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Reduction in HPV16/18 prevalence among young women with high-grade cervical lesions following the Japanese HPV vaccination program

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Abstract

The Japanese government began a human papillomavirus (HPV) vaccination program for girls aged 12-16 years in 2010 but withdrew its recommendation in 2013 because of potential adverse effects, leading to drastically reduced vaccination uptake. To evaluate population-level effects of HPV vaccination, women younger than 40 years of age newly diagnosed with cervical intraepithelial neoplasia grade 1-3 (CIN1-3), adenocarcinoma in situ (AIS), or invasive cervical cancer (ICC) have been registered at 21 participating institutes each year since 2012. A total of 7709 women were registered during 2012-2017, of which 5045 were HPV genotyped. Declining trends in prevalence of vaccine types HPV16 and HPV18 during a 6-year period were observed in CIN1 (50.0% to 0.0%, P_{trend} < .0001) and CIN2-3/AIS (83.3% to 45.0%, P_{trend} = .07) only among women younger than 25 years of age. Overall, HPV vaccination reduced the proportion of HPV16/18-attributable CIN2-3/AIS from 47.7% to 33.0% (P = .003): from 43.5% to 12.5% as routine vaccination (P = .08) and from 47.8% to 36.7% as catch-up vaccination (P = .04). The HPV16/18 prevalence in CIN2-3/AIS cases was significantly reduced among female individuals who received their first vaccination at age 20 years or younger (P = .02). We could not evaluate vaccination effects on ICC owing to low incidence of ICC among women aged less than 25 years. We found HPV vaccination to be effective in protecting against HPV16/18-positive CIN/AIS in Japan; however, our data did not support catch-up vaccination for women older than 20 years. Older adolescents who skipped routine vaccination due to the government's suspension of its vaccine recommendation could benefit from receiving catch-up vaccination before age 20 years.

KEYWORDS
adenocarcinoma in situ, cervical cancer, cervical intraepithelial neoplasia, human papillomavirus, vaccination

1 | INTRODUCTION

Clinical studies of human papillomavirus (HPV) virus-like particle-based vaccines have reported close to 100% protection against vaccine-type infection and its associated diseases in the per-protocol populations.[1,2] In Japan, a bivalent vaccine against HPV16 and HPV18 was licensed in October 2009, and a quadrivalent vaccine against HPV6, HPV11, HPV16, and HPV18 was licensed in July 2011. In Japan, routine (government-funded) HPV vaccination targets girls aged 12-16 years, and catch-up vaccination is recommended for young women up to age 26 years in the Japanese guidelines.[3] A government-funded HPV vaccination program began for girls aged 12-16 years as an urgent high-priority vaccination project in December 2010, and was incorporated into the National Vaccination Program in April 2013. However, the proactive recommendation for HPV vaccination by the government has been suspended since June 2013 because news reports on potential adverse effects of HPV vaccines appeared repeatedly in the media.[4,5] Consequently, the immunization coverage among adolescent girls decreased quickly and dramatically throughout the country.[6] The coverage of HPV vaccines in Sapporo (Hokkaido, Japan) was high (70% for 3-dose completion) in female individuals born between 1994 and 1998, 30%-40% in those born in 1999, but very low (less than 1%) in those born in 2000 or later.[4] A similar decrease in HPV vaccine coverage was reported in Sakai (Osaka, Japan).[7] Suspension of the recommendation for vaccination has continued to the present, despite no scientific or epidemiologic evidence showing a causal link between postvaccination symptoms and HPV vaccines.[6,8] This situation is unique to Japan.

To date, postlicensure evidence of vaccination against HPV-related cervical diseases and genital warts at the population level has been reported in real-world settings.[9] In Australia, an ecological study has shown a decline in the rates of high-grade cervical lesions in targeted populations ahead of other countries.[10] Studies from Australia, the United States, and Scotland have also reported a significant reduction in the prevalence of vaccine-targeted HPV genotypes in vaccinated cohorts.[11-13] In Japan, a prospective cohort study reported a lower incidence of vaccine-type HPV16/18 infections among young vaccinated women.[14] As girls vaccinated at the age of 12-16 years have reached the recommended age for cervical cancer screening, several surveillance studies based on

cervical screening registries have reported lower incidences of abnormal cytology among women aged 20-24 years or vaccinated in the government-funded vaccination program.[15-18] However, these studies were based on cytological data and did not evaluate vaccine type-specific effects on cervical diseases. Moreover, few studies have addressed the population effectiveness of catch-up vaccination in Japan.

To further evaluate the effectiveness of population-based HPV vaccination at an early date, we initiated a collaborative hospital-based research study to monitor the long-term population-level impact of HPV vaccination (the MINT project) in Japan in 2012.[19] The final goal of the MINT project is to determine the vaccine effect on invasive cervical cancer (ICC) (ie, a significant reduction in the incidence of ICC and the number of deaths from ICC) in Japan. However, given the long lead-time from HPV infections to development of ICC, a vaccination-related effect on ICC would take decades to be seen. By contrast, a decrease in HPV16/18-positive rates in cervical diseases is expected to occur more quickly and to thus be one of the earliest measures of vaccine impact.[20] Therefore, we selected HPV16/18 prevalence among young women newly diagnosed with cervical intraepithelial neoplasia grade 1-3 (CIN1-3), adenocarcinoma in situ (AIS), or ICC each year as the primary endpoint. This final analysis (n = 5045) of MINT study I included many more cases than our interim analysis (n = 2402).[21] In addition to updating our previous findings, the larger dataset enabled us to evaluate cross-protection efficacy against nonvaccine types and vaccine impact according to age at first vaccination. The MINT study is the first observation to report the vaccine type-specific impact of the routine and catch-up HPV vaccination on cervical abnormalities in Japan. We hope that our findings will lead to resumption of vaccination recommendation by the Japanese government.

