

Fc functional antibody responses to adjuvanted versus unadjuvanted seasonal influenza vaccination in community-dwelling older adults



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ABSTRACT

Background: Seasonal influenza vaccination with a standard trivalent influenza vaccine (TIV) induces a modest, and cross-reactive, Fc functional antibody response in older adults. Recent improvements to influenza vaccines include a quadrivalent influenza vaccine (QIV) and a TIV adjuvanted with the squalene-based oil-in-water emulsion MF59.

Methods: Pre- and post-vaccination serum samples from older adults vaccinated with QIV (n = 27) and adjuvanted TIV (n = 44) were studied using hemagglutination inhibition (HAI) assays and dimeric Fc-gamma receptor IIIa binding ELISAs, as a surrogate of antibody-dependent cellular cytotoxicity (ADCC).

Results: We found that the unadjuvanted QIV elicited a stronger HAI response against the H1N1 vaccine virus than the adjuvanted TIV. Post-vaccination levels of HA-specific ADCC antibodies were similar for older adults vaccinated with QIV and adjuvanted TIV. The ADCC response to influenza vaccination was largely determined by pre-vaccination or baseline levels of these antibodies, with older adults with low baseline levels of ADCC activity demonstrating greater post-vaccination rises.

Conclusions: In this cohort of community-dwelling older adults, the QIV was at least as good as the adjuvanted TIV in the induction of ADCC and HAI responses. Further studies on how these antibody responses translate to efficacy in preventing influenza infections are warranted.

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1. Introduction

Antibodies are a key component of protective immunity during human influenza infection [1]. Influenza vaccination can induce neutralising antibodies capable of blocking influenza virus entry into cells and preventing infection [2,3]. The seasonal influenza vaccine is updated and administered yearly but provides limited protection when vaccine and circulating strains of influenza virus are mismatched [4,5]. Influenza vaccine responsiveness declines with age [6–8], and older adults demonstrate reduced levels of influenza-specific memory B-cells, long-lived plasma cells and serum antibodies following vaccination compared to younger

adults [9,10]. Immune senescence and underlying comorbidities make older adults more susceptible to influenza-related complications such as pneumonia, hospitalisation and death [11]. Seasonal influenza vaccination is recommended for older adults and decreases influenza infections in this age group [12–15]. Adjuvanted and high dose influenza vaccines can improve vaccine effectiveness in older adults [16–20]. To date, adjuvanted and high dose formulations of the seasonal influenza vaccine are trivalent, containing two subtypes of influenza A virus and one influenza B virus. The standard-dose unadjuvanted seasonal influenza vaccine is now available in quadrivalent form with two influenza A viruses as well as two influenza B viruses, one from each influenza B lineage [21]. The current trivalent adjuvanted influenza vaccine may, therefore, elicit a poorer humoral immune response against one lineage of influenza B virus than the quadrivalent standard-dose unadjuvanted influenza vaccine.

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Hemagglutination inhibition (HAI) has been a valuable surrogate assay of vaccine-induced immunity against influenza virus [22]. HAI assays detect a subset of strain-specific influenza antibodies that bind to epitopes surrounding the receptor binding site of hemagglutinin (HA) and block viral entry. Antibody-dependent cellular cytotoxicity (ADCC) is, however, increasingly recognized as a mediator of influenza immunity [23–34]. ADCC is induced when Fc γ receptors (Fc γ R) on innate immune cells interact with the Fc region of IgG bound to viral proteins on the surface of virally infected cells. Cross-linking of Fc γ R on the immune cell leads to activation and the release of antiviral cytokines and cytotoxic granules. ADCC is mainly mediated by human Fc γ RIIIa found on natural killer (NK) cells, monocytes, and macrophages [35]. Antibodies with ADCC activity can be highly cross-reactive and frequently target more conserved epitopes [23,24], which is advantageous for protection against different subtypes and vaccine mismatched strains of influenza virus. We have previously shown that seasonal influenza vaccination with a standard-dose trivalent influenza vaccine (TIV) induced a modest, but heterogeneous, ADCC response in a cohort of older adults [36]. Whether the use of adjuvanted influenza vaccines can improve this ADCC response in older adults remains uncertain.

Two new TIVs were licensed in Australia for older adults (≥ 65 years) in 2018: a TIV adjuvanted with the squalene-based oil-in-water emulsion MF59 (TIV-A), and a TIV containing four times more antigen than the standard influenza vaccine [37]. In adults, MF59-adjuvanted influenza vaccines increased the magnitude and breadth of the neutralising antibody response [38–40]. Previous studies have also shown that TIV-A can improve protective immunity in some groups of older adults [19,41,42], but whether the protective advantage afforded by this vaccine outweighs the lack of a second influenza B virus remains unclear in the context of healthy older adults. Herein, we compare the HAI and ADCC antibody responses elicited by TIV-A with the standard quadrivalent influenza vaccine (QIV) in community-dwelling older adults.

2. Materials and Methods

2.1. Influenza vaccination cohort

This study was approved by the Human Research Ethics Committee at the Women's and Children's Health Network in Adelaide, South Australia. A cohort of 71 adults aged 65 years and older were vaccinated with either the 2017 QIV (Fluarix Tetra from GSK) or the 2018 TIV-A (Fluad from Seqirus). Pre-vaccination serum samples were taken prior to vaccination and post-vaccination serum samples were collected 28 days after vaccination. In 2017, a group of 27 older adults were vaccinated with the 2017 Southern Hemisphere QIV composed of 15 μ g of inactivated A/Michigan/45/2015 (H1N1) pandemic-like 2009 virus, A/Hong Kong/4801/2014 (H3N2) virus, B/Phuket/3073/2013 virus (B/Yamagata/16/88 lineage) and B/Brisbane/60/2008 (B/Victoria/2/87 lineage) virus. In 2018, 44 older adults were vaccinated with the 2018 Southern Hemisphere TIV-A vaccine composed of 15 μ g of inactivated A/Michigan/45/2015 (H1N1) pandemic-like 2009 virus, A/Singapore/INF16H-16-0019/2016 (H3N2) virus and B/Phuket/3073/2013 virus (B/Yamagata/16/88 lineage) as well as the MF59 adjuvant. As the H3N2 virus component of the 2017 QIV and 2018 TIV-A seasonal influenza vaccines differed, no comparisons were made by HAI or Fc γ RIIIa dimer binding ELISA. The ages of the two vaccine groups were not significantly different (QIV median age = 69 years and TIV-A median age = 68 years). Furthermore, there was no significant difference in the number of self-reported seasonal influenza vaccinations administered to the groups in the past four years.

