Clinical and Translational Report

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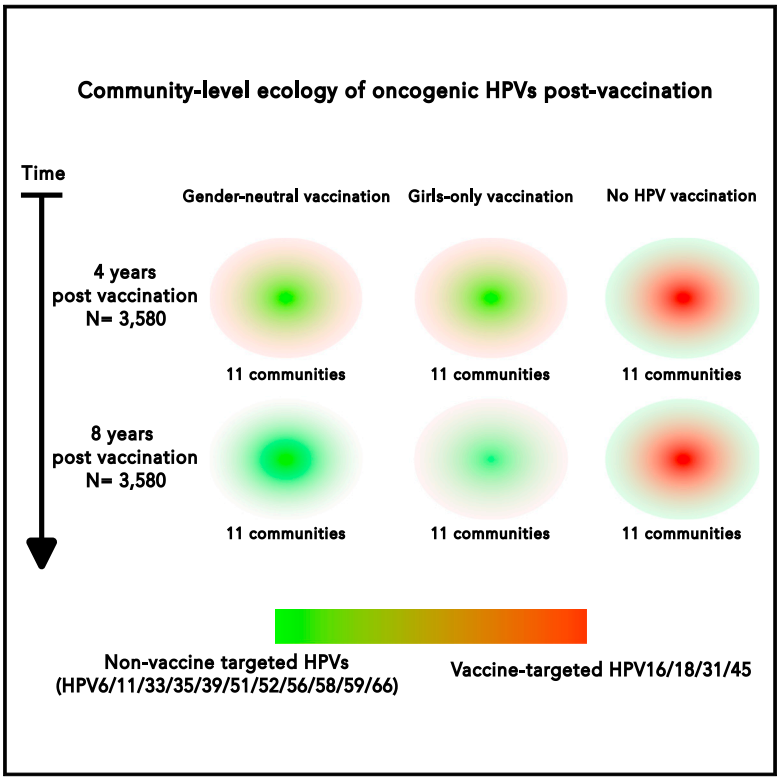
Ecological diversity profiles of non-vaccine-targeted HPVs after gender-based community vaccination efforts

Graphical abstract



Highlights

- Examination of the long-term effect of HPV vaccination on the ecology of untargeted HPVs
- Community-level depletion of vaccine-targeted HPV types occurs 4 years post-vaccination
- HPVs' ecological diversity increases 8 years post gender-neutral vaccination alone
- Observed ecological diversity increases despite the clearance of vaccine-targeted HPVs

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In brief

Depletion of oncogenic HPV types 16/18/31/45 through population-level vaccination is effective, but the niche occupation by the non-vaccine-targeted HPVs is poorly comprehended. Pimenoff et al. employ an ecological niche approach using community-randomized HPV vaccination data, which show a significantly rising ecological diversity of low oncogenic HPVs in gender-neutral vaccination communities 8 years post-vaccination.

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Ecological diversity profiles of non-vaccine-targeted HPVs after gender-based community vaccination efforts

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SUMMARY

The long-term effect of population-level human papillomavirus (HPV) vaccination on the viral ecology of the untargeted HPVs is poorly understood. We performed an 8-year follow-up of 33 communities randomized to gender-neutral HPV16/18 vaccination, girls-only HPV16/18 vaccination, and control communities without HPV vaccination. The 1992/93 and 1994 birth cohorts were invited in school years 2007/8 and 2008/9. Follow-up cervico-vaginal sampling at 18 and 22 years of age, 4 and 8 years post-vaccination, respectively, were attended by 11,396 and 5,602 participants. HPV types 6/11/16/18/31/33/35/39/45/51/52/56/58/59/66/68 were genotyped and used for the community-level ecological diversity estimations. Gender-neutral vaccination communities with a stronger herd immunity than girls-only vaccination communities show a significantly increased HPV α-diversity ($p = 1.1 \times 10^{-8}$) from 4 to 8 years post-vaccination, despite the clearance of the vaccine-targeted HPVs in these communities. This likely sign of niche occupation by the non-vaccine-targeted HPVs will potentially affect the future cervical cancer screening programs but should not interfere with the WHO mission to eliminate cervical cancer.

INTRODUCTION

Current comprehensive human papillomavirus (HPV) vaccine implementation is essentially changing the ecological conditions of this virus-host interaction worldwide.[1] Notable, albeit variable proportions of adolescent and early adult birth cohorts now possess a strongly protective and sustainable vaccine-induced immunity against a number of prevalent HPV genotypes.[2–5] Furthermore, differential herd effects from girls-only and gender-neutral vaccination will with different effectiveness prevent infections also in unvaccinated adolescent and young adult women and men.[6–9] This new era in the persistent HPV infection and host population interplay warrants close surveillance of vaccine-induced evolutionary responses such as type replacement in HPV ecology.[10,11] Observations from HPV vaccination programs[12,13] suggest changes in HPV type distribution in cervical infections and pre-cancer but this has not been systematically confirmed nor tested at ecological diversity level in randomized implementation studies.

Over time changes in ecological opportunities play a major role in infectious agent evolution.[14–16] Major drivers that define these pathogen population dynamics are specific characteristics of the host environment, e.g., vaccine-induced niche availability for particular remaining infections and pathogen antigenic variation often driven by diversifying selection of the host immune system.[15,17] One important factor here is that the repertoire of available statistical methods for estimating infectious agent ecology and evolution and documenting its effects on human health is extensive.[14,18] To add, we applied a graphical independence network (GIN) method to generate simulated synthetic data from observed HPVs infections up to 8 years post-vaccination for further dissemination of HPVs ecological structure dynamics.[19]

Here, we have examined community-level occurrence of genital HPV types up to 8 years post moderate (up to 50%) coverage girls-only or gender-neutral vaccination beyond pairwise HPVs comparison using generalized linear model (GLM) framework and ecological diversity indices. Simulation of synthetic data from the observed girls-only- or gender-neutral-community-randomized vaccination trial data was done as a sensitivity analysis. We also evaluate how our ecological approach reveals the altered stage for the combination of HPV vaccination and screening.[20–24] Here, the possible risk is that when the prevalence of high-oncogenicity HPV types is abruptly altered by the vaccination, the positive predictive value of current screening test fails to distinguish

Table 1. HPV type community prevalence differences between HPV vaccination trial arms

				HPVs community prevalence between arms			
				At community level[b]		At HPV type level[c]	
Vaccine arm comparison[a]	N	Time since vaccination	Birth cohort	LTR	p value	Vaccine types	Non-vaccine types
A-B	2,767	4 years (18 years old)	1992/1993/1994	12.1	0.656	Ns	Ns
A-C	2,250			142.1	0.001	16[d]/18[d]/31[d]/45[d]	Ns
B-C	2,143			158.6	0.001	16[d]/18[d]/31[d]/45[d]	Ns
A-B	2,005	4 years (18 years old)	1993/1994	11.8	0.68	Ns	Ns
A-C	1,518			111.3	0.001	16[d]/18[d]/31[d]/45[d]	Ns
B-C	1,455			108.4	0.001	16[d]/18[d]/31[d]/45[d]	Ns
A-B	2,767	8 years (22 years old)	1992/1993/1994	29.3	0.027	Ns	52[e]
A-C	2,250			172.7	0.001	16[d]/18[d]/31[d]/45[d]	52[d]/66[d]
B-C	2,143			182.8	0.001	16[d]/18 [d]/31[d]/45[e]	Ns
A-B	2,005	8 years (22 years old)	1993/1994	31.8	0.008	Ns	52[e]
A-C	1,518			111.6	0.001	16[d]/18[d]/31[d]/45[d]	66[e]
B-C	1,455			110.2	0.001	16[d]/18[d]/31[d]	Ns
A-B	984	8 years (22 years old)	1994	11.6	0.751	Ns	Ns
A-C	655			63.0	0.001	16[d]/18[d]/45[e]	Ns
B-C	631			61.9	0.001	16[d]/18[d]	Ns

Generalized linear modeling of the cohort data of the 18-year-old gender-neutral arm A, girls-only arm B, and control arm C communities of the women who participated in the follow-up both at the ages of 18 and 22 (n = 3,580). Ns, non-significant results.

