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Differences in incidence and co-occurrence of vaccine and nonvaccine human papillomavirus types in Finnish population before human papillomavirus mass vaccination suggest competitive advantage for HPV33

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To understand likelihood of type replacement after vaccination against the high-risk human papillomavirus (HPV) types, we evaluated competition of the seven most common genital HPV types in a population sample of unvaccinated, fertile-aged Finnish women. First trimester sera from two consecutive pregnancies were retrieved from 3,183 Finnish women (mean age, 23.1 years) of whom 42.3% had antibodies to at least one HPV type (6/11/16/18/31/33/45) at the baseline. Antibody positivity to more than one HPV types by the second pregnancy was common among the baseline HPV seropositives. However, compared to baseline HPV-seronegative women, significantly increased incidence rate ratios (IRRs), indicating an increased risk to seroconvert for another HPV type, were consistently noted only for HPV33 among baseline HPV16 or HPV18 antibody (ab)-positive women: $HPV_{16ab\ only} \rightarrow 16\&33ab$ IRR 2.9 [95% confidence interval (CI) 1.6–5.4] and $HPV_{18ab\ only} \rightarrow 18\&33ab$ IRR 2.5 (95% CI 1.1–6.0), irrespectively of the presence of antibodies to other HPV types at baseline: $HPV_{16ab} \rightarrow 16\&33ab$ IRR 3.2 (95% CI 2.0–5.2) and $HPV_{18ab} \rightarrow 18\&33ab$ IRR 3.6 (95% CI 2.1–5.9). Our findings suggest a possible competitive advantage for HPV33 over other genital HPV types in the unvaccinated population. HPV33 should be monitored for type replacement after HPV mass vaccination.

There are at least 40 genital human papillomavirus (HPV) types classified into oncogenic and nononcogenic types.[1] Transmission probability of the most common type, HPV16, has been estimated to be up to 60%.[2] The presence of multiple types in the sexually active population is common, and the epidemic state of some (*e.g.*, HPV16) but not all HPV types is dynamic (increasing) at the population level in Finland.[3–5] Due to high transmission probability and tendency to persist concomitant infections by high-risk (hr) HPVs are common.[5–10] Furthermore, multiple infections are associated with an even higher increased risk of developing cervical neoplasia.[10]

Two highly efficacious HPV vaccines have now been licensed world wide.[11–14] By diminishing the pool of HPV-susceptible individuals and preventing transmission, HPV vaccination could rapidly change the ecosystem of genital HPV types. Consistent crossprotection provided by the current HPV6/11/16/18 and HPV16/18 vaccines against a number of closely HPV16- or HPV18-related HPV31 and HPV45, respectively,[15–17] makes the situation even more challenging. Replacement of vaccine hrHPV types in an ecological niche induced by mass vaccination may be possible.[18] The vaccine manufacturers already have more polyvalent HPV vaccines under development to tackle possible situations.

We have previously reported the dynamic nature of epidemic caused by HPV16 in Finland[3] and about increased risk of acquiring HPV16 and HPV18 coinfections over time.[4,5] Replacement of vaccine-covered pneumococcal types with nonvaccine types predicted 15 years ago, has been verified after implementation of pneumococcal vaccination and threatens to jeopardize the effectiveness of related vaccination programmes.[19] To better understand the likelihood of possible mass vaccination-induced HPV type replacement, we now

Key words: competition, human papillomavirus, incidence rate ratio, population, serology

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Table I. Random subsample sizes (*n*) and number of all women (*N*) with a minimum of two pregnancies within 5 years during 1995–2003 in the Finnish Maternity Cohort (FMC) by estimated age (in years) and calendar time at the midpoint of two consecutive pregnancies

	1995–1997		1998–2000		2001–2003	
Age	*n*	*N*	*n*	*N*	*n*	*N*
<20	162	1920	159	1,878	168	2,026
20–22	187	7625	190	7,841	171	7,513
23–25	367	13,600	364	13,248	340	13,203
26–28	362	20,413	357	17,105	356	17,401

$N = 123{,}773$ women <29 years of age with a minimum of two pregnancies within 5 years in 1995–2003. Stratified random sample of 3,600 women. 417 women were excluded. *n* (total) = 3,183 women with paired sera analyzed for HPV6, 11, 16, 18, 31, 33, 45 antibodies.

report on the competition of the seven most common genital low-risk (lr) HPV and hrHPV types in a large, random sample of fertile-aged Finnish female population before HPV mass vaccination.

