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Age-related differences in antibody avidities to pertussis toxin and filamentous hemagglutinin in a healthy Japanese population

Rei Fumimoto [a,b], Nao Otsuka [a], Tomimasa Sunagawa [c], Keiko Tanaka-Taya [c], Hajime Kamiya [c], Kazunari Kamachi [a,*]

[a] Department of Bacteriology II, National Institute of Infectious Diseases, Japan
[b] Department of Pediatrics, St Marianna University School of Medicine, Japan
[c] Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Japan

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ABSTRACT

To gain insights into the current Japanese pertussis immunization schedule, we examined the distributions of antibody titers and avidities to pertussis toxin (PT) and filamentous hemagglutinin (FHA) in 460 Japanese healthy subjects (aged 1–60 years) based on age category. Our avidity enzyme-linked immunosorbent assays revealed that young children aged 1–2 years, which corresponded to ages after receiving primary and/or booster pertussis vaccinations, had relatively high-avidity anti-PT IgG (mean avidity index [AI], 40.5%) compared with other age groups (AI, 26.5–31.9%); however, they had relatively low-avidity anti-FHA IgG (AI, 41.8%). In contrast, children aged 3–6 years had both low-avidity anti-PT IgG (AI, 26.5%) and low-avidity anti-FHA IgG (AI, 40.4%). A significant age-related difference in anti-PT IgG avidity was observed between children aged 1–2 years and 3–6 years ($P < 0.05$); however, the difference in anti-FHA IgG avidity was not significant. The anti-PT IgG avidity was positively correlated with the antibody titer, especially among children aged 1–15 years ($r_s = 0.508–0.685$; $P < 0.01$), indicating that the avidity of vaccine-induced anti-PT IgG decreases with decreasing IgG antibody titer to PT. Our findings strongly suggest that vaccine-induced anti-PT IgG avidity rapidly wanes after vaccination, but this is not observed for anti-FHA IgG avidity. Because children aged 3–6 years have both low-quantity and low-quality antibodies against PT, an additional booster vaccination with acellular pertussis vaccines is required for such children in Japan.

1. Introduction

Pertussis (whooping cough) is a highly contagious disease caused by the bacterial pathogen *Bordetella pertussis* [1]. This disease is associated with severe acute respiratory illness among children and a persistent cough among adolescents and adults. Since adolescents and adults constitute the primary reservoir for this pathogen, they play a crucial role in its transmission to children, especially infants and those who are unvaccinated [2,3]. In Japan, pertussis vaccination was introduced in 1950 using whole-cell pertussis vaccines (WCVs); thereafter, acellular pertussis vaccines (ACVs) were introduced in 1981. ACVs have been used according to the routine Japanese immunization schedule, which entails three primary doses (at the ages of 3, 4, and 5 months) and a single booster dose (at the age of 18–23 months) [4]. Vaccination is the most effective method for preventing and controlling pertussis, but pertussis vaccines are unable to provide lifelong immunity [5,6]. Older children, adolescents, and adults as well as prevaccine-aged infants, are at risk for pertussis. A recent meta-analysis estimated that the duration of protective immunity from pertussis is approximately 3 years after the last dose of ACV, assuming 85% vaccine efficacy [7]. Waning immunity after receiving ACVs was a major contributor to the recent resurgence of pertussis among older children and adolescents [8,9], which has been observed in several industrialized countries that have high vaccination coverage [10–13]. In Japan, the incidence of pertussis among adolescents and adults has significantly increased since the early 2000 s [14].

Pertussis toxin (PT) and filamentous hemagglutinin (FHA) derived from *B. pertussis* are the major antigens in ACVs. Therefore, the serum titers of pertussis IgG antibodies against PT and FHA are assessed to monitor vaccine-induced herd immunity [15–18]. Vaccine-induced anti-PT IgG titers rapidly wane over time, whereas anti-FHA IgG titers do not [15,16,18]. To assess the quality

* Corresponding author at: Department of Bacteriology II, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan.
E-mail address: kamachi@nih.go.jp (K. Kamachi).

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of pertussis antibodies, antibody avidities of anti-PT IgG and anti-FHA IgG for their antigens can also be evaluated using enzyme-linked immunosorbent assays (ELISAs) with dissociating agents [19,20]. Avidity ELISA measures the strength of antibody-antigen binding, thereby providing a measure of the quality of the antibodies (i.e., antibody affinity maturation). In previous studies of *Haemophilus influenzae* type b and *Streptococcus pneumoniae* type 6B and 23F vaccines, higher antibody avidities were correlated with higher bactericidal activities [21–23]. As for ACVs, booster vaccinations with ACVs induced an increase in the avidity of anti-PT IgG and anti-FHA IgG among children, adolescents, and pregnant women [19,20,24–27]. The enhanced antibody avidity to PT persist for 1 year following the booster vaccination in children [25], but not for 2 years [28]. However, in our previous study, no significant age-related difference in anti-PT IgG avidity was observed between children and adults [29]. Serum anti-PT IgG titers waned rapidly after ACV vaccination; however, little is known about the waning of anti-PT IgG avidity, including anti-FHA IgG avidity.