[a]A-B indicates estimation of HPV's community prevalence differences between eleven gender-neutral arm A and eleven girls-only arm B communities; A-C indicates estimation of HPV's community prevalence differences between eleven gender-neutral arm A and eleven control arm C communities; B-C indicates estimation of HPV's community prevalence differences between eleven girls-only arm B and eleven control arm C communities. A note of caution here is that we compared the 22-year-olds group A and group B HPV-type community prevalence to the 18-year-old group C HPV type community prevalence and not the 22-year-old group C, as group C had already as 18-year-olds received the HPV cross-vaccinations (See more details in the methods section).

[b]HPV types 6,11,16,18,31,33,35,39,45,51,52,56,58,59,66 were analyzed in all the multivariate community prevalence comparisons with the likelihood ratio test (LRT).[25]

[c]Individual HPV type level differences were estimated as univariate differences within the multivariate HPVs community structure.[25] See also Data S1.

[d]$p < 0.05$: HPV types showing significant difference in community prevalence.

[e]$p < 0.1$: HPV types showing marginally significant difference in community prevalence.

between true and false positive pre-cancer cases.[20] Understanding how the newly established ecological diversity distribution of the remaining HPV types is formed following different vaccination strategies is pivotal in this context and will set the stage for improved screening strategies for cervical cancer.

RESULTS

Elimination of high-oncogenicity HPVs by vaccination strategy

Four years post-HPV vaccination, a comparison of HPV type community prevalence distribution using GLM did not reveal significant differences between the gender-neutral arm A and girls-only arm B communities at the community or type level (arm A and B, Table 1). This remained true even after exclusively selecting the 1993–1994 birth cohorts for the strongest difference in strength for herd effect between arm A and arm B communities from available data while ensuring a reasonable sample size ($n < 2,000$) for the GLM analysis between the arm A and arm B communities (Table 1). Nonetheless, 4 years post-vaccination, a significant depletion of high-oncogenicity vaccine-targeted HPV genotypes 16/18/31/45 was observed for both gender-neutral communities (arms A–C, likelihood ratio test [LRT] = 142.1, p = 0.001, Table 1) and for girls-only communities as compared with control communities (arms B and C, LRT = 158.6, p = 0.001, Table 1) for the full cohort and within the 1993–1994 birth cohorts.

Eight years post-HPV vaccination, we found comparable depletions of the high-oncogenicity vaccine-targeted HPV types 16/18/31/45 in the gender-neutral communities (arms A–C, LRT = 172.7, p = 0.001, Table 1) and types 16/18/31 in the girls-only (arms B and C, LRT 182.8, p = 0.001, Table 1) communities compared with the control communities. Significant depletion of HPV type 45 after vaccination was observed only for the gender-neutral arm A communities both for the full cohort and within the 1993–1994 birth cohorts.

Resurgence of lower-oncogenicity HPVs by vaccination strategy

Eight years post-HPV vaccination and apart from the vaccine-targeted HPV depletion, we found a significant increase of non-vaccine-targeted HPV types 52 and 66 exclusively among the gender-neutral communities compared with the control communities (arms A–C, LTR = 172.7, p = 0.001, Table 1).

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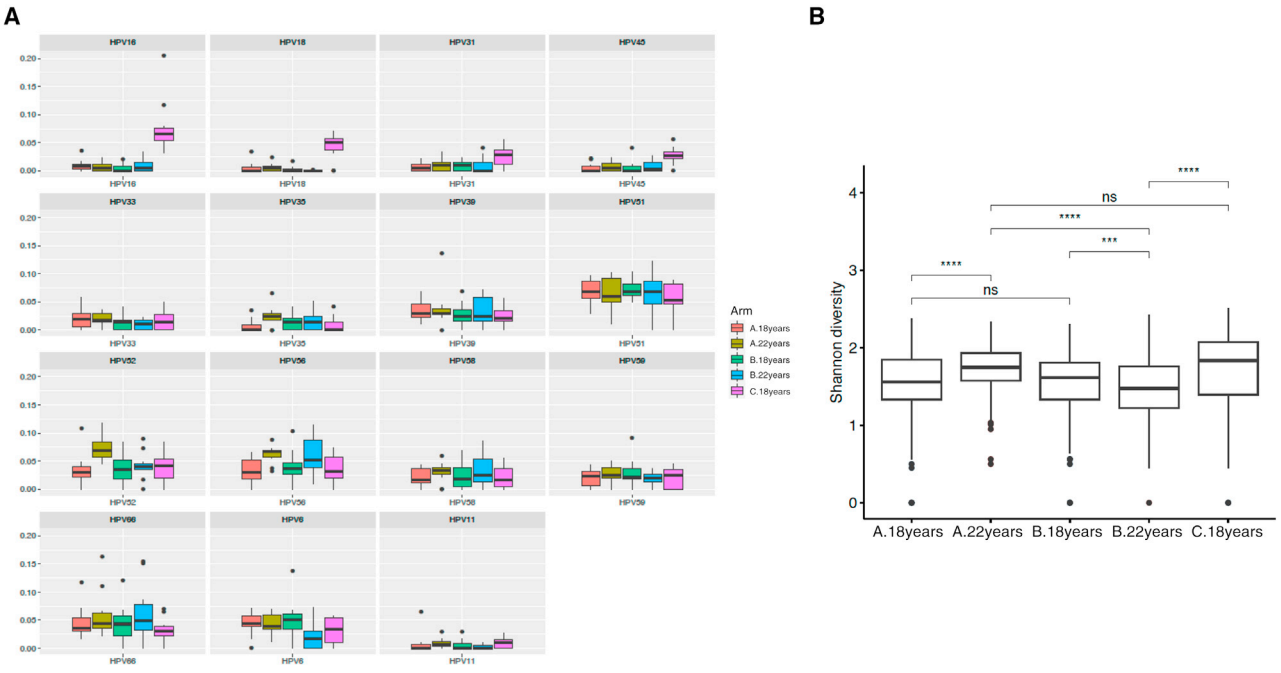

Figure 1. HPV type community prevalence and α-diversity distribution

(A) HPV type-level community prevalence estimated among the 18 and 22 year olds (N = 3,580).

(B) Shannon diversity distribution estimated for the 1992–1994 birth cohorts within each randomized trial arm community and adjusted for equal community size across the same individuals as 18 and 22 year olds. Each arm consisted of eleven communities rarefied without replacement and randomly re-sampled 25 times for the equal community size of the smallest community sample (N = 24).

A.18years, arm A 18 year olds; A.22years, arm A 22 year olds; B.18years, arm B 18 year olds; B.22years, arm B 22 year olds; and C.18years, arm C 18 year olds. p value after FDR correction (****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; ns, non-significant). Note that group C 22 year olds is not used in the comparisons due to the HPV cross-vaccination for group C as 18 year olds (see the details in the methods section).

Moreover, in the overall comparison between the gender-neutral and girls-only communities 8 years after HPV vaccination, the HPV prevalence distribution was significantly different (arms A and B, LRT = 29.34, p = 0.027, Table 1). This difference was particularly driven by the increase of HPV type 52 among the gender-neutral compared with girls-only communities. This increase of HPV 52 contributing to the significant difference between gender-neutral (arm A) and girls-only (arm B) vaccination communities was consistent also among the 1993–1994 birth cohorts with maximized herd effect and ample sample size (N > 2,000) for the estimation (arms A and B, LRT = 31.78, p = 0.008, Table 1).

