國立臺灣大學公共衛生學院流行病學研究所

博士論文

Graduate Institute of Epidemiology

College of Public Health

National Taiwan University

Doctoral Dissertation

人類乳突病毒感染與子宮頸癌變之分子流行病學研究：

人類乳突病毒基因型別、病毒量、嵌入宿主細胞與持續感染對子宮頸癌變之角色探討

Molecular Epidemiology of Human Papillomavirus Infection and Cervical Neoplasia：

Roles of HPV Genotype, Viral Load, Integration and Persistence in the Development of Cervical Neoplasia

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中華民國九十七年七月

July 2008


國立臺灣大學
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國立臺灣大學博士學位論文
口試委員會審定書

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本論文係陳慧祺君（D90842001）在國立臺灣大學流行病學研究所完成之博士學位論文，於民國九十七年七月十九日承下列考試委員審查通過及口試及格，特此證明

口試委員：

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Molecular Epidemiology of Human Papillomavirus Infection and Cervical Neoplasia: Roles of HPV Genotype, Viral Load, Integration and Persistence in the Development of Cervical Neoplasia

人類乳突病毒感染與子宮頸癌變之分子流行病學研究：人類乳突病毒基因型別、病毒量、嵌入宿主細胞與持續感染對子宮頸癌變之角色探討


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Acknowledgements

「如果說我看的比別人更遠，那是因為我站在巨人的肩膀上。」（**If I have seen farther than others, it is because I was standing on the shoulders of giants**）－**1676**，牛頓。

感謝陳老師，謝教授與山林學長收集了這麼珍貴的研究題材，領導優秀的研究團隊前仆後繼地完成收案、實驗與分析的工作，加上金車公司與林景堉博士成功開發的 HPV 晶片，才能讓這一份研究報告問世。感謝禮聰在大川冰庫逐隻核對了 2 萬多隻的檢體，感謝眉虹逐筆核對並連結檢體與抹片資料，感謝曉雯、玉玲、雅玲與國鈞拼了一年半以完成實驗，秋綿與瑞文嚴格的監控實驗品質，感謝怡均逐筆的實驗紀錄鍵入與反覆核對，感謝志真把各種不同的檢體與報告都整理得井然有序，感謝柏亨與久焯在病毒量實驗上的相挺，感謝立德與員山同仁的協助，感謝濬如在準備論文的階段分攤了 HPV group 的工作。感謝 HPV group 所有的同仁與同學(瓊芳、蕙竹、琇詩與彥珍)。感謝陳老師的信任、包容與指引，讓這一群人追隨著您，願意默默地不辭辛勞，確實核對經手的檢體，資料與結果，奉行著把研究做好的信念。把事做好的心，有如巨人的肩膀般寬大而厚實，是研究工作中追求真實的本念。

感謝樹人、日心與郁柔，公婆與爸媽，弟妹們在這七年來給我的支持與協助，因著您們的寬容，接受這個太太、媽媽、媳婦、女兒與姐姐，一直有忙不完的事與熬夜，隨時出動補位，分攤我在每個角色上的不足，才能在婚後完成博士修業。

感謝流病所的老師，同學與 CJ Lab 其他研究小組的同仁，除了傳授知識與實務的討論，祈玲、鈴宜、吟曲、懷壹、碧華、春妃、韻元、婉倫、錦得、禮聰、雅如、美璇、雅堂、士儀與曜任，在這條漫長的路上不斷給我鼓勵！謝謝您們，希望還沒畢業的人早日完成學業唷！感謝何英剛院士當年的鼓勵，堅定了我求學的決心！感謝~感謝上天的恩賜，給我這麼多的愛與幸運！

有一首歌，很貼切地表達我心中的感謝與感動。謹將這一份研究報告與這首歌獻給您！ With my heart~~

慧祺 2008.07.10

(When I am down and, oh my soul, so weary;
When troubles come and my heart burdened be;
Then, I am still and wait here in the silence,
Until you come and sit awhile with me.
You raise me up, so I can stand on mountains;
You raise me up, to walk on stormy seas;
I am strong, when I am on your shoulders;
You raise me up... to more than I can be.
There is no life - no life without its hunger;
Each restless heart beats so imperfectly;
But when you come and I am filled with wonder,
Sometimes, I think I glimpse eternity.
You raise me up... to more than I can be. by Brondan Grahan)


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Abstract in Chinese

子宮頸癌為婦女重要的癌症之一，在台灣歷年來向居婦女癌症首位。人類乳突病毒(HPV)被廣泛認為是子宮頸癌的必要因子，但是過去的研究大多以橫斷式或病例對照研究的設計進行，如此一來，病毒感染狀態很難區分是病毒持續感染或暫時性感染，且與子宮頸病變的因果時序性不易辨明。惟以長期追蹤研究與重複採樣的研究設計方有助於闡明 HPV 感染與其他危險因子誘發子宮頸癌的相關。

本研究係以於 1991 年間所建立的 11923 名參與社區性癌症篩檢研究的婦女世代為研究對象，所有自願參加者分別於 1991-3 及 1993-5 年兩段期間分別接受邀請參加基線健康檢查及追蹤健康檢查，除了簽署同意書外，由訪視人員依標準化問卷收集社會人口學等基本資料、家族疾病史及多種危險因子的暴露資料，並接受醫師進行子宮頸抹片檢查及子宮頸細胞檢體之採集。疑似罹患子宮頸病變的個案進一步轉診輔以陰道鏡採集組織切片進行確診及例行追蹤。HPV 感染之檢測以子宮頸細胞進行 HPV DNA 及型別檢測。病毒量及病毒嵌入宿主細胞的檢測則以同時參加兩次檢查的對象中且於基線檢查感染 HPV16、18、 52 及 58 等四型中任一型者之檢體進行檢測。在追蹤期間，與全國癌症登記系統與死亡登記系統進行資料連結，以獲得子宮頸癌的新發病例。

透過長期追蹤研究設計及 HPV 感染的重複測量，本研究發現在 30-65 婦女之 HPV 盛行率為 24.5%，以 HPV16, 18, 52, 58 與 11 等型為最常見型別。於抹片正常婦女在平均追蹤 1.4 年間，以新偵感染任一型別而言，平均新偵感染率為 8.4%，兩次檢查感染同一型的平均持續感染率為 27.7%。已感染 HPV 者、初次性交年齡較早者、性交後陰道有灌洗習慣者與離婚或寡居者較易獲致新偵感染；年齡較高、高陰道產次(≥4)、曾使用子宮內避孕器者及已停經者等因素則與持續感染有高度相關。在追蹤 15 年後，感染 HPV 或高危險型別 HPV 的子宮頸癌發生率分別為每十萬人年 171 及 265 人，相對危險性則分別是 12.8 及 19.6 倍。與台灣婦女子宮頸癌高度相關的主要高危險型別為 HPV16,18,52,58，預防這四型的感染估計可減少 63%

的子宮頸癌，疫苗型別(HPV16/18)則可預防 51%。持續感染在子宮頸癌發生自然史中居重要關鍵地位，其危險性高達 44.3 倍，一旦病毒清除，則其危險性即未達統計顯著(2.4,0.6-9.2)。HPV16/18/52/58 之病毒量與持續感染呈有明顯劑量效應，高病毒量(>10^4 copies/50ng DNA)並可預測子宮頸癌罹癌風險達 3.4 倍，追蹤期間病毒量降低，後續罹癌風險即明顯降低達十分之一。病毒量與持續感染及子宮頸癌之相關，在停經後婦女尤著。病毒嵌入與是否患有子宮頸病變具統計相關(p=0.0076)，卻無法於正常婦女驗證其預測子宮頸癌之發生。上述結果將有助於子宮頸癌的預防，早期偵測以接受治療，提昇婦女健康及免於重大疾病之死亡。

關鍵字：人類乳突病毒、盛行率、新偵感染、持續感染、病毒量、病毒嵌入、子宮頸癌

Abstract in English

Cervical cancer is the second common cancer in the world and has been the leading female cancer in Taiwan over than a decade. Human papillomavirus (HPV) is well documented as the necessary cause of cervical cancer. But most previous studies were based on the cross-sectional case-control design, which can neither differentiate transient and persistent infection nor clarify the causal temporality of risk factor exposure and health outcome. The best way to examine the causation between HPV infection and various cancers should be based on a long-term follow-up study with repeated measurement of HPV infection.

In this study, 11,923 women were recruited as cohort members from a community-based cancer screening project (CBCSP) since 1991. Participants received health examinations in two bi-annual cycles in 1991-1993 and 1993-1995. After giving their informed consent, cohort members were personally interviewed according to a structured questionnaire to obtain information on socio-demographical characteristics and history of exposures to various cancer risk factors. Virapaps were used to collect cervical cells. Pap smear and health examination were performed. All women with suspected squamous intraepithelial lesions were further examined by colposcopy-guided biopsy to confirm the diagnosis. They were referred to intensive follow-up examinations every four months. The cervical cell samples and Pap smear

were collected at baseline and follow-up were tested for HPV DNA by polymerase chain reaction and genotyping by EasyChip. For cohort members infected with HPV types 16, 18, 52 and 58 further tests on viral load and integration into host genome will be carried out. During follow-up, cases of newly-diagnosed cervical cancers and cervical neoplasia will be ascertained through data linkage with profiles of National Cancer Registry and National Death Certification Registry.

Through this long-term follow-up study in CBCSP woman cohort with repeated measurements of HPV infection, we found HPV prevalence was 24.5% and HPV16, 18, 52, 58 and 11 were common types. Among normal women with a mean follow-up duration of 1.4 years, the summarized acquisition rate was 8.4% for any type and the mean of type-specific persistence rate was 27.7%. Women had HPV infection, earlier age at initial coitus, douching use after sexual intercourse, or not currently married had higher risk to acquire newly infection. The determinants for HPV persistence were higher age, high frequency of vaginal delivery, IUD user or post-menopause. With 15-year follow-up, the incidence of cervical cancer for HPV infection and persistence were 171 and 265 per 100000 person-year, the corresponding hazard ratio were 12.8 and 19.6, respectively. HPV16, 18, 52, 58 were the major high-risk types associated with cervical cancer in Taiwan. Around 63% of cervical cancer could be attributed with these 4 types and 51% for HPV16 and/or 18, which vaccine against. In our study, HPV

persistence was confirmed the pivotal role in the natural history of cervical cancer with a 44.3-fold risk, once the virus was cleared, the risk was non-significant (HR=2.4, 0.6-9.0). The viral load of HPV16, 18, 52 or 58 associated with persistence in a dose-response relationship and higher viral load (>10^4 copies/ 50ng DNA) was more likely to develop cervical cancer during follow-up; lowering viral load had a protective effect (HR=0.1, 0.03-0.3) with cancer incidence. The integration was associated with cervical neoplasia in the cross-sectional study, however, it couldn't predict the risks of HPV persistence and cervical cancer in the longitudinal study.

The findings will be useful for the primary prevention, early detection and intervention for cervical cancer.

Key word: HPV, prevalence, acquisition, persistence, viral load, integration and cervical cancer


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Abbreviations

AR%	attributable risk percentage (attributable fraction)
ASCUS	atypical squamous cells of undetermined significance
CBCSP	community-based cancer screening project
C.I.	confidence intervals
CIN	cervical intraepithelial neoplasia
CIS	carcinoma *in situ*
CXC	cervical cancer
DNA	deoxyribonucleic acid
F/U	follow-up visit
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
HC II	Hybrid Capture II
HSIL /HSIL+	high grade squamous intraepithelial lesion/HSIL or worse
HPV	human papillomavirus
HR-HPV	high-risk HPV (HR-17; HR-13; major HR-6; major HR-4; vaccine types)
ICC	invasive cervical cancer
IUD	intra-uterine device
LSIL/LSIL+	low grade squamous intraepithelial lesion/LSIL or worse
LR-HPV	low-risk HPV
OC	oral contraceptives
OR (aOR; mOR)	odds ratio (age-adjusted ; multiple adjustment)
ORF	open reading frame
Pap smear	Papanicolaou smear
PCR	polymerase chain reaction

PAR%	population attributable risk percentage (population attributable fraction)
RNA	ribonucleic acid
RT-PCR	real-time PCR
HR (aHR; mHR)	hazard ratio (age-adjusted ; multiple adjustment)
SIL	squamous intraepithelial lesion
SSO	sequence-specific oligonucleotide
TBS	the Bethesda system

Chapter I: Introduction

Background and context

Cervical cancer was the second common female cancer in the world; there were about 493,000 new cases and 273,000 mortality cases (Ferlay J 2004). In Taiwan, cervical cancer has been the leading cause of female neoplasia over a decade. According a recent annual report, a total of 5,725 women affected with cervical cancer, including 2,107 of invasive cancer and 3,618 of carcinoma *in situ*. The annual age-adjusted incidence (using the world population in the year of 2000 as standardized population) of invasive cancer was 17.2 per 100,000 person-years, which was around twofold to the western world. The mortality was 7.8 per 100,000 person-years due to 941 women died a cervical cancer death (Cancer registry annual report, 2002).

Human papillomavirus (HPV) infection is now regarded as the necessary cause for cervical cancer (Bosch, Manos et al. 1995; Walboomers, Jacobs et al. 1999). Both epidemiological and molecular evidences strongly support the casual relationship between HPV infection and cervical neoplasia. HPV DNA could be detected in almost 100% of cervical tumor. Moreover, the causal criteria (Hill 1965) are fulfilled as follows: 1) Strength: There is a very strong association between the presence of HPV DNA in cervical cells and the subsequent development of cervical cancer; 2) Consistency: The

association is consistent in a large number of studies in different countries and populations; 3) Specificity : The association between HPV DNA and cervical cancer is fairly specific; 4) Temporality: HPV infection precedes cervical precancerous lesions by a substantial number of years; 5) Biological gradient: The risk of cervical cancer seems to be related to viral load; 6) Plausibility: *In-vitro* and animal studies and epidemiological observation in humans provide plausible evidences; 7) Coherence: The association is coherent with previous knowledge; 8) Experimental evidence: There is increasing molecular evidence for the direct interaction between HPV and essential regulatory mechanisms of cellular growth; and 9) Analogy: HPV and cervical cancer model is analogous to many other papilloma virus-induced papillomas and carcinomas, as well as cancers caused by other viruses including EBV and HBV (Bosch, Lorincz et al. 2002).

HPV 16 was the predominant type among cases affected with high-grade lesions. However, HPV types 52 and/or 58 were the most common types among prevalent cases affected with low-grade lesions and unaffected controls (Liaw, Hsing et al. 1995; Liaw, Hsing et al. 1997) based on the cross-sectional case-control design. HPV-16 seropositivity was strongly associated with cervical cancer (OR=6.33; 95% CI 3.45-11.62) in a nested-case control study with 9- year follow-up (Naucler, Chen et al. 2007). However, serology test of HPV presents a biomarker of cumulative exposure for

passive infection. In a preliminary study, HPV DNA in exfoliated cervical cells collected from 101 cases of cervical cancer and 485 matched controls were tested. The age-residence-adjusted relative risk of developing cervical cancer was 73.9 for HPV-16 alone and 121.3 for HPV-58 alone in comparison with uninfected women. In the multivariate analysis using unconditional logistic regression, single infection with HPV type 16, 18, 52, 58, 31, and 33 was strongly associated with cervical cancer showing an adjusted ORs (95% CI) of 125.5 (42.3-371.9), 32.6 (6.3-168.1), 44.1(14.1-137.3), 137.9(28.8-660.1), 18.0(2.6-123.7) and 62.7(14.8-226.5), respectively. The estimated population attributable fraction for HPV16 and/or HPV18 was 48%, and it increased sharply to 83% for the combination of HPV types16, 18, 52, and 58 (Chen, Lin et al. 2005).

Even though the evidences were copious, it is still unclear that HPV infection was common and asymptomatic, whereas only few of infected would develop cervical neoplasia. To delineate the ingredients of HPV infection on cervical neoplasia is essential and a long-term follow-up study is required. A large-scale population study to understand HPV infection profile in Taiwan is imperative for the campaign against cervical cancer. Considering that the impending HPV vaccine, exigencies of the epidemiologic data for HPV infection in Taiwan is expectable. In the natural history of HPV infection before the occurrence of dysplasia, it is unclear whether both high viral

load and integration of viral DNA into host genome may lead to persistent infection of HPV, or vice versa. The puzzling temporality may be delineated only by a population-based long-term cohort study in which repeated samples is required to elucidate the HPV infection profile (genotype, viral load and host genome integration included) in order to estimate the risk related to cervical neoplasia. There were only few long-term follow-up studies on HPV and cervical neoplasia in other countries, and no long-term follow-up study has ever been carried out in Taiwan.

Specific Aims:

The specific aims of this study are giving as follows:

1) To estimate the HPV type-specific prevalence, persistence and acquisition rate and define their determinants in CBCSP women cohort.
2) To estimate the HPV type-specific incidence of cervical cancer in CBCSP women cohort during a period from 1991 to 2006.
3) To delineate the temporality among persistent infection, viral load and integration of viral DNA into host genome of HPV 16, 18, 52 and 58 by testing markers.
4) To differentiate relative importance of various HPV infection markers including persistent infection, viral load and viral DNA integration into host genome in the

development of cervical neoplasia.

Overview of this Dissertation:

In order to fulfill these aims, three projects of my research work include Project 1: Epidemiology and determinants of HPV infection, persistence and acquisition; Project 2: Natural history of HPV infection, viral load, integration, persistence and cervical neoplasia; and Project 3: Long-term risks and impacts of cervical cancer due to HPV infection and persistence. In Project 1, repeated measurements of HPV DNA and Pap smear from both baseline and follow-up visits were performed. Viral load of major HPV types (HPV16/18/52/58) from two visits were examined in the Project 2. Regarding Project 3, the HPV related health outcomes of cervical neoplasia were obtained through data linkage with nation-wide registries for long-term follow-up. The results were organized into four chapters in the following sections, consisting of chapter III: HPV genotype prevalence and determinants in a large-scale community-based study in Taiwan; chapter IV: Wide-spectrum HPV type-specific acquisition & persistence/clearance in a large-scale community cohort with follow-up; chapter V: Long-term risks and impacts of HPV-associated cervical cancer; and chapter VI: Viral load and integration associated HPV persistence and cervical cancer among normal in a

long-term prospective study.

Table I-1. Summary of this study

Chapter	Aim	Project	Design	Measurement	Estimates
III	1	1	Cross-sectional study	HPV DNA genotype at baseline visit	HPV Prevalence and their determinants
IV	1	1	Longitudinal study using repeated measurements	HPV DNA genotype at baseline & follow-up visits	HPV acquisition & persistence rate and their determinants
V	2	3	Longitudinal study using data linkage	cervical cancer during long-term follow-up	Incidence of CIN and cervical cancer
VI	3,4	2	Longitudinal study using data linkage	HPV viral load & integration	Risk to persistence and cervical cancer

Chapter II: Reviews of Literature

II.1 Characteristics of HPV

II.1.1 HPV genome

HPV genome is a double-stranded DNA in circular form containing approximately 8,000 base pairs and organized in three regions: early genes, late genes (L1 and L2), and upper regulatory regions (URR)(Figure II-1 & Table II-1) (Prendiville and Davies 2005). E1 and E2 genes encode regulatory proteins to modulate transcription and replication in viral life cycle. The E2 gene encodes two proteins involving the inhibition and activation of early gene transcription, respectively (Ward, Coleman et al. 1989). The E4 protein is considered to play an important role for maturation and replication of the virus (Brown, Fan et al. 1994). It also induces the collapse of the cytoplasmic cytokeratin network in human keratinocytes which may assist the releasing of virions from infected cells (Doorbar, Ely et al. 1991).

Two early proteins E6 and E7, particularly of the high-risk HPV types, play important roles in cellular transformation and tumor formation both in vitro and in vivo. The cooperation of E6 and E7 results in a higher capability in cell transformation and immortalization compared with the capability of single protein alone. E6 interacts with p53 (Werness, Levine et al. 1990) and E7 interacts with pRB (Dyson, Howley et al. 1989) to block the pathway of tumor suppression. Degradation of P53 and pro-apoptotic

protein BAK and subsequently inhibited apoptosis increase the instability of chromosome. E6 has also been postulated to activate telomerase and inhibit the degradation of SRC-family kinase (Veldman, Horikawa et al. 2001). E7 protein degrades pRB and blocks the binding of RB protein to the transcription factors E2F family, which activates genes involving DNA synthesis and cell cycle progression (Dyson 1998).

L1 and L2 genes encode two viral capsid proteins during the late stages of virons assembly (Park, Fujiwara et al. 1995). The protein encoded by L1 gene is highly conserved among different HPV types. Therefore, the taxonomic status of HPV types, subtypes, and variants is based on the difference of >10%, 2-10% and <2% sequence of their L1 genes.

II.1.2 HPV Genotypes

Up to now, more than 200 subtypes have been identified based on the phylogenic relationship of sequence in L1 region. Some regions or mRNA, such as E6, E7, were also used for phylogenic classification in few studies. More than 30 types of human papillomavirus are known to have mucosal affinity and related to genital infection.

Detection of HPV infection

To detect HPV DNA in cervical exfoliated cell samples, a widely used commercial kit- Hybrid Capture II (HC II, Digene Corp. USA) directly captured a group of 13

HR-types HPV DNA using a probe-cocktail of HPV type-specific RNAs. Multiple conjugated antibodies bind the DNA/RNA hybrids and magnify signal without amplification of virus genome. Polymerase chain reaction (PCR) based method was commonly performed for target amplification of a small fragments of viral genome in certain conservative region of all HPV genotypes using consensus primers (MY11/09, PGMY11/09, GP5+/6+ and SPF) (Manos, Ting et al. 1989; de Roda Husman, Walboomers et al. 1995; Kleter, van Doorn et al. 1998; Gravitt, Peyton et al. 2000). Real-time PCR method also was performed to detect HPV DNA, moreover, quantitative measures of viral loads were obtained.

Antibodies against the bovine papilloma virus have been use to identify HPV L1 capsid proteins (Dillner, Heino et al. 1991; Naucler, Chen et al. 2007). Detection of HPV DNA in peripheral blood lymphocytes were reported by very few studies (Pao, Lin et al. 1991; Chiou, Wu et al. 2003; Bodaghi, Wood et al. 2005; Ho, Yang et al. 2005). PCR-based method and in-situ hybridization were usually executed for archive Pap smears or biopsy.

II.1.3 Methods for HPV Genotyping

In most studies, HPV genotyping was based on two-stage design of experiment, which was performed for those samples with HPV DNA positivity. Since genotyping by dot blot hybridization necessitated heavy work-load to dot DNA products on membrane

and subsequently perform hybridization with probe of sequence-specific oligonucleotide (SSO). Blots of isotopic/non-isotopic signals were read. These work-load multiplied by the number of types to be detected. Direct sequencing was also documented the usage of HPV genotyping and the drawback limited its practical utility due to loss of delectability on concomitant types in samples. Recently, convenient and rapid chips/arrays coupling chemical binding primers and reversed dot blots hybridization with generic probes on glass/nylon membrane were designed to detect multiple types simultaneously. The HPVDNAChip ® (Biomedlab Co., Korea)(Choi, Kim et al. 2003; Hwang, Jeong et al. 2003; Kim, Jeong et al. 2003), PreTect® (NorChip, Norway)(Cuschieri, Cubie et al. 2005), Roche line blot (Gravitt, Peyton et al. 1998) , AMPLICOR® (Roche) (Monsonego, Bohbot et al. 2005; van Ham, Bakkers et al. 2005) and EASYchip (KingCar Co., Taiwan) (Huang, Huang et al. 2004; Huang, Chao et al. 2004; Lin, Chen et al. 2007) were adapted for epidemiological and clinical research (Table II-2). Genechip had the advantages of lowering the detection limits of viral copies than gel electrophoresis (Huang, Chao et al. 2004) and detecting a wide-spectrum of HPV types in one reaction.

II.1.4 High-risk types of HPV

Oncogenic types are generally classified according to their potential to induce

malignant transformation. HPV16, 18, 31, 33, 35, 45, 51, 52, 56, and 58 are considered as high-risk (HR) types because they are detectable in genital carcinomas and dysplasia (Lorincz 1992). A pooled analysis of HPV DNA in cervical cells of 1918 cases of cervical squamous cell carcinoma and 1928 controls enrolled in 11 case-control studies from 9 countries has been carried out. According to the ORs of developing cervical squamous cell carcinoma, 15 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82) are classified as HR-types, which are consistent with previous classification based on phylogenic relationship (Van Ranst, Kaplan et al. 1992). In addition, HPV types 26, 53 and 66 were suggested to be considered as HR-types in according to epidemiological classification (Munoz, Bosch et al. 2003).

II.1.5 HPV prevalence in Taiwan and Asia

HPV DNA can be detected in virtually all (99.7%) cervical cancers (Walboomers, Jacobs et al. 1999) HPV16 is the most common type found in these tumors (~50%), followed by HPV18 (~15%), HPV45 (~8%), and HPV31 (~5%). The distributions of HPV types in cervical cancers vary across geographic regions, HPV 16 accounted for 60% in European/northern America, 50% in African/Australia, and less than 50% in Asia (Clifford, Smith et al. 2003; Smith, Lindsay et al. 2007). In all regions, HPV18 was the second common type. The importance of HPV52 and 58 was shown in Asia,

which differed with other regions by HPV31, 33 or 45.

In a cross-sectional case-control study (Liaw, Hsing et al. 1995)), which studied on HPV infection and cervical neoplasia in our CBCSP cohort using a sensitive method to detect a broad spectrum of HPV types (Manos, Ting et al. 1989). A total of 88 biopsy-confirmed prevalent cases (including 40 cases of cervical intraepithelial neoplasia (CIN) 1, 9 cases of CIN 2, 36 cases of CIN 3 and 3 cases of invasive cancer) and 261 cytologically normal controls were selected. HPV DNA was found in 92% of cases affected with CIN 2-3 and invasive cancer, 54% of cases affected with CIN 1, and 9% of matched controls. In consistence with findings reported in Western countries, HPV 16 was the predominant type among cases affected with high-grade lesions. A later case-control study on our study subjects reported a high prevalence of HPV52 and HPV58 among cases affected with low-grade lesions and unaffected controls in Taiwan (Liaw, Hsing et al. 1997). HPV types infected Chinese women are different those infected Western women was documented (Bosch, Manos et al. 1995; Schiffman and Brinton 1995). HPV 52 and 58 were also prevalent in women of Hong Kong (Chan, Li et al. 1999; Lo, Wong et al. 2002) and China (Huang, Afonina et al. 1997; Lin, Yu et al. 1998; Lo, Wong et al. 2002).

In Chinese and Japanese, the prevalence of HPV52 has been reported to be 1.4-3.1% and that of HPV58 1.1-3.3% (Chan, Mak et al. 2002; Jeng, Phdl et al. 2005;

Sasagawa, Tani et al. 2005; Lin, Ma et al. 2006; Bao, Li et al. 2008; Chao, Hsu et al. 2008; Huang, You et al. 2008) (Table II-3). The prevalence of HPV varies from even country to country and even from region to region (Clifford, Gallus et al. 2005). In addition to the geographic variation, HPV types and the proportion of women affected with cervical pre-cancerous lesions and their age composition also differ in different populations (Franceschi, Herrero et al. 2006).

The prevalence of HPV increases with cervical cytology grade and age so the results should be stratified for both of these factors.

II.2 Natural history HPV infection

Most genital HPV infection was asymptomatic.

II.2.1 Acquisition

HPV 16 was the most common newly-acquired type from previous studies (Table II-4.) Among young woman, the HPV 16 acquisition rate were 10.5% (within 3 years) , 8.7%, or 7% (within 2.2 years) in the UK, Korea, USA and Canada (Woodman, Collins et al. 2001; Winer, Hughes et al. 2006; Oh, Ju et al. 2008). A lower rate (~1%) was observed in Costa Rica (Richardson, Kelsall et al. 2003). HPV18 was followed as second commonly acquired type in UK, Korea, USA, and Colombia. The acquisition rate of HPV52 and 58 were much lower than HPV16 or 18 in UK (Woodman, Collins et

al. 2001). But in Brazil and Colombia, HPV52 and 58 were also commonly incident type (Franco, Villa et al. 1999; Rousseau, Pereira et al. 2001; Munoz, Mendez et al. 2004; Trottier, Mahmud et al. 2008).

II.2.2 Persistence and clearance

In a nested-case control study based on cytologically normal American women at enrollment, women having HPV DNA in their cervical cells collected at both enrollment and diagnosis had an increased risk (OR, 13.2; 95% CI, 6.2-27.0) of atypical squamous cells of undeterminated significance (ASCUS) and risk (OR, 100.6; 95% CI, 37.7-268.4) of low-grade squamous intraepithelial lesion (Schneider-Maunoury, Croissant et al. 1987; Guo, Sneige et al. 2007) compared with those who were HPV DNA-negative in their cervical cells (Liaw, Glass et al. 1999). A consistent finding (OR, 213.4; 95% CI, 18.1-16000) was reported among Swedish women using the same study design (Wallin, Wiklund et al. 1999). In another study with a follow-up period over five years, an OR of 11-12 folds was reported for women who had type-specific persistence of HPV 16 or 18 at two visits with a time interval of four months compared with those who were negative on both tests (Schlecht, Kulaga et al. 2001).

Most studies with repeated HPV testing have been conducted with young women or college/university girls, and the persistence or acquisition rates were estimated almost exclusively for HPV 16 or -18 (Table II-5). The persistence rate or clearance rate

were used to described the HPV whether the HPV was detectable or not at repeated measurements. In a prospective study of female college students, approximately 70% of women had no detectable HPV DNA within 12 months after new HPV infection. After 18 months, over 80% of women had cleared their infections (Ho, Bierman et al. 1998). Most women infected with a specific HPV type did not sustain the same HPV type 6-12 months after infection (Hildesheim, Schiffman et al. 1994; Hinchliffe, van Velzen et al. 1995; Franco, Villa et al. 1999; Cuschieri, Cubie et al. 2005). Other cohort studies have reported a median duration of HPV DNA detectability of approximately one year (Hildesheim, Schiffman et al. 1994; Evander, Edlund et al. 1995; Franco, Villa et al. 1999; Woodman, Collins et al. 2001). Women aged over 30 years had higher persistence rate than aged less than 24 years (65% vs. 32%) after follow-up of 12 months (Hildesheim, Schiffman et al. 1994).

HPV 16 has been reported to have a longer clearance time than other HPV types (Liaw, Hildesheim et al. 2001; Richardson, Kelsall et al. 2003). Type-specific persistence of HPV has been shown to increase with age and such findings support the utility of HPV screening in older women (Castle, Schiffman et al. 2005).

II.2.3 Viral load

Quantification of HPV viral load is available and has recently been reported using different methods. The different methods (cut-off) of viral load level were used as

qualitative dot blot signals (percentile), semi-quantitative methods of PCR-EIA (percentile) and HC II (pg/ml). Quantitative real-time PCR (Moberg, Gustavsson et al. 2003) improved the estimation of viral load.

High-viral load has been considered as a potential risk factor for cervical neoplasia in some cross-sectional case-control studies (Morrison, Ho et al. 1991; Ho, Bierman et al. 1998; Sun, Liu et al. 2002; Tsai, Wu et al. 2005; Flores, Papenfuss et al. 2006; Cheung, Cheung et al. 2008). However, HPV viral load could not sufficiently predict the risk of subsequent CIN (Ylitalo, Sorensen et al. 2000; Dalstein, Riethmuller et al. 2003; Castle, Schiffman et al. 2005; Monnier-Benoit, Dalstein et al. 2006). There was no standard method or cut-off was used (Table II-6). In longitudinal studies, the incident cervical lesion or cervical cancer was associated with previous high viral load (van Duin, Snijders et al. 2000; Gravitt, Kovacic et al. 2007), and a dose-response relationship was observed (Josefsson, Magnusson et al. 2000; Schlecht, Trevisan et al. 2003; Castle, Schiffman et al. 2005; Moberg, Gustavsson et al. 2005), but other studies have found that viral load could not predict viral clearance and CIN3+ (Lorincz, Castle et al. 2002; Molano, Van den Brule et al. 2003).

II.2.4 Integration into host genome

Cervical cancer cells often contain chromosomally integrated HPV DNA or a mixture of both integrated and episomal forms (Durst, Kleinheinz et al. 1985; Schwarz,

Freese et al. 1985; Yee, Krishnan-Hewlett et al. 1985; Cullen, Reid et al. 1991; Kalantari, Blennow et al. 2001) (Durst, Kleinheinz et al. 1985) . In contrast, HPV DNA is present generally as an episomal form without integration into host genome in cervical warts. The episomal form of HPV DNA present in cervical tumors may be polymeric or contain mutations in the LCR (May, Dong et al. 1994; Rose, Thompson et al. 1997). These findings suggest that integration of HPV DNA into host genome or other events that disturb the organization and/or expression of HPV DNA such as polymerization or mutation are determinant steps in HPV-induced carcinogenesis. Cells that contain integrated HPV16 DNA have a selective advantage for growth (Jeon and Lambert 1995). It is presumed that the integrated HPV genome retains E6 and E7 ORFs, which are capable of over-producing E6 and E7 oncoproteins. Deregulated expression of these oncoproteins is thought to lead to cell transformation and eventually to cancer. The sites in human genome at which HPV DNA integrates are not a significant factor in oncogenesis although the integration often occurs at common fragile sites (Kalantari, Karlsen et al. 1997; Thorland, Myers et al. 2000). However, few integration sites have been mapped in detail and further studies might reveal locations associated with an increased cancer risk or variations in tumor aggression (Kalantari, Karlsen et al. 1997; Lopez-Borges, Gallego et al. 1998). Chromosomal integration of the HPV genome commonly results in the disruption of viral E2 ORF and, as a consequence, the loss of

E2 protein (Baker, Phelps et al. 1987; Corden, Sant-Cassia et al. 1999; Gallo, Bibbo et al. 2003). In addition, the disruption of E2 ORF has been shown to increase the ability of HPV16 to immortalize cells (Romanczuk and Howley 1992). These observations suggest that the loss or inactivation of E2 protein might play some role in cervical carcinogenesis (Schneider-Maunoury, Croissant et al. 1987). The integration of HPV DNA into host genome is associated with the progression of CIN from polyclonal to monoclonal status, and these events play a fundamental role in the progression from low-grade to high-grade cervical neoplasia (Ueda, Enomoto et al. 2003).

The difference of viral load between E2 and E6 was applied for detecting HPV integration due to the loss of E2 while genome integration. A purely integrated form was defined when no E2 was detected. The rapid progression of CIN lesions was observed in patients with a high viral load of integrated HPV16 (Peitsaro, Johansson et al. 2002). Among cancer, the percentage of purely integrated form was varied from 20%~82%. Most previous studies have been based on cross-sectional designs and only a few studies have included normal women (Briolat, Dalstein et al. 2007; Chan, Cheung et al. 2007; De Marco, Gillio-Tos et al. 2007). The percentage of pure integration forms in previous reports was 20%~36% among normal, whether on a cytological or histological basis and whether cervical cells or paraffin-embedded tissue were used (Table II-7). A common limitation of integration studies was the small sample size in each grade of

cervical lesion. The only one longitudinal study among 13 LSIL women with mixed form, only one woman progressed to HSIL; there is no evidence for physical status in disease progression (Ho, Cheng et al. 2006). However, the definitive cut-off of integration using the E2/E6 ratio by RT-PCR has been different in different studies, and the definition of pure integration (no E2) has been common.

The performance of HPV16 integration assay was evaluated (Ruutu, Kulmala et al. 2008). Ratios of E2 to E6 of less than 0.5 indicated the presence of integrated form dominant. Where the ratio was between 0.5 to 1.1, both integrated form and episomal form were observed (mixed). The episomal form dominant was defined as dominant when the ratio was greater than 1.1. The copy numbers of integrated E6 were calculated from the total copy numbers of E6 (both episomal and integrated) subtracting the copy numbers of E2 (episomal).

II.3 Association between HPV infection and cervical cancer

Epidemiological studies based on case-control design provided approximation to relative risk to differentiate the relative importance. The drawback of temporality was remedied by nested-case control design. There only few important studies on HPV infection and the development of cervical neoplasia in subsequent. The relative index facilitated judging the priority among risk factors in comparison to their references. If we obtain the absolute risk/incidence which reflects substantial demand or burden, both

etiological fraction and population attributable proportion were readily.

Using Hybrid Capture II, infection of HPV high risk type was a strong predictor for cervical abnormality or cervical intraepithelial neoplasia based in long-term follow-up (Castle, Wacholder et al. 2002; Kjaer, Hogdall et al. 2006; Cuzick, Szarewski et al. 2008). Based on nested-case control design, HPV16 and HPV18 were investigated their important role about cervical neoplasia (Liaw, Glass et al. 1999; Wallin, Wiklund et al. 1999; Ylitalo, Sorensen et al. 2000; Peto, Gilham et al. 2004). These data indicate that HPV typing at least for HPV 16 and 18 may identify women who would benefit from more aggressive management than women with other HPV types. Accordingly, vaccination against HPV16 and 18 may suggest promising results for prevention of cervical cancer (Harper, Franco et al. 2004; Villa, Costa et al. 2005). However, the role of HPV52 and 58 were addressable in Taiwan or in eastern Asia; their risks were rarely studied due to unpopular in western world.

The significant difference of cumulative incidences between HPV positive and negative was report after 6-year and 12-year of follow-up (Sherman, Lorincz et al. 2003; Huang, Chao et al. 2008). Nevertheless, the variable risk among different high risk types was observed in two women cohort (age under 40) studies for 3-4 years of follow-up, HPV16 was the most oncogenic type (Woodman, Collins et al. 2001;

Naucler, Ryd et al. 2007). The ALTs study reported genotype-specific HPV testing is helpful to identify high or low risk of women with ASCUS to develop more severe lesion (Wheeler, Hunt et al. 2006).

Among young women had repeated positive of HC II, the increased risk associated with SIL during 4-year follow-up and 20% of them developed CIN2+ in duration of 10.6 years (Kjaer, van den Brule et al. 2002; Kjaer, Hogdall et al. 2006). In a nested-case control study, detectable HPV DNA both at baseline and at diagnosed specimen was reported a risk of 213.4 (18.1-16000) compared with repeated negative. HPV16 repeated positive at last two examinations before CIS diagnosed had a risk of 31.2 (10.6-91.8) (Ylitalo, Sorensen et al. 2000). Type-specific persistence of HPV16 or 18 had 11-fold risk to develop any SIL during 5 years and the cumulative incidence was 40%, only 5% for negative and 26% for HPV16 or 18 positive at baseline, which pointed out the important role of HPV persistence in cervical carcinogenesis (Schlecht, Kulaga et al. 2001).

These important results reported the association HPV infection and HPV persistence in relation to subsequent cervical neoplasia using longitudinal study design (Table II-8 & II-9). However, there was only one young women cohort on type-specific basis (Kjaer, van den Brule et al. 2002).


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Chapter III: HPV genotype prevalence and determinants in a large-scale community-based cohort in Taiwan

Introduction

Human papillomavirus (HPV) is now widely accepted as the cause of cervical cancer. The virus is detected in almost 100 percent of women with invasive cervical cancer (Bosch, Manos et al. 1995; Walboomers, Jacobs et al. 1999). More than 200 types of HPV have been identified and 18 have been classified as high-risk in relation to cervical neoplasia (Munoz, Bosch et al. 2003). There is increasing epidemiological and molecular evidence strongly supporting a causal relationship between HPV infection and cervical neoplasia. HPV16 and HPV18 are detected in a high percentage of invasive cervical cancer cases worldwide (Clifford, Smith et al. 2003; Smith, Lindsay et al. 2007). The vaccines Ceravix and Gardasil against HPV types 16 and 18 have recently become available (Harper, Franco et al. 2004; Villa, Costa et al. 2005).

An earlier case-control study on our study subjects reported a high prevalence of HPV52 and HPV58 in Taiwan (Liaw, Hsing et al. 1995; Liaw, Hsing et al. 1997). In Chinese and Japanese, the prevalence of HPV52 has been reported to be 1.4-3.1% and that of HPV58 1.1-3.3% (Chan, Mak et al. 2002; Jeng, Phdl et al. 2005; Sasagawa, Tani et al. 2005; Lin, Ma et al. 2006; Bao, Li et al. 2008; Chao, Hsu et al. 2008; Huang, You

et al. 2008). The prevalence of HPV varies from even country to country and even from region to region (Clifford, Gallus et al. 2005). In addition to the geographic variation, HPV types and the proportion of women affected with cervical pre-cancerous lesions and their age composition also differ in different populations (Franceschi, Herrero et al. 2006). The prevalence of HPV increases with cervical cytology grade and age so the results should be stratified for both of these factors. The prevalence in the control group in case-control studies may reflect only a small portion of the population, generally reflecting that in the same age group as the cases, which may be different from that in the general population. Hospital studies or health clinic studies may result in a population with a higher proportion of women complaining of cervical abnormalities or a group at high risk of HPV infection, thus overestimating the prevalence in the general population.

Cervical cytological examination (the Papanicolaou (Flores, Papenfuss et al.) test) is the main tool for cervical cancer screening. In Taiwan, since 1995, National Health Insurance has reimbursed the costs of annual Pap smear screening for women aged over 30. However, before that, women received Pap screening only after delivery or when they visited a hospital and there was little publicity about Pap screening. A nationwide database of the results of Pap screening was established as the National Cervical Neoplasia Screening registry. HPV genotyping has become much more meaningful, as

the epidemiological profile of HPV genotypes is a major concern in introducing the use of HPV vaccines for the public. There have been some large-scale studies in the Western world, but only limited data are available from Asia. More input to the HPV database from Asia, based on large scale studies, is needed to help in the global campaign against cervical neoplasia.

Using a wide-spectrum HPV blot to detect HPV DNA of 39 types, we carried out a large-scale community-based cohort study with two measurements of HPV DNA (baseline and follow-up) and followed-up the outcome using the national registries. In this report of the baseline profile of HPV infection and its determinants, we aimed to examine the type-specific prevalence in Taiwan and to investigate determinants of HPV infection and performance of HPV testing in cervical cancer screening in Taiwan.

Material and Methods

Study cohort

Recruitment of the Taiwan CBCSP cohort and study design

The study cohort members were enrolled in the Community-Based Cancer Screening Program (CBCSP) in 1990. Forty-one thousand three hundred and eighty

women aged from 30 to 65 years old listed in local household registration offices from seven urban and rural townships in Taiwan were invited to participate in the CBCSP by undergoing two bi-annual regular health examinations in 1991-1992 (baseline examination) and 1993-1995 (follow-up examination). A total of 11,923 women were enrolled, of whom 6,923 women attended for the follow-up examination (Figure III-1). At the baseline examination, 10,615 women who had undergone a Pap smear test and HPV testing and completed a questionnaire were eligible for inclusion; these included 31 patients from a previous study (Liaw et al., 1995 & 1997), for whom HPV data were available, but specimens for HPV genotyping were not. The criteria for non-eligibility were lack of a specimen (n=1,170) and reported hysterectomy according to the questionnaire (n=138). After exclusion of 13 women with cervical cancer diagnosed prior to enrollment, 10,602 women were eligible as HPV cohort members.

At recruitment, well-trained public health nurses gave detailed information about the study to each participant. Written informed consent was obtained from each participant who agreed to participate on a voluntary basis. A structured questionnaire was administered to obtain information on socio-demographic characteristic, cigarette smoking, Pap smear history, reproductive and sexual history, and personal and family history of cancers. Pap smears and cervigrams, magnified photographic images of the cervix taken after application of acetic acid, were taken by gynecologists to screen each

participant for cervical neoplasia. The gynecological examinations were performed at the same time as the health examination or later if the woman was menstruating at the time. A Cervex-Brush (Rovers, The Netherlands) was used to obtain exfoliated cervical cells for the Pap smear. Cervical cells were collected from all participants for HPV DNA testing using ViraPap kits (Digene Diagnostic, Silver Spring, MD), consisting of a Dacron swab for scraping cells and 1 mL of Digene standard transport medium for DNA preservation. The cells were stored at -80℃ until HPV DNA testing.

Pap smears were graded according to the Bethesda system, which categorizes cytology grades into normal, atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and cancer. The cytologically abnormal group consisted of all patients with ASCUS or worse. Participants whose Pap smear or cervigram were scored as low-grade or more severe SIL were referred to study clinics for colposcopy-guided biopsy for confirmatory diagnosis. Endocervical curettage was performed if there was no visible lesion on colposcopic examination.

Bi-annual regular follow-up and four-month intensive follow-up

All participants in this study cohort were invited to be followed bi-annually by a Pap smear and the collection of blood and cervical cells. Cohort members with positive

findings on the Pap smear were referred for colposcopy-guided cervical biopsy according to the standard operational procedures used at recruitment and were intensively followed-up every four months with the collection of a Pap smear and blood and cervical cells using the same standard operational procedures.

Laboratory methods for HPV DNA and genotyping

HPV genotyping was performed from July, 2004 through December, 2005.

Quality assurance:

Ninety-six samples were tested in each experimental batch, including 7 controls for quality-monitoring. Two aliquots, S1 and S2, from a stock of cervical cells infected with a known HPV type were used as control samples to monitor reproducibility. Three cell line controls, HeLa (HPV18-integrated), CaSki (HPV16-integrated), and Jurkat cells (American Type Culture Collection, Manassas, VA), were also used to determine the accuracy and effectiveness of the test. As a PCR positive control, a male blood sample was processed using GAPDH forward and reverse primers. A sterile water control was used to monitor contamination in both the HPV and GAPDH reactions. PCR was re-performed if negative GAPDH results were obtained. The excellent reliability and reproducibility of this method has been documented (Lin, Chen et al. 2007).

DNA extraction:

The thawed cervical cell samples were vigorously shaken in medium and spun the cervical cells down from the swab. Pre-washing of specimen was done before DNA extraction. A 100 μl aliquot of sample was placed in a 1.5 ml tube and was washed with 900 μl of 10 mM Tris buffer, pH 7.4, centrifuged at 8000 rpm for 1 min at room temperature, 900 μl of the supernatant removed, and the rest of the sample frozen at -20℃. Ninety-six samples were processed simultaneously using a Qiamp 96 DNA blood kit (Qiagen Inc., Venlo, Netherlands); these consisted of 89 test samples, 2 HPV-type control samples S1 and S2, 3 cell line samples, and 2 PCR controls (GAPDH and water). Each of the 96 samples was digested by adding 120 μl of proteinase solution (20 μl of the kit proteinase in 100 μl of 10 mM Tris buffer, pH7.4) and 200 μl of the AL buffer and incubation at 5℃ for one hour. The digested sample was added to 200 μl of 100% absolute ethanol, mixed well, and applied to the column, which was then washed with buffers AW1 and AW2, and the DNA eluted with 105 μl of the AE buffer.

HPV PCR assay:

HPV DNA was amplified by using primers MY11/biotinylated GP6+ targeting L1 regions (Lin CY et al., 2007). DNA quantity and integrity were monitored through the amplification of a part of GAPDH (human glyceraldehyde-3-phosphate dehydrogenase)

gene in replicate tubes. The volume of PCR reaction was 25μL in whole experiment. The PCR reaction cocktail contained 15 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM MgCl2, 0.25mM dNTP, 0.5U polymerase (HP^{TM} High Performance HotStart Tag DNA Polymerarse; DNA Technologies Ltd., UK), and 0.6 μM primers. PCR was conducted in a Perkin-Elmer model 9700 thermocycler with following amplification profile: a single cycle of denaturation at 95 ℃ for 10 minutes, 40 cycles of denaturation at 95℃ for 30 seconds, annealing at 45℃ for 40 seconds and elongation at 72℃ for 30 seconds, a final extension at 72℃ for 5 minutes and cooled down to 25℃ for 5 minutes. There were 0.2 μM GAPDH forward and reverse primers was instead in GAPDH PCR cocktail. The GAPDH PCR was performed by 40 cycles of 15 seconds at 95℃, I minute at 57℃ and seconds at 72℃. A 2 μl aliquot of purified DNA was used in each PCR mixture. Before hybridization of HPV blot, the major band of controls was confirmed for successful PCR using electrophoresis. Electrophoresis of other samples was done after HPV blot hybridization to avoid contamination by aerosol while open-close. The reading of gel and Blot were undergone blindly.

HPV genotyping using HPV Blot (Easychip®):

Duplicate samples of oligonucleotide probes (20- to 30-mers with an approximately 100-200 poly-T-tail) for 39 types of HPV (6, 11, 16, 18, 26, 31, 32, 33,

35, 37, 39, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 72, 74, 82, CP8061[71], CP8304[81], L1AE5, MM4[82], MM7[83], and MM8[84]) were deposited symmetrically on a 1.2 x 1.5 cm nylon membrane. Fifteen microliters of PCR product and 5 μL of GAPDH product were then hybridized simultaneously using an HPV blot (Easychip® HPV Genotyping Array, KingCar, Taiwan). There were also four samples of systematic controls (biotin-labeled PCR products), two of the internal control (GAPDH), and two of the negative control (plant dihydroflavonol-4 reductase). The sensitivity of this method (10^{-4} to 10^{-3} ng of DNA) is 10 times higher than that using visible band on gels (Huang, Chao et al. 2004) (Lin, Chen et al. 2007).

The HPV Blot membranes were rinsed with 2X sodium chloride and sodium citrate (SSC) at room temperature for 10 minutes, prehybridized by shaking at 35℃ for 30 min in prehybridization buffer (2X SSC, 0.5% blocking reagent, 5% dextran sulfate, and 0.1 % SDS) containing denatured salmon sperm DNA (50 mg/ml), then hybridized by shaking at 35℃ for overnight in 500 ul of hybridization buffer (2X SSC, 0.5% blocking reagent, 5% dextran sulfate, 0.1% SDS, and 50 mg/ml of denatured salmon sperm DNA) containing 15 ul of denatured amplicons. The chips were washed for 5 minutes at room temperature in washing buffer 1 (2X SSC and 0.1% SDS), then twice for 5 minutes at 35℃ in washing buffer 2 (0.2X SSC and 0.1 % SDS). Following this stringent washing, the chips were rinsed twice with buffer 1 [1X phosphate-buffered saline (PBS), pH 7.4,

0.05% Tween-20, 0.1% SDS] by shaking at room temperature for 5 minutes, then incubated for 60 minutes at room temperature in 500 ul of buffer 2 (1X PBS, pH 7.4, 0.05% Tween-20, 0.1% SDS, and 0.5% blocking reagent) containing Streptavidin-AP (Calbiochem; alkaline phosphatase conjugates for binding with biotinylated GP6+ primer, 1:1000 dilution). After alkaline phosphatase conjugation, the chips were washed in buffer 1 and rinsed for 5 min at room temperature with buffer 3 (0.1 M Tris-HCl, pH 9.5 and 0.1 M NaCl), then 80 ul of substrate (5-bromo-4-chloro-3-indolyl-phosphate and nitro blue tetrazolium) was added and the sample incubated for 30 minutes at room temperature. The reaction was stopped by aspiration of the substrate solution and addition of distilled water. After drying, the results were read and recorded following standard protocols.

Definition of high-risk HPV infection

According to the epidemiologic classification (Munoz 2003), fifteen HPV types are classified as high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82), 3 as probable high-risk types (26, 53, and 66), and 12 as low-risk types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108). We used different groupings in this study, as described below.