2 | MATERIALS AND METHODS

2.1 | Study design

We prospectively undertook a collaborative hospital-based study to monitor the long-term population-level impact of HPV vaccination in Japan. Details of the design and methods have been provided elsewhere.[19,21] Briefly, study participants consist of all women aged 16-39 years (age at registration) newly diagnosed with ICC, CIN, or AIS, without a history of treatment for cervical diseases, at 21 participating institutions as from 2012. These institutes were recruited because they rank highly for the number of ICC cases registered with the Japan Society of Obstetrics and Gynecology (JSOG) Oncology Committee. According to the 2016 JSOG oncology statistics (437 registration hospitals), these 21 institutes covered approximately 19.1% of all JSOG-registered ICC cases. All participants enter the study only after voluntarily providing signed informed consent. Participants are registered together with their vaccine history, each year commencing on January 1 and ending on December 31. Unfortunately, the present study relied on self-reported information about vaccination status and official vaccination records were not available to ascertain vaccination status. Also, information on sexual history was not collected.

This monitoring project was planned for a total period of 21 years, which was divided into 3 phases of 7 years each: phase I, August 2012 to December 2018; phase II, January 2019 to December 2025; and phase III, January 2026 to December 2032.[19] Phase I of this research (MINT study I) was originally planned from 2012 to 2018. However, a research grant obtained from the Foundation for Advancement of International Science was stopped in 2018. Therefore, we undertook a 6-year final analysis of the MINT I study (2012-2017).

The study participants are divided into the following 3 groups: category A, women newly diagnosed with ICC at participating facilities each year (registration and HPV genotyping test are necessary for all women diagnosed with ICC); category B, women newly diagnosed with CIN2-3 or AIS (registration is necessary for all women diagnosed with CIN2-3 or AIS; however, HPV genotyping tests were carried out until the total number of participants tested reaches 600); and category C, women newly diagnosed with CIN1 (each year, registration and HPV genotyping are carried out until the total number of subjects tested at all facilities reaches 100). In addition, the number of women treated for ICC within the previous 10 years at participating institutes who die from the disease is also monitored each year (HPV genotyping test will not be carried out).

In the MINT project, the primary endpoint of phase I is the proportion of HPV16/HPV18-positive results among participants with ICC and CIN2-3/AIS for women aged less than 25 years. Other major endpoints are as follows: (i) the number of women aged less than 25 years who develop ICC or CIN2-3/AIS; and (ii) the proportion of HPV16/HPV18-positive results among women aged less than 25 years with CIN1.

Institutional ethical and research review boards of the participating institutions have approved the study protocol. This study was registered in the UMIN Clinical Trials Registry as UMIN000008891.

2.2 | Human papillomavirus genotyping procedures

Human papillomavirus DNA in cervical samples was determined using the Linear Array (LA) assay (Roche Molecular Systems), a commercialized L1 consensus primer-based PCR method that uses a primer set designated PGMY09/11. The LA test was carried out according to the manufacturer's recommended protocol. Briefly, exfoliated ecto- and endocervical cells were stored in ThinPrep PreservCyt solution (Hologic) until DNA extraction. Total cellular DNA was extracted using a QIAamp MinElute Media kit (Qiagen). Amplicons were subjected to reverse line blot hybridization for detection of 37 individual HPV genotypes (HPV6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51 to 56, 58, 59, 61, 62, 64, 66 to 73, 81-84, 82v, and 89). Linear Array does not directly detect HPV52 but combines a set of probes that detects HPV33, 35, 52, and 58 combined (HPVmix). Specimens that tested negative for HPV33, 35, and 58 individually but were positive for the HPVmix were considered to be HPV52 positive. Thus, LA cannot discriminate HPV52 infection when it is co-infected with HPV33, 35, and 58, suggesting that

HPV52 prevalence might be underestimated in the present study. All of the HPV DNA assays were carried out by individuals who were masked to the results in the clinical profile of each project.

2.3 | Statistical methods

Changes in the positive rates for HPV16 or HPV18 were analyzed according to HPV vaccine status and age group (20-24, 25-29, 30-34, and 35-39 years). Linear regression analysis was used to compare year-on-year trends of HPV16/18 prevalence stratified by age (younger than 25 or 25-39 years) and disease severity (CIN1 or CIN2-3/AIS). We also analyzed the data using the Cochrane-Armitage trend test to evaluate time trends in HPV16/18 prevalence during a 6-year period. Binary comparisons between vaccinated and unvaccinated women were done with a Fisher's exact probability test. The *P* values obtained in all tests were considered significant at less than .05. We used the R version 3.5.1 statistics packages (R Foundation for Statistical Computing) for statistical analysis.

