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Association of HLA class II haplotypes with antibody concentrations after diphtheria-tetanus acellular pertussis booster vaccination in four age groups of Finnish participants

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ABSTRACT

Background: Despite widespread use of whole-cell (wP) and acellular (aP) pertussis vaccines, outbreaks persist in many countries. Polymorphisms in HLA class II molecules, which present antigens to $CD4^+$ T cells, may play a vital role in vaccine responses. Emerging evidence suggests that HLA-DR/DQ variants can significantly influence wP vaccine responses. This study investigated the possible association of HLA class II haplotypes with antibody concentrations after aP booster vaccination.

Materials and methods: Healthy Finnish children (7–10y, $n = 37$), adolescents (11–15y, $n = 37$), young adults (20–34y, $n = 25$), and older adults (60–70y, $n = 25$) received a Tdap3-IPV booster. Serum antibodies against pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), diphtheria toxoid (DT), and tetanus toxoid (TT), as well as PT-neutralizing antibodies (PTNA), were measured before, one month, and one year after the booster. Participants were HLA-typed with a stepwise HLA-DR/DQ screening system using PCR followed by allele-specific probe hybridization. The frequency of HLA haplotypes was compared to the control cohort from the Finnish Pediatric Diabetes Register, which was constructed from parental haplotypes not passed down to diabetic children.

Results: The HLA DR-DQ haplotype frequencies in our cohort did not differ from the control group. Two associations survived FDR correction: (DR7)-*DQA1*02:01-DQB1*02* with lower anti-PT IgG and (DR15)-*DQB1*06:02* with a time-dependent anti-FHA IgG response with higher pre-booster concentrations. Nominally, (DR8)-*DQB1*04* and (DR7)-*DQA1*02:01-DQB1*02* carriers had lower PTNA; (DR9)-*DQA1*03-DQB1*03:03*, (DR15)-*DQB1*06:02*, and *DRB1*04:01-DQA1*03-DQB1*03:02* carriers had higher anti-PT and anti-Prn concentrations; and (DR13)-*DQB1*06:03* carriers had consistently lower anti-DT across all timepoints.

Conclusions: The present study highlights a potential role of certain HLA haplotypes in modulating immune responses to aP vaccination. These findings emphasize the importance of further research to clarify how HLA class II haplotypes influence aP vaccine responses in various populations.

Abbreviations: aP, acellular pertussis vaccine; BERT, Booster Pertussis Vaccine Study; DT, diphtheria toxoid; FHA, filamentous hemagglutinin; HLA, human leukocyte antigen; PRN, pertactin; PT, pertussis toxin; PTNA, PT neutralizing antibodies; Tdap3-IPV, tetanus toxoid with reduced diphtheria toxoid and acellular pertussis vaccine conjugated with inactivated whole polio virus; TT, tetanus toxoid; wP, whole-cell pertussis vaccine.

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1. Introduction

Pertussis, a highly contagious respiratory disease caused by the gram-negative bacterium *Bordetella pertussis (Bp)*, remains a persistent challenge despite widespread vaccination efforts. Vaccine-mediated protection against the disease is achieved through administering a three-dose primary vaccination series and additional booster doses with either whole-cell (wP) or acellular (aP) vaccines. Despite increased vaccination coverage, the disease remains endemic in many countries worldwide. Before the COVID-19 pandemic, the normal circulation of pertussis averaged around 38,000 cases yearly in the European Union and the European Economic Area, with 34,000 cases in 2019 [1]. The Finnish Institute of Health and Welfare reported 557 cases in the same year. The post-pandemic era has brought about a resurgence of pertussis, reporting a record-high number of 32,000 cases from January 1 to March 31, 2024, compared to the 25,000 cases reported in the whole year of 2023 in Europe [1]. Similarly, there were 2820 cases in Finland in 2024 versus 130 cases in the previous year. Although this resurgence may have been driven, at least in part, by the lifting of social distancing measures and other non-pharmaceutical interventions implemented during the pandemic, recent European seroprevalence studies also revealed ongoing pertussis circulation among adults aged 20 to 59 years, evidenced by serum anti-pertussis toxin (PT) IgG antibody concentrations of 50 to >100 IU/mL, indicating a recent infection within the past few years [2,3].

This ongoing resurgence can be attributed to many factors, including large inter-individual variations in vaccine responses, with antibody and cell-mediated immune responses differing up to 100-fold, depending on the vaccine type [4]. Notably, 2–10% of healthy individuals do not generate adequate antibody levels following routine vaccination in general [5]. Similar patterns of variability in antigen-specific antibodies, PT neutralizing antibodies (PTNA), and B-memory cells have been documented in Finnish, British, and Dutch cohorts who received diphtheria-tetanus-acellular pertussis (Tdap) booster [6–8]. Genetics play a role in the variability of vaccine responses among different ethnic groups living in the same region. Twin studies indicate that the heritability of humoral responses ranges from 36% to 90%, and cellular responses range from 39% to 90%, depending on the vaccine [4]. For pertussis, twin studies have shown a 53–65% degree of heritability for humoral and cell-mediated immune responses with aP [9]. This can be associated with genetic polymorphisms in the genes encoding for the Human Leukocyte Antigen (HLA) on chromosome 6, considered the most polymorphic region in the human genome [10]. The HLA class II molecules are highly involved in the antigen presentation of extracellular peptides to $CD4^+$ helper T cells. A scoping review of immune determinants on vaccine responses found that various class I and class II HLA alleles and/or their haplotypes were positively or negatively associated with vaccine immunogenicity, depending on the allele, including hepatitis B, influenza, measles, rubella, and rotavirus vaccines [11].

The HLA genes are divided into three regions, of which HLA class II genes encode for HLA-DR, -DQ, and -DP molecules that are co-dominantly expressed on the surface of antigeen presenting cells and are directly involved in extracellular antigen presentation to $CD4^+$ T cells. Each person inherits two haplotype blocks, with a maximum of two alleles per locus, resulting in six HLA class II antigens per person. Each antigen consists of an α and β chain divided into four subunits, with the α1 and β1 subunits forming the peptide-binding groove. This highly polymorphic region, combined with various allelic combinations, has an extended capacity to present a diverse range of peptides of different lengths for maximum protection. Concurrently, this can also be attributed to variable antibody response to vaccination. Hypothetically, an allele with less or more optimal antigen recognition and presentation will culminate in decreased or increased generation of antibodies after infection and/or vaccination [12,13].

Research on the influence of HLA on pertussis immunity is limited. Previous studies on human PBMCs showed that *Bp* infection down-regulates genes associated with antigen presentation, including those that code for HLA class II molecules [14]. Further analyses indicated that a possible mechanism of immune escape by *Bp* is through the decrease of cell-surface HLA-DR levels through intracellular redistribution, partly mediated by PT. In a case-control study of self-reported pertussis cases in British adults, genetic variation in the HLA class II locus was associated with susceptibility to *Bp* infection [15]. While there are limited studies regarding the effect of HLA alleles on pertussis vaccine responses, a recent study on wP-vaccinated African infants discovered that DRB1 variants with arginine at amino acid position 233 were associated with increased PT-specific T follicular helper cell activity and antibodies [16]. Given the significant role that HLA alleles play in shaping immune responses to various vaccines and the increasing use of Tdap booster vaccinations in different age groups, understanding their influence on pertussis vaccine responses is crucial. In a recent phase IV longitudinal intervention and multicenter trial conducted in Finland, the Netherlands, and the UK, we examined antibody responses following an aP booster across various age groups [6,7]. This study evaluated the possible influence of polymorphisms in HLA class II on antibody and PTNA concentrations post-booster from the respective cohort of different-aged Finnish participants.

