010). The role of the father as a transmitter is not clear. In the Finnish Family HPV study the dynamics of the HPV infection among family members has been studied and subclinical HPV infection of the father was not among the independent risk factors for the newborn up to age 24 months (Rintala et al., 2005b). HPV seropositivity is markedly higher in children and teenagers than adults (Muller et al., 1995), implicating that non-sexual transmission early in life may be a significant way of acquiring the infection (Rintala et al., 2005a; Rintala et al., 2005b; Syrjänen, 2010).

2.3.2 Natural immunity

In the course of natural immunity, HPV infected epithelial cells produce E2 and E6 proteins associated with viral genome persistence and replication, and L1 and L2 capsid proteins. The Langerhan's cells and B-cells recognize these products, which induces activation of T cells, which further limits the infection, and activates the humoral immunity of B-cells (Stanley, 2010). The cellular immunity turns on in a matter of weeks, while stimulation of the humoral immunity may take months; in some individuals humoral HPV specific immunity is totally missing (Kjellberg, 1999; Wikström et al., 1995; Stanley, 2010). Serologic testing for IgG antibodies to HPV may measure cumulative exposure, thus, indicating the past infection of the persons who have no history or current signs of infection. The detection of HPV antibodies may be more common in persons who have had macroscopically visible lesions, such as anogenital warts (Carter et al., 2000). However, not all individuals with genital warts have antibodies to HPV (Wikström et al., 1995a; Wikström et al., 2005b; Wikström et al., 1997; Dunne et al., 2006; Stanley, 2010).

There is very little information on the dynamics of HPV serology in men. The seroprevalence of HPV16 and HPV18 in asymptomatic heterosexual males varies from 11% to 17%, and 6% to 18%, respectively, while the serological dynamics of HPV6 and HPV11 is less well known (Dunne et al al., 2006; Dunne et al., 2009; Lu et al., 2011). According to previous literature, men have a lower level of antibodies across all ages compared to women; this implicates stronger immunity among women (Svare et al., 1997; Kreimer et al., 2004; Markowitz et al., 2009). According to a large cohort of both genders (n=4,303), increasing age and life time number of sexual partners were associated with higher levels of antibodies against HPV6 and 11 (Markowitz et al., 2009). More rapid clearance and stronger immune response may reduce the likelihood of asymptomatic HPV infections to progress to lesions (Anic et al., 2012).

2.3.3 Progression

Fortunately, most HPV infections clear, but a minority of them persist and this increases the possibility of cellular transformation, which ultimately may lead to immortal cells and invasive lesion (**Figure 8**). Several co-factors contribute to the progression of HPV infections: herpes simplex virus, cytomegalovirus, human herpes virus 6, human immunodeficiency virus, hormonal factors (e.g. oral contraceptives), recurring vulvovaginitis, UV-radiation, cigarette smoking, nutritional factors (deficiency of ascorbic acid or B-carotine) and a number of chemical carcinogens (Giuliano et al., 2003; Esquenazi et al., 2010). Of these, especially oral contraceptives, smoking and HIV-infection have generally been acknowledged as contributing to the risk. High viral load and integration increase the risk of cervical cancer (Kulmala et al., 2006; Josefsson et al., 2000; Ylitalo et al., 2000). Samples taken from 42 men (including 2,094 specimens from 7 different anatomical sites) had a much lower viral

load than samples taken from the uterine cervix (Flores et al., 2008b). Samples from the penile shaft and anus had a significantly higher viral load than the other anatomical sites of men. A lower viral load among men may implicate that the cervical epithelium is a more favorable site for viral replication than the skin of the male genital organs (Flores et al., 2008b).

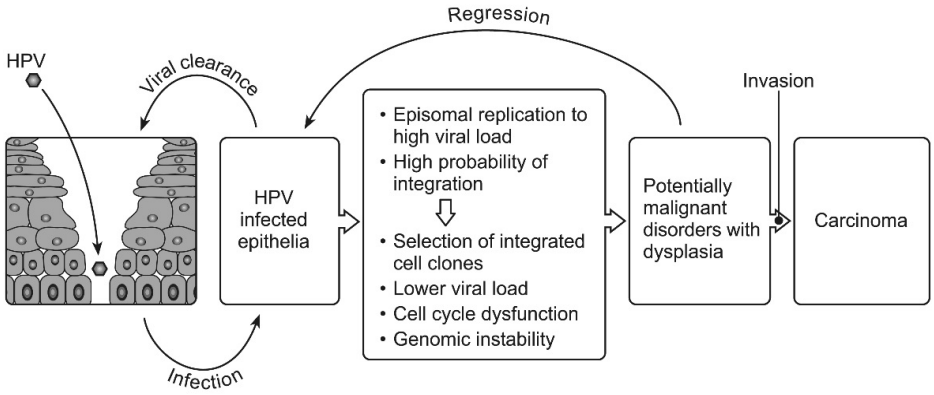

Figure 8. Progression of HPV infection.

2.4 Detection of HPV infections

2.4.1 Gross appearance

The diagnosis of a clinical HPV infection in men is based on visual observations. Adequate light and magnification with a lens or peniscope are required. The urinary meatus may be visualized with an otoscope or a small speculum. Proctoscopy is best avoided until all perianal lesions have been removed to prevent inoculation to anal canal (von Grogh, 1997).

Peniscopy may be used to diagnose penile HPV lesions (von Krogh et al., 2000). Application of acetic acid (3% or 5%) can be used with peniscopy to identify flat lesions and subclinical changes of the mucosa or skin. Parakeratosis with surface nuclear activity and increased cellular density is visualized as acetic whiteness in suspicious locations (Hippeläinen et al., 1991; Hippeläinen et al., 1993b; von Krogh, 1997; von Krogh, 2000). This guides the clinician to take biopsies at appropriate sites of the infected mucosa. However, acetic acid whiteness is not specific only for HPV lesions but may appear also in chronic inflammation and thickening of the epithelium. Abrasions may also appear as acetic acid whiteness. Acetic acid staining cannot be used for examination of mucosal changes in the mouth (Kellokoski et al., 1990; Praetorius, 1997).

2.4.2 Cytology (pap smear)

Cytologically, koilocytosis is the most descriptive cytopathic effect of HPV infections in samples from the female genital tract (Koss and Durfee, 1956). In contrast, cytologic samples from male genitalia are often scanty and unsatisfactory for appropriate analysis; koilocytes are usually absent and often there is only anucleated epithelial material (Krebs, 1989). Sampling may be painful. Although cytology is an inferior detection method of asymptomatic male HPV infection, it is of some value in diagnosing anal HPV infections (Carvalho et al., 2011).

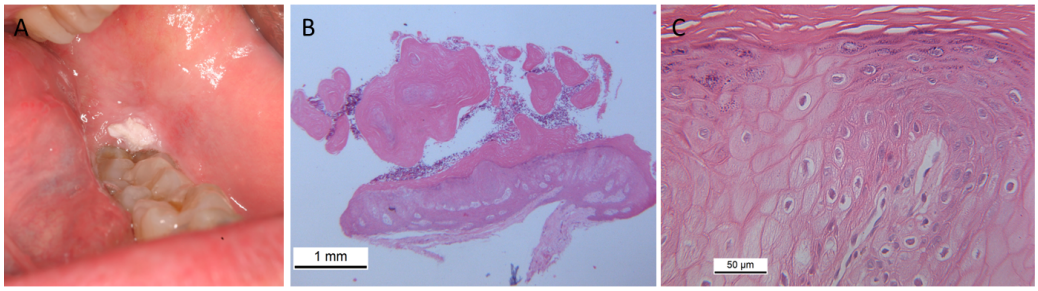

Figure 9. Squamous cell papilloma of oral mucosa, 39 year old male. A. clinical picture B. overview of the histology C. koilocytes in the epithelium.

2.4.3 Histopathology

Koilocytosis is a sign of productive HPV infection in epithelium. In koilocytosis, intermediate layer cells appear with enlarged nuclei and perinuclear cytoplasmic vacuolization, akanthosis and parakeratosis (abnormal keratinization) (**Figure 9**). (Purola and Savia, 1977).

Penile intraepithelial lesion (PIN) is a term used to describe the various grades of intraepithelial lesions of the penis. In these dysplasias, disorganized epithelial layers and cellular polarity, nuclear atypia and abnormal mitoses are seen. PIN is graded as 1 - 3 by the level of dysplasia (**Figure 10**): in grade 1 there are changes only in the basal 1/3 of the epithelium, in grade 2 in 2/3 and in grade 3 there are changes almost throughout the entire epithelium (Wikström et al., 2012; Wickström et al., 2013).

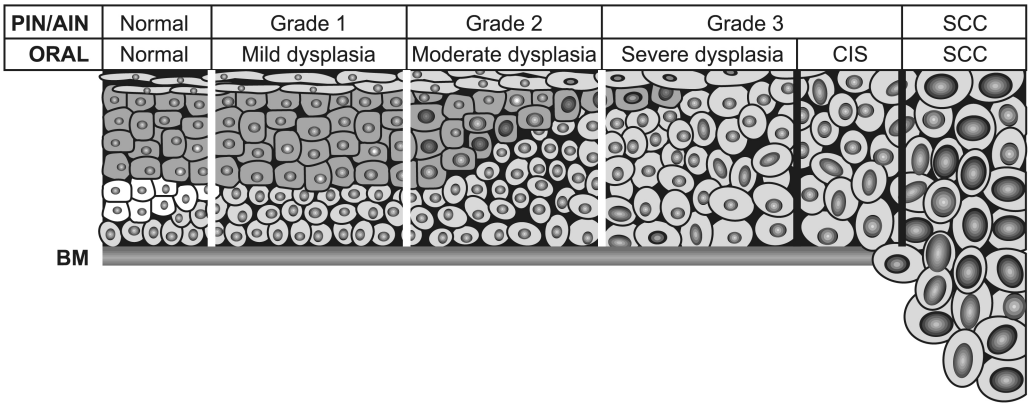

Figure 10. Schematic presentation of HPV infection and intraepithelial lesions caused by HPV. (PIN=penile intraepithelial neoplasia; AIN=anal intraepithelial neoplasia)

2.4.4 HPV DNA detection

To accurately assess HPV infection in males, exfoliated cytology or biopsy specimens and molecular techniques must be employed. HPV tests currently in use rely on the detection of viral nucleic acids in infected tissue. The critical issue is to get an adequate number of infected epithelial cells for HPV DNA/RNA detection.

Nucleic acid hybridization
All nucleic acid hybridization methods rest on HPV DNA or RNA detection with a labeled HPV probe which identifies the analogous sequence in the sample. Southern blot hybridization technique goes back to the 1980's and has been recognized as the gold standard method for HPV detection. Later, in situ hybridization (ISH) was used to localize the HPV DNA specific signals in different lesions. At present, PCR-based techniques, which confirm the hybridization of the PCR product with HPV specific probes, have become routine (Rautava and Syrjänen, 2011).

Polymerase chain reaction (PCR)
PCR is one of the most revolutionary methods of molecule biology. In PCR, a gene or a part of DNA is copied exponentially in vitro in just a few hours. Theoretically, PCR produces more than one billion copies (2^{30}) of a single-stranded DNA molecule after 30 cycles of amplification. PCR assays are sensitive to environmental contamination; previously amplified material may contaminate negative specimens and this may cause false positive results. This problem can be avoided by a set of different procedures (Iftner and Villa, 2003). Usually it is the HPV L1 gene that is targeted for PCR amplification, as it exhibits the least variation among the different mucosal HPV types. There are three commonly used primer pairs for HPV detection:

1) GP5/6 and GP5+/6+ (Van den Brule et al., 1990)
2) MY09/11 pair of degenerate primers and its modified version PGMY09/11 (Gravitt et al., 2000)
3) SPF10 system (Kleter et al., 1998)

Nested PCR
In samples containing a low number of HPV DNA copies or samples with a limited number of cells, nested PCR can be of special value. The nested PCR method includes an additional round of PCR amplification and exploits special internal primers. The same region is targeted by both inner and outer primers. Thus, in buccal smears HPV DNA was detected only in 19% with PGMY09/11 primers while nested PCR yielded a positive result in 74% of the cases (Kay et al., 2002). Higher sensitivity increases the risk of contamination.

2.4.5 Multiplex HPV genotyping (MPG)

MPG is a quantitative and sensitive, high-throughput hybridization method for identification of multiple HR- and LR-HPV genotypes in a single reaction (Schmitt et al., 2006). MPG is based on amplification of HPV DNA by general primer PCR (GP5+/6+) and by further detection of the PCR-products with type-specific oligonucleotide probes coupled to fluorescence polystyrene beads. This Luminex® suspension array technology allows detection and genotyping of up to 100 different HPV genotypes **(Figure 11).** Luminex® is only one of the technologies available for multiplex HPV genotyping.

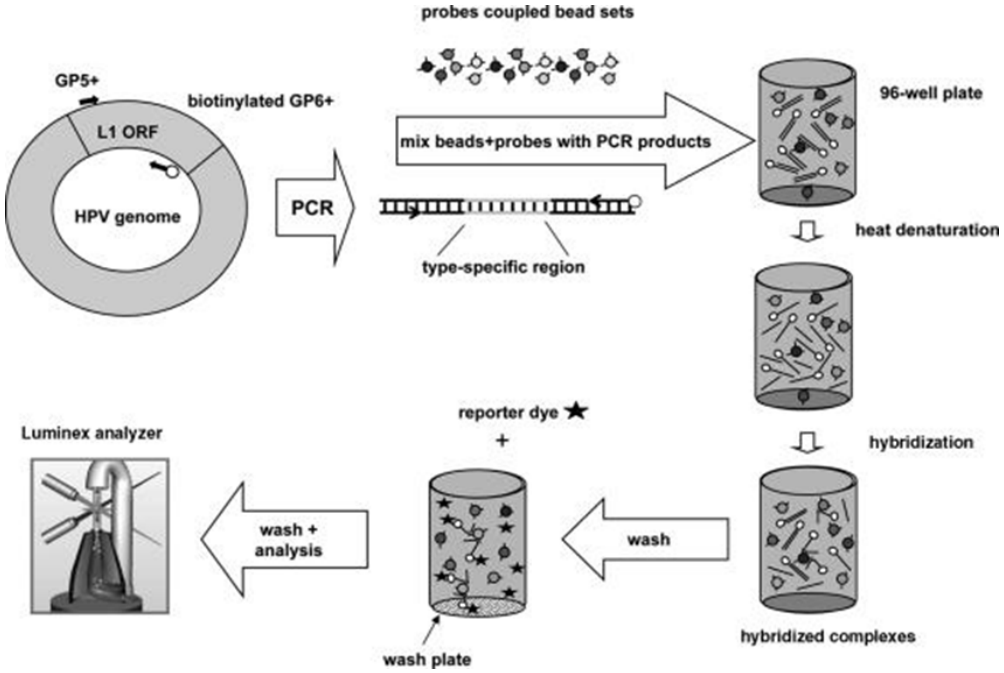

Figure 11. Schematic overview of HPV genotyping of GP5+/6+ PCR products by bead-based multiplex HPV genotyping. ORF, open reading frame (Schmitt and Pawlita, Nucleic Acids Res 2009;37(18):e119).

2.4.6 HPV RNA detection

There are only few commercial HPV RNA tests available. These tests are designed to detect mRNA transcripts of E6 and E7 (Burger et al., 2011; Castle and al., 2013). Since E6 and E7 expressions are thought to increase in proportion to the severity of the dysplastic lesions, the detection of E6/E7 mRNA may be of better prognostic value (compared with that of the DNA detection) in that it could improve the specificity and positive predictive value of screening programs when clinically significant lesions are being sought (Burger et al., 2011). Detection of HPV E6/E7 mRNA and the presence of oncogene activity can be performed by reverse-transcriptase (RT)-PCR or by nucleic acid sequence-based amplification (NASBA) (Snijders et al., 2010). However, in a study by Castle et al. (2013) genotype-specific DNA and E6/E7 mRNA detection methods were compared (2013), and it was found that RNA was only slightly more specific than DNA for identification of anal precancer among 363 HIV positive attendees.

