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OCCURRENCE OF HIGH RISK HUMAN PAPILLOMAVIRUSES AND CERVICAL CANCER AMONG FERTILE-AGED WOMEN IN FINLAND

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Abstract

High risk human papilloma virus (hrHPV) infection is a necessary but not a sufficient cause of cervical cancer. In Finland, since 1990 the incidence of cervical cancer has increased among women younger than 40 years of age despite a nationwide screening programme. In this thesis, the overall objective is to address the role of possible, earlier hrHPV epidemic in this increased incidence of cervical cancer.

The target population includes all fertile-aged women in Finland during 1983–2006. The actual study population comprised all women with a minimum of two pregnancies within five years and under 32 years of age in 1983–1997 and under 29 years of age in 1995–2003 identified from the Finnish Maternity Cohort (FMC). From this subpopulation, two subcohorts were selected for hrHPV antibody analysis by random sampling stratified by age and calendar time. All cases of cervical cancer diagnosed for women under 50 years of age during 1983–2002 and 1986–2006 were identified from the Finnish Cancer Registry. The case-cohort design, used for estimating population attributable fractions (PAF) associated with hrHPV, included the cases of cervical cancer and the first subcohort of FMC.

A steady annual increase of 0.7% per year in the incidence of HPV16 was estimated to have taken place in Finland from 1983 to 1997 among the 23–31-year-old women with at least two pregnancies. The estimated seroprevalence of HPV16 increased from 17% to 24%, respectively. The PAF of hrHPV exposures in squamous cell carcinoma of the uterine cervix (SCC) was estimated as 73% (95% CI: 13% to 93%). For 26–31-year-old women born in the 1960s and 1970s the incidence of SCC was roughly double compared with women born in the late 1950s. Mathematical modelling indicated that changes in the sexual behaviour partly accounted for the increase seen in the incidence of cervical cancer in the 1990s.

The findings of this thesis indicate that growth in the background exposure to HPV16 preceded the increase of incidence of cervical cancer in Finland. At younger birth cohorts, the increase of the incidence of SCC is visible among fertile-aged women in Finland. Whether overall screening starting at 25 years of age, higher participation rate for cervical screening or HPV vaccination of early adolescents is the future solution to lowering the incidence of cervical cancer among young women remains to be seen.

Keywords: cohort studies, human papillomavirus 16, incidence, papillomavirus infections, squamous cell carcinoma, stastitical models, uterine cervical neoplasms

Laukkanen, Päivi, Lisääntymisikäisten naisten korkean riskin ihmisen papilloomavirusten ja kohdunkaulan syövän esiintyvyys Suomessa.
Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta, Terveystieteiden laitos, PL 5000, 90014 Oulun yliopisto; Oulun yliopisto, Luonnontieteellinen tiedekunta, Matemaattisten tieteiden laitos, PL 3000, 90014 Oulun yliopisto; Terveyden ja hyvinvoinnin laitos, PL 310, 90101 Oulu; Suomen syöpärekisteri, Pieni Roobertinkatu 9, 00130 Helsinki; Tampereen yliopisto, Terveystieteiden yksikkö, 33014 Tampereen yliopisto
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Tiivistelmä

Ihmisen papilloomaviruksen (HPV), erityisesti korkean riskin tyypin (hrHPV), aiheuttama infektio on kohdunkaulan syövän välttämätön, mutta ei riittävä syytekijä. Suomessa vuoden 1990 jälkeen kohdunkaulan syövän ilmaantuvuus on valtakunnallisesta seulonnasta huolimatta noussut alle 40-vuotiailla naisilla. Tämän väitöskirjan tavoitteena on osoittaa, mikä rooli mahdollisella aiemmalla hrHPV-epidemialla on kyseiseen kohdunkaulan syövän ilmaantuvuuden kasvuun.

Tutkimuksen kohdeväestöön kuuluvat kaikki lisääntymisikäiset suomalaiset naiset. Varsinainen tutkimusväestö koostui kaikista vuosina 1983–97 alle 32-vuotiaana ja vuosina 1995–2003 alle 29-vuotiaana kaksi kertaa raskaana olleista naisista, jotka identifioitiin Suomen äitikohortista (FMC). Tästä joukosta valittiin satunnaisotannalla kaksi alikohorttia hrHPV-laboratorioanalyysejä varten. Kaikki vuosina 1983–2002 ja 1986–2006 kohdunkaulan syöpädiagnoosin alle 50-vuotiaana saaneet naiset poimittiin Suomen syöpärekisteristä. Tapaus-kohorttiasetelma, jota käytettiin hrHPV altistukseen liittyvien väestösyyosuuksien (PAF) estimoinnissa, sisälsi kohdunkaulan syöpätapaukset ja ensimmäisen alikohortin.

Suomalaisten 23–31 -vuotiaiden, vähintään kahdesti raskaana olleiden, naisten vuosittainen HPV16-ilmaantuvuus kasvoi tasaisesti 0.7 % per vuosi ajanjaksolla 1983–1997. Vastaavasti HPV16:n vallitsevuus kasvoi 17 prosentista 24 prosenttiin. Kohdunkaulan levyepiteelisyövän hrHPV-altistukseen liittyvän PAF:n estimoitiin olevan 73 % (95 %:n luottamusväli 13–93 %). Levyepiteelisyövän ilmaantuvuus oli suunnilleen kaksinkertainen 1960- ja 1970-luvulla syntyneillä naisilla, heidän ollessaan 26–31 -vuotiaita, verrattuna 1950-luvulla syntyneisiin samanikäisiin naisiin. Matemaattisen mallinnuksen tulosten perusteella kohdunkaulan syövän ilmaantuvuuden nousu 1990- luvulla selittyy ainakin osittain sukupuolikäyttäytymisen muutoksilla.

Tämän väitöskirjan tulokset osoittavat, että kasvanut HPV16-virukselle altistuminen edelsi kohdunkaulan syövän ilmaantuvuuden nousua Suomessa. Levyepiteelisyövän ilmaantuvuuden nousu nuorimmissa syntymäkohorteissa on nähtävissä lisääntymisikäisillä naisilla Suomessa. Tulevaisuudessa nähdään, onko seulonnan aloittaminen 25-vuotiaana, korkeampi seulontaan osallistumisosuus vai nuorten aikuisten HPV-rokottaminen ratkaisu nuorten naisten kohdunkaulan syövän ilmaantuvuuden vähentämiseksi.

Asiasanat: epidemiologia, kohdunkaulansyöpä, kohorttitutkimus, papilloomavirus, tilastolliset mallit

To Pirianna, Tiia, Pinja and Aato

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Abbreviations

DNA	Deoxyribonucleic acid
CI	Confidence interval
CIS	Cervical carcinoma in situ
CIN3	Cervical intraepithelial neoplasia grade III
ELISA	Enzyme-linked immunosorbent assay
FCR	Finnish Cancer Registry
FMC	Finnish Maternity Cohort
HIV	Human immunodefiency virus
HPV	Human papilloma virus
HPV16	Human papilloma virus type 16
hrHPV	High risk human papilloma virus
HSIL	High squamous intraepithelial lesion
ICC	Invasive cervical cancer
ICD	International Statistical Classification of Diseases and Related Health Problems
LSIL	Low squamous intraepithelial lesion
SCC	Squamous cell carcinoma of the uterine cervix
PAF	Population attributable fraction
Pap smear	Papaniculaou-stained cytological cervico-vaginal smear
PCR	Polymerase chain reaction
RR	Relative risk, rate ratio

List of original articles

This thesis is based on the following articles referred to in the text by Roman numerals:

I Laukkanen P, Koskela P, Pukkala E, Dillner J, Läärä E, Knekt P & Lehtinen M (2003) Time trends in incidence and prevalence of human papillomavirus type 6, 11 and 16 infections in Finland. J Gen Virol 84(Pt 8): 2105–2109.

II Laukkanen P, Läärä E, Koskela P, Pukkala E, Virkkunen H & Lehtinen M (2010) Population fraction of cervical neoplasia attributable to high-risk human papillomaviruses. Future Oncol 6(5): 709–716.

III Laukkanen P, Lehtinen M, Pukkala E, Surcel H-M & Läärä E (2012) Incidence trends of invasive cervical cancer in Finland with reference to seroprevalence trends of human papillomavirus type 16, 18 and 31/33. Manuscript.

IV Barnabas RV, Laukkanen P, Koskela P, Kontula O, Lehtinen M & Garnett GP (2006) Epidemiology of HPV 16 and cervical cancer in Finland and the potential impact of vaccination: mathematical modelling analyses. PloS Med 3(5): e138

Also unpublished data (III) is presented in this thesis.

Contents

1 Introduction

Human papillomaviruses (HPV) are common viruses that cause warts and have been established as the sexually transmitted agents that cause the majority of invasive cervical cancers and their associated precancerous lesions (IARC 1995, Baseman & Koutsky 2005). The causal relationship between the HPV infections and cancer of the uterine cervix was first suggested by Nobel Laureate of 2008, Harald zur Hausen in the 1970s (zur Hausen 1976). In 1983, HPV type 16 (HPV16) was established as the leading candidate in the pathogenesis of cervical cancer (Dürst *et al.* 1983). The causal relationship became extensively confirmed later, when it was found that virtually all cases of cervical cancer examined worldwide have at least one of the 15 high risk oncogenic types of HPV (Walboomers *et al.* 1999). HPV is a very common infection, although in most cases traces of the virus are eliminated in a relatively short time and hence infected individuals never develop clinically recognized manifestations. Thus, eventually very few HPV infected individuals contract cervical cancer.

A well-established factor that partially explains differential cervical cancer risk is the HPV type (Baseman & Koutsky 2005). In the 1980s and 1990s, type-specific HPV serology was the key method in the elucidation of the importance of HPV exposure for cancer development (Bjørge *et al.* 1997, Lehtinen *et al.* 1996, Mork *et al.* 2001, Wang *et al.* 1997). Later, HPV was also identified as a major aetiological agent of some other cancers, namely 70% of anal cancers, 50% of vaginal cancers, and it was found that about 25% of head and neck squamous cell carcinomas contain HPV DNA, mostly of type 16 (Pagliusi & Garland 2007).

Cancer of the uterine cervix is the third most common cancer among women worldwide with an estimated 529 000 new cases and 274 000 deaths in the year 2008 (Ferlay *et al.* 2010). Almost 80% of the cases occur in developing countries where, in many regions, it is the most common cancer among women (Parkin *et al.* 2001). In developed countries, where population-based screening has been routinely practiced, substantial declines in both incidence and mortality of cervical cancer have been reported (Bray *et al.* 2005a). In Finland, the direction of the trend has changed. The comprehensive drop in incidence of cervical cancer over the 1970s and the 1980s was replaced by an upturn during the 1990s, especially among women younger than 40 years of age (Engholm *et al.* 2012).

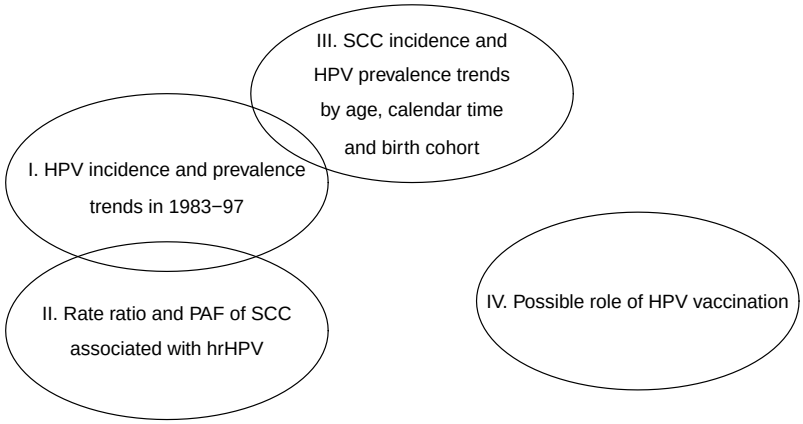

Fig 1. Original studies I, II and III are based data from 1983 to 1997. Original study IV, role of HPV vaccination is based on mathematical modelling analyses.