3 | RESULTS

A total of 7709 women aged less than 40 years who were newly diagnosed with CIN1 (n = 589), CIN2-3/AIS (n = 5828), or ICC (n = 1292) at the 21 participating institutes were registered between 2012 and 2017. Because we started the present study in August 2012, the number of study participants was smaller in 2012 than in other years. For CIN2-3/AIS and ICC, although all cases diagnosed with these diseases at the 21 participating institutes were registered, we could not find any significant trends in the number of cases in women aged 20-24 years between 2013 and 2017 (Figure 1).

We obtained HPV type-specific data from 5045 women (CIN1, n = 573; CIN2-3/AIS, n = 3342; ICC, n = 1130). In the present study, HPV genotyping assays were undertaken for CIN1 and CIN2-3/AIS until the total number of samples tested reached 100 and 600 each year, respectively. Although all ICC cases were to be tested for HPV genotype, HPV typing results were lacking among 162 early stage ICC cases because of ICC diagnosis after conization; their characteristics are summarized in Table 1. The mean age ± SD of study participants was 32.4 ± 4.6 years: 30.6 ± 5.3 years for CIN1, 32.1 ± 4.6 years for CIN2-3/AIS, and 34.0 ± 3.8 years for ICC.

Among women younger than 25 years, HPV16/18 prevalence decreased from 50.0% to 0.0% in CIN1 (P_{trend} < .0001 Figure 2A) and from 83.3% to 45.0% in CIN2-3/AIS (P_{trend} = .07, Figure 2B) during a 6-year period; no similar decline was observed in older age groups. In women younger than 25 years (n = 92), HPV16/18 prevalence in CIN1 was 50.0% (4/8) in 2012, 29.4% (4/17) in 2013, 35.3% (6/17) in 2014, 14.3% (3/21) in 2015, 11.8% (2/17) in 2016, and 0 (0/12) in 2017. Using a linear regression model, attribution of HPV16 and HPV18 to CIN1 decreased in women younger than 25 years by 8.9% (95% confidence interval [CI], 7.4%–10.4%) per year, but there was no change in those aged 25 years or more between 2012 and 2017 (trend = −0.3% per year; 95% CI, −1.2%-0.7%)

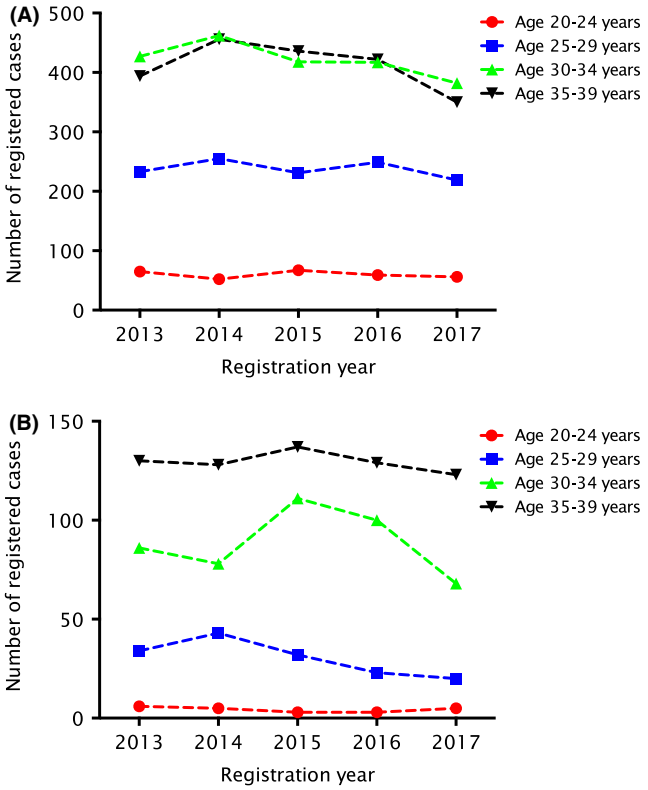

FIGURE 1 Changes in the registered numbers of women with cervical intraepithelial neoplasia grade 2-3 (CIN2-3)/adenocarcinoma in situ (AIS) and invasive cervical cancer (ICC) by age group. Year-on-year trends (dotted lines) of the registered number of Japanese women with CIN2-3/AIS (A) and (ICC) (B) are shown for 4 age groups (20-24 [red], 25-29 [blue], 30-34 [green], and 35-39 [black] y). Although all newly diagnosed cases of these diseases each year at the 21 participating institutions were registered, we could not find any significant trends in the number of CIN2-3/AIS or ICC cases in women aged 20-24 y between 2013 and 2017

(Figure 2C). The difference in these linear trends reached statistical significance (*P* = .001). Similarly, HPV16/18 prevalence in CIN2-3/AIS decreased among women younger than 25 years by 4.5% (95% CI, 2.6%-6.4%) year by year; we found no change in HPV16/18 positivity among women aged 25 years or more across the registration years (trend = −0.2% per year; 95% CI, −0.7%-0.4%) (Figure 2D). The difference in these linear trends was marginally significant (*P* = .06). For ICC cases, however, we could not analyze changes in the HPV16/18 prevalence because only a very small number of ICC cases among women aged 20-24 years were registered each year (Figure 1B).