2.4.7 Serology

The interest in serological techniques to identify HPV has been increasing recently in parallel with the emergence of prophylactic HPV vaccines. The most common

technique for analyzing HPV IgG serology is enzyme linked immunosorbent assay (ELISA) where virus-like particles (VLPs) are used as antigens. Serological tests measure, in principle, past HPV exposure and these tests are not site-specific (Schmitt et al., 2006).

2.4.8 Sampling

Anogenital region

There are three sites in the male genital tract which provide a possible reservoir for HPV: the penis, the urethra and the prostate. According to Giuliano et al. (2007), the best anatomical sites for anogenital sampling in males appear to be the glans, the corona, the prepuce and the shaft of the penis. Sampling in these sites is simple and painless. The penile shaft has been reported to carry the highest viral loads (Flores et al., 2008a; Flores et al, 2008b). Urine sampling would be a painless method to obtain a biological sample, but the prevalence of HPV infection in the urine is lower than in the anatomical sites (Forslund et al., 1993; Lazcano-Ponce et al., 2002; Weaver et al., 2004). Obtaining samples of semen and urethral swabs is challenging. Nevertheless, there are reports according to which semen and the urethral site may be a source of reservoir HPV and need therefore special attention (Monsonego et al., 1993; Rintala et al., 2002).

Oral region

A biopsy of an oral mucosal lesion is the best source of HPV DNA in oral region. Only for research purposes, biopsies are not allowed on ethical grounds: a biopsy is invasive and the reservoir site of HPV in the oral mucosa is not known. Thus, sampling of oral epithelial cells for HPV research is done either by brushing or by rinsing/gargling. Detection of asymptomatic HPV infection requires collection of infected basal and parabasal cells and therefore brushing of the non-keratinized oral mucosal sites is the best way to collect a representative sample (Rautava and Syrjänen, 2011). More than 100,000 cells is an adequate number of cells even when less sensitive methods are used (Kellokoski et al., 1992a; Kellokoski et al., 1992b). The oral rinse/gargle method may provide high quality samples although it is challenging to target the infected basal cells with this method. Saliva is heavily loaded by microbes and this may result in an inadequate amount of human DNA. Rinsing tends also to collect cells from the oropharynx, and thus rinsing is not specific to any given site in the oral region (Rautava and Syrjänen, 2011).

2.5 Clinical manifestations

Describing the natural course of HPV infection is challenging, and the definitions of subclinical, latent or chronic infection are not clear (Syrjänen and Syrjänen, 2000).

Subclinical infection may be used for the lesions which turn into visible ones after application of acetic acid. *Latent* infection refers situations where the infection cannot be detected clinically but where HPV DNA is identified in the epithelium. *Chronic* infection refers to persistent infection which may be clinical or subclinical. Generally, as discussed in Chapter 2.3, HPV infections may have a fluctuating course from subclinical or latent infection to clinically manifest infection. **Table 1** shows some of the clinical manifestations caused by HPV infection and the common HPV genotypes related to the lesions.

Table 1. HPV genotype and clinical manifestations.

Disease		Typical HPV genotypes
Common warts		HPV 2, 4, 7
Flat plane warts		HPV 3, 10
Plantar warts		HPV 1, 2, 4
Anogenital warts	External warts	HPV 3, 6, 10, 11
	Buschke-Lowenstein tumor	HPV 6
	Bowenoid papulosis	HPV 16, 55
Anogenital cancers and premalignant lesions	High-risk types	HPV 16, 18, 31, 33, 45, 51, 52
	Probably high risk types	HPV 68
	Possible high risk types	HPV 26, 53, 64, 65, 66, 67, 69, 70, 73, 82
Oral lesions	Oral papillomas	HPV 2, 6, 7, 11, 16, 18, 32, 57
	Laryngeal papillomas	HPV 6, 11
	Focal epithelial hyperplasia	HPV13, 32
	Head and neck cancers	HPV16, 18, HPV6, 11

Modified from Cubie HA. Diseases associated with human papillomavirus infection. Virology 2013;445(1-2):21-34

2.5.1 Benign lesions

Anogenital sites

Following vegetative replication of HPVs, productive HPV infection ensues and this induces proliferation of the epithelial squamous cells and finally benign tumors. This process is a continuum. Anogenital warts are common clinical manifestations of HPV infection (Winer et al., 2005). In addition to warts, condyloma accuminatum, giant condyloma and bowenoid pigmented papulosis are HPV induced anogenital lesions.

Oral cavity

In the oral cavity, HPV infections may cause papillomas, condyloma accuminatum, verruca vulgaris and focal epithelial hyperplasia (Heck's disease) (Praetorius, 1997; Syrjänen and Syrjänen, 2000; Rautava and Syrjänen, 2012; Prabhu and Wilson, 2013). In the head and neck region, LR-HPV infection causes recurrent respiratory papillomatosis which is a benign, although sometimes life-threatening condition mainly affecting young children and young adults. In recurrent respiratory

papillomatosis, HPV11 has been linked to even more severe clinical conditions than HPV6 (Syrjänen, 2010).

2.5.2 Malignant lesions

Epidemiological studies have firmly shown that there is a causal relation between HPV infections and cancers of certain anatomical sites. In 1995, the International Agency of Research on Cancer (IARC) monograph working group announced that there was sufficient evidence to fullfill Hill's criteria of causality and announced that HPV16 and HPV18 infection are carcinogenic conditions (zur Hausen, 2002; zur Hausen, 2009).

For HPV-associated malignancy, chronic HPV infection, i.e. HPV persistence, is a condition *sine qua non* for malignant transformation. The crucial step in carcinogenesis seems to relate to episomal viral DNA becoming integrated into the DNA of the infected cell. Disruption of the E2 region and expression of oncoproteins E6 and E7 inhibit the tumor suppressors, which can induce progession of malignant cells (zur Hausen, 2002). The percentages of HPV-associated cancers at different anatomical regions of man are illustrated in **Figure 13**.

Anogenital sites
Clinically, among men, HPV is associated with penile (PIN) and anal (AIN) intraepithelial neoplasias graded as low, moderate and severe dysplasias (PIN 1-3, AIN 1-3). If these premalignant lesions are not treated properly and in good time, malignant transformation caused by the infection may lead to invasive squamous cell carcinoma (SCC).

Penile cancer is a rare disease and accounts for less than 0.5% of all cancers among males (Parkin and Bray, 2006). Approximately 40-50% of all invasive penile carcinomas are attributable to HPV (Kayes et al., 2007; Moscicki et al., 2012).

The most crucial causative factor for cancer induction in the anal cancer is HPV infection by the oncogenic virus HPV16. Although anal cancer is an uncommon malignancy affecting middle-aged adults, its incidence has increased among both men and women since the 1980's (Palefsky, 2007; Palefsky, 2008; Mocicki et al., 2012).

Oral cavity
There is growing evidence that HPV is associated with head and neck cancers, including neoplasias of the oral cavity, the oropharynx, the hypopharynx and the larynx (Syrjänen et al., 1982; Syrjänen et al., 1983; Kreimer et al., 2005; Gillison et al., 2001). It is estimated that the HPV attribution is 33-72% for oropharyngeal cancers, 24% for oral cancers and 24% for laryngeal cancers (Isayeva et al., 2012). There is growing evidence of synergism between HPV infection and cigarette smoking in increasing the

risk of oral cancer (Schabath et al., 2012; Smith et al., 2012), which contradicts a previous notion by Fakhry and Gillison (2006).

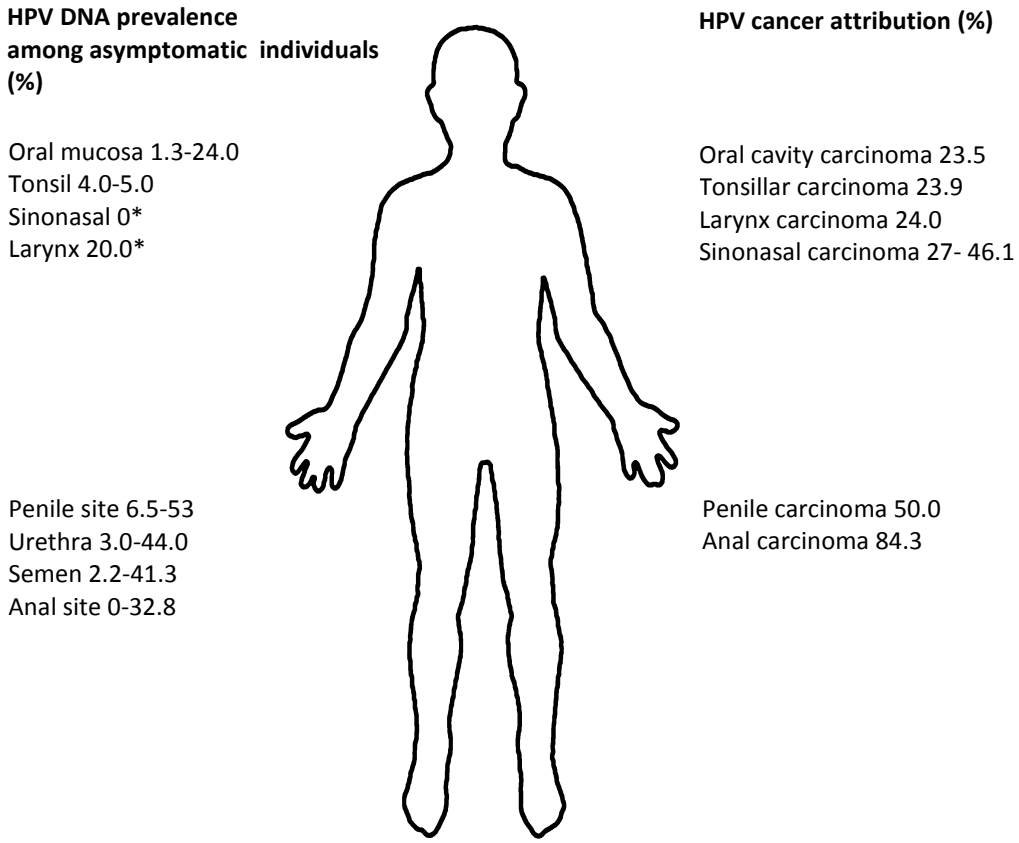

Figure 13. HPV attribution by anatomical site. *Only one study available.

2.6 Asymptomatic HPV infection

2.6.1 Anogenital infections

Prevalence of anogenital infections

Anogenital HPV infections are highly prevalent among sexually active men. The overall detection rate varies from 20% to 73% among healthy males, as shown in **Table 2** (Hippeläinen et al., 1993; Franceschi et al., 2002; Weaver et al., 2004; Kjaer et al., 2005; Dunne, 2006; Giuliano et al., 2010). The detection rates depend on which cohort is examined, which anatomical site is sampled and which method is used to detect HPV. Generally, the prevalence of anogenital HPV infections among men has been estimated even to exceed that of women (Dunne et al., 2006).

Incident anogenital infections

One of the main weaknesses of the data on the incidence of HPV infections among men is a short follow-up time. The annual cumulative risk of a new genital HPV infection in a cohort of the US males was 29.2 % (Giuliano et al., 2008). In the large Human Papillomavirus Infection in Men (HIM) -cohort study with six months of follow-up, the acquisition rate of anal HPV infection was several times higher among MSM than MSW (Nyitray et al., 2011).

Clearance and persistence of anogenital infection

HPV infections may be less likely to persist in men than in women (Giuliano et al., 2010). In men, the mean time to clearance of any HPV infection ranges from 5.9 to 7.5 months; 75% of the infections clear within 12 months (Giuliano et al., 2010; Giuliano et al., 2011). Hippeläinen et al. (1994b) studied 318 men at risk (94.3% partners of HPV infected women) and reported a median clearance time for HPV-positive men of 15.2 months (95% CI 12.2-21.3 months). The clearance of anogenital HPV infections of women has, by comparison, been reported to range between 5.1 and 15.4 months (Trottier et al., 2008).

Infections caused by HR-HPV types, especially by HPV16, persist among women longer than the ones associated with LR-HPV types (Munoz et al., 2004; Trottier et al., 2008; Louvanto et al., 2010). Also, immunodeficiency and MSM have been linked with prolonged persistence (Parisi et al., 2011). Anal HPV seems to persist by its natural course longer among MSM than among MSW. In a large multicenter study with a follow-up of six months, 16.0% of MSM had a persistent oncogenic HPV infection (5.1% HPV16); among MSW the figure was 1.6% (Nyitray et al., 2011). In a 4-year-follow-up-study of MSM by Nyitray et al. (2011), 73% of the HPV16 and 53% of the HPV6 infections persisted.

Predictors of anogenital HPV infections

Sexual behavior

The risk factors for anogenital HPV infection in males associated with sexual behavior include young age at sexual debut; large number of regular, lifetime, and recent sexual partners; and large number of sexual partners before and during marriage (Dunne et al., 2006). The incidence of HPV detection among males is also linked to their female sex partners' sexual history: their lifetime number of sex partners and their frequency of sexual intercourse. Furthermore, males with more than three sex partners and males practicing anal intercourse with men are also independently associated with HPV acquisition. Thus, in a male cohort of 1,305 men, 12% of MSW had anal HPV DNA, while in MSM group the prevalence was 47% (Nyitray et al. 2011). An observation made by Hippeläinen et al. (1994a) was that sexual promiscuity is a risk factor for anogenital HPV infection.

Condom use

The effect of condom use has been evaluated in several cross-sectional analyses (Hippeläinen et al., 1994, Franseschi et al., 2002; Castellsague et al., 2002; Baldwin et al., 2004; Lajous et al., 2005; Repp et al., 2012; Pierce Campbell et al., 2013). In 2000, the US National Institutes of Health concluded that there was insufficient epidemiologic evidence that condom use reduces the risk of HPV transmission (Fitch et al., 2002). It has been estimated that the protection provided by regular condom is only 60%. Although the highest prevalence of HPV DNA is at the penile site, HPV can be transmitted by unprotected areas of the skin, as well, e.g. HPV on the skin of the scrotum (Winer, 2006). There are many additional factors that affect the effectivity of condoms to prevent STD: user experience, STD infectivity, cumulative risk, user failure, method failure and mode of transmission of the STD (Fitch et al., 2002). In the HIM Study, condom use practices differed by country. In the US, men who reported that they always use condoms had the strongest association between condom use and reduced detection of HPV, but in a Brazilian cohort condom use was borderline protective, while in a Mexican cohort, there was no association with reported condom use and protection against HPV infection (Repp et al., 2012). In a large cohort study on 1,471 Finnish military conscripts, condom use turned out to be protective against HPV infection (Hippeläinen et al., 1993a).

Circumcision

Circumcision reduces the frequency of genital HPV infections (Auvert et al., 2009; Gray et al., 2010; Gray et al., 2010; Anic and Giuliano, 2011; Larke et al., 2011; Albero et al., 2012; Tobian et al., 2013). However, there is a strong cultural bias relating to circumcision (Frisch et al., 2013). The mechanism by which circumcision protects against HPV infection is not fully understood. The outer surface of the prepuce is covered by keratinized stratified squamous epithelium providing a protective barrier against HPV infection, but the inner aspect of the foreskin is lined with a non-keratinized mucosa which is more susceptible to the virus. During intercourse, the foreskin is pulled back and minute abrasions occur in inner part of the prepuce. This is how vaginal discharge is exposed to this susceptible site.

Smoking

Smoking has been linked to an increased risk of anogenital HPV infection and HPV disease burden in general (Nyitray et al., 2011). Despite the fact that almost 1/3 of men worldwide are smokers and HPV infection is highly prevalent among men, the attribution of anogenital HPV infection and tobacco smoking has not been studied as much among men as among women (Schabath et al., 2013). In a study by Schabath et al. (2013), current smoking was associated with an increased risk for oncogenic HPV infection at the anogenital site. In a study by Hippeläinen and co-workers (1993a) involving 432 men, smoking was more frequent among those with a HPV infection ($p<0.03$).

Table 2. Prevalence of male genital HPV infection by different studies.