2.2. Hemagglutinin proteins and Hemagglutination inhibition assays

The recombinant HA proteins of the A/Michigan/45/2015 (H1N1), A/California/07/2009 (H1N1) and B/Phuket/3073/2013 influenza viruses as well as the gp140 envelope protein from HIV-1 (expressed in HEK293 cells) were purchased from Sinobiological (Shanghai, China). The neuraminidase (NA) protein of B/Phuket/3073/2013 (expressed in Baculovirus-Insect cells) was also purchased from Sinobiological. The HA protein of the B/Brisbane/60/2008 influenza virus was obtained from BEI Resources (expressed in Sf9 insect cells). HAI assays were performed using turkey red blood cells as previously described by Kristensen et al. [43].

2.3. Dimeric recombinant soluble Fc γ RIIIa binding ELISA

To model the requirement for ADCC antibodies to cross-link Fc γ RIIIa receptor molecules, dimeric recombinant soluble Fc γ RIIIa (Fc γ RIIIa dimer) binding ELISAs were performed as previously described [44]. Briefly, 96-well ELISA plates were coated with 50 ng/well of purified influenza HA or HIV-1 gp140 control protein overnight. Serum dilutions of 1:80 (as previously described for older adults [36]) were added, incubated for 1 h, washed, and 50 μ L of 0.1 μ g/mL biotinylated Fc γ RIIIa dimer (V176 high affinity variant) added for 1 h. Streptavidin-horseradish peroxidase (HRP) (1:10,000, ThermoFisher Scientific, Waltham, MA) was added for 1 h, washed, blotted dry, then 50 μ L of 3,3',5,5'-tetramethylbenzidine (TMB) substrate (Sigma Aldrich, St Louis, MO) was added and the plate was developed. The reaction was stopped, and absorbance read at 450 nm. Intragam 5 (5 μ g/mL, Seqirus, Melbourne, Australia) was used as a positive control and allowed for normalization between plates. Percentage (%) increase was calculated by subtracting the pre-vaccination absorbance from the post-vaccination absorbance and dividing by the pre-vaccination absorbance then multiplying by 100 [% increase = ((post-vaccination absorbance - pre-vaccination absorbance)/pre-vaccination absorbance) \times 100].

2.4. IgG ELISA

Briefly, 96-well plates were coated with 100 ng/well of HA or HIV-1 gp140 protein overnight, blocked with 5% bovine serum albumin (BSA) for 2 h at 37 $^{\circ}$ C and washed. Serial 4-fold dilutions of sera were incubated at room temperature for 2 h, then washed. Rabbit anti-human IgG horseradish peroxidase (1:4000 dilution, Aligent) was incubated for 1 h at room temperature then washed. TMB was added, colour developed, and the reaction was stopped with 0.16 M sulfuric acid. The absorbance of each well was read at 450 nm.

3. Results

3.1. HAI titres pre- and post-vaccination with QIV and TIV-A in older adults

We first compared the HAI response induced by the two different influenza vaccine formulations using HAI assays to the A/Michigan/45/2015 (H1N1) and B/Phuket/3073/2013 influenza viruses, which were common to both the QIV and TIV-A. Pre-vaccination HAI titres were not significantly different between the QIV (median HAI titre = 10) and TIV-A (median HAI titre = 20) vaccinated groups ($p = 0.96$). The standard QIV and the adjuvanted TIV-A induced a significant increase in HAI titre post-vaccination against the H1N1 and B/Yamagata lineage vaccine viruses in older adults (Fig. 1A and B). Interestingly, vaccination with the QIV

resulted in significantly higher HAI titres against the H1N1 vaccine strain (median HAI titre post-QIV = 80) than the TIV-A vaccine (median HAI titre post-TIV-A = 30, $p = 0.0006$; Fig. 1A). The post-vaccination fold increase in HAI titre against the H1N1 vaccine virus was also greater for the QIV group than the TIV-A vaccinated group (7.6-fold increase for QIV vs 1.9-fold increase for TIV-A, $p = 0.0008$; Fig. 1C). Further, 48% of QIV vaccinees demonstrated at least a 4-fold increase in HAI titre post-vaccination against the H1N1 vaccine virus compared to only 14% of the TIV-A vaccinees ($p = 0.0027$; Supplementary Table 1). In contrast, post-vaccination HAI titres, fold increases in HAI and the percentages of vaccinees who had ≥ 4 -fold increase in HAI titre were not significantly different between the QIV and TIV-A for the B/Phuket virus (Fig. 1B and D; Supplementary Table 1).

3.2. Fc γ R1IIa dimer binding antibodies pre- and post-vaccination with QIV and TIV-A in older adults

To determine whether the different influenza vaccines induced antibodies with ADCC activity in older adults, we performed Fc γ R1IIa dimer binding ELISAs to study serum antibodies with ADCC potential. The ability of antibodies to engage two or more Fc γ R1IIa receptors is critical to the initiation of ADCC and we have

previously shown that this higher throughput Fc γ R1IIa dimer ELISA correlates well with cell-based functional assays [45,46]. The pandemic A/California/07/2009 (H1N1) virus was a component of the influenza vaccine from 2009 to 2016, until it was replaced by the A/Michigan/45/2015 (H1N1) virus in the 2017 Southern Hemisphere vaccine. These two H1N1 viruses have a high amino acid sequence identity (97% in HA) but are not antigenically identical [47]. Thus, HA proteins from both A/Michigan/45/2015 and A/California/07/2009 were examined for ADCC activity since the A/Michigan/45/2015 vaccine component may boost responses from prior A/California/07/2009 infection or vaccination. Pre-vaccination levels of HA-specific Fc γ R1IIa dimer binding antibodies were highly variable within each group, but not significantly different between the two vaccine groups. Vaccination with QIV and TIV-A induced Fc γ R1IIa dimer binding antibodies in older adults against the H1N1 vaccine HA (A/Michigan/45/2015) as well as the H1N1 2009 pandemic virus HA (A/California/07/2009; Fig. 2A and B). The QIV resulted in a greater mean post-vaccination increase in Fc γ R1IIa dimer binding antibodies against both H1N1 HAs than the TIV-A (A/Michigan: QIV 243% vs. TIV-A 79% increase, $p = 0.01$; A/California: QIV 251% vs. TIV-A 74% increase, $p = 0.0067$; Fig. 2C and D). Over 59% of QIV vaccinees demonstrated at least a 2-fold increase in Fc γ R1IIa dimer binding post-vaccination against