The time course data of HPV16/18-positive CIN/AIS lesions among women aged 20-24 years during 2012-2017 were also analyzed by the Cochrane-Armitage trend test to evaluate vaccine type-specific impact of routine HPV vaccination. Using the Cochrane-Armitage trend test, the declining trend in HPV16/18 prevalence during a 6-year period was still statistically significant in CIN1 among women aged 20-24 years (P_{trend} = .002), but did not reach statistical significance in CIN2-3/AIS (P_{trend} = .12).

TABLE 1 Characteristics of human papillomavirus (HPV) type-specific analysis among Japanese women

	CIN1 (N = 573)	CIN2-3 or AIS (N = 3342)	ICC (N = 1130)
History of HPV vaccination			
Vaccinated	29 (4.8)	106 (3.1)	22 (1.9)
Bivalent	11 (1.8)	35 (1.0)	12 (1.0)
Quadrivalent	5 (0.8)	30 (0.9)	5 (0.4)
Unknown	13 (2.2)	41 (1.2)	5 (0.4)
Unvaccinated	543 (90.2)	3213 (93.2)	1099 (95.4)
Missing	1 (0.2)	23 (0.7)	9 (0.8)
Registration year			
2012	60 (10.5)	178 (5.3)	28 (2.5)
2013	100 (17.5)	610 (18.3)	215 (19.0)
2014	98 (17.1)	614 (18.4)	223 (19.7)
2015	104 (18.2)	632 (18.9)	256 (22.7)
2016	104 (18.2)	671 (20.1)	216 (19.1)
2017	107 (18.7)	637 (19.1)	192 (17.0)
Age at registration, y			
20-24	92 (16.1)	204 (6.1)	20 (1.8)
25-29	137 (23.9)	718 (21.5)	126 (11.2)
30-34	187 (32.6)	1221 (36.5)	404 (35.8)
35-39	157 (27.4)	1199 (35.9)	580 (51.3)
Birth cohort			
1973-75	44 (7.7)	276 (8.3)	99 (8.8)
1976-78	86 (15.0)	660 (19.7)	321 (28.4)
1979-81	99 (17.3)	726 (21.7)	301 (26.6)
1982-84	113 (19.7)	725 (21.7)	242 (21.4)
1985-87	88 (15.4)	503 (15.1)	119 (10.5)
1988-90	77 (13.4)	288 (8.6)	35 (3.1)
1991-93	47 (8.2)	125 (3.7)	10 (0.9)
1994-96	19 (3.3)	39 (1.2)	3 (0.3)
HPV genotype			
Oncogenic[a]	403 (70.3)	3085 (92.3)	1004 (88.8)
HPV16	78 (13.6)	1387 (41.5)	652 (57.7)
HPV18	34 (5.9)	247 (7.4)	258 (22.8)
Nononcogenic	87 (15.2)	110 (3.3)	26 (2.3)
Negative	83 (14.5)	147 (4.4)	100 (8.8)

Data are shown as n (%).
AIS, adenocarcinoma in situ; CIN, cervical intraepithelial neoplasia; ICC, invasive cervical cancer.
[a]Oncogenic HPV types include HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.

Of 5045 study participants tested for HPV genotype, only 3.1% (157/5045) previously received HPV vaccination; the great majority (96.2% [4855/5045]) reported no history of HPV vaccination (Table 1). Information about HPV vaccination history was missing for 33 women (0.7%). The mean age ± SD of those vaccinated was 30.1 ± 6.3 years at the registration date. Overall, HPV16/18 prevalence in CIN2-3/AIS was significantly reduced among vaccinated women compared with unvaccinated women (33.0% [35/106] vs 47.7% [1535/3213], P = .003). To further evaluate vaccine effectiveness of the routine and catch-up vaccination separately, we compared HPV16/18 prevalence between vaccinated and unvaccinated women across birth cohorts (Table 2). Of CIN2-3/AIS cases in women reporting an HPV vaccination history, 15.1% (16/106, 1994-1996 birth cohorts) were immunized in the government-funded vaccination program whereas the remainder (84.9% [90/106], 1973-1993 birth cohorts) received HPV vaccine as catch-up vaccination. For the 1994-1996 birth cohorts, attribution of HPV16 and HPV18 to CIN2-3/AIS was reduced from