Author	Country	Anatomical site	Assay	Cohort	Age (range)	Sample size	Any type HPV (%)	HR-HPV (%)
Hippeläinen et al., 1993a	Finland	Coronal sulcus, glans, prepuce, urethral meatus – brush	PCR (MY09/11), RFLP	Military conscripts	20	285	17	
Forslund et al., 1993	Sweden	Urethra - wet brush	Type-specific PCR (6, 11, 16, 18, 33)	Military conscripts	21 (20–23)	138	8.7	
Lazcano-Ponce et al., 1998	Mexico	Coronal sulcus, urethra swabs	PCR (GP5+/6+), EIA	Sexually active males	14-55	120	43	19.8
Olatunbosun et al., 2001	Canada	Semen	PCR (MY09/11)	Healthy volunteers	27 (20-41)	40	8.0	
Franceschi et al., 2002	Colombia California Spain Thailand Philippines 1985–1987	Coronal sulcus. Distal urethra, glans - wet swabs	PCR (MY09/11+)	Partners of women with normal cytology	45	717	13	8.3
Rintala et al., 2002	Finland 1998–1999	Vas deferens - biopsy	PCR (MY09/11, GP5+/6+)	Clinically healthymales undergoing vasectomy	40 (33–49)	27	18.5	
Takahashi et al., 2003	Japan	Coronal sulcus, glans, prepuce - self-sampled wet swab	HC II (Digene)	University students	22 (18-35)	75	1.3	1.3
Shin et al., 2004	South Korea	Coronal sulcus, glans, scrotum, and tip - wet brush, urethra - wet swab	Type-specific PCR (6, 11, 16, 18, 31, 33–35, 39, 40, 42–45, 51–54, 56, 68, 59, 66, 68, 73, 70, 74)	University students	22 (18–28)	381	8.7	4.5

Author	Country	Anatomical site	Assay	Cohort	Age (range)	Sample size	Any type HPV (%)	HR-HPV (%)
Weaver et al., 2004	USA, Washington	Glans, prepuce, scrotum, shaft - emery paper, wet swab	PCR (MY09/11+) HMB01	University students	20.5 (18–25)	318	32.8	
Bleeker et al., 2005	Netherlands	penile scrapings, glans corona, prepuce, frenulum	PCR	Partners of women with CIN	22-57	181	72.9	58.6
Hernandez et al., 2006	USA, Hawaii	Coronal sulcus, glans, shaft - wet swab	PCR (MY09/11+) HMB01	University students-physician collected sample	28 (18–63)	136	41.3	
Kjaer et al., 2005	Denmark	Coronal sulcus, and glans - wet swab	PCR (GP5+/6+)	Soldiers	20 (18–29)	374	33.8	
Lajous et al., 2005	Mexico	Coronal sulcus, scrotum, and shaft -swab/brush urethra, and urethral meatus swab	BGH20 /BPCO4	Soldiers	23 (16–40)	1030	44.6	34.8
Rintala et al., 2005	Finland	Distal urethra-brush	PCR (MY09/11, GP5+/6+)	Husbands of third trimester pregnant women	28 (19–43)	76	16.0 (urethral) 20.0 (semen)	
Aquilar et al., 2006	Mexico	Coronal sulcus, glans, scrotum, shaft, tip - wet brush and / distal urethra - wet swab, or meatus urethralis -swab	PCR (MY09/11, L1)	Soldiers	24 (16–50)	582	46.4	
Gupta et al., 2006	India	Coronal sulcus, distal and intrameatal urethra, glans - wet swab, urine	PCR (L1)	Husbands of women with normal cytology	47	30	26.7	

Author	Country	Anatomical site	Assay	Cohort	Age (range)	Sample size	Any type HPV (%)	HR-HPV (%)
Nielson et al., 2007	USA, Florida	Glans/coronal sulcus, shaft, scrotum, perianal area, anus - wet swab	PCR (PGMY 09/11)	Heterosexual men (general public, college) students, military, STD clinic attendees)	18-40	463	65.4	29.2
Patridge et al., 2007	USA, Washington	Glans, shaft, scrotum-emery paper, wet swab	QIAamp PCR	University students	19 (18–20)	240	25.8	20.0
Smith et al., 2007	Kenya	Glans/coronal sulcus, urethra - wet swab	PCR (GP5+/6+)	Sexually active male participants in clinical trial of circumcision	22 (17–25)	98	54	
Ng'ayo et al., 2008	Kenya	Coronal sulcus, shaft, scrotum, perianal - wet swab	QIAamp DNA	Fishermen along Lake Victoria short	31 (18–63)	250	57.6	42.4
Auvert et al, 2009	South Africa	Urethral swab	Roche Amplicor HPV test	Men enrolled in trial of male circumcision	18–24	1,264		18.5
Nyitray et al., 2011	Brazil Mexico USA	coronal sulcus, glans, prepuse, penile shaft, scrotum	PCR (PGMY 09/11) Roche Linear Array	HIM-Study, healthu volunteers	18-70	3326	53.1	30.0
He et al., 2013	China	Penile shaft, glans penis, coronal sulcus, and scrotum	PCR (SPF1/GP6+)	Healthy individuals	25-65	2236	17.5	6.3
Vera-Uehara et al., 2013	Mexico	Penis, glans, sulcus, swab	PCR (PGMY09/11) Roche Linear Array	First and second year medical students		253	19.4	17.4

2.6.2 Oral infections

Prevalence of oral infections

There are less reliable data on the prevalence of oral HPV infections among men than women. Meta-analyses by Miller and Johnstone (2001) and Syrjänen et al. (2012) reported an overall likelihood of detecting HPV at the oral site of more than 10% (both genders). In a recent review of 2,385 males in the US, the prevalence rate was 11% on the basis of rinse and gargle samples (Sanders et al., 2012). The few studies on the occurrence of asymptomatic male oral HPV infection are presented in **Table 3**.

Incident oral infections

In a cohort study from the US, Mexico and Brazil (n=1,626) involving males, incident oral HPV infections were rare (Kreimer et al., 2013). During a median follow-up time of 12.7 months, 4.4% of the men acquired an incident oral HPV infection (95% CI 3.5-5.6; n=115 incident infections), 1.7% (1.2-2.5; n=53 incident infections) an oral oncogenic HPV infection and 0.6% (0.3-1.1; n=18 incident infections) an oral HPV16 infection. During a follow-up of two years, 10% of a male cohort (n=13/131) acquired a new oral HPV infection (Rintala et al., 2006).

Clearance and persistence of oral infection

There are only a few follow-up studies on the clearance of oral HPV infections among men (Rintala et al., 2006; Kreimer et al., 2013). In a recent study by Kreimer et al. (2013) with a follow-up time of 12.7 months, most of the oral HPV infections cleared within one year; the median time of duration of any type of infection was 6.9 months.

As with clearance, there are only a few studies on the persistence of oral HPV infections in healthy men. Parisi and co-workers (2011) reported that 75% of 68 men infected with the human immunodeficiency virus (HIV) had a persistent oral HPV infection at six months of follow-up. Rintala et al. (2006) reported 7% persistence of oral HR-HPV infection among 131 men followed-up for 24 months.

According to recent results of the HIM-Study (n=1626) involving males aged 18-73 years, the median duration of oral HPV infections was 6.9 months (95% CI 6.2-9.3; n=45 cleared) for any HPV genotype; the median duration of HPV16 infections was 7.3 months (6.0-not evaluable; n=5 infections) (Kreimer et al., 2013).

Risk factors for oral HPV infections

Sexual behavior

The association between sexual behavior, especially oral sex, and oral HPV infection is controversial. In the HIM Study, there was no significant association between any measure of oral sexual behavior and acquisition of oral oncogenic HPV (Kreimer et al., 2013). However, according to another study on young men, performing oral sex more than once a week was associated with a significantly increased risk of an incident oral infection (Edelstein et al., 2012). The possible abstinence time was not given. Deep kissing might also contribute to oral HPV transmission. Along with a study by Pickard et al. (2012) involving a college-aged cohort (n=1,000 males and females), open-mouth-kissing was reported as a risk factor for incident oral HPV infection. Increasing age, the lifetime number of kissing or vaginal or oral sex partners and the frequency of marijuana use were also significantly associated with prevalent oral HPV infection by univariate analysis. Sampling was by rinsing, and was thus not specific to oral cavity infection.

Smoking

According to the HIM-Study, the risk for acquiring an oncogenic oral HPV infection is nearly three times (HR=2.80) higher for current smokers and more than two times (HR=2.31) higher for former smokers than for never smokers (Kreimer et al., 2013).

Gender

Data on the prevalence of oral HPV infection among men and women are controversial. In a review by Sanders et al. (2013) on the national prevalence of oral HPV infection and related risk factors in the US adult population, men had a threefold risk (95% CI 2.2 - 4.6) of oral HPV compared to women. In contrast, Miller et al. (1996) reported that HPV positive samples obtained from the healthy oral mucosa were more 1.5-fold more common among females than males.

Age

The prevalence of HPV in men differs significantly between age groups but not linearly (Kreimer et al., 2011). In a cohort of 1,688 healthy men aged 18-75 years the average HPV detection rate was 4%, but among older men the rate was 6% (Kreimer et al., 2011). In another study, a bimodal age pattern was described with peaks at 30-34 years (7.3%; 95% CI 4.6%-11.4%) and at 60-64 years (11.4%; 95% CI 8.5%-15.1%) (Gillison et al., 2012).

Ethnicity

In a study by Pickard et al. (2012), the highest prevalence of oral HPV was detected among non-Hispanic black males (11.4%) who had a 2.7-fold risk compared to non-

hispanic white men. Interestingly and maybe paradoxically, head and neck cancers associated with HPV are less prevalent among non-Hispanic black men than non-Hispanic white men (4% versus 34%) (Settle et al., 2009).

HIV-infection

Kreimer et al. (2004) reported a higher prevalence of oral high-risk HPV infections among HIV-seropositive than HIV-seronegative individuals (13.7% vs. 4.5%; P<.001). In the HIV-seropositive group, the risk of oral HPV positivity increased for persons who had a CD4 cell count below 200 cells/mL, who were HSV (herpes simplex virus)-2 seropositive, who had oral mucosal abnormalities and who had had oral sex with more than one sex partner during the previous year.

Table 3. Prevalence of oral HPV infection among males by different studies.

Reference	Country	Sampling	Assay	Cohort	Age	N	Any type HPV N (%)	HR-HPV N (%)	Genotypes
Summersgill et al., 2001	USA	Saline solution rinse	PCR, dot blot hybridization	Boys in Family Practice or Pediatric outpatient clinics	13-20	97	5 (5.2)	3 (3.1)	16, undetermined type
Kurose et al., 2004	Japan	Cytobrush inside the cheek	Puregene DNA Isolation kit MY09/11 and sequence analysis	Healthy dental clinic visitors	3-85	277	4 (0.6)	1(0.2)	12, 53, 71
Smith et al., 2004	USA	Oral rinse with normal saline	MY09/11, dot blot hybridization	Spouses of pregnant women	18-45	68	4 (5.9)	3 (4.4)	13, 31, 39,
Rintala et al., 2006	Finland	Brush of oral mucosa (5 FU-points)	MY09/11 and GP5+/6+ and Southern blot hybridization	Spouses of pregnant women	28.8 (+/-5)	131		(18–25)	
Smith et al., 2007	USA	Oral rinse with sterile water	QiAamp DNA mini kit (Qiagen) My09/11 and hybridization		16-20	101	0		
D'Souza et al., 2009	USA	Oral rinse and gargle	Puregene based protocol (Qiagen), Roche Linear Array	College-aged men	18-23	210	5 (2.4)	5 (2.4)	16, 35, 39, 51, 66, 84

Reference	Country	Sampling	Assay	Cohort	Age	N	Any type HPV N (%)	HR-HPV N (%)	Genotypes
Kreimer et al., 2011	Brazil, Mexico, USA	Oral rinse / gargle	Puregene based protocol (Qiagen), Roche Linear Array	Multinatinal study (HIM)	18-70	1680	67 (4.0)	(1.3)	16, 31, 35, 39, 51, 52, 56, 58, 59 6, 11, 53, 55, 61, 62, 64, 66, 69, 70, 71, 82, 84, 89
Edelstein et al., 2012	USA	Gargle / rinse and selfcollected oropharyngeal swabs	QiAamp DNA mini kit (Qiagen), MY 09-MY11-HMB01, Luminex®	Male HPV cohort study at the University of Washington	18–24	212	16 (7.5)		6, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 55, 59, 62, 66, 81, 83, 84
Pickard et al., 2012	USA	Oral rinse	DNA extraction PCR with PGMY primers, Roche Linear Array HPV genotyping test	male and female convenience sample from Ohio State University	18–30	463	15 (3.2)		cp6108, 11, 16, 35, 39, 42, 45, 51, 53, 56, 59, 62, 66, 71, 72, 81, 82, 84
Gillison et al., 2012	USA	30-second oral rinse and gargle with mouthwash	DNA extraction PCR with PGMY primers, Roche Linear Array HPV genotyping test	Part of the NHANES 2009-2010	14-69	2748	278 (10.1)		

2.7 Prevention of HPV infection

At present, there are two prophylactic HPV vaccines available, one bivalent (HPV16, 18) Cervarix® (GlaxoSmithKline, London, UK) and the other quadrivalent (HPV6, 11, 16, 18) Gardacil® (Merck & CO, USA). Both vaccines are highly effective and well-tolerated and provide prevention against clinical genital manifestations of HPV (Lehtinen and Paavonen, 2003; Harper, 2009) for women. The efficacy of vaccination with the quadrivalent HPV vaccine of males to prevent genital HPV infection has also been studied (Petäjä et al., 2009; Hillman et al., 2012; Schiller et al., 2012). Efficacy has been documented against genital warts and AIN-lesions (Macartney et al., 2013). In the autumn of 2013, Australia introduced a nationwide vaccination program to cover also boys 12-13 years of age (with catch-up to 15 years of age) (Wilkinson, 2012).

As to the head and neck site, oral anti-VLPs are detectable in vaccinated subjects (Rowhani-Rahbas et al., 2009). According to a four-year-study randomized clinical trial in Costa Rica by Herrero et al. (2013), the prevalence of oral HPV was reduced four years after bivalent vaccination. Although more information is clearly needed on the efficacy of vaccination to prevent oro-pharyngeal HPV infections, these results suggest that primary prevention of these diseases may be possible by appropriate vaccination programs.

3 AIMS OF THE STUDY

The Finnish Family HPV Study was designed to elucidate the dynamics of oral and genital HPV infections among the parents and their newborn. The present study focused on the men (fathers) of this cohort. They were followed up for seven years. The main objective was to describe the natural history of oral HPV infections and the predictors by outcomes. In addition, genital HPV infection among the spouses and the potential influence of this on male HPV infection was examined.

The specific aims were:

to clarify the genotype prevalence of HPV infections in the male genital tract and in semen.

to describe the genotype distribution of oral HPV infections of males and the outcome of these infections during a follow-up time of seven years.

to assess the genotype-specific concordance and associated predictive factors of the HPV infections between the spouses.

4 SUBJECTS, MATERIALS AND METHODS

4.1 Subjects and study design

The Finnish Family HPV study is a longitudinal cohort study, conducted at the Department of Oral Pathology, University of Turku and at the Department of Gynecology and Obstetrics, Turku University Hospital, Turku, Finland. The study was designed to focus on the dynamics of the HPV infections among parents and their offsprings. Pregnant women at the third trimester were enrolled, together with their spouses. The study protocol and its amendments (#2/1998 and #2/2006, #5/2010) have been approved by the Research Ethics Committee of the Turku University Hospital. The members of altogether 329 families were enrolled: 329 mothers, 131 fathers and 331 neonates. The subjects of the present analysis are the 131 fathers (mean age 28.7 years, range 19-46 years) who were followed-up for seven years (**Figure 14**, flowchart). In the original study design, follow-up was scheduled for three years (median 36.8 months; range 32.9-47.3), but was subsequently extended with additional three years (median 77.0 months; range: 50.4-91.5). The 7-year-follow-up visit was completed by 58 fathers. A flowchart of the study summarizes the follow-up visits, the number of male attendees and the samples taken at each visit (**Figure 14**). The specific objectives of the original studies (I-IV) are presented in **Table 4**.

Table 4. Objectives of the original studies included in this thesis.