2. Methods

2.1. Study design

This study is a sub-analysis of the Booster Pertussis Vaccine Study (BERT), a phase IV longitudinal intervention trial conducted in Finland to assess adaptive immune responses following Tdap booster vaccination. The clinical trial was registered in the EU Clinical Trial database (EudraCT number 2016–003678-42) and received approval from the Ethics Committee of the Hospital District of Southwest Finland (ETMK Dnro: 129/1800/2017). Detailed methods and antibody and PTNA results from the study have been previously published [6,7]. In summary, 124 participants were divided into four age-based cohorts: 37 children aged 7–10 years, 37 adolescents aged 11–15 years, 25 young adults aged 20–34 years, and 25 older adults aged 60–70 years. All participants received a Tdap3-IPV vaccine (Boostrix™-IPV by GlaxoSmithKline) at day 0 (baseline), which contains tetanus toxoid (TT), reduced antigen diphtheria toxoid (DT), and three pertussis antigens PT, filamentous hemagglutinin (FHA), and pertactin (PRN) adsorbed onto aluminum. Serum samples were collected at baseline (before vaccination) and on days 28 and 365 after vaccination. The demographics of these participants are detailed in Table 1.

2.2. Analysis of vaccine response

The concentrations of serum IgG and IgA antibodies against the

Table 1
Study population demographics*.

	Study Groups			
	Children, (n = 37)	Adolescents, (n = 37)	Young Adults, (n = 25)	Older Adults, (*n* = 25)
Age (year), median [IQR]	9.2 [8.2–9.7]	13.8 [12.7–14.7]	30.8 [28.9–32.7]	64.20 [62.8–65.4]
Female sex, *n* (%)	18 (50)	19 (51)	21 (84)	21 (91)
Primary vaccination				
wP *n* (%)	0 (0)	18 (49)	25 (100)	23 (100)
aP *n* (%)	36 (100)	19 (51)	0 (0)	0 (0)

* In Finland, wP vaccine was replaced by aP vaccine in 2005. wP, whole cell pertussis vaccine; aP, acellular pertussis vaccine.

vaccine antigens (PT, FHA, and PRN), as well as anti-DT and -TT IgG were quantified at days 0, 28, and 365 using a fluorescent-bead-based multiplex immunoassay (MIA), as reported previously [6]. Antibody concentrations were expressed in international units per milliliter (IU/mL). Additionally, PTNA titers were previously measured at each time point using the Chinese hamster ovary (CHO) cell assay [7]. PTNA titers were reported as the reciprocal of the highest dilution at which no cluster formation of CHO cells was observed.

2.3. HLA typing

All Finnish participants were HLA-typed using a stepwise screening system based on a panel of HLA alleles common in the Finnish population [17]. Briefly, DNA extraction from 250 μL of whole blood was carried out according to E.Z.N.A.® Tissue DNA Kit (Omega Bio-tek, Inc. Norcross Georgia, U.S.) protocols. A stepwise method based on asymmetric PCR and allele-specific probe hybridization was used to detect HLA-DQB1 alleles *02, *03:01, *03:02, *03:03, *04, *05:01, *06:02, *06:03 and *06:04. The presence of *05:02, *05:03, *06:01 and *06:09 alleles (as well as the homozygosity of a sample) was confirmed by sequencing. The DQA1 alleles *02, *03, and *05 were typed in relevant samples using a homogeneous method based on switchable lanthanide luminescence probes [18]. *DRB1*04* subtypes were screened with a DELFIA assay as previously described [19,20]. This method enabled the identification of all common haplotypes of European origin.

2.4. Data and statistical analyses

All statistical analyses were performed with R (version 4.5.1; R Core Team, 2025) [21], using the following packages: lme4 [22] and lmerTest [23] for linear mixed-effects models, emmeans [24] for estimated marginal means and pairwise contrasts, tidyverse [25] for data manipulation, and ggplot2 [26], patchwork [27], and cowplot [28] for visualization.

Data were checked for normality using the Shapiro-Wilk test; non-normally distributed data were $\log_{10}$-transformed prior to analysis. HLA class II haplotypes were coded as a binary carrier variable, with both heterozygous and homozygous carriers classified as carriers to account for the low frequency of homozygous subjects across most haplotypes. Only haplotype carrier frequencies of at least 5% in the cohort ($n \geq 7$) were included in the primary analysis, yielding 12 haplotypes for testing. Haplotypes meeting only the minimum carrier threshold (n = 7–9) were retained for completeness but should be interpreted with caution, as associations based on groups of this size are only hypothesis-generating signals.

Chi-square or Fisher's exact tests were used to compare the frequencies of BERT HLA haplotypes with those in the general population, using the Finnish Pediatric Diabetes Register (FPDR) control group, which represents parental haplotypes not transmitted to children [29]. Haplotypes absent in either cohort were not formally tested. Differences in frequencies between age groups and sexes within the BERT cohort were assessed using Chi-square tests, with Monte Carlo simulations for age-group differences and Fisher's exact tests for sex differences when expected cell counts fell below 5. Haplotypes with carriers observed in only one age group, or with three or fewer total carriers, were not tested as there is insufficient variation across groups to make a meaningful comparison. All frequency comparisons were corrected for multiple testing using the Benjamini-Hochberg false discovery rate (FDR) method.

The association between HLA haplotypes and vaccine antibody concentrations was evaluated using a linear mixed-effects model, fitted separately for each haplotype-antibody combination to account for repeated measures. Each model included fixed effects for time and carrier, a time × carrier interaction, age group and sex as covariates, and a random intercept for each participant to account for the repeated-measures structure of the data. The carrier main effect reflects overall differences in pooled antibody concentrations between carriers and non-carriers across the three time points. In contrast, the time-carrier interaction captures changes in antibody concentrations over time by carrier status. Two secondary models were additionally fitted for each haplotype-antibody combination to formally test whether the HLA-antibody association was modified by sex or age group. The sex interaction model extended the primary model with a sex-carrier interaction term, whereas the age group interaction model extended the primary model with an age group-carrier interaction term.

Least-squares (LS) means and 95% confidence intervals were derived from each model. Pairwise contrasts between carrier and non-carrier groups were computed in two ways. First, as an overall contrast by averaging the model-predicted carrier vs non-carrier difference across all three timepoints (D0, D28, and D365) using proportional weighting. Second, as timepoint-specific contrasts, computed separately at D0, D28, and D365 to identify at which timepoints the carrier effect was most pronounced, with Tukey's correction applied across the overall and the three timepoint comparisons within each model. Estimated differences are reported on the $\log_{10}$ scale alongside back-transformed fold-differences and 95% CIs. To control for multiple testing across the 12 haplotypes tested for each antibody, p-values for the carrier main effect and interaction p-values were adjusted using the FDR method within each antibody. Associations with FDR < 0.05 were considered statistically significant; associations with raw $p < 0.05$ that did not survive FDR correction are reported as nominally significant and interpreted as exploratory.