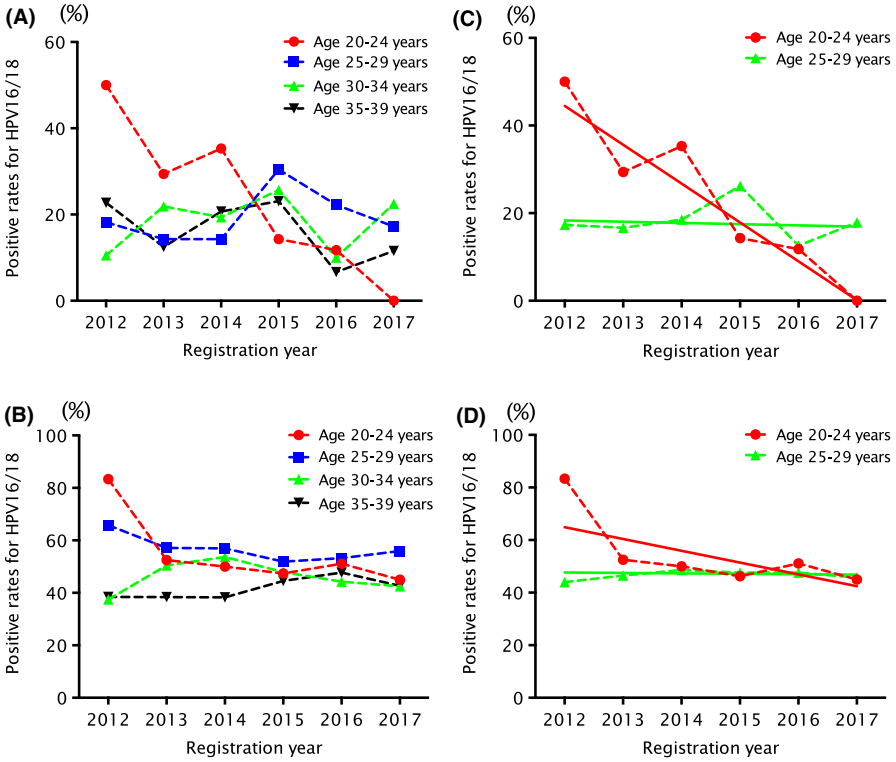

FIGURE 2 Changes in human papillomavirus type 16 or 18 (HPV16/18) prevalence among Japanese women with cervical intraepithelial neoplasia grade 1 (CIN1) or CIN2-3/adenocarcinoma in situ (AIS) by age group. A, B, Year-on-year trend of HPV16/18 prevalence (dotted lines) among Japanese women with CIN1 (A) and CIN2-3/AIS (B) are shown for 4 age groups (20-24 [red], 25-29 [blue], 30-34 [green], and 35-39 [black] y). Among women aged 20-24 y, HPV16/18 prevalence decreased from 50.0% to 0.0% for CIN1 (P_{trend} < .0001) (A) and from 83.3% to 45.0% for CIN2-3/AIS (P_{trend} = .07) (B) during a 6-year period. No similar decline was observed for older age groups. C, D, Year-on-year trend of HPV16/18 prevalence (dotted lines) and estimated prevalence trends (solid lines) among Japanese women with CIN1 (C) and CIN2-3/AIS (D) shown for 2 age groups (20-24 [red] and ≥25 [green] y). Using a linear regression model, the difference in linear trends of HPV16/18 prevalence between women aged 20-24 and ≥25 y was statistically significant for CIN1 (*P* = .001) (C) and marginally significant for CIN2-3/AIS (*P* = .06) (D)

43.5% (10/23, unvaccinated) to 12.5% (2/16, vaccinated) for the national vaccination, but this difference did not reach statistical significance (*P* = .08), probably owing to limitations imposed by the sample size. The vaccination rate among CIN2-3/AIS case in women born during 1994-1996 was much lower than that estimated in previous reports (41% vs approximately 70%), suggesting that unvaccinated women in the 1994-1996 birth cohorts were more likely to develop CIN2-3/AIS. For the 1973-1993 birth cohorts, attribution of HPV16 and HPV18 to CIN2-3/AIS decreased from 47.8% (1525/3190, unvaccinated) to 36.7% (33/90, vaccinated) with catch-up vaccination (*P* = .04). The vaccination effect was observed for HPV16 or HPV18 alone, although it did not reach statistical significance for each type owing to the small sample size. Similar vaccine effects were also observed among CIN1 cases (Table 2). In the 1994-1996 birth cohorts, there were 3 women that developed HPV16/18-positive CIN2-3/AIS (n = 2) or ICC (n = 1) following routine HPV vaccination. We note that these women were born in 1994 and aged 16 years at first vaccination, but no information about their sexual history was available.

We also addressed the effectiveness of HPV vaccinations according to age at first vaccination. The youngest age at the time of first vaccination was 13 years. The HPV16/18 prevalence in CIN2-3/AIS was 12.5% for women who received their first dose at ages 13-16 years (n = 16), 14.3% for those (n = 7) aged 17-20 years at first vaccination, 35.3% for those aged 21-25 years at first vaccination (n = 17), 39.4% for those older than 25 years at first vaccination (n = 66), and 47.8% for those who were unvaccinated (n = 3212) (Figure 3). Of vaccinated women, attribution of HPV16 and HPV18 to CIN2-3/AIS was significantly different between women aged 13-20 years and older than 20 years at first vaccination (13.0% vs 38.6%, *P* = .02). Of women with CIN2-3/AIS, HPV16/18 prevalence among women older than 20 years at first vaccination was not significantly different from that among unvaccinated women (38.6% vs 47.8%, *P* = .18). When women received a first vaccine dose at the age of 13-16 years, the HPV16/18 prevalence in CIN2-3/AIS was similar between women vaccinated with 3 doses (n = 7) and 1-2 doses (n = 9) (14.3% vs 11.1%).