Objective	Original study
HPV genotypes in male genitalia	I, II
Concordance of HPV genotypes in genital samples among the spouses	I
Changes in sexual habits and their impact on HPV status	II
Point prevalences of oral HPV infection	III
Incidence of oral HPV infections of males	III
Clearance of oral HPV infections	IV
Persistence of oral HPV infections	IV
Risk factors for male HPV infection	I, II, III, IV

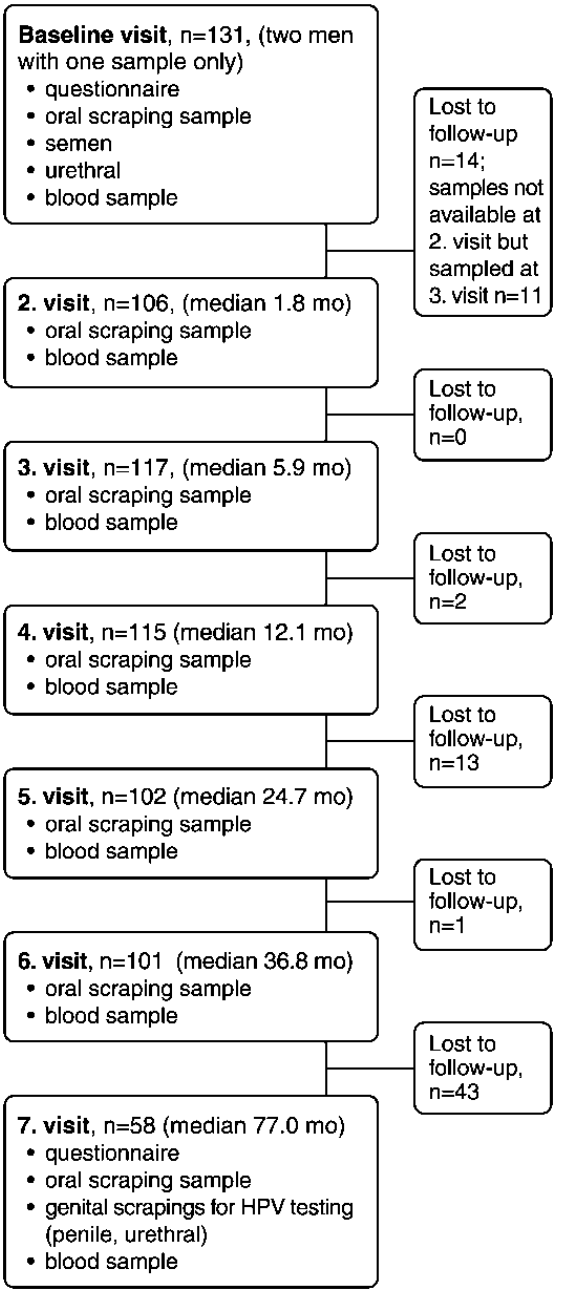

Figure 14. Flowchart of the study (IV)

4.2 Demographic data and sample collection

Demographic data

Demographic data were collected with structured questionnaires at the baseline and 7-year-follow-up visit. All data were collected separately from both partners, treated

confidentially and neither partner had any access to the data of their spouse. The questionnaires addressed the respondents' age, marital status, education and sexual behavior.

Samples

Genital
Urethral (men) and cervical (women) scrapings for HPV testing were taken with a brush (Cytobrush®, MedScand, Malmö, Sweden). Urethral scrapings were directed at the distal part of the urethral mucosa 1 cm deep from the urethral meatus with a small Cytobrush®. After sampling, the brush was placed in a test tube containing 70% ethanol and immediately frozen at -20°C and stored at -70°C.

Cervical samples were collected from the uterine cervix with a brush (Cytobrush®, MedScand, Malmö, Sweden). The brush was placed in a test tube containing 0.05 M phosphate buffered saline with 100 μg of gentamycin and immediately frozen at -20°C and stored at -70°C until analyzed.

Semen
At enrollment, the male subjects delivered a semen sample taken into a plastic container by masturbation. At least two days of abstinence was required. If taken at home, the sample was transported to the laboratory within two hours after ejaculation. Samples were centrifuged in a Sorval MC12V (Sorval Instruments, Zurich, Switzerland) at 3,500 rpm for 15 minutes. Plasma (-20°C) and semen (70°C) were stored separately.

Oral
An oral brush sample (Cytobrush, CooperSurgical, Trumbull; CT, USA) was taken from the buccal mucosa of both cheeks of both spouses with three gentle back-and-forth scrapes in the superior and inferior vestibular areas. The brushes were placed into 70% ethanol. The samples were immediately frozen and stored at -70°C until analyzed.

Clinical examination
At the seven-year visit, two dentists (Jaana Willberg DDS, PhD and Lilli Wideman DDS) performed a careful examination of oral mucosa. Altogether 58 males and their spouses were available for this. Mucosal changes were recorded and photographed using a digital intraoral camera (Planmega, Helsinki, Finland). All lesions in the skin of the face and hands (e.g. warts) were also carefully inspected and photographed. In addition, a dermato-venerologist (Outi Kortekangas–Savolainen MD, PhD) examined the external genitalia of 46 males and the samples were taken from the penile and urethral area.

4.3 HPV DNA detection

4.3.1 DNA isolation

HPV DNA was extracted from the oral and genital scrapings with the high salt method of Miller et al. (1988). A lysis buffer (10mM Tris, 400mM NaCl, 100mM EDTA, 1% SDS) was used for lysing the samples which were further digested overnight with proteinkinase K (10 µl/ml) at 37°C. After this procedure, proteins were precipitated with saturated NaCl and ethanol. DNA was dissolved in 50 µl water, mixed for 15-30 minutes and stored at -20°C.

4.3.2 PCR

HPV-testing was done with nested PCR using MY09 and MY11 as external primers and GP05+/GP06+ as internal primers for the scrapings (Snijder et al., 1990). PCR was done in a 50 µg reaction mixture using Amplitaq Gold DNA polymerase (Perkin Elmer, NJ, USA). The sensitivity of the PCR method was approximately 20 copies of HPV.

4.3.3 HPV genotyping

HPV-genotyping was performed with a Multimetrix kit (Multimetrix, Progen Biotechnik GmbH, Heidelberg, Germany). Altogether 24 LR- and HR-HPV genotypes were identified: LR-HPV6, 11, 42, 43, 44, 70 and HR-HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82. The tests were performed as described in the protocol, except that the earlier nested PCR-products (amplified by MY09/MY11 as outer primers and GP05+/GP06+ as inner primers) were re-amplified for biotinylation using GP05+ and bioGP06+ primers and only half of the volume was used to perform the test, except in the final step where the volyme was 100 µl. The labelled hybrids were analyzed with a Luminex® LX-100 analyzer (Bio-Plex 200 System, Bio-Rad Laboratories, Hercules, CA, USA). A median fluoresence 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 1.5x background MFI (negative control) +5MFI. If any sample was positive for HPV16, the protocol was repeated from the original sample using nested PCR and bead-based HPV16 genotyping assay. This was performed to rule out the possibility of contamination with HPV16 from previous tests. The outline of HPV testing is summarized in **Figure 11.**

4.4 Outcomes of oral sampling

The outcomes of the oral HPV infections are illustrated in **Figure 15**.

At the first level, the outcomes of oral HPV infections in each subject were assessed by comparing the baseline HPV status with that of the last follow-up visit. Outcomes were classified into 6 outcome categories.

- *Always negative* (n=56) and *incident* HPV (n=47) are clear-cut outcomes.
- *Genotype-specific persistence* (n=8) includes cases with two or more consecutive samples testing positive for the same genotype, including the same genotypes as a part of multiple type infections.
- *Non-genotype-specific persistence* (n=7) denotes all cases with two or more consecutive samples testing positive for different HPV genotypes.
- *Fluctuation* (n=2) includes all cases with consecutive samples testing intermittently HPV-positive and HPV-negative, without any two consecutive samples positive for the same or different HPV genotype.
- *Clearance* (n=9) denotes the cases who were baseline positive and cleared the infection during the FU.
- There were seven men with only one sample and who were excluded from the outcome assessments (the 7th category).

The second level was used to refine particularly HPV persistence, because the first-level outcomes number 2-6 were further stratified into three categories of persistence.

- *The three types of persistence*: genotype-specific persistence (n=18), non-genotype-specific persistence (n=10) and persistence of multiple-type infections (n=11).
- *Incident* HPV (n=1)
- *Fluctuation* (n=5)
- *Clearance* (n=53) including all HPV positive cases (at any visit) which were cleared by the last follow-up visit (transient infections).

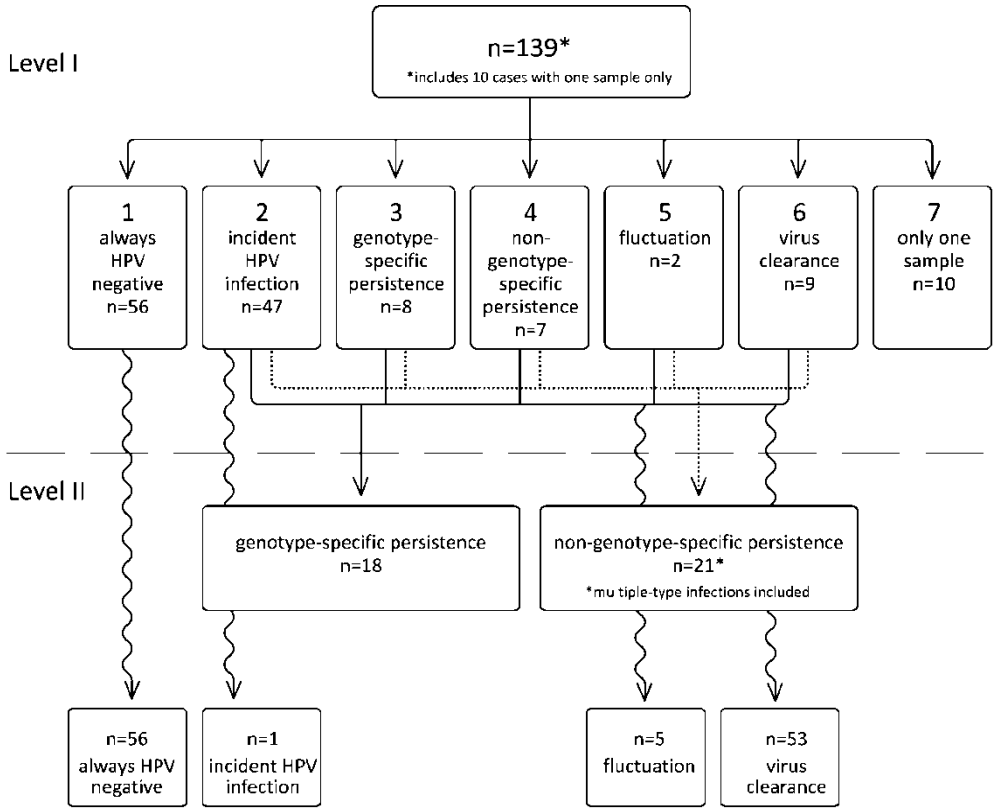

Figure 15. Outcomes of the oral HPV infection during the follow-up (mean 43.8 months, median 36.8 months, range 6.3-36.6 months). (IV)

4.4.1 Actuarial and crude incidence times and rates (III)

The time, in months, from baseline to the first incident infection was recorded separately for actuarial and crude incidence times. For calculation of the actuarial time, all baseline HPV negative men were included in Kaplan-Meier analysis, with cases sensored at the first incident event or at the end of the study for those who did not exhibit an incident event. For calculation of crude incidence times, only those men (n=47) with an incident event were included.

Both actuarial and crude genotype-specific incidence rates (IR) were calculated as the number of incidences per 1,000 male months at risk (mmr), following the same principle. The number of incident events was divided by the total mmr for all baseline HPV-negative men (2,778 mmr) and this included also the men with no incident infection to assess the actuarial incidence rate. To calculate the crude incidence rates, only men with incident events were included, and the number of incident events for each individual genotype was divided by the mmr of those men only. To compare the individual IRs, the rate ratio (RR) statistics (STATA) was used and test-specific 95% confidence intervals (95% CI) were calculated.

4.4.2 Actuarial and crude persistence and clearance times and rates (IV)

The duration of genotype-specific persistence was calculated as the number of months between the first HPV-positive follow-up visit and the last follow-up visit at which the subject still tested positive for that same genotype.

The time, in months, to the first clearance event were calculated from the first HPV-positive visit to the first clearance event, separately for both actuarial and crude times. To calculate the actuarial clearance time, all men (n=74) testing HPV-positive at any time point (at risk for clearance) were included in Kaplan-Meier analysis, i.e., the men who showed clearance as well as the men who did not. To calculate the crude (genotype-specific) clearance times, only the 53 men who exhibited clearance were included. Both actuarial and crude clearance rates were calculated, expressed as events per 1,000 mmr. To compare the individual clearance rates, the RR statistic was used and test-specific 95% confidence intervals (95% CI) were calculated.

4.5 Statistical analyses

All statistical analyses were run using the SPSS® (IBM, NY, USA) and STATA (Stata Corp., College Station, TX, USA) software packages (IBM SPSS Statistics for Windows, version 19.0.1 and STATA/SE 12.0). Frequency tables were analyzed using the $\chi 2$-test, and differences in the means of continuous variables were analyzed with non-parametric (Mann-Whitney or Kruskal-Wallis) tests.

Gee-modelling (I, II)

To analyze the predictors of genotype-specific HPV-persistence, the focus was on persistence of the key HR-HPV-types, i.e., Species 7 (HPV-types 18, 39, 45, 59, 68, 70, 85) and Species 9 (HPV-types 16, 31, 33, 35, 52, 58, 67), similarly as among the mothers (Louvanto et al., 2011). A generalized estimating equation (GEE) modeling was used, clustered by male-ID, with the follow-up time as the time variable and run in univariate and multivariate mode. In the univariate GEE-models, all covariates recorded at baseline were tested and some selected variables from the follow-up questionnaire, previously implicated as potential risk factors of HPV infections in the Finnish Family HPV Study cohort (Rintala et al., 2006; Louvanto et al., 2010). In the final multivariate GEE-model, only the variables that were significant in the univariate model were entered after adjustment for age.

Poisson regression test (III, IV)

We analyzed the covariates of HPV clearance only for Species 7 and Species 9 genotypes using population-averaged (PA) Poisson regression models for panel data, clustered by male-ID, with the follow-up visit as the time variable, an independent within-group correlation structure as the covariance pattern and a robust variance

estimator to account for the within-subject correlation (95% CI). In constructing the univariate and multivariate models, the principles used for GEE (above) were followed.

Intra-class correlation coefficient test (II)

The responses of both spouses at baseline were compared with the responses at the 72-month visit, and, whenever appropriate, the recorded variables were graded as a) unchanged, b) upward or c) downward change. The concordance in the responses between the spouses was calculated using the weighted kappa (intra-class correlation coefficient, ICC) test, with a parallel-mode and two-way random effects model; consistency was assumed and an average measures option was used to interpret ICC and related 95% CI. The impact of all longitudinal changes in the sexual habits of the men as covariates associated with the outcomes (incident, persistent, cleared) of their genital and oral HPV infections was analyzed using univariate regression models. Results are expressed as crude odds ratios (OR) with the 95% confidence intervals (95% CI). All tests were two-sided and considered significant at the p=0.05 level.

4.6 Ethics

The studies were conducted according to the guidelines laid down in the Declaration of Helsinki. The studies were approved by the Ethics Committee of the Hospital District of Southwest Finland. Written informed consent was obtained of the attendees.

5 RESULTS

5.1 Demographic data

Baseline (n=131) (I, II)
At baseline, the mean age of the fathers-to-be was 28 years (range 19-46 years). The child-to-be-born was the first offspring of most of the men (74%). Three or more life-time sexual partners (range 3-30) were reported by 87% of men. Most of the men (79.2%) had never practiced anal sex while 63% had oral sex practices occasionally and 11.7% regularly. Past genital, oral or skin warts was reported by 16.8% (19/113), 7.1% (8/112) and 46.1% (53/115), respectively.

7-year-follow-up visit (n=46) (II)
At the 7-year-follow-up visit, 46 men attended the extended follow-up of the study. 91% reported having the same partner as at baseline, while 9.1% had divorced. 26% had married their partner during follow-up. Almost all men (96%) were employed at the 7-year-follow-up visit.