Since the early 2000s it has been known that HPV infection (and hence cervical cancer) can be prevented by prophylactic vaccination (Koutsky *et al.* 2002). In Finland, two HPV vaccines have been available for private use since 2007. In future, HPV vaccination may have the greatest value in developing countries, where most of the global burden of cervical cancer occurs each year and where Pap smear screening has been largely ineffective or unavailable (Franco & Harper 2005).

In the 1990s, research work was activated (Carter *et al.* 1996, af Geijersstam *et al.* 1998a, Silins *et al.* 1999, Wideroff *et al.* 1999) in order to find answers to questions related to HPV DNA and HPV serology, but many of those studies were cross-sectional, therefore population-based longitudinal data on HPV incidence trends are lacking. In the present study, the role of possible oncogenic HPV epidemics preceding the observed increase in the incidence of cervical cancer in Finland was evaluated among fertile-aged women (Figure 1). Recent changes in the incidence of cervical cancer among different birth cohorts were estimated after the identification of all cases of cervical cancer from the population-based Finnish Cancer Registry. Additionally, scenarios for the possible role of HPV vaccination in Finland were predicted using mathematical modelling.

2 Review of the literature

2.1 Definitions – Cervical cancer and human papillomavirus detection

International Classification of Diseases, ICD-10, is used as basis of coding new cases of cancer at the Finnish Cancer Registry (FCR). In this thesis ICD-10 code C53 "malignant neoplasm of cervix uteri", is referred to shortly as cervical cancer. The FCR classifies the malignancy of cervical cancer in the following categories: invasive cervical cancer (ICC), microinvasive cervical cancer, cervical intraepithelial neoplasia grade III (CIN3), and carcinoma in situ (CIS).

The Bethesda System for reporting the results of cervical cytology was introduced in 1988 as a uniform system of terminology. The Bethesda 2001 Workshop (Solomon *et al.* 2002) updated the terminology. Epithelial cell abnormalities are divided to squamous cell and grandular cell abnormalities. *Squamous cell abnormalities* include the following categories: atypical squamous cells, low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and squamous cell carcinoma (SCC). LSIL encompasses human papillomavirus infection, mild dysplasia, and CIN1. HSIL encompasses moderate and severe dysplasia, CIS, CIN2, and CIN3. *Grandular cell abnormalities* include categories of atypical glandular cells, atypical glandular cells – favor neoplastic, endocervical adenocarcinoma in situ, and adenocarcinoma. (Solomon *et al.* 2002).

Papillomaviruses belong to the Papovaviridae family. Papovaviruses are named for papillomas (warts), polyomas (tumors) and vacuoles (produced by some of these viruses) (Tortora *et al.* 2002). Papillomaviruses are small (40-57 nm), nonenveloped, double-stranded DNA viruses that within the animal kingdom cause a variety of epithelial lesions, ranging from benign warts to invasive cancers (Pagliusi & Garland 2007). Some papilloma virus types are capable of transforming cells and causing cancer. Viral DNA is replicated in the nucleus of the host cell. Transformed host cells may proliferate, resulting in a tumor. (Tortora *et al.* 2002). Of the known cancers associated with human papilomaviruses (HPVs), cancer of the uterine cervix accounts for 90%, whilst other anogenital cancers (vulvae, vagina, penis, anus) for 5%, with cancers of the head and neck corresponding to about 1% (Pagliusi & Garland 2007).

Since 1907 it has been known that genital warts are caused by human papillomaviruses (Tortora *et al.* 2002). Two main types, HPV6 and HPV11, are found in genital warts (Condyloma acuminata) (zur Hausen 1999). Morphologically, some warts are extremely large with multiple fingerlike projections resembling cauliflower. Others are relatively smooth or flat. The incubation period is usually a few weeks or months. (Tortora *et al.* 2002).

As the HPV life cycle takes place entirely within the squamous epithelial cell, it is effectively 'hidden' from the host immune system (Carter *et al.* 2011, zur Hausen 1999). Therefore, presentation of viral antigens to the host immune system is limited (Dillner *et al.* 2011). Unlike other infections, there is no systemic response, no viraemia and no blood-borne phase. The infected epithelial cells undergo 'non-lytic cell death' and as such are not destroyed. They are able to release newly synthesised virus but do not always evoke an inflammatory reaction but rather block the release of inflammatory cytokines, which normally appear in response to an invader. (Carter *et al.* 2011).

There are basically three classes of detectable markers directly derived from HPVs: 1) molecular markers based on the detection of nucleic acid sequences, 2) serological markers based on the detection of antibodies against viral proteins, and 3) cellular markers based on the detection of proteins expressed intracellularly, upon either infection or carcinogenesis. An increasing demand exists to develop standard tools for assessing the quality of HPV detection systems for regulatory and clinical management purposes. (Pagliusi & Garland 2007). International standard reagents for HPV are crucial for improving the sensitivity of antibody assays and the consistency of the results worldwide (Ferguson *et al.* 2006). They will also allow to assure that laboratory services used to evaluate the disease burden, the therapeutic effects of HPV vaccines, and other cancer prevention strategies are accurate and comparable worldwide (Pagliusi & Garland 2007).

Natural HPV infection of the genital tract gives rise to a slow and modest but measurable serum antibody response in most, but not all, infected individuals (Dillner *et al.* 2011). Type specific HPV antibodies can be measured by means of HPV serology (Carter *et al.* 1996, Dillner *et al.* 1996). The intensity of the antibody response depends upon viral load and persistence (Dillner *et al.* 2011). The presence of HPV antibodies is longlasting and stable over time (af Geijersstam *et al.* 1998a) but does not contribute to the clearance of established infections (Dillner *et al.* 2011). *HPV serology* is an important tool in epidemiological studies to assess past exposure (Dillner *et al.* 2011).

The enzyme-linked immunosorbent assay (ELISA) is a widely used method because it is fast and highly automated. The ELISA used in HPV serology, detects anti-HPV

antibodies of the IgG or IgA isotopes. Many ELISA tests are available for clinical use in the form of commercially prepared kits. Procedures are often highly automated, with the results read by a scanner and processed by a computer. (Tortora *et al.* 2002).

2.2 Aetiology and natural history of cervical cancer

Because of its central role in the aetiology of practically all cases, HPV is a (virtually) necessary but (generally) not sufficient cause of cervical cancer (Schiffman *et al.* 2011). New HPV infections acquired at any age are mostly benign, but persistent infections with at least one of the oncogenic, high risk HPV types (hrHPV) explain virtually all cases of cervical cancer (Ho *et al.* 1998, Schiffman *et al.* 2011). In the absence of a persistent hrHPV infection, the risk of cervical cancer is extremely low (Schiffman *et al.* 2011). The incubation time from HPV infection to the diagnosis of cervical cancer varies from 10 to 20 years (Gravitt 2011, Schiffman *et al.* 2011). The interval from first HPV infection to HSIL is usually less than the time from HSIL to cervical cancer, which has been estimated to be about 7-10 years (Carter *et al.* 2011).

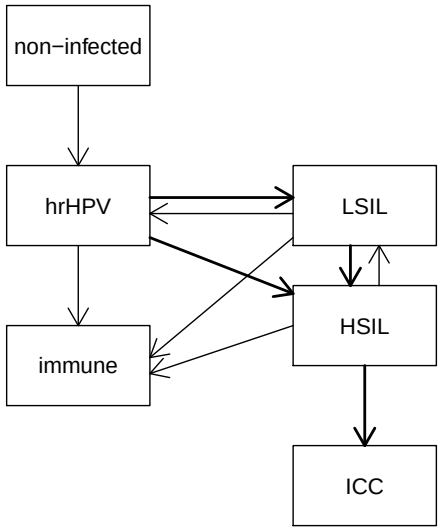

Fig 2. A diagram of the progression from hrHPV infection to invasive cervical cancer (Barnabas & Garnett 2004, Gravitt 2011, Schiffman *et al.* 2011).

A hrHPV infection can progress to LSILs, HSILs and ICC, although most infections regress spontaneously and an immune state follows (Figure 2). The historical literature suggests that between one- and two-thirds of women with HSIL will develop invasive cancer if left untreated (Baseman & Koutsky 2005). The mean age of women with invasive cervical cancer at diagnosis is approximately 50 years, while the mean age of women with HSIL is approximately 28 years (Baseman & Koutsky 2005). This long premalignant course of HPV infection provided the screening programmes an opportunity to detect and treat early disease and prevent progression to cervical cancer.

HPV infection is a common sexually transmitted infection (zur Hausen 1999, Muñoz *et al.* 2003, Walboomers *et al.* 1999). Altogether 118 HPV types have been completely described, and an even higher number of presumed new types have been identified (de Villiers *et al.* 2004). At least 30 different HPV types infect the genital area, of which 15 HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 are classified as high risk HPV (hrHPV) types, and HPV types 26, 53, and 66 as probable high-risk types (Muñoz *et al.* 2003, Baseman & Koutsky 2005, Schiffman *et al.* 2011). In a large meta-analysis, the prevalence of any hrHPV type among cases of invasive cervical cancer was 90% while those of HPV types 16 and 18 were 57% and 16%, respectively (Li *et al.* 2011). The overall prevalences of single and multiple HPV infections were 79% and 11%, respectively (Li *et al.* 2011).

Whilst hrHPV infection is necessary, other associated co-factors are required for the development of malignancy. These may include smoking, long-term oral contraceptive pill use, human immunodeficiency virus (HIV) co-infection, high parity and *Chlamydia trachomatis* infection, absence of male circumcision, immune suppression as well as some dietary factors (Carter *et al.* 2011, Castellsagué *et al.* 2011). These cofactors in cervical cancer may act in at least three ways: 1) by influencing the acquisition of HPV infection, 2) by increasing the risk of HPV persistence, and 3) by increasing the risk of progression from HPV infection to HSIL and cancer (Castellsagué & Muñoz 2003). An explanation of the role some of these co-factors may take in HPV persistence follows. Smoking is immunosuppressive, affecting the metabolism of female hormones and causing genetic damage from tobacco-related carcinogens. The use of oral contraceptive pills enhances HPV gene expression in the cervix and may promote the integration of the virus into the host chromosome. (Carter *et al.* 2011). *Chlamydia trachomatis* is associated with an inflammatory response that has been linked with the generation of free radicals and immune perturbation (Koskela *et al.* 2000, Carter *et al.* 2011).

Regular Pap smear screening reduces the risk of cervical cancer (Castellsagué & Muñoz 2003)). Based on a large pooled analysis (Castellsagué *et al.* 2011), it is proposed that the use of intrauterine device might act as a protective cofactor in cervical carcinogenesis. A protective association was noted for squamous-cell carcinoma, adenocarcinoma and adenosquamous carcinoma, but not among HPV positive women. Cellular immunity augmented by the device might be one of several mechanisms that could explain the findings. (Castellsagué *et al.* 2011).

2.3 Prevention of cervical cancer

Over the past decades, prevention programmes for cancer of the cervix have been essentially based on the detection and treatment of precursor lesions (high grade cervical dysplasia) or early cancer by screening women at risk using the cytological method described by Papanicolau, commonly known as the Pap smear (Pagliusi & Garland 2007). In Europe and Northern America the burden of cervical cancer has disminished due to the introduction of screening in the 1960s, with data from several long-standing cancer registers showing that the incidence rates in most of these countries have more than halved since then (Barken *et al.* 2012). For *conventional screening* (i.e. conventional cervical cytology), studies using cohort, case-control or geographical correlation (before/after analysis) designs indicate substantial effects in reducing the incidence and mortality rates of cervical cancer, the impact exceeding 80% among women screened in various organized settings (IARC Working Group 2005).

Since the mid 1960s both the incidence and mortality rates of cervical cancer have reduced by 80% in Finland (van der Aa *et al.* 2008, Kotaniemi-Talonen *et al.* 2007). The success of the Finnish screening programme was based on multiple organisational and societal factors: a) the invitational coverage of the target population is high, as a nationwide population registry based on unique personal identifiers is used for invitations, b) individual invitation letters with scheduled appointment times are widely used, c) the screening visits are free of charge for the attendees, d) the screening samples are collected by trained registered nurses or midwives, e) smears are analysed mainly in laboratories specialised in cervical cytology, f) histological confirmation and treatment procedures are conducted by highly qualified professional personnel, and g) reporting the screening results to a nationwide mass screening register within the FCR is mandatory for quality assurance purposes (Kotaniemi-Talonen *et al.* 2007).