HR-17 (17 high-risk types) and LR (22 low risk types) groups: In the 39

types detected by HPV blot, there were 14 high-risk types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 82) and 3 probable high-risk types (HPV26, 53, and 66). The low-risk group consisted of the 22 low-risk or undetermined risk types.

HR-13 (13 high-risk types) group: 13 HPV types were grouped together as Hybrid Capture II detected (HC II, (Digene Corp, Gaithersburg, MD , USA); these were HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.

Major HR-6 (major 6 high-risk types) group: this consisted of HPV16, 18, 52, 58, 31, and 33.

Major HR-4 (major 4 high-risk types) group: this consisted of HPV16, 18, 52, and 58.

Vaccine types group: This consisted of HPV16 and 18, against which vaccines are available.

Determination of confirmed cases of cervical cancers

Using data from the National Cancer Registry and National Death Certification Registry, 56 cohort women were found to have histological-confirmed cancer of the cervix uteri within 1 year after enrollment. The criteria of cervical cancer from computerized database were linked as diagnosis with ICD-code of 180 and ICD-O-code

of C-53.

Statistics

The HPV infection genotypes in cervical cells collected at baseline were used to calculate the prevalence of HPV infection in Taiwan. Prevalence stratified by cytology grade and age group was calculated. Various risk factors associated with HPV infection were analyzed. In the univariate analysis, logistic regression was used to test the association and the odds ratios (OR) and age-adjusted OR with 95% CIs were calculated. The trend test was also carried out for exposure in continuous scale. Multiple logistic regression models were used to estimate multivariate-adjusted ORs with 95% CIs for various risk factors associated with HPV infection using SAS 9.1.3. The attributable risk fraction (AR%) and population attributable risk fraction (PAR%) were calculated as (OR-1)/OR x 100% and {[prevalence x (OR-1)]/[prevalence x (OR-1)]+1} x 100% (Miettinen 1974).

Results

Prevalence by cytology grade

At baseline, of the 10602 women, 2594 were HPV-positive (24.5%): these consisted of, 2524 with definite HPV typing, 17 with equivocal typing (inconsistent

typing on repeated testing) and 53 untyped (band not corresponding to one of the 39 tested types). The overall cytologically abnormal rate and squamous intraepithelial lesion rate were 3.9% and 2.6%, respectively. The HPV-positive rates were 22.3%, 50.7%, 81.0%, 98.5%, or 100%, respectively, for the cytological grades of normal, ASCUS, LSIL, HSIL, or SCC (Table III-1). Of the 56 confirmed cervical cancer cases, 55 were HPV-positive (98.2%). The HPV-positive rate increased significantly with cytological grade ($p<0.0001$). The proportion of multiple-type infection increased with cytology grade, but the number of infected HPV types showed the opposite trend ($p=0.0025$). The positive rate for the HR-17 and HR-13 groups, the major HR-6, HR-4, and vaccine types group showed a similar increasing trend (Figure III-2).

HPV type-specific prevalence according to cytological grade

The most common types (>20%) in confirmed cases were HPV16 (49.5%), followed by HPV58 (25.5%) and HPV52 (20.0%). Among cervical cancer cases on cytological basis, the positive rate of HPV16, 52 and 58 were 52.6%, 21.1% and 21.1%, respectively. On the basis of cytological grade, the common types (>10% prevalence) in the HSIL group were HPV16 (32.4%), 58 (25.7%), 52 (23.5%), 33 (11.8%), and 31 (10.3%), the most common types (>8%) in the LSIL group were HPV52 (14.0%), 16 (13.2%), 53 (9.1%), 39 (8.3%), 51 (8.3%), and 58 (8.3%), and the most common types

(>5%) in the ASCUS group were HPV52 (16.2%), 16 (6.6%), 18 (6.6%), 58 (5.9%), and 31 (5.1%). Among the cytologically normal group, a type-specific prevalence greater than 1% was seen for HPV11 (6.1%), 52 (2.2%), 56 (2.0%), 18 (1.9%), 16 (1.7%), 33 (1.3%), and -53 (1.1%). The cumulative percentage of HPV genotypes in the 56 confirmed cervical cancer cases using the order of 'frequent HPV genotypes among cervical cancer in the world' (Bosch et al.,1995) is shown in Figure III-3A and compared with that using the order of 'frequent HPV genotypes among cervical cancer in Taiwan', shown in Figure III-3B. The corresponding results for the cytologically abnormal group are shown in Figure III-3C & D and demonstrate the importance of HPV52 and HPV58 in addition to HPV16.

Age-trend of prevalence according to cytology grade and major HPV types

The age trend showed the prevalence of the HR-17 group was consistently higher than 50% in all age groups in the cytological groups of LSIL, HSIL, and SCC (Figure III-4A). Among the ASCUS group, a higher prevalence (~50%) was seen in the youngest group (30-34) and older groups (50-54, 55-59, and 60+). In the normal group, HR-17 prevalence was slightly increased in the older groups (55+). Figure III-4B shows that a different pattern was observed for the low-risk grouping (the other 22 types), with a similar low and flat age-trend in each of the abnormal grades and a fluctuating curve

in normal due to the small number of infected subjects in each age group. The prevalence of HPV16 or HPV16/18 showed no obvious age-related trend (Figure III-4C). When HPV52 and HPV58 were also included, the prevalence was increased among the 55-59 age group and was much higher in 60+ group. The positive rate for the HR-17 grouping or all types of HPV combined closely paralleled the prevalence of the HPV16/18/52/58 grouping. Similar patterns were seen for cytologically normal women (Figure III-4D).

Determinants of HPV infection

As shown in Table III-2, in the univariate analysis, HPV infection was associated with older women aged 55+, with an OR (95%CI) of 1.4 (1.2-1.6) and 1.3 (1.1-1.6) for the age groups of 55-59 and 60+, respectively. Marital status and number of marriages were not associated with baseline HPV infection. Women who had ever had the habit of cigarette smoking (>4 day per week) or alcohol drinking (>4 days per week) had an OR of 1.9 (1.3-2.9) or 2.0 (1.2-3.3), respectively, but there was no significant correlation with betal chewing (OR: 1.6 (0.6-4.2), which may be due to only 6 women reporting this habit. In terms of experience of pregnancy, delivery and contraception use, aside from an increasing trend between the number of births and HPV infection, those who had undergone several caesarian operations (≥ 3) had a 1.2-fold higher risk than those

who had never had a caesarian. Around 60% of women reported having used an intra-uterus device (IUD) and this was significantly associated with HPV infection. No association was observed between number of vaginal deliveries, abortions, or use of oral contraceptives and HPV infection. Menopausal women had a 1.3-fold higher risk of HPV infection than pre-menopausal women. Age at menarche or menopause was not related to HPV infection. Sexual behavior-related factors were strongly associated with HPV infection. The earlier the age at initial coitus, the higher the risk of having HPV infection was. In our population, only 2.5% of women reported having had more than one sexual partner in her lifetime, but this was significantly associated with a 1.9-fold higher risk. However, similar low risks were seen for women who did not report their age at initial coitus, number of sexual partners, and premarital sex. Previous Pap smear screening experience was not related to HPV infection. Baseline abnormal cytology was highly associated with HPV infection, with an OR of 12.2 (95%CI: 9.6-15.4). In the multiple adjusted model using the stepwise method, abnormal cytology (OR and 95% CI: 12.8, 10.0-16.3), cigarette smoking (OR and 95% CI: 1.8, 1.2-2.7), number of lifetime sexual partners (OR and 95% CI: 1.8, 1.4-2.3), menopause (OR and 95%CI: 1.2, 1.1-1.5), and IUD use (OR and 95%CI: 1.2, 1.1-1.3) were major determinants for baseline HPV infection.

Impact of HPV infection

Using cytological LSIL+ or cytological abnormality as outcome, the AR% was calculated using the aOR of overall HPV, HR-17, major HR-6, major HR-4, and vaccine types group, respectively. For the cytological LSIL+ , the AR% associated with the groupings of 6 or 4 major HPV types was 97-99%, but 73% for the 22 low risk types when any HR-17 types infection were not included. For the abnormal cytology, the AR% was 92-95%. Taking prevalence into consideration, the impact of cytological abnormality related HPV infection was estimated by PAR% calculation. And there will be 74% of cervical abnormality in our population reduced if major HR-17 or HR-13 HPV infection was eradicated, which was close to 70% or 72% to remove major high-risk HPV infection of 4 or 6 types. The similar importance of major high-risk types was determined in relation to cytological LSIL+. However, only 51% of cervical abnormality was attributed to vaccine types in our population.

Discussion

Using a comprehensive cervical cytology test and wide-spectrum HPV testing of the entire women cohort (>10000) in this study, we profiled the HPV infection and obtained a more precise estimation of the genotype-specific HPV prevalence based on this large study sample. It was confirmed that the HPV positive rate and

type-distribution varied in different cytology grades. It is therefore essential to determine the cytology status in investigating HPV prevalence.

In the normal population, ~22% women were HPV-infected. This prevalence is higher than in all previous reports (9.5-14.3%) (Liaw, Hsing et al. 1997; Clifford, Gallus et al. 2005; Bao, Li et al. 2008; Chao, Hsu et al. 2008; Huang, You et al. 2008), with the exception of Jeng's and Lin's studies (19.3~19.85%) (Jeng, Phdl et al. 2005; Lin, Ma et al. 2006). Firstly, in these studies, about 40% of HPV-positive subjects showed no visible band on gel electrophoresis after PCR. This may be due to the low viral load, resulting in an undetectable signal. The higher sensitivity of our chip method has been documented (Huang, Chao et al. 2004). In most previous studies, genotyping was performed only on two stage PCR-positive samples. If the same applied to our study, this would reduce the overall and HR-17 prevalence rate to 13.2 % and 8.6%, respectively. The second reason was probably an epidemic of HPV11 infection in 1991~2. Because that we had routed out possible contaminations from experiment (by repeating HPV11 positive sample) and sample taking (by examined the clustering of geographical area, date of collection/experiment batch and physician). Furthermore, after ignoring HPV11 infection, the overall HPV prevalence was lowered to 12.3%, which is closer to the previous reports. In addition to the above, composition in terms of age distribution, cytology, and geographical factor may explain the variation between

studies. Another interesting phenomenon is that multiple infections were common (~27%) in normal in our study which may depend on how many types were detected by the method used. It is worth mentioning that, during the study period, women only had a Pap smear occasionally and, because of this, HPV detection was an emerging test available only in recent years. So they participated this study or not be causing they had HPV infection or they know about HPV, that the prevalence wouldn't be confounded.

In the past, HC II gave results as positive or negative for high-risk HPV infection, but no indication of the type. Furthermore, multiple type infections were commonly observed, making it difficult to correlate type-specific infection with cervical SILs. For example, women may have HPV16 and co- infection of other high risk type(s), such as HPV18, 31, 33, 52, or 58. We used a combination of major high risk types to evaluate the impact of prevention of cervical abnormality or SILs. The attributable or etiological fraction (AR%) in cases with LSIL+ for HPV infection was about 97%, similar to those for the HR-17, HR13, major HR-6, major HR-4, and vaccine types groups of 98-99%, the corresponding values for cytological abnormality being 92% for HPV infection and 95-97% for the type groups. Considering the burden or population attributable fraction (PAR%), if we remove infection by HPV16/18, HPV16/18/52/58, or HPV16/18/52/58/31/33, the reduced HPV-related PAR% for the LISL+ subjects was 76%, 87%, or 88%, respectively. For the cervical abnormality group, the corresponding

values were 51%, 70%, or 72%, respectively. Except HPV16/18, the impacts in population were similar with using HPV-positive or HR17-positive. A more important concern, the major HR-4 or HR-6 prevalence (6~7%) in normal were much smaller than HR-13 (11%), HR-17 (12%) and any HPV (22%). Around half of HPV positive women, even more than two third women were not at high-risk to developing cancer but acquired unnecessary anxiety. That's the significance of HPV genotyping used as a screening tool in population to differentiate the major high-risk group. Limited by a cross-sectional study, we used confirmation of cervical cancer (<1 year after enrollment) as outcome. One of the 56 confirmed cases was HPV-negative and another Pap-negative, showing equal sensitivity of the methods (0.98) and a lower specificity for HPV testing (0.96 vs. 0.76). However, this study was not designed to compare HPV testing and Pap as the primary tool for screening of cervical abnormality/neoplasia in a randomized control trial.

Some studies have reported that HPV prevalence shows a trend to a decline with age and to show two peaks, either J- or U-shaped (Chan, Mak et al. 2002; Herrero, Castle et al. 2005; Franceschi, Herrero et al. 2006; Lin, Ma et al. 2006), but this was not observed in our population, in which a steadily increasing trend was seen from 22% to 27% going from the younger group (aged 30 to 34) to the older group (aged over 55). No first peak was observed in the younger group, which may be because women aged

under 30 were not included. In this population, most women were currently married (>92%), only 1.4% were remarried, and most women were monogamous (> 96%). The stable sexual relationship during marriage may result in the flat age-trend. An increase in the age-specific positive rate for HPV was observed in older women, which might be due to infection with HPV52 or HPV58 and other high-risk types (Figure III-4). Possible reasons are that HPV52/58 may be more persistent, a cohort effect, or activated latent infection. After controlling for major cytological abnormality, cigarette smoking, increased number of lifetime sexual partners, post-menopausal age, and IUD use increased the risk associated with HPV infection. Undoubtedly, current HPV infection (prevalence) is a pool of clearance and acquisition in a dynamic balance, which virus goes and comes in. The results for the number of sexual partners directly demonstrate that cervical HPV infection is sexual transmitted.

The use of archive specimens collected in 1991 may be questioned. We performed a pilot study and compared our present HPV results with previous results from 1993 (Liaw, Hsing et al. 1995; Liaw, Hsing et al. 1997)and found them to be highly consistent, demonstrating the feasibility of using these specimen collected in 1991-2. One limitation was that the results may not be representative of the population in now. Though the HPV infection profile was applied to women aged 30 to 65 in 1991, it represents HPV infection in women with a stable sex life and a less promiscuous

population. On the other hand, these women were at risk age of cervical neoplasia developing.

In conclusion, infection with HPV 16/58/52/18 or 16/58/52/31/33/18 (major high risk types) is strongly associated with cervical neoplasia in Taiwan. The preventable fraction is ~50% for HPV16/18, but ~70% for major types (HR-4 or HR-6), and an apparent difference was also seen using commutative prevalence. The results indicate that the current bivalent and tetravalent HPV vaccines may be less effective in reducing the incidence of cervical neoplasia in Asia or China than in Western populations. Additional studies on cost-effectiveness of HPV primary screening or programmed HPV vaccination of the population are needed.

Acknowledgements

The authors are grateful to Drs Mark Shciffman, Ann Hsiang Wu, KL Liaw and colleagues for the first stage in subject recruitment. We are also grateful to our research assistants for the genotyping work and for kind help from colleagues at the Yuan-Shan Institute of King-Car Co. The work was supported by the Bureau of Health Promotion, Department of Health (Grant No. 93-028) and the National Science Council (Grant No. NSC 94-2314-B-001-011).

Chapter IV: Wide-spectrum HPV type-specific acquisition & persistence/clearance in a large-scale community cohort with follow-up

Introduction

Human papillomavirus (HPV) infection is the necessary cause of cervical neoplasia and cancer (Bosch, Manos et al. 1995; Walboomers, Jacobs et al. 1999). The understanding of HPV prevalence is a way to estimate the burden of HPV related disease. However, the prevalence only provides us with a one-shot estimate at a certain time-frame, which is the consequence of the persistence of previous infections and the dynamic status of newly-acquired infections. Logically, preventing acquisition of the virus is the best way to protect against possible cervical neoplasia or preventing current virus infection becoming persistence as well. Furthermore, the sustained HPV persistence would increase the possibility to develop cervical neoplasia. In nested-case control studies based on cytologically normal American women at enrollment, women having HPV DNA in their cervical cells collected at both enrollment and diagnosis had an increased risk of atypical squamous cells of undeterminated significance (ASCUS) and low-grade squamous intraepithelial lesions (SIL) compared with those who were HPV DNA-negative in their cervical cells (Liaw, Glass et al. 1999; Wallin, Wiklund et al. 1999). In a follow-up study of longer than five years, an increase in OR of 11-12

folds was reported for women who had type-specific persistence of HPV 16 or 18 at two visits with a time interval of four months compared with those who were negative on both tests (Schlecht, Kulaga et al. 2001).

Most studies with repeated HPV testing have been conducted with young women or college/university girls, and the persistence or acquisition rates were estimated almost exclusively for HPV 16 or 18. The persistence rate or clearance rate were used to describe the HPV whether the HPV was detectable or not at repeated measurements. In a prospective study of female college students, approximately 70% of women had no detectable HPV DNA within 12 months after new HPV infection. After 18 months, over 80% of women had cleared their infections (Ho, Bierman et al. 1998). Most women infected with a specific HPV type did not sustain the same HPV type 6-12 months after infection (Hildesheim, Schiffman et al. 1994; Hinchliffe, van Velzen et al. 1995; Franco, Villa et al. 1999; Cuschieri, Cubie et al. 2005). Other cohort studies have reported a median duration of HPV DNA detectability of approximately one year (Hildesheim, Schiffman et al. 1994; Evander, Edlund et al. 1995; Franco, Villa et al. 1999; Woodman, Collins et al. 2001). Women aged over 30 years had higher persistence rate than aged less than 24 years (65% vs. 32%) after follow-up of 12 months (Hildesheim, Schiffman et al. 1994).

HPV 16 has been reported to have a longer clearance time than other HPV types

(Liaw, Hildesheim et al. 2001; Richardson, Kelsall et al. 2003). Type-specific persistence of HPV has been shown to increase with age and such findings support the utility of HPV screening in older women (Castle, Schiffman et al. 2005).

Repeated HPV testing in a prospective cohort was required to observe the dynamic status of HPV infection. The type-specific rate for other high-risk HPV was less evaluated because of the large sample size and repeated testing was required. In this study, 6877 women from community-based study with repeated HPV testing were enrolled to study HPV type-specific persistence/clearance and acquisition, and related determinants were explored.

Material and methods

Recruitment and study design of a Taiwan CBCSP cohort

The study cohort members were enrolled in the Community-Based Cancer Screening Program in 1990. There were 41,380 women aged from 30 to 65 invited. A total of 11,923 participating women were enrolled in 1991-1992 at baseline examination. At baseline, 10,602 women with completed Pap smears, HPV testing and questionnaires were eligible for the HPV cohort, after exclusion of lack of specimens, hysterectomy and cervical cancer diagnosed before enrollment. Informed consent was signed after a detailed explanation by well-trained public health nurses. The detailed information is

described in Chapter III.

Selection of subjects

Women participating in both baseline and follow-up (F/U) examinations were included in this study for repeated HPV measurements. Among the 10,602 HPV cohort members, 10,532 subjects had intact HPV testing at baseline and 6877 (65.3%) of them participating in the F/U examination were included for analysis (Figure VI-1).

Repeated measurements at follow-up examination

At the F/U examination, all cohort members were invited again from 1993-1995. Most procedures followed the same protocol at baseline examination. The socio-demographic characteristic, cigarette smoking, Pap smear history, reproductive and sexual history, and personal and family history of cancers were collected again using a structured questionnaire. Additional questions regarding recent exposure (in the past year) of sexual behavior, such as number of sexual partners, intra-uterus device (IUD) use, oral contraceptives (OC) use, douching after intercourse and treatment of diseases or cancers were collected. A Cervex-Brush (Rovers, the Netherlands) was used to obtain exfoliated cervical cells for the Pap smear. ViraPap (Digene Diagnostic, Silver Spring, MD) kits were used for collecting and preserving cervical cells. The cells were stored at -80°C until HPV DNA testing. Pap smears were read according to the Bethesda system, which categorizes cytology grades into normal, atypical squamous

cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL) and cancer. The cytology abnormal group combined all the results with ASCUS or worse.

HPV DNA and genotyping

The DNA preparation was done using Qiamp 96 DNA blood kits (Qiagen Inc., Venlo, Netherlands). Polymerase chain reaction (PCR), using MY11/biotinylated GP6+primers was performed to target the L1 gene and an HPV blot (Easychip® HPV Genotyping Array, KingCar, Taiwan) was used to detect thirtynine types of HPV (6, 11, 16, 18, 26, 31, 32, 33, 35, 37, 39, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 72, 74, 82, CP8061[71], CP8304[81], MM4[82], MM7[83], MM8[84] and L1AE5). The HPV blot was based on reverse dot hybridization on a nylon membrane for the above HPV types simultaneously. In addition to the seven embedded control dots on the blot membrane, we designed several control and check points as standard protocol to avoid contamination (Chapter III). Briefly, two controls were used in the PCR stage, including a male human genome with GAPDH forward and reverse primer designed as a PCR positive control. Sterile water control was used to monitor the contamination in both HPV and GAPDH reactions. PCR was re-performed if negative GAPDH results were obtained. Another five controls were triple-blindly used for the whole process to monitor type-specific results and reproducibility. The

quality assurance was examined. Excellent reliability and reproducibility was observed (Lin, Chen et al. 2007).

HPV type-specific acquisition, persistence, clearance

Acquisition cases were defined as a newly acquired HPV type being observed at the F/U visit for women who weren't infected with the same type at baseline. Persistence cases were defined as women being infected with the same type at both baseline and F/U visit. Likewise, clearance cases were recognized if women were infected with a certain type at baseline and that type was absent at the F/U visit. For example, women infected with HPV 16 and -52 at baseline and HPV 16, -33 positive at baseline, will be defined as acquisition of HPV 33, clearance of HPV 52 and persistence of HPV 16 simultaneously. Women infected with a certain HPV type at baseline will definitely be either a persistence case or clearance case for such HPV type. Persistence means the infection was not cleared and clearance means the infection was non-persistent, contrariwise. The overall persistence and acquisition were defined as a pool on a type-specific basis.

Statistics

All calculation and statistical analysis were executed using SAS (version 9.1.3; SAS Institute Inc). The HPV type-specific acquisition rate and persistence rate were calculated based on definition. The clearance rate was equal to 1- persistence rate. In

order to compare acquisition rate and persistence rate of the same type or between types, age-standardization was performed using the truncated (aged from 30 to 65) female world population in 2000, and the female population in Taiwan in 1992, respectively. For the sparse data, the crude rate, age-adjusted rate (age-adj. rate) and the 95% confidence intervals (95% CI) were estimated using Poisson regression. For an equitable concern in rate estimation, another analysis was performed on conservative criteria restricted to those subjects who had the duration of two visits within one to two years. Tests for risk factors associated were evaluated using logistic regression models, and the age-adjusted odds ratio (aOR) and multiple adjusted odds ratio (mOR) were also estimated.

Results

Among 6877 subjects, the mean and median follow-up duration between two visits were 1.58 and 1.40 years, respectively. Women with an abnormal cervix at baseline had either a higher risk of HPV acquisition at F/U (aOR & 95% CI: 2.8, 2.0-4.0), or a higher risk to be persistent at F/U (aOR & 95% CI: 2.2, 1.5-3.1). The acquisition rates in the normal and abnormal groups were 8.4% and 20.8%, respectively. The persistence rates in the normal and abnormal groups were 27.7% and 42.9%, respectively. The number HPV infections had an increasing dose-response with statistical significance (see table

IV-1). In table IV-2, the results are sorted in descending order of adj. rates using the world population on conservative criteria. In the normal group, the type-specific acquisition rate was lower than 1% for every type. After age-adjustment, using the world population in 2000 or Taiwan population in 1992, the age-adj. rates were similar with the crude rates. After further analysis based on the conservative criteria, the results were similar with each other, even after age-adjustment. Consistently, HPV 16 had the highest acquisition rate at 0.8%, followed by HPV 52 (0.7%) and HPV 18 (0.6%). A further stratification analysis by HPV infection status at baseline showed that HPV 16, 18, MM8, 51, 52 and 58 were commonly (>0.4%) acquired in HPV negative women, thus, HPV 55, 51, 16, 53, 43, MM7 were frequently acquired (>1.0%) among HPV positive women at baseline.

The estimation of type-specific persistence rates was confined by the sample size of infected subjects at baseline for each type. The sorting on world age-adj. persistence rates in descending order was made for two strata, for which the baseline positive number was greater or less than 30 (Table IV-3). This is arbitrarily to compare rates based on a relatively large sample size. The estimation of type-specific rates were similar to each other among the crude rate or adjustments, whether using age adjustment by two different standard populations or using conservative criteria. The age-adjusted persistence rates of the most persistent HPV types were; HPV 58 (51.6%), 52 (49.9%),

70 (47.4%), 39 (44.8%), 72 (42.1%) followed by HPV 68, 44, MM8, 53, 54, 51, and CP8304 with persistence rates greater than 30%, then, HPV 16 and 18 estimated at 17.8% and 19.4% , respectively. Although, HPV 11 had a high prevalence at baseline, the virus was mostly (99.2%) cleared after one year. Some low risk or probable risk types (HP V70, 72, 44, MM8, 54 and CP8304) had higher persistence rates than HPV 16 or 18. The age-specific acquisition rates and persistence rates for HPV 16, 18, 52, 58 were plotted and no marked trend was noticed (Figure IV-2).

There are some concerns in comparing acquisition and persistence rates of types. The calculation basis for persistence were subjects with infection at baseline, however, the age distribution of different types may be different. To compare the relative force of the persistence and acquisition rates, the type-specific ratio was calculated using age-adj. rates (Table IV-4). For the ratios indicated at a given period (during the second year after recruitment), the persistence rate was conspicuously greater than the acquisition rate, except for HPV 11.

A more conservative concern that some baseline positive cases cleared their virus and acquired new infections during the two tests would be taken for granted as persistence cases. As the pool of observed persistence might come from a proportion of acquisition cases, a further correction was made to take the portion of acquisition into account. It was presented as ‘Persistence rate $_{observed}$= Persistence rate $_{true}$+

(100-Persistence rate $_{true}$)*Acquisition rate'. The conservative persistence rates were corrected as (persistence rate-acquisition rate)/(100-acqusition rate)*100. The new persistence rates were not markedly different from the original rates.

In normal women, risk factors associated with acquisition were sexual experience related, women who were not currently married (11.7% vs. 8.2%), remarriage (14.9% vs. 8.2%), early age at initial coitus (10.0% vs. 8.0%) and more than one life sexual partner (16.5% vs.8.3%), as expected (see table IV-5). In recent experience, the number of sexual partners in the past year presented a dose-response trend with HPV acquisition. Ever use of vaginal douching had higher acquisition rate (10.5% vs. 8.0%). Often or always use of condoms had a lower rate compared with never or seldom use (6.3% vs. 8.9%), non-significant difference due to sample size. Women reporting no sexual intercourse still had 6.8% HPV acquisition. There were 16.7% of HPV positive women who acquired new infections, however, only 6.2% of HPV negative women were observed. In multiple adjustment models, women not currently married (mOR & 95% CI: 2.6, 1.7-3.9) compared with currently married, previous HPV infection with HR 17 (mOR & 95% CI: 3.6, 2.9-4.6) and other types (mOR & 95% CI: 2.3, 1.7-3.0) compared with HPV negative, and vaginal douching ever use compared with never use increased the risk of HPV acquisition. Lower risks were observed among women who initiated sexual coitus after 24 years of age (mOR & 95% CI: 0.8, 0.6-1.0) as compared to early

sexual coitus between 14-21 years of age, and no sexual intercourse in the past year (mOR & 95% CI: 0.4, 0.3-0.7) compared with never or seldom condom user, (mOR & 95% CI: 0.8, 0.6-1.0). Remarriage and the number of lifetime or recent sexual partners were highly correlated with not currently married, which did reach statistical significance in multiple regression models.

For HPV persistence, the elder women couldn't clear their HPV infection, were more likely to sustain their HPV infection with a significantly increasing trend. More vaginal delivery, OC use, IUD use, post-menopause, and later age at menopause increased the risk of virus persistence. Women who had their initial sexual experience later than 24 years of age had a lower risk to be persistent. High vaginal delivery, IUD use, and menopause significantly predict women who had a lower chance to clear her HPV infection after multivariate adjustment.

Discussion

The repeated HPV testing of two visits was completed in this study using wide-spectrum HPV blot. We confirmed some previous results and made some revisions about HPV acquisition and persistence in women aged over 30. The characteristics and limitations of this large sample size cohort (>6600) are that there were only two visits, and the second testing was performed over one year after the first testing. According to

previous reports, a median duration of HPV DNA detectability of approximately one year was reported and 70% and 80% of infections were cleared at 12 and 18 months (Ho, Bierman et al. 1998). In this study, 76.6% (5110/6667) of women had their second HPV testing after one year and before two years, during which time the rapid clearance period of the infection had been passed through to observe a relatively stable persistent infection. Age adjustment using the world women population and Taiwan population of the year of recruitment, were similar to each other for the crude rate, whatever the acquisition rate or the persistence rate. The conservative estimation of persistence rate was also corrected by the possible acquisition.

Based on the current design, the definition of persistence ruled-in a population who had a previous infection at baseline and the infection was sustained at F/U. But who had the virus infection not long before the first visit and cleared the virus so fast to become undetectable at the F/U visit would be treated as clearance. This is the reason to explain why elder women or post-menopause women were less likely to clear their virus, which they may have been infected with for a long time and once the infection wasn't cleared in the first one to two years, they became long-term persistent. In addition, according to the study diagram, the acquisition rate was only inferred to those newly-acquired HPV infections which were not cleared yet over one year later. Those HPV acquisition and rapid clearance within one year wouldn't be taken into account, so that the overall

acquisition rate may be underestimated. However, the underestimation may not influence the persistence rate since the apparently different (P/A ratio) and the quickly transient infections wouldn't contribute to persistence.

Consistent with previous reports, HPV 16 was the most common newly-acquired type in our population. Although the HPV 16 acquisition rate (0.8%) was much lower than those young women in the UK, Korea, USA and Canada, it's similar with women of comparable age in Costa Rica. However, acquisition of HPV 52 and 58 were followed HPV16 in our population, which reasonably explained that there were more infectious reservoirs (high prevalence) in our population rather than in western populations. The inverse order of common incident types was also applied for HPV18, as well as 31 and 33. A decreased trend was observed in Costa Rica and Colombia (Munoz et al., 2004; Castle et al., 2005), but not observed in our study, which may be the result of young women not being included in our subjects. In women with cytologically abnormal or with previous HPV infections had a higher risk to gain new infections, which may be explained as those women were high-risk group (high exposure probability) for HPV infection or had high-risk sexual partner (high sexual promiscuity). The sexual behavior related factor was highly correlated with HPV acquisition. The number or sexual partners apparently predicted the risk of HPV acquisition. Some highly correlated factors including, not currently married, remarriage,

early age at initial coitus, more than one sexual partner in her life or recently, vaginal douching may result from sexual promiscuity of her or her sexual partners. The increased risk of vaginal douching may be due to those unmeasured residuals of high risk behavior for HPV infection. One mouse model proposed that use of nonoxynol-9 as spermicide may increase the susceptibility of infection by HPV pseudovirus challenge due to chemical disruption of epithelium (Roberts, Buck et al. 2007). Chemical ingredients in douching fluid may facilitate HPV infection establishment.

In previous reports, the outcome of HPV infection, whether detectable or not at follow-up was reported as ‘persistence’ or ‘clearance’ and varied in different follow-up durations and calculations basis using percentages or women-month . The persistence rate increased with age and abnormal cytology was confirmed in our study by some previous reports (Castle, Schiffman et al. 2005; Bulkmans, Berkhof et al. 2007). Multiple type infection was also at an increased risk of persistence.

Inconsistent type-specific persistence or clearance rates were observed. There were 72% of young women in the USA who cleared their HPV 16 infection with a mean duration of 2.2 years (Ho, Bierman et al. 1998), however, 62% of young women in Canada sustained their HPV 16 infection after one year. Among normal cytology women, the persistence of aged >=30 was higher (65%) than <=24 years (32%) after a median follow-up period of 14.9 months (Hildesheim, Schiffman et al. 1994). In studies

with a wide range of age, HPV persistence rates have ranged from 25 ~ 51% (Castle, Schiffman et al. 2005; Cuschieri, Cubie et al. 2005; Bulkmans, Berkhof et al. 2007). The wide-range estimation from different populations may be because of sample size, the proportion of age distribution, the percentage of abnormal women and different follow-up durations of tests. The HPV 16 persistence rate after age-adjustment was about 20% in our study. The above factors may result in the difference, but we could not exclude the virus factors, such as viral load and variants, which may contribute to the variation. The European variants of HPV 16 and HPV 18 appear to persist longer in white women, and African variants persist longer in African American women (Xi, Kiviat et al. 2006).

The variations in HPV type-specific persistence rates other than HPV 16 were observed across studies. In young women studies, higher percentages of remaining infection were observed for HPV 18 (35%), 58 (33%), 16 (27%), 52 (15%), 33 (14%), 45 (11%), 31 (9%) and HPV 35 (0%) (Ho et al., 1998), however, HPV 16, 31, 53 (62%) had a higher rate than HPV 18 (40%) in Canada (Richardson, Kelsall et al. 2003). Based on a time-to-event calculation in a Ludwig-McGill cohort in Brazil, women with HPV 16 infection had a similar clearance rate (82.4 per 1,000 women-month) with infection of HPV 52 (81.0), 31 (79.8), 33 (78.9) and 58 (72.1), but a higher rate (91.9) of HPV 18 infection. In other words, the persistence rates were HPV 58> HPV 33> HPV 31> HPV

52> HPV 16> HPV 18 (Trottier, Mahmud et al. 2008). In the PROBASCAM study in the Netherlands, the descending order and percentage of persistence for HPV 16, 18, 31, 33, 52 and 58 were HPV 16 (51%)> HPV 31, 33 (50%) > HPV 18 (45%) > HPV 52 (33%) > HPV 58 (22%) (Bulkmans, Berkhof et al. 2007).

In our study, as with other reports, HPV 11 infection was rapidly cleared (Trottier, Mahmud et al. 2008). The age-adjustment type-specific rate after correction were HPV 58 (51.5%) and 52 (49.5%), which were the most persistence types in our population. The rates for HPV 16 and 18 were 17.1% and 18.9%, respectively. Interesting variations have been observed in different populations. HPV 52 and 58 are more likely to be persistent or not cleared in Brazilian women and Asian women. HPV 16 is most persistent in Netherlander and Canadian women. Banished from the age distribution, viral load level and HPV intratypic variants may be a possible reason. Probably, host genetic susceptibility may play a role in recognition of different types of virus and clearance of the infected cells. These viral and host factors might be the whys and wherefores of low clearance rate which was responsible that HPV 52 or 58 are more prevalent in Asia or South America than in Europe (Chapter III; (Clifford, Gallus et al. 2005)).

Age-adjustment rates and conservative criteria were used in our study to compare type-specific rates intra-population on a relatively equitable basis, which is more

important for an inter-population comparison. Furthermore, overcoming a common limitation of two test studies, without the information of the time when the infection happened, we presented a relatively stable estimation of long-term persistence rate and acquisition rate. We explored personal life experience as determinants to HPV acquisition and persistence. HPV 52 and HPV 58 being prevalent in Asian women could be explained by the high acquisition rate and low clearance rate.

In conclusion, we represented the important type-specific acquisition and persistence in Asian women, which recognized that there is a higher acquisition rate and persistence rate of HPV 52 and 58 than in western countries. The results support the high prevalence and burden in Asia. In the future, the risk of cervical neoplasia in relation to HPV 52 or 58 should be examined in a longitudinal study to clarify the role of cervical neoplasia. Moreover, HPV vaccine against HPV 52 or 58 will be anticipated for HPV 52 or 58 epidemic regions.


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Chapter V: Long-term risks and impacts of cervical cancer associated HPV infection and persistence

Introduction

Human papillomavirus (HPV) infection is considered as a necessary cause of cervical cancer (Bosch, Manos et al. 1995; Walboomers, Jacobs et al. 1999). The high risk HPV type for cervical carcinogenesis was documented by pooling 1918 cervical cancer cases worldwide (Munoz, Bosch et al. 2003).

Epidemiological studies based on case-control design have provided an approximation of relative risk to differentiate the relative importance. The drawback of temporality has been remedied by nested-case control design. There have only been a few important studies on HPV infection and the subsequent development of cervical neoplasia. The relative index facilitates judging the priority among risk factors in comparison to their references. If we could obtain the absolute risk/incidence which reflects substantial demand or burden, both etiological fraction and population attributable fraction were helpful.

Using a Hybrid Capture II, infection of HPV high risk type was a strong predictor for cervical abnormality or cervical intraepithelial neoplasia based on long-term follow-up (Castle, Wacholder et al. 2002; Kjaer, van den Brule et al. 2002; Kjaer, Hogdall et al. 2006). Based on nested-case control design, HPV 16 and HPV 18 were

investigated for their importance in cervical neoplasia (Liaw, Glass et al. 1999; Wallin, Wiklund et al. 1999; Ylitalo, Sorensen et al. 2000; Peto, Gilham et al. 2004). The significant difference of cumulative incidences between being HPV positive and negative has been reported after 6 years and 12 years of follow-up (Sherman, Lorincz et al. 2003; Huang, You et al. 2008). Nevertheless, the variable risk among different high risk types was observed in two women cohort (age under 40) studies for 3-4 years of follow-up, with HPV 16 being the most oncogenic type (Woodman, Collins et al. 2001; Naucler, Ryd et al. 2007). The ALTs study reported that genotype-specific HPV testing is helpful to identify high or low risk women with ASCUS to develop more severe lesions(Wheeler, Hunt et al. 2006).

Among young women who were repeatedly positive for HC II, there was an increased risk associated with SIL during a 4-year follow-up and 20% of them developed CIN2+ with a longer follow-up to 10.6 years (Kjaer, van den Brule et al. 2002; Kjaer, Hogdall et al. 2006). In a nested-case control study, detectable HPV DNA both at baseline and at diagnosed specimen reported a risk of 213.4 (18.1-16000) compared with repeated negatives. HPV 16 repeated positive at least two examinations before CIS was diagnosed had a risk of 31.2 (10.6-91.8) (Ylitalo, Sorensen et al. 2000). Type-specific persistence of HPV 16 or 18 had an 11-fold risk to develop any SIL during 5 years and the cumulative incidence was 40%, only 5% for negative and 26%

for HPV 16 or 18 positive at baseline, which points out the important role of HPV persistence in cervical carcinogenesis (Schlecht, Kulaga et al. 2001).

In Taiwan, a CBCSP female cohort was conducted from 1991, with a follow-up of more than 15 years. Through the comprehensive health information system including nationwide registry system of the National Cancer Registry and National Death Certification Registry, histologically confirmed cases and mortality cases were ascertained almost entirely.

Material and methods

Recruitment and study design of a Taiwan CBCSP cohort

The study cohort members were enrolled in a Community-Based Cancer Screening Program in 1990. There were 41,380 women aged from 30 to 65 invited. A total of 11,923 participating women were enrolled in 1991-1992 at baseline examination and 6,923 women received follow-up examination in 1993-1995. Only women with completed Pap smears, HPV testing and questionnaire were eligible for the HPV cohort. As a result, the total number of subjects was 10,602. Informed consent was signed after a detailed explanation by well-trained public health nurses. The detailed information is described in Chapter III & IV.

Repeated measurements at baseline and F/U visits

Both at baseline and follow- up (F/U) examinations, all cohort members were invited to participate in a health examination. Most procedures followed the same protocol at both visits. The socio-demographic characteristics, cigarette smoking, Pap smear history, reproductive and sexual history, and personal and family history of cancers were collected again using a structured questionnaire. Additional questions regarding sexual behavior, number of sexual partners, intra-uterus device (IUD) use, oral contraceptives (OC) use douching after intercourse and treatment of diseases or cancers were collected at follow-up for the recent exposure during the two visits. A Cervex-Brush (Rovers, the Netherlands) was used to obtain exfoliated cervical cells for the Pap smear. ViraPap (Digene Diagnostic, Silver Spring, MD) kits were used for collecting and preserving cervical cells. The cells were stored at -80°C until HPV DNA testing. Pap smears were read according to the Bethesda system, which categorizes cytology grades into normal, atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL) and cancer. The abnormal cytology group combined all results with ASCUS or worse.

Inclusion criteria of subjects in long-term follow-up

Those women with normal cytology at baseline and intact HPV testing were

included for analysis. A total of 10,123 subjects were recruited.

HPV DNA and genotyping

The DNA preparation was done using Qiamp 96 DNA blood kits (Qiagen Inc., Venlo, Netherlands). Polymerase chain reaction (PCR), using MY11/biotinylated GP6+primer was performed and an HPV blot (Easychip® HPV Genotyping Array, KingCar, Taiwan) was used to detect thirty-nine types of HPV (6, 11, 16, 18, 26, 31, 32, 33, 35, 37, 39, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 72, 74, 82, CP8061[71], CP8304[81], L1AE5, MM4[82], MM7[83] and MM8[84]). A male human genome with GAPDH forward and reverse primer was designed as a PCR positive control. Sterile water control was used to monitor the contamination in both HPV and GAPDH reactions. PCR was re-performed if negative GAPDH results were obtained. Another 5 controls were triple-blindly used for the whole process to monitor type-specific results and reproducibility. Excellent reliability and reproducibility was observed (Lin CY, 2006).

High-risk groups of HPV infection

According the epidemiologic classification, fifteen HPV types were classified as high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82); three were classified as probable high-risk types (26, 53, and 66); and 12 were classified as

low-risk types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108)(Munoz 2003).

HR-17 (17 high-risk type) infection: Among the 39 types detected by HPV Blot, there were 14 high-risk types, including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 82, and three probable high-risk types, which were HPV 26, 53, and 66. The low-risk type infection was defined with another 22 low- or undetermined-risk types.

HR-13 (13 high-risk type) infection: Another group of 13 HPV types were 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 which Hybrid Capture II detected (HC II, Digene Corp, Gaithersburg, MD , USA)

Major HR-6 (Major 6 high-risk types, HPV 16/18/52/58/31/33) infection: Defined as being positive for any of these HPV types: 16, 18, 31, 33, 52, and 58.

Major HR-4 (Major 4 high-risk types, HPV 16/18/52/58) infection: Defined as being positive for any of these HPV types: 16, 18, 52, and 58.

Vaccine types (HPV 16/18) infection: Defined as being positive for either of these HPV types: HPV 16 or HPV 18.

HPV type-specific persistence, clearance and all clearance

Considering HPV infection at F/U visit, persistence was defined as women sustaining the same HPV infection at both visits. Clearance was recognized if the women had cleared the HPV infection before the F/U visit. All clearance was defined if all HPV infections at baseline had been cleared and become negative at F/U.

Acquisition was defined as those who were negative at baseline but had acquired HPV infection at F/U visit (Chapter IV).

Follow-up of cervical neoplasia

Through data linkage with computerized registries of the National Cancer Registry and National Death Certification Registry, cohort members affected with newly-diagnosed cancers of the cervix uteri up until December 31, 2006 were identified. The criteria of cervical cancer from computerized database were linked as diagnosis with ICD-code of 180 and ICD-O-code of C-53. All the diagnoses of cancers were based on histological basis, furthermore, carcinoma in situ (CIS) and invasive cervical cancer (ICC) were stratified according to histological diagnoses.

Statistics

Risk factors and HPV infection associated with the incident of cervical cancer during follow-up was estimated using the Cox proportional hazard model, as well as age and multiple adjustments. A proportionality test was performed as well. The incidence of cervical cancer was estimated on genotype-specific and groups of risk types for HPV infection and HPV persistence. The attributable risk fraction (AR%) and population attributable risk fraction (PAR%) were calculated as (mHR-1)/mHR x 100% and {[prevalence x (mHR-1)]/[prevalence x (mHR-1)]+1} x 100% (Miettinen 1974). The

Nelson-Aalen estimator of cumulative hazard was calculated and plotted for the cumulative incidence of cervical cancer by groups of HPV infection or persistence. The log rank test using the Kaplan-Meier method was performed to test the median of time to event for exposure groups. All computation and statistic were performed using SAS 9.1.3.

Results

There were 10,123 normal women enrolled for baseline HPV exposure with a mean age of 46.3 years, the mean follow-up duration was 14.5 years. One subject who registered as cervical cancer before the F/U examination was excluded for HPV persistence analysis (Figure V-1). A total of 69 incident cervical cancer (CXC) cases, including 35 ICC and 34 CIS, were diagnosed before December 31st, 2006. In univariate analysis, baseline characteristics including age group (55+), number of vaginal deliveries (4+), post-menopause and HPV infection were associated with cervical cancer (Table V-1). However, only age, number of vaginal deliveries and HPV infection were independently associated with cervical cancer in a multiple adjusted Cox regression model, therefore, for the estimation of risk of cervical cancer for any type of HPV infection, both age and high vaginal delivery were adjusted. There were 54 CXC cases (78.3%) with HPV infection at baseline, the proportion of HR-17, LR, HR-13,

major HR-6, major HR-4 and vaccine type infections were 67%, 36%, 62%, 56%, 49% and 30%, respectively (Table V-2). The proportions among ICC were generally higher than among CIS. Multiple type infections were common (52%). The most common type among CXC were, in order, HPV 16 (18.8%), HPV 52 (15.9%), HPV 58 (13.0%) and HPV 18 (11.6%). There were no cases with infection of HPV 6, 26, 37, 55, 59, 62, 66, 74 or MM7. The genotype-specific incidence was listed in order of high or low risk groups and case numbers. Compared to HPV negative, the mHR were 40.3, 27.0, 49.2, 20.9, 49.7, 13.0, 14.6 and 17.9 for HPV 16, 52, 58, 18, 31, 56, 33, 53, and the corresponding mHR were 174.1, 58.9, 104.0, 63.8, 136.6, 214.4, 172.3 and 59.7 for persistent infection. The risk of cervical cancer after HPV clearance was not observed in most types. Taking the relationship with ICC into consideration, a strong prediction was observed for high risk HPV infection and persistence. However, lower risks were observed in relation with CIS, and only a few cases had persistent infection of some high risk types (HPV 52, 31, 56, 33 and 51) and low risk types (CP8304, 70, 54, 43, 69 and 72). The attributable risk percentage for each type was higher than 90%, except for HPV 11. The highest population attributable percentage was 50% for HPV 16, with HPV 58 (43%), HPV 18 (39%), HPV 52 (36%) and HPV 31 (29%) following.

In relation to HPV infection at baseline, the cervical cancer incidence was 170.8 per 100,000 person-year, and the mHR was 12.8 compared to HPV negative (Table V-4).

The PAR% was 72%, 74% and 70% for CXC, ICC and CIS, respectively. Women who had infection of HR-17 type had increased risks of 19.6 (CXC), 23.3 (ICC) and 16.1 (CIS), however, the risks were much lower with low risk type infection alone (mHR=4.2, 1.8-10.0). According to the groups of high-risk types, the PAR% of cervical cancer were 51%, 63%, 66%, 68% and 69% for vaccine types, major HR-4, major HR-6, HR-13 and HR-17 infection, respectively, corresponding to ICC, which were 63%, 71%, 72%, 73% and 73%, respectively. A lower PAR% was observed in correspondence to CIS. The increasing trend of cervical cancer risk was associated with the number of infected HPV types, and the number of infected HR-HPV types as well. The higher risks of multiple types to developing cervical cancer were 2.7 (1.6-4.7) and 1.7 (0.9-3.2) for HPV and HR-HPV infection compared to a single type infection. Women who had persistent HPV infection (type-specific basis) at F/U had elevated risks (mHR=44.4, 19.0-102.9) of cervical cancer compared to persistently negative women. There was no significant risk observed with all clearance at F/U.

The cumulative incidence of cervical cancer after a follow-up of 15 years was 2.8% for HPV infection and 7.7% for HPV persistence (Figure V-2A-1 & V-2A-2). For HR-17 infection and persistence, the cumulative incidences were 4.4% and 10.0%, which indicated that 10% of women who had HR-17 HPV persistence might proceed to cervical cancer within 15 years. The corresponding percentage for HPV persistence of

major HR-6, major HR-4 and vaccine types were 13.1%, 10.9% and 20.1%, respectively (Figure V-2).

Discussion

With complete HPV testing at baseline and F/U visits of 10,123 women with normal cytology, this is the first study to report the risk of cervical cancer as predicted by baseline HPV infection and HPV persistence on a type-specific basis at F/U. We confirmed the important role of HPV infection in relation to subsequent cervical cancer in Taiwanese women. Compared with our previous cross-sectional study reporting the association between cervical cancer and HPV infection at baseline, we confirmed that the major high risk types were HPV 16, 52, 58, and 18 (Chapter III). The HPV positive rate of cancer cases in a cross-sectional study was 98.2%, which is comparable with 96.6% in a large-scale case series of ICC in Taiwan (Lai, Huang et al. 2007), however, only 78.3% of CXC and 80.0% of ICC were HPV positive in this longitudinal study. The lower positive rate in a longitudinal study than in cross-sectional could be due to new infections during the long-term follow-up. HPV 16, 52, and 58 were also reported as leading types among HSIL in a large-scale CIN study (Chen, Liu et al. 2006). Since the high proportion of multiple infections (51.9%) and spare number of each type with single infection, it is helpful to use major HR-4 types as an indicator, which was 49.3%

in the longitudinal study and 85.5% in the cross-sectional study. The attributable proportion in the population was 63%, which is higher than 51% for HPV 16 and 18 (vaccine types). The importance of HPV 16, 58, 52 and 18 was consistent with the distribution in Asia (Clifford, Smith et al. 2003; Smith, Lindsay et al. 2007). In our longitudinal analysis, HPV 18 was detected among 11.6% of cervical cancer cases but was only detected among 3.6% of cases in cross-sectional analysis, which was lower than 14.5% or 15.3% or 17.8% from cross-sectional studies on invasive cancer cases using biopsy tissues (Clifford, Smith et al. 2003; Lai, Huang et al. 2007; Bao, Li et al. 2008). In the meta-analysis of worldwide cervical cancer cases, HPV 18 was more prevalent in adeno/adenosquamous carcinoma. The use of exfoliated cervical cells may collect fewer cells of endocervix than biopsy, where the adenocarcinoma occurred.

Interestingly, there were a few cases with infection of low risk type, which were found not only in the longitudinal study but also in the cross-sectional study, such as HPV 54, 70, CP8304 [81], MM4 [82], MM8 [84] ((Lai, Huang et al. 2007); Chapter III). HPV 70 is a high-risk type according to phylogenic classification, but was classified as low-risk based on epidemiological data (Van Ranst, Kaplan et al. 1992; Munoz, Bosch et al. 2003). However, in both cross-sectional studies, HPV 70 single infection was detected among cervical cancer cases. This needs to be further examined by collecting more cases.

HPV 16, 18 and 58 seem to be more important for incident ICC and HPV 52 seems to be more correlated with incident CIS. HPV 52 had a longer median of duration than HPV 16, 18 and 58 to develop HSIL (Woodman, Collins et al. 2001), which may result in a longer incubation for ICC occurrence. However, it was confined by the sparse number of cases leading to a wide-range of confidence intervals to draw any conclusion. The follow-up time was perhaps not long enough for normal women to develop cancer.