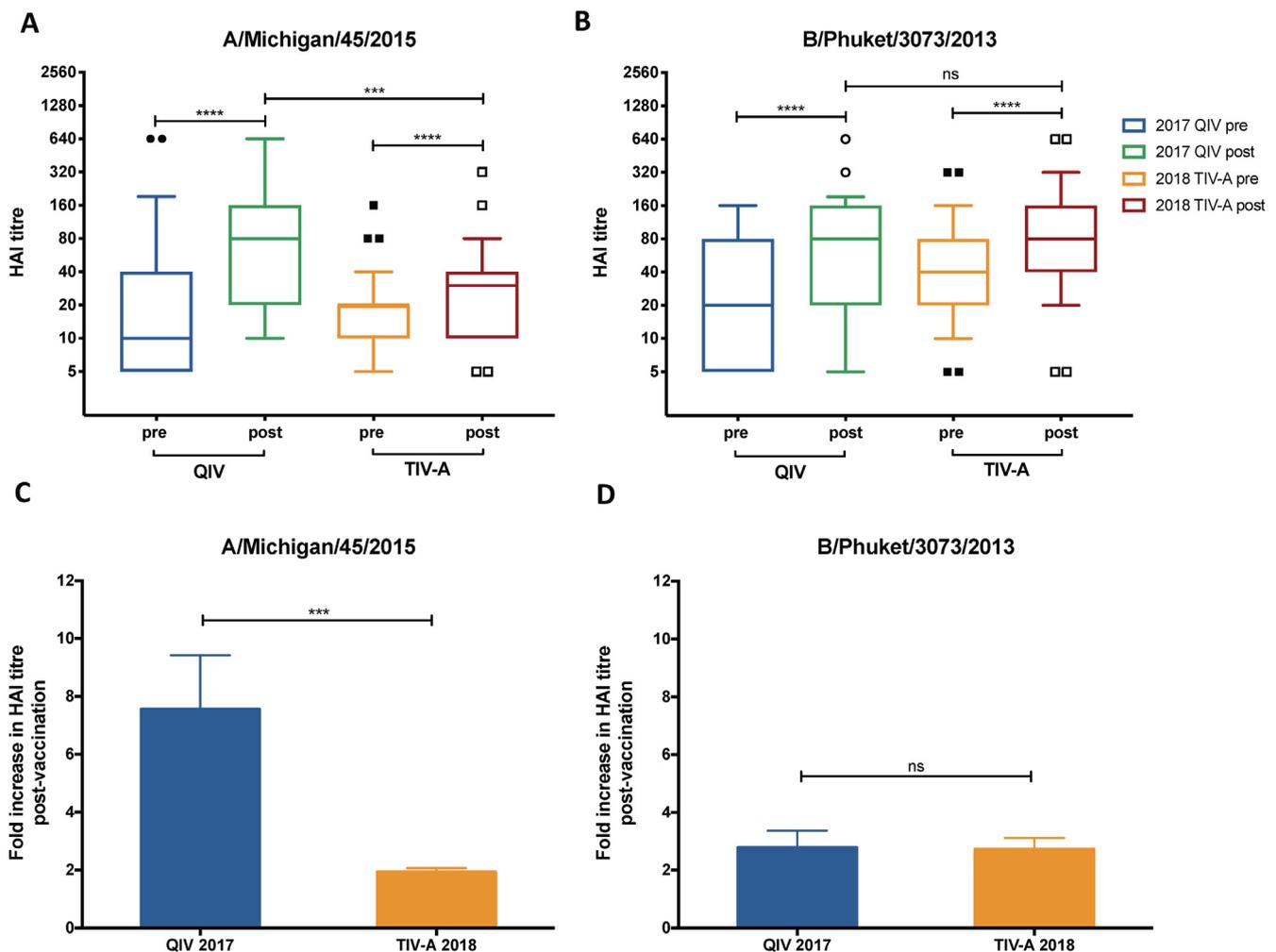


Fig. 1. Hemagglutination inhibition (HAI) antibody response induced by seasonal QIV and TIV-A in community-dwelling older adults. Pre- and post-vaccination HAI titres against the A/Michigan/45/2015 (H1N1) virus (A) and the B/Phuket/3073/2013 Yamagata-lineage virus (B) are shown for older adults vaccinated with the seasonal QIV and TIV-A. In the box and whisker plots, the line represents the median and the whiskers represent the 10–90 percentile, with symbols shown for subjects outside that range. Wilcoxon matched-pairs signed rank tests were used to compare pre- and post-vaccination HAI titres within groups. Mean post-vaccination fold increases (with SEMs) in HAI titre against the A/Michigan/45/2015 (H1N1) virus (C) and the B/Phuket/3073/2013 Yamagata-lineage virus (D) are also shown. Mann Whitney U tests were used to compare HAI titres between the two different vaccine groups. **** = $p < 0.0001$, *** = $p < 0.001$, ns = not significant.

the H1N1 vaccine HA, with only 27% of TIV-A vaccinees reaching this threshold post-vaccination ($p = 0.01$; [Supplementary Table 1](#)). Total anti-HA IgG correlated positively with FcγRIIIa dimer binding antibodies, as previously reported [44], and displayed similar trends for increased responses in the QIV group compared to the TIV-A group ([Supplementary Fig. 1](#)). In this study, the QIV (29% increase) and TIV-A (22% increase) elicited similar ADCC responses against the HA of B/Phuket vaccine virus in older adults ($p = 0.73$; [Fig. 3A](#) and [C](#); [Supplementary Table 1](#)). Antibodies targeting the influenza NA protein also correlate with influenza protection [48] and demonstrate ADCC activity [23]. Both QIV and TIV-A vaccination induced FcγRIIIa dimer binding antibodies against the NA of the B/Phuket vaccine virus, but the response was not significantly different between the QIV and TIV-A groups (QIV 51% vs. TIV-A 33% increase, $p = 0.21$; [Fig. 3B](#) and [D](#)).

3.3. Correlation between HAI titres and FcγRIIIa dimer binding antibodies in influenza vaccinated older adults

Previous studies have given conflicting results regarding the interplay between HAI and ADCC antibody responses following influenza vaccination [36,49]. In this study, we found that there was a strong positive correlation between HAI titre and FcγRIIIa

dimer binding antibodies (A/Michigan $r = 0.60$ $p < 0.0001$ and B/Phuket $r = 0.68$ $p < 0.0001$) in vaccinated older adults ([Fig. 4A](#) and [B](#)). There was a moderate but significant negative correlation between the pre-vaccination level and the post-vaccination increase in FcγRIIIa dimer binding antibodies (A/Michigan $r = -0.39$ $p = 0.0009$ and B/Phuket $r = -0.61$ $p < 0.0001$) following vaccination of older adults ([Fig. 4C](#) and [D](#)). A similar observation has previously been made for the HAI antibody response in other studies [50,51].