The screening programme in Finland was first targeted for the 30-50-year-old, however, since 1992 it has covered 30-60-year-old women. As a rule, invitations are sent every fifth year. (Kotaniemi-Talonen *et al.* 2007, van der Aa *et al.* 2008). All cytological samples taken within the programme are classified according to the Papanicolaou classification system, where class I is normal, class II atypical but non-malignant (corresponds to reactive changes or LSIL in the Bethesda 2001), class III suggestive of malignancy (LSIL or HSIL; mild to moderate dyskaryosis), class IV strongly suggestive of malignancy (HSIL; moderate to severe dyskaryosis), and class V conclusive for malignancy. All women with classes III-V cytology are referred to colposcopy. Most women with Pap class II are recommended for cytological follow-up after 6-12 months (risk group screening), and those with persistent abnormal smears are referred to colposcopy. (Kotaniemi-Talonen *et al.* 2007). The attendance rate of the Finnish screening in 1990-2010 has been approximately 70%, but among women aged 30-35 years the rate has been only approximately 50-60% (Mass Screening Registry 2011).

However, not all untreated CINs would progress to cancer, and at present no reliable method exists for distinguishing the progressing CINs from those nonprogressing ones. As a consequence, the reduction in the burden of cervical cancer for some women has been achieved by treating nonprogressive CINs in other women, and CIN treatment may potentially lead to adverse obstetric outcomes. Even for high grade CIN such as CIN3, the proportion without progression to cancer appears to be substantial. Estimates of the extent of overtreatment have ranged from about three to about six CIN3 treatments per one prevented case of cervical cancer. (Barken *et al.* 2012).

The preventive effect of conventional screening is based on repeated Pap smear tests, although one single test already provides some protection against cervical cancer. Alternative technologies such as liquid based cytology and HPV DNA screening have been developed to improve the prevention of cervical cancer. *Liquid-based cytology* belongs to the same main class as conventional cytology (IARC Working Group 2005). It is used for primary screening of cervical cancer. Liquid-based cytology techniques create more uniform slides, and computer assisted cytology evaluation systems have been adopted to achieve greater laboratory productivity. As yet, however, there is no evidence that they would detect CIN3 more accurately than conventional cytology (Schiffman *et al.* 2011).

Despite great progress in cancer control through screening programmes based on cervical cytology, high coverage and quality control and assurance are crucial in achieving a significant reduction in the burden of disease.However, in developing

countries appropriate resources and continuous quality assurance programmes are not always available. The natural history of HPV infection is such that the lifetime risk for development of cervical cancer without involvement of any screening programme is about 3% and with annual Pap smears, less than 1% (Pagliusi & Garland 2007). The success of screening programmes is related in part to the fact that cervical cancer takes several decades to develop from a chronic oncogenic HPV infection. Although HPV is the commonest genital viral infection in healthy sexually active subjects, the presence of persistent oncogenic HPV types in tissue samples may constitute a prognostic marker of underlying disease in the future. (Pagliusi & Garland 2007).

Randomized clinical trials now provide overwhelming evidence that *HPV DNA screening* is more sensitive than cytological screening for detecting histological CIN3 (Schiffman *et al.* 2011). Together with conventional cytology which has a low sensitivity and a high specificity, the tests could be used to complement each other. A negative HPV DNA test provides a long-term risk stratification: among HPV DNA negative women 5-10 years of reassurance of not developing CIN3, and even stronger reassurance of not developing invasive cervical cancer. High negative predictive value permits the safe and cost-effective lengthening of the cervical screening interval when HPV testing is used. A major challenge will be to implement programs that do not overtreat HPV DNA positive women who do not have obvious long-term persistence of HPV or treatable lesions at the time of initial evaluation. One possibility is to restrict carcinogenic HPV DNA testing to women aged 30 years or older, who are past the peak age of acute HPV infections and have a higher proportion of HPV infections that are persistent. (Schiffman *et al.* 2011).

The conventional screening has been shown to effectively detect SCC in early stages, whereas adenocarcinomas have been reported being less detectable by such methods (Bray *et al.* 2005a, Zappa *et al.* 2004, Mitchell *et al.* 1995). It is worth noting that HPV DNA testing might be especially useful for the detection of adenocarcinomas (Schiffman *et al.* 2011). In Finland, among women under 50 years of age with at least one pregnancy 75% of invasive cervical cancers are SCCs.

It has been known for 20 years that cervical HPV infection and hence, cervical cancer can be prevented by vaccination (Crum 2002). In future, cervical cancer will most likely be largely controlled by *HPV vaccination* (Koutsky & Harper 2006, Lehtinen *et al.* 2006a, FUTURE II Study Group 2007, Paavonen *et al.* 2009). This would be of particular importance for cervical cancer control in developing countries with low resources for screening (Lehtinen *et al.* 2006a, Crum 2002). However, direct evidence for the effectiveness of HPV vaccination against cervical cancer is still lacking. The

latent period for cervical cancer is up to 20 years, thus the results of ongoing vaccine trials will only be seen in the future. In general, cervical intraepithelial neoplasia grade 2 or greater (CIN2+) is the accepted clinical endpoint to evaluate HPV vaccine efficacy (Wheeler *et al.* 2012). Population-based vaccination that incorporates the HPV-16/18 vaccine with high coverage of early adolescents is optimistically anticipated to have the potential to substantially reduce the incidence of cervical cancer (Lehtinen *et al.* 2012). The randomized controlled trials have demonstrated clear efficacy of HPV-based screening and vaccination, but questions about best practices for implementation remain (Gravitt 2011, Katki *et al.* 2009).

Passive follow-ups of HPV vaccination cohorts, recruited for the currently conducted Phase III trials in a population-based fashion, are ongoing in the Nordic countries which have population-based cancer registries (FUTURE II Study Group 2007, Paavonen *et al.* 2009, Lehtinen *et al.* 2012). This will, ultimately, yield data on the efficacy of HPV vaccine against cervical cancer (Lehtinen *et al.* 2006a). In the meantime, data on better surrogate end points than CIN2+ are needed. High vaccine efficacy, 93% (95% confidence interval (CI): 78.9% to 98.7%) against all CIN3 lesions has been recently reported in the total vaccinated HPV-naive cohort and vaccine efficacy against all adenocarcinomas in situ was 100% (95% CI: 31.0% to 100%) (Lehtinen *et al.* 2012).

Currently, there are two vaccines available: the HPV-16/18 AS04-adjuvanted vaccine (Cervarix, GlaxoSmithKline Biologicals) and the quadrivalent HPV-6/11/16/18 vaccine (Gardasil, Merck) (Wheeler *et al.* 2012). Both vaccines are very efficient in reducing HPV16 and HPV18 disease of the lower genital tract; in addition, the quadrivalent vaccine will also significantly reduce genital lesions related to HPV6 and HPV11 infections (Carter *et al.* 2011).

In future, preventing women from being infected with high-risk HPVs before they become sexually active might be the major preventive method against cervical cancer, however, conventional cytological screening will still be needed for non-vaccinated women for several decades. As vaccination will not protect against all cervical cancers, nor against pre-existing HPV infections, and as the duration of protection is not yet known, routine Pap smear screening should be continued (Carter *et al.* 2011). Whilst vaccination will not protect against all hrHPV types, these vaccinated young women are at a significantly lower risk of developing dysplasia, and considerations for modification of the screening programme need to be given a high priority. Options that should be considered may include a) increasing the age of first screening, b) increasing the interval

between screenings, and c) the use of new technologies such as HPV testing (Carter *et al.* 2011).

2.4 Incidence of cervical cancer 1970-2010

In developed countries, the incidence rates of cervical cancer are generally low which is generally attributed to effective screening. The age-incidence curve of cervical cancer in European populations has the following common features: it begins to rise from the age of 20 years onwards, increasing rapidly until reaching a peak usually around 45-49 years of age (Parkin *et al.* 2001, Bray *et al.* 2002). Recently (during 2005-2009) in the Nordic countries the peak of the incidence has been around 35-40 years of age (Engholm *et al.* 2012). In 2008, the cumulative incidence rates (per 100) of cervical cancer up to 75 years of age were 1.0 in Europe whereas in Africa the rate was 3.4 and in South East Asia 2.6, respectively (Ferlay *et al.* 2011).

The dominant histologic subtype of cervical cancer is the SCC, which currently accounts for 75% to 90% of cervical neoplasms in developed countries. Large reductions in incidence of SCC have occurred in several northern and western European countries since the 1970s: around 4% per year in Finland, Sweden, France, and Switzerland. (Bray *et al.* 2005b). In Southeastern England, the cumulative rates showed a similar decline of 4% per year from those diagnosed during 1985-90 to 2000-04 (Robinson *et al.* 2009). While other factors, such as decreasing fertility, may be important in explaining the declining incidence of cervical cancer (Franco *et al.* 2001), comprehensive screening programmes made a major contribution to the diminishing caseload at least in the Nordic countries and the UK (van Ballegooijen *et al.* 2000, Peto *et al.* 2004, Robinson *et al.* 2009).

It is notable that similar birth cohort patterns have emerged across several European countries. A declining incidence is found across generations born in the first three decades of the 20th century, however, this has often been replaced by successive increases in women born thereafter. The timing of the turning point has varied between countries, but in each the risk began to shoot up in generations born in the early 1930s through those of the late 1940s. In northern Europe, this pattern is evident in the cohort trends in Denmark and Finland, whereas in Estonia and the United Kingdom the increased risk among the younger birth cohorts is noticeable somewhat earlier. (Bray *et al.* 2005b).

In Finland, the direction of the trend has changed. The large drop in cervical SCC over several decades (the 1970s and the 1980s) was replaced by an upturn of over 8% per year in the 1990s (Bray *et al.* 2005b). The cumulative incidence rate of cervical cancer up to 75 years of age rapidly decreased from 1.25 per 100 in 1970 to 0.31 per 100 in 1991 (Engholm *et al.* 2012). The low incidence is due to the effective nationwide screening program, which started gradually from 1963 for 30-50-year-old women with recommended screening intervals of five years (Anttila *et al.* 1999, Nieminen *et al.* 2002). During the 1990s, the cumulative incidence rate of cervical cancer rose to 0.40 per 100. Since 2000, the cumulative incidence rates have been between 0.34-0.41 per 100 each year (Engholm *et al.* 2012).

The rise in the level of incidence across successive birth cohorts is evident in Finland in women born since 1945 (Bray *et al.* 2005b). The increasing incidence since 1990 among Finnish women younger than 55 has been attributed to changing sexual lifestyles and smoking habits (Anttila *et al.* 1999) as well as a grown transmission of papillomaviruses in younger generations of women and to screening attendance shortfalls (Bray *et al.* 2005b).

In Finland, since 1990 the incidence of cervical cancer has risen especially among women younger than 40 years of age (Engholm *et al.* 2012). The age-standardised (Nordic standard population), three years average incidence of cervical cancer among women 15-39 years of age was 2.1 per 100 000 woman-years in the late 1980s, but it has risen up to 5.1-5.8 per 100 000 woman-years by 2006-08 (Engholm *et al.* 2012), to almost the same level in this age group as in the late 1960s, when organised cervical cancer screening in Finland was in its infancy (Figure 3). Similar changes are not seen in other Nordic countries nor among women older than 40 years of age, albeit in Finland during the 1990s a slight increase in the incidence of cervical cancer also occurred among older women.

In conclusion, when this study started a rapid increase had already occurred in the incidence of cervical cancer in Finland during the 1990s especially among women younger than 40 years. A possible explanation was that the prevalence of the biological background risk factors of cervical cancer had increased (Anttila *et al.* 1999). Changes or inadequacies in the effectiveness of the organised screening program especially among young target ages might also have contributed to the upward trend (Anttila *et al.* 1999).

In 1969, the age-adjusted seroprevalence of human papillomavirus type 16 (HPV16) among pregnant women was 16% in Stockholm (Sweden) and had increased to 21% by

1989 (af Geijersstam *et al.* 1998b). In Helsinki (Finland), the age-adjusted seroprevalence of HPV16 was 24% among primiparous women in both 1983 and 1990 (Kibur *et al.* 2000a). In Tallinn (Estonia), the comparable seroprevalence of HPV16 was as high as 36% during 1996-97 (Kibur *et al.* 2000b). Thus, before this study it was known that HPV16 seroprevalence may vary both by country and by time period, but population based data on HPV16 incidence trends were lacking.