To further assess the ecological changes in HPVs community prevalence following gender-neutral (arm A) or girls-only (arm B) HPV vaccination vs. control vaccination (arm C), we estimated the HPV type α- (Figure 1) and β-diversity (Figure 2) measures using the available prevalence distribution data of the 15 HPV types by trial arm communities for the 18 year olds (N = 11,396) or among the 18 year olds with also a follow-up at the age of 22 years (N = 3,580).

We found that 18 year olds in the control communities had the highest HPV types α-diversity while the HPV-vaccinated 18 year olds in the gender-neutral and girls-only communities showed significantly lower α-diversity indices (Figure 1B). Significant increase in the α-diversity (false discovery rate [FDR]-corrected $p = 1.1 \times 10^{-8}$) from the same 18- to 22-year-old ones, 4 to 8 years post-vaccination was observed only in the gender-neutral communities. Also, among the 22 year olds, the gender-neutral communities had a significantly higher α-diversity (FDR-corrected $p = 5.0 \times 10^{-17}$) compared with girls-only communities (Figure 1B). This HPV types α-diversity distribution eight years post-vaccination among the gender-neutral communities reached levels observed among the control communities (Figure 1B). The results were sustained also with different community size adjustments or using Simpson's diversity index (Data S2 and S3).

To further estimate differences in HPV type community-level prevalence distribution between gender-neutral and girls-only vs. control communities, β-diversity clustering was analyzed over time (Figure 2). Twenty-six percent differentiation along the first principal component (x axis) for the full cohort data among the 18 year olds (Figure 2A) was mostly driven by the apparent prevalence depletion of the vaccine-targeted HPV16/18/31/45 types. Seventeen percent differentiation along the second principal component (y axis) between communities was mostly driven by the association of HPV39/51/52/56/58/59/66 both in the gender-neutral (A) and girls-only (B) vaccination communities and the clustering of HPV16/18/31/35/45 types in the control arm (C) communities (Figure 2A). The HPV39/51/56/58/59/66 types associated consistently to the intervention arm communities both in the full 18-year-old cohort data (Figure 2A, N = 11,396) and among the 18 year

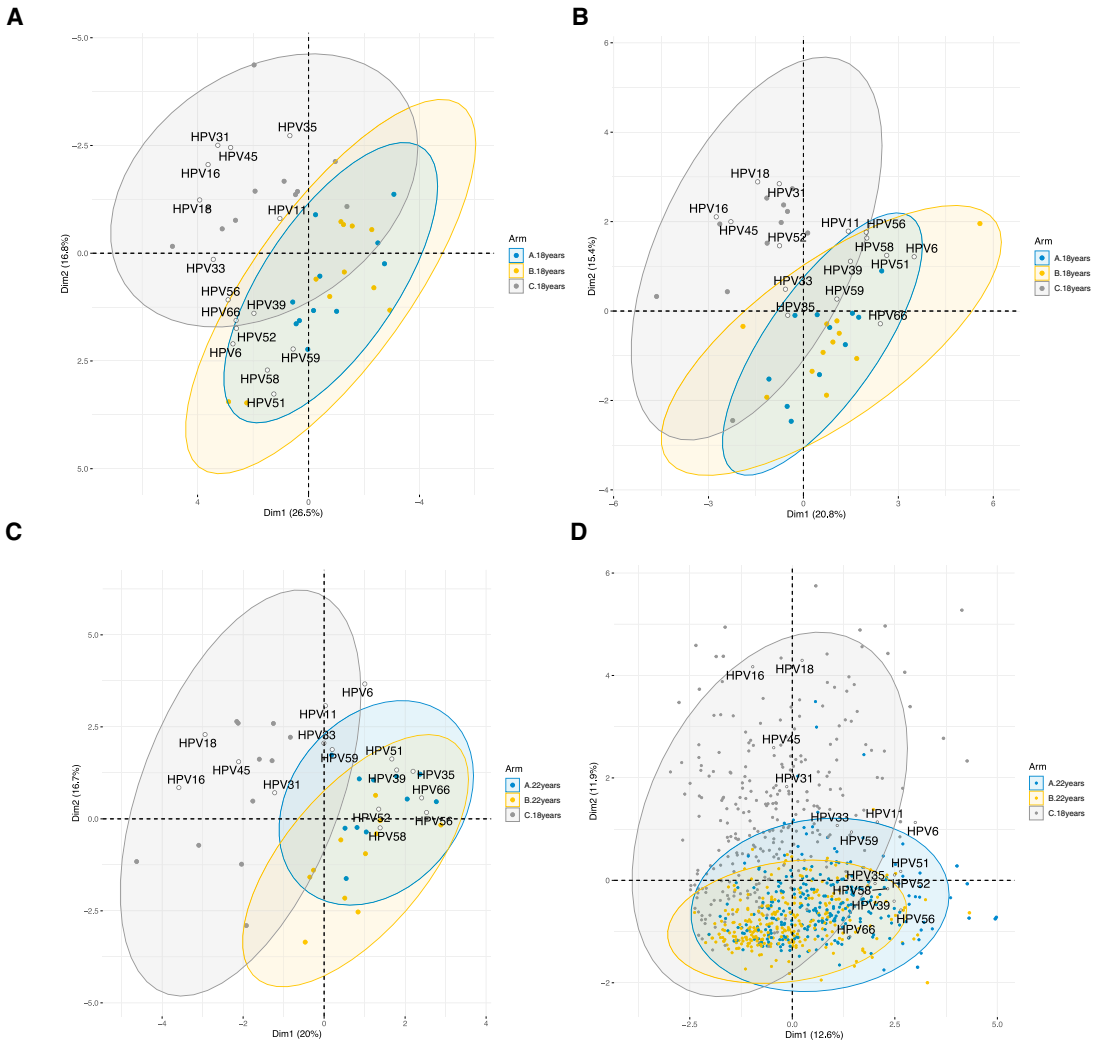

Figure 2. Community-level HPV types β-diversity distribution across 33 Finnish randomized trial communities

Difference in β-diversity estimated (A) among the full cohort of 18 year olds (N = 11,396), (B) among the 18 year olds with follow-up at the age of 22 years (N = 3,580), (C) among the cohort of 22 year olds in arm A and arm B compared with 18 year olds in control arm C (N = 3,580), and (D) among the 22 year olds in arm A and arm B compared with 18 year olds in control arm C (N = 3,580) rarefied and re-sampled 25 times without replacement. White dots represent HPV type prevalence in two dimensions of the ordination plot with the blue (A), yellow (B), and gray (C) dots representing each of the 11 communities (or the re-sampled communities) in each trial arm. The elliptic circles represent the overall diversity among the gender-neutral (A) or girls-only (B) HPV-vaccinated and control (C) communities, respectively. Significant difference between the three arms was observed ($p < 0.001$) using both Bray-Curtis and Jaccard distances in (A)–(D). Note that group C 22 year olds is not used in (C) and (D) comparisons due to the HPV cross-vaccination for group C as 18 year olds (see the details in the methods section).

olds who also participated the follow-up at the age 22 (Figure 2B, N = 3,580).

Among the 22 year olds, the gender-neutral (A) and girls-only (B) vaccination communities clustered separately (20% differentiation in the x axis) from the control (C) communities mostly due to depletion of high-oncogenicity vaccine-targeted HPV types 16/18/31/45 and an association of the non-vaccine-targeted HPV types 35/51/52/56/58/59/66 (Figure 2C).