Material and Methods

Finnish Maternity Cohort

Since 1983, pregnant Finnish women (810,000) have participated in screening for congenital infections during the first trimester. Over 98% of all the pregnant women donate a serum sample at week 12 of gestation (range 10–149) to the Finnish Maternity Cohort (FMC), serum bank held by the National Institute for Health and Welfare (THL). By June 2009, the FMC serum bank had approximately 1,700,000 serum samples stored at −25°C.

All 123,773 women, under 29 years of age, and with a minimum of two available first trimester serum samples drawn within a 5-year period in 1995–2004 were identified for the determination of seroconversions between the two consecutive pregnancies as described previously in detail.[5] A random subcohort of 3,600 women was stratified by calendar time at the midpoint of two consecutive pregnancies in three 3-year time bands and by age at the midpoint in four 3-year age bands (Table 1). In our previous studies, few HPV seroconversions were discovered after the age of 30 years.[5] Due to lack of sample or inadequate serum sample volume, 417 subjects were excluded from the analyses, leaving final study cohort of 3,183 women.

Most HPV-infected women acquire the virus early in sexual life,[20] and the rate of seroconversions in older age bands was expected to be low. To maximize study power, we selected twice as many random subjects in the older age groups between 23 and 28 years of age (Table 1).[5] The THL has permission from the Finnish Parliament to use the FMC serum bank samples for public health research (Laws on KTL/THL 828/1981 and 327/2001: 1a §). Since 2001, an informed consent from the donor has been obtained for research use of the serum samples.

Serology

HPV IgG antibody (ab) analyses for types 6, 11, 16, 18, 31, 33 and 45 were done using virus-like particles (VLPs) kindly provided by Dr. K. Jansen (HPV types 6, 11 and 16, Merck Research Laboratories, Philadelphia, PA), Dr. F. Dessy (HPV types 18, 33 and 45, GlaxoSmithKline Biologicals, Rixensart, Belgium) and Dr. R. Kirnbauer (HPV type 31, University of Vienna, Austria) in a standard, direct ELISA as previously described.[3,21] A pool of serum samples from adolescent virginal females comprised the negative reference by which the absorbance cutoff levels were determined. Seroconversion between the first and the second pregnancy samples was defined by doubling of the absorbance level. To control interassay drift, a set of antibody-positive samples were tested on each plate.

Sensitivity and specificity of the HPV VLP serology has been evaluated using HPV DNA detection by PCR as the gold standard. These studies have shown 50–75% sensitivity and 95–99% specificity for different HPV antibody analyses.[22–26] The HPV IgG antibodies persist in >90% of seropositive individuals for at least 5 years, and seroreversions are rare.[23]

Chlamydia trachomatis IgG antibodies, an indicator of past chlamydial infection and a validated surrogate of lifetime number of sexual partners,[21] were determined by a commercial ELISA using a *C. trachomatis* major outer membrane protein-derived peptide.[21]

Statistical methods

Time elapsed between withdrawals of the two consecutive serum samples varied from 9 months to 5 years by subject. Hence, we used person-time-based statistical analysis approach to evaluate if natural infection as indicated by specific antibodies with HPV16 or HPV18 protects against infections with HPV types 6, 11 (18 or 16), 31, 33 and 45. Seroconversion between the two pregnancies for HPV6, HPV11 (HPV18 or HPV16), HPV31, HPV33 and HPV45 after the initial, antibody-producing HPV16 or HPV18 infection was used as an indicator of a new infection. Time of the seroconversion was assumed to be the middle point of the serum samplings at two consecutive pregnancies.