We also evaluated the prevalence of nonvaccine HPV types HPV31, HPV33, HPV45, HPV52, and HPV58 between vaccinated and unvaccinated women (Table 2). The prevalence of HPV52 and HPV58 in cervical lesions was higher among vaccinated women than among unvaccinated ones (37.9% vs 27.8% for CIN1 and 44.3%

TABLE 2 Comparison of human papillomavirus (HPV) type prevalence between vaccinated and unvaccinated Japanese women by disease severity and birth cohort

	1994–1996 birth cohorts			1973–1993 birth cohorts			All birth cohorts		
	Vaccinated (routine vaccination)	Unvaccinated	*P* value[a]	Vaccinated (catch-up vaccination)	Unvaccinated	*P* value[a]	Vaccinated	Unvaccinated	*P* value[a]
CIN1	n = 13	n = 5		n = 16	n = 538		n = 29	n = 543	
HPV16/18	1 (7.7)	1 (20.0)	.49	1 (6.3)	105 (19.5)	.33	2 (6.9)	106 (19.5)	.14
HPV16	1 (7.7)	1 (20.0)	.49	1 (6.3)	75 (13.9)	.71	2 (6.9)	76 (14.0)	.41
HPV18	0 (0.0)	0 (0.0)	1.00	0 (0.0)	34 (6.3)	.61	0 (0.0)	34 (6.3)	.41
HPV31/33/45	0 (0.0)	0 (0.0)	1.00	0 (0.0)	44 (8.2)	.63	0 (0.0)	44 (8.1)	.16
HPV31	0 (0.0)	0 (0.0)	1.00	0 (0.0)	22 (4.1)	1.00	0 (0.0)	22 (4.1)	.62
HPV33	0 (0.0)	0 (0.0)	1.00	0 (0.0)	12 (2.2)	1.00	0 (0.0)	12 (2.2)	1.00
HPV45	0 (0.0)	0 (0.0)	1.00	0 (0.0)	11 (2.0)	1.00	0 (0.0)	11 (2.0)	1.00
HPV52/58	4 (30.8)	0 (0.0)	.28	7 (43.8)	151 (28.1)	.17	11 (37.9)	151 (27.8)	.29
HPV52	1 (7.7)	0 (0.0)	1.00	4 (25.0)	78 (14.5)	.27	5 (17.2)	78 (14.4)	.59
HPV58	3 (23.1)	0 (0.0)	.52	3 (18.8)	73 (13.4)	.47	2 (9.1)	57 (5.2)	.76
CIN2–3/AIS	**n = 16**	**n = 23**		**n = 90**	**n = 3190**		**n = 106**	**n = 3213**	
HPV16/18	2 (12.5)	10 (43.5)	.08	33 (36.7)	1525 (47.8)	**.04**	35 (33.0)	1535 (47.8)	**.003**
HPV16	2 (12.5)	9 (39.1)	.09	28 (31.1)	1332 (41.8)	.05	30 (28.3)	1341 (41.7)	**.007**
HPV18	0 (0.0)	2 (8.7)	.50	6 (6.7)	239 (7.5)	1.00	6 (5.7)	241 (7.5)	.580
HPV31/33/45	1 (6.3)	2 (8.7)	1.00	15 (16.7)	525 (16.5)	1.00	16 (15.1)	527 (16.4)	.790
HPV31	1 (6.3)	1 (4.4)	1.00	12 (13.3)	397 (12.5)	.75	13 (12.3)	398 (12.4)	1.000
HPV33	0 (0.0)	1 (4.4)	1.00	2 (2.2)	101 (3.2)	1.00	2 (1.9)	102 (3.2)	.770
HPV45	0 (0.0)	0 (0.0)	1.00	1 (1.1)	37 (1.2)	1.00	1 (0.9)	37 (1.2)	1.000
HPV52/58	9 (56.3)	9 (39.1)	.34	38 (42.2)	1265 (39.7)	.66	47 (44.3)	1274 (39.7)	.360
HPV52	4 (25.0)	2 (8.7)	.21	16 (17.8)	673 (21.1)	0.51	20 (18.9)	675 (21.0)	0.720
HPV58	5 (31.3)	7 (30.4)	1.00	22 (24.4)	592 (18.6)	.17	27 (25.5)	599 (18.6)	.100
ICC	**n = 1**	**n = 2**		**n = 21**	**n = 1097**		**n = 22**	**n = 1099**	
HPV16/18	1 (100.0)	2 (100.0)	1.00	14 (66.7)	855 (77.9)	.29	15 (68.2)	857 (78.0)	.30
HPV16	0 (0.0)	2 (100.0)	.33	10 (47.6)	633 (57.7)	.38	10 (45.5)	635 (57.8)	.28
HPV18	1 (100.0)	0 (0.0)	.33	4 (19.1)	251 (22.9)	.80	5 (22.7)	251 (22.8)	1.00
HPV31/33/45	0 (0.0)	0 (0.0)	1.00	2 (9.5)	70 (6.4)	.64	2 (9.1)	70 (6.4)	.65
HPV31	0 (0.0)	0 (0.0)	1.00	2 (9.5)	46 (4.2)	.23	2 (9.1)	46 (4.2)	.24
HPV33	0 (0.0)	0 (0.0)	1.00	0 (0.0)	9 (0.8)	1.00	0 (0.0)	9 (0.8)	1.00
HPV45	0 (0.0)	0 (0.0)	1.00	0 (0.0)	16 (1.5)	1.00	0 (0.0)	16 (1.5)	1.00