Furthermore, a differential clustering driven by the lower-oncogenicity, non-vaccine-targeted HPV types 35/39/51/52/56/58/59/66 was observed. This clustering occurred not only between the intervention and control communities but also between the gender-neutral and girls-only communities among the 22 year olds (y axis in Figure 2C), and it sustained significance even after repeatedly rarefying to equalize the community size of the smallest community sample (x axis in Figure 2D). Sensitivity testing for a variety of equal size community parameters also supported these results (Data S4).

Simulation of HPV community prevalence distribution by vaccination strategy

Finally, to comprehensively evaluate the community-level HPV type diversity distribution between the trial arms, we applied GIN modeling[19] for the observed HPV type prevalence data and generated a synthetic dataset from each original community-randomized trial arm. First, the GIN model was fitted to

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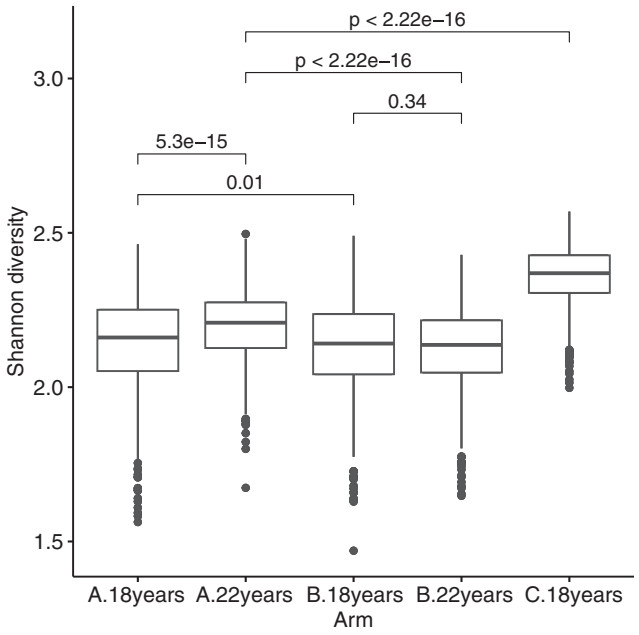

Figure 3. Synthetic community-level α-diversity distribution

Shannon diversity index assessed for the rarefied 1,000 synthetic communities estimated for each gender-neutral, girls-only, and the control communities with equal community size (N = 100) among the 18 and 22 year olds. A.18years, arm A 18 year olds; A.22years, arm A 22 year olds; B.18years, arm B 18 year olds; B.22years, arm B 22 year olds; and C.18years, arm C 18 year olds.

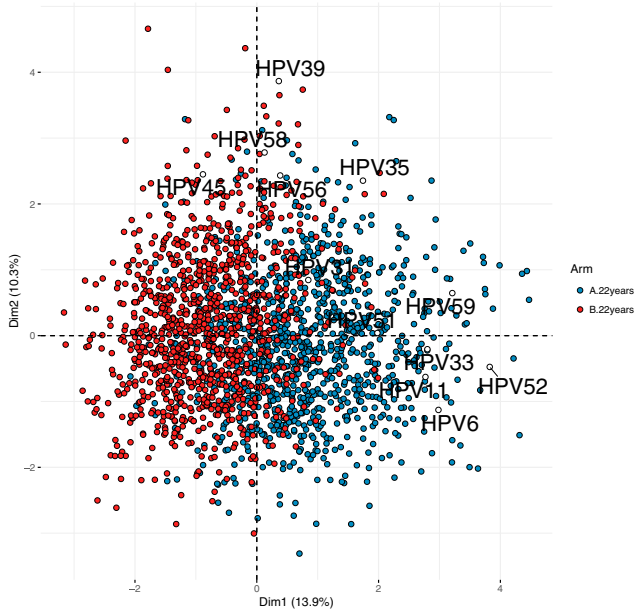

Figure 4. Community-level HPV type β-diversity distribution using synthetic data

β-diversity estimated for the synthetic 22 year olds in gender-neutral arm A and girls-only arm B communities (1,000 communities with N = 100 for each trial arm) and visualizing the significant ($p < 0.001$) differential clustering between the arms. A, arm A; B, arm B; 22years, 22 year olds. Significant difference between the two arms was observed ($p < 0.001$) using both Bray-Curtis and Jaccard distances.

each observed trial arm HPV type distribution over time, from which a synthetic HPV type prevalence distribution for 100,000 individuals was simulated for each arm and time point. For community-level α- and β-diversity estimation, synthetic HPV type prevalence distribution for each trial arm data (N = 100,000) was randomly re-sampled 1,000 times without replacement for equal (N = 100) community size (Data S5). Community-level HPV type α-diversity was estimated using the re-sampled synthetic communities for 18 and 22 year olds (Figure 3). A significant increase in α-diversity (Shannon index, $p = 5.3e{-}15$) was observed from the 18 to 22 year olds in the synthetic gender-neutral communities. In contrast, a significantly lower α-diversity (Shannon index, $p = 2.2e{-}16$) was sustained in the synthetic girls-only communities among the 22 year olds.

Community-level differences in HPV type distribution were further evaluated by estimating β-diversity measures (excluding HPV16/18) for the synthetic trial arm data (Figure 4). A significant differential clustering ($p < 0.001$) between synthetic gender-neutral and girls-only vaccination communities was observed among the 22 year olds as demonstrated by the 13.9% differentiation observed along the first principal (x axis) component (Figure 4). This differentiation was driven by no-oncogenicity HPV types 6 and 11 and lower-oncogenicity HPV types 33 35, 51, 52, and 59 (Figure 4).

DISCUSSION

In this study, we comprehensively evaluate long-term effect of community-randomized gender-neutral and girls-only HPV vaccination on the ecology of the remaining oncogenic HPVs among young adult women vaccinated as early adolescents at population level. We observed a significant depletion of high-oncogenicity vaccine-targeted HPV types 16/18/31/45 in gender-neutral and girls-only vaccination communities 4 years post-vaccination, but this depletion was consistent 8 years post-vaccination only among gender-neutral vaccination communities. Our most important finding, however, was the significantly increased ($P_{FDR} = 1.1 \times 10^{-8}$) oncogenic HPVs ecological diversity from 4 to 8 years post-vaccination exclusively in gender-neutral vaccination communities despite the clearance of the vaccine-targeted types in these communities (Figure 1B, see also Video S1). This rising ecological diversity of the oncogenic HPVs in gender-neutral vaccination communities with a stronger herd immunity compared with girls-only vaccination communities is likely the first recorded sign of ecological niche occupation by the non-vaccine-targeted HPV post-population-level vaccination (Video S2).

HPV vaccination has changed the ecological conditions of this virus-human host interaction. Depending on the vaccine used, the community-level vaccination coverage, and the implemented strategy, the unvaccinated females and males will be protected by strong, vague, or no herd effect against a variable number of oncogenic HPV types. The imminent risk of viral evolutionary responses to diminish the impact of HPV vaccination may introduce problems. Such vaccine-induced evolutionary responses have been observed for a list of viruses, e.g., hepatitis B virus[26] and severe acute respiratory syndrome

coronavirus 2 (SARS-CoV-2),[27] and also predicted for HPVs.[28] The rationale is that the vaccine-induced immunity pivotally reduces the number of susceptible hosts for the viral strains targeted by the vaccine.[1] Differential sustained immune recognition of and response against specific virus types subsequently favor the viral strains not targeted by the vaccines or selection of certain immune escape mutants.[1,17,29,30] Concomitant reduction in the number of susceptible/naive new hosts and rapid increase of immune individuals by gender-neutral HPV vaccination may increase the likelihood of HPV type replacement.