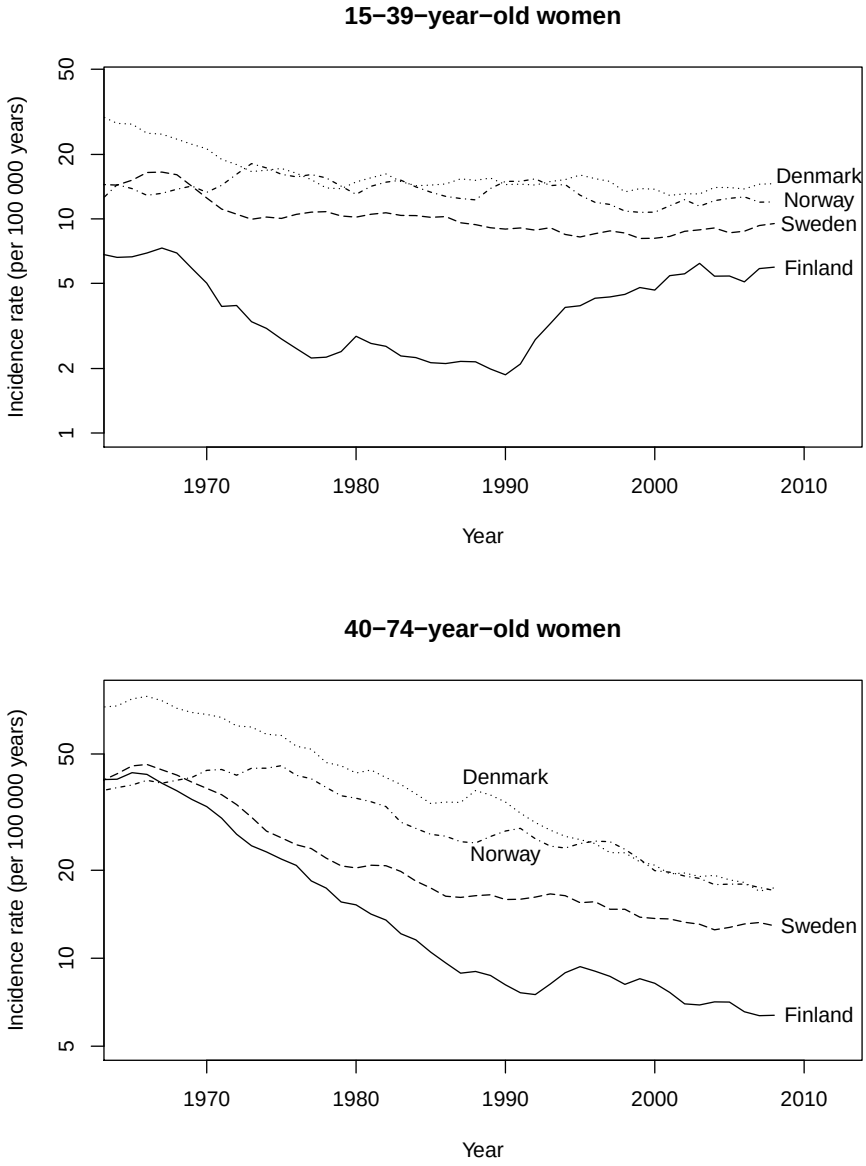

Fig 3. The age-standardised (Nordic standard population) incidence trends of cervical cancer in Denmark, Norway, Sweden and Finland among 15-39 and 40-74-year-old women (Engholm *et al.* 2012). The rates have been smoothed using 3 years average.

3 Aims of the study

High risk human papilloma virus (hrHPV) infection is a necessary but not a sufficient cause of cervical cancer. In Finland, since 1990 the incidence of cervical cancer had an upward trend among women younger than 40 years of age despite the screening programme. The overall objective is to address the role of possible, earlier hrHPV epidemic in this increased incidence of cervical cancer.

The specific aims of the study were:

1. to identify possible oncogenic human papillomavirus type 16 (HPV16) epidemics preceding the increase in incidence of cervical cancer by assessing trends of age-specific incidence and prevalence of HPV16 infections among fertile-aged Finnish women during 1983-1997 (original study I).
2. to obtain valid relative risk estimates of invasive cervical cancer associated with hrHPV, and misclassification-corrected seroprevalence of hrHPV data, for assessing lifetime hrHPV exposure associated population attributable fraction among fertile-aged Finnish women (original study II).
3. to improve our knowledge regarding how age at diagnosis, calendar time, birth cohort and number of pregnancies are associated with the incidence of cervical cancer among Finnish women under 50 years of age during the recent decades (original study III).
4. to obtain estimates of the possible effects of different HPV-vaccination scenarios: a) vaccinating men and women compared with vaccinating women only, b) vaccination before and after sexual debut, and c) immunisation through national programmes versus opportunistic vaccination (original study IV).

4 Materials and methods

4.1 Data sources, study populations and sampling

The study population includes fertile-aged women in Finland. All under 32 years of age in 1983-1997 and under 29 years of age in 1995-2003 pregnant women were identified for subcohort selection and hrHPV antibody analysis. All cases of cervical cancer diagnosed for women younger than 50 years of age during 1983-2002 and 1986-2006 were identified and linked with the subcohorts of pregnant women. In mathematical modelling, virtual population was formed using rates of birth and death, based on the demography of Finland until 2002. More detailed description of the various data sources and study populations as well as sampling procedures follows.

The Finnish Maternity Cohort and subcohort selection

The Finnish Maternity Cohort (FMC) comprises women, whose sera have been collected during the first trimester of pregnancy for the screening of syphilis, human immunodeficiency virus (HIV) and hepatitis B virus infections and rubella immunity at municipal maternity clinics. Two thirds of the serum samples are collected at 8 to 12 weeks of pregnancy. Since 1983, all serum samples have been stored at -25 °C in polypropylene cryovials in a well-protected biorepository at the National Public Health Institute (KTL) of Finland; nowadays the National Institute for Health and Welfare (THL) (Toriola *et al.* 2011). More than 98% of pregnant women in Finland have donated serum samples to the FMC, and currently over 1.6 million samples are kept in storage. Each year, around 60 000 new serum samples are added to the repository (Toriola *et al.* 2011). The National Institute for Health and Welfare has a legal entitlement to use the FMC serum samples for public health research in Finland (KTL 828/1981 and THL 327/2001: 1a §). Since 2001, an informed consent from the donor has been obtained for research use. About 50 percent of the donors became pregnant again within five years.

In February 1998, after identifying all the 230 998 women under 32 years of age and with a minimum of two pregnancies within five years, a subcohort within the FMC was randomly sampled as follows. All eligible women were divided into 25 strata jointly by estimated age (<20, 20-22, 23-25, 26-28 and 29-31 years) and calendar time (1983-85, 1986-88, 1989-91, 1992-94 and 1995-97) of possible seroconversion between the two

pregnancies (Table 1). The estimated time of possible seroconversion was calculated as the mean of two serum sampling dates (any two consecutive pregnancies within five years). In each stratum, a subsample of 200 or 400 women was selected randomly. Four hundred 23-31-year-old women per stratum were selected because their human papillomavirus (HPV) incidence was expected to be low (Kibur *et al.* 2000a). Because of the total loss or inadequate volume of a serum sample for HPV ELISAs, 138 serum sample pairs from the subcohort were excluded. Paired sera (median sampling interval 23.9 months, quartiles 17.9 and 33.5 months) were finally available for 7862 women (Table 1). Original studies I and II are based on this first subcohort.

Table 1. Size (*n*) of random subsamples and absolute numbers (*N*) of women belonging to the first subcohort of FMC by estimated age and calendar time of possible seroconversion. (Reprinted with permission from Society for General Microbiology.)

Age (y)	1983-85	1986-88	1989-91	1992-94	1995-97	Total
	n (N)	n (N)	n (N)	n (N)	n (N)	n (N)
<20	197	192	200	193	200	982
	(1046)	(2285)	(2189)	(2063)	(218)	(7801)
20-22	195	195	193	199	200	982
	(4249)	(8870)	(9267)	(7899)	(717)	(31002)
23-25	390	392	388	394	399	1963
	(7020)	(16177)	(18298)	(16169)	(1153)	(58817)
26-28	394	397	385	390	399	1965
	(7848)	(19300)	(24483)	(22758)	(1549)	(75938)
29-31	396	392	393	389	400	1970
	(5342)	(14001)	(18396)	(18461)	(1240)	(57440)
Total	1572	1568	1559	1565	1598	7862
	(25505)	(60633)	(72633)	(67350)	(4877)	(230998)

The women had had two pregnancies within 5 years during 1983-97.

As the identification took place in February 1998, the availability of two serum samples in the first subcohort of FMC was right censored, for the last three calendar years (1995-1997). Moreover, very low numbers of seroconversions were discovered among 29-31-year-old women. Thus, in 2006, a second subcohort from FMC was selected in the same way as the first one, now from those women under 29 years of age who were pregnant during 1995-2003 (Table 2). Before the random selection, altogether 123,773 eligible women were divided into 12 strata according to estimated age (<20, 20-22, 23-25 and 26-28 years) and calendar time (1995-1997, 1998-2000, 2001-2003)

of possible seroconversion. The second subcohort has been described in detail (Kaasila *et al.* 2009, Merikukka *et al.* 2011). Because of an inadequate volume of a serum sample for HPV ELISAs, 417 serum sample pairs from the second subcohort were excluded. Finally, paired sera were available for 3183 women (Table 2). In the original study III, data from the first subcohort covering calendar years from 1986 to 1994 were included and merged with those of the second subcohort covering calendar years 1995 to 2003.

Table 2. Size (*n*) of random subsamples and absolute numbers (*N*) of women belonging to the second subcohort of FMC by estimated age and calendar time of possible seroconversion.

Age (y)	1995-1997	1998-2000	2001-2003	Total
	n (N)	n (N)	n (N)	n (N)
<20	170	155	164	489
	(1920)	(1878)	(20269	(5824)
20-22	196	186	166	548
	(7625)	(7841)	(7513)	(22979)
23-25	382	364	325	1071
	(13600)	(13248)	(13203)	(40051)
26-28	376	360	339	1075
	(20413)	(17105)	(17401)	(54919)
Total	1124	1065	994	3183
	(43558)	(40072)	(40143)	(123773)

The women had had two pregnancies within 5 years during 1995-2003.

The Finnish Cancer Registry

The Finnish Cancer Registry (FCR) has collected data on all incident cases of cancer in Finland since 1953. Hospitals, medical practitioners, and pathological laboratories notify the FCR about each newly diagnosed cancer. Moreover, Statistics Finland reports to the FCR whenever cancer is mentioned in a death certificate. In cases of cancer with only laboratory notifications or death certificate information, the FCR requests clinical data from the hospital where the primary diagnosis or treatment took place. (Pukkala *et al.* 2010).

Linkage of the FMC with the FCR

In May 2002, the first linkage of the FMC data with the population-based FCR was performed based on the permission of the Finnish Ministry of Health and Welfare, and

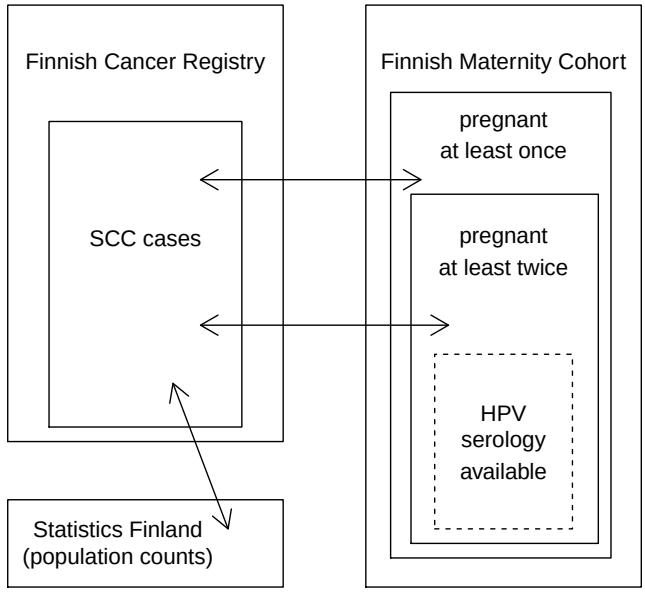

Fig 4. Identification of SCC diagnosed during 1986-2006 in Finland for women under 50 years of age from Finnish Cancer Registry and linkage with Finnish Maternity Cohort and Statistics Finland.

the use of the serum samples was approved by the FMC steering group. As a result of the linkage, 114 incident cases of invasive cervical cancer (ICC), 471 cases of carcinoma in situ (CIS) (registered from 1983 to 1994) and 156 cases of cervical intraepithelial neoplasia grade III (CIN3) (registered since 1995) were identified. Of these, 15 cases (five ICCs, nine CISs and one CIN3 case) belonged to the first subcohort (Table 1). Due to incomplete registration before 1995, 16 CIN3 cases diagnosed during 1991-94 were excluded. Cases with at least a lag of one year between the serum sampling of the second pregnancy and the diagnosis were eligible. In original study II, all 114 ICC and 140 cases of CIN3 and a random sample of 246 cases of CIS were included in the laboratory analyses.