(Continues)

TABLE 2 (Continued)

ICC	n = 1	n = 2		n = 21		n = 1097		n = 22		n = 1099	
HPV52/58	0 (0.0)	0 (0.0)	1.00	3 (14.3)		112 (10.2)	.47	3 (13.6)		112 (10.9)	.49
HPV52	0 (0.0)	0 (0.0)	1.00	1 (4.8)		55 (5.0)	1.00	1 (4.6)		55 (5.0)	1.00
HPV58	0 (0.0)	0 (0.0)	1.00	2 (9.5)		57 (5.2)	.31	2 (9.1)		57 (5.2)	.32

Data are shown as n (%). Bold values indicate significance.
AIS, adenocarcinoma in situ; CIN, cervical intraepithelial neoplasia; ICC, invasive cervical cancer.
[a]*P* values calculated using Fisher's exact probability test.

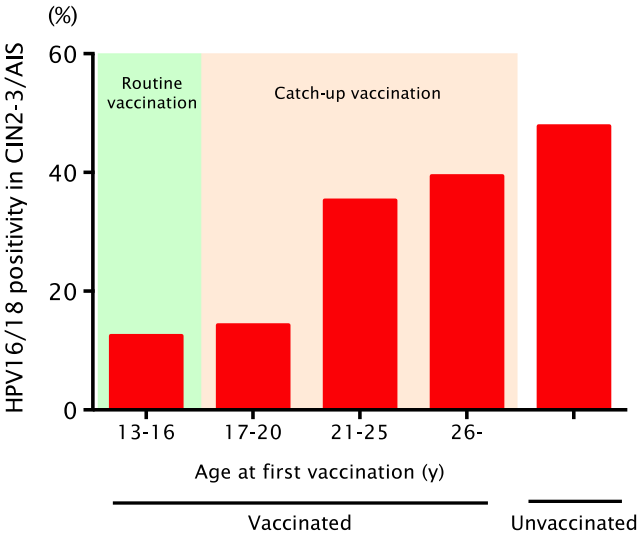

FIGURE 3 Attribution of human papillomavirus type 16 or 18 (HPV16/18) to cervical intraepithelial neoplasia grade 2-3 (CIN2-3)/adenocarcinoma in situ (AIS) by age at first vaccination. Prevalence of HPV16/18 in CIN2-3/AIS was 12.5% for women who received their first dose at ages 12-16 y (n = 16), 14.3% for those aged 17-20 y at first vaccination (n = 7), 35.3% for those aged 21-25 y at first vaccination (n = 17), 39.4% for those aged >25 y at first vaccination (n = 66), and 47.8% for those unvaccinated (n = 3213). Among vaccinated women, attribution of HPV16 and HPV18 to CIN2-3/AIS was significantly lower among those aged 13-20 y at first vaccination than among those aged >20 y at first vaccination ($P = .02$)

vs 39.5% for CIN2-3/AIS, respectively). Interestingly, however, the prevalence of HPV31/33/45 in cervical lesions was not increased among vaccinated women (0.0% vs 8.1% for CIN1 and 15.1% vs 16.4% for CIN2-3/AIS, respectively). When the data were analyzed separately for the 1994-1999 and 1973-1993 birth cohorts, similar results were observed for CIN1 and CIN2-3/AIS.

4 | DISCUSSION

In this study, declining proportions of HPV16/18-positive CIN/AIS lesions among vaccinated cohorts provided more specific evidence than previous studies reporting a declining incidence of abnormal Pap results among young women in Japan.[15-18] In addition, we also found that HPV vaccination was significantly effective against HPV16/18-attributable CIN2-3/AIS in women aged 13-20 years at the time of first vaccination, but not in women older than 20 years at first dose. The limited effectiveness of catch-up vaccination in women older than 20 years was first observed in Japan but similar findings have been observed in other countries.[9,22-24] Using registration data from Kaiser Permanente Northern California, a population-based US case-control study of over 25 000 women reported that significant protection against CIN2 or worse (CIN2+) was observed in women who had received their first HPV vaccine dose aged 14-20 years, but not for women aged 21 years and older at first vaccination, in keeping with our finding.[22] In Australia, with

multiple age-cohort and gender-neutral vaccination programs and very high vaccination coverage, catch-up vaccination with 3 doses significantly protected against high-grade cervical lesions among women aged 15-18 and 19-22 years at first vaccination, but not among women aged 23-27 years at first vaccination.[23] In Scotland, with girls-only vaccination and high uptake, protective effect of 3-dose catch-up vaccination against CIN2+ was significant among women first vaccinated at age 14-17 years, but not among women first vaccinated at age 18 years or older.[24] These differences could be explained in part by differences in vaccine herd effects (ie, reduced transmission of HPV infections) between countries. A recent meta-analysis found that HPV immunization programs with multiple age-cohort vaccination and high vaccination coverage contribute to herd protection.[9] Therefore, the age range of women who benefit from catch-up vaccination might be country-specific. The effectiveness of catch-up vaccination for women older than 21 years is one of the most important issues in HPV vaccination, especially in Japan, because the vaccination rate in women born in or after 2000 is extremely low (less than 1%).[4-7] Therefore, additional studies will be warranted to confirm the limited effectiveness of catch-up vaccination in women older than 20 years.