In most industrialized countries, the pertussis booster vaccination has been included in immunization schedules for adolescents and adults. However, in Japan, no additional booster vaccination has been introduced into the routine pertussis immunization schedule, not only for adolescents and adults, but also for preschool- and school-aged children. In the present study, we therefore examined distributions of antibody avidities of anti-PT IgG and anti-FHA IgG in a healthy Japanese population with a wide age range (1–60 years of age), to gain insight into the current Japanese pertussis immunization schedule. We also evaluated the correlations between the antibody titers and avidities in subjects based on age.

2. Materials and methods

2.1. Serum samples

A total of 460 serum samples were obtained from the National Serum Reference Bank of the National Institute of Infection Disease (Tokyo, Japan). The samples had been collected from 460 healthy subjects (1–60 years) from 2015 to 2016 in Japan: 236 and 224 samples were collected in 2015 and 2016, respectively. The 460 serum samples were divided into nine groups based on the subjects age (20, 21, 35, 43, 34, 77, 77, 77, and 76 samples were included in the 1–2, 3–6, 7–10, 11–15, 16–20, 21–30, 31–40, 41–50, and 51–60 years age categories, respectively) (Table 1). The National Serum Reference Bank prohibits the handling of personal information, with the exception of age, sex, residential area, and date of serum collection. Hence, the subjects' medical and vaccination histories, which were considered personal information, were not available. The present study's design was reviewed and approved by the Human Ethics Committee of the National Institute of Infectious Diseases (approval number 846). All subjects provided informed consent.

2.2. In-house ELISA for anti-PT IgG and anti-FHA IgG

Serum titers of anti-PT and anti-FHA IgG were measured using an in-house ELISA as previously described, but with minor modifications [29]. Briefly, 96-well ELISA plates were coated with PT (The Chemo-Sero-Therapeutic Research Institute [Kaketsuken], Japan) or FHA (Enzo Life Sciences, USA). After blocking with a skim milk solution, a 1:200 diluted serum without heat treatment was added and incubated. The bound antibodies were reacted with alkaline phosphatase-labeled secondary antibody, and *p*-nitrophenyl phosphate was used to develop the color. Absorbance at 405 nm was measured using 650 nm as a reference. Standard curves for anti-PT IgG and anti-FHA IgG were generated from a dilution series (1–160 EU/mL) of Japanese human reference serum (JNIH-10, working standard) in each plate. Serum samples with low- and high-antibody titers were assayed at 1:20 and 1:800 serum dilutions, respectively. The antibody titers were calculated in international units (IU)/mL using the following unit conversions: anti-PT IgG, IU/mL = 1.19 × EU/mL; anti-FHA IgG, IU/mL = 1.01 × EU/mL [29].

2.3. Avidity ELISA for anti-PT IgG and anti-FHA IgG

The avidity values for anti-PT IgG and anti-FHA IgG were measured using 1.5 M NH_4SCN as the dissociating agent [19,26,29]. The avidity assay was performed in the same manner as our in-house ELISA, and an NH_4SCN incubation step was added after the serum incubation [29]. The avidity index (AI) was calculated as: ([sample OD_{405} − blank OD_{405} in the presence of NH_4SCN]/[sample OD_{405} - − blank OD_{405} in the absence of NH_4SCN]) × 100%.

The reference serum JNIH-10 was used as a positive control in each plate (160 EU/mL for both anti-PT IgG and anti-FHA IgG). The mean AI value of JNIH-10 for anti-PT IgG was 51.6% with an 8.2% inter-assay coefficient of variation (14 determinations), and that for anti-FHA IgG was 66.0% with a 6.5% inter-assay coefficient of variation (13 determinations).

2.4. Statistical analyses

The Mann-Whitney *U* test was used to evaluate differences in antibody titer and avidity. *P*-values of <0.05 were considered statistically significant. The Spearman's rank test was used to evaluate the correlations between antibody titer and avidity.

3. Results

3.1. Antibody titers and avidities of anti-PT IgG and anti-FHA IgG

Table 1 summarizes the geometric mean titers and avidities of anti-PT IgG and anti-FHA IgG based on age group. The mean anti-PT IgG titer was the highest in the 1–2 years age group (45.1 IU/mL) and the lowest in the 51–60 years age group (7.9 IU/mL).