Lastly, to assess the robustness of the reported associations against extreme values, a post-hoc sensitivity analysis was performed. Outliers were identified within each antibody × timepoint group on the log10-transformed scale using two standard criteria: observations deviating by more than ±3 SD from the group mean or falling below Q1–3 × IQR or above Q3 + 3 × IQR. Observations meeting either criterion were excluded, and primary linear mixed-effects models were refit on the resulting cleaned dataset to verify the stability of the direction of effect and statistical significance.

3. Results

3.1. Antibody responses

The antibody concentrations following aP booster were previously reported and are shown in Supplementary Fig. 1 [6,7]. IgG antibodies against all vaccine antigens and PTNA concentrations increased across all age groups. Although antibody levels declined one year after vaccination compared with day 28, they remained significantly elevated relative to baseline, as reported earlier [6]. The rise in IgA levels was less pronounced than that of IgG in all age groups. Additionally, there were noticeable differences in vaccine response among individuals.

3.2. HLA haplotype frequencies

The frequency of each haplotype in the BERT cohort was compared with that in the FPDR control group. The BERT cohort HLA haplotype frequencies in the total population did not differ from the FPDR control group, indicating that the frequencies observed in BERT reflect those of the Finnish population (Table 2). Frequencies of haplotypes between age groups (Supplementary Table 1) and sexes (Supplementary Table 2) also did not differ significantly. Subsequently, each HLA haplotype was stratified based on participants who were homozygous, heterozygous, or those without the haplotype (Table 3). Participants who are homozygous for a specific haplotype are rare. Thus, all homozygous participants were combined with heterozygous participants and collapsed as either carrier or non-carrier for all subsequent analyses.

Table 2
HLA-DR/DQ haplotype frequencies in the BERT cohort and the Finnish Pediatric Diabetes Register cohort (control) with statistical comparison.[a,b,c]

HLA Haplotype	BERT					Finnish Pediatric Diabetes Register $N = 2991$ (%)	p-value	p-value (FDR)
	Children, n = 37 (%)	Adolescents, $n = 37$ (%)	Young adults, n = 25 (%)	Older adults, n = 25 (%)	Total Population, $N = 124$ (%)			
(DR1/10) - *DQB1*05:01*	17 (23.0)	21 (28.4)	6 (12.0)	13 (26.0)	57 (23.0)	1177 (19.7)	0.2	0.42
(DR15) - *DQB1*06:02*	11 (14.9)	9 (12.2)	6 (12.0)	7 (14.0)	33 (13.3)	836 (14.0)	0.766	0.893
(DR11/12/13) – *DQA1*05 - DQB1*03:01*	3 (4.1)	11 (14.9)	7 (14.0)	2 (4.0)	23 (9.3)	488 (8.2)	0.53	0.695
(DR13) - *DQB1*06:03*	6 (8.1)	7 (9.5)	4 (8.0)	6 (12.0)	23 (9.3)	559 (9.3)	0.97	1
(DR3) – *DQA1*05 - DQB1*02*	5 (6.8)	4 (5.4)	6 (12.0)	4 (8.0)	19 (7.7)	548 (9.2)	0.421	0.59
(DR7) – *DQA1*02:01 - DQB1*02*	5 (6.8)	6 (8.1)	3 (6.0)	2 (4.0)	16 (6.5)	261 (4.4)	0.118	0.35
(DR8) - *DQB1*04*	7 (9.5)	2 (2.7)	5 (10.0)	1 (2.0)	15 (6.0)	583 (9.7)	0.053	0.222
*DRB1*04:01 – DQA1*03 - DQB1*03:02*	2 (2.7)	3 (4.1)	3 (6.0)	3 (6.0)	11 (4.4)	359 (6.0)	0.307	0.537
*DRB1*04:04 – DQA1*03 - DQB1*03:02*	2 (2.7)	1 (1.4)	4 (8.0)	2 (4.0)	9 (3.6)	227 (3.8)	0.893	0.988
(DR13) - *DQB1*06:04*	2 (2.7)	2 (2.7)	0 (0)	3 (6.0)	7 (2.8)	210 (3.5)	0.563	0.695
(DR7) – *DQA1*02:01 - DQB1*03:03*	3 (4.1)	1 (1.4)	1 (2.0)	2 (4.0)	7 (2.8)	114 (1.9)	0.34	0.548
(DR9) – *DQA1*03 - DQB1*03:03*	4 (5.4)	1 (1.4)	1 (2.0)	1 (2.0)	7 (2.8)	236 (3.9)	0.371	0.556
(DR14) - *DQB1*05:03*	3 (4.1)	1 (1.4)	1 (2.0)	1 (2.0)	6 (2.4)	73 (1.2)	0.133	0.35
*DRB1*04:01 – DQA1*03 - DQB1*03:01*	1 (1.4)	3 (4.1)	0 (0)	1 (2.0)	5 (2.0)	0 (0)	–	–
*DRB1*04:08 – DQA1*03 - DQB1*03:01*	2 (2.7)	1 (1.4)	0 (0)	0 (0)	3 (1.2)	0 (0)	–	–
(DR13) - *DQB1*06:09*	0 (0)	1 (1.4)	1 (2.0)	0 (0)	2 (0.8)	14 (0.2)	0.131	0.35
(DR15) - *DQB1*06:01*	1 (1.4)	0 (0)	0 (0)	0 (0)	1 (0.4)	7 (0.1)	0.278	0.53
*DRB1*04:04 – DQA1*03 - DQB1*02*	0 (0)	0 (0)	1 (2.0)	0 (0)	1 (0.4)	0 (0)	–	–
*DRB1*04:02 – DQA1*03 - DQB1*03:02*	0 (0)	0 (0)	1 (2.0)	0 (0)	1 (0.4)	4 (0.1)	0.184	0.42
*DRB1*04:03 – DQA1*03 - DQB1*03:02*	0 (0)	0 (0)	0 (0)	1 (2.0)	1 (0.4)	38 (0.6)	1	1
*DRB1*04:07 – DQA1*03 - DQB1*03:01*	0 (0)	0 (0)	0 (0)	1 (2.0)	1 (0.4)	0 (0)	–	–
*DRB1*04:05 – DQA1*03 - DQB1*03:02*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (0.6)	–	–
*(DR16) - DQB1*05:02*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	48 (0.8)	–	–
Others	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (0.1)	–	–
Total	74 (100)	74 (100)	50 (100)	50 (100)	248 (100)	5982 (100)		

[a] Frequencies expressed as number of haplotype copies and percentage of total haplotype copies (n_subjects x 2).
[b] Chi-square test used when all expected counts ≥5; Fisher's exact test used otherwise. FDR correction applied across all haplotypes tested.
[c] – indicates haplotype was not observed in either BERT or FPDR cohorts; comparisons not performed.

