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Seroprevalence atlas of infections with oncogenic and non-oncogenic human papillomaviruses in Finland in the 1980s and 1990s

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Vaccines against high-risk (hr) human papillomaviruses (HPVs) causing cervical cancer may soon be licensed. Thus, nature of HPV epidemics needs to be studied now. Random sampling for studies on HPV epidemiology was done from all 230,998 women belonging to the population-based Finnish Maternity Cohort and having a minimum of 2 pregnancies between 1983 and 1994. First pregnancy serum specimens were retrieved for 7,805 subjects, and were analyzed for antibodies to HPV6/11, 16 and 18 with standard ELISAs. HPV16 seroprevalence almost doubled from the 1980s to the 1990s, and the epidemic spread to new areas in 23–31 year olds, i.e. the bulk of pregnant female population in the southwest part of the country. The HPV16 epidemic in the 14–22 year olds in 1983–1988 (1961–1974 birth cohorts) and in the 23–31 year olds in 1989–1994 (1958–1971 birth cohorts) overlapped with strong clustering of HPV16 and HPV18 infections in the latter (odds ratio 8.0, 95% confidence interval 6.6–9.7). Similar clustering of HPV16 and HPV6/11 infections was not found. The epidemic and the clustering may be due to high transmission probability of the hrHPV types and increase in sexual activity of the index birth cohorts.

Key words: cervical cancer; vaccination; population-based follow-up; cohort study; cervical neoplasia; cancer registry; human papillomavirus

Genital infections with human papillomaviruses (HPVs) peak shortly after the beginning of sexually active life between 18 and 22 years of age.[1,2] There are about 40 HPV types causing genital infections. The types in Finland are identical to those seen in the other western/European countries: HPV6/11, 16, 18, 31, 33 and 45.[3] Co-infection of HPV types is not uncommon,[4,5] but factors affecting their acquisition differ between the high risk (hr) and the low risk (lr) HPV types.[6–9] Moreover, there is antagonism between hrHPVs and lrHPVs in cervical carcinogenesis.[10,11]

During the past 30 years, sexual behavior has considerably changed in Finland with increases both in sexual activity and risk-taking behavior among females.[12] This has resulted in increasing trends of hrHPVs (e.g., HPV16) but not lrHPVs (e.g., HPV6/11) incidence in pregnant Finnish women since 1980s.[13] Also, during the last 10 years, the incidence of cervical cancer (CxCa) among fertile-aged women has rapidly increased in Finland.[14,15] Moreover distribution of hrHPV types has changed in the CxCa cases, and a new hrHPV type (HPV45) emerged during the 1990s (data on file).[16,17] This suggests that hrHPV epidemics in Finland have been in a dynamic state for the past 30 years.

Vaccines against the major hrHPV types (HPV16 and HPV18) may be licensed during year 2006, and use of the HPV16/18 vaccine may considerably change the epidemic situation in the young, turning smaller or larger groups of susceptible (vaccine implementation at private or societal level) to group of immunes. Size of the immune group, in the vaccination era, will be determined both by vaccine coverage and changes in sexual behavior, and determines herd immunity against HPV16 and 18.[18] What happens to HPV-type distribution following population level vaccination is open.

Before the vaccination gets started in Finland, we studied the local-specific cumulative exposure to (seroprevalence of) HPV types 6/11, 16 and 18 over time in pregnant Finnish women with special emphasis on co-infections.

Material and methods

Finnish maternity cohort (FMC)

Since 1983, >98% of pregnant Finnish women (altogether 750,000) have participated screening for congenital infections organized by the National Public Health Institute (KTL).[19] During the first trimester (between 10 and 12 weeks of gestation) of each pregnancy, the women donate sera to the FMC-serum bank of KTL. By 1997, the FMC-serum bank contained about 600,000 serial serum samples (stored at −25°C) of about 300,000 women with a minimum of 2 pregnancies.

For a previous study on HPV16 incidence, serum samples of all 230,998 women with a minimum of 2 available pregnancy, withdrawn within 5 years before 1998 and under 32 years of age (at the midpoint of the 2 serum withdrawals), were identified for the determination of seroconversions between 2 consecutive pregnancies.[13] The chosen random subcohort of 7,862 women stratified by calendar time (by 3 years, 1983–1985, 1986–1988, 1989–1991, 1992–1994 and 1995–1997) and age was reorganized for this study according to age at time of serum withdrawal at the first pregnancy (by 3 years 14–19, 20–22, 23–25, 26–28, 29–31, Table I).[13] Mean age of the women at the time of serum withdrawal was 24 years (range 14–31 years).