Reported changes in sexual behavior among spouses during follow-up (II)
Responses to the questionnaires given at baseline by both spouses were compared with those at the 7-year-follow-up visit and the recorded variables were graded as a) unchanged, b) upward or c) downward. The frequency of intercourse and oral sex had decreased in 63% and 23% of the men, respectively. The consumption of alcohol had increased for both spouses during follow-up. Five men and four women had divorced during the follow-up.

5.2 Genital HPV infection in men

Prevalence of HPV in genital samples (I, II)
Totally, 35.9% of the genital male samples tested positive at baseline while 33% were positive at the 7-year-follow-up visit.

Baseline (semen / urethral samples)
Male urethral and semen samples were taken at baseline. Urethral and/or semen samples were HPV positive in 35.9% (47/131) of the men. Semen samples were more frequently positive (n=28/90, 31.1%) than the urethral samples (n=29/128, 22.7%). There were 87 men with both semen and urethral samples available. HPV16 was the most common genotype present in 39.3% of HPV positive semen samples. Multiple type infection was detected at the urethral site in 24.1% and in the semen in 21.6% of

the men. The percent distribution of HPV genotypes in urethral and semen samples are given in **Figures 16** and **17**.

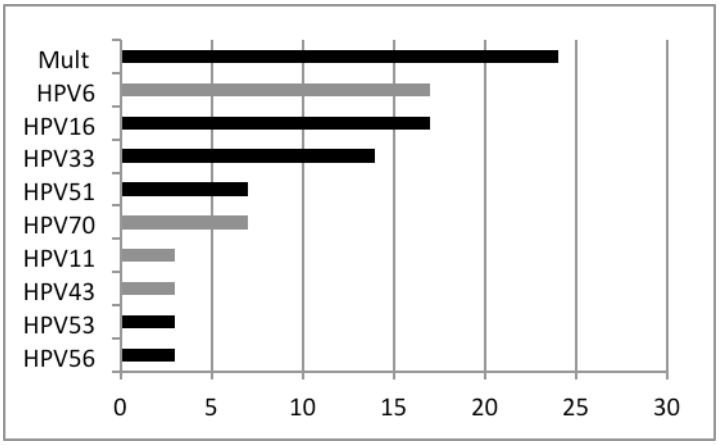

Figure 16. Percent distribution of HPV genotypes among HPV positive urethral samples at baseline. Total HPV positivity was 22.7% (29/128).

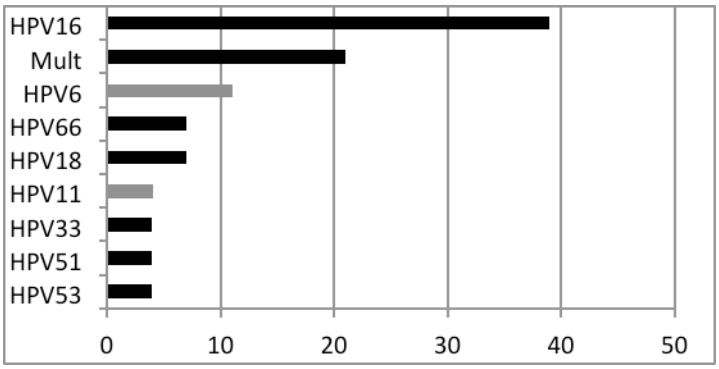

Figure 17. Percent distribution of HPV genotypes among HPV positive semen samples at baseline. Total HPV positivity was 31.1% (28/90).

7-year-follow-up (penile / urethral samples) (II)

46 men were available for genital sampling at the seventh visit. Penile and urethral samples tested HPV positive for 24% (11/46) and 22% (10/45) of the men, respectively. The genotype distribution in these samples is summarized in **Figures 18** and **19**. HPV16 was the single most common genotype in the urethral samples, HPV16 and HPV70 in the penile samples.

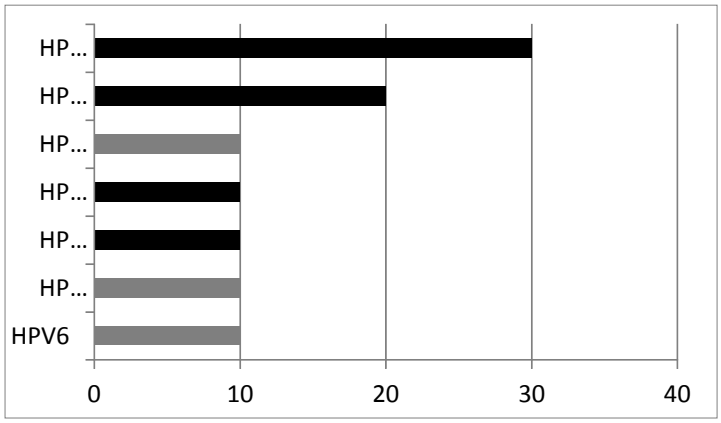

Figure 18. Percent distribution of HPV genotypes among HPV positive urethral samples at the 7-year-follow-up visit. HPV positivity was 22.2% (10/45).

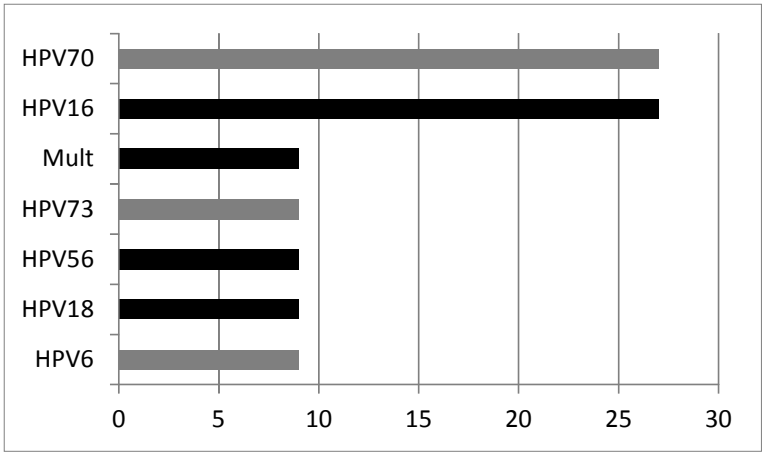

Figure 19. Percent distribution of HPV genotypes among HPV positive penile samples at the 7-year visit. HPV positivity was 23.9% (11/46).

Incident genital infections (II)

Genital samples were collected at baseline and at the 7-year-follow-up visit. Totally, 43 men were available at the two time points for genital sampling. An incident infection was found in 10 (23.3%) men.

Clearance of genital infection (II)

Genital sampling was performed at two distinct time-points. Among the 43 men with two samples (baseline and 7-year-follow-up), eight out of 13 baseline HPV positive men had cleared their any type genital HPV infection by the end of the study.

Genital HPV persistence (II)

Among the 43 men, only 1 had a genotype-specific persistent genital HPV infection (HPV53). A decrease in the number of sexual intercourse reported by the female spouse was negatively associated with (=protected for) any-type of persistent HPV infection of the spouse, i.e., none of the spouses of these women had HPV infection at 7-year-FU-point (p=0.032).

5.3 Oral HPV infection in men

Prevalence of oral HPV infections

Species-specific prevalence (oral samples) (II, III)

The most dominant Species among oral HPV infections throughout the follow-up was Species 9 (the following HPV genotypes were found HPV16, 31, 33, 35, 52, 58, 67) with the peak prevalence at 2-month-follow-up visit. The point prevalences of the different Species by follow-up visits are presented in **Figure 20.**

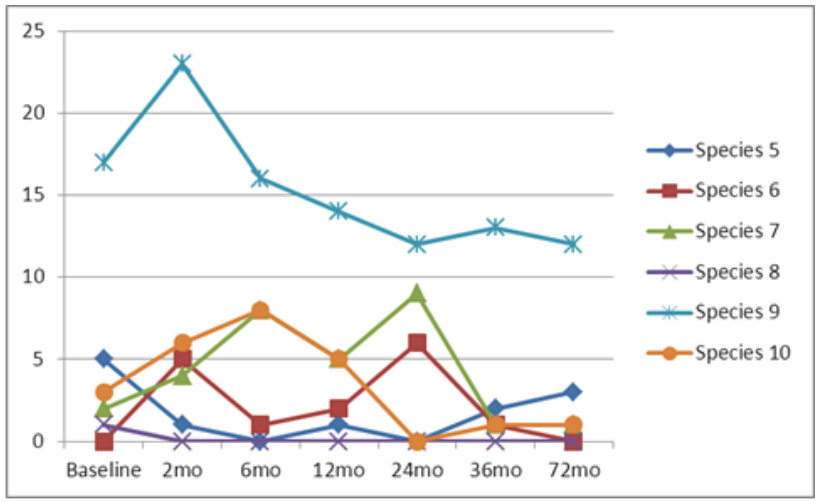

Figure 20. Species-specific point prevalences of HPV during follow-up. Species 9 marked with light blue line and stars

Genotype-specific prevalence (II, III)

The point prevalence of oral HPV infection fluctuated between 15.1% and 31.1% during follow-up of seven years. In total, 17 genotypes were identified. The genotype distributions at different follow-up points are presented in **Figure 21**. The single most frequent genotype was HPV16 throughout follow-up. The peak prevalence of oral HPV infections in terms of percentage (31.1%) occurred at the second visit, i.e., two months after delivery.

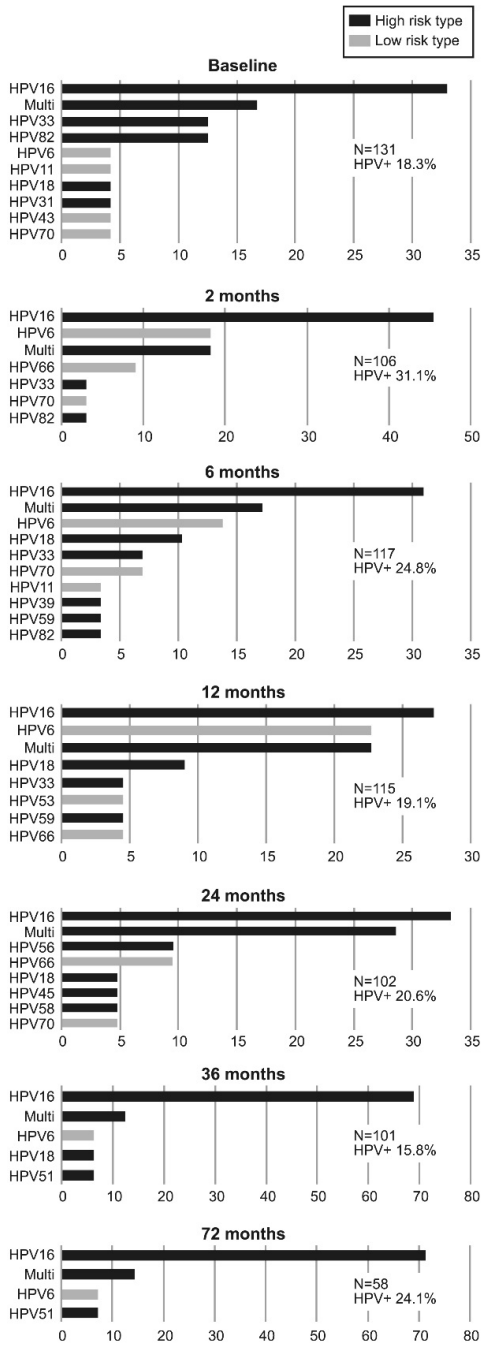

Figure 21. Genotype distributions in oral mucosal samples for HPV during follow-up

Incident oral infections (III)

Oral sampling was performed at seven follow-up visits during the 7-year-follow-up. Of the 107 men who were HPV-negative at baseline, 51 presented with an incident oral

infection during the study. The mean time to the first incident infection ranged from 3.9 months (HPV82) to 25.7 months (HPV56).

Species-specific incident oral infections (III)
The most frequent Species was Species 9 (HPV16, 31, 33, 35, 52, 58, 67) covering 42.1% of the incident infections. Species 7 was identified in 8 (21.1%), Species 10 in 7 (18.4%), Species 6 in 6 (15.8%) and Species 5 in 1 (2.6%) men.

Genotype-specific incident oral infections (III)
Incident infections were most often caused by HPV16 (n=14, 29.8%), followed by HPV 6 (n=6, 12.8%), HPV18 (N=4, 8.5%) and HPV66 (n=4, 8.5%). Multiple type infections comprised 19.1% (n=9) of the cases.

Incidence times for oral infection (III)
The longest time for incident oral HPV infection was detected for Species 6 (14.7 months, n=6, range 4.9-24.4) and Species 7 (11.2 months, n=8, range 5.2-17.2). Shorter times were recorded for Species 10 (5.4 months, n=7, range 2.6-8.1), Species 9 (6.6 months, n=16, range 1.4-11.8) and Species 5 (3.9 months, n=1).

At the genotype level, the longest time for an incident oral HPV infection was recorded for HPV56 (25.7 months, n=1), followed by HPV66 (13.5 months, n=4, range 0.1-27.9) and HPV70 (13.5 months, n=2, range 0.1-35.4). The mean incident time for multiple HPV infections was 13.6 months (n=9, range 4.7-22.4).

Incident rates for oral infection
Actuarial incident rates were calculated for all HPV-negative men at risk at baseline. Thus, the number of months at risk until the first incident event was recorded. For those with no incident event, the total number of follow-up months was recorded.

The highest actuarial incident rate (IR) was detected for HPV16 5.04/1,000mmr (95% CI 2.41-7.67). HPV6 had the second highest rate with 2.15/1,000mmr (95% CI 0.43-3.88). Species 9 (HPV16, 31, 33, 35, 52, 58, 67) had the highest IR among the Species due to a HPV16 rate of 5.75/1,000mmr (95% CI 2.94-8.57).

Crude incidenence rates were considered only for men with incident events (months at risk until the first incident event). The highest crude incidence rate was detected for HPV82 (n=1), 256.4/1,000mmr, followed by HPV39 (208.3/,1000 mmr, n=1), HPV6 (162/1,000 mmr, n=6) and HPV11 (161.2/1,000 mmr, n=1).

Predictors of incident oral HPV infections (III)
The predictors of Species-specific incident oral infections (Species 5, 6, 7, 9) were analyzed with the Poisson regression model. All covariates previously implicated as potential risk factors of HPV in the present study were first tested by univariate

Poisson models, followed by the final multivariate model including all significant or borderline significant univariates. It turned out that none of the questionnaire-recorded covariates was significantly linked with incident high-risk HPV infections.

Oral HPV clearance (IV)

Of the 139 (10 had one sample only) men recruited to this study, 56 tested HPV negative throughout the study. Totally, 72% of the men with a HPV positive oral sample (53/74) cleared their oral HPV infection during follow-up. The mean follow-up time of the men with oral HPV clearance was 43.8 months (SD 26.7 months, median 36.8, range 6.3-87.9)

Species-specific clearance (IV)

All men with Species 8 (1/1) and Species 5 (4/4) infections cleared the infection. The clearance of Species 10 (6, 11, 13, 44, 55, and 74) and Species 9 (16, 31, 33, 35, 52, 58, and 67) was 54.5% (6/11) and 66.7% (20/30), respectively.

Type-specific clearance of oral HPV infection (IV)

By the last follow-up point at the 7-year visit, all of the HPV18 (4/4), HPV33 (5/5), HPV39 (1/1), HPV56 (1/1), HPV70 (2/2) and HPV82 (4/4) infections had cleared. In contrast, only 58.3% (14/24) of the HPV16 and 78.6% (11/14) of the multiple infections had cleared by that time.

Clearance rates

To calculate the actuarial clearance rates, the number of clearance events for each individual genotype (and Species) was divided by the total mmr (male months at risk, which equalled 3450 months accumulated by all 74 men testing positive for HPV); this included the men who did not clear the infection. Hence, the actuarial clearance rate reflects the rate (per 1000 mmr) at which the individual genotypes or Species accumulate the clearance events among all HPV positive men.

HPV11, HPV31, HPV39, HPV43 and HPV56 showed the lowest actuarial clearance rates (0.28 clearance events /1,000 mmr), while HPV16 had the highest rate of 4.05/1,000 mmr; for multiple infections the rate was 3.18/1000 mmr.