Table 1
Geometric mean titers and avidities of anti-PT IgG and anti-FHA IgG based on age grouping.

Age group (years)	Mean age (years)	No. of serum samples	Anti-PT IgG		Anti-FHA IgG	
			IU/mL (95% CI)	AI (%) (95% CI)	IU/mL (95% CI)	AI (%) (95% CI)
1–2	1.4	20	45.1 (31.2–65.3)	40.5 (33.7–48.7)	35.5 (25.2–50.0)	41.8 (35.4–49.4)
3–6	4.5	21	8.2 (4.4–15.3)	26.5 (20.6–34.2)	19.2 (10.9–33.8)	40.4 (31.2–52.3)
7–10	8.7	35	10.4 (6.7–16.1)	29.0 (23.5–35.8)	30.9 (22.0–43.5)	50.0 (43.1–58.0)
11–15	12.6	43	11.5 (8.1–16.5)	30.6 (25.9–36.2)	28.6 (20.0–40.8)	49.1 (43.7–55.1)
16–20	18.2	34	10.4 (7.3–14.8)	31.9 (27.4–37.1)	30.2 (21.1–43.2)	50.4 (43.5–58.3)
21–30	25.5	77	10.1 (7.8–13.0)	30.0 (26.7–33.6)	28.1 (22.8–34.7)	47.3 (43.6–51.4)
31–40	35.4	77	9.1 (6.9–12.0)	30.4 (27.2–34.1)	27.3 (22.2–33.7)	46.5 (42.2–51.3)
41–50	45.3	77	13.0 (9.8–17.2)	28.0 (23.9–32.9)	22.1 (17.5–27.9)	40.9 (37.2–44.9)
51–60	55.3	76	7.9 (5.9–10.5)	29.1 (25.7–33.0)	17.1 (14.0–20.9)	39.4 (36.0–43.2)

IU, international unit; AI, avidity index; CI, confidence interval.

Significant age-related differences were observed between the 1–2 years age group and other age groups ($P < 0.01$) (Fig. 1A). Similarly, the mean anti-FHA IgG titer was the highest in the 1–2 years age group (35.5 IU/mL) and the lowest in the 51–60 years age group (17.1 IU/mL). A significant age-related difference in the titers was observed between the age groups ($P < 0.01$) (Fig. 1B). Notably, the children aged 3–6 years exhibited much lower mean titers of anti-PT IgG and anti-FHA IgG when compared with the young children aged 1–2 years.

To assess the quality of pertussis antibodies, we evaluated antibody avidities of anti-PT IgG and anti-FHA IgG based on age (Table 1, Fig. 1C and D). The 1–2 years age group exhibited the highest mean AI value for anti-PT IgG (AI, 40.5%), whereas the other age groups exhibited much lower mean AI values (AI, 26.5–31.9%); the lowest AI value was among the 3–6 years age group (AI, 26.5%). There were significant age-related differences in the AI values between the 1–2 years age group and the other age groups ($P < 0.05$), with the exception of the 11–15 years age group (Fig. 1C). In contrast, the highest AI value for anti-FHA IgG was observed in the 16–20 years age group (AI, 50.4%), and the lowest AI value was in the 51–60 years age group (AI, 39.4%) (Fig. 1D). Significantly higher AI values for anti-FHA IgG were observed in the 7–10 and 16–20 years age groups than in the 1–2 years age group ($P < 0.05$). Of note, when compared with the young children aged 1–2 years, the children aged 3–6 years exhibited a much lower-avidity anti-PT IgG (AI, 26.5% versus 40.5%; $P < 0.01$), but exhibited the same level of anti-FHA IgG avidity (AI, 40.4% versus 41.8%; $P = 0.715$).

3.2. Correlation between antibody titer and avidity of anti-PT IgG

First, we evaluated the correlation between antibody titer and avidity for anti-PT IgG using all 460 serum samples from subjects aged 1–60 years. As shown in Fig. 2A, the AI value was positively correlated with the anti-PT IgG titer (correlation coefficient $r_s = 0.498$; $P < 0.01$). Next, we evaluated the correlation based on age groupings. As shown in Fig. 3, all age groups exhibited positive correlations ($r_s = 0.288$–0.753) which were statistically significant ($P < 0.01$), with the exception of the 16–20 years age group. Moderate correlations were observed in the 1–2, 3–6, 7–10, and 11–15 years age groups ($r_s = 0.508$–0.685), and a strong correlation was observed in the 41–50 years age group ($r_s = 0.753$). With the exception of the 41–50 years age group, the correlations tended to decrease with increasing age.