Ecological theory recognizes the definition of an ecological niche, which refers to the specific role and position of pathogen species and their strains within an ecosystem.[31] With a notable antigenic variability, each pathogen species strain has a unique set of host adaptation mechanisms that allow it to interact with its host environment and occupy a particular ecological niche.[17] Antigenic diversity of pathogen strains stems from multiple ecological and evolutionary mechanisms of host-pathogen interaction, often particularly from diversifying selection by the host immune system.[17]

In the case of five alpha-papillomavirus species studied here and encompassing the sixteen oncogenic HPV strains (i.e., HPV types 6/11/16/18/31/33/35/39/45/51/52/56/58/59/66/68), we estimate that there is little antigenic variation within a single host but a notable standing phylogenetic diversity for the viral population. In this HPV strain diversity landscape, we showed the clearance of the ecological niche for the vaccine-targeted HPV types 16 and 31 (both belonging to alpha-9 species) and HPV types 18 and 45 (both belonging to alpha-7 species) already 4 years post-vaccination. Furthermore, we suggest that the change in the ecological niche for alpha-9 and alpha-7 papillomavirus species 8 years post-gender-neutral vaccination may have provoked sufficient niche availability and escape from the vaccine-induced selection pressures for the occupation for the phylogenetically alpha-9 species-related types 33/35/52/58 and types 56/66 (i.e., alpha-6 species types) phylogenetically close to alpha-7 species, respectively. This may be the earliest sign of ecological niche occupation by the non-vaccine-targeted HPVs post population-level HPV vaccination now observed in our study.

Altogether, up to 15 years of national HPV vaccination implementation warrants a thorough examination for signs of evolutionary response of non-vaccine-targeted HPV types, i.e., type replacement, in the established HPVs ecological niche.[9,12,13,23,24]

We recently addressed vaccine-targeted HPV eradication particularly within 1994 birth cohort with a rapid clearance of high-oncogenicity vaccine-targeted HPV types under gender-neutral intervention[9] and subsequent HPV type replacement[5,10] in a community-randomized trial implemented in HPV-vaccine-naive population. We reported the decrease of vaccine-targeted HPVs at individual level up to 12 years after vaccine implementation and prevalence fluctuation of certain non-vaccine-targeted oncogenic HPVs (i.e., HPV types 39/51/66/68/73). However, no consistent type-specific epidemiological evidence was observed for type replacement either using HPV DNA[10,32] or serology.[11,23] Subsequently, our previous modeling study suggested that the follow-up time up to 12 years in epidemiological studies may be too short to detect type replacement.[33]

Here, we present data on significant changes in the overall ecological diversity of circulating oncogenic vaccine-targeted and non-targeted HPVs in gender-neutral vaccinated communities as the earliest signs of niche occupation by sustaining HPVs. Comprehensive evaluation of the ecological diversity of HPVs—particularly the lower-oncogenicity non-vaccine-targeted HPV types—using community-randomized HPV vaccine trial data, extensive diversity inference and modeling are consistent. We observed significant changes in oncogenic HPV type diversity distribution within and between communities 4–8 years post-vaccination.

We found that at the age of 22, 8 years post-vaccination, the gender-neutral vaccination communities showed more sustained clearance of all the high-oncogenicity vaccine-targeted or cross-protected HPV types (particularly HPV45, see Table 1), a significant increase of α-diversity ($p < 0.00001$) and also a significant deviation in β-diversity for the sustaining HPV types compared with the girls-only vaccination communities (Figure 1B). An important note here is that to systematically minimize the possible sampling effect in the diversity estimations as demonstrated by Cameron et al.,[34] we repeatedly rarefied without replacement the equal community size for each of the eleven communities in each trial arm. Despite the sustained clearance of vaccine-targeted HPV types, the gender-neutral vaccination communities returned between 4 and 8 years post-vaccination to the similar level of HPV types α-diversity as observed for the control communities. Importantly, our findings of these profound changes in ecological diversity of the HPVs are in agreement with previous findings that the overall 26%–27% prevalence of oncogenic HPVs is sustained in a population regardless of the vaccination strategy.[22] This rebounced total prevalence and ecological diversity of oncogenic HPVs post-vaccination also suggest sustained diversifying evolutionary forces affecting the remaining HPV strains. However, the long-term effects of the HPV vaccination on the antigenic variation and possible virulence shifts of the remaining oncogenic HPVs are not known.

To further test the robustness of our findings, we modeled a large synthetic population of 1,000 communities with 100 individuals in each for gender-neutral, girls-only, and control community setting from the observed trial arm data. Our synthetic data analyses sustained the original findings of significant ecological shift in non-vaccine-targeted HPV type diversity distribution 8 years post-vaccination and the clearance of the vaccine-targeted HPV types.

Most notably, our extensive synthetic data sustained the original result on the increased ecological diversity of HPV types not targeted by the bivalent HPV vaccine exclusively in the gender-neutral vaccination communities (Figures 1 and 3). We suggest that our ecological diversity inference analyses and simulations of the community-randomized trial HPV type data may have, in the gender-neutral vaccination arm, provided the glimpse of what the future might be after the eradication of the HPV-vaccine-targeted types. Our previous modeling already showed that very-high-coverage girls-only HPV vaccination programs would eventually also lead to HPV eradication.[9] However, it would probably take much longer time until any viral strain replacement could be observed following girls-only vaccination.

Despite of the community-randomized controlled trial design being the optimal setup to estimate viral ecological responses at community-level post-vaccination in a systematic way,[1,35] our study suffers from some limitations. Indeed, sampling

bias such as community size heterogeneity and heterogeneity in baseline risk may have affected our empirical observations. However, we rarefied the observational data for equal community size and extensively tested these parameters with resampling (Figure 2D) and modeling the observed data (Data S3). Moreover, the synthetic datasets were used to perform sensitivity test for original findings. No differences in risk-taking sexual behavior between the intervention and control communities were observed using individual *Chlamydia trachomatis* screening data available for the cohort.

Conclusions

Previously, we modeled from observed HPV vaccine trial data and showed the need to opt for gender-neutral HPV vaccination strategies to realistically meet the WHO-targeted eradication of the oncogenic HPVs.[9,36,37] To control oncogenic HPVs and related cancers, we need to understand the evolutionary dynamics of these oncoviruses especially under long-term vaccine-induced selective pressure. Here, we estimate that low-to-moderate coverage HPV vaccination causes between 24.5%–36.7% change in the genital HPV type distribution (Figures 2C and 2D). In HPV vaccination communities, the change mostly stems from a decrease in the occurrence of high-oncogenicity vaccine-targeted HPV types, but particularly when implemented gender-neutral also a 12.6%–20% shift of low oncogenicity not vaccine-targeted HPV types is observed. While eradication of HPV16/18/31/45 will eliminate most of cervical cancers, it is tempting to suggest that an increase of HPV33/35/51/52/56/66[38] or the like with increased virulence might cause a risk of HPV-related cancers in the future.[25] Taken together, understanding the differences in the transmissibility, antigenic variation and oncogenic potential of the remaining HPV types in the newly established ecological niche following different vaccination strategies creates new basis for future cancer screening of HPV-vaccines and the herd effect protected non-HPV-vaccinated women and men.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Study subjects and HPV genotyping
- METHODS DETAILS
  - Data analysis, simulations and statistics
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.chom.2023.10.001.

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AUTHOR CONTRIBUTIONS

Conceptualization, V.N.P. and M.L.; methodology, software, and analysis, V.N.P.; resources, V.N.P., K.L., J.D., and M.L.; sample processing, T.E., P.G., C.L., and A.S.-S.; writing – original draft, V.N.P. and M.L.; writing – review & editing, V.N.P., P.G., K.L., C.L., A.S.-S., J.D., and M.L.; funding acquisition, V.N.P., M.L., and J.D.; visualization, V.N.P.