The sensitivity of HPV serology is dependent on the lag between dates of HPV infection and serum sampling.[20–22] Thus, to maximize study power, we selected twice as many random subjects for the older age-groups between 23 and 31 years of age, as compared to subjects who were under 23 years of age. The distribution of the subjects was almost even with regard to calendar time albeit for the period 1995–1997, which was redundant due to lack of available material at the time of the serum withdrawal. These subjects were excluded from the study, and the final study material consisted of 7,805 subjects (Table I).

Microbial serology

HPV IgG-antibody analyses for types 6/11, 16 and 18 were done applying virus-like particles (VLPs) kindly provided by Dr. Kathrin Jansen (HPV types 6, 11 and 16, Merck Research Laboratories, Philadelphia, PA), and Dr. Francis Dessy (HPV type 18, GlaxoSmithKline Biologicals, Rixensart, Belgium) in a standard, direct ELISA as previously described.[13,20] The specificity/sensitivity of the assays have been tested in many studies using sera from concurrently HPV DNA (PCR) positive women with cervical neoplasia and/or persistent HPV infection, and vary between 95–99%, and 50–75%, respectively.[20–22] The HPV IgG-antibodies persist in >90% of infected individuals for at least 5 years.[23]

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TABLE I – DISTRIBUTION (*N*) OF A RANDOM SUBSAMPLE OF 7,862 PREGNANT FINNISH WOMEN BY AGE AND CALENDAR TIME AT THE TIME OF WITHDRAWAL OF THE FIRST SERUM SPECIMENS

Age in years	Calendar time					
	1983–1985	1986–1988	1989–1991	1992–1994	1995–1997	Total
14–19	338	269	264	368	16	1255
20–22	413	249	251	395	8	1316
23–25	572	473	459	626	6	2136
26–28	574	434	439	613	17	2077
29–31	277	200	208	383	10	1078
Total	2174	1625	1621	2385	57	7862

As a reliable surrogate of risk-taking sexual behavior, herpes simplex virus type-2 (HSV-2) IgG-antibodies were determined by a type-specific glycoprotein G ELISA.[24,25]

Mapping method

Spatio-temporal variation of the HPV seroprevalence rates were visualized by a series of maps, where community level input data were smoothed to 2 × 2 km^2 raster layer. The rates for biggest cities (with 100 or more study subjects) were shown as such using colored circles (size indicating population of the city). This method has been used in many cancer mapping studies.[26,27] The rate of each pixel on the map was calculated as weighted average of rates in communities with population centers within 250 km from the middle of the grid. The pregnant female population of target age group was a direct weighing coefficient, whereas distance was used for inverse weighting. The latter weights were halved at a distance of 25 km. The seroprevalence rates were visualized using 19 colors varying from blue and green for low rates to yellow and red for high rates. To get comparable series of maps, a classification using absolute scales[28] suitable for all maps was used. The classification using 15% relative increase between each step in the scale was selected to cover about 10-fold variation of rates on the map. The random sampling of 7,805 subjects resulted in coverage of almost all the 440 communities in Finland with percentages of study subjects out of the total number of pregnant women varying between 0% (22 communities) and 10% per community (Fig. 1). According to standard procedures,[27] areas with less than one inhabitant per kilometer square were masked.