We also evaluated the efficacy against nonvaccine HPV types HPV31, HPV33, HPV45, HPV52, and HPV58. Among vaccinated women, positive rates of HPV types other than HPV16 and HPV18 should be relatively increased to compensate the prevalence reduction of HPV16/18 due to HPV vaccination. As expected, the prevalence of HPV52 and HPV58 in cervical lesions was increased among vaccinated women compared to unvaccinated women. The relatively increased attribution of HPV52 and HPV58 to cervical abnormalities among vaccinated women suggests that a 9-valent HPV vaccine that covers HPV52 and HPV58[25] should be licensed early in Japan. Interestingly, however, the prevalence of HPV31/33/45 in cervical lesions was not increased among those vaccinated. This might imply cross-protection effects of HPV vaccines against HPV31/33/45, in keeping with other clinical and population-based studies reporting cross-protection effects of HPV vaccines against HPV31/33/45.[9,11,13,14,26] However, women positive for HPV31/33/45 were very few in number compared to HPV16/18 and HPV52/58. To evaluate cross-protection effects of HPV vaccines against HPV31/33/45 in Japan, larger studies will be required.

This study has several limitations. First, we could not determine the vaccine impact on ICC due to the low incidence of ICC among women younger than 25 years. In the present study, only 20 cases of ICC were registered among women aged less than 25 years. Second, information on sexual history was not available in the present study. In the 1994-1996 birth cohorts, 3 women had HPV16/18-positive CIN2-3/AIS (n = 2) or ICC (n = 1) following prior HPV vaccination. All 3 women had received 3 doses but were 16 years old at the time of first vaccination. These data support the Japanese guidelines recommending HPV vaccination most highly for girls younger than 15 years of age,[3] but it remains unclear whether these breakthrough cases received HPV vaccination after first sexual intercourse. Third, the data about vaccination status were from self-reports. A recent study reported that approximately 20% of young Japanese women incorrectly reported their HPV vaccination status.[27] Therefore, possible misclassification of vaccination status could have affected the results. In addition, the type of HPV vaccines received (bivalent or quadrivalent) was unknown in 37.6% (59/157) of vaccinated women. Thus, we could not analyze the data separately for bivalent and quadrivalent HPV vaccines owing to the small sample size.

The present study also provided baseline data for the next phase of the MINT study, MINT study II, which started in April 2019. Briefly, MINT study II uses almost the same study designs and registration of women with CIN/AIS and ICC will be resumed in August 2019. In MINT study I, only 20 cases of ICC were registered among women younger than 25 years of age, whereas 126 ICC cases were registered among women aged 25-29 years. Therefore, in the MINT study II, we might be able to confirm a vaccine impact on ICC in the next 5 years because the 1994-1999 birth cohorts with high vaccination coverage will soon reach the age range of 25-29 years. Among women aged 25-29 years, HPV16/18 prevalence in ICC cases was 83.3% at baseline in MINT study I, but is expected to decrease in MINT study II. Long-term changes in the number of women diagnosed with CIN2-3/AIS or ICC, those receiving hysterectomy for these diseases, and deaths from ICC and positive rates for vaccine types and nonvaccine types will also be monitored. We will be able to assess whether the HPV vaccination "crisis" in Japan might again raise the HPV16/18 prevalence among young women.[8,9] Sexual history information, such as age at first sex, will be collected in MINT study II.

In conclusion, the present study provided the first information on the vaccine type-specific impact of routine and catch-up HPV vaccinations on cervical abnormalities in Japan. Older adolescents born in or after 2000 and who missed HPV vaccination because of the Japanese government's suspension of its vaccine recommendation will soon be 20 years old. Our data suggested that women who did not receive HPV vaccination at age 12-16 years can still benefit from considerable protection if they receive catch-up vaccination by the age of 20 years, but did not support catch-up vaccination of women older than 20 years. Therefore, older adolescents born in 2000 or later and who did not receive routine HPV vaccination at age 12-16 years should receive catch-up vaccination as early as possible. To encourage catch-up vaccination, the Japanese government will need to immediately resume its proactive recommendation for HPV vaccination.

4.1 | Participating institutions

The participating institutions are as follows: Hokkaido University Graduate School of Medicine and Faculty of Medicine; Tohoku University Graduate School of Medicine; Jichi Medical University; University of Tsukuba; Saitama Cancer Center; Saitama Medical University International Medical Center; National Cancer Center Hospital; Cancer Institute Hospital; Keio University School of Medicine; Kanagawa Cancer Center; Kyoto University Graduate

School of Medicine; Kindai University Faculty of Medicine; Osaka International Cancer Institute; Hyogo Cancer Center; Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences; National Hospital Organization Shikoku Cancer Center; Kyusyu University; NHO Kyusyu Cancer Center; Kurume University School of Medicine; Kumamoto University; and University of the Ryukyus.

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DISCLOSURE

The authors have no conflict of interest relevant to this article.

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