Table 3
Distribution of homozygotes, heterozygotes, and subjects without the haplotype*.

Haplotypes	Haplotype Groupings, n		
	Homozygote	Heterozygote	Without the Haplotype
(DR1/10) - *DQB1*05:01*	7	43	74
(DR15) - *DQB1*06:02*	1	31	92
(DR11/12/13) – *DQA1*05 - DQB1*03:01*	1	21	102
(DR13) - *DQB1*06:03*	1	21	102
(DR3) – *DQA1*05 - DQB1*02*	1	17	106
(DR7) – *DQA1*02:01 - DQB1*02*	2	12	110
(DR8) - *DQB1*04*	0	15	109
*DRB1*04:01 - DQA1*03 - DQB1*03:02*	0	11	113
*DRB1*04:04 - DQA1*03 - DQB1*03:02*	0	9	115
(DR13) - *DQB1*06:04*	0	7	117
(DR7) – *DQA1*02:01 - DQB1*03:03*	0	7	117
(DR9) – *DQA1*03 - DQB1*03:03*	0	7	117

* There are 124 BERT participants, of which 248 haplotype copies were detected. Twelve haplotypes were excluded as their frequencies were below 5.

3.3. HLA haplotype associations with post-booster antibody concentrations

An overview of all carrier effects across the 12 haplotypes and antibody outcomes is shown in Fig. 1, with a full *p*-value summary of all 108 tests of both carrier effect and time × carrier interaction in Supplementary Fig. 2. Of the 108 haplotype-antibody associations tested, only two survived FDR correction: (DR7)-*DQA1*02:01-DQB1*02* was associated with lower anti-PT IgG concentrations (carrier FDR = 0.008), and (DR15)-*DQB1*06:02* was associated with a time-dependent anti-FHA IgG response (carrier FDR = 0.018; time × carrier FDR = 0.007). Depending on the haplotype and antibody, carriers had either lower or higher antibody concentrations compared to non-carriers, with associations observed most consistently for anti-PT antibodies.

3.4. Associations of HLA with anti-PT antibody concentrations

Carriers of (DR7)-*DQA1*02:01-DQB1*02* had significantly lower anti-PT IgG concentrations across time (carrier $p < 0.001$, FDR = 0.008; Fig. 2A). Averaged across all three timepoints, carriers had 2.25-fold lower anti-PT IgG concentrations than non-carriers ($p = 0.002$, 95% CI [1.34–3.78]), with significant differences observed at D0 ($p < 0.001$) and D28 ($p = 0.015$), but not at D365. Concordantly, (DR7)-*DQA1*02:01-DQB1*02* carriers also had lower PTNA concentrations (carrier $p = 0.016$, FDR = 0.066; Fig. 2B), with carriers having 1.85-fold lower PTNA overall ($p = 0.019$, 95% CI [1.11–3.08]). The PTNA difference was significant at D0 ($p = 0.017$) and D365 ($p = 0.006$), but not

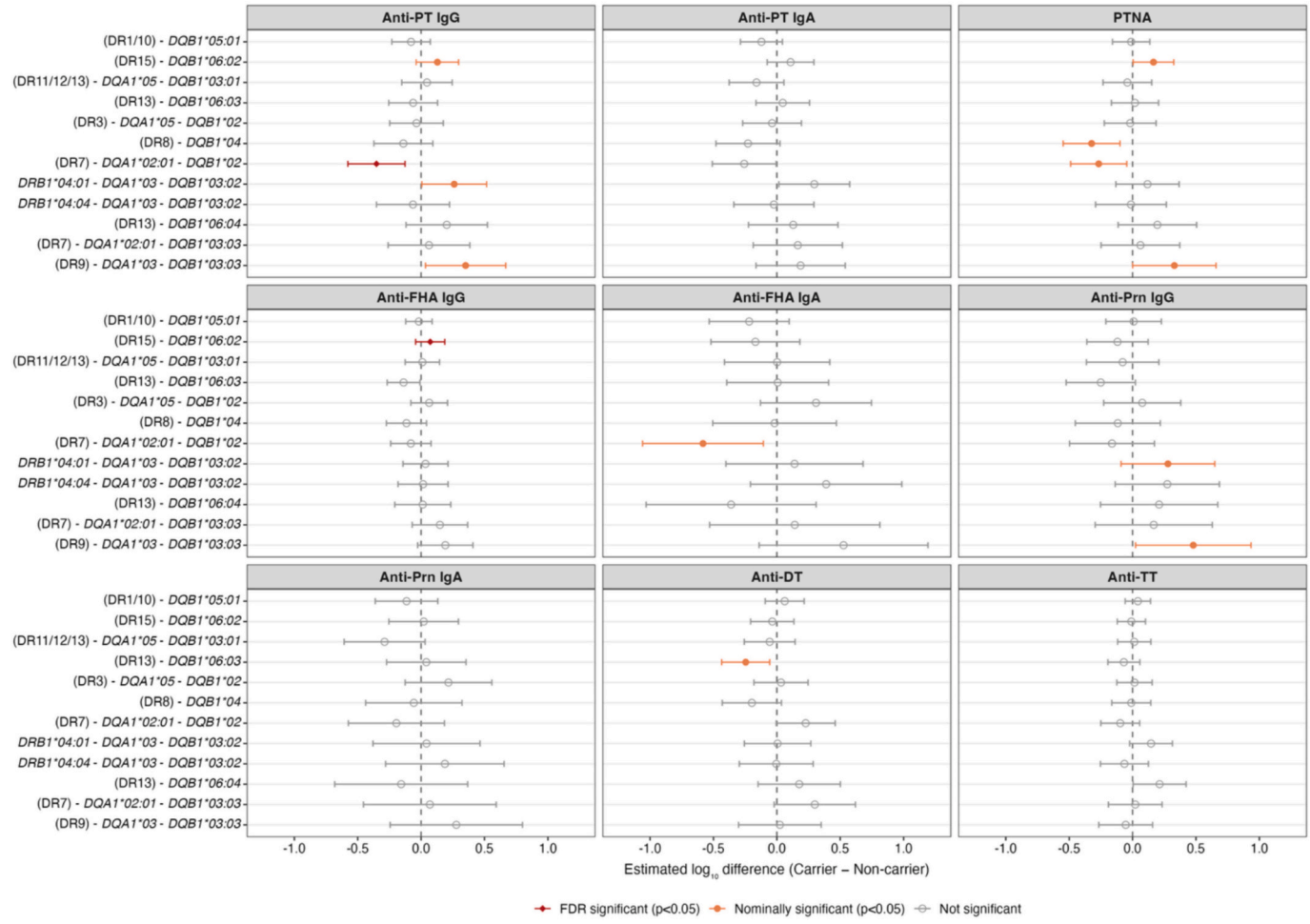

Fig. 1. *Overview of all associations of HLA class II haplotype carrier effects on aP antibody concentrations.* Forest plot showing estimated differences in antibody concentrations between carriers and non-carriers for all 12 HLA class II haplotypes across nine pertussis vaccine antigens (108 tests total). Each point represents the overall least-squares mean difference (carrier minus non-carrier) on the $\log_{10}$ scale, marginalized across the three study timepoints (D0, D28, D365) using proportional weighting from the primary linear mixed-effects model: $\log_{10}$ (antibody) ~ time × carrier + age group + sex + (1|subject). Horizontal bars indicate 95% confidence intervals. The dashed vertical line at zero denotes no difference between carrier and non-carrier groups. False discovery rate (FDR) correction significant associations (Benjamini-Hochberg correction within each antibody across 12 haplotypes, FDR < 0.05) are shown as dark red diamonds; nominally significant associations (raw $p < 0.05$) are shown as orange circles; non-significant associations are shown as grey circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