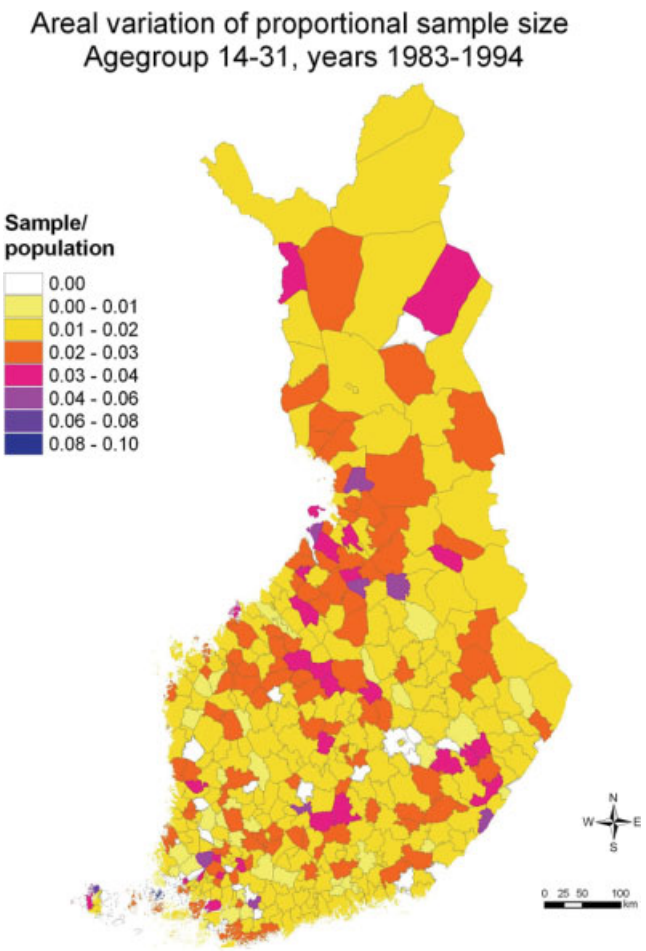

FIGURE 1 – AREAL variation of sample sizes by community in a random sub-sample of 7,805 pregnant (first available pregnancy) Finnish women between the ages of 14 and 31 years in 1983–1994.

Statistical analysis

The logistic regression models were fitted and relative risks (estimated as odds ratios, OR and 95% confidence intervals, CI) calculated using SPSS 13.0. The analyses were done separately for 2 age groups (14–22 years and 23–31 years). We estimated adjusted ORs separately by calendar period (1983–1988/1989–1994) at the time of withdrawal of the first serum specimens, HSV-2 seropositivity, and their combination.

Results

HPV seroprevalences in the random samples of pregnant women

HPV seroprevalences were the highest for HPV16 and about equal for HPV6/11 and HPV18 (Table II). Overall, the HPV16 seroprevalences were very high in all age groups (range 16–20%). Also the HPV6/11 and HPV18 seroprevalences (range 9–12%) were relatively high and evenly distributed by age at serum withdrawal. It is remarkable that already 14–22 year old subjects had as high HPV seroprevalences for single HPV types as the older subjects. On the other hand, the proportion of subjects with concomitant HPV16 and HPV18 seropositivity were higher in the older age-group (327 of 5,240, 6.2%, vs. 111 of 2,531, 4.4%, $p < 0.01$). Hence, we separated the 2 age-groups in further analyses. To further increase power the first calendar time periods (1983–1985 and 1986–1988) and the last periods (1989–1991 and 1992–1994) were combined in the geographic analyses.

Spatio-temporal variation and trends of HPV seroprevalence

Moderate HPV6/11 seroprevalences were seen in parts of southwest Finland both in the 1980s and 1990s among the women below 23 years of age (Figs. 2*a* and 2*b*). In the 1990s moderate to high HPV6/11 seroprevalences were seen for pregnant women between 23 and 31 years of age both in the southern and western parts of the country (Fig. 2*d*). In the 1980s moderate HPV6/11 seroprevalence was found only sporadically in the older age-group (Fig. 2*c*).

High rates of HPV16 seroprevalences were seen in southern Finland both in the 1980s and 1990s among the women below 23 years of age (Figs 3*a* and 3*b*). In the 1990s very high HPV16 seroprevalences were seen among women between 23 and 31 years of age all over southern Finland (Fig. 3*d*). There appeared to be high HPV16 seroprevalences in restricted areas in Lapland in the 1980s in all the age groups. In the 1990s high to very high HPV16 seropositivity seemed to have spread to larger geographic areas in northern Finland in the older but not in the younger age-group. The latter 2 spatio-temporal analyses were, however, subject to low population density (Fig. 3).

Moderate HPV18 seroprevalences were seen in the 1990s in women below 23 years of age (Fig. 4*b*) but less so in the 1980s (Fig. 4*a*). Moderate HPV18 seroprevalences were seen in southern Finland both in the 1980s and 1990s in women between 23 and 31 years of age (Figs 4*c* and 4*d*).

TABLE II – HUMAN PAPILLOMAVIRUS SEROPREVALENCE (PERCENT OF SEROPOSITIVE SUBJECTS % (POS.)) AND PERCENT OF DOUBLE SEROPOSITIVE SUBJECTS (NUMBERS IN PARENTHESES) BY AGE IN A RANDOM SUBSAMPLE OF 7,805 PREGNANT FINNISH WOMEN IN 1983–1994