3.4. HAI and FcγRIIIa dimer binding antibody responses against an influenza B virus from the Victoria lineage

The QIV contained a Victoria lineage influenza B virus (B/Brisbane/60/2008), whereas the TIV-A vaccine did not. We therefore examined the humoral immune response induced against the B/Brisbane virus by the two different influenza vaccines. There was no significant difference in post-vaccination HAI titre between QIV and TIV-A vaccinated groups (median post-vaccination HAI = 40 for both), however the QIV vaccinated group started with a significantly lower pre-vaccination HAI titre (pre-vaccination HAI titre = 10) than the TIV-A vaccinated group (pre-vaccination HAI titre = 30, $p = 0.02$; [Fig. 5A](#)). Further examination of the HAI

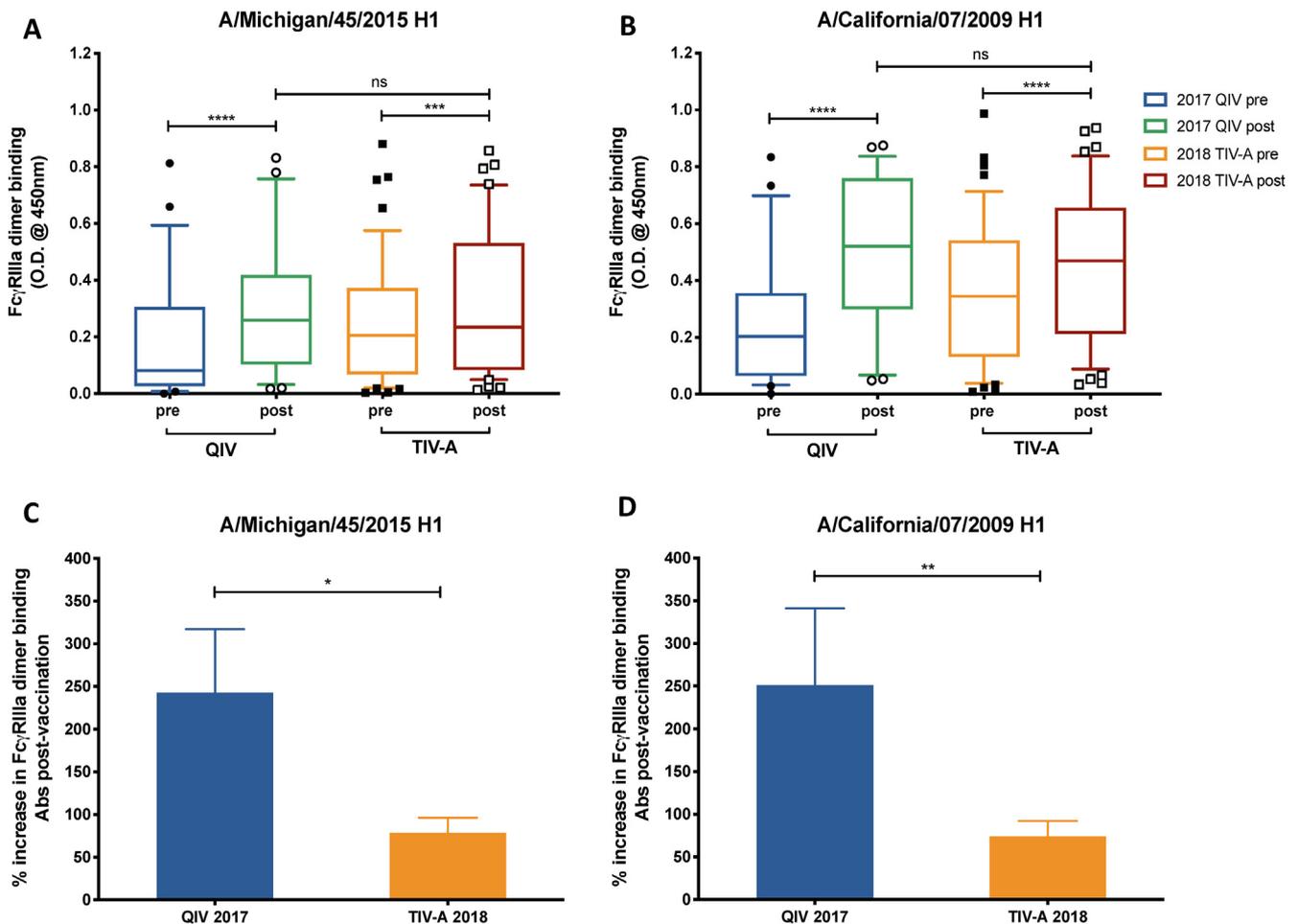


Fig. 2. FcγRIIIa dimer binding antibody response induced against H1N1 HA proteins by seasonal QIV and TIV-A in community-dwelling older adults. Pre- and post-vaccination FcγRIIIa dimer binding antibodies targeting the A/Michigan/45/2015 (H1N1) HA (A) and the A/California/07/2009 (H1N1) HA (B) are shown for older adults vaccinated with the seasonal QIV and TIV-A. In the box and whisker plots, the line represents the median and the whiskers represent the 10–90 percentile, with symbols shown for subjects outside that range. Wilcoxon matched-pairs signed rank tests were used to compare pre- and post-vaccination FcγRIIIa dimer binding antibodies within groups. Mean post-vaccination percentage increases (with SEMs) in FcγRIIIa dimer binding antibodies against the A/Michigan/45/2015 (H1N1) HA (C) and the A/California/07/2009 (H1N1) HA (D) are also shown. Mann Whitney U tests were used to compare FcγRIIIa dimer binding antibodies between the two different vaccine groups. **** = $p < 0.0001$, *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, ns = not significant.