Multiple type infection had an increased risk with cervical cancer when our cross-section study was observed, and a dose-response trend was found consistently in the longitudinal study. An increasing number of HPV infected types with an increasing risk of cytological HSIL was reported both at cross-sectional and prospective analysis among Brazilian women (Trottier, Mahmud et al. 2006). Including normal and abnormal women together, the risk was around 2.5 and 4.5 for 2-3 types and 4-8 types in comparison with single infection, respectively. In our study, women infected with more than one type had 2.7-fold risk and no synergistic effect was observed, which may be explained because only normal women were enrolled in our study and histologically confirmed cancer cases were follow-up. We previously reported that more types of infection are more likely to be HPV persistence, and that this is the pivotal role of cervical carcinogenesis (Chapter IV).

Our longitudinal study reported a dose-response relationship for high frequency of

vaginal delivery with cervical cancer (p=0.004). The mHR was 1.9 (1.1-3.4) for the 4+ group compared to the 0-3 group after adjustments for age and HPV infection. There were 6.2-fold and 3.5-fold risks reported for high frequency (>7) and medium frequency (3-6) compared to low frequency (0-2) (Hsieh, You et al. 1999). Despite this, an independent effect to cervical cancer was observed. Trauma of the cervix caused during vaginal delivery has been proposed as a risk factor in cervical carcinogenesis (Richart 1967), however, the mechanism for neoplasia is still unclear. From our previous reports, high vaginal delivery was not correlated with HPV infection at baseline and acquisition, but increased the risk of HPV persistence (Chapter III & IV). It is plausible that a traumatic cervix may facilitate the preexisting virus to expand loci of infection at the basal layer and become persistent.

We confirmed the imperative role of HPV persistence in cervical cancer development. The apparent difference between HPV infection at baseline and type-specific persistence indicates a casual relationship. Among most types, no cases happened once the virus had been cleared at F/U. Only three cases from 867 women who had viral clearances at F/U developed subsequent cancer. Moreover, we can't exclude possible new infections and clearance during follow-up, which is constrained by only two tests in the longitudinal study. Viral clearance is another way to protect women suffering from cervical cancer for resulting from HPV infection.

Although the acquisition rate was low from our previous report (Chapter IV), the under-estimation of HPV related risk and over-estimation of HPV persistence related risk might be possible. The left truncation of exposure time (without exact time of infection) may result in an under-estimation of relative risk. Another limitation for the longitudinal study was outcome detection bias among exposure and non-exposure groups. With the comprehensive National Cancer Registry and National Death Certification Registry, it's not a concern in our study.

In this substantial study of HPV infection and persistence in relation to the development of cervical cancer, we conclude that the major important types are HPV 16, 18, 52 and 58 in Taiwan. HPV persistence could be a pivotal role or necessary cause for cervical cancer. Clearing the virus may be useful to prevent development of cervical cancer in HPV infected women. This should be further examined with suitable designs.


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Chapter VI: Viral load and integration associated HPV persistence and cervical cancer among normal women in a long-term prospective study

Introduction

HPV infection has been strongly suggested to be a causative agent of cervical cancer, but it is important to note that HPV infection alone is probably insufficient to induce cancer. HPV persistence is considered to play a pivotal role (Chapter V).

High-viral load has been considered as a potential risk factor for cervical neoplasia in cross-sectional case-control studies (Morrison, Ho et al. 1991; Ho, Bierman et al. 1998; Sun, Liu et al. 2002; Tsai, Wu et al. 2005; Flores, Papenfuss et al. 2006; Ho, Cheng et al. 2006). However, HPV viral load could not sufficiently predict the risk of subsequent CIN (Ylitalo, Sorensen et al. 2000; Castle, Wacholder et al. 2002; Dalstein, Riethmuller et al. 2003; Monnier-Benoit, Dalstein et al. 2006). In longitudinal studies, the incident cervical lesion or cervical cancer was associated with previous high viral load (van Duin, Snijders et al. 2000; Gravitt, Kovacic et al. 2007), and a dose-response relationship was observed (Josefsson, Magnusson et al. 2000; Schlecht, Trevisan et al. 2003; Castle, Schiffman et al. 2005; Moberg, Gustavsson et al. 2005), but other studies have found that viral load could not predict viral clearance and CIN3+ (Lorincz, Castle et al. 2002; Molano, Van den Brule et al. 2003).

The inconsistency might be due to small sample size or the accuracy of the test method (HC II) to quantify viral load, and that only one random sample of cervical cells was collected. Repeated samples at multiple points over time is a more accurate quantitative method.

Cervical cancer cells often contain chromosomally integrated HPV DNA or a mixture of both integrated and episomal forms (Durst, Kleinheinz et al. 1985; Schwarz, Freese et al. 1985; Yee, Krishnan-Hewlett et al. 1985; Cullen, Reid et al. 1991; Kulmala, Syrjanen et al. 2006). In contrast, HPV DNA is present generally as an episomal form without integration into the host genome in cervical warts. These findings suggest that integration of HPV DNA into the host genome or other events that disturb the organization and/or expression of HPV DNA such as polymerization or mutation are determinant steps in HPV-induced carcinogenesis. Chromosomal integration of the HPV genome commonly results in the disruption of viral E2 ORF and, as a consequence, the loss of E2 protein (Baker, Phelps et al. 1987; Corden, Sant-Cassia et al. 1999; Gallo, Bibbo et al. 2003). In addition, the disruption of E2 ORF has been shown to increase the ability of HPV16 to immortalize cells (Romanczuk and Howley 1992) These observations suggest that the loss or inactivation of E2 protein might play some role in cervical carcinogenesis (Schneider-Maunoury, Croissant et al. 1987). The integration of HPV DNA into the host genome is associated with the progression of CIN from

polyclonal to monoclonal status, and these events play a fundamental role in the progression from low-grade to high-grade cervical neoplasia (Ueda, Enomoto et al. 2003). The difference of viral load between E2 and E6 was applied for detecting HPV integration due to the loss of E2 while genome integration. A purely integrated form was defined when no E2 was detected. The rapid progression of CIN lesions has been observed in patients with a high viral load of integrated HPV16 (Peitsaro, Johansson et al. 2002).

Quantification of HPV viral load is available and has recently been reported using different methods. The different methods (cut-off) of viral load level were used as qualitative dot blot signals (percentile), semi-quantitative methods of PCR-EIA (percentile) and HC II (pg/ml). Quantitative real-time PCR (Moberg, Gustavsson et al. 2003) improved the estimation of viral load. Additionally, different specimens (cervical cell and stained Pap smear) and different types (mostly for HPV16) were studied. The integration status of the HPV genome could be estimated using the viral load difference of E2 and E6 (Peitsaro, Johansson et al. 2002).

From previous studies, HPV viral load and integration were associated with cervical cancer in case-control studies. In the natural history of HPV infection before the occurrence of dysplasia, it is unclear whether both high viral load and integration of viral DNA into host genome lead to persistent infection of HPV, or vice versa. The

puzzling temporality may be delineated only by following-up with a women cohort with normal cervixes.

Therefore, this prospective study enrolled cytologically normal women with infection of major high-risk HPV type of 16, 18, 52 or 58 from a community based cohort, then the prediction of HPV persistence and risk of cervical cancer were examined.

Material and methods

Recruitment and study design of Taiwan CBCSP cohort

The study cohort members were enrolled in a Community-Based Cancer Screening Program in 1990. There were 41,380 women aged from 30 to 65 invited. A total of 11,923 participating women were enrolled in 1991-1992 at baseline examination and 6,923 women received follow-up examinations in 1993-1995. Only women with completed Pap smears, HPV testing and questionnaire were eligible for the HPV cohort. As a result, the total number of enrolled subjects was 10,602. Informed consent was signed after a detailed explanation by well-trained public health nurses. The detailed information is described in chapters III & IV.

Repeated measurements at baseline and F/U visits

Both at baseline and follow-up (F/U) examinations, all cohort members were invited to participate in a health examination. Most procedures followed the same protocol at both visits. The socio-demographic characteristics, cigarette smoking, Pap smear history, reproductive and sexual history, and personal and family history of cancers were collected again using a structured questionnaire. Additional questions regarding sexual behavior, number of sexual partners, intra-uterus device (IUD) use, oral contraceptives (OC) use, douching after intercourse and treatment of diseases or cancers were collected at follow-up for the recent exposure during the two visits. A Cervex-Brush (Rovers, the Netherlands) was used to obtain exfoliated cervical cells for the Pap smear. ViraPap (Digene Diagnostic, Silver Spring, MD) kits were used for collecting and preserving cervical cells. The cells were stored at -80°C until HPV DNA testing. Pap smears were read according to the Bethesda system, which categorizes cytology grades into normal, atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL) and cancer.

Selection of subjects for HPV viral load and integration into host genome

Cohort members who had consecutive cervical cell samples and were infected with one type of either HPV 16, -18, -52 or -58 at baseline were selected as study subjects for the study of HPV viral load and integration into the host genome. All of their cervical

cell samples collected at bi-annual and four-month follow-up examinations were tested. There were 822 women infected with one type of either HPV 16, 18, 52 or 58 at baseline, and 15 subjects were excluded due to a lack of adequate specimens. A total of 807 subjects were therefore included for viral load analysis (Figure VI-1).

HPV DNA and genotyping

The DNA preparation was done using Qiamp 96 DNA blood kits (Qiagen Inc., Venlo, Netherlands). Polymerase chain reaction (PCR), using MY11/biotinylated GP6+primer was performed and an HPV blot (Easychip® HPV Genotyping Array, KingCar, Taiwan) was used to detect thirty-nine types of HPV (6, 11, 16, 18, 26, 31, 32, 33, 35, 37, 39, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 72, 74, 82, CP8061[71], CP8304[81], MM4[82], MM7[83], MM8[84] and L1AE5). Detailed information is described in chapter III. Human genomes from male blood samples with GAPDH forward and reverse primer were designed as a PCR positive control. Sterile water control was used to monitor the contamination in both HPV and GAPDH reactions. PCR was re-performed if negative GAPDH results were obtained. Quality assurance was ensured by using seven control samples in each batch, and excellent reliability and reproducibility was observed (Lin, Chen et al. 2007).

HPV viral load

Real-time PCR (RT-PCR) was used to examine the HPV DNA viral load (Ho, Cheng et al. 2006). DNA amplifications were carried out in a 96-well reaction plate format in an ABI Prism 5700 Sequence Detection System (Applied Biosystems). Amplification and quantification of the E2 and E6 genes were carried out simultaneously in separate reaction tubes. Both the HPV E2 PCR and E6 PCR reactions were carried out in triplicate. Multiple HPV negative human genomic DNA controls were included in every analysis. The reaction was performed in a 25 μl mixture containing 1x reaction buffer (HP^{TM} HotStart Taq SYBR Green Kit Cat No.PTM767B, Protech) and 100 nM of primers for both E2 and E6 regions. Then 2.5 μl of total DNA were added to the reaction mixture.

The sizes of the E2 and E6 amplimers were 101 and 107 bp (HPV 16), 97 bp and 107 bp (HPV 18), 94 bp and 107 bp (HPV 52), 108 bp and 108 bp (HPV 58), respectively. The amplification conditions were: 10 min at 95°C, a two step cycle at 95°C for 10s and 60°C for 1 min for a total of 45 cycles. The specificity was verified by dissociation curves. Two standard curves were obtained by amplification of serial dilutions of cloned partial-length HPV-16 (from base 28 to base 3890) or HPV-18 (from base 45 to base 3993) or HPV-52 (from base 95 to base 3895) or HPV-58 (from base 45 to base 3994) plasmid DNA in pGEM T-Easy vector (Promega) containing equivalent amounts of E2 and E6 genes from 10 to 10,000,000 copies per μl. Numbers of the threshold cycle obtained from the E2 and E6 PCR were equivalent in each run. Linear plots of log of

copy number vs. number of threshold cycles were consistently obtained for both genes, and their correlation coefficient was between 0.995 and 1.00 in each run.

Viral load of HPV DNA was expressed as copies of HPV genome in 50 ng of cellular DNA. The viral load was grouped as low (L), medium (M) and high (H), while the viral copies were lower than 1,000 ($<10^3$), 1,000-10,000 (10^3-10^4) and higher than 10,000 ($>10^4$), respectively.

HPV Integration

Integration status was further classified among viral load groups as medium and high. If there was no E2 detected, the purely integrated form was observed. Ratios of E2 to E6 of less than 0.5 indicated the presence of integrated form dominant (Ruutu, Kulmala et al. 2008). Where the ratio was between 0.5 to 1.1, both integrated form and episomal form were observed (mixed). The episomal form dominant was defined as dominant when the ratio was greater than 1.1. The copy numbers of integrated E6 were calculated from the total copy numbers of E6 (both episomal and integrated) subtracting the copy numbers of E2 (episomal).

HPV type-specific, persistence and clearance

Considering HPV infection at F/U visit, persistence was defined as women sustaining the same HPV infection at both visits. Clearance was recognized if the

women had cleared the HPV infection before the F/U visit. Acquisition was defined if a newly acquired HPV type was observed at F/U visit (Chapter IV).

Follow-up of cervical neoplasia

Through data linkage with computerized registries of the National Cancer Registry and National Death Certification Registry, cohort members affected with newly-diagnosed cancers of the cervix uteri up until December 31, 2006 were identified. The criteria of cervical cancer from computerized database were linked as diagnosis with ICD-code of 180 and ICD-O-code of C-53. All the diagnoses of cancers was based on histological basis.

Statistics

Characteristics associated with HPV persistence were examined for their association with viral load and integration status using the Mantel-Haenszel chi-square test. The association with HPV persistence was examined using logistic regression, and the age-adjusted odds ratio (aOR) and multiple adjusted odds ratio (mOR) were also estimated. The risk of incident cervical cancer during follow-up was estimated using the Cox proportional hazard model after age and multiple adjustments. The Nelson-Aalen estimator of cumulative hazard was calculated and plotted for the cumulative incidence of cervical cancer by groups of viral load. The log rank test using the Kaplan-Meier

method was performed to test the median of time to event for viral load groups.

Results

The baseline cytology was strongly associated with baseline viral load and integration status (Table VI-1.) The percentage of high viral load was increased with severity of cervical abnormality ($p<0.0001$). The percentage of integration form dominant was varied among the different cytology grades, which were 25.5% (normal), 8.8% (ASCUS), 9.8% (LSIL) and 22.8% (HSIL) (Figure VI-2). The association between viral load and persistence was limited among 391 subjects who had normal cytology at baseline and received the F/U examination. Characteristics, such as age, menopause, multiple sexual partners (>1) and IUD, associated with HPV persistence, but no association was observed with viral load and integration. The number of vaginal deliveries was associated with viral load.

The percentages of persistence were 22.2%, 57.7%, 74.4% for the low, medium and high viral load groups, respectively (Table VI-2). The risk of persistence was increasing increased with increased viral load. In compared with group L ($<10^3$), the mOR were 4.3 (2.4-7.7) and 8.4 (4.8-14.8) for group M (10^3-10^4) and group H ($>10^4$) after adjustment for menopause (mOR: 1.9, 1.2-3.0) and multiple sexual partners (mOR: 7.9: 2.0-31.5). The association between viral load and persistence was not confounded whether in univariate or in multivariate models after adjustment for menopause and

number of sexual partners. Using group L as the reference, the episomal, mixed and integration dominant were highly correlated with HPV persistence. However, in each stratum of viral load, using the episomal group as reference, there were no significant association observed between integration status and persistence. Only two subjects were observed had purely integrated status and they were kept persistent at F/U.

A significant dose-response relationship of HPV viral load in prediction of HPV persistence was observed both among pre- and post-menopausal women (Table VI-3.) Among pre-menopausal women, the percentage of persistence increased with increased viral load, and was 17.0% (group L), 51.1% (group M) and 68.9% (group H), and the 4.8–fold (group M) and 11.7-fold (group H) using group L as reference with adjustment for multiple sexual partners (>1 vs. ≤ 1). The menopause status and number of sexual partners were independently associated with viral load in prediction of HPV persistence. Besides viral load, multiple sexual partners was highly associated (mOR: 12.8, 2.5-66.0) with HPV persistence among pre-menopausal women. However the persistence rate of group L in post-menopausal women was much higher than in pre-menopausal women (30.3% vs. 17%), hence, the risks of persistence were 3.9 (1.6-9.7) and 6.1 (2.7-13.6) for group M and group H, respectively, among post-menopausal women. The association of multiple sexual partners and persistence was no longer observed among post-menopause women. To compare post- and pre-menopausal women in each viral

load group the significant 2.1-fold risk was observed among group L, but not among groups M or H. The changes of viral load to the L (10^3) group at F/U were studied. There was a 4-fold risk of becoming persistent for pre-menopausal women lowering their viral load from group M to L in comparison with a persistent low. This pattern was also observed among post-menopausal women (mOR: 3.1, 0.9-10.3) at borderline significance.

Among 391 subjects, 17 cervical cancer cases were diagnosed from linkage with the National Cancer Registry. One case was diagnosed between two visits, who was not included in the analysis for association of cervical cancer. The incidence of cervical cancer increased with viral load groups from 181.0 (group L), 193.4 (group M) and 669.4 (group H) per 100,000 person-year. In a multiple Cox regression model, women who had a high (>10^4) viral load at baseline had a 3.4 –fold risk to develop cervical cancer during follow-up with adjustment for menopausal status. If the viral load was lowered from medium or high to low at F/U, a significantly protective effect was observed with mHR of 0.1 (0.03-0.3), and persistence at F/U strongly predicted cervical cancer development with mHR of 14.4 (2.3-45.3).

However, among those pre-menopausal women, there was no obvious risk of high viral load, and a protective effect of lowering viral load and an increased risk for persistence were observed, but which were not statistically significant. The markedly

increasing dose-response was examined among post-menopausal women in prediction of cervical cancer (p=0.007). The incidences of cervical cancer were 160.9, 562.0 and 1291.0 per 100,000 person-year for groups L, M and H, respectively. Compared to group L, the mOR were 3.9 (0.6-28.2) and 8.1 (1.7-39.1) for groups M and H, respectively. The lowering viral load had a strong protective effect (mOR: 0.04, 0.01-0.3) with cervical cancer occurrence. Once virus was cleared was achieved, there were no occurrences of cervical cancer.

The cumulative hazards of groups L, M and H were 0.029, 0.042 and 0.105, respectively, after a follow-up to 16 years, in which a significant difference among the curves was observed (p=0.02) (Figure VI-3A). The marked difference between the cumulative incidences (0.143 vs. 0.024) for the 'lowered' and 'not lowered' groups was examined (p<0.0001) (Figure VI-3B).

Discussion

Based on a definite temporality, this study examined the role of viral load and integration of HPV in prediction of HPV persistence and cervical cancer among normal women and followed their subsequent HPV persistence and incident cervical cancer during long-term follow-up.

In the natural history, HPV persistence was a major stage in cervical carcinogenesis (Chapter V). We founded a strong dose-response relationship of viral load in prediction of HPV persistence. HPV viral load could be an important predictor to HPV persistence, both in pre- and post-menopausal women. More than half of the women who had a viral load level of 10^3-10^4 sustained their HPV infection and almost 70% of women who had a viral load level of higher than 10^4 did. Although, only one study ever studied the dose-relationship with HPV clearance using PCR-EIA, no linear-trend was observed due to possible misclassification of semi-quantitative methods of viral load (Molano, Van den Brule et al. 2003). The false-association between integration and persistence was revealed when stratification by viral load levels was made, but 100% of purely integrated HPV genome into the host genome were persistent cases (n=2). We can not rule out that this is one of the possible ways to be persistence. The number of sexual partners seems to be a surrogate marker with long-term infection or long-term persistence, since 15 women had more than one sexual partner, 10 of who were had persistent infection and nine of whom had reported an early initial exposure of sexual coitus.

Based on the definite temporality in our study, only the highest viral load ($>10^4$) could predict the risk of cervical cancer in long-term prediction of cervical cancer, either at baseline or at F/U examination. The pattern of association was similar with

previous studies using quantitative RT-PCR with different cut-offs (copies), even if diversified designs, materials and methods were used (Josefsson, Magnusson et al. 2000; van Duin, Snijders et al. 2000; Ylitalo, Sorensen et al. 2000; Schlecht, Trevisan et al. 2003; Moberg, Gustavsson et al. 2005). Furthermore, high viral load at F/U predicted the increase of cervical cancer risk. Once the viral load was lowered to low, the risk apparently decreased. If the virus was not cleared (persistent), the adjusted hazard ratio (14.4) of cervical cancer was significantly higher than for clearance. The results indicated that lowering the viral load presents a potential protection of cervical neoplasia. Persistence and clearance could be treated as a dichotomous status of the viral load lowering process, which implies that repeated HPV viral load testing among infected women is helpful for their risk assessment. Older women still possessing high or medium viral loads ($>10^3$), whether at baseline or at F/U, had a higher risk of cervical cancer occurrence. Hence, for post-menopausal or older women, at least once viral load testing for HPV for infected women is needed. Lowering the viral load had a strong protective effect. This finding may present the possibility of anti-viral therapy for HPV infected women and may be useful for arresting the occurrences of cervical cancer.

The integration status was associated with cervical neoplasia in cross-sectional analysis, but was not associated with cancer incidence in longitudinal analysis on normal women. Most previous studies have been based on cross-sectional designs and

only a few studies have included normal women (Briolat, Dalstein et al. 2007; Chan, Cheung et al. 2007; De Marco, Gillio-Tos et al. 2007). The only one longitudinal study among 13 LSIL women with mixed form, only one progressed to HSIL; there is no evidence for physical status in disease progression (Ho, Cheng et al. 2006; Ho, Chien et al. 2006). However, the definitive cut-off of integration using the E2/E6 ratio by RT-PCR has been different in different studies, and the definition of pure integration (no E2) has been common. The percentage of pure integration forms among cancer in our study was 23%, which is consistent with previous reports (20%~36%), whether on a cytological or histological basis and whether cervical cells or paraffin-embedded tissue were used. A common limitation of integration studies was the small sample size in each grade of cervical lesion. Among normal women recruited in our study, HPV integration was not infrequent (26%), but not correlated with cervical cancer development. The early event of viral integration prior to cervical lesion may be cleared by the host immune system or cell apoptosis by activation of the tumor suppression system. Only mono-clone or few clones were selected in the natural history in cervical carcinogenesis. The role of integration of HPV DNA is associated with the progression of CIN from polyclonal to monoclonal status and the progression from low-grade to high-grade cervical neoplasia (Ueda, Enomoto et al. 2003). The integration of viral load is a biomarker associated with severe cervical lesions, but not sufficient for cancer

occurrence. However, the E2/E6 ratio in exfoliated cervical cells was an indirect method (compare to in situ hybridization on biopsy tissue) for HPV integration that we can not exclude the possibility of the initiation role being played by the increasing host genome instability.

In conclusion, HPV viral load of types 16/18/52/58 predicts HPV persistence and cervical cancer. Among older or post-menopausal women, viral load testing may be helpful to determine the most important high-risk group of women. Lowering viral load could be a way to prevent cervical cancer among HPV infected women.


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Chapter VII: Conclusion and perspectives:

Through this long-term follow-up study in CBCSP woman cohort with repeated measurements of HPV infection, we found HPV prevalence was 24.5% and HPV16, 18, 52, 58 and 11 were common types. Among normal women with a mean follow-up duration of 1.4 years, the summarized acquisition rate was 8.4% for any type and the mean of type-specific persistence rate was 27.7%. Women had HPV infection, earlier age at initial coitus, douching use after sexual intercourse, or not currently married had higher risk to acquire newly infection. The determinants for HPV persistence were higher age, high frequency of vaginal delivery, IUD user or post-menopause. With 15-year follow-up, the incidence of cervical cancer for HPV infection and persistence were 171 and 265 per 100000 person-year, the corresponding hazard ratio were 12.8 and 19.6, respectively. HPV16, 18, 52, 58 were the major high-risk types associated with cervical cancer in Taiwan. Around 63% of cervical cancer could be attributed with these 4 types and 51% for HPV16 and/or 18, which vaccine against. In our study, HPV persistence was confirmed the pivotal role in the natural history of cervical cancer with a 44.3-fold risk, once the virus was cleared, the risk was non-significant (HR=2.4, 0.6-9.0). The viral load of HPV16, 18, 52 or 58 associated with persistence in a dose-response relationship and higher viral load (>10^4 copies/ 50ng DNA) was more

likely to develop cervical cancer during follow-up; lowering viral load had a protective effect (HR=0.1, 0.03-0.3) with cancer incidence. The integration was associated with cervical neoplasia in the cross-sectional study, however, it couldn't predict the risks of HPV persistence and cervical cancer in the longitudinal study.

In this large scale cohort, completed cytology examination and personal exposure were collected and those incident cervical cancer cases with histological confirmation during follow-up were ascertained from National registries. After 15 years, more than 18000 cervical cells from all women were examined using a HPV blot to detect 39 types of HPV, we confirm the role of HPV infection in prediction of cervical cancer, but also determine the major high risk types (HPV16, 18, 52 and 58) among Taiwanese women, in cross-section study and longitudinal study. In addition, we explore the sexual behavior and multiple sexual partners were strongly correlated with HPV acquisition. The type-specific rate of acquisition and persistence were estimated by HPV testing at two visits. HPV persistence almost is the necessary cause for cervical cancer. The viral load predicts viral persistence and subsequent cervical cancer.

If HPV testing is used as a tool for cervical cancer screening whether used as primary or adjacent, typing is very important for finding the major high risk subjects, repeating testing for finding HPV persistence is imperative, and viral load is also helpful

to determine the most susceptible subjects for cervical cancer. This scenario is more useful for the elder women or post-menopausal women.

After the epidemiologic profile of Taiwanese women was delineated, the use of HPV vaccine could be considered on further cost-effectiveness study.

Some interesting and unsolved scientific questions could be answered in the future work, such as what is the role of menopause in HPV persistence, the role of HPV intratypic variation in HPV persistence and carcinogenesis, how the host genetic polymorphisms to help the virus clear or not, the interaction of viral factors and host factors to HPV persistence or clearance… et. Any possible way to change the pivot turning the virus to lower viral load to clearance will give women a hope being away from cervical cancer.


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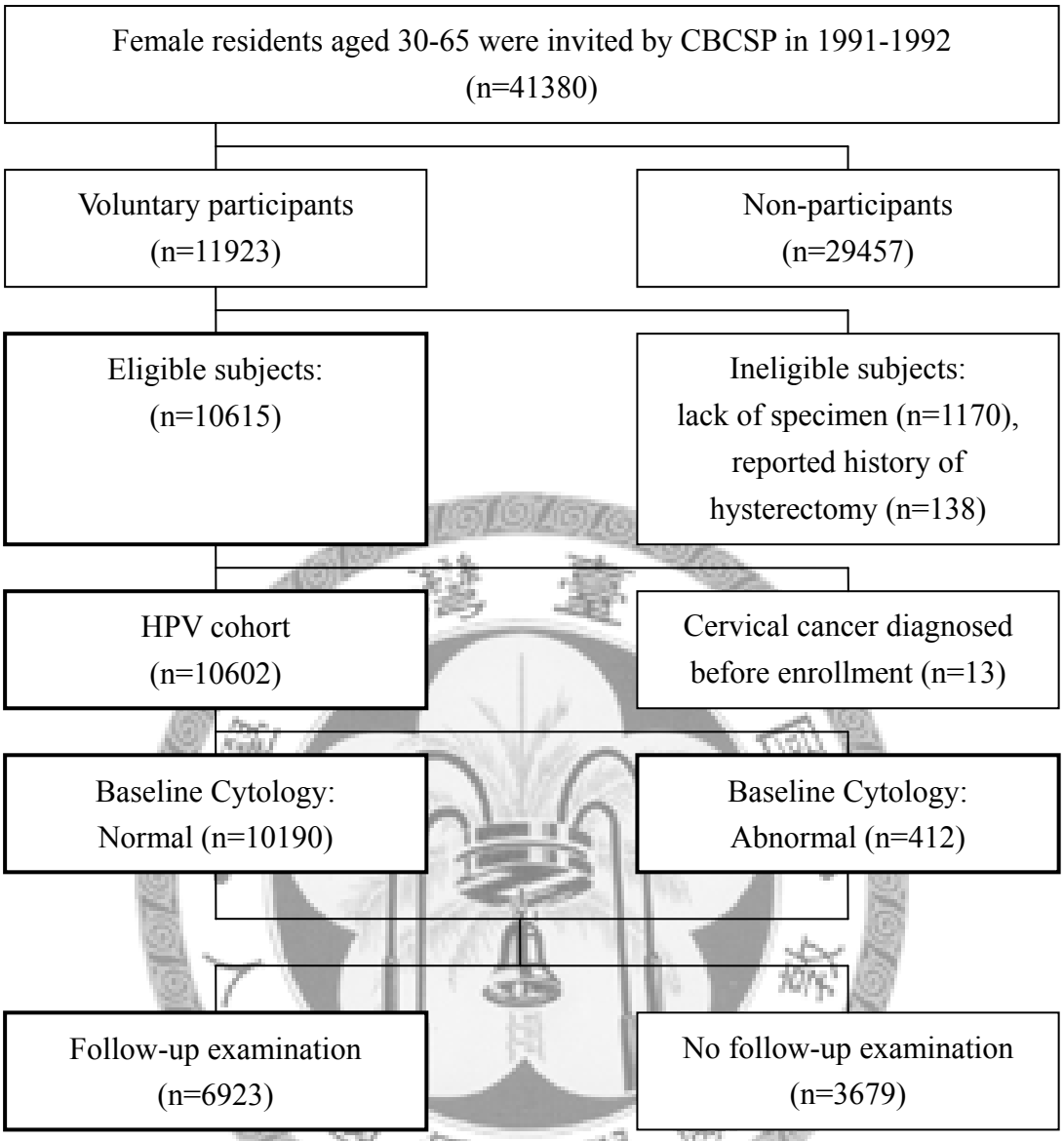


Figure I-1: Inclusion of study participants at two visits.

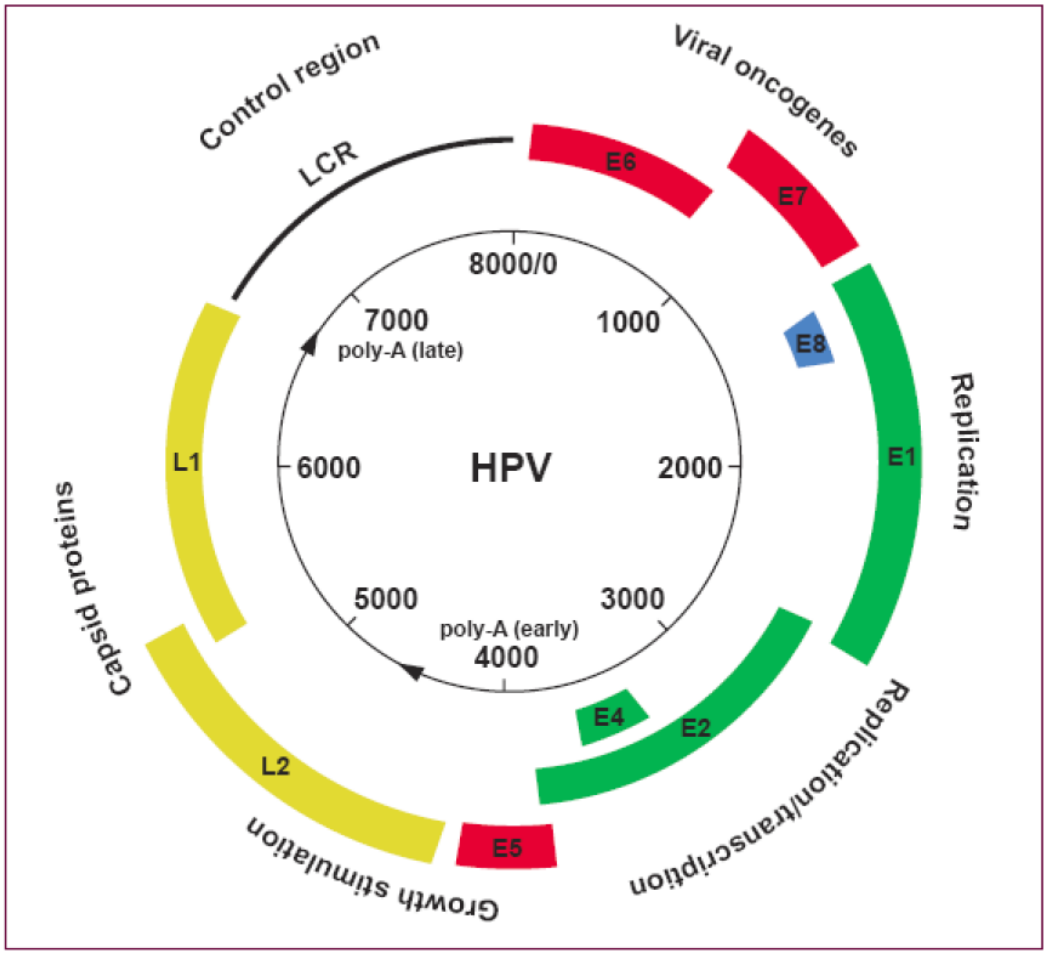


Figure II-1. Genome organization of human papillomavirus

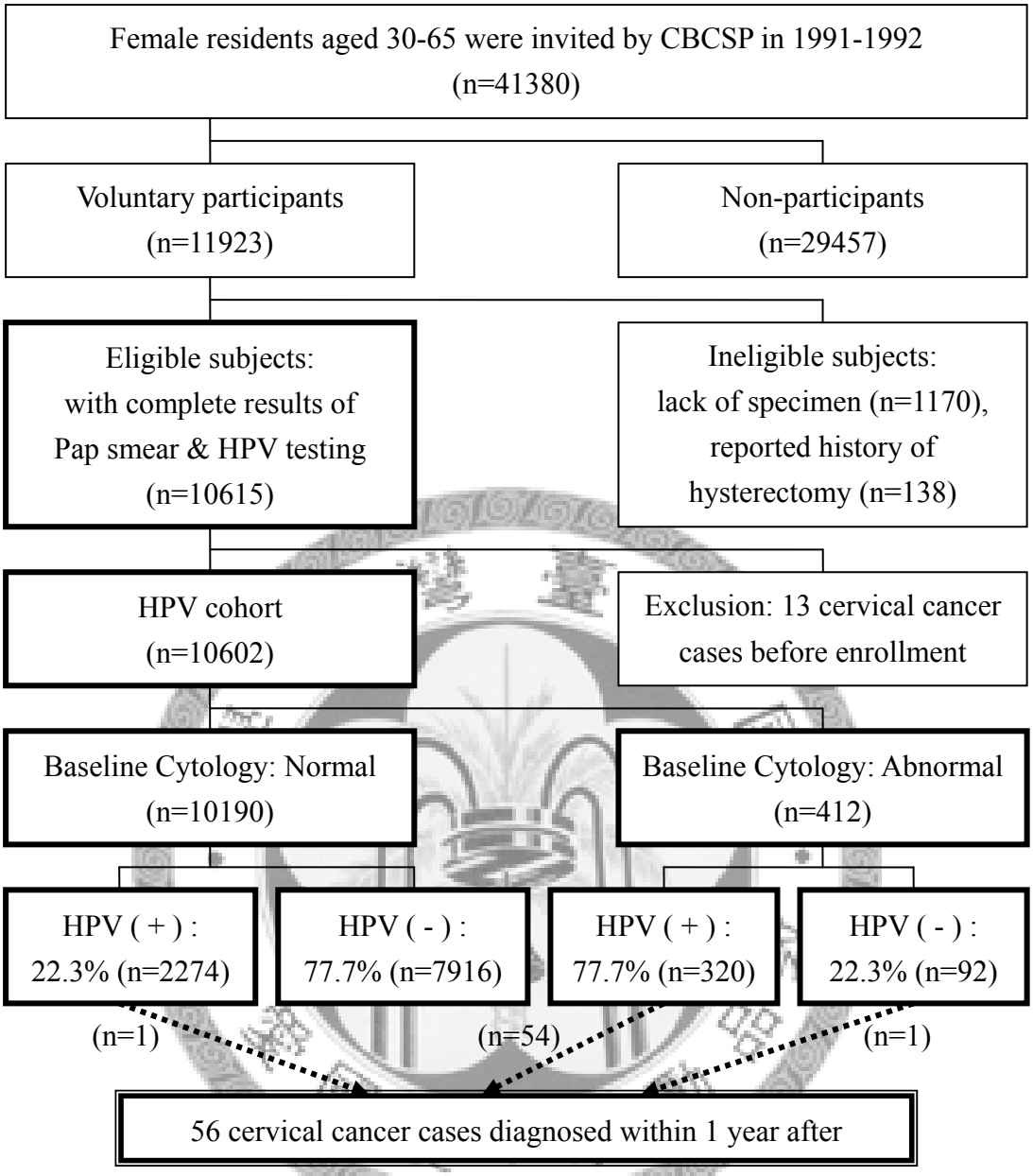


Figure III-1. Flowchart of study participants at study entry.

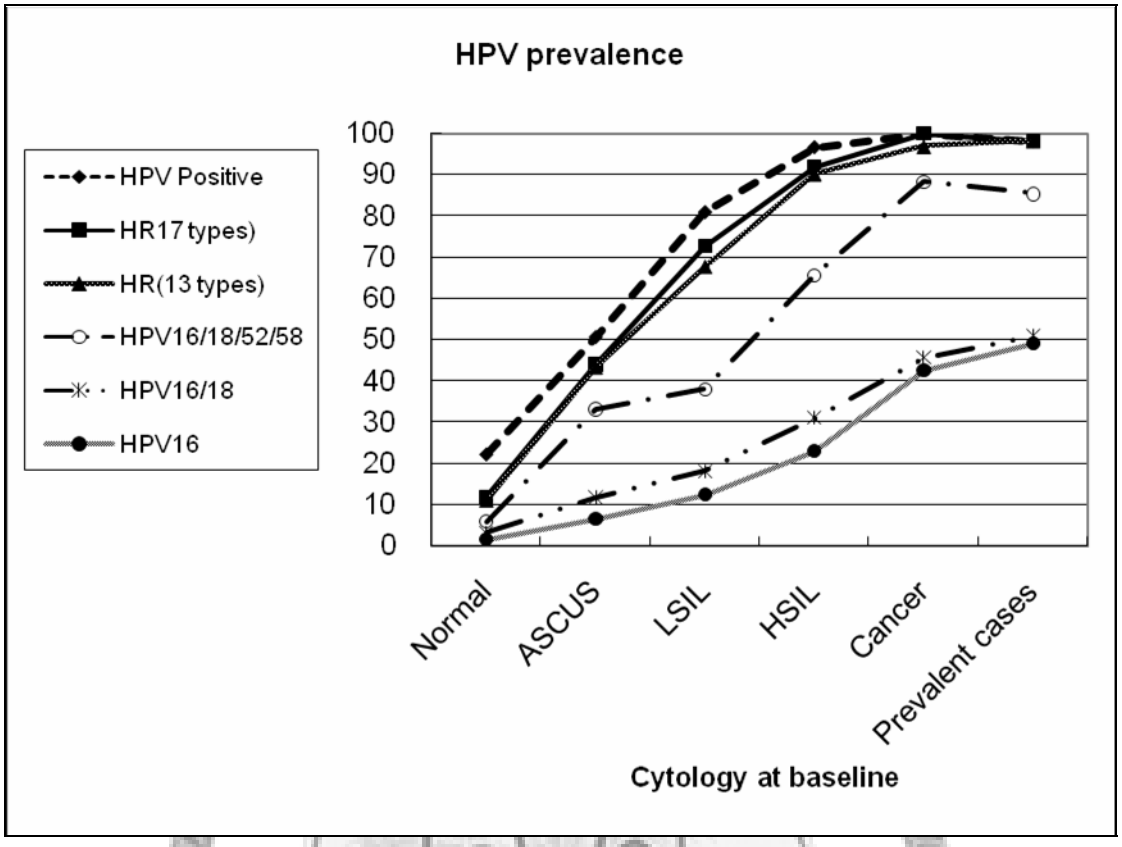


Figure III-2. Prevalence of different HPV genotypes by cylogical findings at study entry.

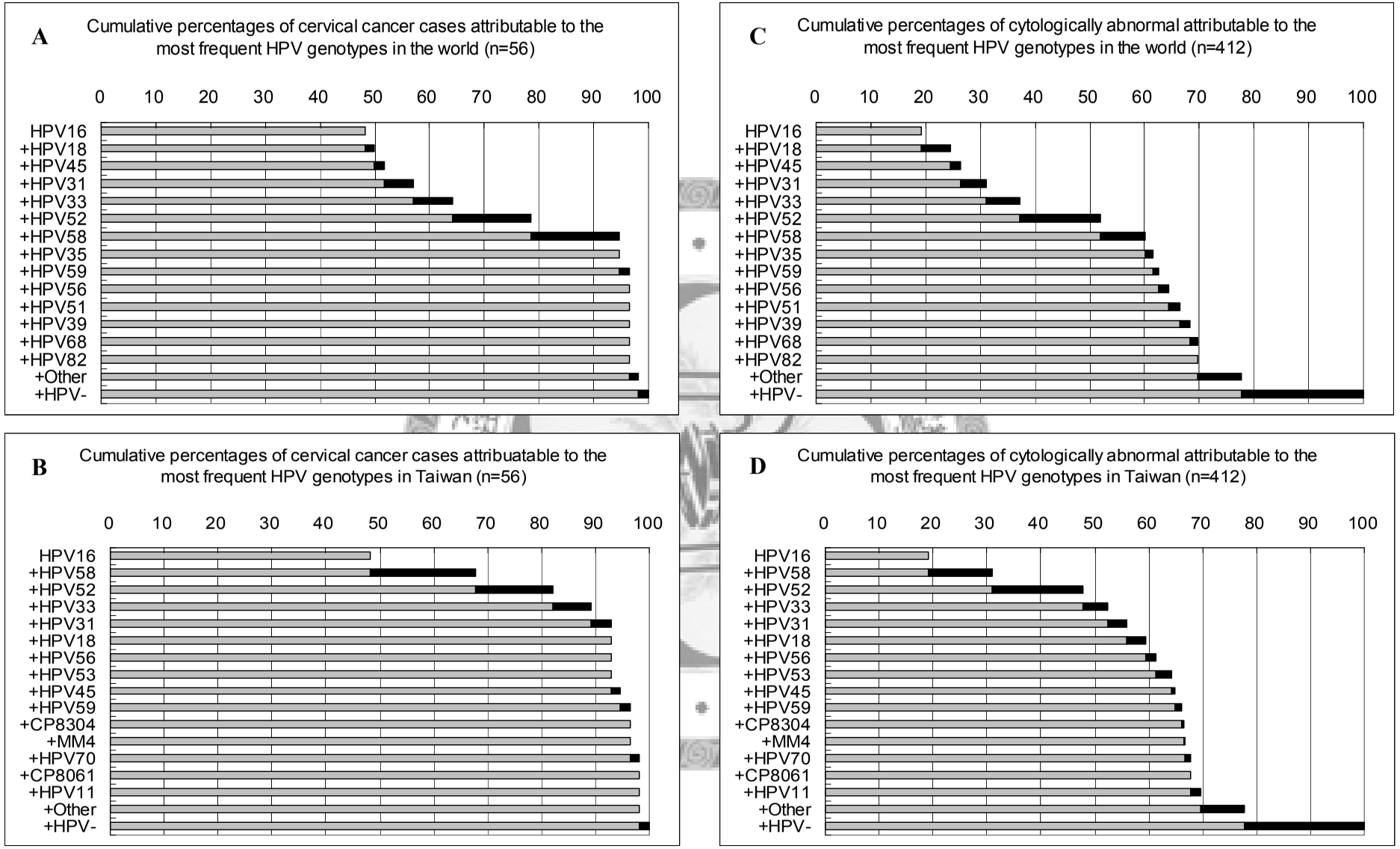


Figure III-3: Cumulative percentage of cervical cancer or cytological abnormality for an additional HPV type was added. (A) cancer cases using the order of frequent HPV genotypes in the world, (B) cancer cases using the order of frequent types in Taiwan, (C) cervical abnormality using the order of frequent HPV genotypes in the world, (D) cervical abnormality using the order of frequent HPV genotypes in Taiwan.

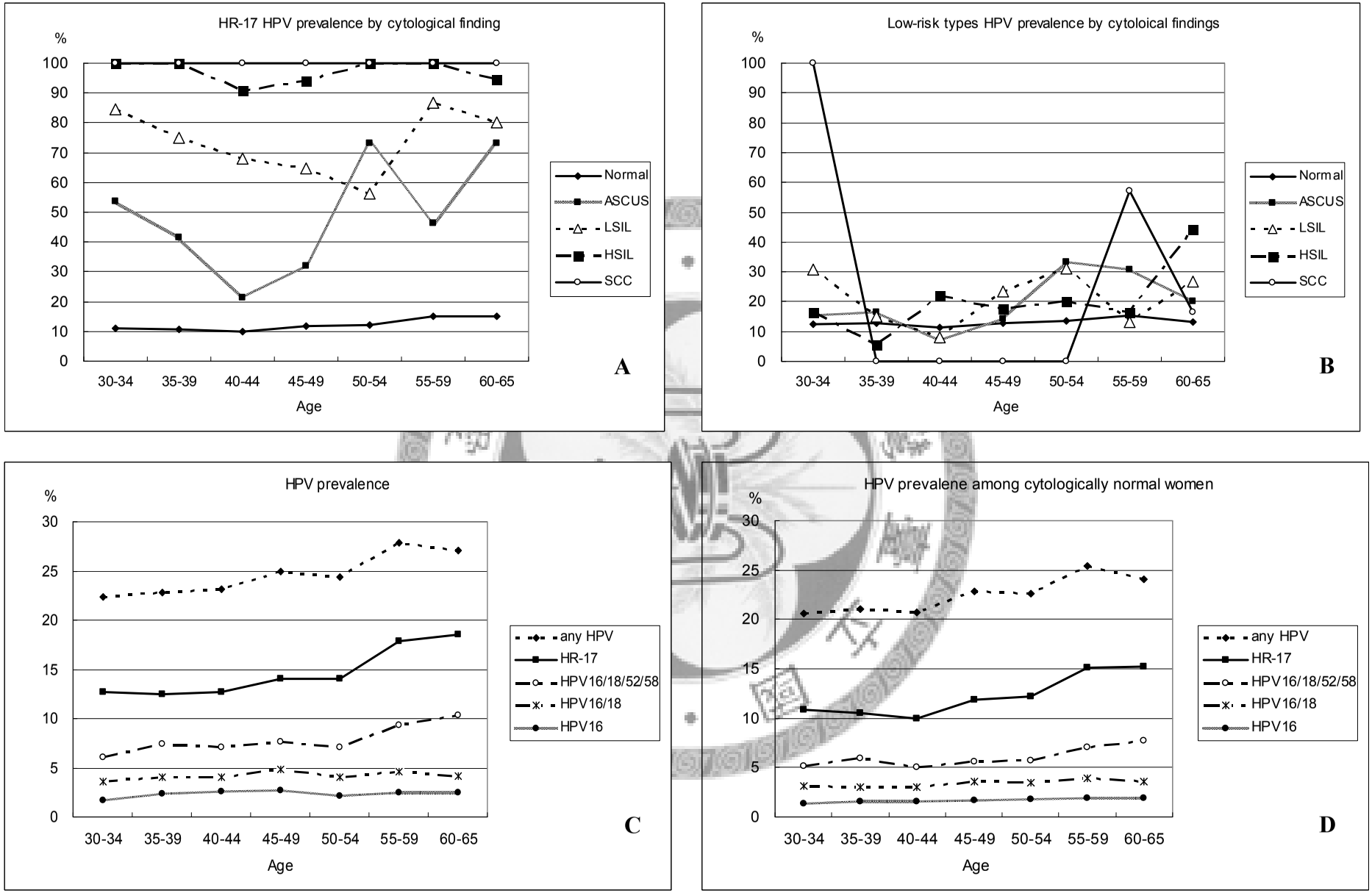


Figure III-4. Age-specific HPV prevalence. (A) age-specific HR-17 HPV prevalence by cytological findings, (B) age-specific low-risk HPV prevalence by cytological findings, (C) age-specific prevalence of different combinations of HPV genotypes in the entire cohort, (D) age-specific prevalence of different combinations of HPV genotypes among cytologically normal women.

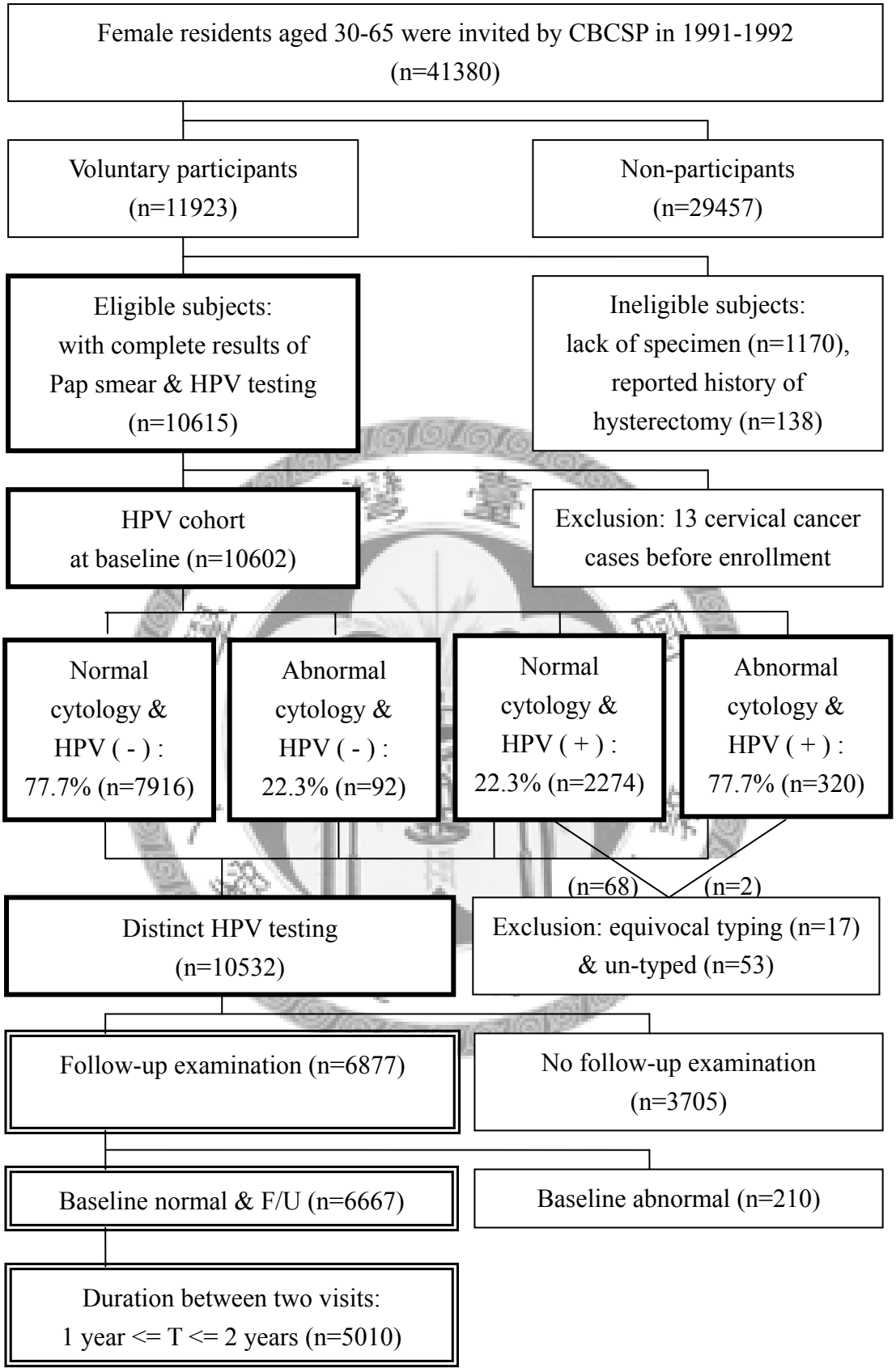


Figure IV-1. Flowchart of study participants for follow-up study on HPV acquisition, persistence and clearance study.