at D28. Similarly, carriers of (DR8)-*DQB1*04* had lower PTNA concentrations (carrier $p = 0.014$, FDR = 0.066; Fig. 2C), with carriers showing a 2.10-fold lower PTNA overall (p = 0.005, 95% CI [1.26–3.53]). This was observed at all time points (D0 p = 0.014; D28 p = 0.010; D365 p = 0.014). For both haplotypes, lower antibody concentrations were observed across all time points, indicating consistently lower anti-PT IgG and/or PTNA levels, independent of the booster response.

Three haplotypes were nominally associated with higher anti-PT antibody concentrations, though none of these associations survived FDR correction (Fig. 3). Carriers of (DR9)-*DQA1*03-DQB1*03:03* had higher anti-PT IgG concentrations overall (carrier $p = 0.039$, FDR = 0.116), with carriers having 2.22-fold higher concentrations than non-carriers averaged across all timepoints ($p = 0.030$, 95% CI [1.08–4.65]). The difference was significant at D0 (p = 0.039) but not at D28 or D365, where the trend remained consistent. A similar pattern was observed for PTNA in (DR9)-*DQA1*03-DQB1*03:03* carriers (carrier p = 0.030, FDR = 0.091), with carriers having 2.14-fold higher PTNA overall ($p = 0.049$, 95% CI [1.00–4.55]), driven primarily by a significant difference at D0 (p = 0.030). Moreover, carriers of (DR15)-*DQB1*06:02* also had nominally higher anti-PT IgG (carrier $p = 0.029$, FDR = 0.114), particularly at D0 (p = 0.029), with no significant difference at D28 or D365. (DR15)-*DQB1*06:02* carriers also had nominally higher PTNA concentrations overall (carrier $p = 0.016$, FDR = 0.066; overall $p = 0.044$, 95% CI [1.01–2.11]), again driven by a D0 difference (p = 0.016). Lastly, carriers of *DRB1*04:01-DQA1*03-DQB1*03:02* had nominally higher anti-PT IgG (carrier effect $p = 0.015$, FDR = 0.088; overall $p = 0.045$, 95% CI [1.01–3.29]), with a significant difference at D0 (p = 0.015) that did not persist at subsequent timepoints. However, given the critically small carrier group sizes underlying the (DR9)-*DQA1*03-DQB1*03:03* ($n = 7$ heterozygous carriers) and *DRB1*04:01-DQA1*03-DQB1*03:02* ($n = 9$ heterozygous carriers) associations, these findings must be interpreted as hypothesis-generating signals only, not as reliable associations.

3.5. Associations of HLA with anti-FHA, anti-Prn, and anti-DT antibody concentrations

A time-dependent association between (DR15)-*DQB1*06:02* carriage and anti-FHA IgG was observed, which survived FDR correction for both the main carrier effect (p = 0.002, FDR = 0.018) and the time × carrier interaction (p < 0.001, FDR = 0.007; Fig. 4D). Carriers had significantly higher anti-FHA IgG concentrations at D0 (p = 0.002, 95% CI