As previously described,[5] we calculated incidence rates (IRs) per 1,000 person-years. Incidence rate ratios (IRRs) with 95% confidence intervals (CIs) were used to estimate the risk of seroconversion for different HPV types after initial infection indicated by antibodies for only one HPV type ($HPV_{16ab\ only}$ or $HPV_{18ab\ only}$) or at least one HPV type (HPV_{16ab} or HPV_{18ab}) compared to those individuals without antibodies to any of the seven HPV types. We repeated the crude IRR analyses for age groups <20, 20–25 and 26–28, and *C. trachomatis* antibody positives and antibody seronegatives separately to evaluate possible interactions between initial HPV seropositivity and age or *C. trachomatis* antibody status at baseline.

Poisson regression models were fitted to adjust confounding factors: age (<20, 20–25 and 26–28) and risk-taking sexual behavior (C. *trachomatis* antibody status was used a surrogate marker) and to test the significance of interaction between initial HPV seropositivity and age at the baseline. Interaction analyses were performed only if differences between age groups warranted further exploration. We included only variables with two-sided p value <0.07 in the interaction models according to Wald χ^2 test for individual regression coefficient. A deviance test was used to evaluate the fit of the main effect Poisson models. All the statistical analyses were done using SPSS 15.0 (SPSS, Chicago, IL) and the Genmod procedure of SAS 9.1 (SAS Institute, Cary, NC).

Results

Baseline characteristics

The overall baseline seropositivity rates for HPV types 6, 11, 16, 18, 31, 33 and 45 in the subcohort of 3,183 women were 11.9% ($n = 380$), 9.9% ($n = 316$), 19.2% ($n = 610$), 13.9% ($n = 442$), 12.9% ($n = 411$), 10.8% ($n = 344$) and 5.1% ($n = 162$), respectively. The corresponding rates of baseline single seropositives for only HPV types 6, 11, 16, 18, 31, 33 or 45 were 2.8% ($n = 90$), 1.4% ($n = 45$), 6.2% ($n = 197$), 2.9% ($n = 93$), 4.5% ($n = 142$), 2.7% ($n = 86$) and 0.7% ($n = 23$), respectively.

Seroconversions by baseline HPV16 seropositivity

Numbers of seroconversions for different HPV types varied between 16 and 48 among the baseline HPV-seronegative women (Table 2). In the baseline HPV16-seropositive women, the number of HPV6 seroconversions was low, whereas HPV18, HPV33 and HPV45 seroconversions were not infrequent. Among those only HPV16 seropositive, HPV18 and HPV33 seroconversions were the most frequent (Table 2). After adjusting for age and *C. trachomatis* antibody status, baseline HPV16-seropositive women had increased risk for HPV18 (adjusted IRR: 2.6, 95% CI: 1.1–6.0), HPV33 (adjusted IRR: 3.2, 95% CI: 2.0–5.2) and HPV45 seroconversion (adjusted IRR: 2.4, 95% CI: 1.6–7.1) as compared to baseline HPV-seronegative women. Women, who were baseline seropositive to HPV16 only had increased risk for HPV18 (adjusted IRR: 3.0, 95% CI: 1.1–8.2) and HPV33 seroconversion (adjusted IRR: 2.9, 95% CI: 1.6–5.4; Table 3).

Seroconversions by baseline HPV18 seropositivity

Among baseline HPV18-seropositive women, the number of HPV6 seroconversions was also low. HPV33 and HPV45 seroconversions were not seen infrequently. In baseline HPV18-seropositive women, only HPV33 seroconversions were the most frequent (Table 2). All baseline HPV18-seropositive women had increased risk for HPV33 (adjusted IRR: 3.6, 95% CI: 2.1–5.7) and HPV45 (adjusted IRR: 6.4, 95% CI: 3.0–14) seroconversion as compared to baseline HPV-seronegative women. However, baseline HPV18-seropositive women only had increased risk for HPV33 seroconversion only (adjusted IRR: 2.5, 95% CI: 1.1–6.0; Table 3).