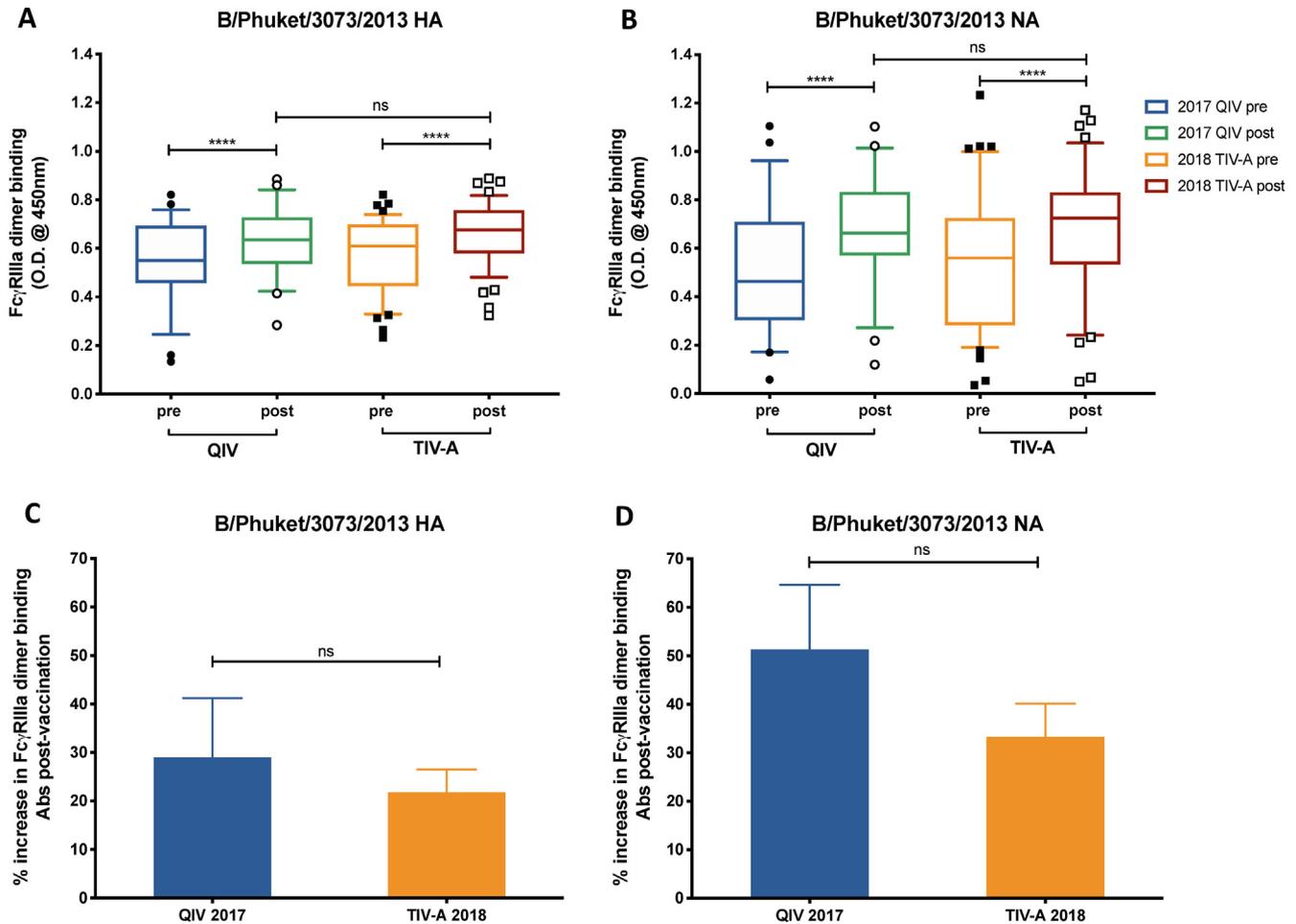


Fig. 3. Fc γ RIIIa dimer binding antibody response induced against B/Phuket/3073/2013 HA and NA proteins by seasonal QIV and TIV-A in community-dwelling older adults. Pre- and post-vaccination Fc γ RIIIa dimer binding antibodies targeting the B/Phuket/3073/2013 HA (A) and NA (B) are shown for older adults vaccinated with the seasonal QIV and TIV-A. In the box and whisker plots, the line represents the median and the whiskers represent the 10–90 percentile, with symbols shown for subjects outside that range. Wilcoxon matched-pairs signed rank tests were used to compare pre- and post-vaccination Fc γ RIIIa dimer binding antibodies within groups. Mean post-vaccination percentage increases (with SEMs) in Fc γ RIIIa dimer binding antibodies against the B/Phuket/3073/2013 HA (C) and NA (D) are also shown. Mann Whitney U tests were used to compare Fc γ RIIIa dimer binding antibodies between the two different vaccine groups. **** = $p < 0.0001$, ns = not significant.

response revealed that 8/27 subjects vaccinated with QIV demonstrated at least a 4-fold increase in HAI titre post-vaccination versus 0/44 in the TIV-A vaccinated group ($p = 0.0002$; [Supplementary Table 1](#)). In addition, the proportion of subjects with a rise in HAI titre against the B/Brisbane virus was higher for the QIV vaccinated group (15/27) compared to the TIV-A vaccinated group (13/44, $p = 0.04$; Fisher's exact test). The fold increase in HAI titre post-vaccination was also significantly higher for older adults vaccinated with the QIV compared to the TIV-A ([Fig. 5B](#)).

To study B/Victoria lineage-specific antibodies with ADCC potential, Fc γ RIIIa dimer binding ELISAs were performed with the HA of the B/Brisbane influenza virus. Immunization with the QIV resulted in a modest but statistically significant increase in HA-specific Fc γ RIIIa dimer binding antibodies against the B/Brisbane vaccine strain. In contrast, there was no increase in HA-specific Fc γ RIIIa dimer binding antibodies against the B/Brisbane virus following TIV-A vaccination in older adults ([Fig. 6A](#)). There was a trend towards higher post-vaccination Fc γ RIIIa dimer binding antibodies for the QIV vaccinated group compared to the TIV-A group ($p = 0.056$; [Fig. 6A](#)). The post-vaccination rise in Fc γ RIIIa dimer binding antibodies against the HA of B/Brisbane virus was also significantly higher for the QIV than the TIV-A ([Fig. 6B](#)).

3.5. Breadth of Fc γ RIIIa dimer binding antibody response to non-vaccine HAs following influenza vaccination in older adults

Previous studies have shown that adjuvanted influenza vaccines can increase the cross-reactivity of the NABs induced, but it is unclear whether the breadth of the ADCC response also increases post-vaccination. Pre- and post-vaccination sera samples were tested against HAs from three non-vaccine Group 1 influenza A viruses: a heterologous H1 (A/Solomon Islands/3/2006), a H2 (A/Japan/305/1957) and a H5 (A/Vietnam/1194/2004). Both influenza vaccines led to a slight post-vaccination rise in Fc γ RIIIa dimer binding antibodies against the heterologous H1 HA, with a statistically significant rise in the TIV-A group ($p = 0.02$; [Fig. 7A](#)). Conversely, there was no increase in the Fc γ RIIIa dimer binding antibody response against the H2 HA in older adults vaccinated with QIV or TIV-A ([Fig. 7B](#)). The majority of older adults had low to undetectable levels of Fc γ RIIIa dimer binding antibodies against the H5 HA since this subtype does not circulate in humans. The H5 Fc γ RIIIa dimer binding antibodies were boosted modestly following vaccination in both vaccine groups, with a trend towards a larger rise in the TIV-A group than the QIV group (TIV-A increase = 103% vs QIV increase = 14%, $p = 0.057$; [Fig. 3C](#)).