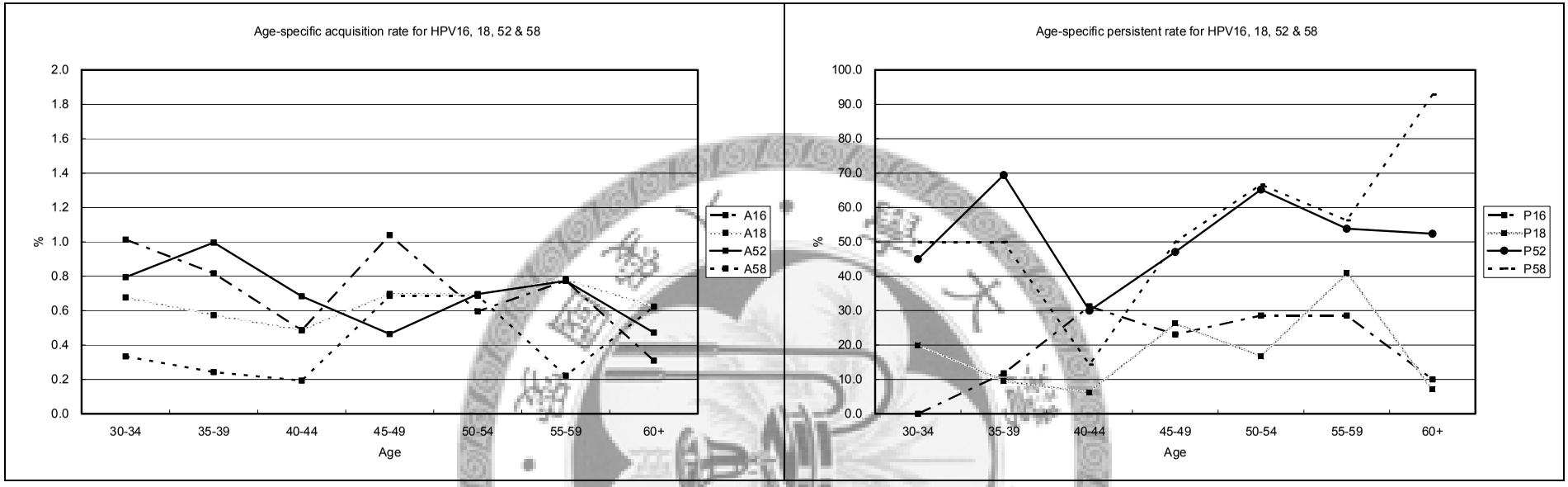


Figure IV-2. The age distribution of acquisition rate (A) and persistence rate (P) of HPV16, 18, 52, and 58.

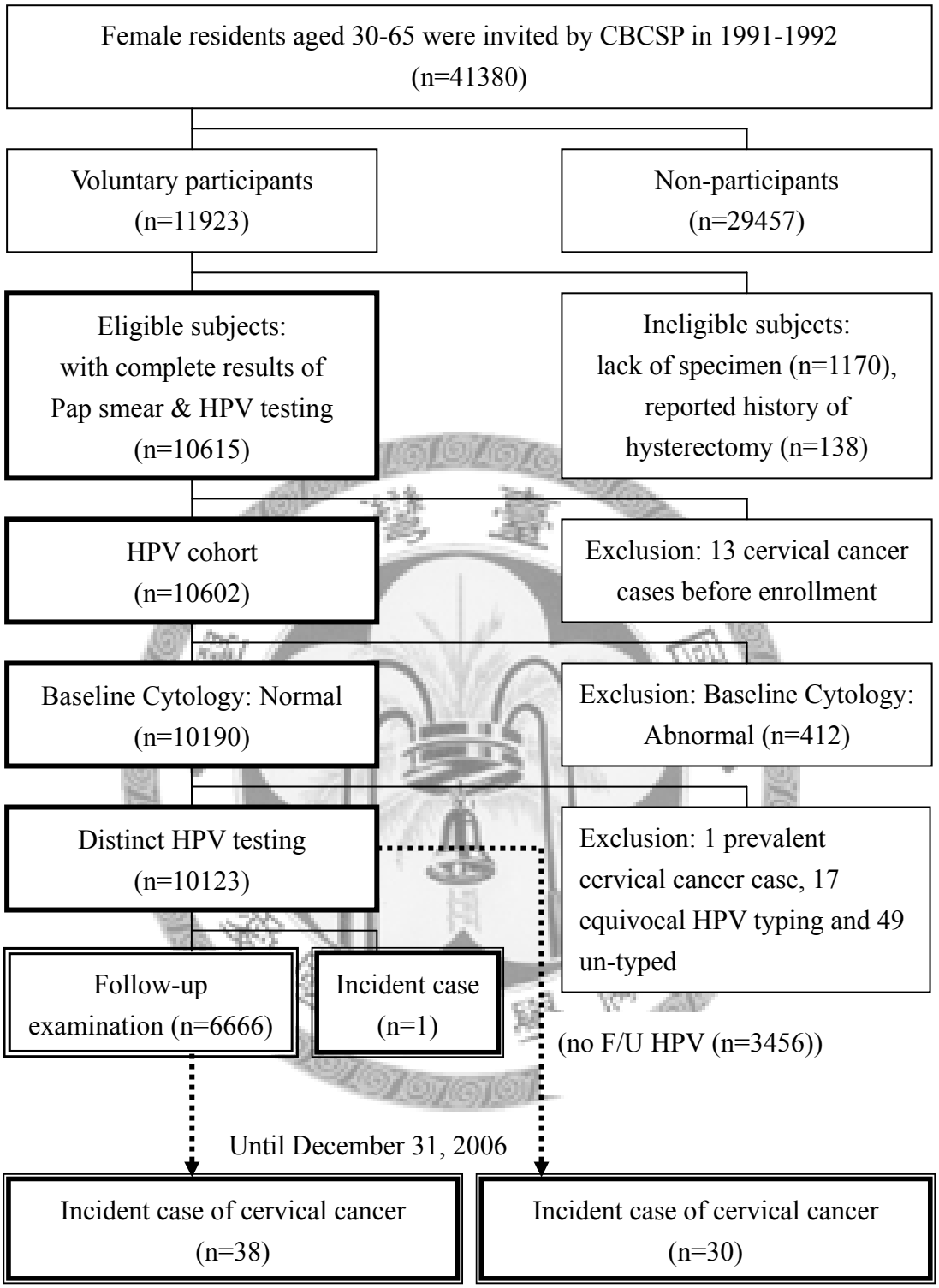
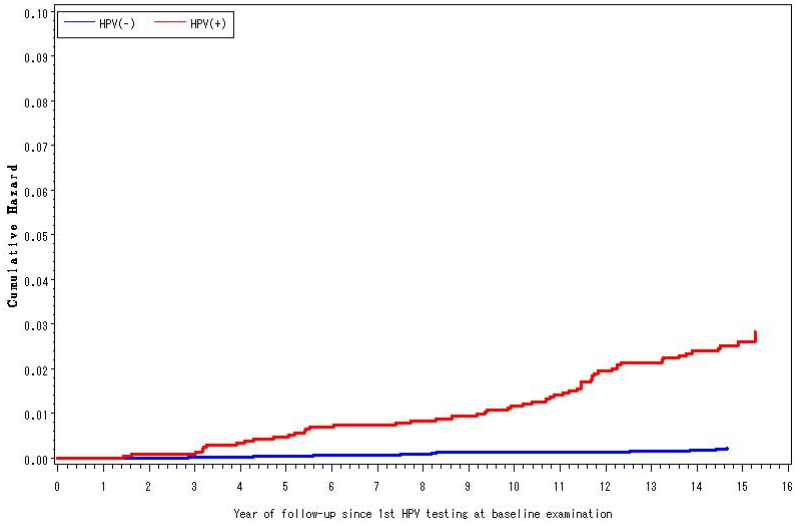


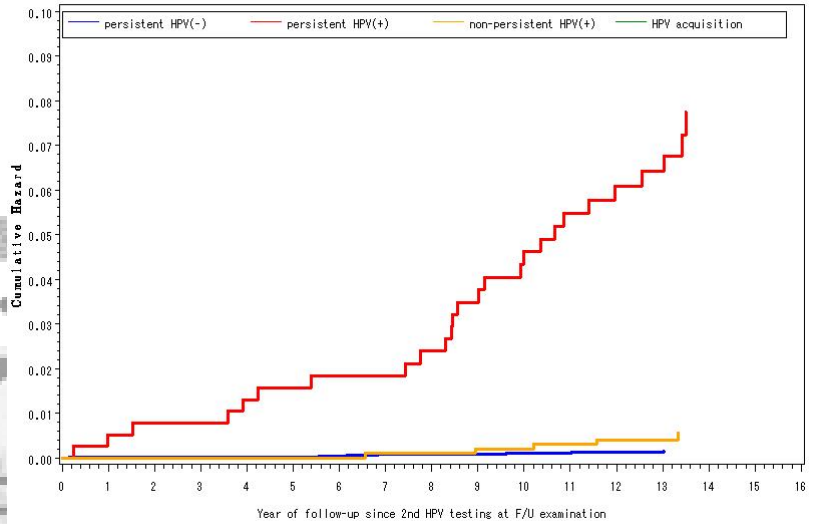
Figure V-1. Flowchart of study participants in long-term follow-up study on cervical neoplasia.

Cumulative hazards of developing cervical cancer by HPV infection status



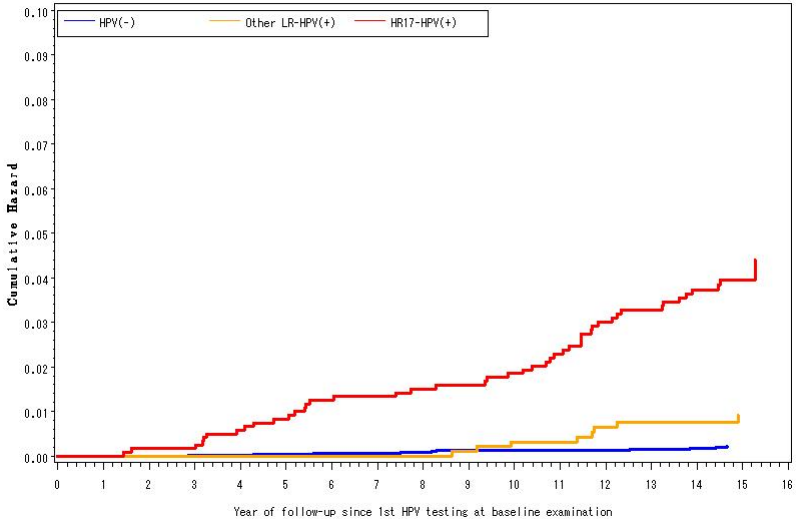
A-1

Cumulative hazards of developing cervical cancer by HPV persistence



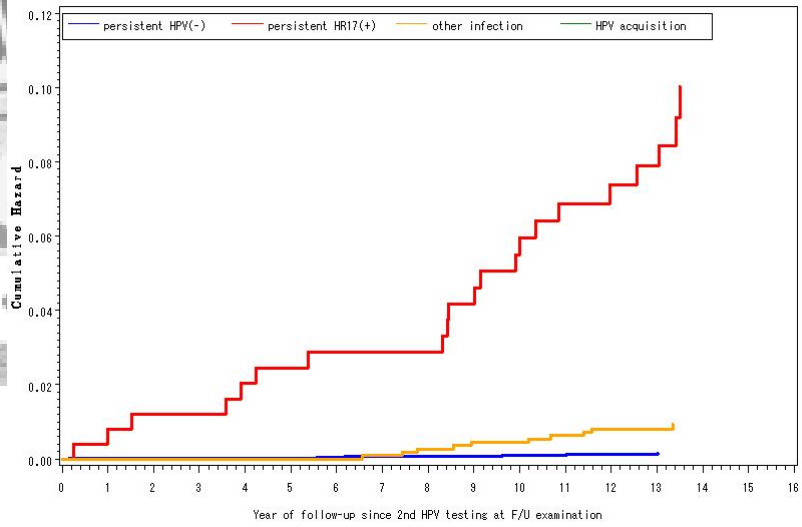
A-2

Cumulative hazards of developing cervical cancer by HPV infection status



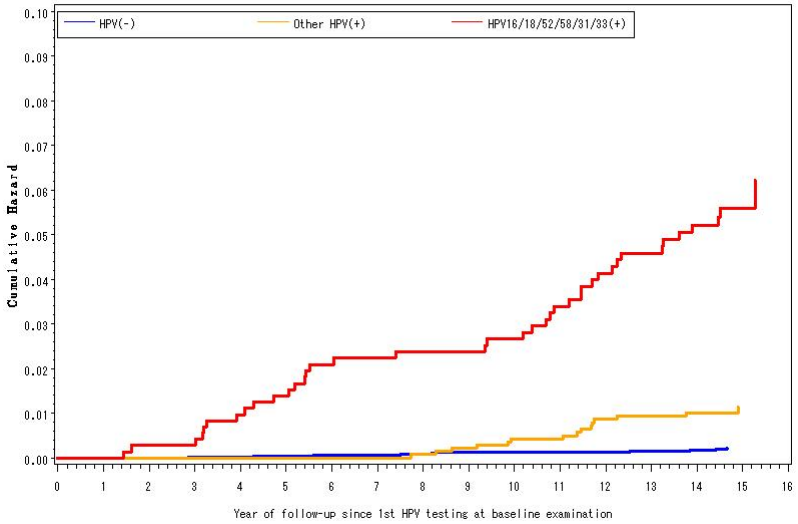
B-1

Cumulative hazards of to developing cervical cancer by persistence of HR-17 HPV



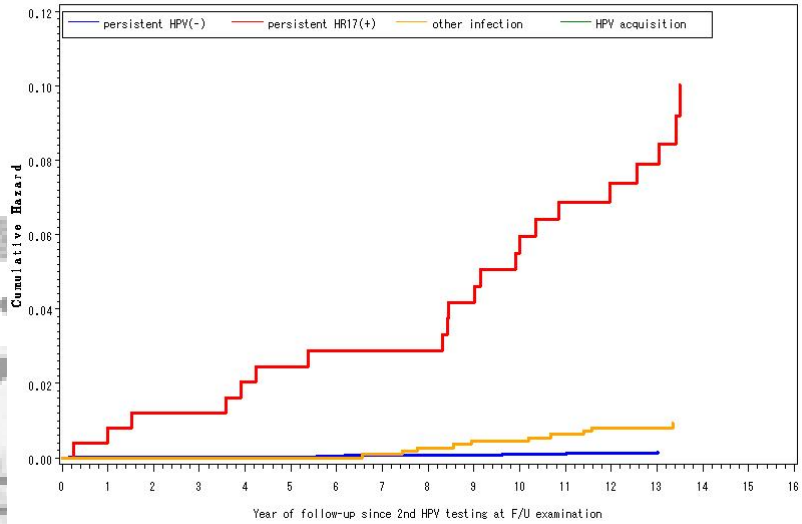
B-2

Cumulative hazards of developing cervical cancer by HPV infection status



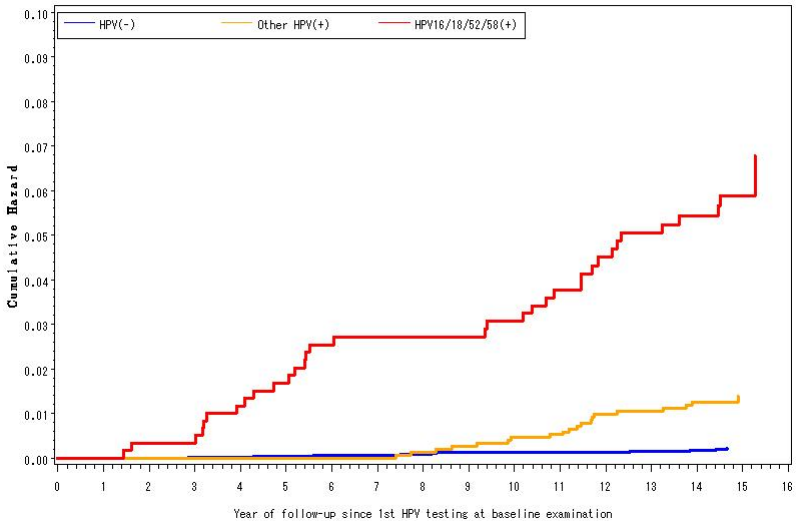
C-1

Cumulative hazards of to developing cervical cancer by persistence of HR-17 HPV



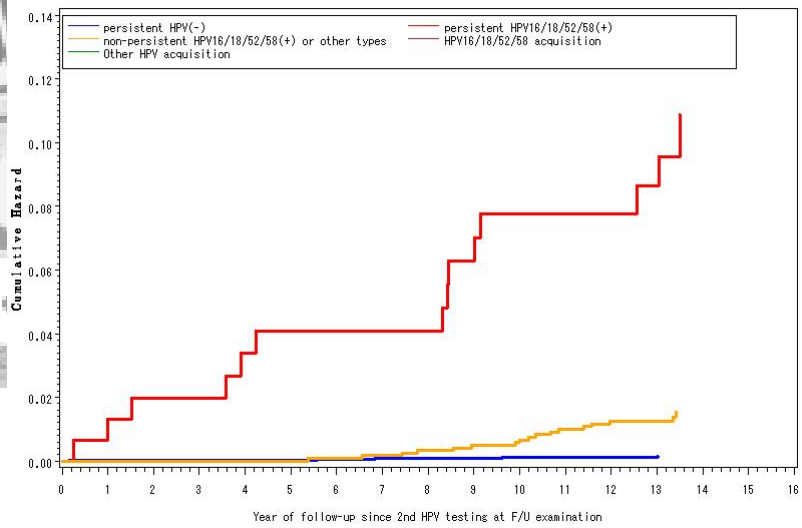
C-2

Cumulative hazards of developing cervical cancer by HPV infection status



D-1

Cumulative hazards of to developing cervical cancer by persistence of various HPV genotypes



D-2

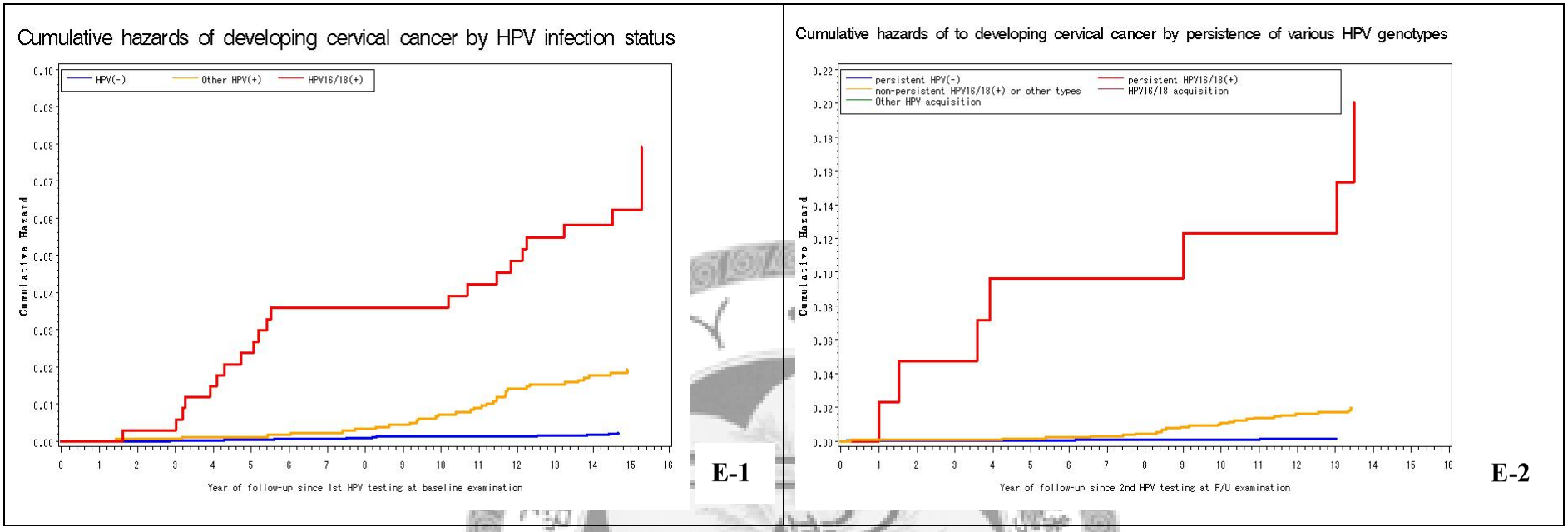


Figure V-2. Cumulative hazards of developing cervical cancer by HPV infection status and persistence. All HPV genotypes (A), HR-17 HPV genotypes (B), HPV16/18/52/58/31/31 genotypes (C), HPV16/18/52/58 genotypes (D), and HPV16/18 genotypes (E).

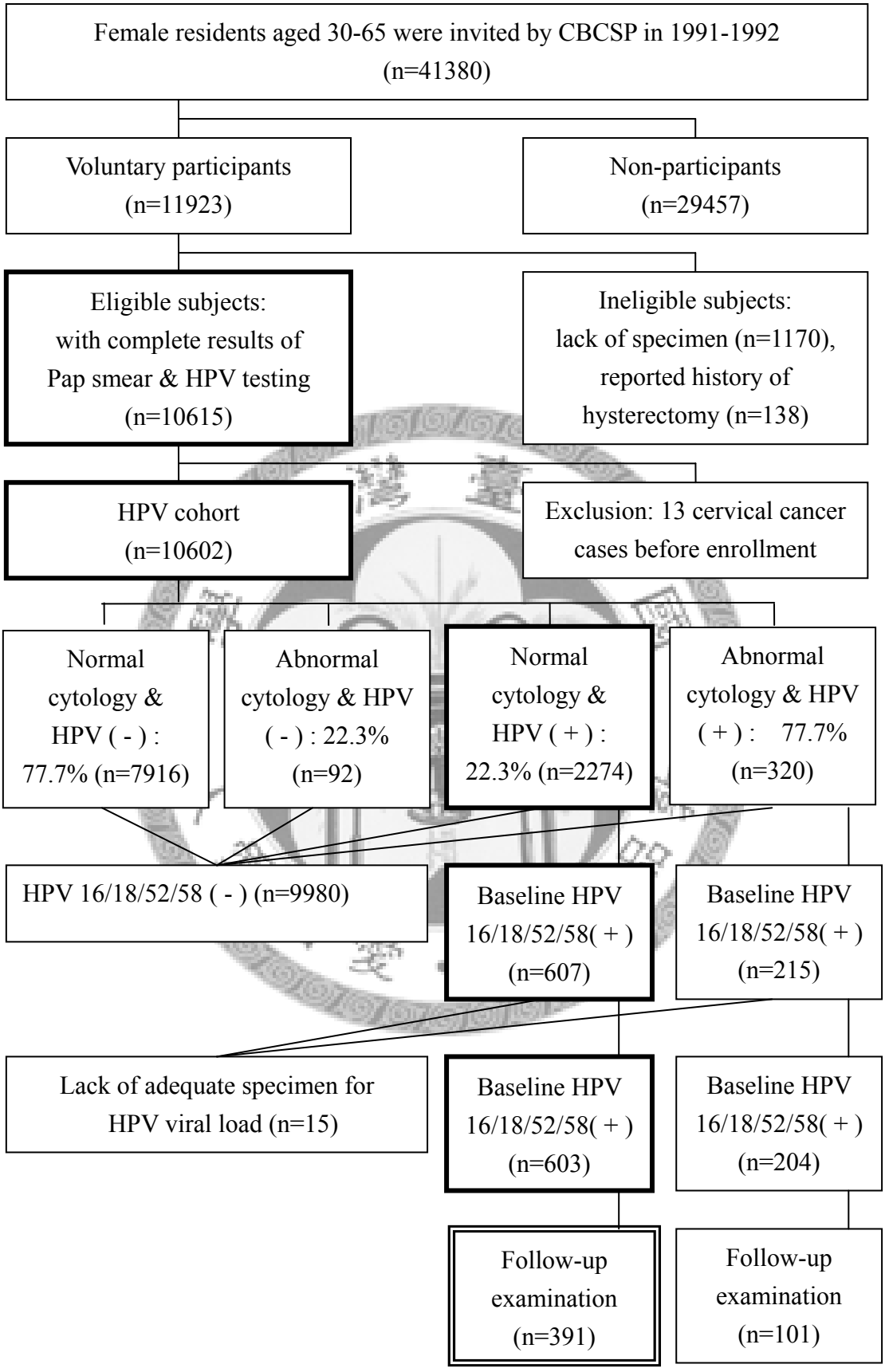


Figure VI-1. Flowchart of study participants for study on HPV viral load and host genome integration.

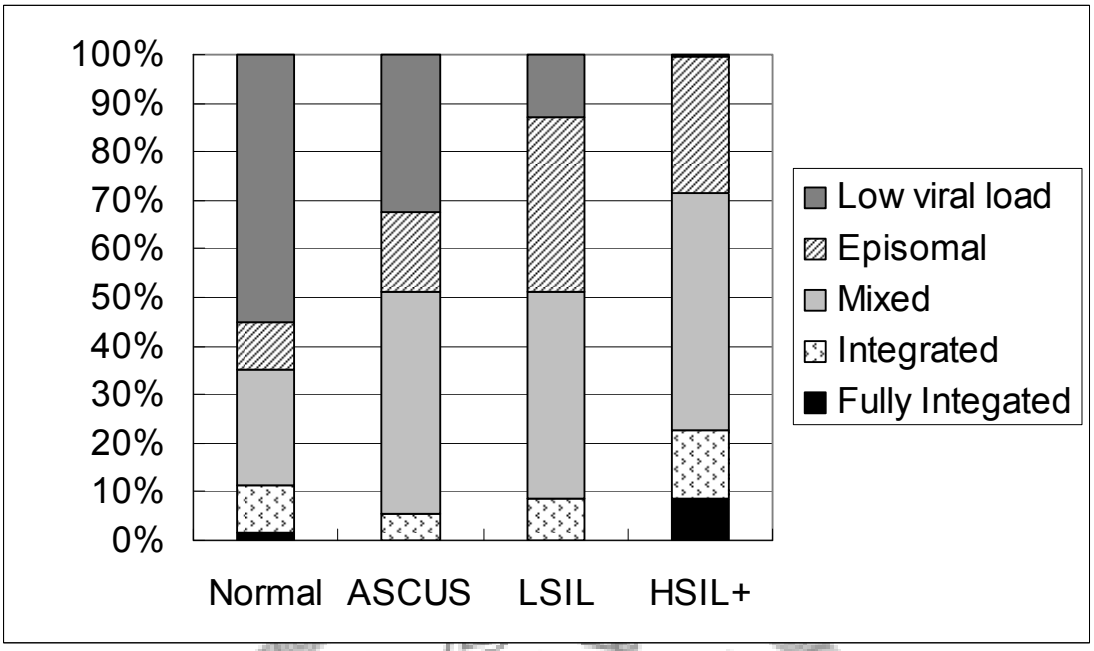


Figure VI-2. Distribution of low viral load and host genome integration of HPV by cytological finding at study entry.

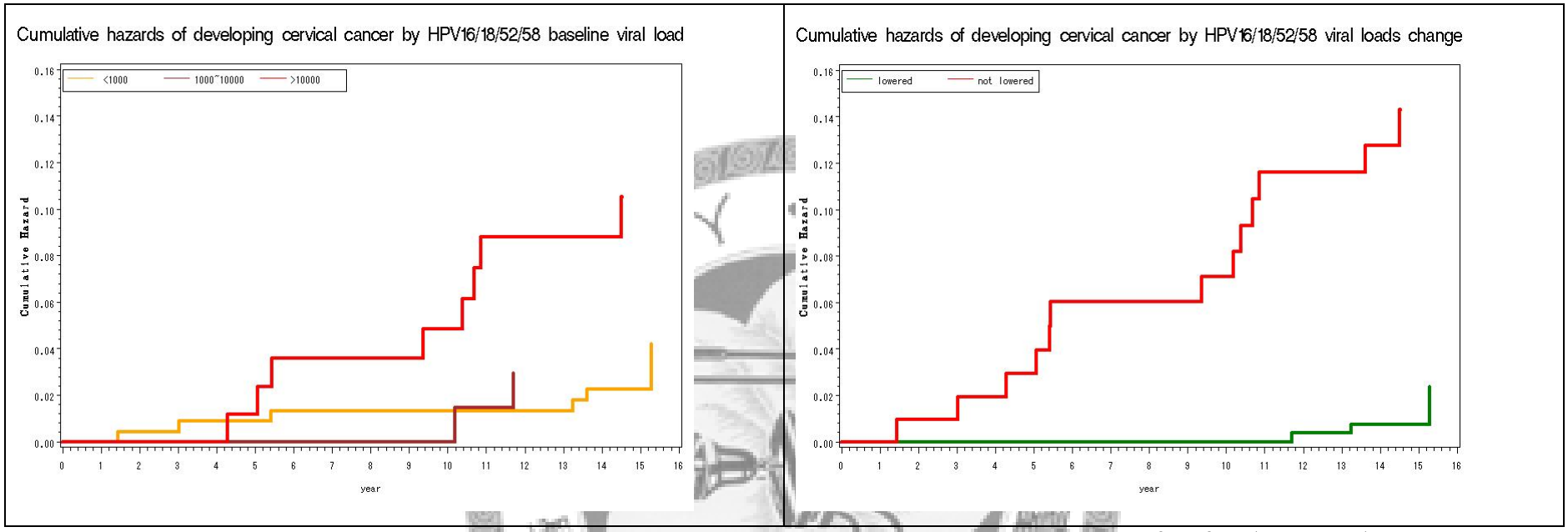


Figure IV-3. Cumulative hazards of cervical cancer by viral load and viral load change. (A) viral load ($<10^3$, 10^3-10^4, and $>10^4$) at study entry; (B)viral load lowered and not lowered at F/U visit.


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Table II-1. Size and functions of papillomavirus proteins

Viral protein/genomic elements	Molecular weight/size	Function
Non-coding elements		
Long control region (LCR)	500-1000 bp	Origin of replication and regulation of HPV gene expression
Early proteins		
E1	65-85 kD	Helicase function; essential for viral replication and control of gene expression; similar among types
E2	48 kD	Viral transcription factor; essential for viral replication and control gene transcription; genome segregation and encapsidation
E3	Unknown	Function not known; only present in a few HPVs
E1^E4	10-44 kD	Binding to cytoskeletal protein
E5	14 kD	Interaction with EGF/PDGF-receptors
E6	16-18 kD	Interaction with several cellular proteins; degradation of p53 and activation of telomerase
E7	~10 kD	Interaction with several cellular proteins; interaction with pRB and transaction of E2F-dependent promoters
E8^E2	20 kD	Long distance transcription and replication repressor protein
Late proteins		
L1	57 kD	Major capsid protein
L2	43-53 kD	Major capsid protein

Table II-2. Comparison of Hybrid Capture II and different genechip for HPV genotyping

Kit	Hybrid Capture II ®	HPVDNACHIP ®	Line blot/ linear array	AMPLICOR®	PreTect®	EASYChip®	INNO-LiPA ® HPV Genotyping	Seeplex® HPV Genotyping kits
Corporation	Digene	Biomedlab	Roche	Roche	NorChip	KingCar	Innogenetics	Gentaur
Country	USA	Korea	Switzerland	Switzerland	Norway	Taiwan	Belgium	Belgium
Target	DNA	DNA	DNA	DNA	mRNA of E6/E7	DNA	DNA	DNA
Types detected	2 kits (non-type specific)	22	27/37	13	5	39	24	5 kits (non-type specific)
HR-HPV	13 types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	15 types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 & 69)	13 types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 & 68)	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, & 68	16, 18, 31, 33 and 45	17 types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 82, 26, 53 & 66	15 types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 53 & 66	Kit 1: 6, 11, 16, 18, 31, 45; Kit 2: 31, 52, 45, 39, 18, 16; Kit 3: 53, 66, 51, 56, 35, 33; Kit 4: 68, 70, 59, 73, 58, 54; Kit 5: 40, 11, 42, 6, 43, 44.
LR-HPV or undermined risk types	5 types (6, 11, 42, 43, 44)	7types (6, 11, 34, 40, 42, 43 & 44)	14 types (6, 11, 26, 40, 42, 53, 54, 55, 57, 66, 73, 82, 83 & 84)			22 types (6, 11, 42, 43, 44, 54, 61, 70, 72, 81, 32, 37, 55, 62, 67, 69, 74, MM4, MM7, MM8, CP8061& L1AE5	9 types (6, 11, 40, 42, 43, 44, 54, 70, 74)	

16	V	V	V	V	V	V	V	V
18	V	V	V	V	V	V	V	V
31	V	V	V	V	V	V	V	V
33	V	V	V	V	V	V	V	V
35	V	V	V	V		V	V	V
39	V	V	V	V		V	V	V
45	V	V	V	V	V	V	V	V
51	V	V	V	V		V	V	V
52	V	V	V	V		V	V	V
56	V	V	V	V		V	V	V
58	V	V	V	V		V	V	V
59	V	V	V	V		V	V	V
68	V	V	V	V		V	V	V
69		V						
82			V			V	V	V
26			V			V	V	V
53			V			V	V	V
66		V	V			V	V	V
06	V		V			V	V	V
11	V		V			V	V	V
32						V		
34		V					V	
37						V		
40		V	V				V	V

42	V	V	V			V	V	V
43	V	V				V	V	V
44	V	V				V	V	V
54						V	V	V
55			V			V		
57			V					
61						V		
62						V		
67						V		
69						V		
70						V	V	V
72						V		
73			V					V
74						V	V	
CP8061[71]						V		
CP8304[81]						V		
MM4[82]			V			V		
MM7[83]			V			V		
MM8[84]			V			V		
L1AE5						V		

Table II-3. Prevalence of HPV16, 18, 52, 58 infections in Taiwan or Asia among cytologically normal women

Author, year	Country	Design	N	HPV testing	Typing	HPV	HR-HPV	HPV16	HPV18	HPV52	HPV58	HPV16/18	Note
Liaw et al., 1997	Taiwan	Case-control	420	MY PCR	Dot blot	9.5		0.7	0.2	1.4	1.2		
Sasagawa et al., 2001	Japan	4 hospital	1562	LCR-E7 PCR	RFLP	9.7		1.2	0.8	3.3	3.7		
Chan et al., 2002	Hong Kong	STD clinic	553	MY PCR	RFLP	30.6	14.8	4.9	0.9	0.2	4.3		HPV11:5.1%
Jeng et al., 2005	Northern Taiwan	Health clinics at medical center	1320	PCR	Korea chip	19.85		4.9	4.7	3.1	3.3		
Lin et al., 2006	Southern Taiwan	Local Clinics	4383	PCR	High risk chip	19.3	11.1	2.4	1.1	2.4	2.1		
Huang et al., 2008	Middle Taiwan	5 cities	1156	PCR	HPV blot	7.6		0.8	0.7	0.8	0.6		
Chao et al., 2008	Northern Taiwan	Survey in TauYuan county	10014	SPF1/GP6+	Easychip	10.8		0.36	1.2	1.4	1.12		not normal only, HPV11(0.1)
Bao et al., 2008	Asia (meta)	Meta-analysis	4872	PCR		14.3		2.6	0.7	1.5	0.9		
Clifford et al., 2005	Asia (pooled)	Pooled analysis	6100	GP5+/6+ PCR		11		1.5	0.5	0.5	0.4		no Chinese

Table II-4. Acquisition rate of HPV16, 18, 52, 58 and 11 among cytologically normal women

Author, year	Country	Cohort	age	N	F/U	HPV testing	Unit	HPV16	HPV18	HPV52	HPV58	HPV11	Remarks
Winer et al., 2006	USA		18-22 (Young)	82	34 mo	PGMY PCR							
Woodman et al., 2001	UK		15-19 (Young)	1075	3y	GP PCR	CI at 3y	10.5%	6.6%	1.0%	2.8%	4.3% (06/11)	
Oh et al., 2008	Korea	Busan	17-26 (Young)	171	NA		%	8.7%	4.1%	2.1%	1.0%		
Ho et al., 1998	USA		20 (Young)	608	2.2y	MY09/11 Southern blot	%	7%	4%	3%	3%		
Franco et al., 1999	Brazil	Ludwig-McGill	M: 33.3y	1425	M: 0.84y	MY09/11 PCR+ RFLP	%	1.4%	0.3%	1.1%	0.9%	0.7% (06/11)	
Rousseau et al., 2001	Brazil	Ludwig-McGill	18-60	1860	1y	MY09/11 PCR+ RFLP		2.5 %		0.9%	0.9%		Negative only
Munoz et al., 2004	Colombia		13-85	1610	5y		%	1.0%	0.7%	0.5%	0.7%	0.2%	
Trottier et al., 2008	Brazil	Ludwig-McGill	18-60	2462, 18555 visits	5y	MY09/11 PCR+ RFLP(39 types)	/1000 women-month	1.81	0.47	0.59	0.62	0.57 (06/11)	
Richardson et al., 2003	Canada	McGill, Montreal		635	M: 1.8y	MY09/11 PCR + Roche RLB	1y %	4.3%	1.6%			1.9%(06)	
Castle et al., 2005	Costa Rica	Guanacaste	35y-65y	7237	Md: 5.6y	MY09/11 PCR		~1%					

Table II-5. Persistence rate of HPV16, 18, 52, 58 and 11 among cytologically normal women

Author, year	Country	Cohort	age	N	F/U	HPV testing	Outcome	unit	HPV16	HPV18	HPV52	HPV58	HPV11
Ho et al., 1998	USA		20 (Young)	608	2.2y	MY09/11 Southern blot	**Clearance (persistence)**	%	72% (28%)	65% (35%)	85% (15%)	67% (33%)	
Trottier et al., 2008	Brazil	Ludwig-McGill	18-60	2462, 18555 visits	5y	MY09/11 PCR+ RFLP (39 types)	**Clearance (persistence)**	/1000 women-month	82.4 68.6% (31.6%)	91.9 76.6% (23.4%)	81.0 67.5% (32.5%)	72.1 60.1% (39.9%)	104.8 (06/11) 87.3% (12.7%)
Richardson et al., 2003	Canada	McGill, Montreal		635	M: 1.8y	MY09/11 PCR + RLB	**Persistence**	1y %	62%	40%			42% (06)
Castle et al., 2005	Costa Rica	Guanacaste	35-65y	7237	Md: 5.6y	MY09/11 PCR	**Persistence**		25-35 %				
Bulkmans et al., 2007	Netherlands	PROBASCAM	30-60	713	1.5y	GP PCR + RLB	**Persistence**		51%	45%	33%	22%	
Cuschieri et al., 2005	UK		<21~>45	126	M: 2.2	GP PCR	**Persistence**		48.5%	20.0%	16.7%	33.3%	
Hinchliffe et al., 1995	UK			366			**Clearance**		93% clearance				
Evander et al., 1995	Sweden		19-25	276	2y	MY09/11 PCR	**Clearance**		80% clearance				
Hildesheim et al., 1994	USA	Kaiser	16-94	393	1.24y	MY09/11 PCR	**Persistence**		Age>30: 65% vs. age<=24: 32%				

Table II-6. Viral load associated with cervical neoplasia in longitudinal study

Author, year		Design	F/U	N	Specimen	Method	HPV type	Baseline status	Outcome	Viral load
Josefsson et al., 2000	+	Nested-case control	7.8y	478/608	Stained-Pap smear	RT PCR	16		CIS	HPV negative: (ref.) Ct(45.26-50.0): OR=1.9 Ct(42.08-45.26): OR=7.2 Ct(38.7-42.08): OR=22.8 Ct(35.9-38.7): OR=18.9 Ct(<35.9): OR=59.0
Ylitalo et al., 2000	+	Nested-case control	15y	478/608	Stained-Pap	RT PCR	16		CIS	H(Ct<39.6): CI=22.7% M(Ct39.6-45.6): CI=6.6%
Van Duin et al., 2002	+	Nested-case control		12/47	Cervical cell	RT-PCR	16		CIN2/3	>2.4*10^6: OR=7.7
Castle et al., 2002	+	Nested-case control	5y		Cervical cell	HCII (pg/ml)		Normal	ASCUS+	<10: CI= 4% 10-10^2: CI=11% >10^2: CI=33%
Moberg et al., 2005	+	Nested-case control	1-28	62/501	Cervical cell	RT PCR	16,31,18/45	Abnormal included	ICC	HPV16: 0: OR=1; 0-0.44: Or=5.6 0.44-2.47: OR=7.7 2.47-18.22: OR=18.5 >18.22: OR=51.0
Peitsaro et al., 2002	+	longitudinal		24		RT-PCR	16			Disease progression

Author, year		Design	F/U	N	Specimen	Method	HPV type	Baseline status	Outcome	Viral load
Lorincz et al., 2002	-	longitudinal	10y	2941	Cervical cell	HCII/RLU (pg/ml)		<CIN3	CIN3+	1-10: RR=1.0 10-10^2: RR=1.7* 10^2-10^3: RR=1.3(NS) >=10^3: RR=0.9(NS)
Dalstein et al., 2003	+	longitudinal	1.5y	647		HCII/RLU (pg/ml)		Normal/ ASCUS/ LSIL	CIN2/3+	<10: CI= 4% 10-10^2: CI=11% >10^2: CI=33%
Monnier-Benoit et al., 2006	+	longitudinal	1.5y	38	Cervical cell	RT-PCR	16	Abnormal included	CIN2/3	<200*10^3: CI=14% <200*10^3: CI=48%
Molano et al., 2003	-	longitudinal	5y	227		PCR-EIA		Normal	Clearance	I: OR=1; II: OR=1.24; III:OR=0.89; IV: OR=0.60(*); V: OR= 1.11
Wang et al., 2004			5-7y			IgG	16			
Gravitt et al., 2007	+	longitudinal	7y	225	Cervical cell	Qualitative index (1-5) of dot blot	16	Normal	CIN2+	High(4-5) vs. Low(1-3) OR=2.6(*)
Castle et al., 2005	+	longitudinal	10y	704	Cervical cell	HCII (pg/ml)	16	Normal	CIN3+	0.6-1.33: RR=1 1.34-4.32: RR=2.4 4.33-21.11: RR=2.1 >21.12: RR=3.6(*)

Author, year		Design	F/U	N	Specimen	Method	HPV type	Baseline status	Outcome	Viral load
Schlecht et al., 2003	+	longitudinal	8y	417	Cervical cell	RT-PCR (copy/cell)		Normal	Any SIL	<1: OR=1; 1-10: OR=1.9(NS); 10-10^2: OR=3.4(*); 10^2-10^3: OR=2.9(*); >10^3: OR=4.5(*)
Ho et al., 2006	+	longitudinal	0.5y	65		RT-PCR	16,18,52,58	LSIL	HSIL	45%
Wensveen et al., 2005	-	longitudinal	1.5y	148		PCR				Viral load associated abnormal , but not predict Pap abnormality
Ho et al.,1995	+	longitudinal					1,5			serology

Table II-7. Integration associated with cervical neoplasia among cytological normal

Author, year	Design	N	Specimen	Method	HPV type	E2/E6 Ratio	Diagnosis	Normal	CIN1	CIN2	CIN3	Cancer
Briolat et al., 2007	Cross-sectional	122	Cervical smear	RT PCR	16	I:0.001-0.003 M:0.004-1 E:>1	Histology	E:71.4% (n=7)	E:33.3% (n=15)	E:34.4% (n=32)	E:26.2% (n=65)	E:0% (n=3)
Huang et al., 2007	Cross-sectional	101	paraffin-embedded tissue	RT PCR	16 (n=69)	PI:0 (pure) M:0-1 E:>1	Histology		PI: 33.3% E: 16.7% (n=6)	PI: 27.3% E: 9.1%(n=11)		PI: 25.7% E: 17.1% (CCI: n=35) PI: 47.1% E: 5.9% (CCII+: n=17)
Huang et al., 2008	Cross-sectional	101	paraffin-embedded tissue	RT PCR	18 (n=32)	PI:0 (pure) M:0-1 E:>1	Histology		PI: 16.7% E: 50.0% (n=6)	PI:44..4% E: 44.4%(n=9)		PI: 35.9% E: 35.7% (CCI: n=14) PI: 33.3% E: 66.9% (CCII+: n=3)
Cricca et al., 2008	Cross-sectional	166	Cervical cell	RT PCR	16	PI: 0 (pure) M:0-0.93 E:>0.93	Histology		E:72.2% PI: 0% (n=72)	E: 20.5% PI: 6.0% (n=83)		E: 0% PI: 81.8% (n=11)
Guo et al., 2007	Cross-sectional	42	paraffin-embedded tissue	RT PCR	16	I:0.6 M:0.7-1 E:>1	Histology			E: 14.0% I: 18.0% (n=22)		E: 0% I: 35% (n=20)
De Marco et al., 2007	Cross-sectional	52	Cervical cell	DIPS PCR	16	PI:0 (pure) M:0-1 E:>1	Histology	E: 92% (n=12)	E: 73% PI: 9% (n=11)	E: 69% PI: 0% (n=13)	E: 61% PI: 15% (n=13)	M: 100% (n=1)

Author, year	Design	N	Specimen	Method	HPV type	E2/E6 Ratio	Diagnosis	Normal	CIN1	CIN2	CIN3	Cancer
Cheung et al., 2008	Cross-sectional	91	Cervical cell	RT PCR 4 regions of E2	52	PI:0 (pure) M:0-1 E:>1	Histology	E: 62.5% PI: 20.8% (n=24)		E: 55.0% PI: 10.0% (n=20)	E: 44.4% PI: 7.4% (n=27)	E: 10.0% PI: 25.0% (n=20)
Chan et al., 2007	Cross-sectional	75	Cervical cell	RT PCR	58	PI:0 (pure)	Histology	(n=15) PI:20%	(n=15) PI:20%	(n=15) PI:6.7%	(n=15) PI:20%	(n=15) PI:20%
Ho et al., 2006	Cross-sectional	152	Cervical cell	RT PCR	16,18,52,58	PI: 0 M:0-1 E:>1	Histology			($n_{16,18,52,58}$=25,12,13, 10) PI:22%(16);16%(18); 16%(52); 0%(58) E:55%(16); 63%(18); 8%(52); 88%(58)		($n_{16,18,52,58}$=23,10,12,8) PI:20%(16);50%(18);10 %(52);12.5%(58) E:17%(16); 0%(18); 75%(52); 88%(58)
Andersson et al., 2005	Cross-sectional	166	paraffin-embedded tissue	RT PCR	16	PI: 0 (pure)	Histology		PI: 25% (n=8)	PI: 7% (n=15)	PI: 36% (n=25)	
Peitsao et al., 2002	Cross-sectional	31	paraffin-embedded tissue	RT PCR	16	PI: 0 (pure) I+M:<1 E:>1	Histology		Development the new method to detect integration Integration associated with progression			

Table II-8. Risks of HPV infection to developing cervical neoplasia among cytologically normal women

Author, year	Country	Cohort	age	N	HPV testing	Year of F/U	F/U	Outcome	OR	Cumulative Incidence	Remark
Liaw et al., 1999	USA	Kaiser	16-94	380/ 1037	MY PCR	5y	Annual cytology	LSIL HSIL	OR: 44.4 (L) OR: 67.1 (H)		
Wallin et al., 1999	Sweden		NA	118 sets CS/CN	GP PCR	5.6 (Md)	Pap bank	Cancer	OR: 16.4		
Ylitalo et al., 2000	Sweden	Uppsala	≥30	140 sets CS/CN	HPV16		Pap bank	Carcinoma in situ	OR: 5.3		
Cuzick et al., 2008	UK		≥35	2111	HCII	9	Data linkage	CIN2+	OR: 6.5		Abnormal included
Castle et al., 2002	USA	Kaiser	16-94	2020	HCII	4.75	Annual cytology	≥ASCUS		HR(+): 16.8% HR(-): 4.2%	
Kjaer et al., 2006	Denmark		22-32	7218	HCII	10.6	Data linkage	CIN2+ CIN3+	OR: 4.4 OR: 4.4		
Kjaer et al., 2006	Denmark		40-50	1305	HCII	10.0	Data linkage	CIN2+ CIN3+	OR: 12.1 OR: 12.5		
Kjaer et al., 2002	Denmark		20-29	742	GP PCR HPV(HR)	4	Data linkage	Atypia, LSIL, HSIL	OR: 3.2 (4.9) OR: 7.5 (9.3) OR: 25.8 (34.5)		
Khan et al., 2005	USA	Kaiser	≥30	13229	HCII HPV16 HPV18	10	Annual cytology	CIN3+(H) or cancer		HPV16: 20.7% HPV18: 17.7% HCII(-): 0.5%	

Author, year	Country	Cohort	age	N	HPV testing	Year of F/U	F/U	Outcome	OR	Cumulative Incidence	Remark
Peto et al., 2004	UK	Manchester	15-69	6128 (232)	MY09/11 PCR	14				HR-HPV: 28%	
Sherman et al., 2003	USA	Kaiser	16-94	20800	MY PCR	12.1	Annual cytology	CIN3+ or cancer	OR: 8.0	HPV(+): 6.92% HPV(-): 0.87%	Abnormal included
Huang et al., 2008	Taiwan		<30~>60	1310	SPF1/GP6 PCR	6	Data linkage	HSIL+	OR: 24.5	HPV(+) :10.0% HPV(-): 0.34%	
Woodman et al., 2001	UK		15-19	1075 HPV+: 40.7% HPV16: 10%	GP PCR	3	Cytology every 6m	HSIL	HPV: 7.8(*) HPV16: 8.5(*) HPV18: 3.3(*) HPV31: 3.5(*) HPV33: 0.6 HPV52: 2.3 HPV58: 2.9 HPV6/11: 3.8(*)	HPV: 43.8% HPV16: 10.5% HPV18: 6.6% HPV31: 3.1% HPV33: 3.3% HPV52: 1.0% HPV58: 2.8% HPV6/11: 3.8(*)	HPV52 had longer median duration High HPV+ and HPV16+
Naucler et al., 2007	Sweden		32-38	5696	GP PCR	4.1	Data linkage	CIN2+	HPV16: 17.4 HPV18: 11.3 HPV31: 20.1 HPV33: 27.8 HPV52: 10.9 HPV58: 13.4		

Table II-9. Risks of HPV persistence infection to cervical neoplasia among cytologically normal women

Author, year	Country	Cohort	age	N	Year of F/U		HPV testing	Outcome	Findings	Cumulative Incidence	
Kjaer et al., 2002	Denmark		20-29	742	4		GP PCR	Atypia, LSIL, HSIL	OR: 7.4 OR: 83.8 OR : 413.9		
Kjaer et al., 2006	Denmark		22-32	7218	10.6	Data linkage	HCII	CIN2+		HR(+): 20% HR(-): 2.3%	
Wallin et al., 1999 (nested-case control study)	Sweden			104 sets CS/CN	5.6		GP PCR		OR: 213.4		
Ylitalo et al., 2000 (nested-case control study)	Sweden	Uppsala	15-49	263sets CS/CN			HPV16	Carcinoma in situ	OR: 31.2		
Schlecht et al., 2001	Brazil			1611	6	Cytology every 6m	MY PCR HPV16/ 18 persistence	SIL+ HISL	11.15 12.27	SIL+: HPV16/18:40% Negative: 5%	SIL+: I: $72.3*10^{-5}$ I: $6.1*10^{-5}$

Table III-1. Genotype-specific HPV prevalence at baseline by cytological findings

HPV status at baseline	Total (n=10602)		Baseline cytology										cancer cases (<=1 yr) (n=56)	
			Normal (n=10190)		ASCUS (n=136)		LSIL (n=121)		HSIL (n=136)		SCC (n=19)			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Negative	8008	75.5	7916	77.7	67	49.3	23	19.0	2	3.3	0	0.0	1	1.8
Positive	2594	24.5	2274	22.3	69	50.7	98	81.0	134	98.5	19	100.0	55	98.2
Any positive of 39 types	2524	23.8	2208	21.7	67	49.3	96	79.3	134	98.5	19	100.0	55	98.2
Equivocal typing	17	0.2	17	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Untyped	53	0.5	49	0.5	2	1.5	2	1.7	0	0.0	0	0.0	0	0.0
Among positve of 39 types	2524		2208		67		96		134		19		55	
PCR (+)	1645	65.2	1344	60.9	58	86.6	90	93.8	134	100.0	19	100.0	55	100.0
PCR (-)	879	34.8	864	39.1	9	13.4	6	6.3	0	0.0	0	0.0	0	0.0
Single type	1776	70.4	1602	72.6	33	49.3	57	59.4	75	56.0	9	47.4	31	56.4
Multiple types	748	29.6	606	27.4	34	50.7	39	40.6	59	44.0	10	52.6	24	43.6
2 types	484	19.2	396	17.9	23	34.3	25	26.0	33	24.6	7	36.8	14	25.5
3 types	167	6.6	132	6.0	6	9.0	9	9.4	18	13.4	2	10.5	6	10.9
4 types	51	2.0	41	1.9	2	3.0	3	3.1	4	3.0	1	5.3	2	3.6
5 types	21	0.8	16	0.7	1	1.5	0	0.0	4	3.0	0	0.0	2	3.6
6 types	12	0.5	9	0.4	1	1.5	2	2.1	0	0.0	0	0.0	0	0.0
7 types	3	0.1	3	0.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
8 types	5	0.2	4	0.2	1	1.5	0	0.0	0	0.0	0	0.0	0	0.0
9 types	2	0.1	2	0.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
11 types	2	0.1	2	0.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
15 types	1	0.0	1	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
High-risk type (17 types)	1527	14.4	1229	12.1	60	44.1	88	72.7	131	96.3	19	100.0	54	98.2
Low-risk type (22 types)	1417	13.4	1335	13.1	24	17.6	24	19.8	28	20.6	11	20.0	11	20.0
High-risk type (13 types)	1421	13.4	1134	11.1	59	43.4	82	67.8	127	93.4	19	100.0	54	98.2
HPV16/18/52/58/33/31	979	9.2	734	7.2	54	39.7	54	44.6	119	87.5	18	94.7	52	94.5
HPV16/18/52/58	822	7.8	607	6.0	45	33.1	47	38.8	107	78.7	16	84.2	47	85.5
HPV16/18	445	4.2	344	3.4	16	11.8	23	19.0	52	38.2	10	52.6	28	50.9
High-risk (HR-) type														
HPV16	249	2.3	170	1.7	9	6.6	16	13.2	44	32.4	10	52.6	27	49.1
HPV18	226	2.1	198	1.9	9	6.6	8	6.6	10	7.4	1	5.3	2	3.6
HPV31	85	0.8	59	0.6	7	5.1	4	3.3	14	10.3	1	5.3	5	9.1
HPV33	164	1.5	134	1.3	6	4.4	7	5.8	16	11.8	1	5.3	5	9.1
HPV35	55	0.5	42	0.4	1	0.7	5	4.1	7	5.1	0	0.0	2	3.6
HPV39	113	1.1	96	0.9	3	2.2	10	8.3	3	2.2	1	5.3	1	1.8

Table III-1 (cont.)