3.3. Correlation between antibody titer and avidity of anti-FHA IgG

As shown in Fig. 2B, the AI value for anti-FHA IgG was positively correlated with the anti-FHA IgG titer among all 460 serum samples ($r_s = 0.653$; $P < 0.01$). All age groups exhibited positive correlations ($r_s = 0.489$–0.782; $P < 0.01$) (Fig. 4). There were strong correlations in the 7–10, 11–15, and 16–20 years age groups ($r_s = 0.728$–0.782), and moderate correlations in the other age groups ($r_s = 0.489$–0.652). The correlations tended to be stronger in preteens and teens.

3.4. Correlation between antibody avidities of anti-PT IgG and anti-FHA IgG

Among all 460 serum samples, a weak positive correlation was observed between the AI values for anti-PT IgG and anti-FHA IgG ($r_s = 0.381$; $P < 0.01$) (Supplementary Fig. 1). The positive correlations were seen among all age groups ($r_s = 0.328$–0.580; $P < 0.05$), with the exception of the 16–20 years age group ($r_s = 0.022$; $P > 0.05$) (Supplementary Fig. 2). There were moderate correlations

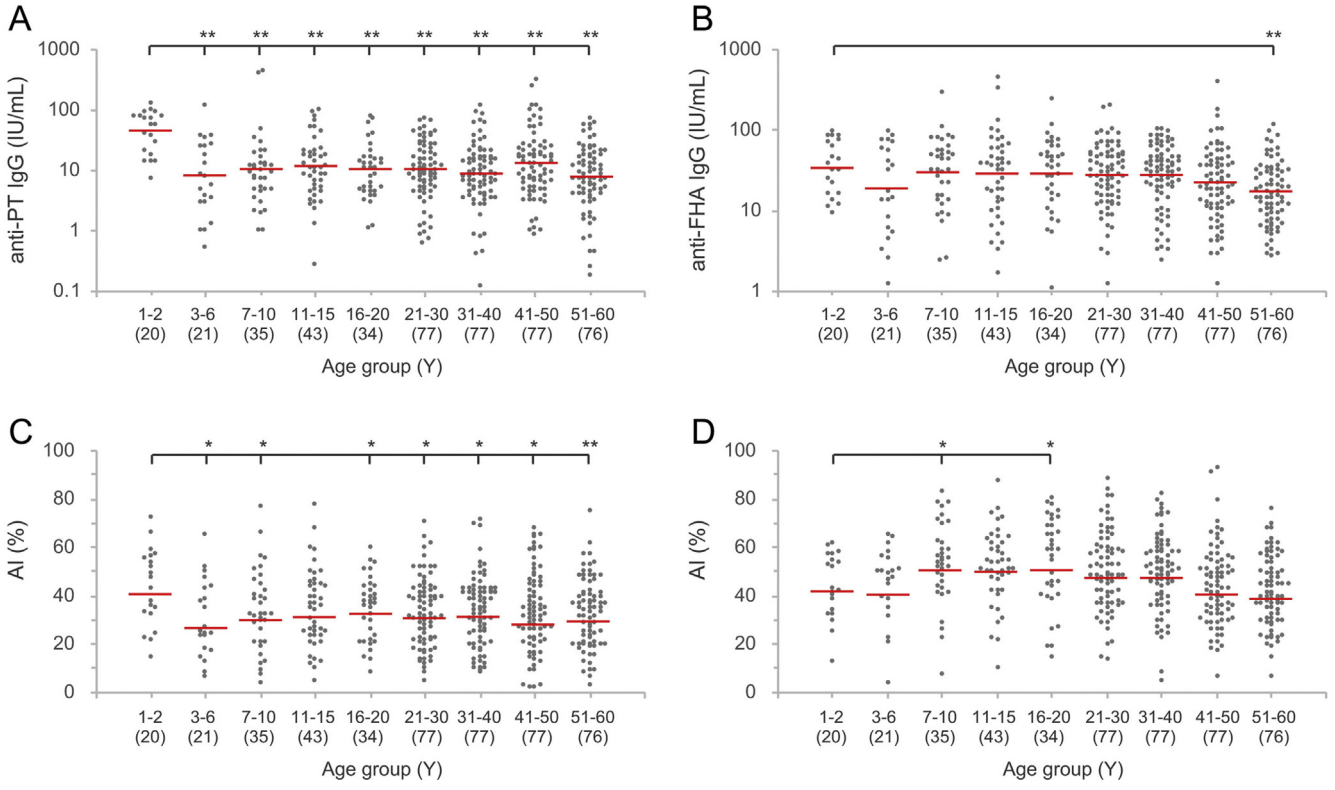

Fig. 1. Distributions of antibody titers and avidities of anti-PT IgG and anti-FHA IgG based on age grouping. (A) anti-PT IgG titer, (B) anti-FHA IgG titer, (C) anti-PT IgG avidity, (D) anti-FHA IgG avidity. Bars indicate the geometric mean values. Numbers in parentheses indicate the number of serum samples that were analyzed. AI, avidity index; Y, years. Statistically significant differences between the 1–2 years age group and the other age groups are indicated by asterisks (*$P < 0.05$; **$P < 0.01$, Mann-Whitney U test).