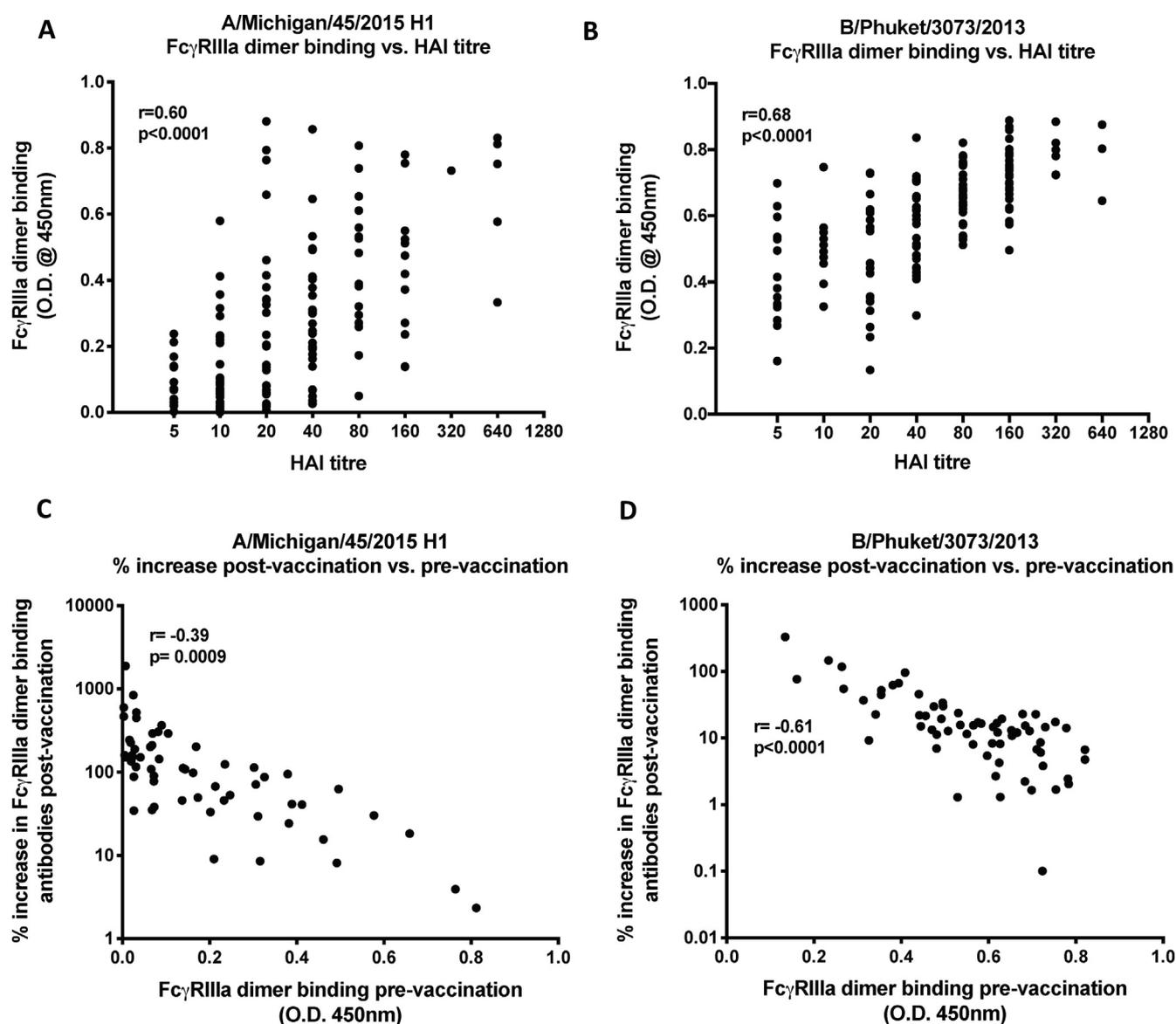


Fig. 4. Correlations between HAI and Fc functional antibody responses in influenza vaccinated community-dwelling older adults. Spearman correlations between pre- and post-vaccination HAI titre and FcγRIIIa dimer binding antibodies for the H1N1 (A) and B/Yamagata lineage (B) vaccine viruses are shown for older adults vaccinated with QIV and TIV-A. Spearman correlations between pre-vaccination FcγRIIIa cross-linking antibodies and post-vaccination percent increase in FcγRIIIa dimer binding antibodies (on a logarithmic scale) for the H1N1 (C) and B/Yamagata lineage (D) vaccine viruses are also shown. Due to the y-axis being graphed on a logarithmic scale, 13 subjects for H1N1 and 6 subjects for B/Yamagata vaccine viruses are not shown as they demonstrated no increase or a decrease in FcγRIIIa cross-linking antibodies post-vaccination, but these subjects were still included in the Spearman correlation analysis.

4. Discussion

The development of novel influenza vaccines for high-risk groups has provided exciting new options for protection against seasonal influenza in older adults. The challenge remains that older adults are a highly heterogeneous population, with a wide variety of ages (65–90 years), comorbidities (including chronic lung disease, cardiac disease and obesity) and living arrangements (community dwelling vs. institutionalised). Due to this heterogeneity, it is important to establish an influenza vaccination strategy that maximises protection while also minimising adverse reactions and cost. Prior studies report that adjuvanted influenza vaccines provide better protection in older adults by inducing superior neutralising antibody responses [18,19,41,42]. Currently, the adjuvanted influenza vaccine is only approved for use in a trivalent form [21], which means that one lineage of influenza B virus is not contained in these vaccines compared to the standard QIV.

To determine whether the TIV-A enhances immunogenicity in older adults compared to the unadjuvanted QIV, we studied the humoral immune response by assessing both HAI and Fc functional antibodies. Surprisingly, we found that the unadjuvanted QIV elicited a higher post-vaccination HAI titre against the H1N1 vaccine strain than TIV-A in older adults. The post-vaccination rise in FcγRIIIa dimer binding antibodies targeting the H1N1 vaccine HA was also significantly higher for the QIV vaccinated group. Meta-analysis of 20 clinical studies found that TIV-A was more effective at inducing antibodies against H3N2 and influenza B virus antigens [41,42], but there was no indication that TIV-A was less immunogenic than unadjuvanted vaccines against H1N1 antigens. Interestingly, we found no difference in the antibody response induced by QIV and TIV-A against the B/Phuket vaccine strain of influenza virus. There was, however, limited evidence that the TIV-A may improve ADCC responses against heterologous strains of influenza A not present in either of the vaccines, with modest increases in