	Age in years					
	14–19 (n = 1239)	20–22 (n = 1308)	23–25 (n = 2130)	26–28 (n = 2060)	29–31 (n = 1068)	Total (7805)
HPV6/11	10 (121)	11 (138)	10 (217)	10 (200)	12 (129)	10 (805)
HPV16	16 (200)	20 (268)	19 (397)	19 (397)	16 (169)	18 (1,431)
HPV18[1]	9 (105)	10 (125)	11 (228)	11 (217)	11 (119)	10 (794)
HPV6/11&16	3 (34)	5 (64)	5 (96)	4 (88)	4 (45)	4 (327)
HPV6/11&18	2 (21)	3 (42)	3 (58)	3 (64)	3 (36)	3 (221)
HPV16&18	4 (44)	5 (67)	6 (124)	7 (139)	6 (64)	6 (438)

[1]Total number of serum specimens that was analyzed for type HPV18 was 7,771 (14–19 years, n = 1,228; 20–22 years, n= 1,303; 23–25 years, n = 2,123; 26–28 years, n = 2,051; 29–31 years, n = 1,066).

Co-infection with low-risk and high-risk HPV types

The estimated relative risk for concomitant infections with both HPV type 6/11 and HPV type 16 in the same individuals is 3–4 times higher than HPV6/11 or HPV16 seronegative individuals (Table III). The point-estimate for the co-infection remained the same after adjusting for calendar time and HSV-2 antibodies, a surrogate of sexual risk-taking behavior. Moreover, there was no material difference in the point-estimates by age at serum withdrawal.

Co-infection with high-risk HPV types

The estimated relative risk for HPV16 and HPV18 co-infections in the same individuals is 5–8 times higher than HPV16 or HPV18 seronegative individuals (Table III). The observed co-infection was not affected by adjusting for calendar time, and adjusting for HSV-2 antibodies had only a little effect for the point estimates among the older age-group, 23–31 year olds (Table III). The older subjects had more than 1.5-times higher point estimates for the concomitant HPV16 and HPV18 seropositivity than the younger subjects. This difference remained after adjusting for calendar time or both calendar time and HSV-2 antibodies (Table III). The 95% confidence intervals of the point estimates for concomitant HPV (HPV 16 and HPV18 vs. HPV16 and HPV6/11) seropositivities did not overlap among the older subjects.

Discussion

We found silent HPV epidemics in fertile-aged women in southwest Finland. Seroprevalence of HPV16 increased from the 1980s to the 1990s, and these infections were found to spread in a very large proportion of the fertile-aged female population in the southwest part of the country. There was also considerable overlap with the appearance of the silent HPV16 epidemic in the adolescent women at the ages of 14–22 years between 1983 and 1988 (the 1961–1974 birth cohorts), and the (high to) very high seroprevalence of (HPV18 and) HPV16 at the ages of 23–31 in 1989–1994 (the 1958–1971 birth cohorts). During the present decade silent hrHPV epidemic has continued among adolescent and young Finnish women in the southernmost part of the country.[29]

Population based Finnish Maternity Cohort is the worlds largest serum bank covering the vast majority of fertile-aged female population of Finland, >98% of all pregnant women, since 1983.[19] The random sub-sample, 7,862 women, of the 230,998 Finnish women with a minimum of 2 pregnancies has previously been used for analysis of incidence trends of HPV16.[13] According to the geographic mapping system approximate mean of 1.9% of pregnant women/community were included in the current random sub-sample of the FMC. However, scarcely inhabited parts of the country (Lapland) were masked according to routine procedures.[27]

Determination of HPV6/11, 16 and 18 IgG-class antibodies was done by internationally standardized ELISAs[20,30] using high quality VLP antigens from the vaccine manufacturers. Serum IgG-antibodies are very stable, and not affected by differences in blood withdrawal, specimen handling or mailing of the serum specimens in our wide country. Following natural infection HPV IgG-antibodies are detectable in 75% of the recently infected individuals, and remain detectable in most cases for at least 5 years after the infection.[21,23] Serum IgG-antibodies to the HPV VLPs are generally considered a highly type-specific marker of cumulative exposure to the different genital HPV types, e.g. 6/11, 16, 18, 31 and 33.[20–22,30] Serological determination of cumulative exposure to the different HPV types is especially suitable for a population-based cohort study expanding over decades, and avoids performance bias characteristic for a follow-up study with multiple visits.[9]