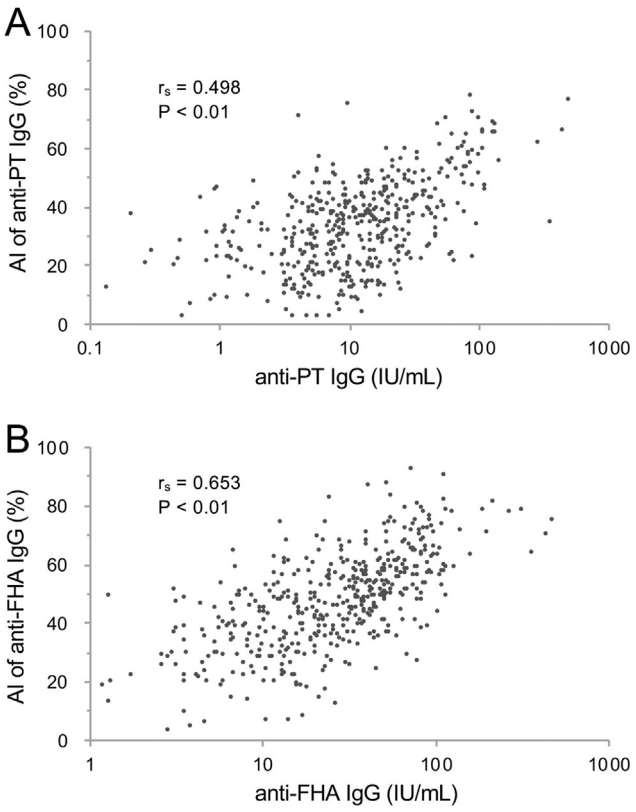

Fig. 2. Correlations between IgG antibody titer and avidity in all healthy subjects. (A) anti-PT IgG, (B) anti-FHA IgG. The IgG titers in subjects' serum samples (n = 460) were plotted against avidity index (AI) values. The correlation coefficients and *P*-values were determined using the Spearman's rank test.

in the 1–2, 3–6, 7–10, and 41–50 years age groups (r_s = 0.475–0.580). With the except of the 41–50 years age group, the correlation tended to decrease with increasing age.

4. Discussion

In the present study, young children aged 1–2 years, which corresponded to the ages after receiving primary and/or booster pertussis vaccinations, showed the highest mean titers of both anti-PT IgG and anti-FHA IgG. They had high-avidity anti-PT IgG when compared with other age groups but had low-avidity anti-FHA IgG. In contrast, children aged 3–6 years (mean age, 4.5 years) had low-avidity for both anti-PT IgG and anti-FHA IgG with low titers of the antibodies. Our findings suggest that vaccine-induced anti-PT IgG avidity rapidly wanes after ACV vaccination; however, this was not observed for the avidity of anti-FHA IgG. To the best of our knowledge, this is the first study to show age-related differences in the pertussis antibody avidities in healthy subjects across a broad age range.

Vaccine-induced pertussis antibodies rapidly wane with age [5,6]. The duration of immunity after vaccination is estimated to be in the range 4–12 years [6,30]. A recent study showed that anti-PT and anti-FHA antibodies waned after 5 years and 5–10 years, respectively [31]. Here, we observed that Japanese children aged 3–6 years exhibited markedly lower mean titers of both anti-PT IgG and anti-FHA IgG, compared to children aged 1–2 years. Unfortunately, vaccine histories of our subjects were unavailable due to privacy protection policies in Japan. However, since childhood immunization rates with ACVs are high in Japan (≥90%), we concluded that the low seroprevalences in the children aged 3–6 years were caused by waning immunity of ACVs. The seroprevalences of anti-PT IgG and anti-FHA IgG (across all age groups) were in good agreement with the previous data from the Japanese national pertussis serosurveillance conducted in 2013 (the National Epidemiological Surveillance of Vaccine-Preventable Disease, https://www.niid.go.jp/niid/ja/y-graphs/1600-yosoku-index-e.html).