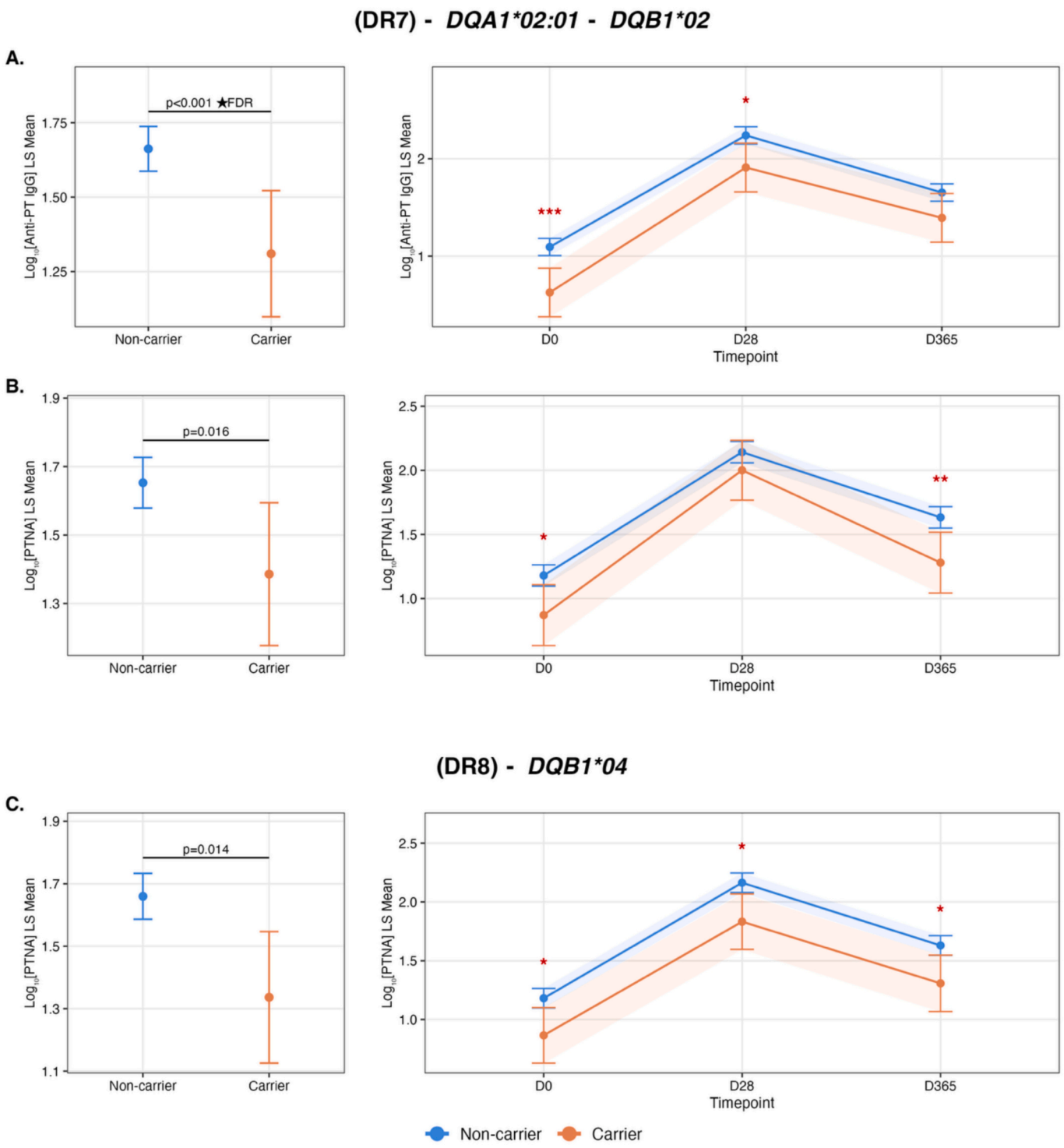

Fig. 2. *HLA class II haplotypes associated with lower anti-PT IgG and PTNA concentrations.* LS mean antibody concentrations in carriers and non-carriers of (DR7)-*DQA1*02:01-DQB1*02* (panels A–B; $n = 14$ carriers) and (DR8)-*DQB1*04* (panel C; $n = 15$ carriers), estimated from primary linear mixed-effects models. For each panel, the left plot shows overall LS means, averaged across all three time points with proportional weighting, and the significance bracket indicates the carrier main effect p-value, and the star represents significant p-values after FDR correction. The right plot shows LS means at each individual time point, with the error bars and shaded bands representing the 95% confidence interval. Red asterisks above the right-hand plots denote timepoint-specific significance from pairwise emmeans contrasts after Tukey's correction (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Blue, non-carriers; orange, carriers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

[1.24–2.45]), but concentrations converged following the Tdap booster with no significant difference at D28 or D365. For anti-FHA IgA, (DR7)-*DQA1*02:01-DQB1*02* carriers had nominally lower concentrations (carrier p = 0.031, FDR = 0.367; Fig. 4A), with carriers having 3.82-fold lower overall anti-FHA IgA (p = 0.017, 95% CI [1.28–11.46]). Significant differences were observed at D0 (p = 0.031) and D365 (p = 0.036). (DR15)-*DQB1*06:02* and (DR9)-*DQA1*03-DQB1*03:03* showed nominally significant time × carrier interactions for anti-FHA IgA (p = 0.036, FDR = 0.223 and p = 0.037, FDR = 0.223, respectively) in the absence of significant carrier main effects (Fig. 4B and E). For DR15, no

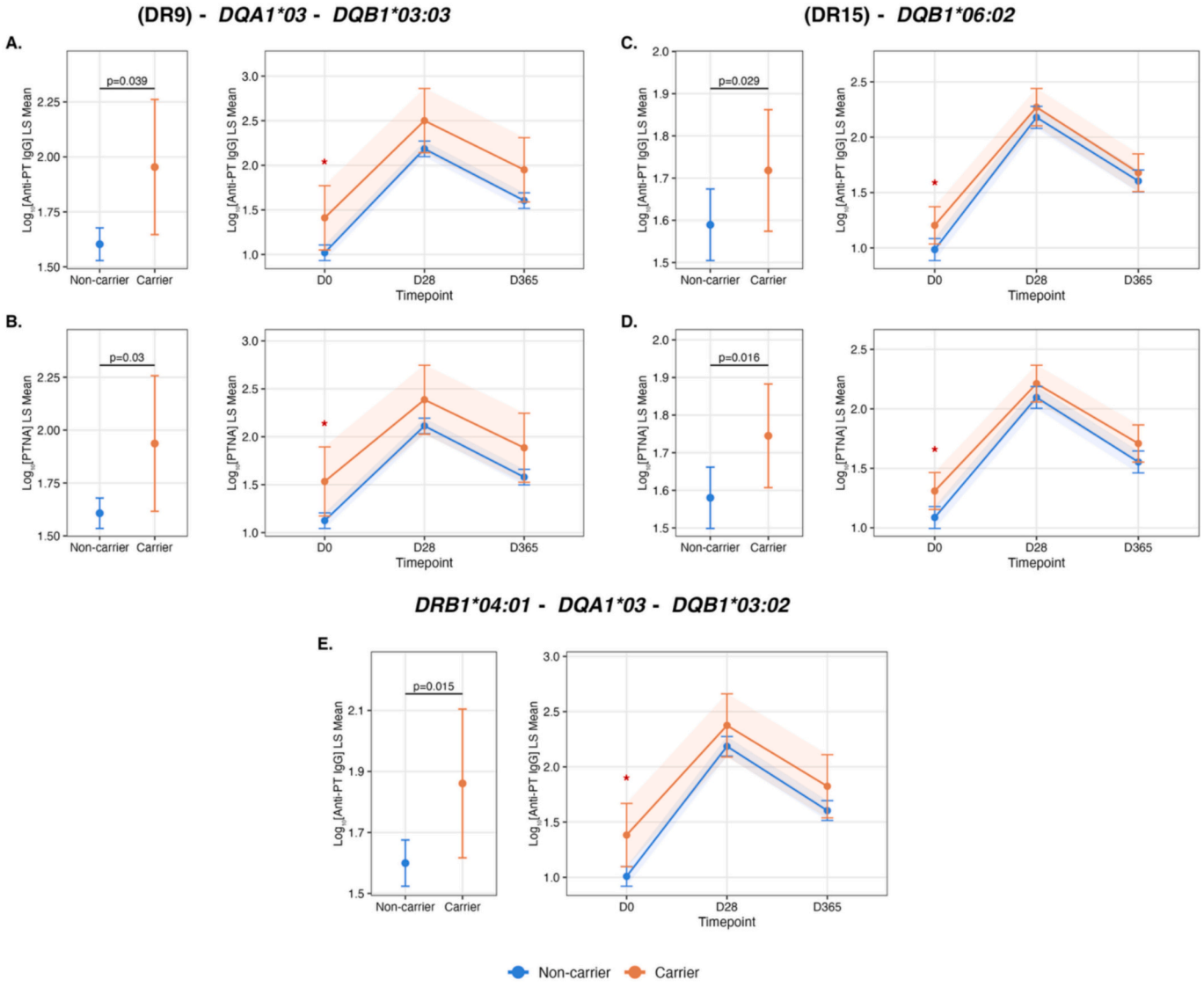

Fig. 3. *HLA class II haplotypes associated with higher anti-PT IgG and PTNA concentrations.* LS mean antibody concentrations in carriers and non-carriers of (DR9)-*DQA1*03-DQB1*03:03* (panels A–B; $n = 7$ carriers), (DR15)-*DQB1*06:02* (panels C-D; $n = 32$ carriers), and *DRB1*04:01-DQA1*03-DQB1*03:02* (panel E, $n = 11$), estimated from primary linear mixed-effects. For each panel, the left plot shows overall LS means, averaged across all three time points with proportional weighting, and the significance bracket indicates the p-value for the carrier main effect. The right plot shows LS means at each time point, with error bars and a shaded band representing the 95% confidence interval. Red asterisks above the right-hand plots denote timepoint-specific significance from pairwise emmeans contrasts after Tukey's correction (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Blue, non-carriers; orange, carriers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significant differences were observed at any time point, although carriers appeared to have lower anti-FHA IgA levels. For DR9, a nominally significant difference emerged only at D365, where carriers had 6.21-fold higher IgA concentrations than non-carriers (p = 0.047, 95% CI [1.02–37.60]).