We noted silent HPV16 epidemic, in Finnish females and to a lesser extent an HPV6/11 epidemic, spreading in time (from the 1980s to 1990s) over probable, adjacent geographic areas. It was remarkable that the silent HPV16 epidemic was not restricted to the young women in the 1980s and 1990s. It spread over time within the birth cohorts studied to new women, who became pregnant between 23 and 31 years of age in the 1990s. This fits with general increase in the sexual activity (e.g. number of intercourses/increased transmission from the partner) of the female birth cohorts' studied,[12] and efficient transmission of HPV16. Based on the FMC data, the transmission probability of HPV16 per contact has been estimated to be 0.6,[31] much higher than that for instance of HSV-2.[24,32] Our findings suggest that sexual risk-taking behavior (e.g. increased number of life-time partners, decreased age at sexual debut) indicated by a highly reliable surrogates serum HSV-2 antibodies or early age at the first pregnancy may not be an extremely strong determinant of HPV16 spread. This seems to be true in the epidemic situation of a widely spread HPV16 infection in southernmost part of Finland in the 1980s and 1990s.[13] Thus, high transmission probability and increased sexual activity, not directly sexual risk-taking behavior, may play an important role in the spreading of HPV16.

Finally, we evaluated double antibody positivity stemming from infections with HPV16 and HPV18 as compared to double antibody positivity stemming from HPV16 and HPV6/11 infections. We noted that the relative risks for cumulative exposures to HPV16 and HPV18 infections and HPV16 and HPV6/11 infections were different (the 95% confidence intervals did not overlap) in the older age-group. The double antibody positivity for HPV16 and HPV18, indicator of cumulative exposure to both viruses, increased significantly over time as the time of sexually active life increased. For the double HPV16 and HPV6/11 antibody positivity this was not observed. The sociology and biology behind these phenomena is complex. Constant change of sexual behavior, e.g., increase in sexual activity of the Finnish females, has taken place in the birth cohorts born, since 1957.[12] This might enable spreading of HPV types with high transmission probability within a birth cohort over time and space. It was seen for HPV16, but to a lesser extent or not at all for HPV6/11 and HPV18, respectively. The transmission probabilities of HPV6/11 and HPV18 may be lower than that of HPV16. Specific ability of the latter 2 types to cause persistent infections, especially of HPV18 following prior exposure with HPV16, might explain the difference.[9,33]

Our findings and those of other authors indicate that numerous hrHPV types may be prone to co-infect an individual over