DECLARATION OF INTERESTS

V.N.P. is also affiliated to Aqsens Health Ltd but not related to this work.

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REFERENCES

1. McLean, A.R. (1995). Vaccination, evolution and changes in the efficacy of vaccines: a theoretical framework. Proc. Biol. Sci. *261*, 389–393. https://doi.org/10.1098/rspb.1995.0164.
2. Artemchuk, H., Eriksson, T., Poljak, M., Surcel, H.M., Dillner, J., Lehtinen, M., and Faust, H. (2019). Long-term antibody response to human papillomavirus vaccines: up to 12 years of follow-up in the Finnish maternity cohort. J. Infect. Dis. *219*, 582–589. https://doi.org/10.1093/infdis/jiy545.
3. Kjaer, S.K., Nygård, M., Sundström, K., Dillner, J., Tryggvadottir, L., Munk, C., Berger, S., Enerly, E., Hortlund, M., Ágústsson, Á.I., et al. (2020). Final analysis of a 14-year long-term follow-up study of the effectiveness and immunogenicity of the quadrivalent human papillomavirus vaccine in women from four Nordic countries. EClinicalMedicine *23*, 100401. https://doi.org/10.1016/j.eclinm.2020.100401.
4. Kann, H., Lehtinen, M., Eriksson, T., Surcel, H.M., Dillner, J., and Faust, H. (2021). Sustained cross-reactive antibody responses after human papillomavirus vaccinations: up to 12 years follow-up in the Finnish maternity cohort. J. Infect. Dis. *223*, 1992–2000. https://doi.org/10.1093/infdis/jiaa617.
5. Mariz, F.C., Gray, P., Bender, N., Eriksson, T., Kann, H., Apter, D., Paavonen, J., Pajunen, E., Prager, K.M., Sehr, P., et al. (2021). Sustainability of neutralising antibodies induced by bivalent or quadrivalent HPV vaccines and correlation with efficacy: a combined follow-up analysis of data from two randomised, double-blind, multicentre, phase 3 trials. Lancet Infect. Dis. *21*, 1458–1468. https://doi.org/10.1016/S1473-3099(20)30873-2.
6. Drolet, M., Bénard, É., Boily, M.C., Ali, H., Baandrup, L., Bauer, H., Beddows, S., Brisson, J., Brotherton, J.M.L., Cummings, T., et al. (2015). Population-level impact and herd effects following human papillomavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect. Dis. *15*, 565–580. https://doi.org/10.1016/S1473-3099(14)71073-4.
7. Lehtinen, M., Luostarinen, T., Vänskä, S., Söderlund-Strand, A., Eriksson, T., Natunen, K., Apter, D., Baussano, I., Harjula, K., Hokkanen, M., et al. (2018). Gender-neutral vaccination provides improved control of human papillomavirus types 18/31/33/35 through herd immunity: results of a community randomized trial (III). Int. J. Cancer *143*, 2299–2310. https://doi.org/10.1002/ijc.31618.

8. Lehtinen, M., Baussano, I., Paavonen, J., Vänskä, S., and Dillner, J. (2019). Eradication of human papillomavirus and elimination of HPV-related diseases - Scientific basis for global public health policies. Expert Rev. Vaccines *18*, 153–160. https://doi.org/10.1080/14760584.2019.1568876.

9. Vänskä, S., Luostarinen, T., Baussano, I., Apter, D., Eriksson, T., Natunen, K., Nieminen, P., Paavonen, J., Pimenoff, V.N., Pukkala, E., et al. (2020). Vaccination with moderate coverage eradicates oncogenic human papillomaviruses if a gender-neutral strategy is applied. J. Infect. Dis. *222*, 948–956. https://doi.org/10.1093/infdis/jiaa099.

10. Gray, P., Palmroth, J., Luostarinen, T., Apter, D., Dubin, G., Garnett, G., Eriksson, T., Natunen, K., Merikukka, M., Pimenoff, V., et al. (2018). Evaluation of HPV type-replacement in unvaccinated and vaccinated adolescent females–post-hoc analysis of a community-randomized clinical trial (II). Int. J. Cancer *142*, 2491–2500. https://doi.org/10.1002/ijc.31281.

11. Gray, P., Kann, H., Pimenoff, V.N., Adhikari, I., Eriksson, T., Surcel, H.M., Vänskä, S., Dillner, J., Faust, H., and Lehtinen, M. (2020). Long-term follow-up of human papillomavirus type replacement among young pregnant Finnish females before and after a community-randomised HPV vaccination trial with moderate coverage. Int. J. Cancer *147*, 3511–3522. https://doi.org/10.1002/ijc.33169.

12. Gargano, J.W., McClung, N., Lewis, R.M., Park, I.U., Whitney, E., Castilho, J.L., Pemmaraju, M., Niccolai, L.M., Brackney, M., DeBess, E., et al. (2023). HPV type-specific trends in cervical precancers in the United States, 2008 to 2016. Int. J. Cancer *152*, 137–150. https://doi.org/10.1002/ijc.34231.

13. Mesher, D., Soldan, K., Lehtinen, M., Beddows, S., Brisson, M., Brotherton, J.M.L., Chow, E.P.F., Cummings, T., Drolet, M., Fairley, C.K., et al. (2016). Population-level effects of human papillomavirus vaccination programs on infections with nonvaccine genotypes. Emerg. Infect. Dis. *22*, 1732–1740. https://doi.org/10.3201/eid2210.160675.

14. Hanski, I. (1999). Matapopulation Ecology (Oxford University Press).

15. Stearns, S.C., and Koella, J.C. (2007). Evolution in Health and Disease, Second Edition (Oxford University Press). https://doi.org/10.1093/acprof:oso/9780199207466.001.0001.

16. Anderson, R., and May, R.M. (1991). Infectious Diseases of Humans: Epidemiology and Control (Oxford University Press).

17. Lipsitch, M., and O'Hagan, J.J. (2007). Patterns of antigenic diversity and the mechanisms that maintain them. J. R. Soc. Interface *4*, 787–802. https://doi.org/10.1098/rsif.2007.0229.

18. Geoghegan, J.L., and Holmes, E.C. (2018). The phylogenomics of evolving virus virulence. Nat. Rev. Genet. *19*, 756–769. https://doi.org/10.1038/s41576-018-0055-5.

19. Højsgaard, S. (2012). Graphical independence networks with the grain package for R. J. Stat. Softw. *46*, 1–26.

20. El-Zein, M., Richardson, L., and Franco, E.L. (2016). Cervical cancer screening of HPV vaccinated populations: cytology, molecular testing, both or none. J. Clin. Virol. *76*, S62–S68. https://doi.org/10.1016/j.jcv.2015.11.020.

21. Méthot, P.O. (2011). Research traditions and evolutionary explanations in medicine. Theor. Med. Bioeth. *32*, 75–90. https://doi.org/10.1007/s11017-010-9167-4.

22. Louvanto, K., Eriksson, T., Gray, P., Apter, D., Baussano, I., Bly, A., Harjula, K., Heikkilä, K., Hokkanen, M., Huhtinen, L., et al. (2020). Baseline findings and safety of infrequent vs. frequent screening of human papillomavirus vaccinated women. Int. J. Cancer *147*, 440–447. https://doi.org/10.1002/ijc.32802.