For calculation of crude clearance rates, only the 53 men with a documented clearance event were included. To calculate the crude clearance rates, the number of clearance events for each individual genotype was divided by the months at risk until the first clearance event of that genotype. Crude clearance rates can be used comparing the clearance of different HPV genotypes and Species. In this cohort, the crude type-specific clearance rates for all genotypes were very similar (12-31/1,000 mmr). Most exceeded 25/1,000 mmr.

Predictors of HPV clearance
No predictors for Species 7/9 clearance could be identified either by univariate or by multivariate models adjusted for age.

Oral HPV persistence
Genotype-specific persistence of HPV infection was defined as any man testing positive for the same genotype on at least in two consecutive follow-up visits.

Persistence times
Of the individual genotypes, HPV51 persisted the longest (mean 30.7 months, range 24.1-37.2), followed by HPV16 (21.7 months, range 1.8-55.1), HPV33 (7.1 months, range 2.2-12.0), HPV39 (6.7 months, n=1) and HPV18 (6.0 months, n=1). The mean persistence time of multiple type infections was 5.0 months (range 1.4-16.2). **Figure 22** summarizes the genotype-specific persistence times.

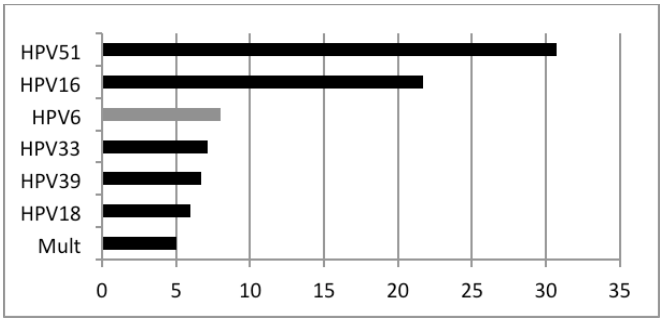

Figure 22. Genotype-specific persistence times of oral HPV infection (mean times, months).

Predictors of persistent oral HPV infection
Predictors of persistent oral HPV infection at the HPV genotype level were analyzed for the key HR-HPV-types, i.e., for Species 7 (18, 39, 45, 59, 68, 70, 85) and Species 9 (HPV16, 31, 33, 35, 52, 58, 67). The univariate GEE-model showed that a history of genital warts was the only significant covariate associated with reduced risk of oral HPV persistence with Species 7/9 (p=0.0001; OR=0.41; 95% CI 0.33-0.51), while smoking was of borderline significance (0.066) for an increased risk of oral HPV persistence. Smoking and genital warts remained significant independent predictors also after adjustment for age.

5.4 Genotype-specific concordance between oral and genital HPV infection at baseline among spouses (I)

The spouses of the men were sampled for cervical and oral sites (**Figures 23** and **24**) at the same time point (third trimester of their index pregnancy (n=128)) as the men at

baseline (**Figures 16** and **17**). HPV DNA was detected in 18.8% of the cervical and 17.2% of oral samples. HPV16 was the most frequent genotype present in 70.8% of HPV positive cervical samples (as a single infection in 29.2% and as a part of multiple infection in 41.7% of HPV positive samples) and in 77.3% of oral samples.

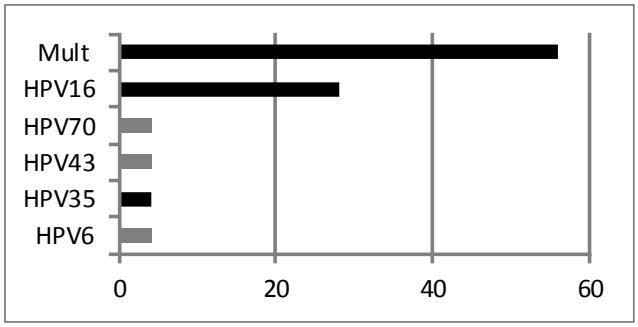

Figure 23. Percent distribution of HPV genotypes among HPV positive genital samples of female spouses at baseline. HPV positivity was 18.8% (24/128).

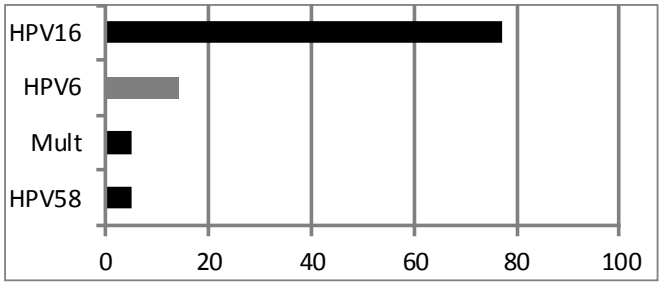

Figure 24. Percent distribution of HPV genotypes among HPV positive oral samples of the female spouses at baseline. HPV positivity was 17.2% (22/128).

Genotype-specific concordance

The genotype-specific concordance was low. It ranged from 0% to 9% among the spouses and varied by anatomical site (**Table 5)**.

Table 5. Concordant HPV genotypes in women and men.

Concordant sample pairs (male/female)	n	Concordant genotypes (n)
Semen / uterine cervix	4	HPV16 (n=3), HPV31 (n=1)
Semen / oral mucosa	0	
Male urethra / uterine cervix	3	HPV6 (n=1), HPV16 (n=2)
Male urethra / oral mucosa	3	HPV6 (n=2), HPV16 (n=1)

Predictors of concordance

All 8 concordant couples were analyzed separately for a risk-profile. There was no significant male HPV-risk profile associated with increased type-specific HPV concordance between the spouses. A higher number (>6) of lifetime sexual partners of the female spouse of the concordant couples was a significant predictive factor for concordance (p=0.030; Fisher's exact test). Otherwise, the profiles were similar for concordant and disconcordant couples.

5.5 Changes of spouses' sexual habits: impact on genital and oral infections of males (II)

The overall outcome (all event categories) of genital HPV infections was significantly predicted by changes in the number of copulations (p=0.023) and changes in marital status (p=0.022). Changes in the number of sexual partners during follow-up was also linked with the overall outcome of oral HPV infections (p=0.047).

Having another partner than at baseline (OR=15.0 (95% CI 1.36-166.1) and changed marital status (particularly divorce, p=0.001) were associated with an increased risk of incident genital HPV infections.

An increase in the frequency of oral sex reported by the female spouse was associated with an increased risk of incident oral HPV infections in the male spouse (p=0.029), while a reduction in the frequency of sexual intercourse reported by the female spouse was related (protective) to a negative genital HPV status of the men at the 7-year-follow-up visit (p=0.032).

6 DISCUSSION

HPV is the single most important etiological factor for cancer of the uterine cervix, which explains why the main attention during the last 30 years, regarding this infection, has been focused on genital HPV infection of women. Men have been considered mainly as reservoirs of HPV which they transmit to their partners (Munoz et al., 1996; Castellsague et al., 1997; Castellsague et al., 2002). Lately, evidence has increased and indicates that persistent HPV infection is associated with benign and malignant lesions at different anatomical sites not only in women but also in men. HPV-associated diseases in both genders include genital and oral warts, recurrent respiratory papillomatosis, cancers of the genitourinary tract and head and neck region (Giuliano et al., 2010; Hartwig et al., 2012; Moscicki et al, 2012). Thus, more data are urgently needed on the natural history of HPV infection among men.

The Finnish Family HPV Study was designed to elucidate the dynamics of HPV transmission in families. The focus of the present thesis was on HPV infection in men, but the data has been retrieved from the Finnish Family HPV Study. The studies presented here supply apparently the longest follow-up of male oral HPV infections and provide insight into the natural history of this disease. The present studies on male genital HPV infection included only two visits, but the interval between the visits was nearly seven years, which allows some estimation of the natural history of male genital HPV infections. The HPV data of the female spouses are only used to assess HPV genotype concordance among the couples and to understand the role of the woman as transmitter of HPV to her partner.

Representativeness of the study population

The participants in the present studies were mainly healthy young adults living in a stable relationship. The couples were recruited when the female spouse was pregnant at her third trimester. This cohort represents a more homogenous group with a stable relationship and with a known HPV status of the spouse than those of large multicenter studies presented earlier (Kjaer et al., 2005; Giuliano et al., 2009; Kreimer et al., 2011; Munc et al., 2012; Kreimer et al., 2013). Factors relating to ethnicity could not been surveyed in the Finnish Family HPV Study because of cohort homogeneity. The mean age of the men at baseline was 28.9 (range 19-46) years and of the women 25.5 years. According to Statistics Finland, the average age of women at first live birth was 28.5 years in 2012; the women in the current cohort were younger than Finnish primiparas in general.

In the present study, 16.8% (19/133) of the men had a history of genital warts at baseline. This is substantially more than in other studies on asymptomatic male attendees. Munk and coworkers found that 7.9 % of 23,000 Danish men aged 18-45 reported ever having genital warts (Munk et al., 2012); the figure in the US is 5.6% for

sexually active men and women aged 18–59 years (Dinh et al., 2008). Maybe men with a history of genital warts were more interested in participating in a study focusing on HPV infections to gain more information on the disease and to be managed professionally.

In the present study, only 34.4% (45/131) of the men answered the question on their history of STDs. Questions on such diseases are, of course, delicate and responders tend rather to underrate than overrate their history of STDs. It is impossible to elicit the missing data of the 86 men who did not answer this question. Totally, 24 of the men reported having had a STD. Fifteen men reported a history of *Chlamydia trachomatis*, three of genital *Herpes simplex* and one of *Gonorrhea.* A history of multiple STDs was stated by five men. In a study on 432 Finnish military conscripts, a history of STD was reported by 10.4% of the men (Hippeläinen et al., 1993a), while in another study of 4,074 men aged 18-70 residing in US, Mexico and Brazil, previous STD was reported by 16% of the men (Giuliano et al., 2011).

Sexual habits have changed over the recent years (Syrjänen et al., 2012). In the present study, 63% of the men reported occasional and 11% regular oral sex practices. In a study by Kreimer et al. (2013) on male HPV infection, oral sex was practiced by 87% of the 1,626 men. In that cohort, 86% of the men were heterosexual. According to what is known about oral sexual practices in the Finnish population, about 40 % of married men have occasional oral sex from their partner, as do two-thirds of cohabiting men and over two-thirds of men in living-apart relationships (Kontula, 2010). Although less frequently practiced, anal sex has known to represent heterosexual repertoire across the centuries (McBridge and Fortenberry, 2010). According to the Finnish Family HPV Study, anal sex practices are less common than in other studies (McBridge and Fortenberry, 2010; Kreimer et al., 2013). Totally, only 21% of the men in our study had ever practiced anal sex, which is less than in the Finnish population in general (50%). McBridge and Fortenberry (2010) estimated in their review that the lifetime prevalence of anal intercourse in heterosexual relationships fluctuates between 6% and 40%. It could be speculated that a stable marital relationship may contribute to the reported lower prevalence of anal sex in the current study. On the other hand, these practices have become more common in the recent years (Kontula, 2010) and the baseline data of the present study were recorded more than a decade ago.

Genital HPV infection in men

Prevalence (I, II)

Factors affecting HPV prevalence

Several cross-sectional studies on the frequency of anogenital HPV infection in men are available and the reported variation is from 1.3% to 72.9% (Dunne et al., 2006; Patridge and Koutsky, 2006). The differences and the number of anatomical sites

sampled, sampling methods and cohorts enrolled can explain these divergent results. Obviously, the HPV DNA detection rate increases if there are clinical findings in the genital tract of the subjects. In the present study, baseline sampling was made from the urethra and semen, while both penile and urethral samples were taken at 7-year-follow-up point. Genital examination was performed by a dermatovenereologist at the last follow-up visit. In the fossa navicularis of the urethra there are two distinct types of epithelia in a specific anatomical location. These transformation sites are vulnerable and susceptible to HPV infection. Therefore, the urethral sample was regarded as the most reliable one. At baseline, the impact of HPV infection on the quality of the semen was of special interest, as previously discussed by Rintala et al. (2004).

HPV prevalence: urethral and penile samples

In the present study, 23% (29/128) of the urethral samples at baseline and 22% (10/45) at the 7-year-follow-up were HPV DNA positive. In previously published studies, HPV PCR-detection rates at the urethral site have varied from 3% to 44% (Kataoka et al., 1991; Forslund et al., 1993; Astori et al., 1995; Aynald et al., 2003; Giuliano et al., 2008). In the current study, penile samples were collected at the 7-year-follow-up visit at which time HPV positivity was detected in 23.9% (11/46) of the men. Previous studies have reported HPV detection rates from the penile site between 6.5% and 53% (Wikström et al., 2000; Lazcano-Ponce et al., 2001; Hernandez et al., 2006; Dunne et al., 2006).

HPV is detected more frequently in penile than urethral samples. In heterosexual men, the penile shaft, glans and coronal sulcus are the most likely HPV positive sites and HR-HPV genotypes and multiple type infections are most probable at these sites (Giuliano et al., 2007). Visible genital warts are most common in the penile shaft. In a study on asymptomatic men from US, Mexico and Brazil, HPV infection was more frequent in the penile shaft (49.9%), the glans (35.8%), and the anal site (17.6%) compared to the urethra (10.1%). Smith et al. (2007) studied 98 Kenyan men and reported a HPV prevalence of 53% for the samples from the penis; 50% from the glans, the coronal sulcus and inner foreskin tissue; and 18% from the urethra.

Not suprisingly, a higher HPV prevalence at the urethral site has been reported in studies on men with symptomatic HPV infection than in men with asymptomatic HPV infections. Shigehara et al. (2010) studied HPV positivity at the urethral site of men with urethritis (n=142). HPV was detected in 48% (68 cases) of the men. The site-specific HPV positivity was 31%, 20% and 24% for the penis, urethra and urine, respectively. These results implicate that clinical HPV infections are also frequent in urethra.

Urethral sampling tends to be painful for the patients and the use of smaller, more patient-friendly brushes, like the interdental brushes used in dentistry, is recommended. Urine sampling would be a painless and facile way to obtain specimens and there have

been efforts to develop urine sampling for HPV screening. However, based on urine sampling only, the prevalence of HPV infection among asymptomatic men produces lower estimates of the infection than brush samples from genital sites. In asymptomatic men, the HPV prevalence in urine is generally below 10% (Forslund et al., 1993; Lazcano-Ponce et al., 2002; Weaver et al., 2004), but among males with clinical urethral warts, the frequency of HPV positive urine samples is 76-88% (Forslund et al., 1993; Lazcano-Ponce et al., 2002; Weaver et al., 2004). Since the urine is not an optimal medium for diagnosis of HPV, urine sampling was not used in the present study for asymptomatic men.

HPV prevalence: semen

In the present study, HPV DNA was found in 31% of the semen samples (n=28/90) of asymptomatic men.

Data on the prevalence of HPV in semen are scanty and the detection rate varies from 2.2 to 41.3% among asymptomatic men (Dunne et al., 2006). Scaled cells of urethral lesions and/or warts might increase HPV DNA positivity in semen samples (Garolla et al., 2011). A recent meta-analysis by Laprise et al. (2013) reported a HPV prevalence rate in semen samples ranging from 0% (18-year-old men with no previous history of sexual activity) to 100% (men with intrameatal warts).

The effect of HPV infection on semen quality has been of special interest. In a recent meta-analysis by Laprise et al. (2013), the prevalence of HPV in the semen of infertile men was higher (16%) than the prevalence in other populations (10%). An association between HPV infection and abnormalities of sperm parameters and sperm motility has been reported (Lai et al., 1997; Garolla et al., 2011). In infertile men, HPV DNA positivity varies from 4% to 36% (Pao et al., 1996; Lai et al., 1997; Rohde et al., 1999; Laprise et al., 2013). The cohort of the present study consisted of spouses of pregnant women, which, of course, implies that the men were fertile. A lack of association between male fertility and HPV positivity is supported by our earlier findings on a subcohort of the Finnish Family HPV Study: HR-HPV was detected in 15% (10/65) of the semen samples tested with PCR and consecutive Southern blot hybridization, but the presence HR-HPV did not affect the quality of semen (Rintala et al., 2005). Only borderline significance was found between HPV and lower semen pH. Earlier, it has been reported that a low pH of the sperm is associated with bacteriological findings in spermatic flora of infertile males (Granouillet et al., 1982). Another cause for concern is the possible transmission of HPV DNA to oocytes by infected sperm (Garolla et al., 2011). Currently, infertility clinics do not test for HPV of the donor sperm.