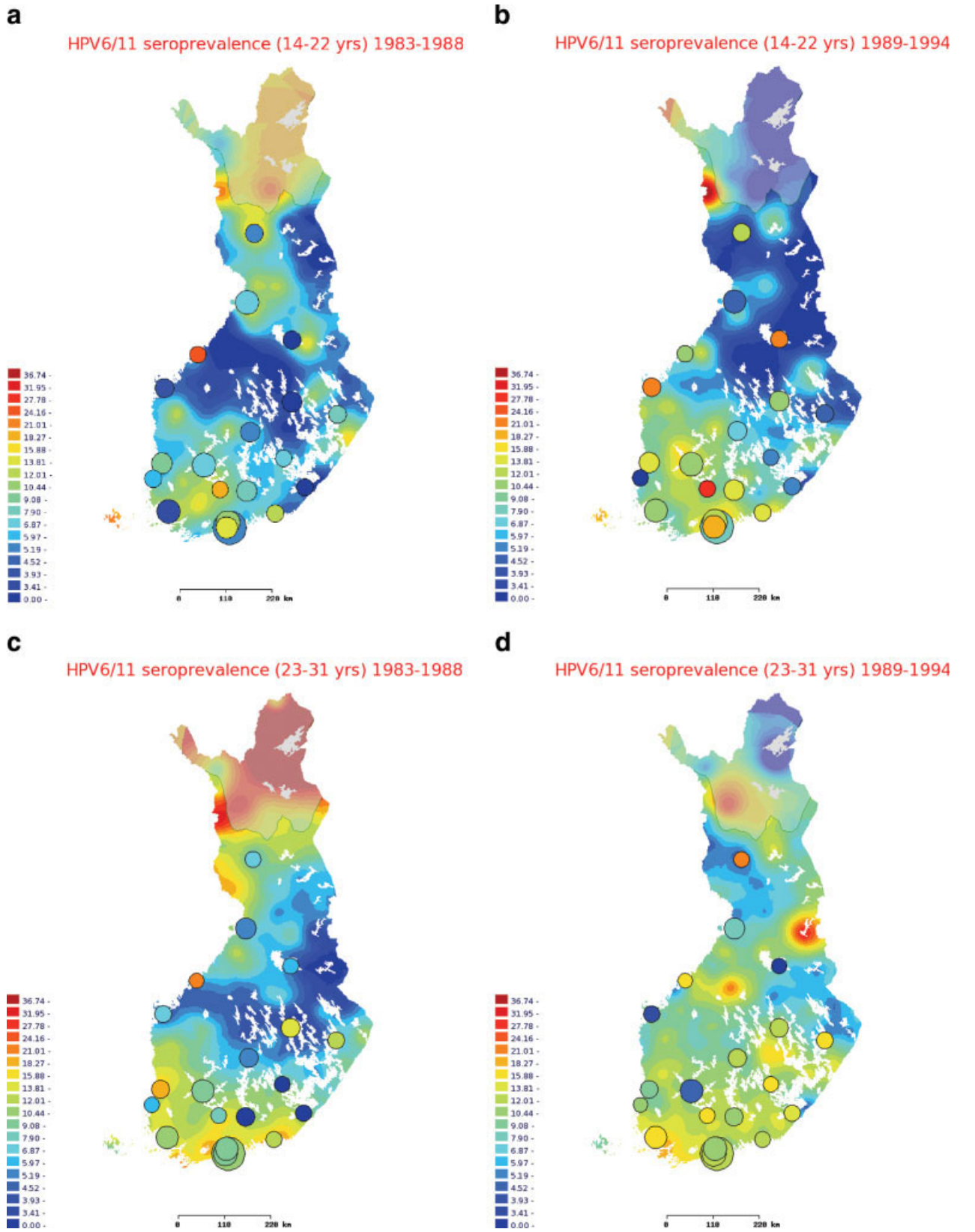

FIGURE 2 – Atlas of human papillomavirus type 6/11 (HPV6/11) seroprevalence in fertile-aged Finnish women. The HPV6/11 seroprevalences (IgG class antibodies) were determined in a random sample of first trimester sera drawn from pregnant (first pregnancy) women between the ages 14 and 22 years (*a* and *b*), and 23 and 31 years (*c* and *d*) between 1983 and 1988 and 1989 and 1994, respectively. Areas covered with hatched lines indicate the scarcely inhabited areas of Finland with less than one inhabitant per kilometer square.