23. Gray, P., Kann, H., Pimenoff, V.N., Eriksson, T., Luostarinen, T., Vänskä, S., Surcel, H.M., Faust, H., Dillner, J., and Lehtinen, M. (2021). Human papillomavirus seroprevalence in pregnant women following gender-neutral and girls-only vaccination programs in Finland: a cross-sectional cohort analysis following a cluster randomized trial. PLoS Med. *18*, e1003588. https://doi.org/10.1371/journal.pmed.1003588.

24. Lehtinen, M., Pimenoff, V.N., Nedjai, B., Louvanto, K., Verhoef, L., Heideman, D.A.M., El-Zein, M., Widschwendter, M., and Dillner, J. (2022). Assessing the risk of cervical neoplasia in the post-HPV vaccination era. Int. J. Cancer *152*, 1060–1068. https://doi.org/10.1002/ijc.34286.

25. Wang, Y., Naumann, U., Wright, S.T., and Warton, D.I. (2012). mvabund - an R package for model-based analysis of multivariate abundance data. Methods Ecol. Evol. *3*, 471–474. https://doi.org/10.1111/j.2041-210X.2012.00190.x.

26. Gandon, S., and Day, T. (2008). Evidences of parasite evolution after vaccination. Vaccine *26*, C4–C7. https://doi.org/10.1016/j.vaccine.2008.02.007.

27. Andreano, E., Piccini, G., Licastro, D., Casalino, L., Johnson, N.V., Paciello, I., Dal Monego, S., Pantano, E., Manganaro, N., Manenti, A., et al. (2021). SARS-CoV-2 escape from a highly neutralizing COVID-19 convalescent plasma. Proc. Natl. Acad. Sci. USA *118*, e2103154118. https://doi.org/10.1073/pnas.2103154118.

28. Murall, C.L., Bauch, C.T., and Day, T. (2015). Could the human papillomavirus vaccines drive virulence evolution? Proc. Biol. Sci. *282*, 20141069. https://doi.org/10.1098/rspb.2014.1069.

29. Holmes, E.C. (2008). Evolutionary history and phylogeography of human viruses. Annu. Rev. Microbiol. *62*, 307–328. https://doi.org/10.1146/annurev.micro.62.081307.162912.

30. Sallinen, S., Norberg, A., Susi, H., and Laine, A.L. (2020). Intraspecific host variation plays a key role in virus community assembly. Nat. Commun. *11*, 5610. https://doi.org/10.1038/s41467-020-19273-z.

31. Schoener, T.W. (2009). Ecological niche. In The Princeton Guide to Ecology, S.A. Levin, S.R. Carpenter, C.J. Godfray, A.P. Kinzig, M. Loreau, J.B. Losos, B. Walker, and D.S. Wilcove, eds. (Princeton University Press), pp. 3–13.

32. Gray, P., Luostarinen, T., Vänskä, S., Eriksson, T., Lagheden, C., Man, I., Palmroth, J., Pimenoff, V.N., Söderlund-Strand, A., Dillner, J., and Lehtinen, M. (2019). Occurrence of human papillomavirus (HPV) type replacement by sexual risk-taking behaviour group: post-hoc analysis of a community randomized clinical trial up to nine years after vaccination (IV). Int. J. Cancer *145*, 785–796. https://doi.org/10.1002/ijc.32189.

33. Man, I., Vänskä, S., Lehtinen, M., and Bogaards, J.A. (2021). Human papillomavirus genotype replacement: still too early to tell? J. Infect. Dis. *224*, 481–491. https://doi.org/10.1093/infdis/jiaa032.

34. Cameron, E.S., Schmidt, P.J., Tremblay, B.J.-M., Emelko, M.B., and Müller, K.M. (2021). Enhancing diversity analysis by repeatedly rarefying next generation sequencing data describing microbial communities. Sci. Rep. *11*, 22302. https://doi.org/10.1038/s41598-021-01636-1.

35. Weinberger, D.M., Malley, R., and Lipsitch, M. (2011). Serotype replacement in disease after pneumococcal vaccination. Lancet *378*, 1962–1973. https://doi.org/10.1016/S0140-6736(10)62225-8.

36. World Health Organization (2020). Global strategy to accelerate the elimination of cervical cancer as a public health problem. https://iris.who.int/bitstream/handle/10665/336583/9789240014107-eng.pdf?sequence=1.

37. Lehtinen, M., and Pimenoff, V.N. (2021). Moral dilemma(s) in human papillomavirus vaccination – revisiting the role of the herd effect. Euro Surveill. *26*, 2101154. https://doi.org/10.2807/1560-7917.ES.2021.26.50.2101154.

38. Combes, J.-D., Guan, P., Franceschi, S., and Clifford, G.M. (2014). Judging the carcinogenicity of rare human papillomavirus types. Int. J. Cancer *136*, 740–742. https://doi.org/10.1002/ijc.29019.

39. Söderlund-Strand, A., and Dillner, J. (2013). High-throughput monitoring of human papillomavirus type distribution. Cancer Epidemiol. Biomarkers Prev. *22*, 242–250. https://doi.org/10.1158/1055-9965.EPI-12-1003.

40. Lehtinen, M., Kaasila, M., Pasanen, K., Patama, T., Palmroth, J., Laukkanen, P., Pukkala, E., and Koskela, P. (2006). Seroprevalence atlas of infections with oncogenic and non-oncogenic human papillomaviruses in Finland in the 1980s and 1990s. Int. J. Cancer *119*, 2612–2619. https://doi.org/10.1002/ijc.22131.

41. Lehtinen, M., Apter, D., Baussano, I., Eriksson, T., Natunen, K., Paavonen, J., Vänskä, S., Bi, D., David, M.P., Datta, S., et al. (2015). Characteristics of a cluster-randomized phase IV human papillomavirus vaccination

effectiveness trial. Vaccine *33*, 1284–1290. https://doi.org/10.1016/j.vaccine.2014.12.019.

42. Willis, A.D. (2019). Rarefaction, alpha diversity, and statistics. Front. Microbiol. *10*, 2407. https://doi.org/10.3389/fmicb.2019.02407.
43. Shannon, C.E. (1948). A mathematical theory of communication. Bell Syst. Tech. J. *27*, 379–423.
44. Simpson, E.H. (1949). Measurement of diversity. Nature *163*, 688. https://doi.org/10.1038/163688a0.
45. Pimenoff, V.N., Tous, S., Benavente, Y., Alemany, L., Quint, W., Bosch, F.X., Bravo, I.G., and de Sanjosé, S. (2019). Distinct geographic clustering of oncogenic human papillomaviruses multiple infections in cervical cancers: results from a worldwide cross-sectional study. Int. J. Cancer *144*, 2478–2488. https://doi.org/10.1002/ijc.31964.
46. Koller, D., and Friedman, N. (2009). Probabilistic Graphical Models: Principles and Techniques (MIT Press).