HPV status at baseline	Total (n=10602)		Baseline cytology: Normal (n=10190)		ASCUS (n=136)		LSIL (n=121)		HSIL (n=136)		SCC (n=19)		cancer cases (<=1 yr) (n=56)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
HPV45	71	0.7	63	0.6	1	0.7	4	3.3	2	1.5	1	5.3	1	1.8
HPV51	100	0.9	77	0.8	6	4.4	10	8.3	7	5.1	0	0.0	2	3.6
HPV52	297	2.8	222	2.2	22	16.2	17	14.0	32	23.5	4	21.1	11	20.0
HPV53	145	1.4	116	1.1	6	4.4	11	9.1	11	8.1	1	5.3	3	5.5
HPV56	222	2.1	205	2.0	2	1.5	9	7.4	6	4.4	0	0.0	2	3.6
HPV58	149	1.4	92	0.9	8	5.9	10	8.3	35	25.7	4	21.1	14	25.5
HPV59	30	0.3	20	0.2	4	2.9	4	3.3	2	1.5	0	0.0	1	1.8
HPV66	37	0.3	27	0.3	3	2.2	6	5.0	1	0.7	0	0.0	0	0.0
HPV68	83	0.8	67	0.7	4	2.9	5	4.1	6	4.4	1	5.3	4	7.3
HPV26	18	0.2	14	0.1	2	1.5	0	0.0	2	1.5	0	0.0	1	1.8
HPV82	7	0.1	7	0.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Low-risk (LR-) type														
HPV11	638	6.0	621	6.1	6	4.4	5	4.1	5	3.7	1	5.3	3	5.5
HPV06	34	0.3	32	0.3	0	0.0	1	0.8	1	0.7	0	0.0	0	0.0
HPV42	22	0.2	20	0.2	2	1.5	0	0.0	0	0.0	0	0.0	0	0.0
HPV43	63	0.6	60	0.6	1	0.7	2	1.7	0	0.0	0	0.0	0	0.0
HPV44	87	0.8	86	0.8	0	0.0	0	0.0	1	0.7	0	0.0	0	0.0
HPV54	103	1.0	88	0.9	4	2.9	5	4.1	4	2.9	2	10.5	3	5.5
HPV61	42	0.4	40	0.4	0	0.0	0	0.0	2	1.5	0	0.0	0	0.0
HPV72	76	0.7	66	0.6	4	2.9	1	0.8	4	2.9	1	5.3	1	1.8
HPV37	2	0.0	1	0.0	0	0.0	0	0.0	1	0.7	0	0.0	0	0.0
HPV32	25	0.2	24	0.2	0	0.0	1	0.8	0	0.0	0	0.0	0	0.0
HPV55	37	0.3	33	0.3	0	0.0	2	1.7	2	1.5	0	0.0	0	0.0
HPV62	70	0.7	66	0.6	1	0.7	2	1.7	1	0.7	0	0.0	0	0.0
HPV67	32	0.3	30	0.3	1	0.7	0	0.0	1	0.7	0	0.0	0	0.0
HPV69	34	0.3	32	0.3	0	0.0	2	1.7	0	0.0	0	0.0	0	0.0
HPV70	100	0.9	90	0.9	5	3.7	2	1.7	2	1.5	1	5.3	2	3.6
HPV74	17	0.2	17	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
MM4	27	0.3	22	0.2	1	0.7	1	0.8	3	2.2	0	0.0	1	1.8
MM7	1	0.0	1	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
MM8	97	0.9	87	0.9	3	2.2	4	3.3	2	1.5	1	5.3	2	3.6
CP8061	78	0.7	73	0.7	2	1.5	2	1.7	1	0.7	0	0.0	0	0.0
CP8304	101	1.0	94	0.9	1	0.7	2	1.7	3	2.2	1	5.3	1	1.8
L1AE5	4	0.0	4	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

Table III-2. Risk factors associated with HPV infection at baseline

Factors	Total (n=10602)		HPV positive at baseline (n=2594)				
	n	%	n	%	OR	aOR (95% CI)	mOR (95% CI)
Demographic characteristics							
Age at baseline							
30-34	1435	13.5	318	22.2	1.0		
35-39	1909	18.0	438	22.9	1.0	0.9 - 1.2	
40-44	1684	15.9	389	23.1	1.1	0.9 - 1.2	
45-49	1347	12.7	334	24.8	1.2	1.0 - 1.4	
50-54	1606	15.1	393	24.5	1.1	1.0 - 1.3	
55-59	1497	14.1	418	27.9	1.4	1.2 - 1.6)**	
60+	1124	10.6	304	27.0	1.3	1.1 - 1.6)*	
p for trend					<0.001		
Marital status at baseline							
Currently married	9798	92.4	2383	24.3	1.0	1.0	
Widowed/Divorced/Others	796	7.5	208	26.1	1.1	1.0 (0.9 - 1.2)	
Unknown	8	0.1	3	37.5	1.9	2.0 (0.5 - 8.3)	
No. of marriage							
0-1	10379	97.9	2537	24.4	1.0	1.0	
2+	153	1.4	46	30.1	1.3	1.3 (0.9 - 1.9)	
Unknown	70	0.7	11	15.7	0.6	0.6 (0.3 - 1.1)	
Lifestyle habit							
Cigarette smoking							
Never	10449	98.6	2541	24.3	1.0	1.0	
Ever	108	1.0	41	38.0	1.9	1.9 (1.3 - 2.9)**	1.8 (1.2 - 2.7)*
Unknown	45	0.4	12	26.7	1.1	1.2 (0.6 - 2.2)	
Alcohol drinking							
Never	10491	99.0	2555	24.4	1.0	1.0	
Ever	67	0.6	26	38.8	2.0	2.0 (1.2 - 3.3)*	
Unknown	44	0.4	13	29.5	1.3	1.3 (0.7 - 2.5)	
Betal chewing							
Never	10538	99.4	2575	24.4	1.0	1.0	
Ever	18	0.2	6	33.3	1.5	1.6 (0.6 - 4.2)	
Unknown	46	0.4	13	28.3	1.2	1.2 (0.7 - 2.4)	
Pregnancy, delivery & contraception							
No. of lifetime childbirth							
0-3	2665	25.1	597	22.4	1.0	1.0	
4	2104	19.8	494	23.5	1.1	1.0 (0.9 - 1.2)	
5	2046	19.3	505	24.7	1.1	1.1 (0.9 - 1.3)	
6	1513	14.3	402	26.6	1.3	1.2 (1.0 - 1.4)*	
7	931	8.8	226	24.3	1.1	1.0 (0.9 - 1.2)	
8	564	5.3	146	25.9	1.0	0.9 (0.8 - 1.2)	
9+	550	5.2	171	31.1	1.6	1.4 (1.1 - 1.8)*	
Unknown	229	2.2	53	23.1			
p for trend					<0.001		
No. of vaginal delivery							
0-2	2121	20.0	501	23.6	1.0	1.0	
3	3021	28.5	683	22.6	0.9	0.9 (0.8 - 1.0)	
4	2380	22.4	626	26.3	1.2	1.1 (0.9 - 1.2)	
5+	2881	27.2	742	25.8	1.1	1.0 (0.8 - 1.1)	
Unknown	199	1.9	42	21.1	0.9	0.8 (0.6 - 1.2)	
p for trend					0.006		
No. of cesarean section							
0	3887	36.7	905	23.3	1.0	1.0	
1	3208	30.3	790	24.6	1.1	1.1 (1.0 - 1.2)	
2	2024	19.1	503	24.9	1.1	1.1 (1.0 - 1.2)	
3+	1257	11.9	343	27.3	1.2	1.2 (1.1 - 1.4)*	
Unknown	226	2.1	53	23.5	1.0	1.0 (0.7 - 1.4)	
p for trend					0.006		
Abortion							
Never	9665	91.2	2389	24.7	1.0	1.0	
Ever	707	6.7	151	21.4	0.8	0.9 (0.7 - 1.1)	
Unknown	230	2.2	54	23.5	0.9	0.9 (0.7 - 1.3)	
Intra-uterine device use							
Never	4286	40.4	963	22.5	1.0	1.0	
Ever	6253	59.0	1621	25.9	1.2	1.2 (1.1 - 1.3)**	1.2 (1.1 - 1.3)**
Unknown	63	0.6	10	15.9	0.7	0.6 (0.3 - 1.3)	
Oral contraceptives use							
Never	7281	68.7	1781	24.5	1.0	1.0	
Ever	3251	30.7	801	24.6	1.0	1.1 (1.0 - 1.2)	
Unknown	70	0.7	12	17.1	0.6	0.6 (0.3 - 1.2)	
Oral contraceptives use (Current)							
Never use+not now	10146	95.7	2481	24.5	1.0	1.0	
Yes	326	3.1	89	27.3	1.2	1.3 (1.0 - 1.6)	
Unknown	130	1.2	24	18.5	0.7	0.7 (0.4 - 1.1)	

Table III-2. (cont.)

Factors	Total (n=10602)	HPV positive at baseline (n=2594)			
	n (%)	n (%)	OR	aOR (95% CI)	mOR (95% CI)
Menstration					
Age at menarche					
14-16	5765 (54.4)	1385 (24.0)	1.0	1.0	
~13	1342 (12.7)	316 (23.5)	1.0	1.0 (0.9 - 1.2)	
17+	3348 (31.6)	861 (25.7)	1.1	1.0 (0.9 - 1.1)	
Unknown	147 (1.4)	32 (21.8)	0.9	0.9 (0.6 - 1.3)	
Menopause					
No	6707 (63.3)	1538 (22.9)	1.0	1.0	
Yes	3857 (36.4)	1050 (27.2)	1.3	1.3 (1.1 - 1.5)*	1.2 (1.1 - 1.5)*
Unknown	38 (0.4)	6 (15.8)			
Age at menopause	3857	1050			
<=45	536 (13.9)	133 (24.8)	1.0	1.0	
46-50	1670 (43.3)	461 (27.6)	1.2	1.2 (0.9 - 1.5)	
51-55	1282 (33.2)	365 (28.5)	1.2	1.2 (1.0 - 1.6)	
56+	164 (4.3)	43 (26.2)	1.1	1.1 (0.7 - 1.6)	
Unknown	205 (5.3)	48 (23.4)	0.9	0.9 (0.6 - 1.4)	
p for trend			0.261		
Sexual behavior					
Age at initial coitus					
14-21	4077 (38.5)	1099 (27.0)	1.0	1.0	
22-23	2762 (26.1)	666 (24.1)	0.9	0.9 (0.8 - 1.0)*	
24+	3655 (34.5)	812 (22.2)	0.8	0.8 (0.7 - 0.9)**	
Unknown	108 (1.0)	17 (15.7)	0.5	0.5 (0.3 - 0.9)*	
p for trend			<0.001		
No. of lifetime sexual partners					
0-1	10250 (96.7)	2480 (24.2)	1.0	1.0	1.0
2+	262 (2.5)	99 (37.8)	1.9	1.9 (1.5 - 2.5)**	1.8 (1.4 - 2.3)**
Unknown	90 (0.8)	15 (16.7)	0.6	0.6 (0.4 - 1.1)	
Premarital sex					
No	10261 (96.8)	2522 (24.6)	1.0	1.0	
Yes	230 (2.2)	55 (23.9)	1.0	1.0 (0.8 - 1.4)	
Unknown	111 (1.0)	17 (15.3)	0.6	0.5 (0.3 - 0.9)*	
Pap smear expirence					
Pap smear					
Never	9118 (86.0)	2225 (24.4)	1.0	1.0	
Ever	1456 (13.7)	360 (24.7)	1.0	1.0 (0.9 - 1.2)	
Unknown	28 (0.3)	9 (32.1)	1.5	1.5 (0.7 - 3.2)	
Cytology at baseline					
Normal	10190 (96.1)	2274 (22.3)	1.0	1.0	1.0
Abnormal	412 (3.9)	320 (77.7)	12.1	12.2 (9.6 - 15.4)**	12.8 (10.0 - 16.3)**

aOR: age-adjusted odds ratio; mOR: multivariate-adjusted odds ratio.
*: p<0.05; **: p<0.001.

Table III-3. Attributable risk percent and population attributable risk percent of cytological LSIL+ or abnormality for different combinations of HPV types at baseline

	Population		Cervical cancer case					LSIL+					Abnormal cytology				
HPV status at baseline	n	%	no	OR	95%C.I.	AR%	PAR%	no	OR	95%C.I.	AR%	PAR%	no	OR	95%C.I.	AR%	PAR%
Negative	8008	75.5	1	1	referent			25	1	referent			92	1	referent		
all types	2594	24.5	55	173	30 - ∞	99	98	251	34	23 - 52	97	89	320	12	10 - 15	92	73
HR-17 type	1527	14.4	54	293	50 - ∞	100	98	238	59	40 - 89	98	89	298	21	16 - 27	95	74
LR-22 type(HR-17 not included)	997	9.1	1	8	0 - 631	88		11	4	2 - 7	73	20	18	1	0 - 1		
HR-13 type	1421	13.4	54	316	54 - ∞	100	98	228	61	40 - 92	98	89	287	22	17 - 28	95	74
HPV16/18/52/58/33/31	979	9.2	52	449	80 - ∞	100	98	191	77	51 - 118	99	88	245	29	22 - 37	97	72
HPV16/18/52/58	822	7.8	47	485	83 - ∞	100	97	170	83	54 - 128	99	87	215	31	24 - 39	97	70
HPV16/18	445	4.2	28	536	88 - ∞	100	96	85	75	48 - 119	99	76	101	25	19 - 34	96	51
HPV16	249	2.3	27	970	158 - ∞	100	96	70	125	77 - 202	99	74	79	40	29 - 56	98	47
HPV18	226	2.1	2	71	4 - ∞	99	60	19	29	16 - 54	97	37	28	12	8 - 19	92	19
HPV52	297	2.8	11	307	44 - ∞	100	90	53	71	43 - 116	99	66	76	30	21 - 41	97	44
HPV58	149	1.4	14	825	124 - ∞	100	92	49	157	93 - 263	99	69	57	53	31 - 79	98	42

AR%: attributable risk percent.
PAR%: population attributable risk percent.

Table IV-1. HPV type-specific acquisition and persistence by cytological findings at baseline

Cytology and HPV at baseline	No. of both visits	HPV status at follow-up: acquisition			
		No.	%	aOR	95%CI
All subjects (n=6877)					
Cytology					
Abnormal	210	43	20.5	2.8	(2.0-4.0)
Normal	6667	563	8.4	1.0	
Cytology and HPV at baseline		**persistence/ not clearance at follow-up**			
		No.	%	aOR	95%CI
All HPV positive (n=1567)					
Cytology					
Abnormal	156	67	42.9	2.2	(1.5-3.1)
Normal	1411	391	27.7	1.0	
Cytologically normal (n=1411)					
No. of HPV infected					
1	1052	217	20.6	1.0	
2	233	93	39.9	2.3	(1.7-3.2)
3	76	48	63.2	6.2	(3.8-10.3)
4	23	13	56.5	4.0	(1.7-9.4)
5	13	10	76.9	11.2	(3.0-42.1)
>5	14	10	71.4	8.7	(2.6-28.8)
trend test				<.0001	

aOR: age-adjusted odds ratio

Table IV-2. Genotype-specific HPV acquisition rates

		Cytologically No.rmal at baseline (n=6667) duration: 1.58y (mean); 1.39 y (median)							1 y<=Duration <= 2 y (n=5010) duration: 1.39y (mean); 1.36 y (median)						Cytologically No.rmal & HPV (-) (n=3934)						Cytologically No.rmal & HPV (+) (n=1076)					
	risk	Negative	Acquisition				age-adj. rate		Acquisition				age-adj. rate		Acquisition				age-adj. rate		Acquisition				age-adj. rate	
HPV		No.	No.	%	lower	upper	world	Taiwan	No.	%	lower	upper	world	Taiwan	No.	%	lower	upper	world	Taiwan	No.	%	lower	upper	world	Taiwan
16	H	6563	48	0.7	0.6	1.0	0.8	0.7	36	0.7	0.5	1.0	0.8	0.7	23	0.6	0.4	0.9	0.6	0.6	13	1.3	0.8	2.2	1.3	1.3
52	H	6517	47	0.7	0.5	1.0	0.7	0.7	34	0.7	0.5	1.0	0.7	0.7	20	0.5	0.3	0.8	0.4	0.4	14	1.5	0.9	2.5	0.6	0.6
18	H	6542	42	0.7	0.5	0.9	0.6	0.6	30	0.6	0.4	0.9	0.6	0.6	21	0.5	0.3	0.8	0.5	0.5	9	0.9	0.5	1.8	0.9	0.9
56	H	6540	37	0.6	0.4	0.8	0.5	0.6	28	0.6	0.4	0.8	0.5	0.6	12	0.3	0.2	0.5	0.3	0.3	16	1.6	1.0	2.7	0.6	0.6
54	L	6612	28	0.4	0.3	0.6	0.4	0.4	26	0.5	0.4	0.8	0.5	0.5	12	0.3	0.2	0.5	0.1	0.2	14	1.4	0.8	2.3	0.2	0.2
MM8	L	6619	39	0.6	0.4	0.8	0.6	0.6	27	0.5	0.4	0.8	0.5	0.5	13	0.3	0.2	0.6	0.5	0.5	14	1.3	0.8	2.3	0.5	0.5
53	H	6603	36	0.6	0.4	0.8	0.5	0.5	24	0.5	0.3	0.7	0.5	0.5	17	0.4	0.3	0.7	0.3	0.3	7	0.7	0.3	1.4	1.2	1.3
44	L	6609	30	0.5	0.3	0.6	0.4	0.5	24	0.5	0.3	0.7	0.5	0.5	12	0.3	0.2	0.5	0.3	0.3	12	1.2	0.7	2.0	0.6	0.6
CP8304	L	6603	34	0.5	0.4	0.7	0.5	0.5	25	0.5	0.3	0.7	0.4	0.5	12	0.3	0.2	0.5	0.0	0.0	13	1.3	0.7	2.2	0.2	0.1
51	H	6616	24	0.4	0.3	0.6	0.4	0.4	18	0.4	0.2	0.6	0.4	0.4	11	0.3	0.2	0.5	0.5	0.5	7	0.7	0.3	1.4	1.5	1.4
45	H	6624	23	0.4	0.2	0.5	0.4	0.4	17	0.3	0.2	0.5	0.4	0.4	11	0.3	0.2	0.5	0.3	0.3	6	0.6	0.3	1.3	0.7	0.6
39	H	6605	27	0.4	0.3	0.6	0.4	0.4	20	0.4	0.3	0.6	0.4	0.4	8	0.2	0.1	0.4	0.1	0.1	12	1.2	0.7	2.0	0.6	0.5
62	L	6625	31	0.5	0.3	0.7	0.4	0.5	22	0.4	0.3	0.7	0.4	0.4	10	0.3	0.1	0.5	0.0	0.0	12	1.1	0.6	2.0	0.4	0.5
72	L	6617	24	0.4	0.2	0.5	0.4	0.4	19	0.4	0.2	0.6	0.4	0.4	15	0.4	0.2	0.6	0.0	0.1	4	0.4	0.1	1.0	0.3	0.3
58	H	6609	27	0.4	0.3	0.6	0.4	0.4	17	0.3	0.2	0.6	0.3	0.3	11	0.3	0.2	0.5	0.0	0.0	6	0.6	0.3	1.3	0.5	0.5
70	L	6607	25	0.4	0.3	0.6	0.4	0.4	16	0.3	0.2	0.5	0.3	0.3	8	0.2	0.1	0.4	0.4	0.4	8	0.8	0.4	1.5	0.3	0.4
33	H	6572	21	0.3	0.2	0.5	0.3	0.3	13	0.3	0.2	0.5	0.3	0.3	10	0.3	0.1	0.5	0.3	0.3	3	0.3	0.1	0.9	0.2	0.2
43	L	6625	16	0.2	0.1	0.4	0.3	0.3	12	0.2	0.1	0.4	0.2	0.2	5	0.1	0.1	0.3	0.3	0.3	7	0.7	0.3	1.4	1.2	1.1
68	H	6623	20	0.4	0.2	0.6	0.3	0.3	12	0.2	0.1	0.4	0.2	0.2	11	0.3	0.2	0.5	0.1	0.1	1	0.1	0.0	0.7	0.1	0.1
CP8061	L	6622	16	0.2	0.1	0.4	0.2	0.2	12	0.2	0.1	0.4	0.2	0.2	8	0.2	0.1	0.4	0.3	0.3	4	0.4	0.1	1.0	0.9	1.1
11	L	6259	15	0.3	0.2	0.5	0.2	0.2	10	0.2	0.1	0.4	0.2	0.2	7	0.2	0.1	0.4	0.2	0.2	3	0.4	0.1	1.2	0.4	0.4
42	L	6649	17	0.3	0.2	0.4	0.3	0.3	10	0.2	0.1	0.4	0.2	0.2	5	0.1	0.1	0.3	0.1	0.1	5	0.5	0.2	1.1	0.6	0.6
MM4	L	6647	10	0.2	0.1	0.4	0.2	0.2	9	0.2	0.1	0.3	0.2	0.2	4	0.1	0.0	0.3	0.0	0.0	5	0.5	0.2	1.1	0.2	0.3
31	H	6632	13	0.2	0.1	0.4	0.2	0.2	9	0.2	0.1	0.3	0.2	0.2	5	0.1	0.1	0.3	0.1	0.1	4	0.4	0.1	1.0	0.4	0.3
55	L	6650	12	0.3	0.1	0.5	0.1	0.2	9	0.2	0.1	0.3	0.1	0.2	6	0.2	0.1	0.3	0.3	0.3	3	0.3	0.1	0.9	1.6	1.6
61	L	6640	10	0.2	0.1	0.3	0.2	0.1	6	0.1	0.1	0.3	0.1	0.1	4	0.1	0.0	0.3	0.2	0.2	2	0.2	0.0	0.8	0.8	1.0
66	H	6646	12	0.2	0.1	0.4	0.2	0.2	7	0.1	0.1	0.3	0.1	0.1	1	0.0	0.0	0.2	0.1	0.1	6	0.6	0.3	1.3	0.2	0.2
59	H	6645	8	0.1	0.1	0.3	0.1	0.1	7	0.1	0.1	0.3	0.1	0.1	1	0.0	0.0	0.2	0.1	0.1	6	0.6	0.3	1.3	0.1	0.1
35	H	6640	11	0.2	0.1	0.3	0.1	0.2	7	0.1	0.1	0.3	0.1	0.1	3	0.1	0.0	0.2	0.1	0.1	4	0.4	0.1	1.0	0.3	0.3
82	H	6658	6	0.1	0.0	0.2	0.1	0.1	6	0.1	0.1	0.3	0.1	0.1	2	0.1	0.0	0.2	0.2	0.2	4	0.4	0.1	1.0	0.4	0.3
06	L	6645	9	0.1	0.1	0.3	0.1	0.1	5	0.1	0.0	0.2	0.1	0.1	1	0.0	0.0	0.2	0.0	0.0	4	0.4	0.1	1.0	0.4	0.4
74	L	6650	8	0.1	0.1	0.3	0.1	0.1	6	0.1	0.1	0.3	0.1	0.1	2	0.1	0.0	0.2	0.0	0.1	4	0.4	0.1	1.0	0.4	0.4
67	L	6646	6	0.1	0.1	0.3	0.1	0.1	5	0.1	0.0	0.2	0.1	0.1	2	0.1	0.0	0.2	0.3	0.3	3	0.3	0.1	0.9	0.1	0.1
26	H	6650	8	0.2	0.1	0.3	0.1	0.1	5	0.1	0.0	0.2	0.1	0.1	1	0.0	0.0	0.2	0.0	0.0	4	0.4	0.1	1.0	0.3	0.3
MM7	L	6662	5	0.1	0.1	0.3	0.1	0.1	5	0.1	0.0	0.2	0.1	0.1	2	0.1	0.0	0.2	0.3	0.3	3	0.3	0.1	0.9	1.2	1.3
32	L	6646	7	0.2	0.1	0.4	0.1	0.1	4	0.1	0.0	0.2	0.1	0.1	1	0.0	0.0	0.2	0.0	0.0	3	0.3	0.1	0.9	0.3	0.3
L1AE5	L	6660	3	0.1	0.0	0.4	0.1	0.0	2	0.0	0.0	0.2	0.1	0.0	1	0.0	0.0	0.2	0.1	0.1	1	0.1	0.0	0.7	0.5	0.5
69	L	6641	7	0.2	0.1	0.4	0.1	0.1	4	0.1	0.0	0.2	0.1	0.1	3	0.1	0.0	0.2	0.2	0.2	1	0.1	0.0	0.7	0.8	0.8
37	L	6661	0	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.2	0.2	0	0.0	0.0	0.0	0.0	0.0

Table IV-3. Genotype-specific HPV persistence rates

		Entire cohort (n=6877) duration: 1.58y (mean); 1.40 (median)							Cytologically normal at baseline (n=6667) duration: 1.58y (mean); 1.39y (median)							Normal & 1 year <=Duration <= 2 years (n=5010) duration: 1.39y (mean); 1.36y (median)						
				PersisteNo.t rate			age-adj. rate				PersisteNo.t rate			age-adj. rate				PersisteNo.t rate(P%)			age-adj. rate	
HPV		No.	p	%	lower	upper	world	TaiwaNo.	No.	p	%	lower	upper	world	TaiwaNo.	No.	p	%	lower	upper	world	TaiwaNo.
58	H	83	45	54.2	40.5	72.6	49.6	49.1	58	35	54.2	40.5	72.6	49.4	49.0	48	30	62.5	43.7	89.4	51.6	52.0
52	H	189	100	52.9	43.5	64.4	51.6	51.0	150	81	52.9	43.5	64.4	51.5	51.3	112	59	52.7	40.8	68.0	49.9	48.6
70	L	60	30	50.0	35.0	71.5	48.4	48.2	55	26	50.0	35.0	71.5	41.9	41.1	42	19	45.2	28.9	70.9	47.4	45.3
39	H	73	24	32.9	22.0	49.1	30.6	31.5	62	23	32.9	22.0	49.1	36.4	37.4	45	19	42.2	26.9	66.2	44.8	44.9
72	L	49	19	38.8	24.7	60.8	33.2	33.6	45	19	38.8	24.7	60.8	36.5	37.6	33	17	51.5	32.0	82.9	42.1	43.0
68	H	51	17	33.3	20.7	53.6	28.6	31.9	44	16	33.3	20.7	53.6	32.5	35.5	33	13	39.4	22.9	67.8	38.3	43.9
44	L	54	27	50.0	34.3	72.9	33.6	38.1	53	26	50.0	34.3	72.9	33.1	37.7	43	22	51.2	33.7	77.7	36.3	41.6
MM8	L	48	17	35.4	22.0	57.0	28.6	30.4	43	15	35.4	22.0	57.0	29.3	30.2	34	12	35.3	20.0	62.1	31.0	32.0
53	H	77	29	37.7	26.2	54.2	30.8	32.9	64	25	37.7	26.2	54.2	34.3	36.2	50	18	36.0	22.7	57.1	30.9	32.8
54	L	65	21	32.3	21.1	49.6	28.8	28.6	55	18	32.3	21.1	49.6	28.3	29.1	42	13	31.0	18.0	53.3	30.0	30.4
51	H	64	17	26.6	16.5	42.7	22.5	22.6	51	13	26.6	16.5	42.7	20.6	21.5	38	10	26.3	14.2	48.9	23.2	22.6
CP8304	L	60	21	35.0	22.8	53.7	20.9	25.1	59	21	35.0	22.8	53.7	21.0	25.3	44	14	31.8	18.8	53.7	21.7	26.5
18	H	137	25	18.2	12.3	27.0	16.6	17.0	125	24	18.2	12.3	27.0	17.1	17.5	92	19	20.7	13.2	32.4	19.4	19.3
16	H	135	29	21.5	14.9	30.9	18.5	20.4	104	21	21.5	14.9	30.9	16.8	19.3	77	17	22.1	13.7	35.5	17.8	20.7
33	H	107	18	16.8	10.6	26.7	13.3	14.5	95	14	16.8	10.6	26.7	11.9	12.7	67	10	14.9	8.0	27.7	12.7	13.2
45	H	48	7	14.6	7.0	30.6	9.2	10.9	43	6	14.6	7.0	30.6	8.4	10.1	33	4	12.1	4.5	32.3	7.6	9.5
56	H	138	16	11.6	7.1	18.9	9.1	9.7	127	10	11.6	7.1	18.9	6.9	7.1	106	8	7.5	3.8	15.1	6.9	6.8
11	L	413	2	0.5	0.1	1.9	0.6	0.5	403	2	0.5	0.1	1.9	0.6	0.5	323	2	0.6	0.2	2.5	0.8	0.7
CP8061	L	42	20	47.6	30.7	73.8	38.5	43.5	40	18	47.6	30.7	73.8	37.1	41.8	29	14	48.3	28.6	81.5	43.5	50.5
31	H	50	22	44.0	29.0	66.8	41.4	40.5	35	17	44.0	29.0	66.8	42.7	41.3	29	13	44.8	26.0	77.2	40.6	39.5
62	L	38	13	34.2	19.9	58.9	34.7	37.7	37	13	34.2	19.9	58.9	34.9	37.8	27	11	40.7	22.6	73.6	30.2	38.3
43	L	39	6	15.4	6.9	34.2	12.9	14.3	37	6	15.4	6.9	34.2	13.7	15.0	23	3	13.0	4.2	40.4	10.3	13.2
61	L	22	11	50.0	27.7	90.3	43.2	37.3	22	11	50.0	27.7	90.3	43.2	37.3	18	8	44.4	22.2	88.9	18.5	20.3
35	H	34	7	20.6	9.8	43.2	11.6	13.1	27	5	20.6	9.8	43.2	7.6	8.4	17	4	23.5	8.8	62.7	10.2	11.3
69	L	22	6	27.3	12.3	60.7	9.2	10.3	21	6	27.3	12.3	60.7	10.0	11.1	13	5	38.5	16.0	92.4	11.4	12.7
32	L	17	6	35.3	15.9	78.6	23.2	29.6	16	6	35.3	15.9	78.6	23.2	29.6	12	5	41.7	17.3	100.1	25.9	32.4
26	H	14	4	28.6	10.7	76.1	26.7	30.4	12	3	28.6	10.7	76.1	25.4	28.9	12	3	25.0	8.1	77.5	25.4	28.9
55	L	19	6	31.6	14.2	70.3	17.6	21.4	17	5	31.6	14.2	70.3	18.1	22.6	12	4	33.3	12.5	88.8	23.3	30.0
67	L	18	4	22.2	8.3	59.2	15.9	20.6	16	3	22.2	8.3	59.2	12.2	14.5	12	3	25.0	8.1	77.5	17.1	19.8
59	H	23	5	21.7	9.0	52.2	20.5	21.3	17	4	21.7	9.0	52.2	20.5	21.3	12	1	8.3	1.2	59.2	8.0	8.5
06	L	19	2	10.5	2.6	42.1	8.2	7.8	17	1	10.5	2.6	42.1	5.3	4.4	12	1	8.3	1.2	59.2	5.3	4.4
66	H	21	4	19.0	7.1	50.8	5.5	5.9	16	3	19.0	7.1	50.8	4.6	5.0	12	2	16.7	4.2	66.6	4.6	5.0
MM4	L	18	9	50.0	26.0	96.1	53.3	55.1	15	7	50.0	26.0	96.1	40.8	47.4	9	4	44.4	16.7	NA	40.0	42.1
42	L	14	5	35.7	14.9	85.8	32.8	26.6	13	5	35.7	14.9	85.8	34.3	28.1	9	4	44.4	16.7	NA	30.6	23.6
74	L	12	3	25.0	8.1	77.5	16.3	22.7	12	3	25.0	8.1	77.5	16.3	22.7	7	2	28.6	7.1	NA	14.4	20.4
82	H	4	1	25.0	3.5	177.5	10.6	8.9	4	1	25.0	3.5	NA	10.6	8.9	2	1	50.0	7.0	NA	21.2	17.7
L1AE5	L	1	1	100.0	14.1	709.9	10.8	15.8	1	1	100.0	14.1	NA	10.8	15.8	1	1	100.0	14.1	NA	10.8	15.8
37	L	1	1	100.0	14.1	709.9	8.0	8.5	1	1	100.0	14.1	NA	8.0	8.5	1	1	100.0	14.1	NA	8.0	8.5

Table IV-4. Ratios and corrected persistence rates of genotype-specific persistence rate (P%) and acquisition rate (A%) of common HPV types

	adjusted (world)		P/A	(P-A)/P	corrected
HPV	P %	A%	ratio	%	P %
58	51.6	0.3	155	99	51.5
52	49.9	0.7	72	99	49.5
70	47.4	0.3	160	99	47.3
39	44.8	0.4	124	99	44.6
72	42.1	0.4	119	99	41.8
68	38.3	0.2	166	99	38.1
44	36.3	0.5	79	99	36.0
MM8	31.0	0.5	62	98	30.6
53	30.9	0.5	67	99	30.6
54	30.0	0.5	61	98	29.6
51	23.2	0.4	57	98	22.9
CP8304	21.7	0.4	49	98	21.3
18	19.4	0.6	32	97	18.9
16	17.8	0.8	23	96	17.1
33	12.7	0.3	49	98	12.5
45	7.6	0.4	21	95	7.3
56	6.9	0.5	13	92	6.4
11	0.8	0.2	4	73	0.6

Table VI-5. Risk factors associated with HPV acquisition & persistence among cytologically normal women at baseline

Variables	HPV Acquisition (n=6667)						HPV persistence (n=563)					
	n	%	OR	aOR	95% CI	mOR	95% CI	n	%	OR	aOR	95% CI	mOR	95% CI
Demographic characteristics														
Age at baseline														
30-34	901	13.5	1.0					179	12.7	1.0				
35-39	1241	18.6	0.9		0.6 - 1.2			235	16.7	1.7		1.0 - 2.8)*		
40-44	1044	15.7	0.9		0.6 - 1.2			211	15.0	1.3		0.7 - 2.1		
45-49	879	13.2	0.9		0.7 - 1.3			180	12.8	1.6		0.9 - 2.7		
50-54	1028	15.4	1.1		0.8 - 1.5			226	16.0	2.4		1.4 - 3.9)**		
55-59	918	13.8	1.0		0.8 - 1.4			229	16.2	3.4		2.1 - 5.5)**		
60+	656	9.8	1.0		0.7 - 1.4			151	10.7	4.5		2.7 - 7.6)**		
p for trend			0.304							<0.001				
Age at baseline (Ⅱ)														
30-44	3186	47.8	1.0			1.0		625	44.3	1.0			1.0	
45-54	1907	28.6	1.1		0.9 - 1.4	1.1	0.9 - 1.4	406	28.8	1.5		1.1 - 2.0)*	0.9	0.6 - 1.4
55+	1574	23.6	1.1		0.9 - 1.4	1.2	0.9 - 1.5	380	26.9	2.8		2.1 - 3.8)**	1.2	0.7 - 2.1
p for trend			0.201							<0.001				
Marital status at baseline														
Currently married	6188	92.8	1.0			1.0		1292	91.6	1.0				
Widowed/Divorced/Others	477	7.2	1.5	1.5	1.1 - 2.0)*	2.6	1.7 - 3.9)**	119	8.4	1.4	1.1	0.7 - 1.7		
No. of marriage														
0-1	6567	98.5	1.0					1380	97.8	1.0				
2+	94	1.4	1.9	1.9	1.1 - 3.3)*			29	2.1	1.9	1.7	0.8 - 3.7		
Unknown	6	0.1						2	0.1	0	0.0			
Lifestyle habit														
Cigarette smoking														
Never	6576	98.6	1.0					1381	97.9	1.0				
Ever	67	1.0	0.9	0.9	0.3 - 2.2			22	1.6	1.5	1.7	0.7 - 4.2		
Unknown	24	0.4						8	0.6	1	12.5			
Alcohol drinking														
Never	6607	99.1	1.0					1393	98.7	1.0				
Ever	37	0.6	1.7	1.7	0.7 - 4.4			10	0.7	1.1	1.5	0.4 - 5.9		
Unknown	23	0.3						8	0.6	1	12.5			
Betal chewing														
Never	6633	99.5	1.0					1401	99.3	1.0				
Ever	9	0.1	-					1	0.1	1	100.0	∞		
Unknown	25	0.4						9	0.6	1	11.1			
Pregnancy, delivery & contracetion														
No. of lifetime childbirth														
0-3	1724	25.9	1.0					336	23.8	1.0				
4	1338	20.1	0.8	0.8	0.6 - 1.1			272	19.3	2.0	1.7	1.2 - 2.6)*		
5	1338	20.1	0.9	0.9	0.7 - 1.1			287	20.3	1.8	1.5	1.0 - 2.2		
6	936	14.0	1.1	1.1	0.8 - 1.4			209	14.8	2.0	1.5	1.0 - 2.4		
7	578	8.7	1.1	1.1	0.8 - 1.5			121	8.6	2.4	1.6	0.9 - 2.6		
8	337	5.1	0.9	0.9	0.6 - 1.4			72	5.1	1.8	1.5	0.8 - 2.6		
9+	318	4.8	0.8	0.8	0.5 - 1.2			93	6.6	3.7	2.1	1.2 - 3.7)*		
Unknown	98	1.5						21	1.5	6	28.6			
p for trend										<0.001				

aOR: age-adjusted odds ratio; mOR: multivariate-adjusted odds ratio.

*: p<0.05; **: p<0.001

Table VI-5. (cont.)

Variables	HPV Acquisition (n=6667)					HPV persistence (n=1411)				
	n (%)	n (%)	OR	aOR (95% CI)	mOR (95% CI)	n (%)	n (%)	OR	aOR (95% CI)	mOR (95% CI)
No. of vaginal delivery										
0-2	1388 (20.8)	111 (8.0)	1.0			288 (20.4)	43 (14.9)	1.0		1.0
3	1957 (29.4)	160 (8.2)	1.0	1.0 (0.8 - 1.3)		382 (27.1)	102 (26.7)	2.1	1.9 (1.2 - 2.8)*	1.7 (1.1 - 2.6)*
4	1514 (22.7)	125 (8.3)	1.0	1.0 (0.8 - 1.4)		344 (24.4)	96 (27.9)	2.2	1.7 (1.1 - 2.6)*	1.5 (1.0 - 2.4)
5+	1725 (25.9)	155 (9.0)	1.1	1.1 (0.8 - 1.5)		382 (27.1)	147 (38.5)	3.6	2.1 (1.3 - 3.3)*	1.8 (1.1 - 3.0)*
Unknown	83 (1.2)	12 (14.5)				15 (1.1)	3 (20.0)			
p for trend				0.314					<0.001	
No. of cesarean section										
0	2423 (36.3)	193 (8.0)	1.0			487 (34.5)	132 (27.1)	1.0		1.0
1	2060 (30.9)	175 (8.5)	1.1	1.1 (0.9 - 1.3)		439 (31.1)	125 (28.5)	1.1	1.0 (0.8 - 1.4)	1.0 (0.7 - 1.3)
2	1296 (19.4)	103 (7.9)	1.0	1.0 (0.8 - 1.3)		275 (19.5)	66 (24.0)	0.8	0.9 (0.6 - 1.2)	0.9 (0.6 - 1.2)
3+	793 (11.9)	78 (9.8)	1.3	1.3 (1.0 - 1.7)		189 (13.4)	62 (32.8)	1.3	1.3 (0.9 - 1.9)	1.2 (0.8 - 1.8)
Unknown	95 (1.4)	14 (14.7)				21 (1.5)	6 (28.6)			
p for trend				0.228					0.488	
Abortion										
Never	6095 (91.4)	512 (8.4)	1.0			1298 (92.0)	372 (28.7)	1.0		
Ever	473 (7.1)	37 (7.8)	0.9	1.0 (0.7 - 1.4)		91 (6.4)	13 (14.3)	0.4	0.5 (0.3 - 1.0)	
Unknown	99 (1.5)	14 (14.1)				22 (1.6)	6 (27.3)			
Oral contraceptives use										
Never	4517 (67.8)	373 (8.3)	1.0			963 (68.2)	264 (27.4)	1.0		
Ever	2144 (32.2)	189 (8.8)	1.1	1.1 (0.9 - 1.3)		446 (31.6)	127 (28.5)	1.1	1.4 (1.0 - 1.8)*	
Unknown	6 (0.1)	1 (16.7)				2 (0.1)	0 (0.0)			
Intra-uterine device use										
Never	2634 (39.5)	210 (8.0)	1.0			498 (35.3)	114 (22.9)	1.0		1.0
Ever	4031 (60.5)	353 (8.8)	1.1	1.1 (0.9 - 1.3)		913 (64.7)	277 (30.3)	1.5	1.5 (1.1 - 1.9)*	1.4 (1.1 - 1.8)*
Unknown	2 (0.0)	0 (0.0)								
Menstration										
Age at menarche										
14-16	3691 (55.4)	299 (8.1)	1.0			756 (53.6)	199 (26.3)	1.0		
<=13	857 (12.9)	75 (8.8)	1.1	1.1 (0.8 - 1.4)		171 (12.1)	40 (23.4)	0.9	1.0 (0.7 - 1.5)	
17+	2062 (30.9)	182 (8.8)	1.1	1.1 (0.9 - 1.3)		468 (33.2)	147 (31.4)	1.3	1.0 (0.7 - 1.3)	
Unknown	57 (0.9)	7 (12.3)				16 (1.1)	5 (31.3)			
Menopause										
No	4318 (64.8)	343 (7.9)	1.0			852 (60.4)	173 (20.3)	1.0		1.0
Yes	2346 (35.2)	220 (9.4)	1.2	1.3 (0.9 - 1.9)		559 (39.6)	218 (39.0)	2.5	1.8 (1.1 - 2.8)*	1.8 (1.2 - 2.7)*
Unknown	3 (0.0)	0 (0.0)								
Age at menopause						559	218			
<=45	320 (13.6)	30 (9.4)	1.0			69 (12.3)	20 (29.0)	1.0		
46-50	1008 (43.0)	90 (8.9)	0.9	1.0 (0.6 - 1.5)		234 (41.9)	91 (38.9)	1.6	1.6 (0.9 - 2.8)	
51-55	805 (34.3)	82 (10.2)	1.1	1.2 (0.7 - 1.9)		208 (37.2)	80 (38.5)	1.5	1.6 (0.8 - 2.9)	
56+	105 (4.5)	10 (9.5)	1.0	1.1 (0.5 - 2.5)		25 (4.5)	17 (68.0)	5.2	4.9 (1.8 - 13.5)*	
Unknown	108 (4.6)	8 (7.4)				23 (4.1)	10 (43.5)			
p for trend				0.564					0.017	

aOR: age-adjusted odds ratio; mOR: multivariate-adjusted odds ratio.

*: p<0.05; **: p<0.001

Table VI-5. (cont.)

Variables	(n=6667)	HPV Acquisition (n=563)					(n=1411)	HPV persistence (n=391)			
	n (%)	n (%)	OR	aOR (95% CI)	mOR (95% CI)		n (%)	n (%)	OR	aOR (95% CI)	mOR (95% CI)
Sexual behavior (lifetime)											
Age at initial coitus											
14-21	2499 (37.5)	250 (10.0)	1.0		1.0		572 (40.5)	191 (33.4)	1.0		
22-23	1717 (25.8)	138 (8.0)	0.8	0.8 (0.6 - 1.0)*	0.8 (0.7 - 1.1)		357 (25.3)	96 (26.9)	0.7	0.8 (0.6 - 1.1)	
24+	2427 (36.4)	173 (7.1)	0.7	0.7 (0.6 - 0.9)**	0.8 (0.6 1.0)*		478 (33.9)	103 (21.5)	0.5	0.7 (0.5 - 0.9)*	
Unknown	24 (0.4)	2 (8.3)					4 (0.3)	1 (25.0)			
p for trend				0.000						<0.001	
No. of lifetime sexual partners											
0-1	6490 (97.3)	536 (8.3)	1.0				1352 (95.8)	370 (27.4)	1.0		
2+	164 (2.5)	27 (16.5)	2.2	2.2 (1.4 - 3.3)**			57 (4.0)	21 (36.8)	1.5	1.7 (1.0 - 3.1)	
Unknown	13 (0.2)	0 (0.0)					2 (0.1)	0 (0.0)			
Premarital sex											
No	6499 (97.5)	549 (8.4)	1.0				1378 (97.7)	384 (27.9)	1.0		
Yes	143 (2.1)	12 (8.4)	1.0	1.0 (0.5 - 1.8)			29 (2.1)	6 (20.7)	0.7	1.0 (0.4 - 2.6)	
Unknown	25 (0.4)	2 (8.0)					4 (0.3)	1 (25.0)			
Sexual behavior & contraception (recent)											
No. of sexual partners											
0	834 (12.5)	57 (6.8)	1.0	1.0			197 (14.0)	76 (38.6)	1.0	1.0	
1	5040 (75.6)	433 (8.6)	1.3	1.5 (1.1 - 2.0)*			1039 (73.6)	269 (25.9)	0.6	0.8 (0.6 - 1.1)	
2+	9 (0.1)	2 (22.2)	3.9	4.8 (1.0 - 23.7)			4 (0.3)	2 (50.0)	1.6	2.4 (0.3 - 18.0)	
Unknown	784 (11.8)	71 (9.1)					171 (12.1)	44 (25.7)			
p for trend				<0.001						<0.001	
OC use											
No	5663 (84.9)	476 (8.4)	1.0	1.0			1193 (84.5)	335 (28.1)	1.0	1.0	
Yes	216 (3.2)	17 (7.9)	0.9	1.0 (0.6 - 1.7)			48 (3.4)	13 (27.1)	1.0	1.3 (0.7 - 2.6)	
Unknown	788 (11.8)	70 (8.9)					170 (12.0)	43 (25.3)			
IUD use											
No	4810 (72.1)	405 (8.4)	1.0	1.0			1007 (71.4)	279 (27.7)	1.0	1.0	
Yes,but remove	278 (4.2)	19 (6.8)	0.8	0.8 (0.5 - 1.3)			60 (4.3)	20 (33.3)	1.3	1.3 (0.7 - 2.3)	
Yes,currently	766 (11.5)	66 (8.6)	1.0	1.1 (0.8 - 1.4)			168 (11.9)	46 (27.4)	1.0	1.4 (0.9 - 2.0)	
Condom use (I)											
Never	4289 (64.3)	381 (8.9)	1.0	1.0			912 (64.6)	244 (26.8)	1.0	1.0	
Seldom	313 (4.7)	27 (8.6)	1.0	1.0 (0.7 - 1.6)			56 (4.0)	14 (25.0)	0.9	1.1 (0.6 - 2.1)	
Often	151 (2.3)	9 (6.0)	0.7	0.7 (0.4 - 1.4)			21 (1.5)	4 (19.0)	0.6	0.9 (0.3 - 2.7)	
Always	279 (4.2)	18 (6.5)	0.7	0.8 (0.5 - 1.3)			51 (3.6)	8 (15.7)	0.5	0.7 (0.3 - 1.5)	
No sexual intercourse	834 (12.5)	57 (6.8)	0.8	0.7 (0.5 - 0.9)*			197 (14.0)	76 (38.6)	1.7	1.3 (0.9 - 1.8)	
Unknown	801 (12.0)	71 (8.9)					174 (12.3)	45 (25.9)			
Condom use (II)											
Never+seldom	4602 (69.0)	408 (8.9)	1.0	1.0	1.0		968 (68.6)	258 (26.7)	1.0	1.0	
Often+always	430 (6.4)	27 (6.3)	0.7	0.7 (0.5 - 1.1)	0.8 (0.5 - 1.2)		72 (5.1)	12 (16.7)	0.6	0.7 (0.4 - 1.4)	
No sexual intercourse	834 (12.5)	57 (6.8)	0.8	0.7 (0.5 - 0.9)*	0.4 (0.3 - 0.7)**		197 (14.0)	76 (38.6)	1.7	1.3 (0.9 - 1.8)	
Unknown	801 (12.0)	71 (8.9)					174 (12.3)	45 (25.9)			

aOR: age-adjusted odds ratio; mOR: multivariate-adjusted odds ratio.