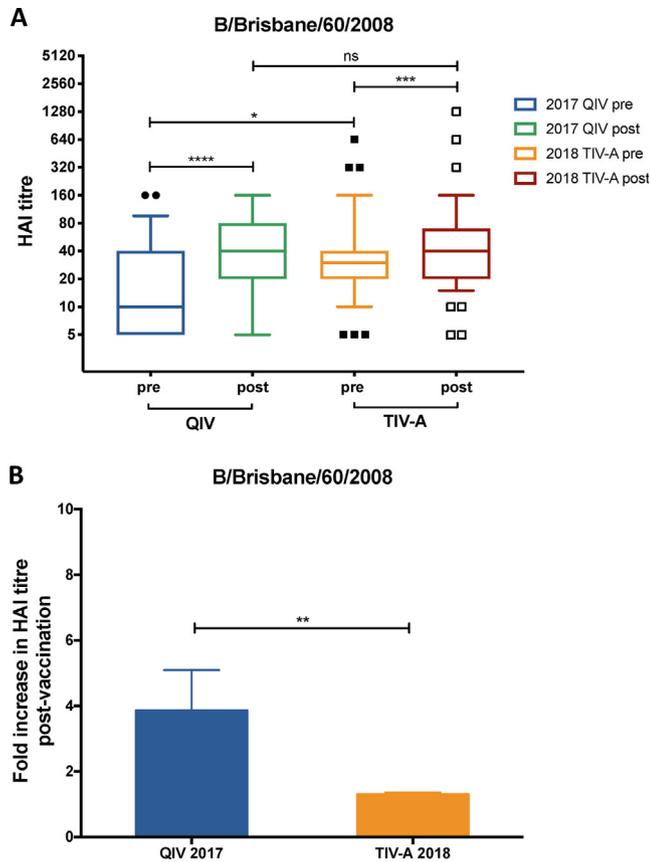


Fig. 5. Hemagglutination inhibition (HAI) antibody response to the B/Brisbane/60/2008 influenza virus elicited by seasonal QIV and TIV-A in community-dwelling older adults. Pre- and post-vaccination HAI titres against the B/Brisbane/60/2008 Victoria lineage virus (A) are shown for older adults vaccinated with the seasonal QIV and TIV-A. In the box and whisker plots, the line represents the median and the whiskers represent the 10–90 percentile, with symbols shown for subjects outside that range. Wilcoxon matched-pairs signed rank tests were used to compare pre- and post-vaccination HAI titres within groups. Mean post-vaccination fold increases (with SEMs) in HAI titre against the B/Brisbane/60/2008 virus (B) are also shown. Mann Whitney U tests were used to compare HAI titres between the two different vaccine groups. **** = $p < 0.0001$, *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, ns = not significant.

the responses to a seasonal H1 and a H5 HA. The lack of immune benefit of adjuvant on the HAI antibody response was not in agreement with some published literature [19,38,39,41,42], but similar findings have been reported. Song *et al.* also found no significant difference in the HAI antibody response (including seroconversion, seroprotection, post-vaccination HAI titre and post-vaccination fold increase in HAI titre) induced by MF59-adjuvanted and unadjuvanted influenza vaccines in a cohort of healthy community-dwelling older adults 28 days post-vaccination [52]. Several factors may contribute to the divergent results reported in older adult vaccination studies including geographic location (which impacts influenza seasonality), strains of influenza virus in the vaccine, average age, relative health, vaccination history and residential care or community dwelling.

In this study, the presence of MF59 adjuvant did not improve the HAI or Fc functional antibody responses against the H1N1 and B/Phuket vaccine strains in community-dwelling older adults. Instead, the increase in Fc functional antibodies following influenza vaccination was largely determined by the pre-vaccination or baseline levels of Fc γ R1IIa dimer binding antibodies. Older adults with low baseline levels of Fc γ R1IIa cross-linking antibodies demonstrated a greater post-vaccination rise in these antibodies. Influenza vaccination studies have reported similar findings for

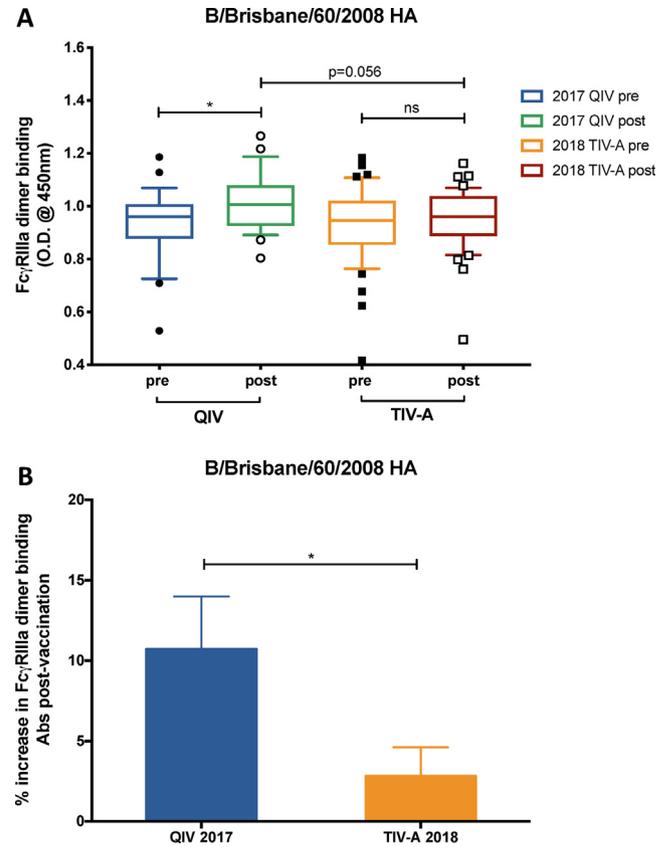


Fig. 6. Fc γ R1IIa dimer binding antibody response against the B/Brisbane/60/2008 HA induced by seasonal QIV and TIV-A in community-dwelling older adults. Pre- and post-vaccination Fc γ R1IIa dimer binding antibodies targeting the B/Brisbane/60/2008 HA (A) are shown for older adults vaccinated with the seasonal QIV and TIV-A. In the box and whisker plots, the line represents the median and the whiskers represent the 10–90 percentile, with symbols shown for subjects outside that range. Wilcoxon matched-pairs signed rank tests were used to compare pre- and post-vaccination Fc γ R1IIa dimer binding antibodies within groups. Mean post-vaccination percent increases (with SEMs) in Fc γ R1IIa dimer binding antibodies against the B/Brisbane/60/2008 HA (B) are also shown. Mann Whitney U tests were used to compare Fc γ R1IIa dimer binding antibodies between the two different vaccine groups. * = $p < 0.05$, ns = not significant.

HAI titre in a variety of cohorts including children, pregnant women, healthcare workers and older adults [50,51,53,54]. The underlying mechanism is unknown, but low pre-vaccination levels of HA antibodies may reduce binding competition for HA-specific B-cells. The observation that older adults with low baseline levels of HA-specific Fc functional antibodies are subject to the strongest post-vaccination increase may reflect a positive outcome of influenza vaccination. Antibodies with ADCC activity have previously been shown to confer protective immunity in murine models of influenza as well as leading to a reduction in viral replication and symptoms in experimental human infection [24,32]. Older adults with low levels of influenza-specific antibodies, both HAI and ADCC, may be more susceptible to influenza virus infection. As such, a strong vaccine-induced antibody response may improve protective immunity in these at-risk individuals.

A clear advantage of the standard QIV over the TIV-A is that it contains an additional strain of influenza B. In this study, the QIV included the B/Brisbane/60/2008 virus and induced a modest post-vaccination increase in Fc γ R1IIa dimer binding antibodies targeting the HA of this virus. In contrast, TIV-A lacked this strain and did not induce a post-vaccination rise in Fc γ R1IIa dimer binding antibodies. The unadjuvanted QIV demonstrated improved seroconversion rates and Fc functional antibody responses against