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Multi-collect specimen collection kit	Abbott	Cat# 9K12-01
Critical commercial assays		
Abbott m2000sp DNA extraction.	This paper	Published also in Söderlund-Strand and Dillner[39].
PCR and MALDI-TOF mass spectrometry for HPV genotyping	This paper	Published also in Söderlund-Strand and Dillner[39]
Deposited data		
Aggregated community-level HPV prevalence data four years post-vaccination (18-year-olds, N=11,396).	This paper	Fairdata IDA: https://doi.org/10.24340/mxdt-m377
Aggregated community-level prevalence data four years (18-year-olds, N=3,580) and eight years (22-year-olds, N=3,580) post-vaccination.	This paper	Fairdata IDA: https://doi.org/10.24340/mxdt-m377
Synthetic HPV vaccination data simulated from the original trial HPV genotype data (N=500,000)	This paper	Fairdata IDA: https://doi.org/10.24340/vqcx-3fbj6h
Software and algorithms		
R program	R and RStudio	R version 4.2.2 (2022-10-31)
Rcpp,	R library	https://github.com/RcppCore/Rcpp
vegan	R library	https://github.com/vegandevs/vegan
FactorMineR	R library	https://github.com/cran/FactoMineR
factoextra	R library	https://github.com/kassambara/factoextra
mvabund	R library	https://github.com/cran/mvabund
reshape2	R library	https://github.com/cran/reshape2
data.table	R library	https://github.com/Rdatatable/data.table
tidyverse	R library	https://github.com/tidyverse/dplyr
vcdExtra	R library	https://github.com/friendly/vcdExtra
gRbase	R library	https://github.com/hojsgaard/gRbase
gRim	R library	https://github.com/hojsgaard/gRim
RBGL	R library	https://github.com/Bioconductor/RBGL
Rgraphviz	R library	https://github.com/cran/Rgraphviz
MASS	R library	https://www.stats.ox.ac.uk/pub/MASS4/
Code and algorithm for the diversity inference and simulation of the synthetic data	GitHub repository	https://github.com/PimenoffV/HPV.ecology

RESOURCE AVAILABILITY

Lead contact

Further information and requests for data and resources should be directed to and will be fulfilled by the lead contact, Ville N. Pimenoff (ville.pimenoff@ki.se).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The original trial community-level HPVs prevalence data and the synthetic genotype data (N=500,000) have been deposited into a corresponding Fairdata IDA (https://etsin.fairdata.fi/) database (community aggregated Fairdata: https://doi.org/10.24340/mxdt-m377 and synthetic HPV vaccination Fairdata: https://doi.org/10.24340/vqcx-3fbj6h, respectively) and are available as of the date

of publication. The DOIs of the database are also listed in the key resources table. All core code has been deposited at GitHub and is publicly available as of the date of publication. DOI is listed in the key resources table. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon reasonable request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study subjects and HPV genotyping

We stratified 33 geographically distinct (> 50 km apart from each other) Finnish communities according to pre-ascertained high, intermediate and low HPV16/18 seroprevalence.[40,41] From sub strata we randomized communities to three arms: arm A gender-neutral HPV16/18 vaccination (11 communities), arm B girls-only HPV16/18 vaccination and hepatitis B-virus (HBV) vaccination of boys (11 communities), and arm C gender-neutral HBV vaccination (11 communities).[41] All resident boys (31,117) and girls (30,139) in the 1992-94 birth cohorts were invited, 4,426 girls and 1,786 boys received three doses of the bivalent HPV16/18 vaccine in arm A communities, 5,056 girls were HPV16/18 vaccinated and 3,664 boys were HBV vaccinated in arm B communities, and 5,037 girls and 2,948 boys were HBV vaccinated in arm C communities.[41] In 2010-13 7,977 HPV16/18 and 4,159 non-HPV vaccinated females, and in 2014-16 5,602 HPV16/18 vaccinated females attended follow up visits for cervico-vaginal sampling at age 18 and 22 years, respectively. This HPV vaccination cohort trials and its long-term follow-up involving human samples have been approved by the pertinent Finnish National Ethical Review Boards (1154/2003, 1174/2004) and the Pirkanmaa Hospital District Ethical Review Board (R07113M/2007, R11058/2011, R13149/ 2014 and R19136/2020). All the participants are healthy volunteers and have signed an informed consent for sampling and analysis covering the intervention and the follow-up phases of the cohort.

All samples were obtained by a trained study nurse during participants study visits. The cervico-vaginal samples were collected as vaginal swab immersed in first-void urine using the Abbott multi-collect specimen collection kit (Abbott, USA) and stored in -80 ∘C before extraction. The tube contained 1.2 ml of transport buffer (guanidine thiocyanate in Tris buffer), and approximately 3ml of urine was added to the tube at the time of sampling. DNA was extracted using the m2000sp system for Chlamydia sample extraction (Abbott, USA) and processing 400μl of each original sample. The extracted DNA samples were stored at −20 ∘C until analysed. All sample extracts were genotyped at the same time for HPV6/11/16/18/31/33/35/39/45/51/56/58/59/66/68 using consensus PCR followed by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry.[39] For quality control each batch included a positive and negative control and the samples were checked using quantitative real-time PCR targeting the HBB gene (haemoglobin, beta).[39] HPV-negative samples were included in the data analysis only if the result of the betaglobin-testing was positive. HPV-positive samples were included in the data analysis regardless of the results of the betaglobin-testing. Study subjects with HPV-negative and betaglobin-negative samples were excluded. This originally recruited and previously described[22,32] women's cervical HPV genotype data across 33 Finnish randomized trial communities for the 18-year-olds (N=11,396) and among the 18-year-olds with follow-up at the age of 22 years (N=3,580) was used in this study for the analyses.

METHODS DETAILS

Data analysis, simulations and statistics

To define differences in HPV type prevalence distribution and diversity within HPV vaccine communities in gender neutral arm A and girls-only arm B vaccination communities and control communities in arm C beyond pairwise, we calculated HPV type ecological diversity distributions within each community and arm using α-diversity[42] with Shannon[43] and Simpson[44] index. Each arm consisted of eleven communities repeatedly rarefied without replacement either 25-times (observed data) or 1000-times (synthetic data) for equal community size.[34,42] Full algorithm of the diversity estimations is available in GitHub.

To measure diversity differences between HPV vaccine communities in arm A and arm B and control communities in arm C, we calculated HPV type diversity distributions between communities using β-diversity[42] with Bray-Curtis and Jaccard distances for both observed and rarefied community data, and visualized using ordination plots as previously demonstrated.[24] Furthermore, we estimated the HPV type community prevalence differences beyond pairwise using the generalised linear models (GLM) framework[25] as previous implemented for HPV type data.[45] Code for the diversity difference estimation is also available in GitHub.

To estimate possible differences in risk-taking sexual behavior between the communities we performed *Chlamydia trachomatis* screening including a questionnaire on behaviour, and sexual health on all group A, B and C communities at age 18. No differences were found between the group A, B and C communities using *Chlamydia trachomatis* infection as a surrogate for sexual risk-taking behaviour that could have implicated differential sexual behavior of the adolescents who initially received HPV vaccines. Moreover, group C individuals were informed during the initial vaccination visits at junior high-school that they will get the still beneficial HPV vaccine at the age of 18. A note of caution here is that therefore we compared the 22-year-olds group A and group B HPV diversity distributions to the 18-year-old group C diversity patterns and not the 22-year-old group C as they had already as 18-year-olds received the HPV cross-vaccinations.

For comprehensive sensitivity analysis of the HPV type diversity estimations between HPV vaccine communities we modeled synthetic HPV type community prevalence data from our girls-only and gender-neutral community-randomized vaccination trial genotype data using Bayesian framework for graphical independence networks (GIN).[46] Exploiting the GIN method,[19] we simulated a

synthetic HPV prevalence dataset from a GIN fitted to our observed trial arm communities HPV prevalence data. Each synthetic trial arm HPV prevalence dataset (N=100,000) for 18- and 22-year-olds was repeatedly rarefied (N=100) and re-sampled (x1000) without replacement for estimating community-level HPV type prevalence distribution. Diversity estimations for the synthetic data among the 22-year-olds included thirteen HPV types, HPV16/18 types were excluded.

QUANTIFICATION AND STATISTICAL ANALYSIS

All analyses and simulations described in this study were performed using R and were based on the fifteen different genital HPV type infections originally observed in the community randomized HPV vaccination trial, unless otherwise indicated. Details of the analysis tools used in R are listed in the key resources table. Please see associated figure legends and methods details for details on statistical analysis.