Genotype-specific prevalence at anogenital sites (I, II)

Different HPV genotypes are known to have a predilection for either mucosa or skin. The types of epithelia vary at different anatomical sites of the male genitalia, and a natural question is whether certain HPV genotypes prefer certain anatomical regions of

the male genitalia. In the present study at baseline, HPV6, 11, 16, 33, 43, 51, 53, 56, and 70 were found in urethral samples and multiple type infection was found in 7/128 samples. In semen samples (n=90), HPV6, 11, 18, 33, 51, 53, and 66 were detected. Multiple types accounted for 21.6% (6/28) of the HPV positive semen samples. At the last follow-up visit (7-year-follow-up, n=45), the following genotypes were recovered: HPV6, 11, 16, 53, 56, 58 and 70. At the penile site, HPV16 and HPV70 were the most frequent genotypes representing both 27.2% (3/11) of the HPV positive samples. All other genotypes were present as single findings (HPV6, 18, 66, 70 and multiple type infection with HPV53 and HPV82).

These results are in agreement with previous reports (reviewed by Dunne et al., 2006). The most frequently detected HPV genotype has consistently been HPV16. Other common HPV types in male genital tract, besides multiple type infections, include HPV6, 11, 18, 31, 33, 42, 52, 53, 54, 59 and 84. Recently, Sichero et al. (2013) identified 86 unique HPV types among 508 of 931 specimens. The results showed the presence of a broad range of α-, β- and γ-HPV genotypes in the male genitals., PGMY09/11, GP5+/6+ or FAP59/64 PCR products were sequenced to identify the HPV types not covered by their usual HPV testing method which identified only 37 α-HPV genotypes. These data clearly implicate that genotype distributions reported from different studies are strictly dependent on the HPV testing method and its HPV genotype coverage. Many new, commercially available HPV tests detect the clinically most important mucosal α-HPV genotypes.

Predictors of genital HPV infections in men

In contrast to previous studies, we could not identify any specific male HPV-risk profile, despite the comprehensive questionnaire used here. Earlier, several risk factors of male anogenital HPV infection have been associated with sexual behavior, e.g., young age at sexual debut; greater number of regular, lifetime and recent sexual partners; greater number of sexual partners before and during marriage (Hippeläinen et al., 1993a; Dunne et al., 2006; Patridge and Koutsky, 2006; Nyitray et al., 2011). The different results from our analysis may be linked with differences in the cohorts, since the present study focuses on men cohabiting with the same partner in a stable relationship. In a study on 1,471 Finnish military conscripts - a totally different study cohort from the present Finnish Family HPV Study - sexual promiscuity was reported as being the most important risk factor for genital HPV infections (Hippeläinen et al., 1993a).

According to a systematic review by Albero and coworkers (2012), there is a robust inverse association between male circumcision and genital HPV prevalence. In Finland, circumcision is not routine and is mainly performed for clinical reasons, e.g. for treating phimosis. This is why none of the men of the present cohort had been circumcised.

Immunodeficiency is associated with an increased prevalence of anogenital HPV infections independent of sexual risk factors. There is a clear association between a progressively lower CD4+ level and increased detection of HPV types at anogenital sites (Palefsky, 2006). The present cohort consisted of healthy males (fathers-to-be) and immunodeficiency was not among factors studied.

Incidence (II)

In the present study, HPV samples from genital sites were collected at baseline and seven years later. Totally, 43 men were available for sampling at the two time points. Infection was present in 10 (23.3%) of the men. Although there are several cross-sectional studies on the prevalence of male genital HPV infection, only few studies on incident HPV infection among men are available (Wikström et al., 2000; Kjaer et al., 2005; Lajous et al., 2005; Giuliano et al., 2008; Giuliano et al., 2011). Patridge and co-workers (2007) performed sampling from genital sites of 240 male university students (age 18-23) at four-month intervals up to 24 months to assess incident genital HPV infections. They reported incident infections in 62.4% of the men. There were no differences between the genital sites (glans, penile shaft or scrotum). In another study of 1,159 men aged 18-70 years with a median follow-up time of 27.5 months (18.0-31.2), an incident infection was detected in 26.8% (Giuliano et al., 2011).

Predictors of incident genital HPV infection (III)

We found that changing the partner increased the risk for incident HPV infections in the male genital tract. In earlier studies, an increased risk for incident genital HPV infection has been linked to having more than three sexual partners during the follow-up (Kjaer et al., 2005), history of other STDs (Hippeläinen et al., 1993a) and a high frequency of intercourse (Baldvin et al., 2004). In line with our results, Padridge et al. (2007) also found that a new sexual partner during the past four months was associated with an increased risk for incident HPV infection. In addition, smoking was linked to an increase in incident infections (Patridge et al., 2007).

The present cohort consisted of only young men. It is worth mentioning that men might have an age-independent risk for genital HPV infection throughout their lives (Giuliano et al., 2011) in contrast to women whose risk for incident cervical infection decreases with age (Munoz et al., 2004; Castle et al., 2005). However, at this stage it is not possible to totally rule out that the reported incident infections in men would not be actually reactivated latent HPV infections, especially since the follow-up in the studies are short. More careful dissection of clearance and incident data might provide new insight to this topic.

Clearance (II)

In the present study, male genital HPV samples were taken only twice. Eight men out of 13 who had been HPV positive at baseline (62%) had cleared their infection and five

had still a HPV infection at the 7-year-follow-up visit. However, only one subject had the identical HPV genotype (HPV53) at baseline and seven years later. Thus, the other four men had cleared their baseline infection and had acquired an incident infection with a different HPV genotype during the follow-up. According to a large prospective cohort study of men, the mean time to clear any genital HPV infection is 5.9-7.5 months; 75% of the infections clear within 12 months (Giuliano et al., 2010; Giuliano et al., 2011).

Persistence (II)

There is no universal definition for HPV persistence; HPV persistence has not been defined on the viral level as has been done for many other viruses [e.g. HSV and HBV (hepatitis B virus)], where the expression on certain viral genes is associated only with viral latency or persistence. In most studies persistence is defined as detection of HPV in two subsequent samples. Occasionally, persistence has been defined by the duration of the HPV infection: only those infections where the same HPV type is detected for 12 or even 24 months are considered to be persistent. In the present study, HPV persistence was defined as detection of HPV positivity in two consecutive samples.

Persistence of genital HPV (II)

HPV persisted in the genital tract of only one male subject (1/43), who had HPV53 both at baseline and seven years later. Interestingly, this subject was the oldest in the study cohort (52 years at the 7-year-follow-up visit). The low number of subjects whose genital HPV persisted is probably due to the fact that only two samples were taken, seven years apart. These healthy, young HPV positive men apparently resolved their infection by the last follow-up visit (seven years), since most HPV infections do clear within two years (Giuliano, 2010; Giuliano, 2011). None of the spouses of the women who reported a decrease in the number of copulations had a genital HPV infection at the 7-year-follow-up visit. Thus, a reduction in the number of copulations was protective against persistance of HPV ($p=0.032$).

Persistence of male genital HPV infection has been evaluated in only a few studies, even these with short follow-up times. Lajous et al. (2005) reported HPV persistence in 29.4% of 336 Mexican healthy military men in a one-year follow-up study. Circumcision was protective against persistent male genital HPV infection (OR=0.10, 95% CI, 0-0.87). In a study by Silva et al. (2011), 72 HIV negative men were followed up for six months, and 66.9% of them had a persistent HPV infection. In a Danish study on healthy military conscripts (n=374) followed-up for six months, nearly one fourth of the HPV positive subjects remained HPV positive at the last follow-up visit (Kjaer et al., 2005). However, since clearance of the HPV infection usually takes more than six months follow-up-times can be considered to have been short in these studies.

Oral HPV infections in men

Prevalence (III)

Factors affecting prevalence in the oral mucosa

The oral mucosa poses a challenge for HPV sampling, since the site of an asymptomatic oral HPV infection cannot be ascertained and viral loads are low (Syrjänen, 1992; Kellokoski et al., 1992). In the present study oral scrapings with a brush were taken from non-keratinized oral mucosa to ensure a proper yield of nucleated cells (Kellokoski et al., 1992a; Kellokoski et al., 1992b; Puranen et al., 1995; Puranen et al., 1996). Our study differs from many other studies which have generally relied on samples obtained by rinsing or gargling (Gillison et al., 2012). Rinsing and gargling have not always produced the required minimum of 100,000 cells needed for a representative sample, especially from smokers (Kellokoski et al., 1992a). As with genital HPV prevalence studies, differences among the cohorts enrolled into various studies probably influence oral HPV prevalence rates, as well.

Prevalence of oral infections

The sampling method to obtain oral material for HPV seems to be crucial. In the present study, HPV was identified in the oral cavity of 15.1% to 31.1% of the healthy male population; the occurrence fluctuated during the 7-year-follow-up and seemed to be substantially higher than the 0-11% reported previously (0-11%). Miller and Johnstone (2001) have estimated that the likelihood of detecting HPV in the oral cavity exceeds 10% (both genders). This is in line with the more recent review by Sanders et al. (2012) concerning a US population with a reported prevalence of 11% in males. In a study by Gillison et al. (2012) involving both men (n=2,748) and women (n=2,831) aged 14 to 69 years, the prevalence of oral HPV in men was 10.1%. In our cohort sampling was performed by brushing, but in the US cohort samples were retrieved by rinsing/gargling. Overall, the detection rate of oral HPV has been increasing lately, as the methods have become more standardized.

HPV genotype distribution in the oral mucosa (II, III)

The HPV spectrum in the oral mucosa was wider than in the anogenital region. In the current study, HPV16 represented the most frequent genotype in the oral mucosa followed by multiple type infections. We also found the following genotypes: HPV6, 11, 18, 31, 33, 39, 43, 45, 51, 53, 56, 58, 59, 66, 70 and 82. All these genotypes have been previously found in anogenital sites. There is a paucity of published data on HPV genotype distribution in the oral mucosa. The following genotypes have been reported: HPV6, 11, 16, 18, 31, 33, 35, 39, 42, 45, 51, 52, 53, 55, 56, 58, 59, 61, 62, 64, 66, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89 and cp6108 (Kellokoski et al., 1990; Syrjänen and Syrjänen, 2000; Kreimer et al., 2011; Edelstein et al., 2012; Pickard et al., 2012). Gillison et al. (2012) reported a prevalence of HPV16 that was significantly higher in

men than in women (1.6% vs 0.3%; $p < 0.00.1$), but no detailed genotype-specific data were given.

Risk factors for oral HPV infection (III)

The present study failed to establish an association between prevalent oral HPV infection and a history of oral sex, which is at variance when compared to some previous studies (D'Souza et al., 2009). Kreimer et al. (2011) found that individuals who reported never performing oral sex had a similar prevalence of oral HPV infections as those who reported ever having oral sex (3.8% vs. 4.1%, respectively). Interestingly, deep kissing has been recently attributed to prevalent oral HPV infection (D'Souza et al., 2009; Pickard et al., 2012). The questionnaire used in the present study did not address kissing habits. An important difference between our oral HPV studies and others is related to the type of cohort studied. We focused on spouses expecting their first newborn at baseline; other reports have studied on men with different sexual behaviors and undetermined partnerships.

Data on the prevalence of oral HPV infection among men and women are controversial. Gillison et al. (2011) reported a three-fold higher HPV infection prevalence (10.1%) among men compared to women (3.2%) (Gillison et al., 2011). This difference in HPV prevalence linked to gender has not been confirmed in other studies. In the Finnish Family HPV Study cohort, the HPV prevalence among women was only slightly lower (15-24%) than among men (15.1%-31.1%), but it did fluctuate within the same range (Rautava et al., 2012). In the present cohort, women were pregnant at study entry. Pregnancy modifies hormonal and immunological factors which may affect the prevalence and persistence of HPV infection (Louvanto et al., 2010; Louvanto et al., 2011). Pregnancy is a state of immunological depression and causes profound changes in hormonal status (Schneider et al., 1987; Armbuster-Morales et al., 2000). Recently, a meta-analysis of 48 studies on the effects of pregnancy, delivery and post-partum on changes in sexual behavior found that pregnancy induces a significant decline in sexual practices, particularly in the third trimester (Serati et al., 2010). The frequency of sexual intercourse of the mother-to-be declines during pregnancy (Serati et al., 2010).

Older age might be associated with oral HPV infections. Gillison et al. (2012) reported a bimodal age-pattern of subjects who have these infections: one peak at 30-34 yeras and another at 60-64 years. The second peak may be due to reactivations of old infections. However, others have not yet confirmed these results. Previously, a bimodal pattern has been described for oral warts and laryngeal papillomas, as well, but the peaks appear much earlier (at younger than 2 years and at over 20 years) (Derkay et al., 2008; Syrjänen, 2010). In addition, age-related changes in immunity may enhance persistence among the older population. Because of the selected age profile of the present cohort (father-to-be), estimation of the age effect on oral HPV infection, if any, was not possible.

The present study did not find an association between smoking and prevalent oral HPV infection. This is in contrast to earlier studies where current tobacco use has been linked to an increased prevalence of oral HPV infections (Kreimer et al., 2011; Gillison et al., 2012). In the National Health and Nutrition Examination Survey (NHANES-study) of the US, use of more than 21 cigarettes per day was associated with a very much higher oral HPV prevalence (20.7%; 95% CI 12.6-32.0) compared to non-smokers (1.1%; 95 % CI 0.6-2.3) (Gillison et al., 2012). In a study by Kreimer et al. (2011) on 1,680 healthy men, the strongest association with oral HPV infection was documented among current cigarette smokers (2.5-fold increased risk of infection; 95% CI 1.4-4.4). The results of the present study may be influenced by differences among cohorts. The attendees of the present study were younger men. At baseline, 63.0% of the men reported having never smoked. Of the 44 smoking men, 26.3% claimed that they were smoking 1-10 cigarettes a day while 43.2% reported 11-20 cigarettes a day. Only 20.5% of the smokers consumed more than 20 cigarettes (or equivalent) per day. The start age for smoking was usually 14-17 years (n=30/44). In our study, participants were relatively young and smoking was rather uncommon, and when someone smoked, consumtion was reasonably limited. Thus, the number of pack years was small compared with the larger population-based studies.

In addition to male gender, older age and current smoking, HIV-infection increases the prevalence of oral HPV infection. In the present cohort all subject were HIV-negative.

Incidence (III)

Incidence of oral HPV (III)

The Finnish Family HPV Study provides the longest follow-up of oral HPV infections in women and men to date. This cohort included 131 men followed up for seven years (median 37.0 months, range 2.13-91.5 months, mean 45.2 months). Incident oral infections were documented in 52.3% of the 107 men negative for HPV at baseline. Most of the incident infections occurred during the first six months of the study. The acquisition time of incident oral HPV varied from 3.9 (HPV82) to 25.7 (HPV56) months during the follow-up. Interestingly, the present data differs from the one reported on a large cohort study (n=1,626) involving Brazilian, Mexican and US men. The results of that study suggested that incident oral HPV infections are rare (Kreimer et al., 2013). However, the follow-up was only 12.7 months (median), and only 4.4% of the participating men acquired an incident oral HPV infection (95% CI 3.5-5.6; n=115 incident infections). Of these infections, 1.7% (95% CI 1.2-2.5; n=53) were caused by high risk HPVs in general and 0.6% (0.3-1.1, n=18 incident infections) were due to HPV16. Piccard and co-workers have reported an incidence of any oral HPV infection of 5.7 per 1,000 person-months in a cohort of 1,000 men and women aged 18-30 years followed up for 3 months (Pickard et al., 2012). There is also another study by Edelstein et al. (2012) on 212 men (18-24 years) followed up for 12 months with a

cumulative incidence of 12% of any oral HPV infection. However, the follow-up times of all these studies available have been at most 12 months, i.e., nearly six years less than in the Finnish Family HPV Study.