Interaction between hrHPV serostatus, age and C. trachomatis antibody status

HPV45 and especially the HPV33 seroconversions tended to increase by age both among HPV16-seropositive women and among HPV18-seropositive women. For HPV33 seroconversion rates, the differences between the youngest (<20 years of age) and the oldest (26–28 years of age) age groups were fourfold and sixfold among the HPV16 and HPV18 seropositives, respectively, but did not reach statistical significance. On the other hand, virtual absence of HPV45 seroconversion (IRR = 0.08, 95% CI 0.0–0.9), due to antagonistic interaction of baseline HPV18 and *C. trachomatis* antibody, was statistically significant ($p = 0.04$).

Discussion

We found significant and consistent excess risk of seroconversion for HPV33 among the baseline HPV16- and HPV18-seropositive women, irrespective of age or presence or absence of antibodies to other HPV types or *C. trachomatis*, the latter being a validated surrogate marker of sexual risk taking behavior.[21]

The population-based FMC serum bank is the world's largest biobank cohort of fertile-aged women. It receives and stores first trimester serum samples from virtually all pregnant Finnish women. We have continued our studies on a large, random subsample of 123,773 unvaccinated fertile-aged females with a minimum of two consecutive pregnancies within 5 years. Our material is representative of the fertile-aged Finnish female population, which has one of highest total fertility rates (1.85/woman) in Europe. Previously, we have used this approach for the identification of HPV prevalence and incidence trends in fertile-aged female population.[3,4] It was now used for the identification of possible competitive advantage of one or more of the seven most common genital HPV types. Our previous study on population dynamics of four genital HPV types[5] indicated that the approach had power to identify excess risks of seroconversions for different HPV types by baseline HPV16 or HPV18 serostatus.

We evaluated seropositivity for the seven most common HPV types, while the other HPV types not considered are rare in Finland.[22,23] However, there are some limitations in HPV serology, and therefore the results should be interpreted with caution. One is misclassification bias, as not all HPV-infected women produce antibodies and some are late responders. The low sensitivity of VLP ELISA reflects this, whereas the identification of HPV DNA has been used as the gold standard. It is possible that transient HPV infections detected by PCR might not result in antibody production. If a proportion of women classified as HPV naïve were not naïve and in addition were not likely to seroconvert (not seroconverting after an initial HPV infection), this would have increased the point estimates. On the other hand, as the infections identified by seroconversions took place within approximately 2.5 years, up to 65–75% sensitivity was reached.[24] As such, this kind of suboptimal sensitivity would at the second sample analysis (definition of the

Table 2. Seroconversions (SCs), incidence rates (IRs per 1,000 person-years) and crude incidence rate ratios (IRRs) with 95% confidence intervals (95% CIs) for HPV6, 11, 16, 18, 31, 33 and 45 in initially HPV16- or HPV18-seropositive women compared to initially seronegative women using Poisson regression main effect models ($N = 3183$, an FMC-serum bank subsample of 123,773 women <29 years of age with a minimum of two pregnancies between 1995 and 2003)

	Follow-up					
Baseline	**HPV6**	**HPV11**	**HPV16/HPV18**	**HPV31**	**HPV33**	**HPV45**
Seronegative						
SC	36	23	48/16	40	44	16
IR	8.4	5.4	11.2/3.7	9.4	10.3	3.7
IRR	1.0	1.0	1.0/1.0	1.0	1.0	1.0
Seropositive						
HPV16						
SC	3	7	n.a./9	8	32	14
IR	2.7	6.1	n.a./10.3	8.0	33.6	11.8
IRR	0.3 (0.1–1.4)	1.1 (0.5–2.7)	n.a./2.8 (1.2–6.3)	0.9 (0.4–1.8)	3.3 (2.1–5.2)	3.2 (1.6–6.5)
HPV16 only						
SC	1	0	n.a./5	1	14	2
IR	2.2	0.0	n.a./11.2	22	32.4	4.5
IRR	0.3 (0.0–1.9)	0.0 (0.0)	n.a./3.0 (1.1–8.3)	0.2 (0.03–1.7)	3.2 (1.7–5.7)	1.2 (0.3–5.2)
HPV18						
SC	3	8	7/n.a.	11	24	14
IR	3.8	9.9	4.3/n.a	15.6	35.4	17.4
IRR	0.4 (0.1–1.5)	1.9 (0.8–4.2)	1.3 (0.6–2.8)/n.a	1.7 (0.9–3.3)	3.4 (2.1–5.7)	4.7 (2.3–9.6)
HPV18 only						
SC	1	3	3/n.a.	3	6	2
IR	4.3	13.1	13.1/n.a.	13.1	26.5	8.7
IRR	0.5 (0.1–3.7)	2.4 (0.7–8.1)	1.2 (0.4–3.7)/n.a.	1.4 (0.4–4.5)	2.6 (1.1–6.0)	2.3 (0.5–10.1)

n.a.: not applicable.