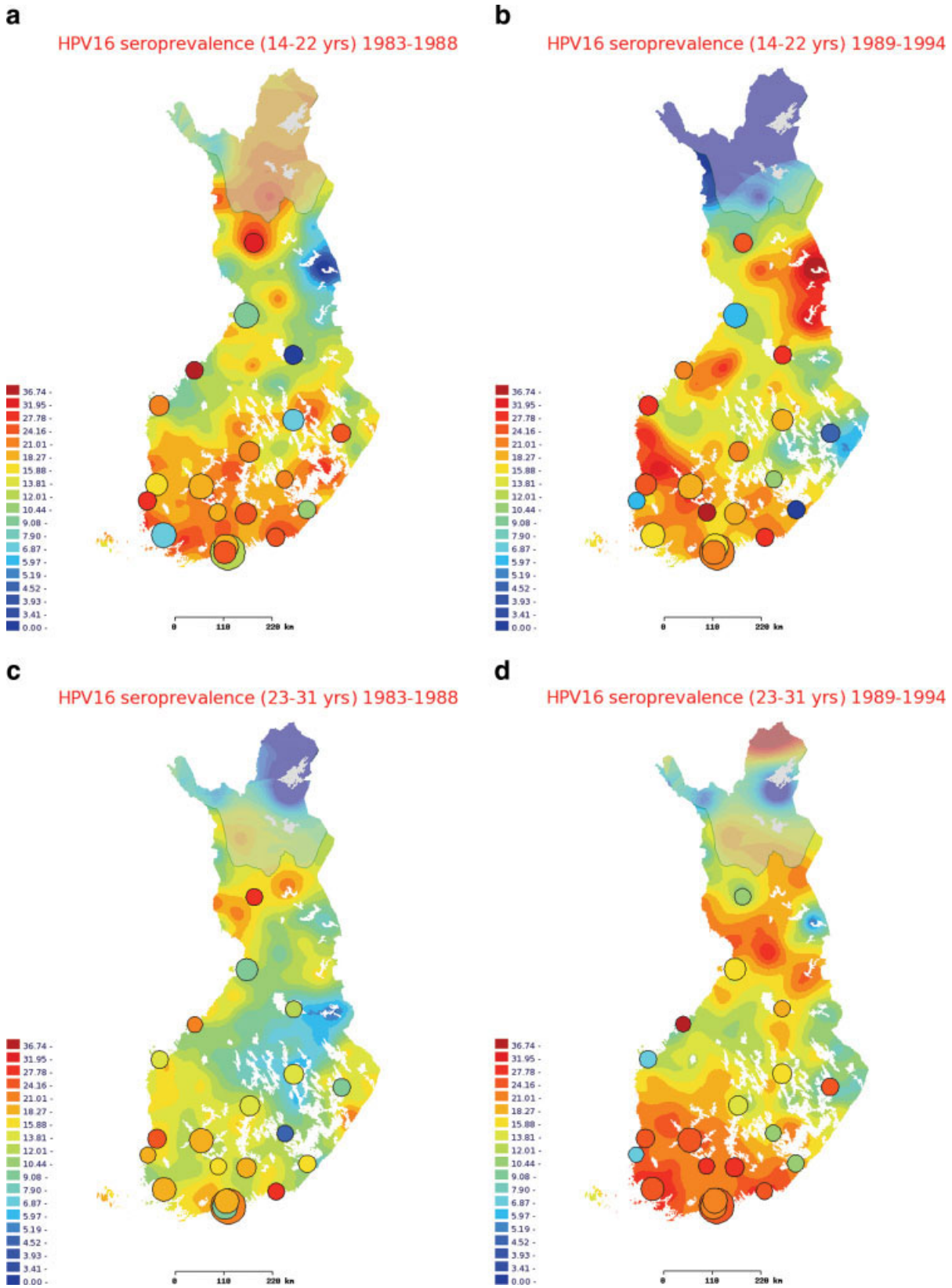

FIGURE 3 – Atlas of human papillomavirus type 16 (HPV16) seroprevalence in fertile-aged Finnish women. The HPV16 seroprevalences (IgG class antibodies) were determined in a random sample of first trimester sera drawn from pregnant (first pregnancy) women between the ages 14 and 22 years (a and b), and 23 and 31 years (c and d) in 1983–1988 and 1989–1994, respectively.