In original study III, we identified all cases of squamous cell carcinoma of the uterine cervix (SCC) from the FCR diagnosed in Finland during 1986-2006 for women under 50 years of age. By second linkage between the FCR and FMC, cases of SCC for women who were pregnant at least once from 1983 were identified (Figure 4).

4.2 Laboratory analyses

The HPV type 6, 11, 16, 18, 31 and 33 IgG antibody analyses were performed by standard ELISA (Dillner *et al.* 1996). Highly purified, yeast-expressed HPV6, 11 and 16 virus-like particles, HPV18 and 33 virus-like particles and HPV31 virus-like particles comprising the L1 proteins (kindly supplied by Dr Kathrin Jansen, Merck Research Laboratories [NJ, USA], Dr Brigitte Colau, GlaxoSmithKline Biologicals [Rixensart, Belgium] and Dr Reinhard Kirnbauer, University of Vienna [Vienna, Austria], respectively) were used as antigens. A pool of serum samples from adolescent virginal females was used as the negative reference by which the absorbance cut-off levels were determined. The HPV6 and HPV11 as well as the HPV31 and HPV33 results were combined (either negative or one/both positive). The sensitivity and specificity of the HPV serology has been evaluated using HPV DNA detection by polymerase chain reaction (PCR) as the gold standard. These studies have shown 50-75% sensitivity and 95-99% specificity for HPV16, 18, 31 and 33 antibody analyses (Kaasila *et al.* 2009, Merikukka *et al.* 2011).

In original study I, incident cases of HPV were defined as seroconversions, i.e. an initially antibody negative person becoming antibody positive in her second sample. Internal control sera were included on each plate. All serum samples of a given age group over the different time periods were analyzed simultaneously. In original study II, IgG antibodies to *Chlamydia trachomatis* were analyzed by a commercial ELISA method (Anilabsystems, Helsinki, Finland). In original study III, the first set of serum samples (years 1986-1994) was analyzed during 2000-2006, and the second set (years 1995-2003) was analyzed in 2007-2008. The information on the dates of serum sample and maternal age was used to standardize the cut-off levels of HPV serology for the two subcohorts, which were analyzed in the laboratory at different times. For the second set the cut-off of 0.4 was used for both HPV type 16 (HPV16) and type 18 (HPV18), and cut-offs 0.51 for HPV type 31 (HPV31) and 0.6 for HPV type 33 (HPV33) were used.

4.3 Statistical methods (I-III)

Incidence and prevalence trends of HPV type 16 (I)

In original study I, because the numbers of seroconversions were lower than expected, the two youngest and the three oldest age groups were combined for the statistical

analyses based on previous observations on the characteristics of the age group-specific seroconversion rates of HPV16 (Kibur *et al.* 2000a). The age groups were combined among six different calendar-time periods separately. The incidence rates of HPV16 per 1000 person years among the susceptibles (i.e. seronegative at the time of first pregnancy) were calculated. We fitted a Poisson regression model with age at the time of possible seroconversion (0=under 23 and 1=23-31 years), linear calendar-time term (-6=1983-85, -3=1986-88, 0=1989-91, 3=1992-94 and 6=1995-97), quadratic calendar-time term and interaction terms between age and both calendar-time terms to evaluate possible time trends. In addition, we fitted Poisson regression with linear (first-degree), quadratic (second-degree) and cubic (third-degree) calendar-time terms for HPV16 incidence in the younger age groups. The models were fitted by the method of maximum likelihood using the Genmod procedure of the SAS program (version 8.2).

To estimate the spread of the epidemic, the prevalences of HPV16 at the time of the first pregnancy/serum sampling were calculated for the two age groups at six different calendar-time periods. In the incidence calculations, age and calendar time were fixed at the mean of the two dates of serum sampling, the median sampling interval being about 2 years. The prevalence was calculated from the first serum samples; therefore the prevalence proportions apply to women who are about one year younger and refer to a calendar-time period about one year earlier than the incidence rates.

Rate ratios and population attributable fractions of SCC and CIN3 associated with HPV (II)

In original study II, to estimate the rate ratio (RR) of invasive SCC and CIN3 associated with incident HPV16, HPV18 and HPV31/33 infection, proportional hazards regression models adopted to the case-cohort design were fitted. The sampling fractions varied across the 25 strata from 0.016 to 0.908 (Table 1). In model fitting, members of the subcohort were weighted by $1/(n_j/N_j)$, where n_j/N_j was the sampling fraction of stratum j ($j = 1,\ldots, 25$). The risk set at each event time consisted of the case being diagnosed plus the members of the subcohort who were at risk at that particular event time. Those 15 members of the subcohort, who were also identified as cases using personal identifiers in the linkage of the FMC and FCR, were included in the subcohort until the event time of their own.

The RRs were adjusted for potential confounders. The presence of *Chlamydia trachomatis* and HPV6/11 antibodies were considered as markers of sexual risk-taking

behavior (Dillner *et al.* 1996). The details of the number of invitations to cervical cancer screening were available through the linkage of the subcohort with the cancer screening invitation matrix of the FCR. The linkage was based on the domicile of each woman during her first pregnancy. The number of screening invitations is a time-dependent covariate and was calculated to refer to the time one year before the cervical cancer diagnosis for the cases and for the respective age-matched subcohort members. Calculations were made in 5-year bands in order to control irregularity of screening invitations for some birth cohorts in the municipalities. To adjust the effect of living in larger urbanized areas, the municipalities were divided according to number of inhabitants ($<$35 000 or $\geq$35 000) (Lehtinen *et al.* 2006b).

The RRs associated with all three hrHPV types (HPV16, HPV18 and HPV31/33), respectively, were estimated, and 95% confidence intervals (CIs) were calculated based on robust variance estimates for case-cohort data (Barlow 1994). The models were fitted using the PHREG procedure of the SAS program (versions 8.2 and 9.1).

The population attributable fraction (PAF) was estimated as:

$PAF = 100 \times P_e(RR-1)/(1+P_e[RR-1])$,

where $P_e = (P_o + Sp - 1)/(Se + Sp - 1)$ is the misclassification-corrected prevalence (Franco 1992) of exposure to hrHPV among the subcohort and RR is the relative risk. P_o is the observed prevalence, the specificity (Sp) of the hrHPV-serology was assumed to be 0.99, and the sensitivity (Se) 0.50 (Dillner *et al.* 1996, Luostarinen *et al.* 1999). The 95% (and, if specifically indicated, 90%) CIs for the PAF were estimated using the method by Natarajan, Lipsitz and Rimm based on Bonferroni inequality using the 97.5% CIs for the prevalence and the RR (Natarajan *et al.* 2007). Similarly, as in Original study I, the HPV seroprevalence was estimated as the proportion of seropositives at the first pregnancy serum samples.

Incidence of SCC by age at diagnosis, period, year of birth and the number of pregnancies (III)

In original study III, to describe the incidence of SCC by age at diagnosis, calendar time (period) and birth cohort four graphical presentations were created (Carstensen 2007):

1. Rates versus age, observations within each period connected,
2. Rates versus age, observations within each birth cohort connected,
3. Rates versus period, observations within each age-class connected and
4. Rates versus cohort, observations within each age-class connected.

To describe the association of the number of pregnancies and the incidence of SCC cohort-specific age-incidence plots were constructed.

The seroprevalences of hrHPV (HPV types 16, 18 and 31/33) were calculated for women up to 22 and 23-28 years of age in three 6-year periods (1986-91, 1992-97 and 1998-2003) in order to get most recent and stable estimates of the seroprevalences. Thus, two younger age groups up to 22 and two older age groups (23 to 25 and 26 to 28) were combined. The original 3-year calendar time periods from 1986 to 2003 were combined into three 6-year periods (1986-91, 1992-97 and 1998-2003) (Table 1 and Table 2). The prevalences of hrHPV for two age groups (up to 22 and 23 to 28 years of age) and three calendar time periods were calculated as weighted averages of the corresponding four prevalences. For example, four prevalences in 1986-88 for women under 20 and 20-22 years of age and in 1989-91 for both age groups, respectively, were combined into a weighted average for the age-group under 23 years and calendar period 1986-91.

4.4 Mathematical modelling analyses (IV)

In original study IV, a deterministic compartmental model of HPV16 infection and progression to cervical cancer was developed. The model was built on a previous model through the addition of type-specific immunity (Barnabas & Garnett 2004). Figure 2 provides a schematic representation of the model for women, however, the model also allows for screening and treatment and accounts for hysterectomy for nonmalignant disease. All non-infected women were susceptible in the model. In women, the model describes the flow of incident cases from the acquisition of asymptomatic HPV infection through premalignant disease to ICC, although most HPV infections regress spontaneously. In men, the model is straightforward from susceptible men to immune via infected. Considering a single virus type (HPV16), we assume that regression results in lifelong acquired immunity. However, HPV 16 serum antibody test sensitivity and specificity and gradual loss of a detectable antibody response are included to allow the comparison of model results with serological data, where false negatives will increase as a function of time since infection (Carter *et al.* 2001, af Geijersstam *et al.* 1998a).

The model equations were solved numerically by a special C++ program. The model population was stratified according to age (0 to 84 years) in 5-year cohorts (0-4, 5-9,. . . , 80-84) and sexual activity class (1,. . . , 4), defined according to the rate of sexual partner change (Garnett & Gregson 2000). With sexual behaviour available only from cross-sectional studies at two time points, each of which included multiple birth cohorts,

Table 3. Distribution of the sexual activity groups by age group for Finland in 1992 (Haavio-Mannila *et al.* 2001). (From original, open access article IV.)

		Sexual activity group			
		Highest	Moderately high	Moderate	Lowest
Gender	Age (y)	(%)	(%)	(%)	(%)
Men	15-19	2	3	35	60
	20-24	2	4	33	61
	25-29	1	7	19	73
	30-34	1	2	27	70
	35-39	1	3	19	77
	40-44	1	4	24	71
	45-49	0.5	4	26	69.5
Women	15-19	1.5	3	13.5	82
	20-24	1.5	2.5	34	62
	25-29	1	2	21	76
	30-34	1	2	9	88
	35-39	1	1	13	85
	40-44	0.5	1	9	89.5
	45-49	0.5	0.5	5	94

an understanding of the changes in sexual behaviour as a function of age (Table 3) and time is difficult, especially in the light of potential social desirability biases (Fenton *et al.* 2001).

We derived an annual number of new partners from the reported number of lifetime partners stratified by age and divided by the estimated duration of activity based on the difference between the age at survey and the average age at sexual debut estimated during that survey (Haavio-Mannila *et al.* 2001) (Table 4). Commonly in surveys addressing sexual behaviour men and women report different average numbers of sexual partners; this difference is most marked in earlier surveys. Thus, behaviours were estimated from the mean of behaviours reported by men and women. For both sexes, the observed age of sexual debut and the number of sexual partners changed between the surveys, which were reflected in the model with a one-off change in the rates of acquisition of sexual partner and the age at sexual debut. The influence of the timing of this step change was explored. The model was run to equilibrium using the data on sexual behaviour from the 1971 survey, with changes in the rates of partner change and the age at sexual debut from the 1992 survey introduced later. The size, sex, and age

Table 4. Mean number of new partners per year for the sexual activity groups by age group for Finland in 1992 (Haavio-Mannila *et al.* 2001). (From original, open access article IV.)