*: p<0.05; **: p<0.001

Table VI-5. (cont.)

Variables	(n=6667)	HPV Acquisition (n=563)					(n=1411)	HPV persistence (n=391)				
	n (%)	n (%)	OR	aOR	(95% CI)	mOR (95% CI)	n (%)	n (%)	OR	aOR	(95% CI)	mOR (95% CI)
Vaginal douching use (I)												
Never	3663 (54.9)	292 (8.0)	1.0	1.0			718 (50.9)	183 (25.5)	1.0	1.0		
Seldom	387 (5.8)	40 (10.3)	1.3	1.4	(1.0 - 1.9)		111 (7.9)	31 (27.9)	1.1	1.2	(0.8 - 1.9)	
Often	349 (5.2)	46 (13.2)	1.8	1.8	(1.3 - 2.5)**		73 (5.2)	20 (27.4)	1.1	1.1	(0.6 - 1.9)	
Always	638 (9.6)	58 (9.1)	1.2	1.2	(0.9 - 1.6)		140 (9.9)	37 (26.4)	1.1	1.1	(0.7 - 1.7)	
No sexual intercourse	834 (12.5)	57 (6.8)	0.8	0.7	(0.5 - 1.0)		197 (14.0)	76 (38.6)	1.8	1.3	(0.9 - 1.9)	
Unknown	796 (11.9)	70 (8.8)					172 (12.2)	44 (25.6)				
Vaginal douching use (II)												
Never	3663 (54.9)	292 (8.0)	1.0	1.0		1.0	718 (50.9)	183 (25.5)	1.0	1.0		
Ever	1374 (20.6)	144 (10.5)	1.4	1.4	(1.1 - 1.7)*	1.3 (1.0 - 1.6)*	324 (23.0)	88 (27.2)	1.1	1.1	(0.8 - 1.5)	
No sexual intercourse	834 (12.5)	57 (6.8)	0.8	0.7	(0.5 - 1.0)		197 (14.0)	76 (38.6)	1.8	1.3	(0.9 - 1.9)	
Pap smear & HPV experience												
Pap smear												
Never	5893 (88.4)	490 (8.3)	1.0				1265 (89.7)	356 (28.1)	1.0			
Ever	760 (11.4)	72 (9.5)	1.2	1.2	(0.9 - 1.5)		140 (9.9)	33 (23.6)	0.8	0.9	(0.6 - 1.4)	
Unknown	14 (0.2)	1 (7.1)					6 (0.4)	2 (33.3)				
HPV at baseline												
Negative	5256 (76.4)	327 (6.2)	1.0									
Positive	1411 (20.5)	236 (16.7)	3.0	3.0	(2.5 - 3.6)**							
HPV at baseline												
Negative	5256 (51.9)	327 (6.2)	1.0			1.0						
HR17	786 (7.8)	152 (19.3)	3.6	3.6	(2.9 - 4.4)**	3.6 (2.9 - 4.6)**						
Others	625 (6.2)	84 (13.4)	2.3	2.3	(1.8 - 3.0)**	2.3 (1.7 - 3.0)**						

aOR: age-adjusted odds ratio; mOR: multivariate-adjusted odds ratio.

*: p<0.05; **: p<0.001

Table V-1. Risk factors associated with developing cervical cancer

Variables	Normal (n=10123)	Cervical cancer (n=69)					
	n (%)	case	person-year	Incidence (10^{-5})	HR	aHR	95% CI
Demographic characteristics							
Age at baseline							
30-34	1368 (13.5)	8	20365.0	(39.3)	1.0		
35-39	1821 (18.0)	8	26935.6	(29.7)	0.8		(0.3 - 2.0)
40-44	1585 (15.7)	9	23240.3	(38.7)	1.0		(0.4 - 2.6)
45-49	1278 (12.6)	8	18712.7	(42.8)	1.1		(0.4 - 2.9)
50-54	1536 (15.2)	12	21966.4	(54.6)	1.4		(0.6 - 3.5)
55-59	1437 (14.2)	16	20415.7	(78.4)	2.0		(0.9 - 4.8)
60+	1098 (10.8)	8	14862.1	(53.8)	1.4		(0.5 - 3.8)
trend test					p=0.0623		
Age at baseline (I)							
30-44	4774 (47.2)	25	70540.9	(35.4)	1.0		
45-54	2814 (27.8)	20	40679.0	(49.2)	1.4		(0.8 - 2.5)
55+	2535 (25.0)	24	35277.8	(68.0)	2.0		(1.1 - 3.5)*
trend test					p=0.0373		
Marital status at							
Currently married	9350 (92.4)	60	135525.2	(44.3)	1.0		
Widowed/Divorced/Others	766 (7.6)	9	10881.3	(82.7)	1.9	1.6	(0.8 - 3.3)
Unknown	7 (0.1)	0	91.3	(0.0)			
No. of marriage							
0-1	9907 (97.9)	68	143487.6	(47.4)	1.0		
2+	149 (1.5)	1	2122.8	(47.1)	1.0	0.9	(0.1 - 6.7)
Unknown	67 (0.7)	0	887.3	(0.0)			
Lifestyle habit							
Cigarette smoking							
Never	9977 (98.6)	68	144415.4	(47.1)	1.0		
Ever	103 (1.0)	1	1446.8	(69.1)	1.5	1.5	(0.2 - 10.9)
Unknown	43 (0.4)	0	635.5	(0.0)			
Alcohol drinking							
Never	10019 (99.0)	68	144998.3	(46.9)	1.0		
Ever	62 (0.6)	1	878.6	(113.8)	2.4	2.4	(0.3 - 17.4)
Unknown	42 (0.4)	0	620.8	(0.0)			
Betal chewing							
Never	10062 (99.4)	69	145610.0	(47.4)	1.0		
Ever	17 (0.2)	0	238.2	(0.0)	-		
Unknown	44 (0.4)	0	649.5	(0.0)	-		
Pregnancy, delivery & contraception							
No. of lifetime childbirth							
0-3	2564 (25.3)	10	37719.3	(26.5)	1.0		
4	2010 (19.9)	12	29213.1	(41.1)	1.6	1.5	(0.7 - 3.6)
5	1945 (19.2)	14	28309.1	(49.5)	1.9	1.8	(0.8 - 4.1)
6	1439 (14.2)	13	20640.4	(63.0)	2.4	2.2	(0.9 - 5.3)
7	898 (8.9)	7	12805.7	(54.7)	2.1	1.9	(0.7 - 5.3)
8	529 (5.2)	6	7564.6	(79.3)	1.3	1.2	(0.5 - 3.3)
9+	520 (5.1)	6	7180.9	(83.6)	3.2	2.9	(1.0 - 8.7)
Unknown	218 (2.2)	1	3064.6	(32.6)			
trend test					p=0.008		
No. of vaginal delivery							
0-2	2040 (20.2)	7	30106.1	(23.3)	1.0		
3	2893 (28.6)	15	42317.8	(35.4)	1.5	1.5	(0.6 - 3.9)
4	2252 (22.2)	21	32626.7	(64.4)	2.8	2.7	(1.1 - 6.9)*
5+	2748 (27.1)	26	38779.6	(67.0)	2.9	2.7	(1.0 - 7.3)
Unknown	190 (1.9)	0	2667.5	(0.0)			
trend test					p=0.004		
No. of vaginal delivery							
0-3	4933 (48.7)	22	72423.9	(30.4)	1.0		
4+	5000 (49.4)	47	71406.3	(65.8)	2.2*	2.0	(1.1 — 3.7)*
Unknown	190 (1.9)	0	2667.5	(0.0)			

HR: hazrad ratio; aHR: age-adjusted hazard ratio.
*: p<0.05; **: p<0.001

Table V-1. (cont.)

Variables	Normal (n=10123)	Cervical cancer (n=69)					
	n (%)	case	person-year	Incidence (10^{-5})	HR	aHR	95% CI
No. of cesarean section							
0	3727 (36.8)	23	53825.5	42.7	1.0		
1	3057 (30.2)	19	44241.5	42.9	1.0	1.0	(0.5 - 1.8)
2	1933 (19.1)	17	28128.6	60.4	1.4	1.4	(0.8 - 2.7)
3+	1191 (11.8)	9	17281.7	52.1	1.2	1.2	(0.6 - 2.6)
Unknown	215 (2.1)	1	3020.5	33.1			
trend test					p=0.345		
Abortion							
Never	9219 (91.1)	68	133320.9	51.0	1.0		
Ever	685 (6.8)	0	10089.0	0.0	-		
Unknown	219 (2.2)	1	3087.8	32.4			
Oral contraceptives use							
Never	6980 (69.0)	45	100694.6	44.7	1.0		
Ever	3076 (30.4)	23	44912.1	51.2	1.1	1.3	(0.8 - 2.1)
Unknown	67 (0.7)	1	891.0	112.2			
Oral contraceptives use (Current)							
Never use+not now	9699 (95.8)	64	140363.3	45.6	1.0		
Yes	297 (2.9)	4	4384.5	91.2	2.0	2.6	(0.9 - 7.2)
Unknown	127 (1.3)	1	1750.0	57.1			
Intra-uterine device use							
Never	4105 (40.6)	24	59387.3	40.4	1.0		
Ever	5958 (58.9)	45	86308.2	52.1	1.3	1.2	(0.8 - 2.1)
Unknown	60 (0.6)	0	802.3	0.0			
Menstration							
Age at menarche							
14-16	5515 (54.5)	35	80139.4	43.7	1.0		
~13	1282 (12.7)	10	18766.6	53.3	1.2	1.3	(0.7 - 2.7)
17+	3184 (31.5)	23	45639.2	50.4	1.2	0.9	(0.5 - 1.6)
Unknown	142 (1.4)	1	1952.6	51.2			
Menopause							
No	6406 (63.3)	33	94413.2	35.0	1.0		
Yes	3683 (36.4)	36	51614.8	69.7	2.1	2.4	(1.0 - 6.1)
Unknown	34 (0.3)	0	469.7	0.0			
Age at menopause	3683						
~45	509 (13.8)	6	7070.0	84.9	1.0		
46-50	1596 (43.3)	17	22394.7	75.9	0.9	0.8	(0.3 - 2.0)
51-55	1216 (33.0)	9	17168.4	52.4	0.6	0.6	(0.2 - 1.6)
56+	160 (4.3)	2	2193.4	91.2	1.1	1.1	(0.2 - 5.6)
Unknown	202 (5.5)	2	2788.3	71.7			
trend test					p=0.498		
Sexual behavior (lifetime)							
Age at initial coitus							
14-21	3860 (38.1)	34	55252.7	61.5	1.0		
22-23	2656 (26.2)	17	38434.1	44.2	0.7	0.7	(0.4 - 1.3)
24+	3504 (34.6)	18	51390.8	35.0	0.6	0.6	(0.4 - 1.1)
Unknown	103 (1.0)	0	1420.1	0.0			
trend test							
Age at initial coitus							
14-15	16 (0.2)	0	225.3	0.0			
16-17	203 (2.0)	1	2852.5	35.1			
18-19	1023 (10.1)	11	14574.8	75.5			
20-21	2618 (25.9)	22	37600.2	58.5			
22-23	2656 (26.2)	17	38434.1	44.2			
24-25	1853 (18.3)	12	27074.4	44.3			
26-27	988 (9.8)	4	14542.6	27.5			
28+	663 (6.5)	2	9773.8	20.5			
Unknown	103 (1.0)	0	1420.1	0.0			

HR: hazrad ratio; aHR: age-adjusted hazard ratio.
*: p<0.05; **: p<0.001

Table V-1. (cont.)

Variables	Normal (n=10123)	Cervical cancer (n=69)					
	n (%)	case	person-year	Incidence (10^{-5})	HR	aHR	95% CI
No. of lifetime sexual partners							
0-1	9790 (96.7)	68	141815.1	47.9	1.0		
2+	249 (2.5)	1	3569.1	28.0	0.6	0.6	(0.1 - 4.3)
Unknown	84 (0.8)	0	1113.5	0.0			
Premarital sex							
No	9799 (96.8)	68	141862.5	47.9	1.0		
Yes	218 (2.2)	1	3171.5	31.5	0.7	0.8	(0.1 - 6.0)
Unknown	106 (1.0)	0	1463.7	0.0			
Sexual behavior & contraception (recent)							
No. of sexual partners							
0	946 (9.3)	9	13499.7	66.7	1.0	1.0	
1	5513 (54.5)	32	80717.1	39.6	0.6	0.9	(0.4 - 2.0)
2+	9 (0.1)	0	133.6	0.0	-		
Unknown	3655 (36.1)	28	52147.3	53.7			
OC use							
No	6222 (61.5)	10	90708.6	11.0	1.0	1.0	
Yes	243 (2.4)	1	3596.0	27.8	0.6	1.1	(0.1 - 8.0)
Unknown	3658 (36.1)	28	52193.1	53.6			
IUD use							
No	5316 (52.5)	31	77424.6	40.0	1.0	1.0	
Yes,but remove	303 (3.0)	2	4404.8	45.4	1.2	1.0	(0.2 - 4.2)
Yes,currently	812 (8.0)	7	11999.5	58.3	1.4	2.6	(1.1 - 6.1)*
Unknown	3692 (36.5)	29	52668.9	55.1			
Condom use							
Never+seldom	5031 (49.7)	30	73554.6	40.8	1.0	1.0	
Often+always	462 (4.6)	0	6879.9	0.0	-		
No sexual intercourse	946 (9.3)	9	13499.7	66.7	1.7	1.2	(0.5 - 2.6)
Unknown	3684 (36.4)	30	52563.5	57.1			
Vaginal douching use							
Never	4036 (39.9)	23	59035.6	39.0	1.0	1.0	
Ever	1471 (14.5)	9	21595.6	41.7	1.1	1.1	(0.5 - 2.5)
No sexual intercourse	946 (9.3)	9	13499.7	66.7	1.7	1.1	(0.5 - 2.6)
Unknown	3670 (36.3)	28	52366.9	53.5			
Pap smear experience							
Pap smear							
Never	8738 (86.3)	61	126473.1	48.2	1.0		
Ever	1358 (13.4)	8	19634.4	40.7	0.8	0.9	(0.4 - 1.9)
Unknown	27 (0.3)	0	390.2	0.0			
HPV at baseline							
HPV infection							
Negative	7916 (78.2)	15	114887.7	13.1	1.0		
Positive	2207 (21.8)	54	31610.0	170.8	13.1	12.9	(7.3 - 22.8)**
single positive	1601 (15.8)	26	23103.1	112.5	8.6	8.6	(4.5 - 16.2)**
multiple	606 (6.0)	28	8521.5	328.6	25.4	24.2	(12.9 - 45.6)**
2 types	395 (3.9)	18	5497.3	327.4	25.3	24.4	(12.2 - 48.5)**
3 types	133 (1.3)	6	1896.2	316.4	24.5	23.3	(9.0 - 60.3)**
4+ types	78 (0.8)	4	1113.3	359.3	27.7	25.1	(8.2 - 76.6)**
Multiple vs single	606 (6.0)	28	8521.5	328.6	3.0	2.8	(1.6 - 4.8)**
HR-HPV at baseline							
Negative	7916 (78.2)	15	114887.7	13.1	1.0		
HR17	1228 (12.1)	46	17338.6	265.3	20.5	20.0	(11.1 - 35.9)**
Other types	979 (9.7)	8	14271.4	56.1	4.3	4.3	(1.8 - 10.1)**
single HR-HPV positive	968 (9.6)	31	13662.8	226.9	17.5	17.1	(9.2 - 31.8)**
multiple							
2 types	186 (1.8)	10	2592.5	385.7	29.9	28.1	(12.6 - 62.9)**
3 types	49 (0.5)	3	719.4	417.0	31.9	31.2	(9.0 - 108.1)**
4+ types	25 (0.2)	2	363.9	549.6	42.2	40.1	(9.1 - 176.0)**
Multiple HR vs single HR	260 (2.6)	15	3675.8	408.1	1.8	1.7	(0.9 - 3.2)

HR: hazrad ratio; aHR: age-adjusted hazard ratio.

*: p<0.05; **: p<0.001

Table V-2. Genotypespecific HPV infection of cervical cancer cases at baseline (n=56) and incident cervical cancer cases (n=69) during follow-up

	Risk group	CXC at baseline (n=56)		Incident cervical cancer (among 10123 normal) CXC (n=69)		ICC (n=35)		CIS (n=34)	
HPV status at baseline		no.	%	no.	%	no.	%	no.	%
Negative		1	1.8	15	21.7	7	20.0	8	23.5
Positive		55	98.2	54	78.3	28	80.0	26	76.5
PCR (+)		55	100.0	48	88.9	25	89.3	23	88.5
PCR (-)		0	0.0	6	11.1	3	10.7	3	11.5
Single type		31	56.4	26	48.1	15	53.6	11	42.3
Multiple types		24	43.6	28	51.9	13	46.4	15	57.7
High-risk type (17 types)		54	98.2	46	66.7	25	71.4	21	61.8
Low-risk type (22 types)		11	20.0	25	36.2	11	31.4	14	41.2
High-risk type (13 types)		54	98.2	43	62.3	25	71.4	18	52.9
HPV16/18/52/58/33/31		52	94.5	39	56.5	24	68.6	15	44.1
HPV16/18/52/58		47	85.5	34	49.3	22	62.9	12	35.3
HPV16/18		28	50.9	21	30.4	15	42.9	6	17.6
HPV16	H	27	49.1	13	18.8	9	25.7	4	11.8
HPV52	H	11	20.0	11	15.9	5	14.3	6	17.6
HPV58	H	14	25.5	9	13.0	7	20.0	2	5.9
HPV18	H	2	3.6	8	11.6	6	17.1	2	5.9
HPV11	L	3	5.5	7	10.1	3	8.6	4	11.8
HPV31	H	5	9.1	6	8.7	4	11.4	2	5.9
HPV56	H	2	3.6	5	7.2	2	5.7	3	8.8
CP8304	L	1	1.8	5	7.2	3	8.6	2	5.9
HPV33	H	5	9.1	4	5.8	2	5.7	2	5.9
HPV53	H	3	5.5	4	5.8	2	5.7	2	5.9
HPV35	H	2	3.6	3	4.3	2	5.7	1	2.9
HPV54	L	3	5.5	3	4.3	2	5.7	1	2.9
HPV70	L	2	3.6	3	4.3	2	5.7	1	2.9
MM8	L	2	3.6	3	4.3	3	8.6	0	0.0
HPV39	H	1	1.8	2	2.9	2	5.7	0	0.0
HPV45	H	1	1.8	2	2.9	2	5.7	0	0.0
HPV51	H	2	3.6	2	2.9	1	2.9	1	2.9
HPV68	H	4	7.3	2	2.9	1	2.9	1	2.9
HPV42	L	0	0.0	2	2.9	1	2.9	1	2.9
HPV43	L	0	0.0	2	2.9	0	0.0	2	5.9
HPV44	L	0	0.0	2	2.9	0	0.0	2	5.9
HPV69	L	0	0.0	2	2.9	1	2.9	1	2.9
MM4	L	1	1.8	2	2.9	1	2.9	1	2.9
CP8061	L	0	0.0	2	2.9	0	0.0	2	5.9
HPV82	H	0	0.0	1	1.4	0	0.0	1	2.9
HPV61	L	0	0.0	1	1.4	1	2.9	0	0.0
HPV72	L	1	1.8	1	1.4	0	0.0	1	2.9
HPV67	L	0	0.0	1	1.4	1	2.9	0	0.0
L1AE5	L	0	0.0	1	1.4	0	0.0	1	2.9
HPV59	H	1	1.8	0	0.0	0	0.0	0	0.0
HPV66	H	0	0.0	0	0.0	0	0.0	0	0.0
HPV26	H	1	1.8	0	0.0	0	0.0	0	0.0
Single type infection									
HPV16	H	13	23.6	6	8.7	5	14.3	1	2.9
HPV18	H	0	0.0	4	5.8	3	8.6	1	2.9
HPV58	H	8	14.5	2	2.9	2	5.7	0	0.0
HPV52	H	5	9.1	2	2.9	0	0.0	2	5.9
HPV56	H	0	0.0	2	2.9	1	2.9	1	2.9
HPV53	H	0	0.0	2	2.9	0	0.0	2	5.9
HPV33	H	2	3.6	1	1.4	1	2.9	0	0.0
HPV31	H	1	1.8	1	1.4	0	0.0	1	2.9
HPV45	H	1	1.8	0	0.0	0	0.0	0	0.0
HPV11	L	0	0.0	3	4.3	2	5.7	1	2.9
CP8304	L	0	0.0	1	1.4	1	2.9	0	0.0
HPV54	L	0	0.0	1	1.4	0	0.0	1	2.9
CP8061	L	0	0.0	1	1.4	0	0.0	1	2.9
HPV70	L	1	1.8	0	0.0	0	0.0	0	0.0

Table V-3. Genotype-specific incidence, hazard ratio, attributable risk percent, and population attributable risk percent of developing cervical cancer by HPV infection status, persistence and clearance

		Normal (n=10123)	Cervical cancer (n=69)					Cervical cancer among persistence subjects					Cervical cancer among clearance subjetcs				
HPV		no. (%)	no. (%)	incidence (10-5)	mHR (95%CI)	AR%	PAR%	no.	case no.	incidence (10-5)	mHR (95%CI)		no.	case no.	incidence (10^{-5})	mHR (95%CI)	
16	H	170 (1.7)	13 (18.8)	**549.3**	**40.3** (19.2 - 84.7)	98	40	21	5	**1894.7**	**174.1** (54.0 - 560.6)		82	1	82.8	**8.5** (1.0 - 69.2)	
52	H	221 (2.2)	11 (15.9)	**352.8**	**27.0** (12.4 - 58.9)	96	36	81	6	**537.7**	**58.9** (19.7 - 175.4)		69	0	0.0	**-**	
58	H	92 (0.9)	9 (13.0)	**708.2**	**49.2** (21.3 - 113.5)	98	30	35	4	**855.1**	**104.0** (28.5 - 379.2)		23	0	0.0	**-**	
18	H	198 (1.9)	8 (11.6)	**285.0**	**20.9** (8.8 - 49.3)	95	28	24	2	**590.5**	**63.8** (12.6 - 323.5)		101	1	68.1	**7.0** (0.9 - 57.1)	
31	H	59 (0.6)	6 (8.7)	**714.6**	**49.7** (19.2 - 128.6)	98	22	17	3	**1302.7**	**136.6** (33.1 - 564.1)		18	0	0.0	**-**	
56	H	205 (2.0)	5 (7.2)	**171.4**	**13.0** (4.7 - 35.8)	92	19	10	3	**2120.5**	**214.4** (50.1 - 917.4)		117	1	58.8	**5.7** (0.7 - 46.5)	
33	H	134 (1.3)	4 (5.8)	**204.2**	**14.6** (4.9 - 44.1)	93	15	14	4	**1969.4**	**172.3** (43.5 - 682.7)		81	0	0.0	**-**	
53	H	116 (1.1)	4 (5.8)	**244.5**	**17.9** (5.9 - 54.0)	94	16	25	2	**585.6**	**59.7** (12.0 - 296.1)		39	0	0.0	**-**	
35	H	42 (0.4)	3 (4.3)	**516.1**	**35.3** (10.1 - 123.2)	97	12	5	1	**1340.4**	**159.5** (15.7 - 1620.1)		22	2	644.6	**74.0** (14.7 - 371.5)	
39	H	96 (0.9)	2 (2.9)	**149.1**	**9.7** (2.2 - 42.5)	90	8	23	2	**606.6**	**53.6** (10.8 - 265.9)		39	0	0.0	**-**	
51	H	77 (0.8)	2 (2.9)	**178.4**	**12.5** (2.9 - 54.9)	92	8	13	2	**1053.9**	**96.0** (19.2 - 479.4)		38	0	0.0	**-**	
68	H	67 (0.7)	2 (2.9)	**214.1**	**15.3** (3.5 - 67.1)	93	9	16	1	**427.4**	**39.1** (4.6 - 330.8)		28	0	0.0	**-**	
45	H	63 (0.6)	2 (2.9)	**221.1**	**16.8** (3.8 - 73.5)	94	9	6	1	**1178.1**	**103.3** (10.9 - 979.8)		37	0	0.0	**-**	
82	H	7 (0.1)	1 (1.4)	**1101.0**	**75.2** (9.7 - 580.8)	99	5	1	0	**0.0**	**-**		3	0	0.0	**-**	
11	L	621 (6.1)	7 (10.1)	**76.8**	**5.9** (2.4 - 14.5)	83	23	2	0	**0.0**	**-**		401	4	67.4	**6.2** (1.8 - 21.5)	
CP8304	L	94 (0.9)	5 (7.2)	**382.4**	**28.5** (10.3 - 78.7)	96	20	15	1	**494.4**	**74.6** (8.3 - 668.1)		28	0	0.0	**-**	
70	L	90 (0.9)	3 (4.3)	**240.8**	**18.4** (5.3 - 63.6)	95	13	26	3	**815.1**	**80.6** (20.2 - 322.3)		29	0	0.0	**-**	
54	L	88 (0.9)	3 (4.3)	**242.8**	**16.9** (4.9 - 58.7)	94	12	18	1	**374.4**	**51.9** (5.8 - 461.4)		37	2	380.9	**41.4** (8.5 - 201.2)	
MM8	L	87 (0.9)	3 (4.3)	**245.5**	**19.7** (5.7 - 68.0)	95	14	1	0	**0.0**	**-**		-	-	-	**-**	
44	L	86 (0.8)	2 (2.9)	**160.8**	**11.0** (2.5 - 48.4)	91	8	25	0	**0.0**	**-**		27	1	249.4	**23.3** (2.8 - 195.2)	
CP8061	L	73 (0.7)	2 (2.9)	**193.1**	**14.5** (3.3 - 63.7)	93	9	7	1	**1019.4**	**95.0** (10.8 - 832.5)		8	0	0.0	**-**	
43	L	60 (0.6)	2 (2.9)	**238.7**	**17.8** (4.1 - 78.0)	94	9	6	0	**0.0**	**-**		31	1	219.2	**21.6** (2.6 - 175.6)	
69	L	32 (0.3)	2 (2.9)	**444.1**	**27.9** (6.3 - 123.4)	96	8	6	1	**1169.8**	**269.0** (23.7 - 3050.8)		15	1	450.7	**41.7** (4.9 - 353.1	
MM4	L	22 (0.2)	2 (2.9)	**630.4**	**11.0** (2.5 - 48.4)	91	2	21	4	**1406.0**	**120.8** (30.8 - 472.9)		38	1	180.6	**21.0** (2.6 - 173.0)	
42	L	20 (0.2)	2 (2.9)	**692.4**	**46.3** (10.5 - 203.7)	98	8	5	1	**1361.9**	**159.7** (18.0 - 1416.4)		8	1	866.9	**96.2** (11.5 - 805.1)	
72	L	66 (0.6)	1 (1.4)	**107.6**	**12.9** (7.3 - 22.9)	92	7	19	1	**358.9**	**47.5** (5.5 - 407.4)		26	0	0.0	**-**	
61	L	40 (0.4)	1 (1.4)	**179.7**	**12.8** (7.2 - 22.6)	92	4	11	1	**615.7**	**92.8** (8.5 - 1014.0)		11	0	0.0	**-**	
67	L	30 (0.3)	1 (1.4)	**231.8**	**12.7** (7.2 - 22.6)	92	3	3	1	**2291.3**	**166.0** (17.6 - 1564.8)		13	0	0.0	**-**	
L1AE5	L	4 (0.0)	1 (1.4)	**1993.3**	**12.5** (7.1 - 22.2)	92	0	18	1	**411.8**	**44.2** (5.2 - 373.7)		22	1	319.3	**32.3** (4.0 - 263.6)	
Single type																	
16	H	70 (0.7)	6 (8.7)	610.1	**45.4** (17.5 - 117.3)	98	23	9	2	1795.3	**178.1** (35.4 - 896.7)		39	1	175.6	**17.6** (2.1 - 144.9)	
52	H	105 (1.0)	2 (2.9)	134.0	**10.5** (2.4 - 46.0)	90	9	45	2	326.8	**37.7** (7.8 - 182.7)		34	0	0.0	**-**	
58	H	33 (0.3)	2 (2.9)	447.8	**34.4** (7.8 - 152.0)	97	10	14	1	565.0	**86.7** (9.5 - 787.3)		7	0	0.0	**-**	
18	H	97 (1.0)	4 (5.8)	293.2	**21.9** (7.3 - 66.0)	95	17	12	1	625.8	**69.0** (8.4 - 569.9)		50	1	139.0	**13.8** (1.7 - 112.7)	
31	H	17 (0.2)	1 (1.4)	407.3	**31.6** (4.2 - 239.2)	97	5	4	1	1901.1	**256.3** (27.7 - 2371.7)		6	0	0.0	**-**	
56	H	122 (1.2)	2 (2.9)	114.3	**9.1** (2.1 - 40.0)	89	9	3	1	2451.0	**227.4** (25.2 - 2052.4)		72	1	95.5	**9.5** (1.2 - 77.1)	
33	H	56 (0.5)	1 (1.4)	120.4	**8.7** (1.2 - 66.4)	89	4	3	1	2277.9	**195.3** (20.7 - 1843.5)		36	0	0.0	**-**	
53	H	50 (0.5)	2 (2.9)	293.9	**23.5** (5.4 - 102.8)	96	10	8	0	0.0	**-**		18	0	0.0	**-**	
11	L	507 (5.0)	3 (4.3)	40.1	**3.0** (0.9 - 10.3)	66	9	1	0	0.0	**-**		331	2	40.7	**3.4** (0.7 - 16.6)	
CP8304	L	42 (0.4)	1 (1.4)	167.1	**12.5** (1.7 - 94.8)	92	5	10	1	699.3	**65.5** (7.4 - 583.2)		17	0	0.0	**-**	
54	L	37 (0.4)	1 (1.4)	182.7	**13.7** (1.8 - 104.0)	93	4	9	1	750.2	**89.3** (9.6 - 833.5)		17	0	0.0	**-**	
CP8061	L	34 (0.3)	1 (1.4)	212.6	**16.7** (2.2 - 126.3)	94	5	11	1	721.5	**89.0** (10.6 - 744.9)		11	0	0.0	**-**	

no case had HPV infection of type 66, -59, 26, 62, 55, 6, 32, 74, 37, MM7, 82, 44, CP8061, 43, 72, and L1AE5

mHR: age and no. of vaginal delivery adjusted; AR%: attributable risk percent; PAR%: populaiton attributable risk percent.

Table V-3. (cont.)

		Normal (n=10089)		Cervical cancer (n=35)							Cervical cancer among persistence subjects						Cervical cancer among clearance subjetcs					
HPV		no.	%	no.	%	incidence (10-5)	mHR	95%CI	AR%	PAR%	no.	case no.	incidence (10-5)	mHR	95%CI		no.	case no.	incidence (10^{-5})	mHR	95%CI	
16	H	166	1.6	9	25.7	385.3	**60.9**	22.7 - 163.7	98	50	21	5	**1894.7**	**418.3**	97.0 - 1803.2		82	1	82.8	**20.6**	2.1 - 198.8	
52	H	215	2.1	5	14.3	163.0	**27.0**	8.5 - 85.1	96	36	77	2	**185.4**	**47.8**	8.0 - 286.7		69	0	0.0	-		
58	H	90	0.9	7	20.0	558.8	**86.0**	29.7 - 249.1	99	43	35	4	**855.1**	**247.8**	52.5 - 1170.8		23	0	0.0	-		
18	H	196	1.9	6	17.1	215.0	**34.0**	11.4 - 101.3	97	39	24	2	**590.5**	**173.4**	25.9 - 1161.1		100	0	0.0	-		
31	H	57	0.6	4	11.4	487.7	**72.8**	21.2 - 250.0	99	29	16	2	**897.3**	**206.5**	31.2 - 1366.2		18	0	0.0	-		
56	H	202	2.0	2	5.7	69.3	**11.3**	2.3 - 54.6	91	17	8	1	**842.5**	**182.3**	17.3 - 1917.8		117	1	58.8	**14.0**	1.5 - 135.0	
33	H	132	1.3	2	5.7	103.4	**15.6**	3.2 - 75.3	94	16	12	2	**1123.6**	**378.6**	43.6 - 3289.1		81	0	0.0	-		
53	H	114	1.1	2	5.7	124.0	**19.9**	4.1 - 96.3	95	18	25	2	**585.7**	**135.4**	21.7 - 842.8		39	0	0.0	-		
35	H	41	0.4	2	5.7	351.1	**54.8**	11.2 - 268.4	98	18	5	1	**1340.5**	**382.0**	24.1 - 6044.7		21	1	334.9	**96.3**	9.0 - 1034.8	
39	H	96	1.0	2	5.7	149.1	**21.1**	4.3 - 102.0	95	16	23	2	**606.6**	**126.4**	20.2 - 790.2		39	0	0.0	-		
51	H	76	0.8	1	2.9	90.2	**13.8**	1.7 - 112.7	93	9	12	1	**561.5**	**130.7**	12.8 - 1329.8		38	0	0.0	-		
68	H	66	0.7	1	2.9	108.4	**17.1**	2.1 - 139.3	94	10	16	1	**427.4**	**99.8**	9.5 - 1047.3		28	0	0.0	-		
45	H	63	0.6	2	5.7	221.0	**36.1**	7.5 - 173.7	97	18	6	1	**1177.9**	**357.8**	21.4 - 5981.1		37	0	0.0	-		
11	L	617	6.1	3	8.6	33.1	**5.3**	1.4 - 20.6	81	21	2	0	**0.0**	-			398	1	16.9	**3.1**	0.3 - 30.4	
CP8304	L	92	0.9	3	8.6	233.2	**37.6**	9.6 - 146.1	97	25	19	2	**758.7**	**146.9**	22.1 - 977.4		38	1	180.6	**54.0**	5.5 - 533.9	
70	L	89	0.9	2	5.7	161.7	**26.9**	5.6 - 129.9	96	19	25	2	**557.3**	**129.0**	20.7 - 804.8		29	0	0.0	-		
54	L	87	0.9	2	5.7	163.5	**24.5**	5.1 - 118.9	96	17	17	0	**0.0**	-			37	2	381.0	**96.2**	16.1 - 575.9	
MM8	L	87	0.9	3	8.6	245.5	**41.8**	10.8 - 161.7	98	26	15	1	**494.3**	**345.2**	28.3 - 4205.0		28	0	0	-		
69	L	31	0.3	1	2.9	227.6	**31.9**	3.9 - 263.9	97	9	5	0	**0.0**	-			15	1	450.7	**131.6**	11.0 - 1567.7	
MM4	L	21	0.2	1	2.9	327.0	**48.6**	5.9 - 397.6	98	9	7	1	**1019.4**	**278.4**	21.9 - 3532.9		8	0	0.0	-		
42	L	19	0.2	1	2.9	360.9	**53.7**	6.5 - 441.5	98	9	5	1	**1362.4**	**481.0**	38.7 - 5977.3		7	0	0.0	-		
61	L	40	0.4	1	2.9	179.7	**27.5**	3.3 - 225.4	96	9	11	1	**615.8**	**444.9**	16.2 - 12182.6		11	0	0.0	-		
67	L	30	0.3	1	2.9	231.9	**33.4**	4.1 - 272.2	97	9	3	1	**2293.6**	**478.5**	29.4 - 7800.5		13	0	0.0	-		
Single type																						
16	H	69	0.7	5	14.3	511.2	**85.4**	27.0 - 270.4	99	37	9	2	1795.3	**497.4**	75.5 - 3275.2		39	1	569.5	**44.7**	4.6 - 438.2	
52	H	103	1.0	0	0.0	0.0	-				43	0	0.0	-			34	0	0.0	-		
58	H	33	0.3	2	5.7	447.8	**75.7**	15.4 - 372.5	99	20	14	1	565.0	**400.6**	34.9 - 4594.2		7	0	0.0	-		
18	H	96	1.0	3	8.6	222.1	**37.0**	9.5 - 143.2	97	25	12	1	625.8	**157.6**	15.9 - 1564.1		49	0	0.0	-		
31	H	16	0.2	0	0.0	0.0	-				3	0	0.0	-			6	0	0.0	-		
56	H	121	1.2	1	2.9	57.5	**10.0**	1.2 - 81.4	90	10	2	0	0.0	-			72	1	1047.1	**22.9**	2.4 - 220.7	
33	H	56	0.6	1	2.9	120.4	**18.3**	2.2 - 149.3	95	9	3	1	2277.9	**711.8**	42.6 - 11905.8		36	0	0.0	-		
11	L	506	5.0	2	5.7	26.8	**4.3**	0.9 - 20.6	77	14	1	0	0.0	-								
CP8304	L	42	0.4	1	2.9	167.1	**26.0**	3.2 - 212.1	96	9	10	1	699.3	**154.4**	14.2 - 1674.4		330	1	4902.0	**3.6**	0.4 - 35.4	

no case had HPV infection of type 66, -59, 26, 62, 55, 6, 32, 74, 37, MM7, 82, 44, CP8061, 43, 72, and L1AE5

mHR: age and no. of vaginal delivery adjusted; AR%: attributable risk percent; PAR%: populaiton attributable risk percent.

Table V-3. (cont.)

HPV		Normal (n=10088) no.	%	Cervical cancer (n=34) no.	%	incidence (10-5)	mHR	95%CI	AR%	PAR%	Cervical cancer among persistence subjects no.	case no.	incidence (10-5)	mHR	95%CI	Cervical cancer among clearance subjetcs no.	case no.	incidence (10^{-5})	mHR	95%CI
16	H	161	1.6	4	5.8	174.1	**23.4**	7.0 - 77.9	96	26	16	0	0.0	-		81	0	0.0	-	
52	H	216	2.1	6	8.7	195.9	**28.0**	9.7 - 80.8	96	36	79	4	366.6	**70.7**	17.6 - 283.5	69	0	0.0	-	
58	H	85	0.8	2	2.9	165.5	**20.5**	4.3 - 97.6	95	14	31	0	0.0	-		23	0	0.0	-	
18	H	192	1.9	2	2.9	72.4	**9.8**	2.1 - 46.3	90	14	22	0	0.0	-		101	1	68.1	**12.4**	1.4 - 110.6
31	H	55	0.5	2	2.9	250.6	**32.4**	6.9 - 153.2	97	14	15	1	487.8	**90.9**	9.6 - 857.7	18	0	0.0	-	
56	H	203	2.0	3	4.3	103.6	**14.6**	3.9 - 55.3	93	21	9	2	1563.7	**486.1**	53.8 - 4389.9	116	0	0.0	-	
33	H	132	1.3	2	2.9	103.5	**14.2**	3.0 - 67.1	93	15	12	2	1135.1	**118.1**	18.7 - 746.2	81	0	0.0	-	
53	H	114	1.1	2	2.9	123.6	**16.5**	3.5 - 77.9	94	15	23	0	0.0	-		39	0	0.0	-	
35	H	40	0.4	1	1.4	179.9	**21.8**	2.7 - 175.6	95	8	4	0	0.0	-		21	1	333.9	**65.3**	6.9 - 614.8
51	H	76	0.7	1	1.4	90.4	**11.7**	1.5 - 93.9	91	7	12	1	570.5	**90.6**	9.8 - 836.9	38	0	0.0	-	
68	H	66	0.6	1	1.4	108.6	**14.2**	1.8 - 113.5	93	8	15	0	0.0	-		28	0	0.0	-	
82	H	7	0.1	1	1.4	1101.3	**129.1**	15.5 - 1072.4	99	8	1	0	0.0	-		3	0	0.0	-	
11	L	618	6.1	4	5.8	44.1	**6.5**	2.0 - 21.6	85	25	2	0	0.0	-		400	3	50.7	**9.0**	2.0 - 40.6
CP8304	L	91	0.9	2	2.9	156.9	**21.7**	4.6 - 103.0	95	16	19	2	753.3	**113.9**	15.9 - 815.5	37	0	0.0	-	
70	L	88	0.9	1	1.4	81.9	**11.6**	1.5 - 93.0	91	8	24	1	291.7	**51.6**	5.6 - 474.8	29	0	0.0	-	
54	L	86	0.8	1	1.4	82.0	**10.6**	1.3 - 85.3	91	7	18	1	374.4	**64.2**	5.9 - 693.6	35	0	0.0	-	
44	L	86	0.8	2	2.9	160.8	**20.3**	4.3 - 96.3	95	14	25	0	0.0	-		27	1	249.4	**32.4**	3.5 - 301.3
CP8061	L	73	0.7	2	2.9	193.1	**27.1**	5.7 - 128.2	96	16	18	1	411.7	**69.9**	7.3 - 666.6	22	1	319.3	**55.9**	6.2 - 503.2
43	L	60	0.6	2	2.9	238.7	**33.5**	7.1 - 157.6	97	16	6	0	0.0	-		31	1	219.2	**37.6**	4.2 - 338.1
69	L	31	0.3	1	1.4	229.0	**25.8**	3.2 - 210.3	96	7	6	1	1169.6	**464.5**	25.3 - 8546.0	14	0	0.0	-	
MM4	L	21	0.2	1	1.4	326.3	**39.9**	5.0 - 321.7	97	7	6	0	0.0	-		8	0		-	
42	L	19	0.2	1	1.4	364.6	**43.9**	5.4 - 353.3	98	7	4	0	0.0	-		8	1	867.3	**191.6**	20.0 - 1839.1
72	L	66	0.6	1	1.4	107.6	**13.8**	1.7 - 111.2	93	8	19	1	358.9	**67.3**	7.0 - 651.7	26	0	0.0	-	
L1AE5	L	4	0.0	1	1.4	1992.0	**305.9**	38.2 - 2446.9	100	11	1	0	0.0	-		0	0	0.0	-	
Single type																				
16	H	65	0.6	1	1.4	106.8	**13.9**	1.7 - 111.4	93	8	7	0	0.0	-		38	0	0.0	-	
52	H	105	1.0	2	2.9	134.0	**20.4**	4.3 - 96.2	95	17	45	2	326.8	**69.3**	12.5 - 383.0	34	0	0.0	-	
58	H	31	0.3	0	0.0	0.0	-				13	0	0.0	-		7	0	0.0	-	
18	H	94	0.9	1	1.4	74.1	**9.8**	1.2 - 78.1	90	7	11	0	0.0	-		50	1	139.0	**25.1**	2.8 - 224.8
31	H	17	0.2	1	1.4	407.3	**63.0**	7.8 - 505.4	98	9	4	1	1901.1	**412.5**	37.3 - 4558.0	6	0	0.0	-	
56	H	121	1.2	1	1.4	57.4	**8.5**	1.1 - 68.1	88	8	3	1	2451.0	**1083.2**	76.9 - 15256.2	71	0	0.0	-	
33	H	55	0.5	0	0.0	0.0	-				2	0	0.0	-		36	0	0.0	-	
53	H	50	0.5	2	2.9	293.9	**44.4**	9.4 - 209.5	98	18	8	0	0.0	-		18	0	0.0	-	
11	L	505	5.0	1	1.4	13.4	**1.9**	0.2 - 14.9	46	4	1	0	0.0	-		330	1	20.4	**3.4**	0.4 - 30.4
54	L	37	0.4	1	1.4	182.7	**28.3**	3.5 - 230.5	96	9	9	1	750.2	**131.6**	11.3 - 1532.0	17	0	0.0	-	
CP8061	L	34	0.3	1	1.4	212.6	**33.7**	4.2 - 270.1	97	10	11	1	721.5	**142.7**	15.5 - 1311.2	11	0	0.0	-	

no case had HPV infection of type 66, -59, 26, 62, 55, 6, 32, 74, 37, MM7, 82, 44, CP8061, 43, 72, and L1AE5

mHR: age and no. of vaginal delivery adjusted; AR%: attributable risk percent; PAR%: populaiton attributable risk percent.

Table V-4. Incidence, hazard ratio, attributable risk percent, and population attributable risk percent of incident cervical cancer by HPV infection status at baseline and type-specific persistence or clearance at follow-up

	Normal	Cervical cancer (n=69)						ICC (n=35)						CIS (n=34)					
	(n=10123)		**incidence**	**mHR**	(95%CI)	AR%	PAR%		**incidence**	**mHR**	(95%CI)	AR%	PAR%		**incidence**	**mHR**	(95%CI)	AR%	PAR%
HPV	no.	no.	**(10-5)**					no.	**(10-5)**					no.	**(10-5)**				
At baseline																			
Negative	7916 (78.2)	15	13.1	1.0				7	6.1	1.0				8	7.0	1.0			
any HPV	2207 (21.8)	54	170.8	12.8	(7.2 - 22.6)	92	72	28	89.3	14.3	(6.2 - 32.7)	93	74	26	82.9	11.6	(5.2 - 25.6)	91	70
HR-17	1228 (12.1)	46	265.3	19.6	(10.9 - 35.1)	95	69	25	145.9	23.3	(10.1 - 53.9)	96	80	21	122.6	16.8	(7.4 - 37.9)	94	65
LR-22	1335 (13.2)	25	129.6	9.7	(5.1 - 18.5)	90	54	11	57.5	9.2	(3.6 - 23.7)	89	62	14	73.0	10.3	(4.3 - 24.7)	90	55
LR-22 (HR-17 excluded)	979 (9.7)	8	56.1	4.2	(1.8 - 10.0)	76	24	3	21.1	3.3	(0.9 - 12.9)	70	25	5	35.1	5.1	(1.7 - 15.5)	80	28
HR-13	1133 (11.2)	43	268.3	19.7	(11.0 - 35.6)	95	68	25	157.6	25.1	(10.8 - 58.1)	96	80	18	113.8	15.5	(6.7 - 35.6)	94	61
HPV16/18/52/58/33/31	733 (7.2)	39	376.1	27.4	(15.1 - 49.8)	96	66	24	234.5	36.9	(15.9 - 85.7)	97	79	15	147.6	19.9	(8.4 - 47.1)	95	57
HPV16/18/52/58	606 (6.0)	34	399.6	29.3	(16.0 - 53.9)	97	63	22	261.7	41.6	(17.7 - 97.4)	98	78	12	144.1	19.5	(8.0 - 47.8)	95	52
HPV16/18	344 (3.4)	21	435.2	31.8	(16.4 - 61.6)	97	51	15	314.0	52.0	(21.2 - 127.5)	98	72	6	127.4	17.1	(5.9 - 49.4)	94	34
single positive	1601 (15.8)	26	112.5	8.5	(4.5 - 16.1)	88	54	15	65.2	10.5	(4.3 - 25.8)	90	69	11	47.9	6.8	(2.7 - 17.0)	85	48
multiple	606 (6.0)	28	328.6	24.0	(12.8 - 45.1)	96	58	13	155.4	24.6	(9.8 - 61.9)	96	68	15	178.8	24.2	(10.2 - 57.4)	96	58
2 types	395 (3.9)	18	327.4	24.0	(12.1 - 47.8)	96	47	7	129.7	20.6	(7.2 - 58.8)	95	53	11	201.7	27.5	(11.0 - 68.6)	96	51
3 types	133 (1.3)	6	316.4	23.7	(9.2 - 61.3)	96	23	3	161.2	26.2	(6.8 - 101.6)	96	33	3	161.1	22.2	(5.9 - 84.0)	95	21
4+ types	78 (0.8)	4	359.3	24.5	(8.1 - 74.6)	96	15	3	271.7	41.4	(10.5 - 162.8)	98	32	1	93.1	11.5	(1.4 - 92.6)	91	7
Multiple vs. single	606 (6.0)	28	328.6	2.7	(1.6 - 4.7)	64	9	13	155.4	2.3	(1.1 - 4.8)	56	10	15	178.8	3.5	(1.6 - 7.7)	71	13
single HR-HPV positive	968 (9.6)	31	226.9	17.0	(9.2 - 31.5)	94	60	15	111.0	17.9	(7.3 - 43.9)	94	71	16	118.1	16.434	(7.0 - 38.4)	94	59
multiple	260 (2.6)	15	408.1	29.1	(14.1 - 59.7)	97	42	10	275.8	43.2	(16.3 - 114.3)	98	62	5	139.7	17.975	(5.8 - 55.3)	94	30
2 types	187 (1.8)	10	385.7	27.3	(12.2 - 61.1)	96	33	6	233.5	36.5	(12.2 - 109.4)	97	49	4	156.0	19.901	(6.0 - 66.5)	95	25
3 types	48 (0.5)	3	417.0	30.2	(8.7 - 104.7)	97	12	2	289.0	46.4	(9.6 - 224.4)	98	24	1	146.8	19.602	(2.4 - 157.4)	95	8
4+ types	25 (0.2)	2	549.6	37.7	(8.6 - 165.4)	97	8	2	549.6	83.2	(17.2 - 402.3)	99	24	0	0.0	-	)		
Multiple HR vs. single HR	260 (2.6)	15	408.1	1.7	(0.9 - 3.2)	41	2	10	275.8	7.1	(2.1 - 23.6)	86	19	5	139.7	3.264	(1.2 - 8.7)	69	5
At F/U																			
Persistence																			
Persistent negative	4902 (73.5)	7	9.7	1.0				3	4.2	1.0				4	5.6	1.0			
any HPV	390 (5.9)	26	475.1	44.3	(19.0 - 102.9)	98	72	13	243.6	50.6	(14.3 - 179.4)	98	74	13	242.7	41.0	(13.2 - 127.3)	98	69
HR-17	252 (3.8)	21	601.1	56.0	(23.7 - 132.6)	98	68	12	352.8	74.7	(20.9 - 266.9)	99	73	9	265.8	44.2	(13.5 - 144.9)	98	61
LR-22	236 (3.5)	12	351.7	31.2	(12.1 - 80.4)	97	52	6	179.3	35.9	(8.8 - 146.2)	97	55	6	179.7	**28.3**	(7.8 - 102.5)	96	48
LR-22 excluding HR-17	131 (2.0)	4	211.8	20.3	(5.9 - 70.2)	95	28	1	53.5	23.5	(3.9 - 143.1)	96	30	2	107.2	18.1	(3.3 - 100.2)	94	25
HR-13	233 (3.5)	21	650.4	60.4	(25.5 - 143.1)	98	68	12	382.6	80.8	(22.6 - 288.6)	99	73	9	288.3	47.8	(14.6 - 156.6)	98	61
HPV16/18/52/58/33/31	177 (2.7)	19	785.8	74.2	(31.0 - 177.5)	99	66	12	511.0	109.9	(30.7 - 392.4)	99	74	7	302.9	50.8	(14.7 - 174.9)	98	55
HPV16/18/52/58	154 (2.3)	14	669.8	63.7	(25.6 - 158.4)	98	59	10	487.1	105.5	(28.9 - 385.1)	99	70	4	199.3	33.8	(8.4 - 135.6)	97	41
HPV16/18	44 (0.7)	7	1192.3	103.8	(36.1 - 298.0)	99	40	7	1192.3	238.3	(61.0 - 931.5)	100	61	0	0.0	-			
Clearance																			
all clearance	**867** (13.0)	**3**	23.6	2.4	(0.6 - 9.2)	58	15	1	7.9	1.8	(0.2 - 17.6)	45		2	15.7	2.8	(0.5 - 15.5)		

mHR: age and no. of vaginal delivery adjusted; AR%: attributable risk percent; PAR%: populaiton attributable risk percent.