For anti-Prn IgG, (DR9)-*DQA1*03-DQB1*03:03* carriers had nominally higher concentrations (carrier p = 0.011, FDR = 0.131; Fig. 4F), with carriers having 3.01-fold higher anti-Prn IgG overall (p = 0.039, 95% CI [1.06–8.59]). The association was driven by a significant difference at D0 (p = 0.011), with non-significant trends at D28 and D365. Carriers of *DRB1*04:01-DQA1*03-DQB1*03:02* similarly showed nominally higher anti-Prn IgG (carrier $p = 0.024$, FDR = 0.143; Fig. 4G), particularly at D0 (p = 0.024). Lastly, carriers of (DR13)-*DQB1*06:03* had nominally lower anti-DT concentrations (carrier p = 0.023, FDR = 0.270; Fig. 4C), with carriers having 1.76-fold lower anti-DT overall (p = 0.011, 95% CI [1.14–2.72]). This effect was stable and significant across all three timepoints (D0 p = 0.023, D28 p = 0.049, D365 p = 0.016).

3.6. Age group and sex as effect modifiers of HLA-antibody associations

To evaluate whether carrier effects differ across age groups, a separate exploratory model was fitted that included an age group × carrier interaction term. Of the 12 nominally significant interactions, only two haplotype–antibody pairs had sufficient carrier numbers to permit meaningful interpretation within each age group (minimum five carriers per age group) and are presented in Supplementary Fig. 3. (DR1/10)-*DQB1*05:01* carriers showed a nominally significant age group × carrier interaction for anti-Prn IgA (p = 0.019, FDR = 0.155; Supplementary Fig. 3 A). Carriers had consistently lower anti-Prn IgA concentrations than non-carriers in children, adolescents, and young adults, while the direction reversed in older adults, where carriers had significantly higher concentrations (p = 0.035). A similar trend was observed with (DR15)-*DQB1*06:02* carriers for anti-DT concentrations (p = 0.008,

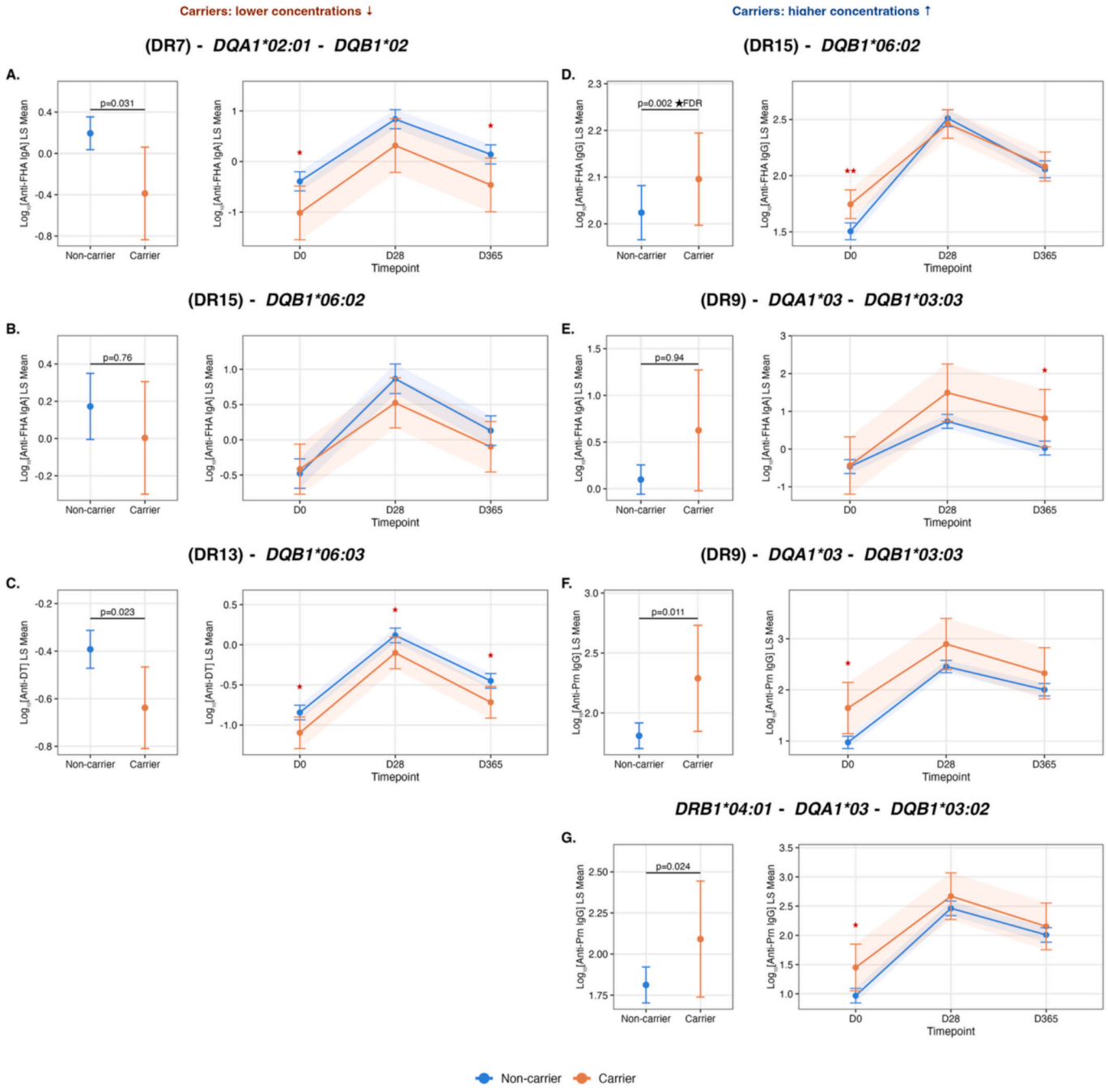

Fig. 4. *HLA class II haplotype effects on anti-FHA, anti-Prn, and anti-DT antibody concentrations.* LS mean antibody concentrations in carriers and non-carriers for seven haplotype-antibody associations. For each panel, the left plot shows overall LS means, averaged across all three time points with proportional weighting, and the significance bracket indicates the p-value for the carrier main effect. The right plot shows LS means at each time point, with error bars and a shaded band representing the 95% confidence interval. Red asterisks above the right-hand plots denote timepoint-specific significance from pairwise emmeans contrasts after Tukey's correction (*p < 0.05, **p < 0.01, ***p < 0.001). Blue, non-carriers; orange, carriers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

FDR = 0.063; Supplementary Fig. 3B). Carriers had significantly lower anti-DT concentrations in children (2.21-fold, p = 0.021), while the opposite was observed in older adults, though these differences were not statistically significant.