		Sexual activity group			
		Highest	Moderately high	Moderate	Lowest
Gender	Age (y)	(%)	(%)	(%)	(%)
Men	15-19	12.5	2.00	1.10	0.38
	20-24	15.0	1.40	0.39	0.14
	25-29	12.5	0.83	0.25	0.08
	30-34	10.0	0.50	0.17	0.06
	35-39	8.5	0.27	0.11	0.04
	40-44	7.5	0.33	0.10	0.04
	45-49	7.5	0.33	0.10	0.03
Women	15-19	15.0	3.50	1.34	0.48
	20-24	17.5	0.96	0.38	0.14
	25-29	15.0	0.67	0.21	0.08
	30-34	10.0	0.35	0.15	0.06
	35-39	7.5	0.45	0.16	0.04
	40-44	7.5	0.45	0.08	0.04
	45-49	7.5	0.45	0.08	0.03

distribution and rates of birth and death of the population were based on the demography of Finland.

5 Results

The results are presented in the order of original studies I-IV. The three first sections rely on the same data (Table 1) from the first subcohort of Finnish Maternity Cohort (FMC) comprising a stratified random sample of around 8000 women under 32 years of age and with a minimum of two pregnancies within five years. The second subcohort of FMC (Table 2) was formed later on, and the results in section three are based on both subcohorts. For the results in the second and third section, two linkages of the subcohorts of FMC with the Finnish Cancer Registry done at different times are presented in Figures 4 and 6. The last section summarizes the results of mathematical modelling.

5.1 Incidence and prevalence trends of HPV type 16 (I)

In the first subcohort (Table 1), during 1983-97 the seroprevalence of HPV type 16 (HPV16) was relatively stable, from 17% to 18% among the 15-22-year-old women (Figure 5). For the 23-31-year-old women the seroprevalence varied between 15% and 17% from calendar period 1983-85 to 1989-91, but increased thereafter to 24% in 1995-97.

The incidence of seroconversions of HPV16 was 1.4- to 5-fold higher among the 15-22-year-old women compared with the 23-31-year-old women (Figure 5). In the younger age group, the incidence was the lowest, 13 per 1000 woman-years, in 1992-94 and highest, 31 per 1000 woman-years, in 1995-97. Yet, there was insufficient evidence of either an upward curvature tendency or a linear trend. In the older age group, a gradually increasing linear trend (P=0.007) of the incidence of seroconversions of HPV16 was observed during 1983-97. The incidence in 1995-97 was 13 per 1000 woman-years, i.e. 2.6-fold higher than in 1983-85.

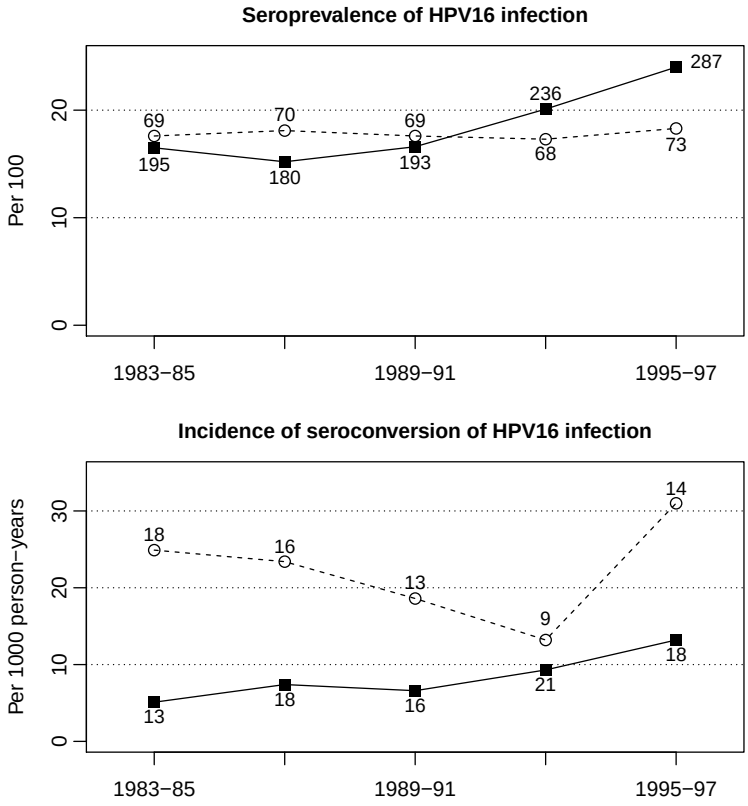

Fig 5. The seroprevalence and incidence of seroconversion of HPV16 infection in women belonging to the Finnish Maternity Cohort by estimated age and calendar time of possible seroconversion. The women had had two pregnancies within five years during 1983-97. Numbers of prevalent and incident cases are shown for the different time periods among women under 22 (- - -) and 23-31 years of age (—), respectively. (Reprinted with permission from Society for General Microbiology.)

5.2 Population fraction of SCC attributable to hrHPV (II)

In the follow-up of women in the FMC with at least two pregnancies within five years during January 1984 to May 2002, we identified 83 cases of squamous cell carcinoma of the uterine cervix (SCC), 18 cases of adenocarcinoma and 13 cases of unspecified carcinoma or unknown histological diagnosis. Only the cases of SCC were included in

the analyses. In addition, 386 cervical intraepithelial neoplasia (CIN) cases, including 246 cases diagnosed as cervical carcinoma in situ and 140 cases diagnosed as CIN grade III (CIN3), were available for the serological analyses. (Figure 6).

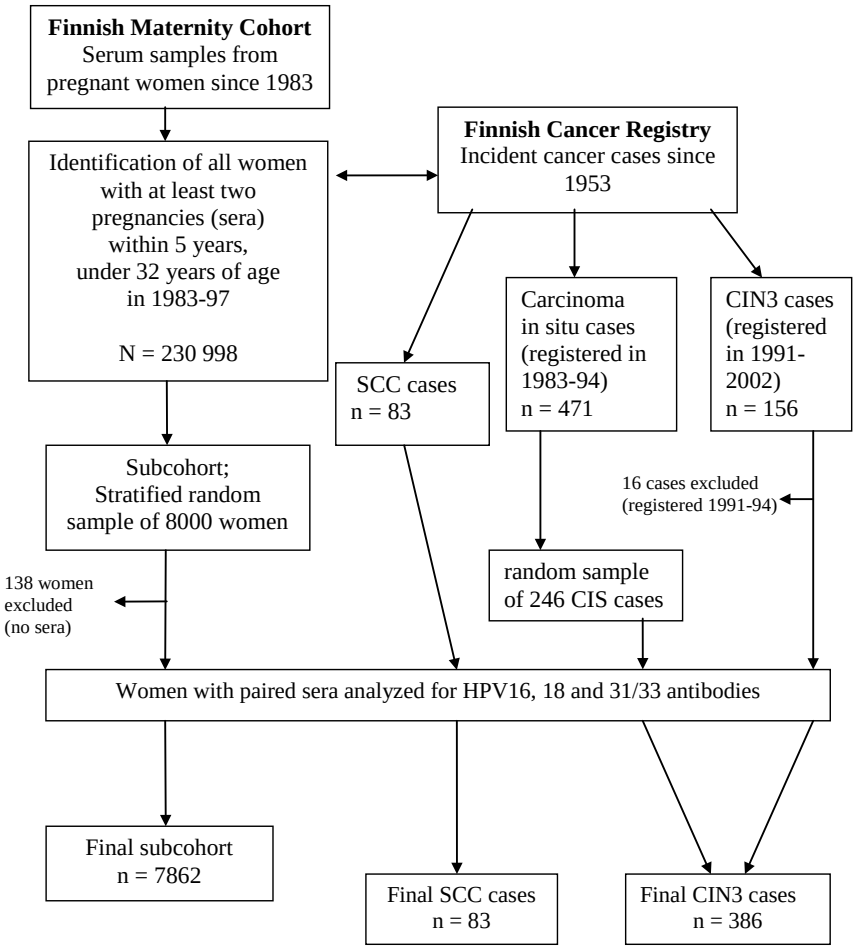

Fig 6. Linkage of the subcohort of FMC with the Finnish Cancer Registry resulting to final subcohort, cases of SCC and CIN3. (Reprinted with permission from Expert Reviewers Ltd.)

The prevalent infections of HPV16 were associated with an increased relative rate of SCC (RR: 2.3, 95% CI: 1.3 to 4.1); an association that remained after adjusting for *Chlamydia trachomatis*, HPV6/11, HPV18, HPV31/33, screening and urban versus rural residence. The rate of SCC was strongly associated with a seroconversion of HPV16 (5.1, 1.8 to 14). The seroconversion of HPV18 was related to an increased rate for CIN3 (2.7, 1.3-5.4) after adjustment, but not to excess relative rate for SCC (1.4, 0.3 to 6.9). The seroconversion of HPV31/33 was associated with an increased rate for SCC (5.1, 1.9 to 13) and possible increased rate for CIN3 (1.3, 0.6 to 3.0). The seroprevalence of any high risk HPV type 16, 18, 31 and/or 33 (hrHPV) was associated with an increased rate of SCC (1.7, 1.0 to 2.8) and CIN3 (1.3, 1.0 to 1.6). Again, an incident infection of any hrHPV was associated with a fivefold increased rate of SCC (5.4, 2.6 to 11). The corresponding relative rate of CIN3 was 1.9 (95% CI: 1.2 to 2.9). In further analyses, the relative rates based on seroconversions, i.e. incident infections, of HPV were used.

The estimated population attributable fraction (PAF) of HPV16 in SCC was 61% (95% CI: 18% to 85%). For the serologically defined hrHPV types the corresponding PAF estimate was 73% (13% to 93%). The estimated PAF of hrHPV infection in CIN3 was 36% (-5% to 65%), but for HPV16 alone it was only 6% (-19% to 35%).

5.3 Incidence trends of SCC by year of birth and the number of pregnancies (III)

In Finland, among 26-31 and 32-37-year-old women the incidence of SCC during periods 1995-2000 and 2001-2006 (mean years 1998 and 2004) was roughly twice as high as that in women of the same ages in earlier periods (Figure 7 a). During 1995-2006 the incidence of SCC increased among women of 26-31 and 32-37 years of age (mean ages 29 and 35 years), but not among younger women (mean age 23 years) (Figure 7 b).

The incidence of SCC increased by age until 40 years of age for women born in the late 1950s (mean birth cohort 1957), while for women born in the late 1960s and during the 1970s, the incidence of SCC rose up to 4 per 100 000 woman-years already when they were 26-31 -years-old (Figure 7 c) and d). Similarly, for women who had been pregnant at least once the incidence of SCC increased at younger ages for the younger birth cohorts (Figure 7 e). For women who had been pregnant at least twice the incidence of SCC in the cohort with mean year of birth 1969 was higher at younger age than in the earlier birth cohorts (Figure 7 f).