Table VI-1. Characteristics associated with HPV viral load and Integration status at baseline

Variables		Viral load						Integration status (among viral load>10^3)							
		Low ($<10^3$)		Medium (10^3 -10^4)		High ($>10^4$)		Eipsomal form (>1.1)		Mixed form (0.5-1.1)		Integrated form predominant (0-0.5)		Integrated predominant (<0.5)	Purely integrated (no E2)
		n	%	n	%	n	%	n	%	n	%	n	%	n	n
Total (n=807)															
HPV16	242	121	50.0	30	12.4	91	37.6	42	34.7	58	47.9	21	17.4	19	2
HPV18	225	164	72.9	27	12.0	34	15.1	7	11.5	36	59.0	18	29.5	13	5
HPV52	290	95	32.8	71	24.5	127	42.7	47	23.7	101	51.0	47	23.7	42	5
HPV58	145	53	36.6	28	19.3	64	44.1	24	26.1	52	56.5	16	17.4	8	8
Baseline cytology															
Normal	603	332	55.0	118	19.6	153	25.4	60	22.1	142	52.4	69	25.5	60	9
ASCUS	37	12	32.4	9	24.3	16	43.2	6	24.0	17	68.0	2	8.0	2	0
LSIL	47	6	12.7	10	21.3	31	66.0	17	41.5	20	48.8	4	9.8	4	0
HSIL	120	6	5.0	12	10.0	102	85.0	32	28.1	56	49.1	26	22.8	16	10
				X^2= 181.9, p<0.0001						**X^2=22.4, p=0.0076**					
Cytologically normal & F/U (n=391)															
HPV16	104	75	72.1	12	11.5	17	16.3	14	48.3	13	44.8	2	6.9	2	0
HPV18	125	109	87.2	9	7.2	7	5.6	3	18.8	11	68.8	2	12.5	2	0
HPV52	149	60	40.3	38	25.5	51	34.2	25	28.1	44	49.4	19	21.3	18	1
HPV58	58	25	43.1	14	24.1	19	32.8	12	36.4	20	60.6	1	3.0	0	1
Age group															
30-44	169	101	59.8	34	20.1	34	20.1	23	33.8	34	50.0	11	16.2	10	1
45-54	112	67	59.8	26	23.2	19	17.0	15	33.3	25	55.6	5	11.1	5	0
55+	110	62	56.4	29	26.4	19	17.3	15	31.3	24	50.0	9	18.8	8	1
				X^2=1.78, p=0.77						X^2=1.62, p=0.95					
Menopause															
No	231	141	61.0	45	19.5	45	19.5	31	34.4	44	48.9	15	16.7	14	1
Yes	160	89	55.6	44	27.5	27	16.9	22	31.0	39	54.9	10	14.1	9	1
				X^2=3.48, p=0.18						X^2=0.68, p=0.88					
no. of labor delivery															
0-2	69	48	69.6	12	17.4	9	13.0	8	38.1	11	52.4	2	9.5	2	0
3	111	61	55.0	27	24.3	23	20.7	22	44.0	22	44.0	6	12.0	6	0
4	95	59	62.1	17	17.9	17	17.9	8	23.5	20	58.8	8	23.5	7	1
5+	116	62	53.4	16	13.8	38	32.8	15	27.8	30	55.6	9	16.7	8	1
				X^2=14.3, p=0.03						X^2=7.4, p=0.59					
no. of sexual partner															
0-1	375	223	59.5	67	17.9	85	22.7	50	32.9	79	52.0	23	15.1	22	1
>2	15	6	40.0	5	33.3	4	26.7	3	33.3	4	44.4	2	22.2	1	1
				X^2=2.89, p=0.23						X^2=7.59, p=0.055					
IUD use															
Never	119	76	63.9	21	17.6	21	17.6	17	40.5	20	47.6	6	14.3	6	0
Ever	272	154	56.6	50	18.4	68	25.0	36	30.5	63	53.4	19	16.1	17	2
				X^2=2.49, p=0.26						X^2=1.79, p=0.62					

Table VI-2. Viral load levels and integration status of HPV16/18/52/58 among cytologically normal at baseline (n=391)

			Persistent at F/U (n=166)				
Viral load levels	Integration status(E6/E2 ratio)	n	no.	%	aOR (95%CI)	mOR (95%CI)	mOR (95%CI)
Low (L, reference)		230	51	22.2	1.0		
Medium (M)		71	40	57.7	4.5 (2..6-8.0)	4.3 (2.4-7.7)	
High (H)		86	63	74.4	8.4 (4.8-14.7)	8.4 (4.8-14.8)	
menopause (post- vs. pre-)						1.9 (1.2-3.0)	
sexual partner (>1 vs.						7.9 (2.0-31.5)	
L (reference)		230	51	22.2	1.0	1.0	
	Eipsomal form (>1.1)	53	35	66.0	4.9 (2.6-9.3)	5.0 (2.7-9.5)	
	Mixed form (0.5-1.1)	83	55	66.3	6.9 (4.0-12.0)	6.8 (3.9-11.9)	
	Integrated form predominant (0-0.5)	25	17	68.0	7.5 (3.0-18.3)	7.7 (3.1-19.0)	
	Integrated predominant (<0.5)	23	15	65.2	5.7 (2.3-14.1)	5.9 (2.4-14.8)	
	Purely integrated form (no E2)	2	2	100.0	∞		
L		230	51	24.8	1.0	1.0	
M	Eipsomal form	30	15	50.0	3.5 (1.6-7.7)	3.6 (1.6-8.0)	1.0
	Mixed form	34	19	55.9	4.4 (2.1-9.4)	4.6 (2.2-9.7)	1.3 (0.5-3.4)
	Integrated form predominant	8	6	75.0	10.5 (2.1-53.8)	11.0 (2.1-56.8)	2.0 (0.5-17.7)
H	Eipsomal form	23	16	69.6	8.0 (3.1-20.6)	7.9 (3.1-20.4)	1.0
	Mixed form	49	36	73.5	9.7 (4.8-19.7)	9.3 (4.6-19.0)	1.2 (0.4-3.6)
	Integrated form predominant	17	11	64.7	6.4 (2.3-18.2)	6.5 (2.3-18.7)	0.8 (0.2-3.1)

aOR: age-adjusted odds ratio; mOR: multivariate-adjusted odds ratio.

Table VI-3. Viral load and viral load change associated with persistence among pre- and post-menopausal women

Viral load level and change	Pre-menopause (n=231)					Post-menopause (n=160)					aOR post- vs. pre-menopause
		Persistence					Persistence				
	N	no.	%	aOR (95%CI)	mOR (95%CI)	N	no.	%	aOR (95%CI)	mOR (95%CI)	
Baseline viral load											
L	141	24	17.0	1.0	1.0	89	27	30.3	1.0	1.0	**2.1(1.1-4.0)**
M	45	23	51.1	5.1 (2.4-10.6)	4.8 (2.2-10.2)	27	17	63.0	3.9 (1.6-9.6)	3.9 (1.6-9.7)	1.6(0.6-4.3)
H	45	31	68.9	10.9 (5.0-23.3)	11.7 (5.3-25.8)	44	32	72.7	6.1 (2.7-13.7)	6.1 (2.7-13.6)	1.2(0.5-3.0)
				trend test: p<0.0001	12.8 (2.5-66.0)				trend test: p=0.0001	1.5 (0.1-21.8)	
Viral load lowered at F/U	179					106					
L to L	130	15	11.5	1.0	1.0	75	17	22.7	1.0	1.0	1.9(0.8-4.3)
M to L	33	12	36.4	4.5 (1.8-11.3)	4.0 (1.5-10.5)	16	7	43.8	3.1 (0.9-10.1)	3.1 (0.9-10.3)	1.2(0.3-4.2)
H to L	16	4	25.0	2.1 (0.5-8.3)	2.3 (0.6-9.3)	15	5	33.3	1.2 (0.3-5.0)	1.1 (0.3-4.7)	1.1(0.2-6.4)
					10.9 (1.7-69.3)					4.2 (0.-74.6)	

aOR: age-adjusted odds ratio; mOR: multivariate-adjusted odds ratio.

Table VI-4. HPV viral load, viral load change and persistence of HPV16/18/52/58 in predicton of developing cervical cancer

Variables	n	Cervical cancer (n=16)											
		overall				pre- menopause				post-menopause			
		case	Incidence 10^{-5}	mHR	(95%CI)	case	Incidence 10^{-5}	aHR	(95%CI)	case	Incidence 10^{-5}	aHR	(95%CI)
at baseline													
viral load													
L	230	6	181.0	1.0		4	193.0	1.0		2	160.9	1.0	
M	71	2	193.4	1.1	(0.2 - 5.5)	0	0.0	-	(-)	2	562.0	3.9	(0.6 - 28.2)
H	89	8	669.4	3.4	(1.2 - 10.0)	1	153.2	0.8	(0.1 - 7.5)	7	1291.0	8.1	(1.7 - 39.1)
adj. menopause				3.3	(1.2 - 9.7)								
at follow-up													
viral load													
L	284	3	72.4	1.0		2	84.8	1.0		1	75.8	1.0	
M	49	2	298.0	3.8	(0.6 - 22.9)	1	283.0	3.4	(0.3 - 37.4)	1	402.9	6.1	(0.4 - 97.4)
H	57	11	1503.7	18.5	(5.1 - 67.0)	2	642.3	8.4	(1.2 - 60.6)	9	2772.6	37.2	(4.7 - 294.8)
adj. menopause				2.5	(0.9 - 7.4)								
Viral load lowered to low													
No	106	13	926.9	1.0		3	399.9	1.0		10	1533.0	1.0	
Yes	284	3	72.4	0.1	(0.03 - 0.3)	2	75.4	0.2	(0.03 - 1.2)	1	67.2	0.04	(0.01 - 0.3)
adj. menopause				2.9	(1.0 - 8.4)								
Persistence													
Clearance	236	2	65.2	1.0		2	99.0	1.0		0	0.0	1.0	
Persistence	154	14	757.9	14.4	(2.3 - 45.3)	3	299.0	3.3	(0.6 - 20.1)	11	1303.9	∞	(-)
adj. menopause				2.4	(0.7 - 8.6)								

aHR: age-adjusted hazard ratio; mHR: multivariate-adjusted hazard ratio.

Appendixes

A	Risk factors associated with HPV infection among cytologically normal at baseline
B	Risk factors associated with HPV infection among abnormal
C	Risk factors associated with HPV acquisition
D	Risk factors associated with HPV acquisition among cytologically abnormal
E	Risk factors associated with HPV persistence
F	Risk factors associated with HPV persistence among cytologically abnormal at baseline
G	Primers used for real-time PCR
H	Disassociation curves and standard curve for HPV viral load
I	Reliability of viral load measurements
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Appendixe A. Risk factors associated with HPV infection among cytologically normal at baseline

Variables	Normal (n=10190)	HPV positive at baseline (n=2274)			
	n (%)	n (%)	OR	aOR (95% CI)	mOR (95% CI)
Age at baseline					
30-34	1395 (13.7)	285 (20.4)	1.0		
35-39	1845 (18.1)	390 (21.1)	1.0	(0.9 - 1.2)	
40-44	1599 (15.7)	332 (20.8)	1.0	(0.9 - 1.2)	
45-49	1284 (12.6)	291 (22.7)	1.1	(0.9 - 1.4)	
50-54	1556 (15.3)	353 (22.7)	1.1	(1.0 - 1.4)	
55-59	1439 (14.1)	365 (25.4)	1.3	(1.1 - 1.6)*	
60+	1072 (10.5)	258 (24.1)	1.2	(1.0 - 1.5)*	
p for trend			<0.001		
Marital status at baseline					
Currently married	9413 (92.4)	2085 (22.2)	1.0		
Widowed/Divorced/Others	770 (7.6)	187 (24.3)	1.1	1.1 (0.9 - 1.3)	
Unknown	7 (0.1)	2 (28.6)	1.4	1.5 (0.3 - 7.6)	
No. of marriage					
0-1	9974 (92.4)	2222 (22.2)	1.0		
2+	149 (7.6)	43 (24.3)	1.4	1.4 (1.0 - 2.0)	
Unknown	67 (0.1)	9 (28.6)	0.5	0.5 (0.3 - 1.1)	
Cigarette Cigarette					
Never	10043 (97.9)	2225 (22.3)	1.0		
Ever	104 (1.5)	38 (28.9)	2.0	2.1 (1.4 - 3.1)**	1.9 (1.3 - 2.9)*
Unknown	43 (0.7)	11 (13.4)	1.2	1.2 (0.6 2.5)	
Alcohol drinking					
Never	10086 (99.0)	2240 (22.2)	1.0		
Ever	62 (0.6)	22 (35.5)	1.9	2.0 (1.2 - 3.3)*	
Unknown	42 (0.4)	12 (28.6)	1.4	1.4 (0.7 - 2.8)	
Betal chewing					
Never	10129 (99.4)	2257 (22.3)	1.0		
Ever	17 (0.2)	5 (29.4)	1.5	1.5 (0.5 - 4.3)	
Unknown	44 (0.4)	12 (27.3)	1.3	1.3 (0.7 - 2.6)	
Pregnancy, delivery & contraception					
No. of lifetime childbirth					
0-4	4605 (43.4)	967 (21.0)	1.0		1.0
5-8	4845 (45.7)	1115 (23.0)	1.1	1.1 (1.0 - 1.2)	1.0 (0.9 - 1.1)
9+	521 (4.9)	146 (28.0)	1.5	1.4 (1.1 - 1.7)*	1.2 (1.0 - 1.5)*
Unknown	219 (2.1)	46 (21.0)			
p for trend			<0.001		
No. of vaginal delivery					
0-2	2058 (20.2)	452 (22.0)	1.0		
3	2912 (28.6)	606 (20.8)	0.9	0.9 (0.8 - 1.0)	
4	2270 (22.3)	538 (23.7)	1.1	1.0 (0.9 - 1.2)	
5+	2760 (27.1)	642 (23.3)	1.1	0.9 (0.8 - 1.1)	
Unknown	190 (1.9)	36 (18.9)	0.8	0.8 (0.5 - 1.1)	
p for trend			0.057		
No. of CS					
0	3745 (36.8)	796 (21.3)	1.0		
1	3079 (30.2)	686 (22.3)	1.1	1.1 (0.9 - 1.2)	
2	1949 (19.1)	443 (22.7)	1.1	1.1 (1.0 - 1.2)	
3+	1201 (11.8)	303 (25.2)	1.3	1.2 (1.1 - 1.5)*	
Unknown	216 (2.1)	46 (21.3)	1.0	1.0 (0.7 - 1.4)	
p for trend			0.006		
Abortion					
Never	9282 (91.1)	2092 (22.5)	1.0		
Ever	688 (6.8)	135 (19.6)	0.8	0.9 (0.7 - 1.1)	
Unknown	220 (2.2)	47 (21.4)	0.9	0.9 (0.7 - 1.3)	
OC use					
Never	7017 (68.9)	1579 (22.5)	1.0		
Ever	3106 (30.5)	685 (22.1)	1.0	1.0 (0.9 - 1.1)	
Unknown	67 (0.7)	10 (14.9)	0.6	0.6 (0.3 - 1.2)	
OC use (Current)					
Never use+not now	9765 (95.8)	2183 (22.4)	1.0		
Yes	298 (2.9)	69 (23.2)	1.0	1.1 (0.9 - 1.5)	
Unknown	127 (1.2)	22 (17.3)	0.7	0.7 (0.4 - 1.1)	
IUD use					
Never	4134 (40.6)	845 (20.4)	1.0		1.0
Ever	5996 (58.8)	1421 (23.7)	1.2	1.2 (1.1 - 1.3)**	1.2 (1.1 - 1.3)**
Unknown	60 (0.6)	8 (13.3)	0.6	0.6 (0.3 - 1.3)	

Appendixe A. Risk factors associated with HPV infection among cytologically normal at baseline

Variables	Normal (n=10190)	HPV positive at baseline (n=2274)			
	n (%)	n (%)	OR	aOR (95% CI)	mOR (95% CI)
Menstration					
Age at menarche					
14-16	5550 (54.5)	1225 (22.1)	1.0		
~13	1291 (12.7)	275 (21.3)	1.0	1.0 (0.8 - 1.1)	
17+	3207 (31.5)	746 (23.3)	1.1	1.0 (0.9 - 1.1)	
Unknown	142 (1.4)	28 (19.7)	0.9	0.8 (0.6 - 1.3)	
Menopause					
No	6448 (60.8)	1352 (21.0)	1.0		1.0
Yes	3708 (35.0)	918 (24.8)	1.2	1.3 (1.0 - 1.5)*	1.2 (1.0 - 1.4)
Unknown	34 (0.3)	4 (11.8)			
Age at menopause	3708	918			
~45	512 (13.8)	114 (22.3)	1.0		
46-50	1607 (43.3)	405 (25.2)	1.2	1.2 (0.9 - 1.5)	
51-55	1227 (33.1)	315 (25.7)	1.2	1.2 (0.9 - 1.6)	
56+	160 (4.3)	39 (24.4)	1.1	1.1 (0.7 - 1.7)	
Unknown	202 (5.4)	45 (22.3)	1.0	1.0 (0.7 - 1.5)	
p for trend			0.280		
Sexual behavior					
Age at initial coitus					
14-21	3888 (38.2)	942 (24.2)	1.0		
22-23	2670 (26.2)	597 (22.4)	0.9	0.9 (0.8 - 1.0)	
24+	3529 (34.6)	721 (20.4)	0.8	0.8 (0.7 - 0.9)*	
Unknown	103 (1.0)	14 (13.6)	0.5	0.5 (0.3 - 0.9)*	
p for trend			<0.001		
No. of lifetime sexual partners					
0-1	9856 (96.7)	2174 (22.1)	1.0		1.0
2+	250 (2.5)	88 (35.2)	1.9	1.9 (1.5 - 2.5)**	1.8 (1.4 - 2.4)**
Unknown	84 (0.8)	12 (14.3)	0.6	0.6 (0.3 - 1.1)	
Premarital sex					
No	9866 (96.8)	2211 (22.4)	1.0		
Yes	218 (2.1)	49 (22.5)	1.0	1.1 (0.8 - 1.5)	
Unknown	106 (1.0)	14 (13.2)	0.5	0.5 (0.3 - 0.9)*	
Pap smear experience					
Pap smear					
Never	8798 (86.3)	1981 (22.5)	1.0		
Ever	1365 (13.4)	285 (20.9)	0.9	0.9 (0.8 - 1.1)	
Unknown	27 (0.3)	8 (29.6)	1.4	1.4 (0.6 - 3.3)	

*p<0.05, **p<0.001
oral contraceptives : OC
Caesarian operations : CS

Appendix B. Risk factors associated with HPV infection among abnormal at baseline

Variables	Abnormal (n=412)		HPV positive at baseline (n=320)								
	n	%	n	%	OR	aOR	95% CI		mOR	95% CI	
Age at baseline											
30-34	40	9.7	33	82.5	1.0						
35-39	64	15.5	48	75.0	0.6		0.2	1.7			
40-44	85	20.6	57	67.1	0.4		0.2	1.1			
45-49	63	15.3	43	68.3	0.5		0.2	1.2			
50-54	50	12.1	40	80.0	0.8		0.3	2.5			
55-59	58	14.1	53	91.4	2.2		0.7	7.7			
60+	52	12.6	46	88.5	1.6		0.5	5.3			
p for trend					<0.05						
Age at baseline (Ⅱ)											
30-44	189	1.8	138	73.0	1.0						
45-54	113	1.1	83	73.5	1.0		0.6	1.7	0.8	0.5	1.5
55+	110	1.0	99	90.0	3.3		1.7	6.7)**	1.2	0.4	3.7
p for trend					<0.05						
Marital status at baseline											
Currently married	385	93.4	298	77.4	1.0						
Widowed/Divorced/Others	26	6.3	21	80.8	1.2	1.1	0.4	3.1			
Unknown	1	0.2	1	100.0	∞						
No. of marriage											
0-1	405	98.3	315	77.8	1.0						
2+	4	1.0	3	75.0	0.9	0.7	0.1	7.3			
Unknown	3	0.7	2	66.7	0.6	0.3	0.0	3.2			
Cigarette smoking											
Never	406	98.5	316	77.8	1.0						
Ever	4	1.0	3	75.0	0.9	1.0	0.1	10.0			
Unknown	2	0.5	1	50.0	0.3	0.2	0.0	4.6			
Alcohol drinking											
Never	405	98.3	315	77.8	1.0						
Ever	5	1.2	4	80.0	1.1	1.4	0.1	13.0			
Unknown	2	0.5	1	50.0	0.3	0.2	0.0	4.6			
Betal chewing											
Never	409	99.3	318	77.8	1.0						
Ever	1	0.2	1	100.0	∞						
Unknown	2	0.5	1	50.0	0.3	0.2	0.0	4.6			
Unknown	3	0.7	2	66.7	0.6	0.3	0.0	3.4			
Pregnancy, delivery & contraception											
No. of lifetime childbirth											
0-3	83	0.8	64	77.1	1.0						
4	81	0.8	60	74.1	0.8	0.8	0.4	1.7			
5	85	0.8	60	70.6	0.7	0.6	0.3	1.3			
6	67	0.6	61	91.0	3.0	2.5	0.9	7.1			
7	29	0.3	23	79.3	1.1	0.8	0.3	2.6			
8	28	0.3	20	71.4	0.2	0.2	0.1	0.6)*			
9+	29	0.3	25	86.2	1.9	0.9	0.3	3.4			
Unknown	10	0.1	7	70.0							
p for trend					0.223						
No. of vaginal delivery											
0-2	63	15.3	49	77.8	1.0						
3	109	26.5	77	70.6	0.7	0.8	0.4	1.7			
4	110	26.7	88	80.0	1.1	1.2	0.5	2.8			
5+	121	29.4	100	82.6	1.4	1.1	0.4	2.6			
Unknown	9	2.2	6	66.7	0.6	0.4	0.1	2.1			
p for trend					0.117			-			
No. of CS								-			
0	142	34.5	109	76.8	1.0			-			
1	129	31.3	104	80.6	1.3	1.2	0.7	2.2			
2	75	18.2	60	80.0	1.2	1.3	0.6	2.7			
3+	56	13.6	40	71.4	0.8	0.7	0.3	1.4			
Unknown	10	2.4	7	70.0	0.7	0.5	0.1	2.3			
p for trend					0.642			-			
Abortion								-			
Never	383	93.0	297	77.5	1.0			-			
Ever	19	4.6	16	84.2	1.5	1.3	0.4	4.8			
Unknown	10	2.4	7	70.0	0.7	0.5	0.1	2.1			

Appendix B. Risk factors associated with HPV infection among abnormal at baseline

Variables	Abnormal (n=412)		HPV positive at baseline (n=320)					
	n	%	n	%	OR	aOR	95% CI	mOR 95% CI
OC use								
Never	264	64.1	202	76.5	1.0			
Ever	145	35.2	116	80.0	1.2	1.6	1.0 - 2.7	
Unknown	3	0.7	2	66.7	0.6	0.3	0.0 - 3.6	
OC use (Current)								
Never use+not now	381	92.5	298	78.2	1.0			
Yes	28	6.8	20	71.4	0.7	0.8	0.3 - 1.9	
Unknown	3	0.7	2	66.7	0.6	0.3	0.0 - 3.2	
IUD use								
Never	152	36.9	118	77.6	1.0			
Ever	257	62.4	200	77.8	1.0	1.0	0.6 - 1.7	
Menstration								
Age at menarche								
14-16	215	52.2	160	74.4	1.0			
~13	51	12.4	41	80.4	1.4	1.6	0.7 - 3.5	
17+	141	34.2	115	81.6	1.5	1.2	0.7 - 2.1	
Unknown	5	1.2	4	80.0	1.4	1.0	0.1 - 9.8	
Menopause								
No	259	2.4	186	71.8	1.0			1.0
Yes	149	1.4	132	88.6	3.0	2.5	0.9 - 7.1	2.8 1.1 - 7.2 *
Unknown	4	0.0	2	50.0				
Age at menopause	149		132					
~45	24	16.1	19	79.2	1.0			
46-50	63	42.3	56	88.9	2.1	1.3	0.3 - 5.9	
51-55	55	36.9	50	90.9	2.6	1.6	0.3 - 7.9	
56+	4	2.7	4	100.0	∞			
Unknown	3	2.0	3	100.0	∞			
p for trend					0.128			
Sexual behavior								
Age at initial coitus								
22-25	171	1.6	124	72.5	1.0			1.0
14-21	189	1.8	157	83.1	1.9	1.8	1.1 - 3.1 *	1.8 1.1 - 3.1
26+	47	0.4	36	76.6	1.2	1.5	0.7 - 3.2	1.6 0.7 - 3.6
Unknown	5	0.0	3	60.0				
No. of lifetime sexual partners								
0-1	394	95.6	306	77.7	1.0			
2+	12	2.9	11	91.7	3.2	3.6	0.5 - 29.0	
Unknown	6	1.5	3	50.0	0.3	0.1	0.0 - 0.8 *	
Premarital sex		0.0						
No	395	95.9	311	78.7	1.0			
Yes	12	2.9	6	50.0	0.3	0.3	0.1 - 0.9 *	
Unknown	5	1.2	3	60.0	0.4	0.2	0.0 - 1.6	
Pap smear experience								
Pap smear								
Never	320	77.7	244	76.3	1.0			
Ever	91	22.1	75	82.4	1.5	1.6	0.9 - 3.0	
Unknown	1	0.2	1	100.0	∞			

oral contraceptives : OC
Caesarian operations : CS

Appendix C. Risk factors associated with HPV acquisition

Variables	Total (n=6877)	HPV Acquisition (n=606)						
	n (%)	n (%)	OR	aOR (95% CI)	mOR (95% CI)	mOR (95% CI)	mOR (95% CI)	
Age at baseline								
30-34	920 (13.4)	83 (9.0)	1.0					
35-39	1273 (18.5)	103 (8.1)	0.9	(0.7 - 1.2)				
40-44	1094 (15.9)	95 (8.7)	1.0	(0.7 - 1.3)				
45-49	920 (13.4)	76 (8.3)	0.9	(0.7 - 1.3)				
50-54	1053 (15.3)	98 (9.3)	1.0	(0.8 - 1.4)				
55-59	942 (13.7)	90 (9.6)	1.1	(0.8 - 1.5)				
60+	675 (9.8)	61 (9.0)	1.0	(0.7 - 1.4)				
p for trend								
Age at baseline (Ⅱ)								
30-44	3287 (47.8)	281 (8.5)	1.0		1.0	1.0	1.0	
45-54	1973 (28.7)	174 (8.8)	1.0	(0.8 - 1.3)	1.0 (0.8 - 1.3)	1.0 (0.8 - 1.3)	1.0 (0.8 - 1.3)	
55+	1617 (23.5)	151 (9.3)	1.1	(0.9 - 1.4)	1.2 (1.0 - 1.6)	1.1 (0.9 - 1.5)	1.1 (0.9 - 1.4)	
p for trend								
Marital status at								
Currently married	6386 (92.9)	548 (8.6)	1.0		1.0	1.0	1.0	
Widowed/Divorced/Others	489 (7.1)	58 (11.9)	1.4	1.4 (1.1 - 1.9)*	2.5 (1.6 - 3.8)**	2.4 (1.6 - 3.7)**	2.5 (1.7 - 3.8)**	
Unknown	2 (0.0)	0 (0.0)						
No. of marriage								
0-1	6775 (98.5)	591 (8.7)	1.0					
2+	96 (1.4)	14 (14.6)	1.8	1.8 (1.0 - 3.1)				
Unknown	6 (0.1)	1 (16.7)						
Cigarette smoking								
Never	6785 (98.7)	597 (8.8)	1.0					
Ever	68 (1.0)	5 (7.4)	0.8	0.8 (0.3 - 2.0)				
Unknown	24 (0.3)	4 (16.7)						
Alcohol drinking								
Never	6815 (99.1)	596 (8.7)	1.0					
Ever	39 (0.6)	6 (15.4)	1.9	1.9 (0.8 - 4.5)				
Unknown	23 (0.3)	4 (17.4)						
Betal chewing								
Never	6843 (99.5)	602 (8.8)	1.0					
Ever	9 (0.1)	0 (0.0)	-					
Unknown	25 (0.4)	4 (16.0)						
Pregnancy, delivery & contraception								
No. of lifetime childbirth								
0-3	1765 (25.7)	158 (9.0)	1.0					
4	1386 (20.2)	110 (7.9)	0.9	0.9 (0.7 - 1.1)				
5	1380 (20.1)	110 (8.0)	0.9	0.9 (0.7 - 1.1)				
6	970 (14.1)	94 (9.7)	1.1	1.1 (0.8 - 1.4)				
7	590 (8.6)	61 (10.3)	1.2	1.1 (0.8 - 1.6)				
8	352 (5.1)	34 (9.7)	1.0	1.0 (0.6 - 1.5)				
9+	332 (4.8)	25 (7.5)	0.8	0.8 (0.5 - 1.3)				
Unknown	102 (1.5)	14 (13.7)						
p for trend								

Appendix C. Risk factors associated with HPV acquisition

Variables	Total (n=6877)	HPV Acquisition (n=606)					
	n (%)	n (%)	OR	aOR (95% CI)	mOR (95% CI)	mOR (95% CI)	mOR (95% CI)
No. of vaginal delivery							
0-2	1415 (20.6)	115 (8.1)	1.0				
3	2020 (29.4)	175 (8.7)	1.1	1.1 (0.8 - 1.4)			
4	1581 (23.0)	138 (8.7)	1.1	1.1 (0.8 - 1.4)			
5+	1775 (25.8)	166 (9.4)	1.2	1.2 (0.8 - 1.6)			
Unknown	86 (1.3)	12 (14.0)					
p for trend							
No. of CS							
0	2498 (36.3)	212 (8.5)	1.0				
1	2121 (30.8)	185 (8.7)	1.0	1.0 (0.8 - 1.3)			
2	1335 (19.4)	111 (8.3)	1.0	1.0 (0.8 - 1.2)			
3+	824 (12.0)	84 (10.2)	1.2	1.2 (0.9 - 1.6)			
Unknown	99 (1.4)	14 (14.1)					
p for trend							
Abortion							
Never	6294 (91.5)	554 (8.8)	1.0				
Ever	480 (7.0)	38 (7.9)	0.9	0.9 (0.6 - 1.3)			
OC use							
Never	4648 (67.6)	399 (8.6)	1.0				
Ever	2223 (32.3)	206 (9.3)	1.1	1.1 (0.9 - 1.3)			
Unknown	6 (0.1)	1 (16.7)					
IUD use							
Never	2715 (39.5)	227 (8.4)	1.0				
Ever	4160 (60.5)	379 (9.1)	1.1	1.1 (0.9 - 1.3)			
Unknown	2 (0.0)	0 (0.0)					
Menstration							
Age at menarche							
14-16	3802 (55.3)	319 (8.4)	1.0				
~13	879 (12.8)	80 (9.1)	1.1	1.1 (0.9 - 1.4)			
17+	2138 (31.1)	200 (9.4)	1.1	1.1 (0.9 - 1.3)			
Unknown	58 (0.8)	7 (12.1)					
Menopause							
No	4463 (64.9)	375 (8.4)	1.0				
Yes	2410 (35.0)	231 (9.6)	1.2	1.3 (0.9 - 1.8)			
Unknown	4 (0.1)	0 (0.0)					
Age at menopause							
~45	329 (13.7)	33 (10.0)	1.0				
46-50	1038 (43.1)	92 (8.9)	0.9	0.9 (0.6 - 1.4)			
51-55	829 (34.4)	87 (10.5)	1.1	1.1 (0.7 - 1.7)			
56+	105 (4.4)	10 (9.5)	0.9	1.0 (0.5 - 2.2)			
Unknown	109 (4.5)	9 (8.3)					
p for trend							

Appendix C. Risk factors associated with HPV acquisition

Variables	Total (n=6877)	HPV Acquisition (n=606)						
	n (%)	n (%)	OR	aOR (95% CI)	mOR (95% CI)	mOR (95% CI)	mOR (95% CI)	
Sexual behavior (lifetime)								
Age at initial coitus								
14-21	2586 (37.6)	269 (10.4)	1.0					
22-23	1765 (25.7)	149 (8.4)	0.8	0.8 (0.6 - 1.0)*				
24+	2501 (36.4)	186 (7.4)	0.7	0.7 (0.6 - 0.8)**				
Unknown	25 (0.4)	2 (8.0)						
Age at initial coitus (Ⅱ)								
22-25	3089 (44.9)	251 (8.1)	1.0		1.0	1.0	1.0	
14-21	2586 (37.6)	269 (10.4)	1.3	1.3 (1.1 - 1.6)*	1.3 (1.0 - 1.6)*	1.3 (1.0 - 1.5)*	1.2 (1.0 - 1.5)*	
26+	1177 (17.1)	84 (7.1)	0.9	0.9 (0.7 - 1.1)	0.9 (0.7 - 1.2)	0.9 (0.7 - 1.2)	0.9 (0.7 - 1.2)	
Unknown	25 (0.4)	2 (8.0)						
No. of lifetime sexual partners								
0-1	6692 (97.3)	577 (8.6)	1.0					
2+	170 (2.5)	29 (17.1)	2.2	2.2 (1.4 - 3.3)**				
Unknown	15 (0.2)	0 (0.0)						
Premarital sex								
No	6701 (97.4)	589 (8.8)	1.0					
Yes	150 (2.2)	15 (10.0)	1.2	1.2 (0.7 - 2.0)				
Unknown	26 (0.4)	2 (7.7)						
Sexual behavior & contraception								
No. of sexual partners								
0	863 (12.5)	59 (6.8)	1.0		1.0	1.0	1.0	
1	5189 (75.5)	465 (9.0)	1.3	1.5 (1.1 - 2.0)*	2.3 (1.5 - 3.4)**	2.3 (1.6 - 3.4)**	2.3 (1.6 - 3.4)**	
2+	9 (0.1)	2 (22.2)	3.9	4.7 (0.9 - 23.5)	4.0 (0.8 - 21.0)	3.3 (0.6 - 17.7)	3.1 (0.6 - 16.7)	
Unknown	816 (11.9)	80 (9.8)						
p for trend								
Condom use (I)								
Never	4428 (64.4)	412 (9.3)	1.0	1.0				
Seldom	316 (4.6)	28 (8.9)	0.9	1.0 (0.7 - 1.5)				
Often	153 (2.2)	9 (5.9)	0.6	0.7 (0.3 - 1.3)				
Always	284 (4.1)	18 (6.3)	0.7	0.7 (0.4 - 1.2)				
No sexual intercourse	863 (12.5)	59 (6.8)	0.7	0.6 (0.5 - 0.9)*				
Unknown	833 (12.1)	80 (9.6)						
Condom use (II)								
Never+seldom	4744 (69.0)	440 (9.3)	1.0	1.0	1.0	1.0	1.0	
Often+always	437 (6.4)	27 (6.2)	0.6	0.7 (0.5 - 1.0)	0.7 (0.5 - 1.1)	0.8 (0.5 - 1.2)	0.8 (0.5 - 1.2)	
No sexual intercourse	863 (12.5)	59 (6.8)	0.7	0.6 (0.5 - 0.9)*	-	-	-	
Unknown	833 (12.1)	80 (9.6)						
Vaginal douching use (I)								
Never	3761 (54.7)	318 (8.5)	1.0	1.0				
Seldom	399 (5.8)	41 (10.3)	1.2	1.3 (0.9 - 1.8)				
Often	363 (5.3)	49 (13.5)	1.7	1.7 (1.2 - 2.4)*				
Always	663 (9.6)	60 (9.0)	1.1	1.1 (0.8 - 1.5)				
No sexual intercourse	863 (12.5)	59 (6.8)	0.8	0.7 (0.5 - 1.0)*				
Unknown	828 (12.0)	79 (9.5)						

Appendix C. Risk factors associated with HPV acquisition

Variables	Total (n=6877)	HPV Acquisition (n=606)						
	n (%)	n (%)	OR	aOR (95% CI)	mOR (95% CI)	mOR (95% CI)	mOR (95% CI)
Vaginal douching use							
Never	3761 (54.7)	318 (8.5)	1.0	1.0	1.0	1.0	1.0
Ever	1425 (20.7)	150 (10.5)	1.3	1.3 (1.1 - 1.6)*	1.2 (1.0 - 1.5)*	1.2 (1.0 - 1.5)	1.2 (1.0 - 1.5)
No sexual intercourse	863 (12.5)	59 (6.8)	0.8	0.7 (0.5 - 1.0)*	-	-	-
Unknown	828 (12.0)	79 (9.5)					
OC use							
No	5828 (84.7)	505 (8.7)	1.0				
Yes	229 (3.3)	22 (9.6)	1.1	1.2 (0.7 - 1.9)			
Unknown	820 (11.9)	79 (9.6)					
IUD use							
No	4967 (72.2)	432 (8.7)	1.0				
Yes,but remove	284 (4.1)	22 (7.7)	0.9	0.9 (0.6 - 1.4)			
Yes,currently	781 (11.4)	70 (9.0)	1.0	1.1 (0.8 - 1.4)			
Unknown	845 (12.3)	82 (9.7)					
Pap smear & HPV experience							
Pap smear							
Never	6056 (88.1)	523 (8.6)	1.0				
Ever	807 (11.7)	82 (10.2)	1.2	1.2 (1.0 - 1.6)			
Unknown	14 (0.2)	1 (7.1)					
Cytology at baseline							
Normal	6667 (96.9)	563 (8.4)	1.0		1.0	1.0	1.0
Abnormal	210 (3.1)	43 (20.5)	2.8	2.8 (2.0 - 4.0)**	2.6 (1.8 - 3.8)**	1.5 (1.0 - 2.2)*	1.3 (0.9 - 2.0)
HPV at baseline							
Negative	5310 (77.2)	332 (6.3)	1.0			1.0	
Positive	1567 (22.8)	274 (17.5)	3.2	3.2 (2.7 - 3.8)**		3.0 (2.5 - 3.6)**	
HPV at baseline							
Negative	5310 (52.5)	332 (6.3)					1.0
HR17	933 (9.2)	189 (20.3)	3.8	3.8 (3.1 - 4.6)**			3.6 (2.9 - 4.6)**
Others	634 (6.3)	85 (13.4)	2.3	2.3 (1.8 - 3.0)**			2.2 (1.7 - 3.0)**

Appendix D. Risk factors associated with HPV acquisition among cytologically abnormal

Variables	Abnormal (n=210)	HPV Acquisition (n=43)					
	n (%)	n (%)	OR	aOR (95% CI)	mOR (95% CI)	mOR (95% CI)	
Age at baseline							
30-34	19 (9.0)	4 (21.1)	1.0				
35-39	32 (15.2)	9 (28.1)	1.5	(0.4 - 5.6)			
40-44	50 (23.8)	13 (26.0)	1.3	(0.4 - 4.7)			
45-49	41 (19.5)	5 (12.2)	0.5	(0.1 - 2.2)			
50-54	25 (11.9)	1 (4.0)	0.2	(0.0 - 1.5)			
55-59	24 (11.4)	6 (25.0)	1.3	(0.3 - 5.3)			
60+	19 (9.0)	5 (26.3)	1.3	(0.3 - 6.0)			
p for trend							
Age at baseline (Ⅱ)							
30-44	101 (48.1)	26 (25.7)	1.0		1.0	1.0	
45-54	66 (31.4)	6 (9.1)	0.3	(0.1 - 0.7)*	0.3 (0.1 - 1.0)*	0.3 (0.1 - 1.0)	
55+	43 (20.5)	11 (25.6)	1.0	(0.4 - 2.2)	0.7 (0.2 - 2.0)	0.7 (0.2 - 2.0)	
p for trend							
Marital status at baseline							
Currently married	198 (94.3)	41 (20.7)	1.0				
Widowed/Divorced/Others	12 (5.7)	2 (16.7)	0.8	0.8 (0.2 - 4.0)			
No. of marriage							
0-1	208 (99.0)	43 (20.7)	1.0				
2+	2 (1.0)	0 (16.7)	-				
Cigarette smoking							
Never	209 (99.5)	43 (20.7)	1.0				
Ever	1 (0.5)	0 (0.0)	-				
Alcohol drinking							
Never	208 (99.0)	42 (20.2)	1.0				
Ever	2 (1.0)	1 (50.0)	4.0	9.1 (0.3 — 297.2)			
Betal chewing							
Never	210 (100.0)	43 (20.5)	1.0				
Ever	0 (0.0)	0 (-)	-				
Pregnancy, delivery & contraception							
No. of lifetime childbirth							
0-3	41 (19.5)	8 (19.5)	1.0				
4	48 (22.9)	13 (27.1)	1.5	1.7 (0.6 - 4.8)			
5	42 (20.0)	7 (16.7)	0.8	1.0 (0.3 - 3.2)			
6	34 (16.2)	5 (14.7)	0.7	1.0 (0.3 - 3.5)			
7	12 (5.7)	4 (33.3)	2.1	2.5 (0.5 - 11.2)			
8	15 (7.1)	4 (26.7)	2.1	2.5 (0.5 - 12.7)			
9+	14 (6.7)	2 (14.3)	0.7	0.6 (0.1 - 3.9)			
Unknown	4 (1.9)	0 (0.0)					
p for trend							

Variables	Abnormal (n=210)		HPV Acquisition (n=43)								
	n	%	n	%	OR	aOR	95% CI	mOR	95% CI	mOR	95% CI
No. of vaginal delivery											
0-2	27	12.9	4	14.8	1.0						
3	63	30.0	15	23.8	1.8	2.0	0.6 - 7.0				
4	67	31.9	13	19.4	1.4	2.1	0.6 - 7.9				
5+	50	23.8	11	22.0	1.6	2.7	0.6 - 11.5				
Unknown	3	1.4	0	0.0							
p for trend											
No. of CS											
0	75	35.7	19	25.3	1.0						
1	61	29.0	10	16.4	0.6	0.6	0.2 - 1.4				
2	39	18.6	8	20.5	0.8	0.8	0.3 - 2.0				
3+	31	14.8	6	19.4	0.7	0.6	0.2 - 1.7				
Unknown	4	1.9	0	0.0							
p for trend											
Abortion											
Never	199	94.8	42	21.1	1.0						
Ever	7	3.3	1	14.3	0.6	0.6	0.1 - 5.0				
Unknown	4	1.9	0	0.0							
OC use											
Never	131	62.4	26	19.8	1.0						
Ever	79	37.6	17	21.5	1.1	1.0	0.5 - 2.0				
IUD use											
Never	81	38.6	17	21.0	1.0						
Ever	129	61.4	26	20.2	1.0	1.0	0.5 - 2.1				
Menstration											
Age at menarche											
14-16	111	52.9	20	18.0	1.0						
~13	22	10.5	5	22.7	1.3	1.4	0.5 - 4.6				
17+	76	36.2	18	23.7	1.4	2.0	0.9 - 4.6				
Unknown	1	0.5	0	0.0							
Menopause (I)											
No	145	69.0	32	22.1	1.0						
Yes	64	30.5	11	17.2	0.7	0.2	0.0 - 2.6				
Unknown	1	0.5	0	0.0							
Age at menopause											
~45	9	14.1	3	33.3	1.0						
46-50	30	46.9	2	6.7	0.1	0.1	0.0 - 1.1				
51-55	24	37.5	5	20.8	0.5	0.4	0.1 - 2.8				
56+	0	0.0	0	-	-						
Unknown	1	1.6	1	100.0							
p for trend											

Variables	Abnormal (n=210)		HPV Acquisition (n=43)						
	n	%	n	%	OR	aOR	95% CI	mOR	95% CI	mOR	95% CI
Sexual behavior (lifetime)											
Age at initial coitus											
14-21	87	41.4	19	21.8	1.0						
22-23	48	22.9	11	22.9	1.1	1.3	0.5 - 3.2				
24+	74	35.2	13	17.6	0.8	0.8	0.4 - 1.8				
Unknown	1	0.5	0	0.0							
p for trend											
Age at initial coitus (Ⅱ)											
22-25	95	45.2	21	22.1	1.0						
14-21	87	41.4	19	21.8	1.0	0.9	0.4 - 1.8				
26+	27	12.9	3	11.1	0.4	0.4	0.1 - 1.5				
Unknown	1	0.5	0	0.0							
No. of lifetime sexual partners											
0-1	202	96.2	41	20.3	1.0						
2+	6	2.9	2	33.3	2.0	2.0	0.3 - 11.7				
Unknown	2	1.0	0	0.0							
Premarital sex											
No	202	96.2	40	19.8	1.0						
Yes	7	3.3	3	42.9	3.0	2.3	0.5 - 11.3				
Unknown	1	0.5	0	0.0							
In the past year											
No. of sexual partners											
0	29	13.8	2	6.9	1.0	1.0					
1	149	71.0	32	21.5	3.7	3.7	0.7 - 18.2				
Unknown	32	15.2	9	28.1							
p for trend											
OC use											
No	165	78.6	29	17.6	1.0	1.0					
Yes	13	6.2	5	38.5	2.9	2.8	0.8 - 9.9				
Unknown	32	15.2	9	28.1							
IUD use											
No	157	74.8	27	17.2	1.0	1.0					
Yes,but remove	6	2.9	3	50.0	4.8	6.0	1.0 - 36.5				
Yes,currently	15	7.1	4	26.7	1.8	1.3	0.4 - 4.6				
Unknown	32	15.2	9	28.1							
Condom use (I)											
Never	139	66.2	31	22.3	1.0	1.0					
Seldom	3	1.4	1	33.3	1.7	1.4	0.1 - 16.2				
Often	2	1.0	0	0.0	-		-				
Always	5	2.4	0	0.0	-		-				
No sexual intercourse	29	13.8	2	6.9	0.3	0.3	0.1 - 1.3				
Unknown	32	15.2	9	28.1							

Variables	Abnormal (n=210)		HPV Acquisition (n=43)											
	n	%	n	%	OR	aOR	95% CI		mOR	95% CI		mOR	95% CI	
Condom use (II)														
Never+seldom	142	67.6	32	22.5	1.0	1.0								
Often+always	7	3.3	0	0.0	-		-							
No sexual intercourse	29	13.8	2	6.9	0.3	0.3	0.1	1.3						
Unknown	32	15.2	9	28.1										
Vaginal douching use (I)														
Never	98	46.7	26	26.5	1.0	1.0								
Seldom	12	5.7	1	8.3	0.3	0.2	0.0	2.0						
Often	14	6.7	3	21.4	0.8	0.7	0.2	2.8						
Always	25	11.9	2	8.0	0.2	0.2	0.0	1.0						
No sexual intercourse	29	13.8	2	6.9	0.2	0.2	0.0	1.1						
Unknown	32	15.2	9	28.1										
Vaginal douching use (II)														
Never	98	46.7	26	26.5	1.0	1.0			1.0			1.0		
Ever	51	24.3	6	11.8	0.4	0.3	0.1	0.9 *	0.3	0.1	0.9 *	0.3	0.1	0.9 *
No sexual intercourse	29	13.8	2	6.9	0.2	0.2	0.0	1.1	0.3	0.1	1.2	0.3	0.1	1.2
Unknown	32	15.2	9	28.1										
Pap smear & HPV experience														
HPV at baseline														
Negative	54	0.8	5	9.3	1.0				1.0					
Positive	156	2.3	38	24.4	3.2	3.6	1.3	10.1 *	3.5	1.1	11.1 *			
HPV at baseline														
Negative	54	0.5	5	9.3	1.0		-					1.0		
HR17	147	1.5	37	25.2	3.3	3.7	1.3	10.3 *				3.6	1.1	11.2 *
Others	9	0.1	1	11.1	1.2	2.1	0.2	23.0				2.8	0.2	32.4

Appendix E. Risk factors associated with HPV persistence

Variables	Total (n=1567)		HPV persistence (n=458)					
	n	%	n	%	OR	aOR	95% CI	mOR 95% CI
Age at baseline								
30-34	195	12.4	35	17.9	1.0			
35-39	259	16.5	62	23.9	1.4		0.9 - 2.3	
40-44	241	15.4	46	19.1	1.1		0.7 - 1.8	
45-49	209	13.3	50	23.9	1.4		0.9 - 2.3	
50-54	246	15.7	81	32.9	2.2		1.4 - 3.5	)**
55-59	250	16.0	104	41.6	3.3		2.1 - 5.1	)**
60+	167	10.7	80	47.9	4.2		2.6 - 6.8	)**
p for trend					<0.001			
Age at baseline (Ⅱ)								
30-44	695	44.4	143	20.6	1.0			1.0
45-54	455	29.0	131	28.8	1.6		1.2 - 2.1	)* 1.1 (0.8 - 1.6)
55+	417	26.6	184	44.1	3.0		2.3 - 4.0	)** 1.6 (1.0 - 2.6)*
p for trend					<0.001			
Marital status at baseline								
Currently married	1439	91.8	412	28.6	1.0			
Widowed/Divorced/Others	128	8.2	46	35.9	1.4	1.1	0.8 - 1.7	
No. of marriage								
0-1	1535	98.0	446	29.1	1.0			
2+	30	1.9	12	40.0	1.6	1.5	0.7 - 3.2	
Unknown	2	0.1	0	0.0				
Cigarette smoking								
Never	1536	98.0	449	29.2	1.0			
Ever	23	1.5	8	34.8	1.3	1.5	0.6 - 3.7	
Unknown	8	0.5	1	12.5				
Alcohol drinking								
Never	1547	98.7	453	29.3	1.0			
Ever	12	0.8	4	33.3	1.2	1.5	0.5 - 5.3	
Unknown	8	0.5	1	12.5				
Betal chewing								
Never	1557	99.4	456	29.3	1.0			
Ever	1	0.1	1	100.0	∞			
Unknown	9	0.6	1	11.1				
Pregnancy, delivery & contraception								
No. of lifetime childbirth								
0-3	366	23.4	65	17.8	1.0			
4	308	19.7	93	30.2	2.0	1.8	1.2 - 2.6	)*
5	314	20.0	90	28.7	1.9	1.5	1.0 - 2.2	)*
6	238	15.2	76	31.9	2.2	1.6	1.1 - 2.5	)*
7	129	8.2	43	33.3	2.3	1.5	0.9 - 2.4	
8	82	5.2	38	46.3	1.8	1.4	0.9 - 2.4	
9+	106	6.8	47	44.3	3.7	2.0	1.2 - 3.4	)*
Unknown	24	1.5	6	25.0				
p for trend					<0.001			
No. of vaginal delivery								
0-2	308	19.7	48	15.6	1.0			
3	426	27.2	119	27.9	2.1	1.9	1.3 - 2.8	)*
4	395	25.2	124	31.4	2.5	1.8	1.2 - 2.8	)*
5+	421	26.9	164	39.0	3.5	1.9	1.2 - 3.0	)*
Unknown	17	1.1	3	17.6				
p for trend					<0.001			
No. of CS								
0	541	34.5	151	27.9	1.0			
1	486	31.0	145	29.8	1.1	1.0	0.8 - 1.4	
2	306	19.5	81	26.5	0.9	1.0	0.7 - 1.3	
3+	210	13.4	75	35.7	1.4	1.4	1.0 - 2.0	)*
Unknown	24	1.5	6	25.0				
p for trend					0.165			
Abortion								
Never	1446	92.3	438	30.3	1.0			
Ever	96	6.1	14	14.6	0.4	0.5	0.3 - 0.9	)*
Unknown	25	1.6	6	24.0				
OC use								
Never	1056	67.4	308	29.2	1.0			
Ever	509	32.5	150	29.5	1.0	1.3	1.0 - 1.7	)*
Unknown	2	0.1	0	0.0				
OC use (Current)								
Never use+not now	1499	95.7	438	29.2	1.0			
Yes	62	4.0	18	29.0	1.0	1.5	0.8 - 2.7	
Unknown	6	0.4	2	33.3				
IUD use								
Never	555	35.4	133	24.0	1.0			1.0
Ever	1012	64.6	325	32.1	1.5	1.5	1.2 - 1.9	)* 1.5 (1.2 - 1.9)*