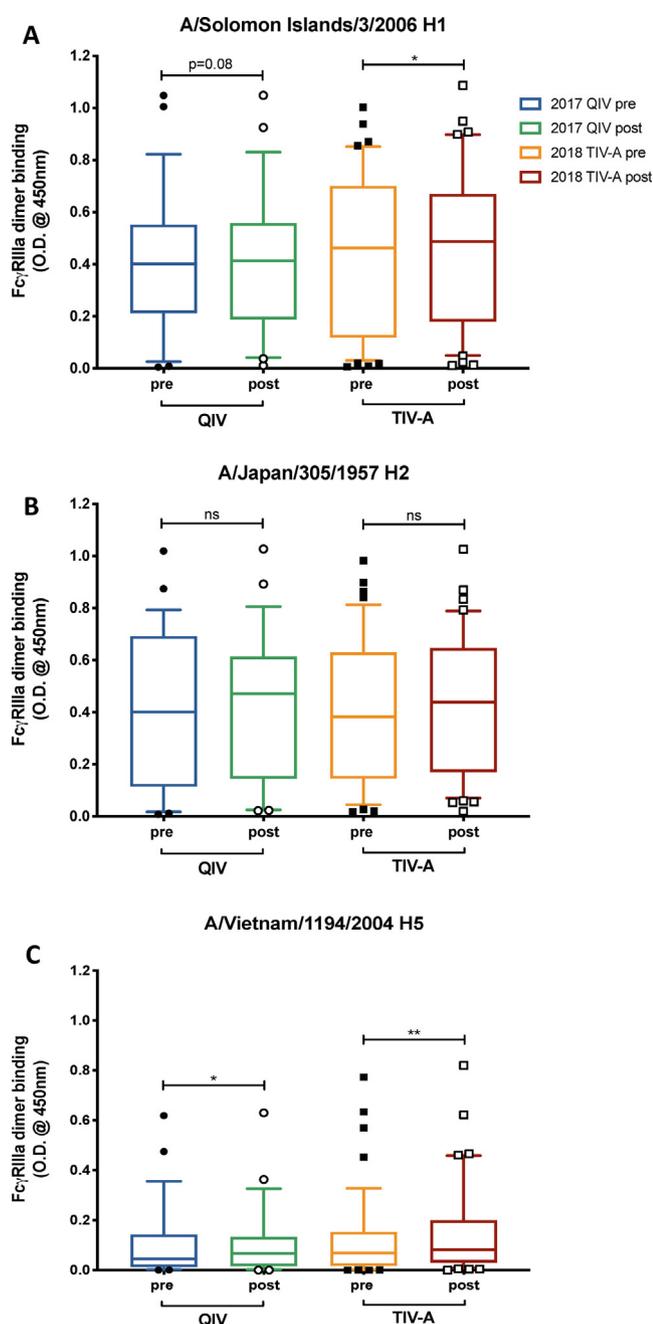


Fig. 7. Breadth of Fc γ RIIIa dimer binding antibody response induced by seasonal QIV and TIV-A in community-dwelling older adults. Pre- and post-vaccination Fc γ RIIIa dimer binding antibodies targeting the A/Solomon Islands/3/2006 (H1N1) HA (A), the A/Japan/305/1957 (H2N2) HA (B) and the A/Vietnam/1194/2004 (H5N1) HA (C) are shown for older adults vaccinated with the seasonal QIV and TIV-A. In the box and whisker plots, the line represents the median and the whiskers represent the 10–90 percentile, with symbols shown for subjects outside that range. Wilcoxon matched-pairs signed rank tests were used to compare pre- and post-vaccination Fc γ RIIIa dimer binding antibodies within groups. ** = $p < 0.01$, * = $p < 0.05$, ns = not significant.

the B/Brisbane virus as well as the H1N1 vaccine virus suggesting that the current TIV-A vaccine does not improve immunogenicity against all vaccine strains in all groups of older adults. This work highlights the necessity for a more comprehensive assessment of the current influenza vaccination strategy considering the variability in the aged population.

We acknowledge limitations of our studies requiring future work. The TIV-A and QIV vaccines were administered in different years and not matched for the H3N2 component, limiting our

ability to compare responses to the H3 HA. Although the subjects were well matched for age and vaccination status over the past four years, we cannot exclude confounding health factors that may have altered the humoral immune response. Since the A/Michigan/45/2015 (H1N1) virus replaced A/California/07/2009 (H1N1) in 2017, older adults in the QIV group may have been naïve to this virus prior to vaccination. In contrast, subjects in the TIV-A group could have been previously vaccinated against A/Michigan/45/2015 (H1N1) in 2017. Older adults with baseline or pre-vaccination HAI titres of ≥ 40 demonstrate reduced vaccine responsiveness (as measured by fold increase in HAI titre post-vaccination) [54], but we found that baseline titres of HAI antibodies against A/Michigan/45/2015 (H1N1) were low (< 40) and not significantly different between the QIV and TIV-A groups. These results suggest that the stronger HAI response observed in the QIV group (compared to the TIV-A group) was not related to baseline HAI titre. The strong correlation between baseline antibody levels and vaccine responsiveness suggests that comprehensive matching of subjects for baseline responses may be of benefit in future vaccine studies. A follow-up study examining the ability of the QIV and TIV-A to overcome “immune imprinting” in older adults by boosting novel HAI responses compared to memory HAI responses is warranted.

Our analysis primarily focused on responses to vaccine strains, but we also studied responses to the 2009 pandemic H1 protein as well as more divergent H1, H2 and H5 HA proteins. The ADCC response to the pandemic A/California/07/2009 HA closely mirrored that of the A/Michigan/45/2015 vaccine HA, which is not surprising given their high degree of similarity. We observed only modest responses to the other heterologous strains (A/Solomon Islands H1, A/Japan H2 and A/Vietnam H5) tested, but there was some evidence that slightly stronger responses were observed in the TIV-A group and even weak responses may provide some protective benefit when vaccine strains are mismatched against circulating strains. Our work analysed community-dwelling, rather than institutionalised older subjects. Institutionalised subjects are at an even greater risk of influenza complications and TIV-A vaccine responsiveness may be different in this group [55]. Our duration of follow up was short (28 days) and future studies in community-dwelling older adults should also include later time points after vaccination since previous reports have suggested that the persistence of HAI antibodies was improved in TIV-A vaccinated subjects [52].

Collectively, this study showed that the MF59-adjuvanted TIV did not elicit a superior ADCC or HAI antibody response compared to the unadjuvanted QIV in an Australian cohort of community-dwelling older adults. In addition to inducing responses to a second influenza B virus, the standard QIV elicited significantly higher post-vaccination titres of HAI antibodies targeting the H1N1 vaccine virus than TIV-A. This highlights the need for influenza vaccine efficacy studies to be performed in different subsets of older adults.

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CRediT authorship contribution statement

Hillary A. Vandervén: Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing - review &

editing, Visualization, Project administration. **Ian Barr:** Conceptualization, Investigation, Writing - review & editing, Supervision. **Arnold Reynaldi:** Formal analysis, Writing - review & editing. **Adam K. Wheatley:** Conceptualization, Resources, Writing - review & editing. **Bruce D. Wines:** Methodology, Resources, Writing - review & editing. **Miles P. Davenport:** Formal analysis, Supervision, Writing - review & editing. **P. Mark Hogarth:** Methodology, Resources, Writing - review & editing. **Stephen J. Kent:** Conceptualization, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2020.01.066>.

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