Antibody avidity represents a functional measure of antibody affinity maturation. High-avidity antibodies are considered to contribute to protection against viral and bacterial infections. Antibody avidity of anti-PT IgG was higher after natural infection with *B. pertussis* than after booster vaccination with an ACV, and healthy individuals (mean age, 15.5 years) had low-avidity anti-PT IgG [20]. Here, we showed that young children aged 1–2 years had high-avidity anti-PT IgG (mean AI, 40.5%) when compared with other age groups (AI, 26.5–31.9%), whereas children aged 3–6 years had low-avidity anti-PT IgG (AI, 26.5%). The anti-PT IgG avidity was positively correlated with the antibody titer, especially among children aged from 1 to 15 years (r_s = 0.508–0.685), indicating that the avidity of vaccine-induced anti-PT IgG decreases with decreasing IgG antibody titer to PT. Our observation is in agreement with a previous study that found that antibody avidity to PT did not persist for 2 years after booster vaccinations were given to children [28]. In this study, we also confirmed that other age groups (7–10, 11–15, 16–20, 21–30, and 41–50 years of age) exhibited slightly higher levels of anti-PT IgG avidity (AI, 28.0–31.9%) when compared with children aged 3–6 years. Moreover, higher mean titers of anti-PT IgG were observed in all the age groups, but there were no statistical differences with children aged 3–6 years ($P > 0.05$). Since the antibody response to PT is specific to *B. pertussis* infection, a plausible explanation for the higher avidity of anti-PT IgG is that the natural infection with *B. pertussis* frequently occurs in older children, adolescents, and adults [29]. Taken together, vaccination with ACVs can induce high-avidity anti-PT IgG; however, the high-avidity antibody rapidly wanes with age. Japanese children aged 1–2 years had high-avidity anti-PT IgG with high titers, but children aged 3–6 years had low-avidity anti-PT IgG with low titers. An additional booster vaccination with ACVs should therefore be required for Japanese children aged 3–6 years.

In the present study, we showed that antibody avidity of anti-FHA IgG was lower in the 1–2 and 3–6 years age groups (mean AI, 40.4–41.8%), when compared with older children and adolescents (AI, 49.1–50.4%). Surprisingly, although a significant age-related difference in anti-PT IgG avidity was observed between the age groups, this was not also observed for anti-FHA IgG avidity. The children aged 3–6 years exhibited a lower mean titer of anti-FHA IgG than the children aged 1–2 years; however, they exhibited a similar mean AI value for anti-FHA IgG. Correlation analysis also confirmed that both the age groups exhibited moderate positive correlations between the antibody titers and the AI values (r_s = 0.587 and 0.604). These observations lead us to the hypothesis that vaccine-induced low-avidity anti-FHA IgG may persist longer in children. A booster vaccination with ACVs induces an increase of anti-FHA IgG avidity [19,26]; however, no previous studies have described the duration of anti-FHA IgG avidity. Further studies are thus needed to test this hypothesis.

Our avidity ELISA showed that older children and adolescents aged 7–10 and 16–20 years, respectively, had significantly higher-avidity anti-FHA IgG when compared with young children aged 1–2 years ($P < 0.05$). Our study also showed that the older children and adolescents exhibited higher mean titers of anti-FHA IgG. Previous studies have demonstrated that the seroprevalence of anti-FHA IgG tends to remain constant or increase with age [15,16]. A possible explanation for this pattern is that antibody response to FHA is not specific to *B. pertussis* infection,