Appendix E. Risk factors associated with HPV persistence

Variables	Total (n=1567)		HPV persistence (n=458)					
	n	%	n	%	OR	aOR	95% CI	mOR 95% CI
Menstraion								
Age at menarche								
14-16	835	53.3	229	27.4	1.0			
~13	190	12.1	47	24.7	0.9	1.0	0.7 - 1.5	
17+	525	33.5	177	33.7	1.3	1.0	0.8 - 1.3	
Unknown	17	1.1	5	29.4				
Menopause								
No	954	60.9	203	21.3	1.0			
Yes	613	39.1	255	41.6	2.6	1.7	1.1 - 2.7)*	1.9 1.3 - 2.8)**
Age at menopause	613		255					
~45	75	12.2	24	32.0	1.0			
46-50	259	42.3	109	42.1	1.5	1.5	0.9 - 2.6	
51-55	230	37.5	94	40.9	1.5	1.4	0.8 - 2.5	
56+	25	4.1	17	68.0	4.5	4.0	1.5 - 10.8)*	
Unknown	24	3.9	11	45.8				
p for trend					0.035			
Sexual behavior (lifetime)								
Age at initial coitus								
14-21	639	40.8	220	34.4	1.0			
22-23	393	25.1	114	29.0	0.8	0.9	0.7 - 1.1	
24+	530	33.8	123	23.2	0.6	0.7	0.5 - 0.9)*	
Unknown	5	0.3	1	20.0				
p for trend					<0.001			
No. of lifetime sexual partners								
0-1	1502	95.9	436	29.0	1.0			
2+	62	4.0	22	35.5	1.3	1.5	0.9 - 2.7	
Unknown	3	0.2	0	0.0				
Premarital sex								
No	1529	97.6	450	29.4	1.0			
Yes	33	2.1	7	21.2	0.6	1.0	0.4 - 2.3	
Unknown	5	0.3	1	20.0				
Sexual behavior & contraception (recent)								
No. of sexual partners								
0	219	14.0	88	40.2	1.0	1.0		
1	1147	73.2	309	26.9	0.5	0.8	0.6 - 1.1	
2+	4	0.3	2	50.0	1.5	2.2	0.3 - 17.0	
Unknown	197	12.6	59	29.9				
p for trend					<0.001			
OC use								
No	1315	83.9	383	29.1	1.0	1.0		
Yes	56	3.6	17	30.4	1.1	1.6	0.9 - 2.8	
Unknown	196	12.5	58	29.6				
IUD use								
No	1124	71.7	324	28.8	1.0	1.0		
Yes,but remove	64	4.1	22	34.4	1.3	1.3	0.7 - 2.2	
Yes,currently	177	11.3	51	28.8	1.0	1.4	1.0 - 2.1	
Unknown	202	12.9	61	30.2				
Condom use (I)								
Never	1013	64.6	282	27.8	1.0	1.0		
Seldom	59	3.8	15	25.4	0.9	1.1	0.6 - 2.0	
Often	23	1.5	4	17.4	0.5	0.7	0.2 - 2.2	
Always	53	3.4	9	17.0	0.5	0.7	0.3 - 1.5	
No sexual intercourse	219	14.0	88	40.2	1.7	1.3	0.9 - 1.7	
Unknown	200	12.8	60	30.0				
Condom use (II)								
Never+seldom	1072	68.4	297	27.7	1.0	1.0		
Often+always	76	4.9	13	17.1	0.5	0.7	0.4 - 1.4	
No sexual intercourse	219	14.0	88	40.2	1.8	1.3	0.9 - 1.7	
Unknown	200	12.8	60	30.0				
Vaginal douching use (I)								
Never	790	50.4	211	26.7	1.0	1.0		
Seldom	121	7.7	32	26.4	1.0	1.1	0.7 - 1.7	
Often	84	5.4	24	28.6	1.1	1.1	0.7 - 1.9	
Always	155	9.9	44	28.4	1.1	1.2	0.8 - 1.7	
No sexual intercourse	219	14.0	88	40.2	1.8	1.3	0.9 - 1.8	
Unknown	198	12.6	59	29.8				
Vaginal douching use (II)								
Never	790	50.4	211	26.7	1.0	1.0		
Ever	360	23.0	100	27.8	1.1	1.1	0.8 - 1.5	
No sexual intercourse	219	14.0	88	40.2	1.8	1.3	0.9 - 1.8	
Unknown	198	12.6	59	29.8				
Pap smear experience								
Pap smear								
Never	1382	88.2	412	29.8	1.0			
Ever	179	11.4	44	24.6	0.8	0.9	0.6 - 1.3	
Unknown	6	0.4	2	33.3				
Cytology at baseline								
Normal	1411	90.0	391	27.7	1.0			1.0
Abnormal	156	10.0	67	42.9	2.0	2.2	1.5 - 3.1)**	2.2 1.5 - 3.1)**

oral contraceptives : OC
Caesarian operations : CS

Appendix F. Risk factors associated with HPV persistence among cytologically abnormal at baseline

Variables	Abnormal (n=156)	HPV persistence (n=67)					
	n (%)	n (%)	OR	aOR (95% CI)	mOR (95% CI)	mOR (95% CI)	
Age at baseline							
30-34	16 (10.3)	7 (43.8)	1.0				
35-39	24 (15.4)	6 (25.0)	0.4	(0.1 - 1.7)			
40-44	30 (19.2)	6 (20.0)	0.3	(0.1 - 1.2)			
45-49	29 (18.6)	9 (31.0)	0.6	(0.2 - 2.0)			
50-54	20 (12.8)	12 (60.0)	1.9	(0.5 - 7.3)			
55-59	21 (13.5)	16 (76.2)	4.1	(1.0 - 16.8)*			
60+	16 (10.3)	11 (68.8)	2.8	(0.7 - 12.0)			
p for trend			<0.001				
Age at baseline (Ⅱ)							
30-44	70 (44.9)	19 (27.1)	1.0		1.0	1.0	
45-54	49 (31.4)	21 (42.9)	2.0	(0.9 - 4.4)	2.2 (1.0 - 4.8)	2.2 (0.9 - 5.6)	
55+	37 (23.7)	27 (73.0)	7.2	(3.0 - 17.8)**	6.7 (2.7 - 16.7)**	7.4 (2.7 - 19.8)**	
p for trend			<0.001				
Marital status at baseline							
Currently married	147 (94.2)	61 (41.5)	1.0				
Widowed/Divorced/Others	9 (5.8)	6 (66.7)	2.8	3.1 (0.6 - 15.6)			
No. of marriage							
0-1	155 (99.4)	67 (43.2)	1.0				
2+	1 (0.6)	0 (0.0)	-				
Cigarette smoking							
Never	155 (99.4)	67 (43.2)	1.0				
Ever	1 (0.6)	0 (0.0)	-				
Alcohol drinking							
Never	154 (98.7)	66 (42.9)	1.0				
Ever	2 (1.3)	1 (50.0)	1.3	1.4 (0.1 - 30.1)			
Betal chewing							
Never	156 (100.0)	67 (42.9)	1.0				
Ever	0 (0.0)	0 (-)	-				
Pregnancy, delivery & contraception							
No. of lifetime childbirth							
0-3	30 (19.2)	8 (26.7)	1.0				
4	36 (23.1)	15 (41.7)	2.0	2.3 (0.7 - 7.6)			
5	27 (17.3)	12 (44.4)	2.2	1.8 (0.5 - 6.5)			
6	29 (18.6)	15 (51.7)	2.9	3.2 (0.9 - 11.6)			
7	8 (5.1)	3 (37.5)	1.7	1.2 (0.2 - 8.6)			
8	10 (6.4)	7 (70.0)	2.2	1.0 (0.2 - 5.3)			
9+	13 (8.3)	7 (53.8)	3.2	0.8 (0.1 - 4.7)			
Unknown	3 (1.9)	0 (0.0)					
p for trend			0.025				
No. of vaginal delivery							
0-2	20 (12.8)	5 (25.0)	1.0				
3	44 (28.2)	17 (38.6)	1.9	2.0 (0.5 - 7.5)			
4	51 (32.7)	28 (54.9)	3.7	3.0 (0.7 - 12.1)			
5+	39 (25.0)	17 (43.6)	2.3	0.7 (0.1 - 3.4)			
Unknown	2 (1.3)	0 (0.0)					
p for trend			0.117				
No. of CS							
0	54 (34.6)	19 (35.2)	1.0		1.0		
1	47 (30.1)	20 (42.6)	1.4	1.3 (0.5 - 3.1)	1.3 (0.5 - 3.1)		
2	31 (19.9)	15 (48.4)	1.7	1.8 (0.7 - 4.8)	1.8 (0.7 - 4.7)		
3+	21 (13.5)	13 (61.9)	3.0	3.2 (0.9 - 11.2)	2.6 (0.8 - 8.3)		
Unknown	3 (1.9)	0 (0.0)					
p for trend			0.035				
Abortion							
Never	148 (94.9)	66 (44.6)	1.0				
Ever	5 (3.2)	1 (20.0)	0.3	0.3 (0.0 - 3.4)			
Unknown	3 (1.9)	0 (0.0)					
OC use							
Never	93 (59.6)	44 (47.3)	1.0				
Ever	63 (40.4)	23 (36.5)	0.6	0.9 (0.5 - 2.0)			
IUD use							
Never	57 (36.5)	19 (33.3)	1.0		1.0		
Ever	99 (63.5)	48 (48.5)	1.9	1.7 (0.8 - 3.6)	1.6 (0.7 - 3.3)		
Menstration							
Age at menarche							
14-16	79 (50.6)	30 (38.0)	1.0				
~13	19 (12.2)	7 (36.8)	1.0	1.1 (0.4 - 3.4)			
17+	57 (36.5)	30 (52.6)	1.8	1.0 (0.5 - 2.4)			
Unknown	1 (0.6)	0 (0.0)					
Menopause							
No	102 (65.4)	30 (29.4)	1.0				
Yes	54 (34.6)	37 (68.5)	5.2	1.6 (0.4 - 7.1)			
Age at menopause							
~45	6 (11.1)	4 (66.7)	1.0				
46-50	25 (46.3)	18 (72.0)	1.3	1.0 (0.1 - 12.4)			
51-55	22 (40.7)	14 (63.6)	0.9	0.5 (0.0 - 5.7)			
56+	0 (0.0)	0					
Unknown	1 (1.9)	1 (100.0)					
p for trend			0.700				

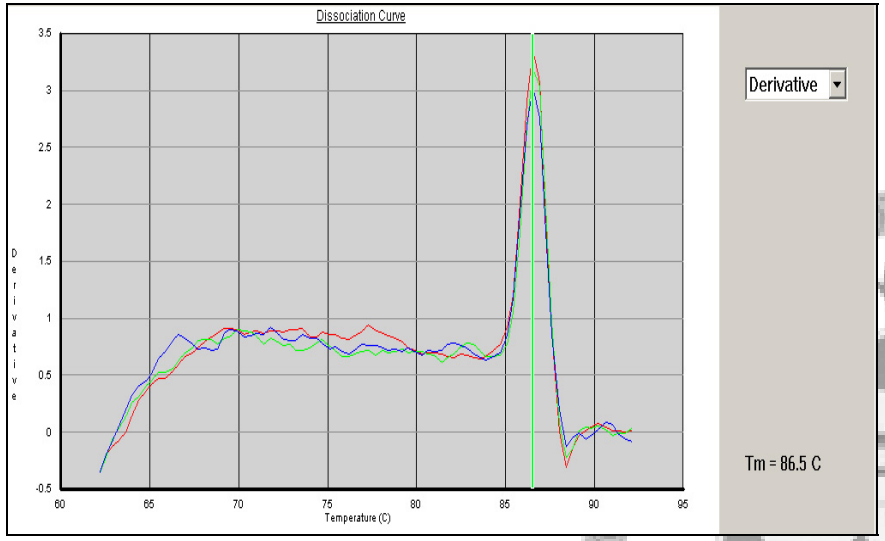
Variables	Abnormal (n=156)	HPV persistence (n=67)					
	n (%)	n (%)	OR	aOR (95% CI)	mOR (95% CI)	mOR (95% CI)	
Sexual behavior (lifetime)							
Age at initial coitus							
14-21	67 (42.9)	29 (43.3)	1.0				
22-23	36 (23.1)	18 (50.0)	1.3	1.4 (0.6 - 3.5)			
24+	52 (33.3)	20 (38.5)	0.8	0.9 (0.4 - 2.2)			
Unknown	1 (0.6)	0 (0.0)					
p for trend			0.640				
No. of lifetime sexual partners							
0-1	150 (96.2)	66 (44.0)	1.0				
2+	5 (3.2)	1 (20.0)	0.3	0.6 (0.1 - 5.9)			
Unknown	1 (0.6)	0 (0.0)					
Premarital sex							
No	151 (96.8)	66 (43.7)	1.0				
Yes	4 (2.6)	1 (25.0)	0.4	0.8 (0.1 - 8.7)			
Unknown	1 (0.6)	0 (0.0)					
Sexual behavior & contraception (recent)							
No. of sexual partners							
0	22 (14.1)	12 (54.5)	1.0	1.0			
1	108 (69.2)	40 (37.0)	0.5	0.9 (0.3 - 2.6)			
Unknown	26 (16.7)	15 (57.7)					
OC use							
No	122 (78.2)	48 (39.3)	1.0	1.0			
Yes	8 (5.1)	4 (50.0)	1.5	2.2 (0.4 - 11.1)			
Unknown	26 (16.7)	15 (57.7)					
IUD use							
No	117 (75.0)	45 (38.5)	1.0	1.0		1.0	
Yes,but remove	4 (2.6)	2 (50.0)	1.6	1.0 (0.1 - 9.8)		1.3 (0.1 - 12.7)	
Yes,currently	9 (5.8)	5 (55.6)	2.0	6.5 (1.4 - 31.0)	*	4.1 (1.0 - 17.5)	
Unknown	26 (16.7)	15 (57.7)					
Condom use (I)							
Never	101 (64.7)	38 (37.6)	1.0	1.0			
Seldom	3 (1.9)	1 (33.3)	0.8	1.2 (0.1 - 17.8)			
Often	2 (1.3)	0 (0.0)	-				
Always	2 (1.3)	1 (50.0)	1.7	1.9 (0.1 - 40.7)			
No sexual intercourse	22 (14.1)	12 (54.5)	2.0	1.2 (0.4 - 3.5)			
Unknown	26 (16.7)	15 (57.7)					
Condom use (II)							
Never+seldom	104 (66.7)	39 (37.5)	1.0	1.0			
Often+always	4 (2.6)	1 (25.0)	0.6	0.6 (0.0 - 6.8)			
No sexual intercourse	22 (14.1)	12 (54.5)	2.0	1.2 (0.4 - 3.5)			
Unknown	26 (16.7)	15 (57.7)					
Vaginal douching use (I)							
Never	72 (46.2)	28 (38.9)	1.0	1.0			
Seldom	10 (6.4)	1 (10.0)	0.2	0.2 (0.0 - 1.9)			
Often	11 (7.1)	4 (36.4)	0.9	2.0 (0.5 - 8.5)			
Always	15 (9.6)	7 (46.7)	1.4	2.3 (0.6 - 8.3)			
No sexual intercourse	22 (14.1)	12 (54.5)	1.9	1.2 (0.4 - 3.8)			
Unknown	26 (16.7)	15 (57.7)					
Vaginal douching use (II)							
Never	72 (46.2)	28 (38.9)	1.0	1.0			
Ever	36 (23.1)	12 (33.3)	0.8	1.3 (0.5 - 3.3)			
No sexual intercourse	22 (14.1)	12 (54.5)	1.9	1.2 (0.4 - 3.9)			
Unknown	26 (16.7)	15 (57.7)					
Pap smear experience							
Pap smear							
Never	117 (75.0)	56 (47.9)	1.0				
Ever	39 (25.0)	11 (28.2)	0.4	0.5 (0.2 - 1.2)			

oral contraceptives : OC
Caesarian operations : CS

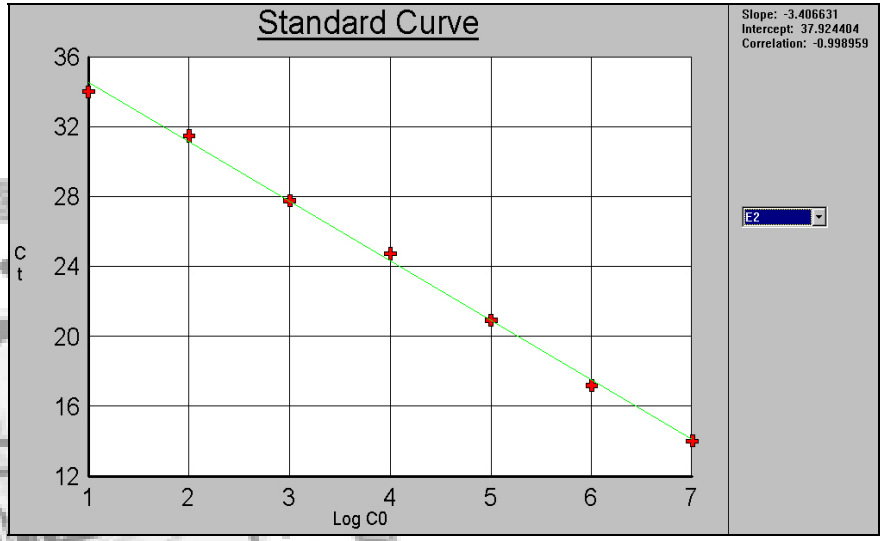
Appendix G. Primers used for real-time PCR

Oligo name	Sequence (5'~3')	Site in HPV genome	
16E2-F	AATTATTAGGCAGCACTTGGCCA	3381 –	3403
16E2-R	ATCTTGGTCGCTGGATAGTCGTCT	3481 –	3458
16E6-F	GAGCGACCCAGAAAGTTACCAC	122 –	243
16E6-R	ACCTCACGTCGCAGTAACTGTTG	228 –	206
18E2-F	CCGCTACTCAGCTTGTTAAACAGCT	3454 –	3478
18E2-R	GCCGACGTCTGGCCGTAG	3550 –	3533
18E6-F	CGGCGACCCTACAAGCTACC	129 –	148
18E6-R	ACCTCTGTAAGTTCCAATACTGTCTTGC	235 –	208
52E2-F	ACTGAAACTGCTGTCCACCTATGC	3361 –	3384
52E2-R	TGACGTCTGGTCGTCGTCG	3454 –	3436
52E6-F	ACACGACCCCGGACCCT	120 –	136
52E6-R	CTTGTATACCTCTCTTCGTTGTAGCTCTTT	233 –	204
58E2-F	CCACTACTGAAACTGCTGACCCAA	3366 –	3389
58E2-R	GGGTGTTGTCTCTGGAGTCTGGTAA	3473 –	3449
58E6-F	GGAGAAACCACGGACATTGCA	127 –	147
58E6-R	ACCTCAGATCGCTGCAAAGTC	234 –	214

HPV16/E2 Dissociation Curve



HPV16/E6 Standard Curve



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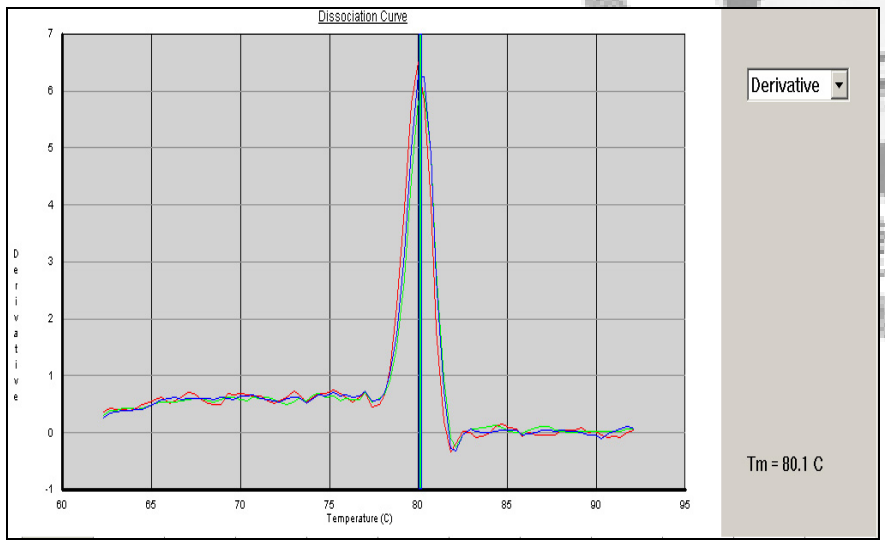

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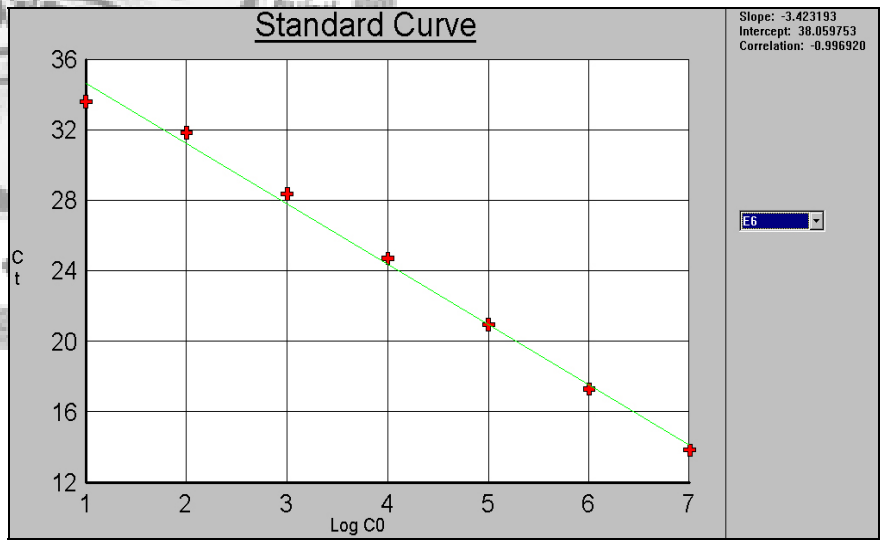

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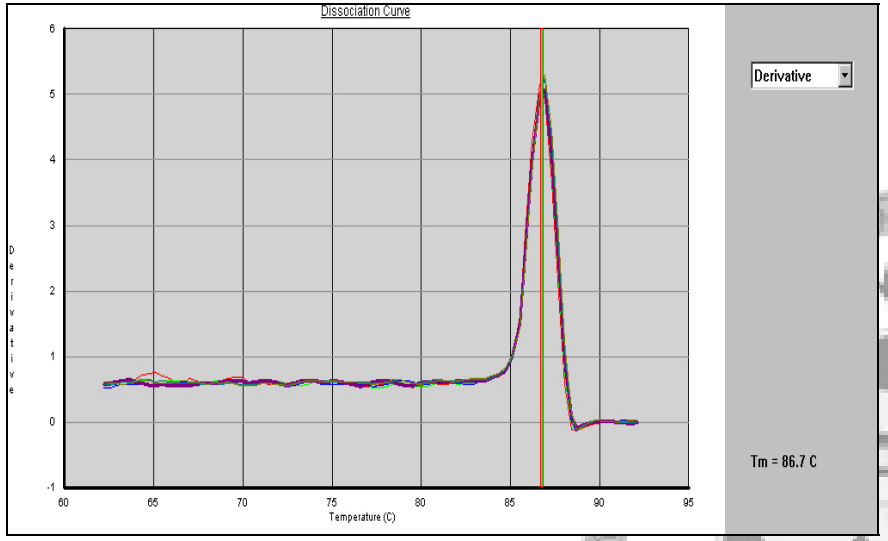

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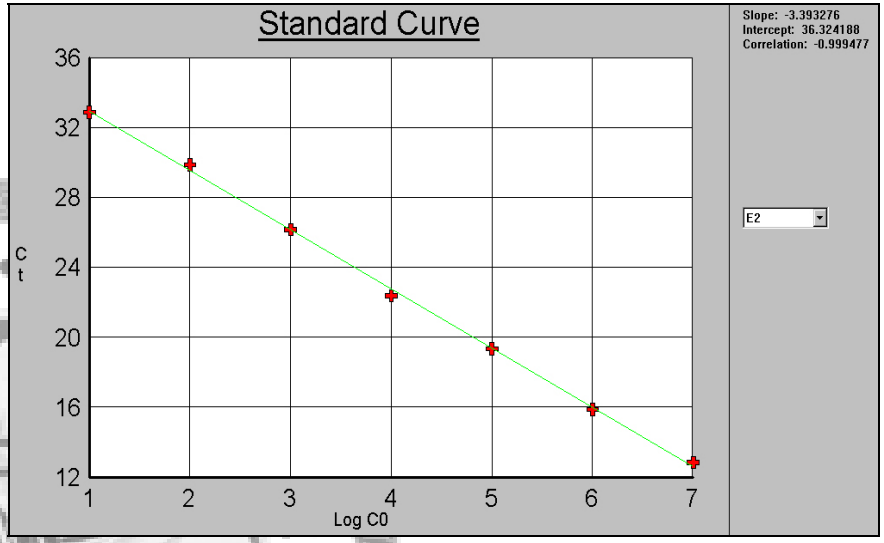

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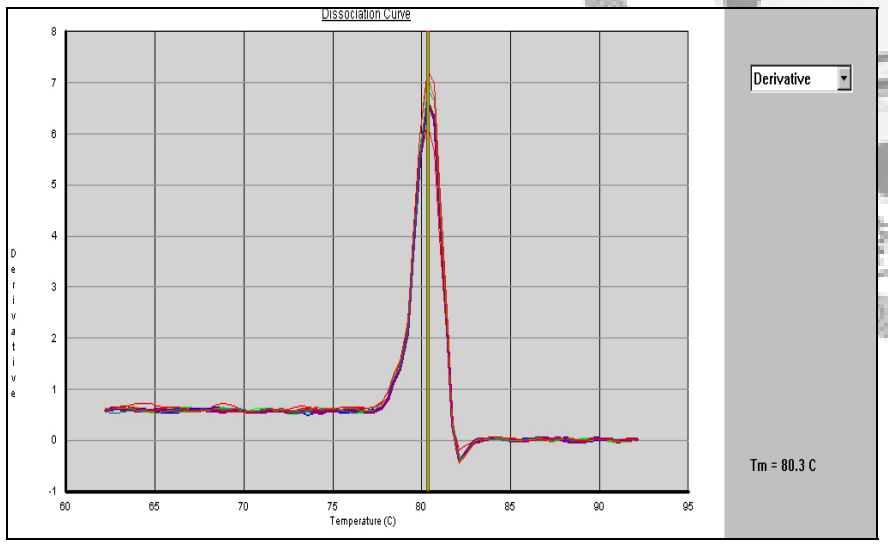

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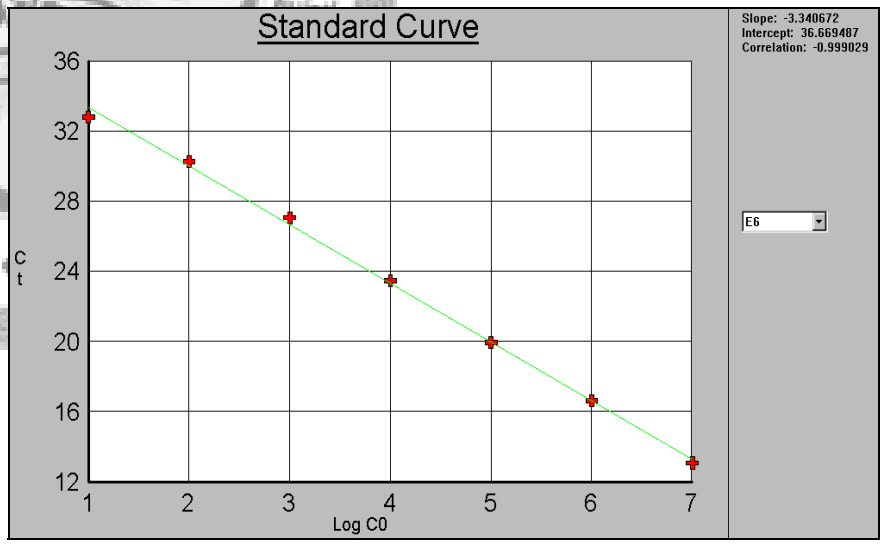
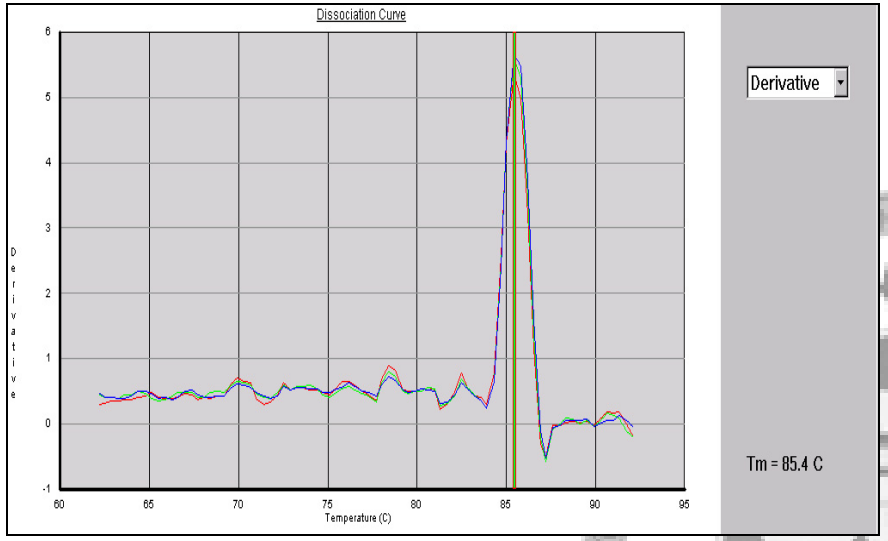


HPV52/E2 Dissociation Curve



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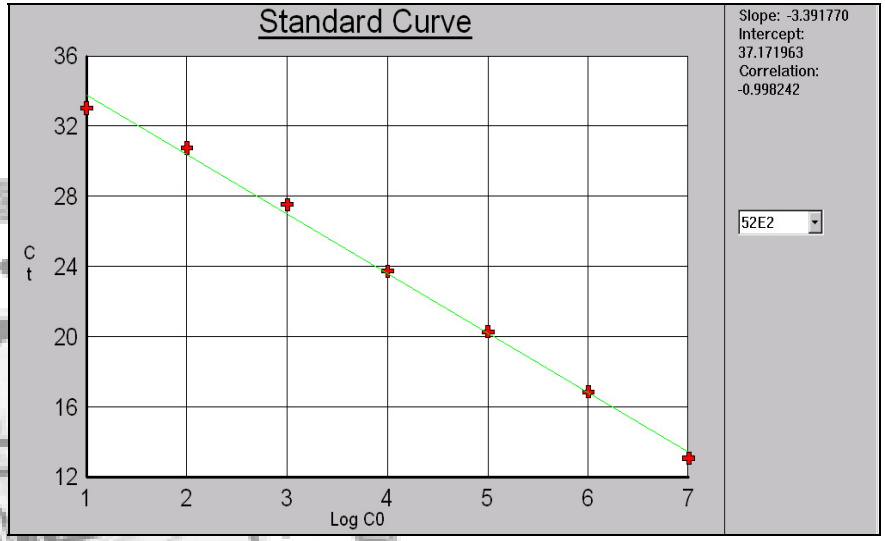

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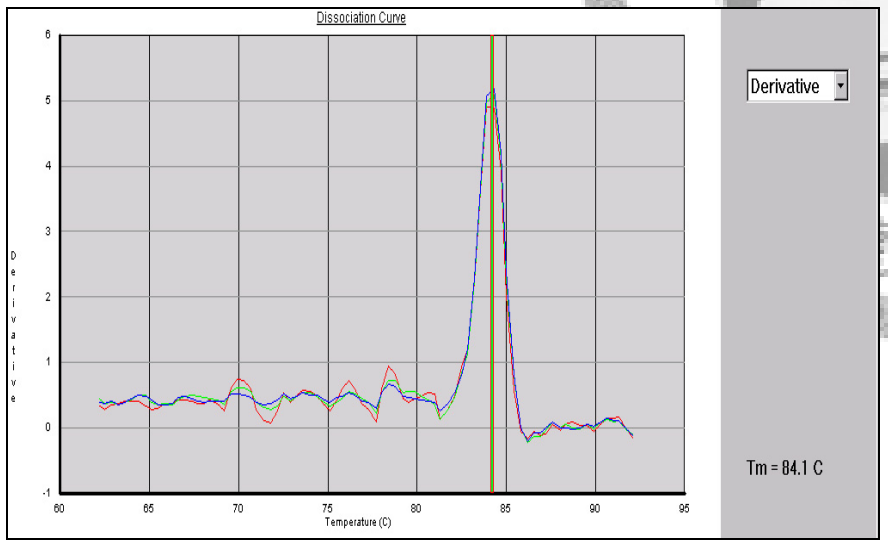

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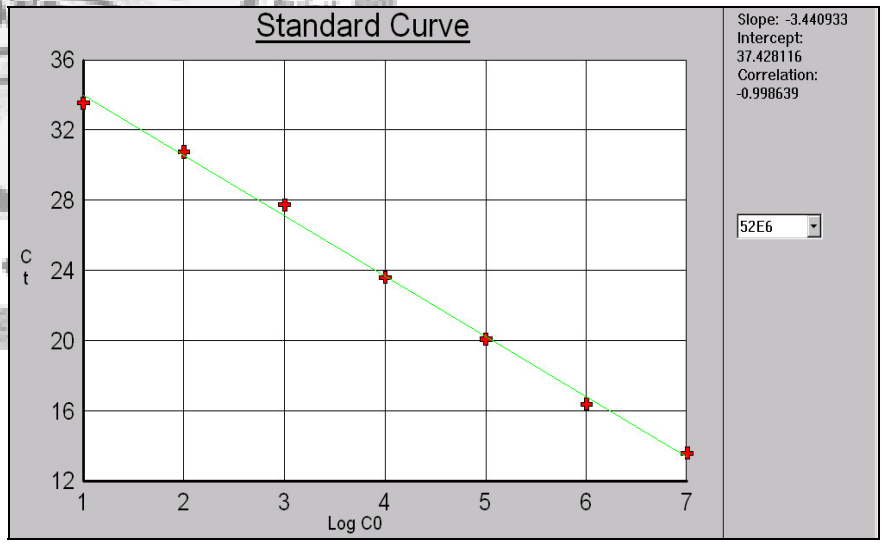
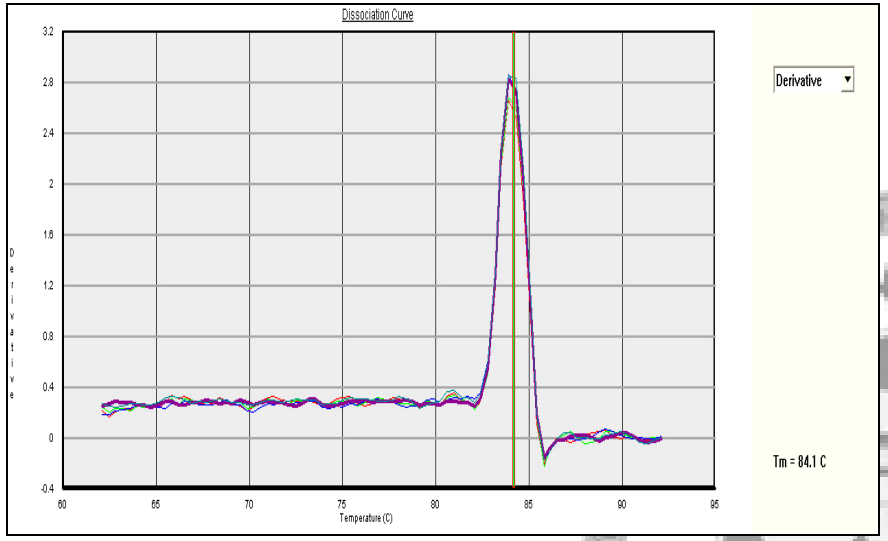


HPV58/E2 Dissociation Curve



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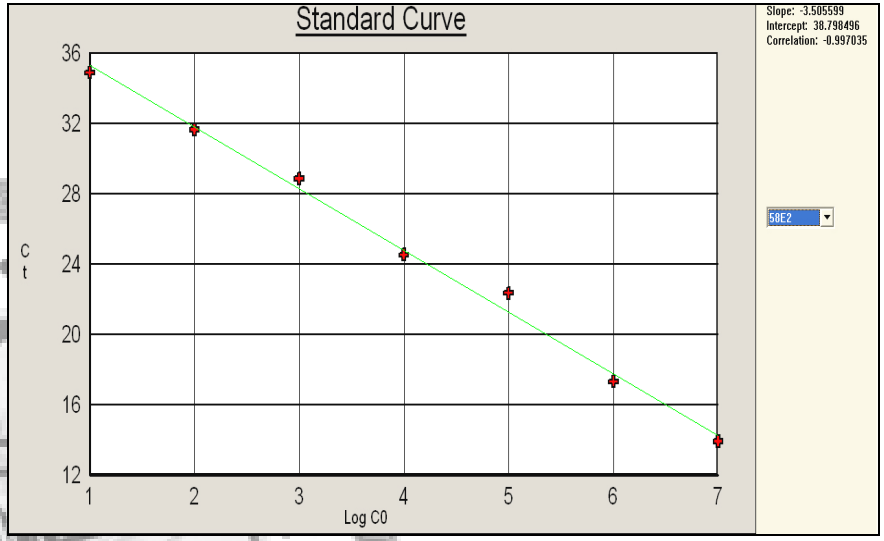

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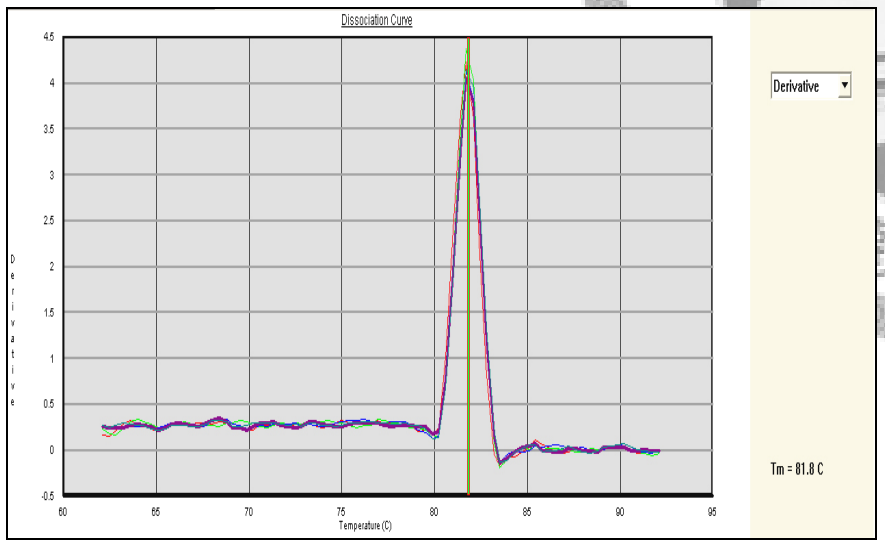

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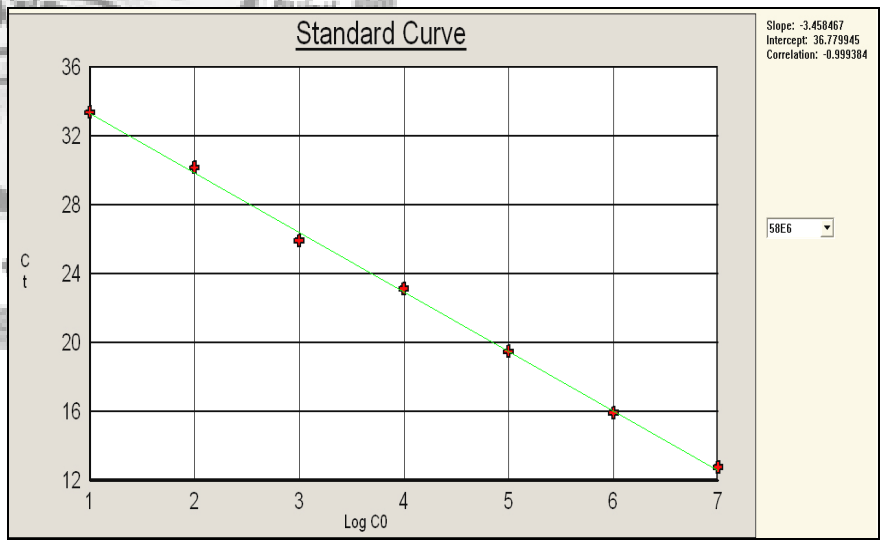

Appendix I. Reliability of viral load measurements

						correlation coefficients among E6 triplicates				correlation coefficients with		
	Median	Mean	SD	Minimum	Maximum	r1	r2	r3	ICC	DNA concentration	no. of cell	log10 (cell)
HPV16(n=170)												
E6	1,657.1	292,228	200,733	7.6	2,597,193	0.99965	0.99888	0.99825	0.991(0.988-0.993)	0.18943	0.202	0.1565
						<.0001	<.0001	<.0001		0.0134	0.008	0.0287
E2	2,103.3	18,260	10,278	0.0	1,300,114	0.99226	0.96602	0.95747	0.936(0.917-0.952)			
						<.0001	<.0001	<.0001				
HPV18(n=86)												
E6	620.9	25,897	75,809	12.7	480,193	0.93884	0.8345	0.79637	0.940(0.913-0.959)	-0.07787	-0.113	0.0684
						<.0001	<.0001	<.0001		0.9141	0.909	0.5413
E2	391.1	18,138	57,440	0.0	330,988	0.96898	0.97582	0.95677	0.983(0.976-0.989)			
						<.0001	<.0001	<.0001				
HPV52(n=324)												
E6	1,001.3	36,880	204,139	10.1	3,020,304	0.97109	0.95134	0.86905	0.964(0.956-0.910)	0.00357	0.005	0.0505
						<.0001	<.0001	<.0001		0.9490	0.93	0.3647
E2	697.1	17,652	74,963	0.0	809,828	0.959	0.8785	0.8081	0.945(0.933-0.954)			
						<.0001	<.0001	<.0001				
HPV58(n=148)												
E6	1,657.1	34,352	125,468	13.0	1,378,086	0.98771	0.93367	0.91906	0.957(0.944-0.968)	0.13893	0.13	0.1891
						<.0001	<.0001	<.0001		0.0922	0.115	0.021
E2	947.5	23,491	73,698	0.0	755,533	0.97957	0.93258	0.93229	0.972(0.963-0.979)			
						<.0001	<.0001	<.0001				

Publications list

Award:

Young investigator Award, 24th International Papillomavirus Conference and Clinical Workshop 2007; Beijing.

Peer-reviewed papers:

1. Naucler P, Chen HC, Persson K, You SL, Hsieh CY, Sun CA, et al. Seroprevalence of human papillomaviruses and Chlamydia trachomatis and cervical cancer risk: nested case-control study. J Gen Virol. 2007 Mar;88(Pt 3):814-22.
2. Lin CY, Chen HC, Lin RW, You SL, You CM, Chuang LC, et al. Quality assurance of genotyping array for detection and typing of human papillomavirus. J Virol Methods. 2007 Mar;140(1-2):1-9.
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6. Lu SN, Chen HC, Tang CM, Wu MH, Yu ML, Chuang WL, et al. Prevalence and manifestations of hepatitis C seropositivity in children in an endemic area. PediatrInfectDisJ. 1998;17(2):142-5.
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8. Tsau YK, Sheu JN, Chen CH, Teng RJ, Chen HC. Decreased urinary epidermal growth factor in children with acute renal failure: epidermal growth factor/creatinine ratio not a reliable parameter for urinary epidermal growth factor excretion. Pediatr Res. 1996 Jan;39(1):20-4.
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10. Chen HC, You SL, Chen CJ, Wang CW, Yang CS. A preliminary study on seroprevalence of Hepatitis C virus infection in Taiwan. Chinese Journal of Public Health. 1992;11 No.3:214-9.

Conference presentation:

1. Pan MH, Chen HC, Lee BH, Lin CY, Chou YC, You SL, et al., editors. HPV types 16/18/52/58 viral load & integration status associated with cervical precancerous lesion in Taiwan. 24th International Papillomavirus Conference and Clinical Workshop 2007; Beijing.
2. Pan MH, Chen HC, Chou YC, You SL, Hsieh CY, Chen CJ, editors. 10 year follow-up of a nation-wide health insurance reimbursed cervical neoplasia screening program: a cohort study. 24th International Papillomavirus Conference and Clinical Workshop 2007; Beijing.
3. Chiang CJ, Chen HC, Pan MH, Lin CY, Chou YC, You SL, et al., editors. Type-specific comparison of Human Papillomavirus DNA genotyping array and dot blot hybridization. 24th International Papillomavirus Conference and Clinical Workshop 2007; Beijing.
4. Chiang CJ, Chen HC, Pan MH, Lin CY, Chou YC, You SL, et al., editors. Multiple repeated measurement of type-specific HPV infection in a Taiwanese female cohort. 24th International Papillomavirus Conference and Clinical Workshop 2007; Beijing.
5. Chen HC, Pan MH, Lin CY, Chou YC, You SL, Hsieh CY, et al., editors. HPV infection associated cervical neoplasia in a long-term follow-up cohort in Taiwan. 24th International Papillomavirus Conference and Clinical Workshop 2007; Beijing.
6. Chen HC, Pan MH, Lin CY, Chou YC, You SL, Hsieh CY, et al., editors. HPV infection and co-infection of HPV types 16, 18, 52 and 58 in a large-scale community study of Taiwanese women. 24th International Papillomavirus Conference and Clinical Workshop; 2007; Beijing.
7. Chen HC, Lee BH, Pan MH, Lin CY, Chou YC, You SL, et al., editors. HPV types 16/18/52/58 viral load & integration status predict viral persistence & progression in Taiwan. 24th International Papillomavirus Conference and Clinical Workshop; 2007; Beijing.
8. Pan MH, Chen HC, You SL, Lin CY, Chou Y, Hsieh C, et al., editors. Acquisition rate and risk factors of type-specific HPV in 6,883 women with repeated HPV genotyping in Taiwan 23rd International Papillomavirus Conference and Clinical Workshop; 2006; Prague.
9. Lin CC, Chen HC, You SL, Lin CY, Pan MH, Chou YC, et al., editors. Association between cervical human papillomavirus infection and female lung and breast cancers in Taiwan: a 14-year follow-up study 23rd International Papillomavirus Conference and Clinical Workshop; 2006; Prague.
10. Chen HC, You SL, Lin CY, Pan MH, Chou YC, Hsieh CY, et al., editors. HPV type-specific prevalence and co-infection rate among 10602 women in Taiwan:

The CBCSP cohort study. 23rd International Papillomavirus Conference and Clinical Workshop; 2006; Prague.

11. Chen HC, You SL, Lin CY, Pan MH, Chou YC, Hsieh CY, et al., editors. Incidence of cervical cancer associated with HPV type-specific infection and persistence in Taiwan: A 14-year follow-up study 23rd International Papillomavirus Conference and Clinical Workshop; 2006; Prague.
12. Chaung LC, Chen HC, You SL, Lin CY, Pan MH, Chou YC, et al., editors. Human papillomavirus as a risk factor for the malignant neoplasm of rectum, recto-sigmoid junction and anus: a cohort study in Taiwan. 23rd International Papillomavirus Conference and Clinical Workshop; 2006; Prague.
13. Chen HC, You SL, Lin CY, Pan MH, Chou YC, Lin RW, et al., editors. Wide-spectrum detection of HPV types and their incidence of cervical neoplasia in a long-term follow-up study in Taiwan. EUROIN 2006; 2006; Paris.
14. You SL, Chen HC, Lin CY, Lin RW, You CM, Shih LY, et al., editors. Genotype-specific HPV prevalence and persistence among women in Taiwan: a community-based cancer-screening project. THE 31st EUROPEAN CONGRESS OF CYTOLOGY; 2005 2005/10; Paris.
15. Chen HC, You SL, Lin CY, Lin RW, You CM, Shih LY, et al., editors. HPV type-specific incidence and association with cervical neoplasia: a 12-year follow-up study in Taiwan. THE 31st EUROPEAN CONGRESS OF CYTOLOGY; 2005 ; Paris.
16. Lin CY, Chen HC, Lin RW, You SL, Chen CJ, editors. Quality Assurance of Genotyping Array for Detection and Typing of Human Papillomavirus. 22nd International Papillomavirus Conference and Clinical Workshop; 2005; Vancouver.
17. Naucler P, Chen HC, Persson K, You SL, Chen CJ, et al., editors. Serologic response to human papillomavirus type 6, 16, 18 and Chlamydia trachoma is: a nested case-control study in Taiwan. 22nd International Papillomavirus Conference and Clinical Workshop; 2005; Vancouver.
18. Chen HC, Lin CY, You SL, Naucler P, Lin RW, You CM, et al., editors. A 10-year follow-up study of HPV type-specific risks for cervical cancer in Taiwan. 22nd International Papillomavirus Conference and Clinical Workshop; 2005 ; Vancouver.