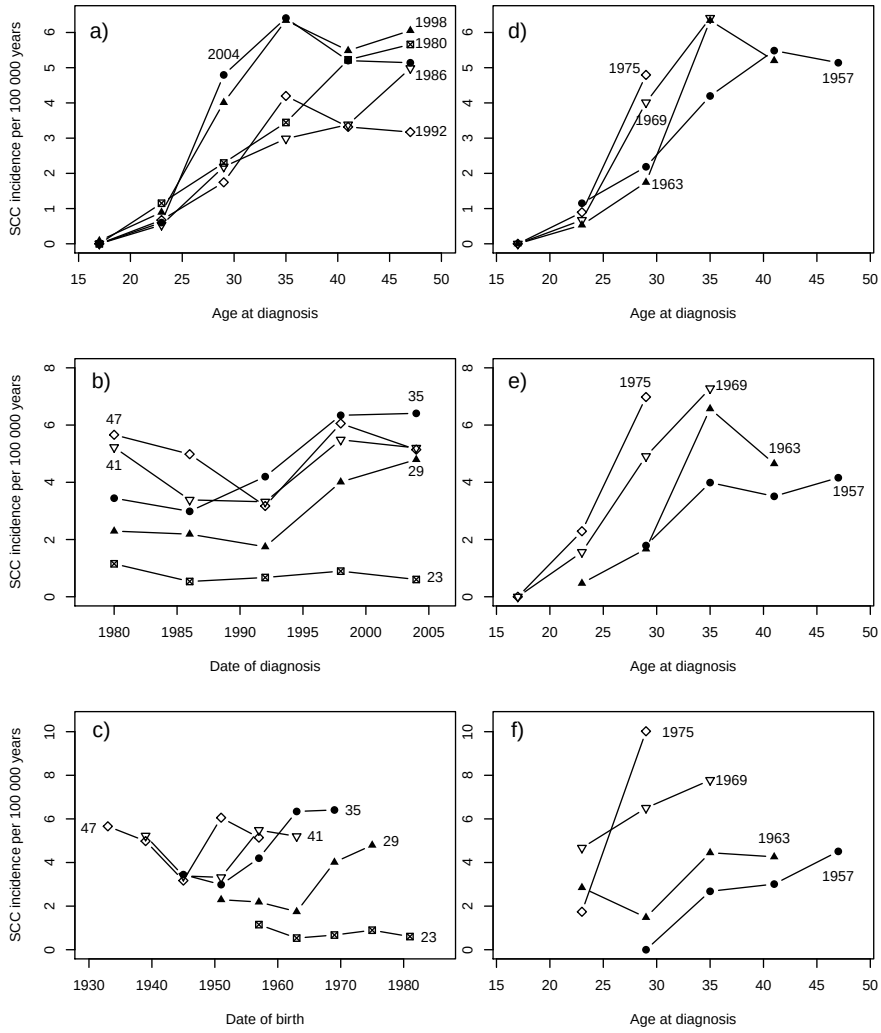

Fig 7. The incidence of SCC for all women younger than 50 years of age diagnosed during 1986-2006 in Finland by a) age at diagnosis in different periods indicated by mean calendar year, b) calendar time in different age groups indicated by the mean age, c) year of birth in different age groups indicated by the mean age and d) age at diagnosis in different birth cohorts indicated by mean year of birth. The incidence of SCC for women diagnosed during 1986-2006 who have been pregnant at least e) once or f) twice within five years before 29 years of age by age at diagnosis in different birth cohorts indicated by mean year of birth.

For women who had been pregnant at least twice and were born in the 1970s (mean year of birth 1975), the seroprevalence of hrHPV was 33% and 36% in the calendar periods with mean years 1995 and 2001, respectively. For 23-28 -years-old women, the seroprevalences of hrHPV were 32%, 34% and 36% in calendar periods 1986-91, 1992-97 and 1998-2003, respectively.

5.4 Modelling the potential impact of HPV16 vaccines (IV)

In the mathematical modelling, using survey-based information on sexual activity by age (Table 3) to model the partner change rate (Table 4), we found that the maximum likelihood (ML) estimate of the transmission probability reached its theoretical upper bound 1, which generated a lower prevalence of HPV16 than that observed in original study I. Statistically, this estimate suggests that a) the estimated HPV prevalence was too high, b) the rates of change of sexual partners were too low, or c) acquired immunity might not give lifelong protection. Assuming that survey data will underestimate sexual activity and that the rates of sexual partner change are doubled, a new ML-estimate of 0.6 per sexual partnership was derived for transmission probability. Clearly, due to the uncertainty in the estimates of sexual activity along with other potential errors in estimating parameter values and model structure, great uncertainty exists in the model-based estimate of the transmission probability per partnership.

The modelled and observed incidence of invasive cervical cancer (ICC) amongst 35-39 -years-old women (Finnish Cancer Registry 2002) is compared in Figure 8, where the changes in the sexual behaviour are insufficient to account for all the change in the disease incidence. The model initially overestimates, and then underestimates, the incidence of HPV16 associated ICC in women aged 35-39. According to the model, the incidence would have increased from 2.8 per 100 000 woman-years in 1985 to 4.1 per 100 000 woman-years in 1999, whereas the real-life estimate from reported cases increased from 2.0 to 4.8 per 100 000 woman-years (Finnish Cancer Registry 2002). Despite the changes in the sexual behaviour being observed not until the 1992 survey (Haavio-Mannila *et al.* 2001) the changes could have occurred earlier, and the change in the incidence of ICC was more consistent with a change in the sexual behaviour in the model in 1985. Moreover, the results from original study I suggest that the seroprevalence of HPV16 started to increase from 1985 (Figure 5).

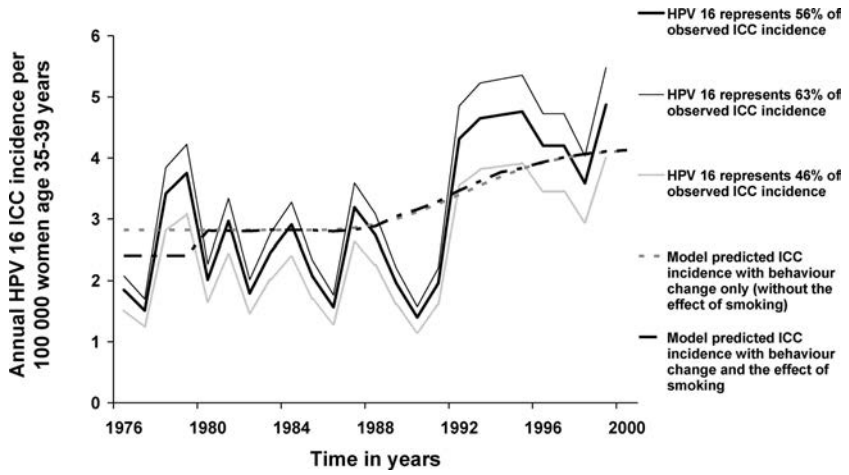

Fig 8. The observed incidence of HPV16 associated ICC (Finnish Cancer Registry 2002) compared with model predictions. The changes in the sexual behaviour, which were reported in 1992 (Haavio-Mannila *et al.* 2001), were implemented in the model in 1985, because they could have occurred before 1992. (From original, open access article IV.)

The results on the impact of vaccination are shown in Figure 9. Here, it is assumed that a) the transmission probability of HPV16 per sexual partnership is 0.6 and b) the new sexual partner rates per year are doubled as previously, and c) sexual debut occurs on average at 16.6 years for women and at 17.7 years for men. Figure 9A illustrates the impact of varying vaccine coverage on the incidence of ICC for vaccinating 15-year-old women alone or both women and men. Vaccinating women and men appears to have a small benefit over vaccinating women only (4% at low and 7% at high coverage). Vaccinating 90% of women alone would reduce the incidence of ICC by 91%. Voluntary vaccination among 10% of 15-year-old women and 30% of susceptible 20-year-old women would reduce the incidence of ICC by 43%. Whilst this scenario would decrease the incidence, its impact is secondary to the reduction which is possible to achieve through widespread vaccination (e.g. 90% coverage of women).

The impact of vaccination on the incidence of ICC associated with HPV16 is shown in Figure 9B when ages for vaccination of 90% of women alone changes. Vaccination at birth and at age 15 years generated the greatest reduction in the incidence of ICC, to 0.6 cases per 100 000 women, with a lag seen if vaccinated at birth. Vaccination at the age of 20 years produced a 63% decrease, and at 25 years, a 41% decrease, in the incidence

of cancer. After the age of sexual debut, the impact of vaccination declines as the age of vaccination increases, because of prior infection.

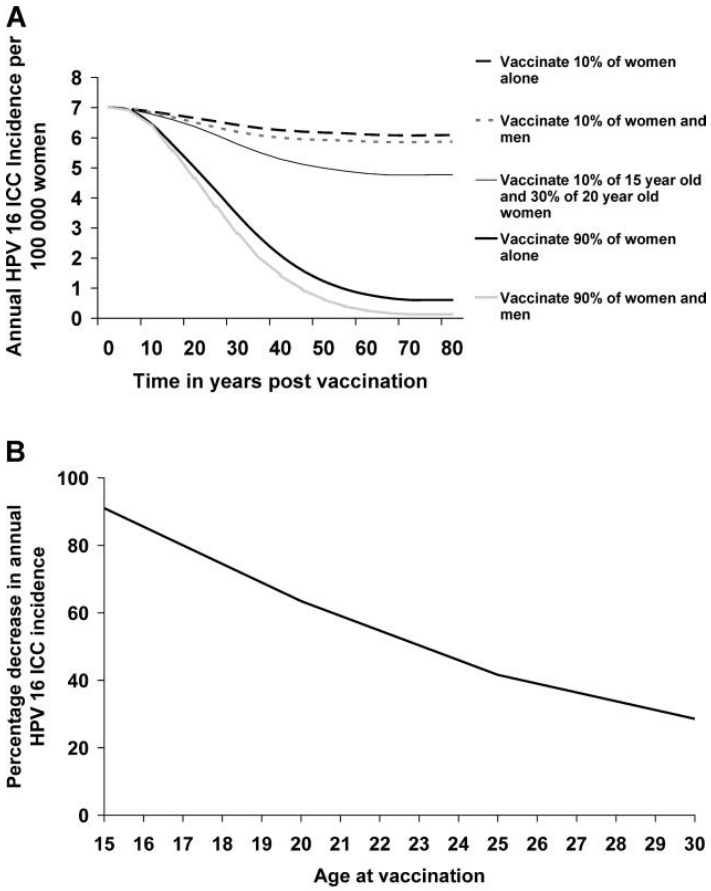

Fig 9. The impact of varying the target population for HPV16 vaccination. (A) The effect of routinely vaccinating successive cohorts of men and women compared to vaccinating women alone at low (10%) and high (90%) coverage. Additionally, the effect of a reasonable expected coverage using private, voluntary vaccination among 10% of 15 year-old women and 30% of susceptible 20 year-old women. (B) The impact of vaccination at different ages on incidence of HPV16 associated ICC for vaccination of 90% of women alone. Sexual debut for women is at 16.6 years and 17.7 years for men. (From original, open access article IV.)

6 Discussion

6.1 Summary of main findings

A steady increase on average in the incidence of human papilloma virus type 16 (HPV16) was estimated to have taken place in Finland from 1983 to 1997 among the 23-31-year-old women with at least two pregnancies within five years. These women represented one-third of fertile-aged Finnish women. During the same period of time, the estimated seroprevalence of HPV16 increased from 17% to 24% in this subpopulation. The epidemic spread of the oncogenic HPV16 throughout the 1980s and 1990s pre-dated the observed increase in the incidence of cervical cancer in fertile-aged Finnish women from 1990.

The population attributable fractions (PAFs) of serologically defined and misclassification-corrected HPV16 in squamous cell carcinoma of the uterine cervix (SCC) was estimated as 61%. The estimated PAF of combined high risk HPV types 16, 18, 31 and 33 (hrHPV) in SCC was 73%. The widths of the error margins for PAFs were substantial, though. Considerably lower HPV16 and hrHPV associated estimates of PAF in cervical intraepithelial neoplasia grade III (CIN3) were obtained.

For 26-31-year-old women born in the 1960s and 1970s the incidence of SCC was roughly double compared to that in women of the same ages but born in the late 1950s. For 23-28-year-old women who had been pregnant at least twice the estimated seroprevalence of hrHPV increased gradually from 32% to 36% during 1986-2003.

Mathematical modelling, used to explore the impact of HPV16 vaccination, indicated that changes in sexual behaviour might have partly accounted for the increase seen in the incidence of cervical cancer in 35-39-year-old women from 1990. Vaccinating 90% of young women before sexual debut would have an the estimated potential to decrease HPV16 associated incidence of cervical cancer by 91%.

6.2 Strengths and limitations

The Finnish Maternity Cohort (FMC) serum bank and Finnish Cancer Registry (FCR) offer a reliable basis for identification of antibodies in the blood serum and of women with a diagnosis of cervical cancer. The coverage of FCR is almost 100%. Cancer

cases were identified by linking the data files of the FMC and the FCR using the unique personal identification code (PIC).

Since the sensitivity of HPV16 serology for a single sample is not greater than 50-65% (Carter *et al.* 1996, Dillner *et al.* 1996), the observed seroprevalences in original study I represent an underestimate of the epidemic. The seroreversions of HPV are previously reported to be negligible (af Geijersstam *et al.* 1998a) and in our study were low as expected, 1.4% for HPV16 within 2.5 years on average. Yet, it is noteworthy that previous occurrence of HPV infections in the population has an effect on the incidence of HPV at each subsequent time point and is not taken into account by conventional statistical methods. This is due to contacts between adjacent birth cohorts and is further modified, for example, by assortative risk-taking sexual behaviour not included in the Poisson regression model either.