Table 3. Adjusted IRR with 95% CI for HPV6, 11, 16, 18, 31, 33 and 45 in initially HPV16 or HPV18 seropositive women compared to initially seronegative women using Poisson regression main effect models ($N = 3183$, an FMC-serum bank subsample of 123,773 women <29 years of age with a minimum of two pregnancies between 1995 and 2003)

	Follow-up					
Baseline	**HPV6**	**HPV11**	**HPV16/HPV18**	**HPV31**	**HPV33**	**HPV45**
Seronegative						
IRR	1.0	1.0	1.0/1.0	1.0	1.0	1.0
Seropositive						
HPV16						
IRR	0.3 (0.1–0.9)	1.2 (0.5–2.7)	n.a./2.6 (1.1–6.0)	0.9 (0.4–1.9)	3.2 (2.0–5.2)	2.4 (1.6–7.1)
HPV16 only						
IRR	0.2 (0.1–1.0)	0.0 (0.0–∞)	n.a./3.0 (1.1–8.2)	0.3 (0.0–1.9)	2.9 (1.6–5.4)	1.2 (0.3–5.4)
HPV18						
IRR	0.4 (0.1–1.3)	1.9 (0.8–4.3)	1.4 (0.6–3.2)/n.a.	1.9 (1.0–3.7)	3.6 (2.1–5.9)	6.4 (3.0–14)
HPV18 only						
IRR	0.5 (0.1–3.3)	2.3 (0.7–7.8)	1.2 (0.4–4.0)/n.a.	1.8 (0.6–5.9)	2.5 (1.1–6.0)	2.9 (0.6–13)

n.a.: not applicable.

seroconversions), if anything, have reduced the point estimates. Finally, HPV seroreversions are rare,[25] type specificity of the VLP serology in natural infection-derived antibodies appears not to be a problem,[26] and HPV antibody positivity is considered as a reliable indicator of cumulative incidence of HPV infection.[21,27]

The probability of HPV33 seroconversions tended to increase by age. This is most likely due to continuing sexual risk-taking behavior and was evident even if controlled for by adjusting for *C. trachomatis*, the seropositivity of which increases linearly with the number of lifetime sexual partners.[21] *C. trachomatis* seropositivity was associated with an increased risk for HPV45 seroconversion among HPV18-seropositive women. This is biologically plausible as cervical metaplastic cells are targets for all these micro-organisms. It also might reflect phylogenetic closeness of these two, clade A7, hr HPV types.

Our findings suggest a competitive advantage for HPV33 over a number of other genital HPV types in the unvaccinated population as no comparable, consistent patterns by baseline HPV16 or HPV18 serostatus were observed for the other hrHPV types. Depending on to which extent HPV vaccine-induced protection resembles that of natural infection-induced immunity, HPV33 needs to be monitored for type replacement after HPV mass vaccination

Changes in the competition of genital HPV infections at population level are difficult to study or monitor. Mathematical modeling suggests that after HPV16 vaccination of females and males with a moderate (35–70%) vaccine coverage, herd immunity results in a marked HPV16 prevalence reduction,[28] but is this creating the required ecological niche for type replacement? On the other hand, it is well known that HPV16/18 VLP vaccines induce 100-fold higher and markedly broader humoral immunity than natural infections.[15–17] Serological crossreaction after vaccination has been reported against HPV31 and 45.[15,17] Interestingly crossreactive antibodies against HPV33 induced by the vaccines have not been consistently detected.

In conclusion, our findings suggest competitive advantage for HPV33 over a number of other genital HPV types in the unvaccinated population. This warrants further research and monitoring for type replacement after HPV mass vaccination.

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