Predictors of oral incident infection (III)

Immunodeficiency, smoking, French kissing and oral sex are linked to increase in risk for incident oral HPV infection both in healthy and HIV-positive men (Edelstein et al., 2012; Kreimer et al., 2004; D'Souza et al., 2009; Beachler et al., 2011). Recently, a study of young men indicated that performing oral sex with women (cunnilingus) more than once a week was associated with a significantly increased risk of incident oral infection (Edelstein et al., 2012). The median follow-up time in that study was 10.7 months. The present multivariate analysis did not result in any significant association between the male sexual habits (or any of the demographic characteristics) and incident oral HR-HPV infections. This is in line with the HIM-Study (Kreimer et al., 2013) where oral sex was not among the risk factors for incident oral HPV infection. However, in the present study, an increased frequency of oral sex reported by the female spouse was associated with an increase in incident oral HPV infections in the male spouse ($p=0.029$). Interstingly, mechanical trauma is related to reactivation of latent HPV infections. Thus, instead of a real new incident infection, also reactivation of a latent infection may have caused the attribution described above. Pickard et al. (2013) found by univariate analysis that an incident infection is associated with having open-mouth kissed a new partner in the previous three months (OR,2.6, 95% CI 1.0-6.4). As mentioned before, French kissing was not among the factors analyzed in our study.

Kreimer et al. (2013) reported a nearly three times higher oral HPV incidence rate for current smokers (HR=2.80) and more than two times higher for former smokers (HR=2.31) compared to never-smokers. In the present study, there was no association between smoking and acquisition of a new oral HPV infection.

The HIM-Study included male subjects residing in the US, Brazil and Mexico (mean age 32 years, range 18-73). Mexican men were at a significantly higher risk for a new incident infection compared to men living in the US (Kreimer et al., 2013). Maybe factors associated with hygiene, nutrition, immunology and sexual behavior are explanatory factors for this finding.

Kreimer et al. (2013) found that men not cohabiting or not being married were at increased risk for a new incident oral infection. This is in agreement with our results regarding genital infections. It has been speculated that the narrower sexual network provided by a stable marital relationship protects against HPV infections. Thus, marital status and sexual orientation (MSW), linked to lower-risk sexual behavior, might be more substantial predictive factors than the number of life-time sexual partners.

Clearance (IV)

Clearance of oral HPV (IV)

There is limited information on the clearance of oral infections in men, since, apart from the Finnish Family HPV Study, there is only one prospective study addressing the question of clearance of oral HPV infection (Kreimer et al., 2013). In the present study 72% (53/74) of the men with HPV positive oral samples cleared their infection by the time of the last follow-up visit at seven years. The mean follow-up time of these men was 43.8 months (SD 26.7 months, median 36.8, range 6.3-87.9) and the mean time to first clearance ranged from 1.4 to 36.6 months in men. For the female participants of the Finnish Family HPV Study, the clearance time was 2.5-37.2 months (Louvanto et al., 2013). Thus, clearance times are practically identical for both genders.

In the present study, clearance of oral HPV infection was dependent on the HPV genotype. Especially HPV16 but also multiple type infections cleared less frequently than the other genotypes. Only 58.3% of HPV16 (14/24) and 78% (11/14) of multiple type infections had cleared by the time of the last follow-up visit. In contrast, all of the other genotypes (HPV18 (4/4), HPV33 (5/5), HPV39 (1/1), HPV56 (1/1), HPV70 (2/2) and HPV82 (4/4)) had cleared. No predictors for Species 7/9 clearance could be identified by univariate or multivariate models adjusted for age.

These results are in accordance with our previous results (Rintala et al., 2006) and a more recent study by Kreimer and co-workers (2013). Rintala et al. (2006) reported results from a two-year follow-up of subcohort of 113 men participating in the Finnish Family HPV Study with six follow-up visits and reported point-prevalences of HR HPV infections between 18% and 25%. HPV was cleared by the end of follow-up by 19 males who at baseline were HR-HPV positive. Virus clearance has started at the 12-month-follow-up time point. No predictive factors could be identified.

In a cohort followed up for a median of 12.7 months and sampled by the rinse-and-gargle method, most of the infections cleared within one year; the median time was 6.9 months (95% CI 6.2-9.3; n=45 cleared infections) for any HPV type, 6.3 months (6.0-9.9; n=18) for oncogenic HPVs and 7.3 months (6.0-not estimated; n=5 cleared infections) for HPV16 (Kreimer at al., 2013). In line with our results, no predictive factors were identified in that study, either.

Persistence (IV)

Persistence of oral HPV infection (IV)

Persistence of HPV infections depends on the type of HPV, follow-up time and compliance of the study population. In the current study, the persistence times ranged from 1.8 to 55.1 months (both HPV16). However, in a study with many follow-up-points, some of the attendees were not compliant for sampling at every follow-up-

point, and this may have influenced the recorded persistence times which were defined as the time between two consecutive positive samples. It is worth noting that the prevalence of HPV persistence is totally dependent on the follow-up time: D'Souza et al. (2007) reported 60% persistence of oral infections in a study with 136 participants and a follow-up of six months (two visits). The higher percentage of persistence in the study by D'Souza et al. is probably due to short follow-up since clearance of HPV infections should take more than six months (Louvanto et al., 2010; Rautava et al., 2013).

Genotype-specific persistence of oral infections (IV)

Since persistent HPV infection may cause malignant transformation, it is essential to understand the meaning of the HPV genotype distribution in persistent infections. HPV16 alone or as one of multiple type infections is the most frequently persisting HPV genotype also in the oral mucosa. In addition to the single most frequent genotype to persist (HPV16), five other genotypes (HPV6, 18, 33, 39, 51) persist in the male oral mucosa; this is different from the situation in women (HPV6, 11, 33, 58, 66) (Louvanto et al., 2010). In the present study, HPV51 persisted the longest in the mouth, for 30.7 months.

In addition to the Finnish Family HPV Study on persistent oral HPV infection, there is one Japanese study of 662 male and female participants who were followed up for 2 years (Kurose et al., 2004). In that study, only four HPV positive individuals were found at baseline, two of them had a persistent HPV infection with the same genotype at the 2-year visit (cutaneous type HPV12, mucosal type HPV71).

Predictors of Species-specific persistence (IV)

In the present study, a history of genital warts was the only significant covariate associated with a decreased risk of Species 7/9 persistence and smoking with an increased risk, respectively. The first exposition to HPV infection seems to induce HPV specific immunity, protecting from infection or aiding to clear it faster (Stanley, 2012; Syrjänen and Syrjänen, 2000). The intriguing question is: Can previous warts induce an immunological response in the oral cavity and reduce the risk of oral HPV persistence? We are not aware on any studies focusing on this question. However, since we have also followed the newborns, we might be able to understand the role of oral and genital HPV infection in HPV specific immunity and vice versa.

Importantly, we found that smoking increases the risk of oral HPV persistence of the 7/9 Species of HPV ($p=0.033$, OR = 1.92, 95% CI 1.05−3.50). Current smoking has been attributed to an increased risk of cervical HPV infection and higher viral loads have been detected in smokers than in non-smokers (Gunnell et al., 2006). The association found between smoking and prevalent oral HPV infection in cross sectional studies might actually be due to persistence of oral HPV infection in the smokers. It would be important to understand how tobacco or smoking facilitates HPV persistence.

It is known that smoking reduces the number of Langerhans cells in the oral epithelium and is associated with local immunosuppression. Similarly, oral infections, e.g., periodontitis, are more prevalent in smokers (Labriola et al., 2000). Also in our study, smoking was linked with clinical oral findings. Along with HPV sampling of the oral mucosa at the 7-year-follow-up visit, a clinical examination of the oral cavity was performed by a dentist. We found that half of the men had minor oral mucosal changes detectable by clinical examination. By univariate analysis, these lesions were associated only with smoking (p=0.046). Unfortunately, biopsy samples from these lesions were not available; had they been, the presence of HPV in the samples could have been assessed.

In the present study, oral sex practices reported by men were not associated with an increased risk for oral HPV persistence (Species 7/9). The same has been shown for the women in the Finnish Family HPV Study (Rautava et al., 2012). Rintala et al. (2006) have previously reported that persistent oral HR HPV is a powerful predictor of persistent HR HPV in the other spouse (odds ratio 10.0; 95% CI 1.46–68.69). This implicates that the spouse with a persistent oral HPV infection might infect his/her spouse by an oro-oral transmission route. As the virus remains persistent, the spouse is all the time exposured to HPV transmission which might progress to a persistent oral infection due a high viral load and/or smoking of the HPV recipient.

Genotype-specific concordance of the spouses at baseline (I)

Study I focused on HPV genotype-specific concordance of the couples. The female spouses were sampled for cervical and oral sites at the third trimester of their index pregnancy (n=128). The baseline male urethral and semen samples were taken at the same time. The genotype-specific concordance ranged from 0% to 9% among the spouses, according to the anatomical sampling sites; four couples had concordant semen and uterine cervix samples, and three couples had concordant urethra and uterine cervix samples, which is lower than reported in the scanty literature previously. Concordance rates varying widely between 2% and 87% have been reported (Hippeläinen et al., 1994b; Castellsague et al., 1997; Bleeker et al., 2005; Reiter et al., 2010). Reiter et al. (2010) published a meta-analysis and showed that there was HPV genotype-specific concordance in 25.5% of the couples (30 studies, 2972 couples). The concordance was higher among female partners of the men with HPV infections than among the male partners of the women with HPV infections. This can be related to the fact that the duration of HPV clearance is longer for women than men. According to the same meta-analysis, the highest concordance rates were recorded for HPV6, 11, 16 and 18, which cause most of the disease burden attributable to HPV infections; HPV6 and 11 are main causative genotypes for highly infectious genital warts, whereas HPV16 and 18 represent common oncogenic HPV genotypes with a longer persistence time. Bleeker and co-workers (2005) reported cross-sectional concordance between female and male sexual partners with the same genotype in 37% of the cases.

Parada et al. (2011) reported a HPV concordance of 6.7% (34/504) in a population of 504 couples residing in rural parts of Mexico; the genotype-specific concordance was 61.8% of the concordant cases (21/34). In their study, an abstinence of three days was required of the attendees before sampling to rule out contamination.

The low concordance in HPV genotypes in the current study may be due to several factors. Firstly, the women of our study were pregnant. As discussed in section 6.2, pregnancy may induce changes in immunology and sexual behaviors. Secondly, partners that had been cohabiting for years had had their personal exposure to the HPV infection of different genotypes already years ago and due to the complex course of HPV infection, factors associating with persistence, clearance, transient infections and natural immunity might influence the results. In addition, although sampling of the partners was performed skilfully from genital and oral mucosal sites, providing even more samples of multiple anatomical sites of the men might have yielded higher concordance rates. However, the significance of multiple sampling in this respect is likely to be quite low, because the same HPV genotype has been detected in different anatomical sites (cervix, vagina, vulva) (Mäenpää et al., 1992).

Risk factors for concordance

In our analysis, all eight concordant couples were analyzed separately for a risk-profile. We could not identify any male risk profile linked with increased type-specific concordance of the couples, whereas a higher number (>6) of lifetime sexual partners of the female spouses was a significant risk factor for concordance ($p=0.030$; Fisher's exact test). Otherwise the profiles were similar for concordant and disconcordant couples. Previously, men have been suggested to represent reservoirs of HPV threatening their female partners (Reiter et al., 2010). Sexual risk behavior of men has also been claimed to be a predictive factor for females to acquire HPV infection. However, in the light of our results it could be speculated that the female oral or genital mucosa is a more important site as a reservoir of HPV than the same anatomic sites in men. Nyitray et al. (2012) documented that type-specific concordance of the partners is inversely associated with the age difference between partners, which could be related to host immunity and life-time exposure to HPV. Concordance of the most prevalent HPV genotypes does not necessarily mean that the current partner has transmitted that HPV genotype to his/her partner. It is also possible that both spouses may have acquired the most prevalent genotypes separately earlier in life. Since HPV16 is the most common genotype to persist, the persistence of “an old HPV infection” might result in false positive concordance between the couples.

In a study by Parada et al. (2011), the risk of HPV infection in female partners was increased among couples where the male partner reported a history of sexual relations with sex workers and inadequate use of condoms. Their cohort differed from ours in many respects. For example, their cohort consisted of Mexicans of whom 85% were Catholic while the Finnish population is largely Protestant. In Catholic countries, the

female spouse may be monogamous and risky sexual behavior of male spouse may be more important in comparison to the Nordic countries, where late-onset partnerships are common and women may act sexually more liberally before settling down in a stable relationship. From an international perspective, Finnish attitudes towards union formation are currently exceptionally liberal. In 1971 Finnish women reported 2.6 partners, in 2007 the number was 10.4. For men, the numbers were 10.4 and 14.7, respectively (Kontula, 2010).

Study strengths and limitations

Due to the complex natural history of HPV, long follow-up studies with multiple sampling points are mandatory for full information on the the dynamics of HPV infection. The prospective the Finnish Family HPV Study is the longest follow-up study on oral HPV infections involving both genders. It provides unbiased estimates of HPV outcomes in the oral mucosa. The study is unique in that it is a 7-year-study with seven-follow-up points with samples of multiple anatomical sites of each participant and demographic data on family members, including the fathers, the mothers and the follow-up of the offsprings. Pregnancy (or pregnancies) may have influenced on the results compared with the other cohorts.

This study has its limitations. The Finnish Family HPV Study was not originally designed to focus strictly on a male cohort but to explore the dynamics of HPV infection among ordinary Finnish families and especially to understand the natural history of HPV infection in children. This sets certain limitations on the present studies, which focus on male subjects. The Finnish Family HPV Study was initiated already ten years ago. It has been a pioneering study on the natural history of HPV infections of asymptomatic family members and is a continuation of previous studies on women's oral HPV infection and mother-child HPV transmission (Kellokoski et al., 1990; Puranen et al., 1996). The focus of the current studies is on men. The cohort is small in size compared with the ongoing follow-up studies in the US (Giuliano et al., 2009; Kreimer et al.; 2011; Kreimer et al., 2013). The extension of the present study was completed only by 58 attendees. The long follow-up time was also challenging in that the motivation of the participants to attend sampling at several time-points was certainly at risk and led to some drop-outs along the way. Also, this cohort of 58 men that attended in the extended follow up of this study might have represented a selected subcohort. Only four of these men had divorced during the follow-up. Thus, there is a possibility of bias relating to the relatively small amount of attendees and a large number of drop-outs. In the future, a larger cohort size with multiple follow-up points would facilitate our understanding of the natural course of HPV infections in men. Still, the Finnish Family HPV Study provides an opportunity to study HPV infection in young unvaccinated boys whose parents' HPV status is known and recorded. Data provided by a long-follow-up study of children and their families are mandatory to improve our understanding of the transmission modes and outcome of HPV infection in adult males.

7 SUMMARY AND CONCLUSIONS

Asymptomatic genital HPV infections are common (35.9% at baseline and 33% at the last follow-up-point) among healthy Finnish men, and 13 different genotypes were identified in samples from the male genitalia. HPV16 was the single most common genotype followed by HPV6.

Asymptomatic oral HPV infection was also prevalent, and - again - HPV16 was the most common genotype. Altogether, 17 HPV genotypes were detected in the oral mucosa during a follow up of seven years. The point prevalence varied from 15.1% to 31.1%. The spectrum of HPV genotypes was wider in the oral mucosa than the genital tract.

Most of the oral HPV infections of the men cleared (71.6% of 74 men). No predictors for clearance from the oral mucosa of HPV Species 7/9 were identified.

14.0% of the male subjects had genotype-specific persistence of oral HPV infection (18/129); the mean persistence time ranged from 6.0 to 30.7 months. Most of the persistent oral HPV infections in men were caused by HPV16. Smoking increased the risk for HR-HPV persistence in the oral mucosa; a history of genital warts protected from HR-HPV persistence.

At the last follow-up visit, 50.1% of the men (28/55) had visible changes in their oral mucosa. Smoking was the only risk factor predictive of these changes (p=0.046).

The genotype-specific concordance among the spouses was low. A female risk profile might enhance the transmission of HPV infection to her male partner and increase genotype-specific HPV concordance between the spouses. A specific risk profile for males that would predict HPV concordance between the spouses was not identified.

Sexual partners living in a stable relationship share the same environment and are exposed to one another's risky sexual behavior, should such occur. Data in the present study implicate that a stable marital relationship protects against oral and genital HPV. Changing the sexual partner (OR=15.00, p=0.028) and marital status (especially divorce) (p=0.001) increases the risk of incident genital HPV infections.

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