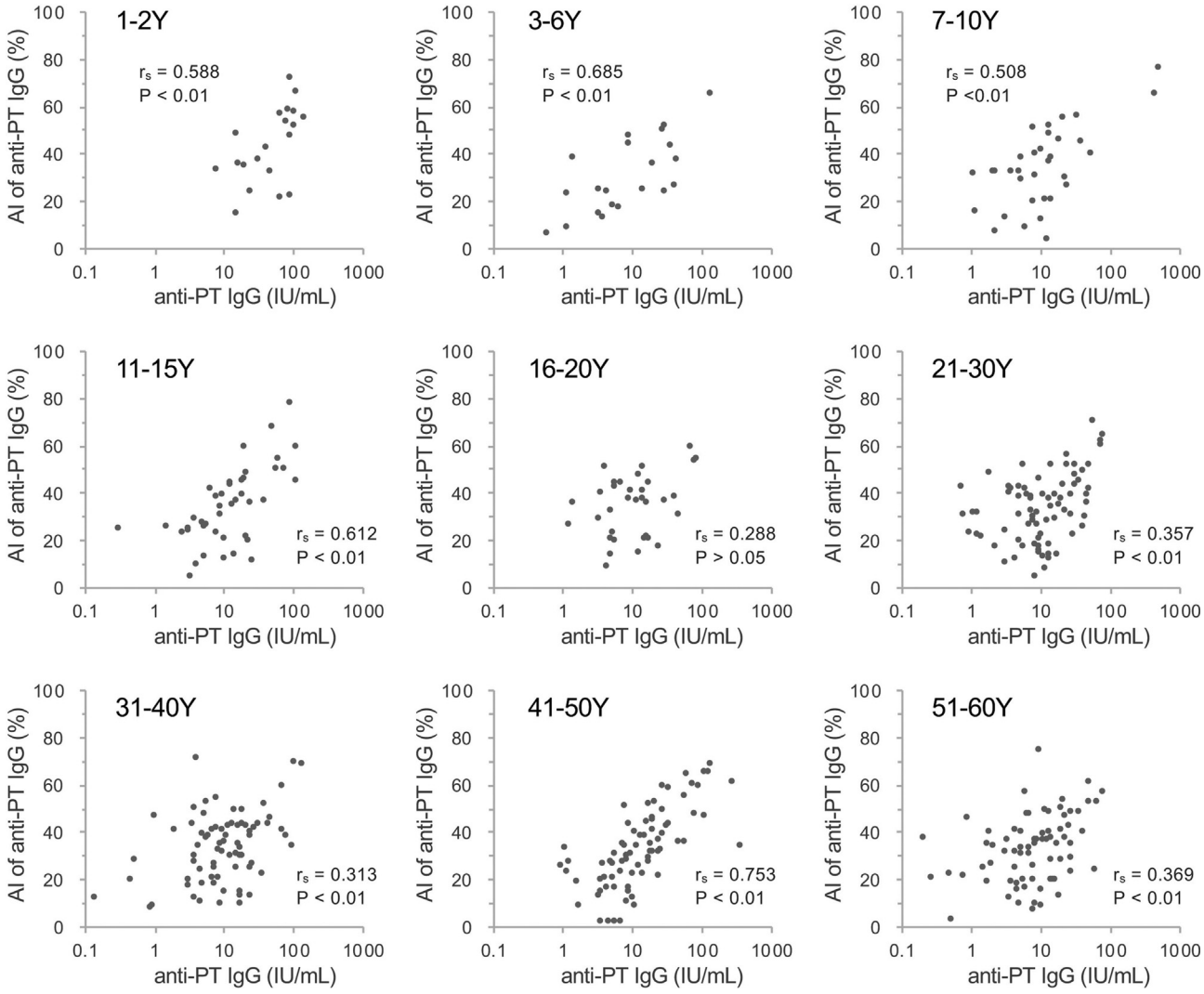

Fig. 3. Correlations between anti-PT IgG titer and avidity based on age grouping. The IgG titers in subjects' serum samples were plotted against avidity index (AI) values: 20, 21, 35, 43, 34, 77, 77, 77, and 76 samples were included in the 1–2, 3–6, 7–10, 11–15, 16–20, 21–30, 31–40, 41–50, and 51–60 years age categories, respectively. Y, years. The correlation coefficients and *P*-values were determined using the Spearman's rank test.

i.e., anti-FHA IgG would be produced not only after contact with *B. pertussis* but could also be induced by contact with other human respiratory pathogens such as *Bordetella parapertussis* and *Mycoplasma pneumoniae* [32–36]. Previously, it was shown that high avidity anti-PT IgG was more frequently induced after natural infection with *B. pertussis* than after ACV booster vaccinations [20]. As there are no reports of changes in anti-FHA IgG avidity after *B. pertussis* infection, we believe that the higher titer and avidity of anti-FHA IgG in the older children and adolescents might be mainly caused by true *B. pertussis* infections.

In Japan, ACVs were introduced in 1981 instead of WCVs. In this study, the subjects aged less than 34 years would have had ACVs during childhood, whereas individuals older than 35 years of age would have had WCVs. Interestingly, it was shown that the antibody avidity of anti-FHA IgG was significantly lower in the older age groups (41–50 and 51–60 years of age, which corresponds to the WCVs ages), when compared with the younger age groups (7–10, 11–15, 16–20, 21–30 years, the ACVs ages) (mean AI, 39.4–40.9% versus 47.3–50.4%; $P < 0.05$). In contrast, no significant difference in the avidity of anti-PT IgG was observed between the older and younger age groups ($P > 0.05$). The vaccine type used in childhood might affect the antibody avidity of anti-FHA IgG in adulthood.

In the present study, young children aged 1–2 years had low-avidity anti-FHA IgG, despite the fact that they had high-avidity anti-PT IgG. ACVs contain both formalin-treated PT and FHA as major antigens. An animal study previously demonstrated that formalin treatment with high concentrations did not affect the production of anti-PT antibodies, but it reduced the production of neutralizing antibodies against PT [37]. The young children aged 1–2 years had low-avidity anti-FHA IgG, suggesting that ACV vaccination is difficult to induce high-avidity antibodies to FHA among this age group. Further studies are required to elucidate the cause of low-avidity anti-FHA IgG in young children aged 1–2 years.