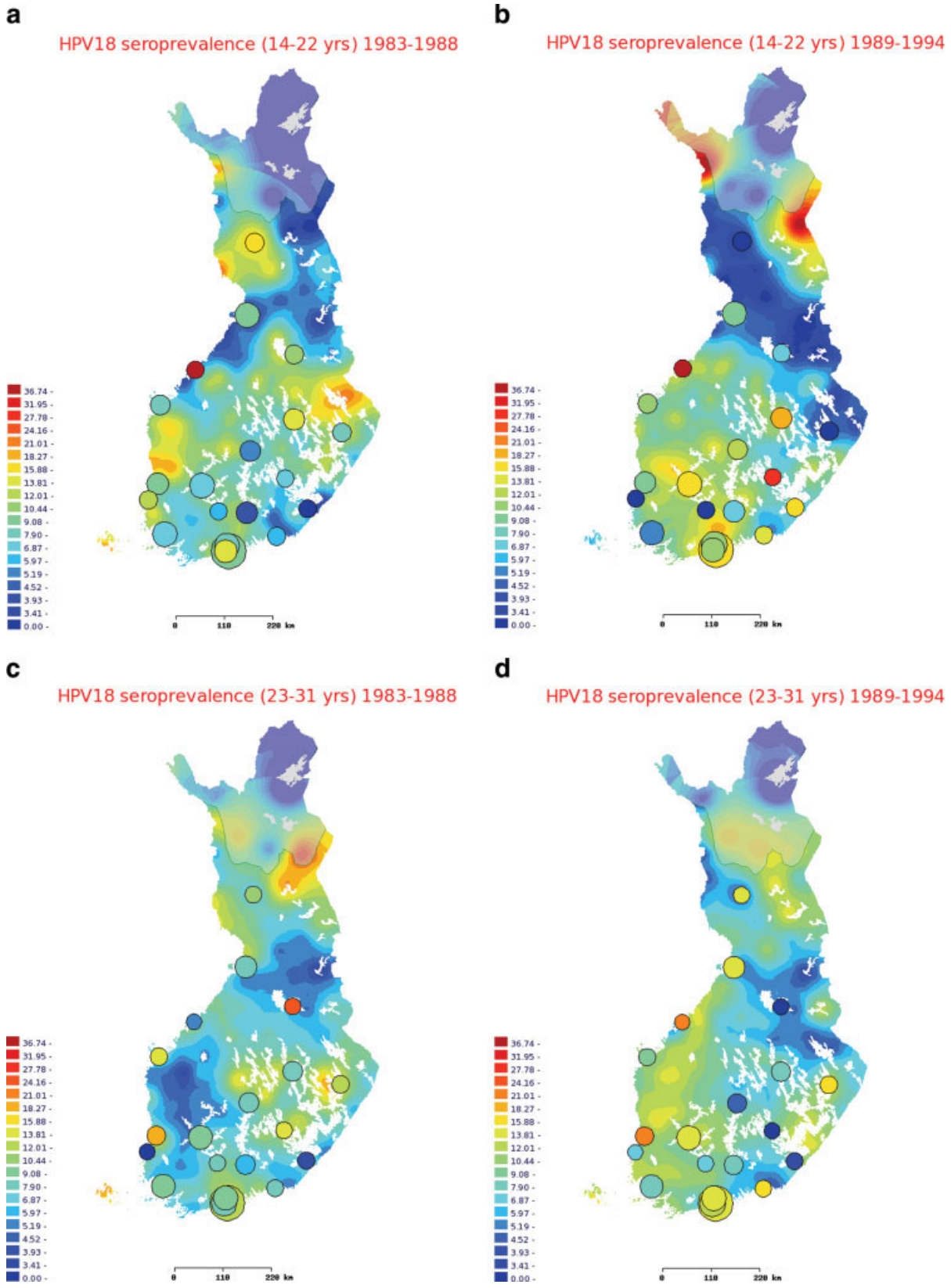

FIGURE 4 – Atlas of human papillomavirus type 18 (HPV18) seroprevalence in fertile-aged Finnish women. The HPV18 seroprevalences (IgG class antibodies) were determined in a random sample of first trimester sera drawn from pregnant (first pregnancy) women between the ages 14 and 22 years (*a* and *b*), and 23 and 31 years (*c* and *d*) in 1983–1988 and 1989–1994, respectively.

TABLE III – FREQUENCY AND RELATIVE RISK (ODDS RATIO, OR, AND 95% CONFIDENCE INTERVAL, CI) OF SEROPOSITIVITY FOR TWO HUMAN PAPILLOMAVIRUS (HPV) TYPES BY AGE IN PREGNANT FINNISH WOMEN

Category	Double HPV seropos[1] (%)	Crude OR (95% CI)	Adjusted[2] OR (95% CI)	Adjusted[3] OR (95% CI)	Adjusted[4] OR (95% CI)
HPV16&6/11					
14–22 years	98 (21%)	3.2 (2.4–4.2)	3.2 (2.4–4.2)	3.1 (2.4–4.1)	3.1 (2.4–4.1)
23–31 years	229 (24%)	3.9 (3.2–4.7)	3.9 (3.2–4.7)	3.7 (3.1–4.5)	3.7 (3.0–4.4)
HPV16&18					
14–22 years	111 (24%)	5.1 (3.9–6.8)	5.2 (3.9–6.8)	5.0 (3.7–6.6)	5.0 (3.7–6.6)
23–31 years	327 (34%)	8.9 (7.4–10.7)	8.8 (7.3–10.7)	8.0 (6.6–9.7)	8.0 (6.6–9.7)

The ORs were calculated to compare the relative risk of an HPV16 seropositive individual and an HPV16 seronegative individual to be positive for another HPV type.Serum HPV6/11/16 IgG antibodies were determined in a random sample of, 2547 14–22-year old women and 5,258 23–31-year old women. Serum HPV18 IgG antibodies were determined in the same random sample of 2,531 14–22-year old women and 5,240 23–31-year old women. The sera were withdrawn between 1983 and 1994 during the first trimester of the first available pregnancy.

[1]Number of double seropositives in HPV16 seropositives.–[2]Adjusted for calendar time.–[3]Adjusted for herpes simplex virus Type 2 antibodies.–[4]Adjusted for both calendar time and HSV2 antibodies.

time.[9,34] It is possible that susceptibility to persistent hrHPV infections is associated with the hrHPV co-infections,[35] but there may not be such common susceptibility to infections with both the hrHPV types and the lrHPV types, due to somewhat different mode of transmission of the latter.[36] Furthermore, in prospective studies hrHPV types and lrHPV types are antagonistic already at relatively early stages of cervical carcinogenesis.[10,11] Thus, interaction of, e.g., HPV16 and HPV6/11 resembles the theoretical superinfection model (by HPV16), while the interaction of, e.g., HPV16 and HPV18 may come closer to the theoretical coinfection model.[37,38]

In conclusion during wide spread epidemics hrHPVs are prone to cause co-infections in individuals with increased sexual activity. In the forthcoming HPV vaccination era new, sexually active birth cohorts form a target for long-lasting sexual health education and screening for the hrHPVs.

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