Risk-taking sexual behavior (as defined by acquired hrHPV infections), living in urban areas involving increased background exposure to the micro-organisms, such as *Chlamydia trachomatis* and HPV6/11, and smoking are known to raise the incidence of SCC and CIN3 (Muñoz *et al.* 2003, Dillner *et al.* 1996, Koskela *et al.* 2000, Plummer *et al.* 2003). However, since the 1960s, organized screening for cervical cancer has considerably decreased the incidence of cervical cancer in Finland (Anttila *et al.* 1999). In original study II, the effect of risk-taking sexual behavior was adjusted for by *Chlamydia trachomatis* and/or HPV6/11 antibodies (Dillner *et al.* 1996). In addition, the effects of the risk factors (except smoking) and screening were adjusted for in the proportional hazards models. The estimates of RR were highest among the incident HPV infections.

A useful statistic to quantify the effect of the prevention of infectious agents on cancer prevention is PAF, defined as the proportion of new cancer cases in a specific population that would have been prevented by a hypothetical intervention in a specific exposure (de Martel *et al.* 2012). Unbiased estimation of the hrHPV-associated PAF relies on accurate estimates of both RR and the prevalence of infection in the target population, both of which are dependent on the sensitivity and specificity of HPV serology. If HPV serology is compared with the detection of HPV DNA with PCR, the sensitivity of HPV16 serology is the highest (65-70%), HPV31 moderate and HPV18 the lowest in incident infections taking place within a few years from serum sampling (Dillner *et al.* 1996, Kjellberg *et al.* 1999, Carter *et al.* 1996). Thus, the fact that the seroconversions were identified between the two pregnancies and took place, on average, within a period of 2.5 years, provided the highest possible sensitivity. After seroconversion,

HPV antibody levels are predominantly stable over five years (af Geijersstam *et al.* 1998a). Our seroprevalence estimates were corrected for misclassification (Franco 1992) taking into account the assumed low sensitivity (50%) of HPV serology for a single serum sample. We made the assumption that we are measuring the same exposure with HPV serology and persistence of HPV DNA as determined by PCR. Following this assumption, we could obtain an estimate of the seroprevalence of hrHPV, a measure of cumulative incidence of hrHPV, in the subcohort of women with a minimum of two pregnancies.

Since HPV serology is more sensitive for incident infections than for prevalent ones, the PAFs based on incident infections with misclassification-corrected prevalences of HPV probably yield as accurate estimates as one can generate using serological observations. HrHPV infections are associated in most cases of SCC, but their role, as defined by HPV serology, in CIN3 appeared to be smaller. Yet, this may be due to the fact that the development of HPV antibodies takes up to 10 years (Wang *et al.* 2003), a very long time for a fertile-aged study population in original study II. The lag in the development of HPV antibodies probably further reduced the sensitivity of this serological approach. To the best of our knowledge, this is the first case-cohort study assessing the PAF of HPV16 in cervical carcinoma that controls both for the effect of risk-taking sexual behavior and for the screening programs for cervical cancer.

The study population was limited to women with a history of at least two pregnancies. Thus, the PAF of HPV16 in cervical cancer in nonpregnant women was not possible to estimate. However, the FMC comprises virtually all (98%) pregnant women in Finland since 1983 (Toriola *et al.* 2011) and, thus, represents a notable proportion of the fertile-aged Finnish women. In most countries, parity has been suggested to be a risk factor of cervical cancer (Muñoz *et al.* 2002, Hinkula *et al.* 2004); yet, no good data are available to suggest that the role of oncogenic HPVs in cervical cancer is different in pregnant compared with nonpregnant women. Thus, some extrapolation of our results to the general population can be made, but with caution.

In original study IV, screening was incorporated into the model by assuming the same mass-screening coverage and efficacy rates as reported in 1999 for the time period starting in 1970, because screening coverage has not remarkably changed during the time period. In 1999 the national screening programme in Finland invited 80% of the 30-year-old women to screening, 60% of those invited were screened – that is, only 49% of those eligible (Finnish Cancer Registry 2002). Low participation in organised screening was found to correlate well with high or increased incidence of cervical

cancer, so improved screening participation should reduce the incidence (Anttila *et al.* 1999). Among the 40-55-years-old women on average 72% of eligible women are screened. With the 75% increase in the incidence of ICC among the 35-39-year-old women, an improved coverage to detect precancerous lesions in 30-year-old women is necessary. For simplicity, changing sexual behaviour practices and regular and/or sporadic screening done in the private sector were not considered in the modelling. Oral contraceptive use and parity are aetiological cofactors that may also account for some of the cervical cancer incidence increase (Castellsagué & Muñoz 2003), whereas organised screening programmes for cervical cancer in Finland have been unchanged since 1990, and changes in registration or diagnostic practices are not thought to be important (Anttila *et al.* 1999).

6.3 Comparison with background knowledge and other evidence

No increase in the seroprevalence of HPV16 was observed in an earlier Finnish study among women with two pregnancies in the Helsinki metropolitan area in 1983-89 and 1990-96 (Kibur *et al.* 2000a). The seroprevalence was 23% for both time periods, remarkably higher than the 15-17% observed in the present study in 1983-91 among all young Finnish women with two pregnancies, but similar to the seroprevalence in 1995-97. In the second subcohort sampled from FMC (Table 2) during 1995-2003, the seroprevalence of HPV16 was 20% for all pregnant women below 29 years (Kaasila *et al.* 2009), which confirms the increase of seroprevalence observed already in 1992-97 (Figure 5). Swedish data from Stockholm show a comparable, albeit earlier, rise in the seroprevalence of HPV16 from 16 to 21% between 1969 and 1980 (af Geijersstam *et al.* 1998b). A series of detailed questionnaire studies have shown that the sexual behaviour of 18-34-year-old Finnish women changed considerably from 1971 to 1999 (Haavio-Mannila *et al.* 2001). The average number of lifetime sexual partners grew from 2.6 to 7.7 and the mean age of sexual debut decreased from 18.9 to 16.6 years, increasing the impact of oncogenic HPV infections due to earlier exposure. An increase in risk-taking sexual behaviour could have exposed more and more susceptible adolescent individuals to the virus, and eventually resulted in the considerable increase of seroprevalence of HPV16 in the new sexually active birth cohorts in the 1990s.

Studies of the natural history of HPV suggest that HPV infection is very common in young women, but that its prevalence declines with time (or age), which reflects elimination of the virus by immunological mechanisms. On the other hand, women who remain infected with HPV until early middle ages (30-50 years) are at an increased risk of developing epithelial abnormalities recognised as precursors of SCC. (Parkin *et al.* 2001). Population-based estimates of the actual prevalence or seroprevalence of HPV infection among Finnish women are rare (Kaasila *et al.* 2009, Merikukka *et al.* 2011, Auvinen *et al.* 2005). Original study III provided estimates of the prevalence for women with a history of at least two pregnancies based on hrHPV serology. The FMC comprises virtually all (98%) pregnant women in Finland since 1983 (Toriola *et al.* 2011) and, thus, represents a significant proportion of the population of Finnish women. Because of overall low sensitivity of hrHPV serology, the current estimates of seroprevalence are underestimates and the real prevalences were most likely higher than the 32 to 36% in different calendar periods observed in the analyses.

In original study II, the highest rate ratios (RRs) of SCC were associated with the seroconversions of HPV16 and combined hrHPVs. Conversely, the RRs of CIN3 associated with seroconversions of HPV16 and hrHPV were low to moderate. In addition, the estimated PAFs were somewhat conflicting with the estimates derived using RR estimates from a comprehensive meta-analysis with CIN2+, high grade squamous intraepithelial lesions (HSIL) and invasive cervical cancer (ICC) as the end point (Koshiol *et al.* 2008). We noted that restricting the statistical analysis to *Chlamydia trachomatis*-positive women only, the point estimate of PAF of hrHPV in CIN3 increased to 50% (95% CI: -3% to 80%). A double infection might give rise to a more pronounced HPV load and detectable hrHPV antibodies.

While some of the increase in incidence of cervical cancer could be explained through observed changes in sexual activity and in the rates of new sexual partners, these were not entirely sufficient in the mathematical models (original study IV). This suggests that other cofactors also contributed to the observed increase. For example, the decrease in age at sexual debut could expose those with immature cervical transformation zones to HPV infection (Crum 2000), adding susceptibility further and enhancing the effect of changes in sexual risk behaviour. Thus, early age at sexual debut could raise disease in young women in two ways: 1) through extension time since infection and 2) through increased vulnerability of the immature transformation zone. The two would be difficult to differentiate epidemiologically, and only the former mechanism was included in the model. As a result, changes in the rates of sexual partner change rather than the age

at sexual debut had a greater effect on the incidence of ICC (unpublished data). This increased risk associated with sexual partners reflects the risk of HPV infection rather than the risk of ICC, once infected.

The impact of vaccination in mathematical modelling analyses (IV) is comparable with findings from previous modelling exercises (Hughes *et al.* 2002, Goldie *et al.* 2004, Kulasingam & Myers 2003, Sanders & Taira 2003, Taira *et al.* 2004), where high coverage of women alone, vaccination before sexual debut, and long-term protection (or boosters) providing three to four decades of protection are required to substantially reduce the incidence of ICC.

A vaccine providing protection of less than 15 years may generate unexpected outcomes by shifting susceptibility in women to an older age group, where they could have increased risk of persistence. However, if age-specific patterns of persistence are derived from following cohorts of infected women, because older women infected at entry are more likely to have a persistent infection, it seems possible that an observed increase in persistent infection with increased age is an artifact. It is possible that type replacement with nonvaccine oncogenic HPV types could have a comparable effect, with a obstinate increase in incidence of cervical cancer. This could be avoided by including new oncogenic HPV types in the booster vaccines.

The results of original study III suggest that the incidence of cervical cancer will continue to have an upward trend in Finland, since the younger birth cohorts seem to have a higher rate of SCC in a given age than the older birth cohorts. This means that the described effect (Bray *et al.* 2005b) for the 1990s, the study period was 1955-1999, has continued from the year 2000 onwards.

In original study III, the younger birth cohorts seemed to have a higher rate of SCC in a given age than the older birth cohorts. The effect first described by Anttila et al (Anttila *et al.* 1999), and later by Bray et al (Bray *et al.* 2005b) for the 1990s (study period was 1955-1999) has continued from the year 2000 onwards.

7 Conclusions

While it is not possible to conclude from the present study that the observed increase of incidence of cervical cancer resulted specifically from the rising incidence of HPV16, the findings indicate that a growth in the background exposure to human papilloma virus type 16 (HPV16) pre-dated the increase of incidence of cervical cancer in Finland. A prophylactic vaccine may eventually reduce the incidence of cervical cancer. However, in countries, like Finland, with well-established and effective cervical cancer screening programmes, questions about how the vaccine will be used need to be addressed. Both naturally occurring and vaccination induced dynamics of background exposure to the oncogenic HPVs should be considered before a national HPV vaccination and the cervical cancer screening programme are combined in cervical cancer prevention.

In developed countries, vaccine coverage, in terms of both the target population and oncogenic HPV types, needs to be high in order to sustain the low incidence of cervical cancer obtained by effective screening. Continuing screening programmes have the potential to detect precancerous lesions in those not vaccinated and lesions associated with nonvaccine HPV types. The most effective strategy apparently is vaccination combined with current screening protocols, compared to both screening alone and vaccination alone. However, cytological screening is costly, and in choosing a cervical cancer prevention strategy, health economic assessments of available options are warranted.

At younger birth cohorts the increase of the incidence of squamous cell carcinoma of the uterine cervix (SCC) is visible among fertile-aged Finnish women despite the number of pregnancies. The increase of incidence of SCC and the slight increase of prevalence of hrHPV indicates that the incidence of SCC for women up to 35 years of age will probably stay at least at the same level as it is currently in Finland. Whether overall screening at 25 years of age, higher participation rate for cervical screening or mass HPV vaccination of early adolescents is the solution to lowering the incidence of SCC among young women in the future remains to be seen.

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