In conclusion, our results confirm that Japanese children aged 3–6 years had significantly lower titers and avidities of anti-PT IgG when compared with young children aged 1–2 years. These findings suggest that high-avidity anti-PT IgG rapidly wanes after ACVs vaccination. Since the children aged 3–6 years have both low-quantity and low-quality antibodies to PT, an additional booster vaccination with ACVs should be required for this age group in Japan.

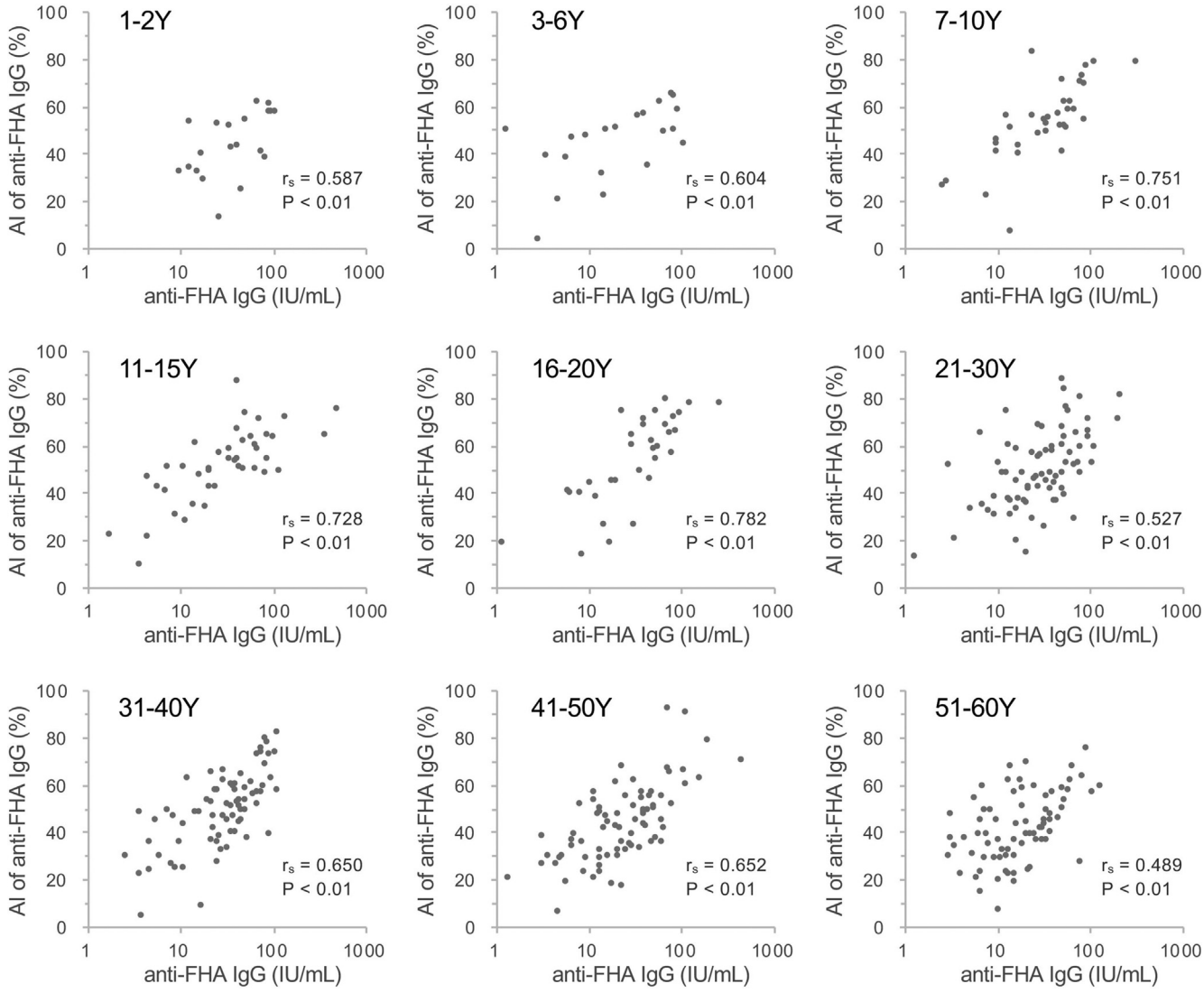

Fig. 4. Correlations between anti-FHA IgG titer and avidity based on age grouping. The IgG titers in subjects' serum samples were plotted against avidity index (AI) values: 20, 21, 35, 43, 34, 77, 77, 77, and 76 samples were included in the 1–2, 3–6, 7–10, 11–15, 16–20, 21–30, 31–40, 41–50, and 51–60 years age categories, respectively. Y, years. The correlation coefficients and *P*-values were determined using the Spearman's rank test.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.03.055.

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