A secondary model including a sex × carrier interaction term was fitted for all haplotype–antibody combinations. One association survived FDR correction: (DR15)-*DQB1*06:02* × anti-DT (p = 0.002, FDR = 0.027). Seven further pairs were nominally significant but did not survive FDR correction. Pairs with fewer than five carriers per sex were excluded from detailed interpretation, leaving five qualifying pairs (Supplementary Fig. 4). All five interactions showed crossover patterns where the carrier effect was in opposite directions in males and females. For (DR15)-*DQB1*06:02*, male carriers had significantly lower anti-DT concentrations than male non-carriers (p = 0.006, 95% CI [1.33–5.06]), while female carriers showed a non-significant trend in the opposite direction (Supplementary Fig. 4D). The same haplotype showed a similar pattern for PTNA, with female carriers having significantly higher PTNA concentrations than female non-carriers (p = 0.002, 95% CI [1.28–3.08]), whereas male carriers showed a trend toward lower PTNA concentrations (Supplementary Fig. 4 A). For (DR13)-

*DQB1*06:03*, female carriers had significantly lower anti-TT concentrations ($p = 0.016$; 95% CI [1.09–2.21]), whereas male carriers trended higher (Supplementary Fig. 4E). Crossover patterns were also observed for (DR1/10)-*DQB1*05:01* with PTNA (Supplementary Fig. 4B) and (DR11/12/13)-*DQA1*05-DQB1*03:01* with anti-Prn IgG (Supplementary Fig. 4C), though neither reached significance within either sex.

3.7. Robustness of findings

Sensitivity analyses of the carrier effect in the primary models were conducted to assess the robustness of the results. Outliers were identified within each antibody × time point group, yielding 16 flagged observations across the full dataset. Refitting the primary models after outlier exclusion did not alter the direction of effect for both FDR-significant and most of the nominally significant associations. An exception was identified where the nominally significant association between (DR13)-*DQB1*06:03* and anti-DT lost significance after exclusion ($p = 0.023$ full data; $p = 0.086$ after exclusion). Full sensitivity analysis results are presented in Supplementary Table 3.

4. Discussion

This study analyzed longitudinal serum samples following Tdap booster vaccination in Finnish participants to assess the possible role of HLA class II alleles in antibody concentrations after a Tdap booster vaccination. Depending on the allele and antigen, several HLA-class II alleles were identified as having either positive or negative associations with antibody concentrations. Specifically, (DR7)-*DQA1*02:01-DQB1*02*, (DR8)-*DQB1*04*, and (DR13)-*DQB1*06:03* haplotypes were associated with low antibody concentrations, while (DR9)-*DQA1*03-DQB1*03:03*, (DR15)-*DQB1*06:02*, and *DRB1*04:01-DQA1*03-DQB1*03:02* were associated with higher antibody concentrations after an aP booster vaccination. It should be noted, however, that the associations involving haplotypes based on critically small group sizes should be considered hypothesis-generating signals rather than reliable associations.

The (DR7)-*DQA1*02:01-DQB1*02* and (DR15)-*DQB1*06:02* haplotypes have been extensively studied in relation to immune responses to several other vaccines (see below). In the present study, these haplotypes showed contrasting patterns, suggesting lower and higher antibody concentrations, respectively, following aP booster. Specifically, carriage of the (DR7)-*DQA1*0201-DQB1*02* haplotype was associated with comparatively lower concentrations of anti-PT IgG, PTNA, and anti-FHA IgA in this cohort. These observations are consistent with earlier reports linking DR7-DQ2-related alleles to reduced vaccine responsiveness to hepatitis B, influenza, and measles vaccines. Martinetti et al. reported that Italian infant hypo-responders (<10 mIU/mL) and non-responders (<2 mIU/mL) had higher frequencies of *DRB1*07:01*, *DQA1*02:01,* and *DQB1*02* alleles forming DR7-DQ2 haplotype compared to responders (50 mIU/mL) and the control group after hepatitis B primary immunization [30]. A study conducted on Spanish older adults undergoing hemodialysis also showed a higher frequency of DR7 and DQ2 alleles in non-responders (<10 mIU/mL) compared to responders and controls after having received a 4-dose hepatitis B vaccine [31]. Similarly, non-Hispanic white American children with haplotypes consisting of DR7 alleles were hyporesponsive after receiving three doses of trivalent inactivated influenza vaccine [32]. In two independent cohorts that analyzed HLA associations with measles antibody responses after vaccination in healthy American children, *DRB1*07:01* and *DQA1*02:01* alleles were consistently negatively associated with measles antibody responses [33]. In contrast, the (DR15)-*DQB1*06:02* haplotype demonstrated a trend toward higher anti-PT IgG and PTNA concentrations in this cohort. Similar patterns have been noted in studies of other vaccines, including COVID-19 and hepatitis B [34,35]. For instance, *DRB1*15:01*, which is commonly inherited in linkage with *DQB1*06:02*, has been associated with enhanced spike-specific T-cell responses following BNT162b2 vaccination in UK healthcare workers, as well as after natural SARS-CoV-2 infection [34]. In another British cohort, participants with *DRB1*15, DQA1*01,* and *DQB1*06*, which are alleles commonly inherited as a haplotype, were highly associated with anti-spike protein antibodies after vaccination [35]. In the same study, participants with *DQB1*06* alleles were also associated with higher antibody levels against the SARS-CoV-2 receptor-binding domain after the ChAdOx1 nCov-19 vaccine and had a lower likelihood of breakthrough infections compared to non-carriers [35]. Similarly, the (DR15)-*DQA1*01-DQB1*06* haplotype was also found in Swedish responders after hepatitis B vaccination [36].

The current study also identified suggestive associations between (DR13)-*DQB1*06:03* and lower anti-DT IgG concentrations; (DR15)-*DQA1*01-DQB1*06* and lower anti-FHA IgA levels; and (DR9)-*DQA1*03-DQB1*03:03* and higher anti-PT IgG, PTNA, and anti-FHA IgA levels. Notably, prior vaccine studies reported heterogeneous or even opposing associations for these haplotypes and related alleles. For instance, the *DRB1*13* and *DQB1*06:03* alleles were associated with protection against hepatitis B and influenza infections. HLA-DR13 alleles were involved in viral clearance against hepatitis B in Spanish and German subjects [37]. Furthermore, the *DQB1*06:03* allele positively influenced Influenza vaccine responses in Italian, British, and American cohorts [11] and in Swedish healthy participants who received the hepatitis B vaccine [36]. A meta-analysis also concluded that *DRB1*13:01* was associated with higher hepatitis B vaccine antibody response [38]. At the same time, a dual role of DR15 was also observed in this study. While DR15 has been associated with enhanced responses to several vaccine antigens as discussed above, it has also been linked to reduced responses to structurally distinct antigens such as anthrax protective antigen and measles-mumps-rubella vaccine antigens. In a large double-blind, placebo-controlled trial, the DR15 haplotype was associated with a 25.5–44.5% lower anthrax protective antigen IgG response in heterozygous and homozygous carriers, respectively, among European-American individuals [35]. Notably, Ovsyannikova et al. observed that the *DRB1*15/16-DQB1*06-DPB1*03* haplotype was associated with both higher measles IgG and lower rubella IgG antibody levels within the same MMR-vaccinated cohort [36], demonstrating that opposing effects of DR15 on antibody responses to different vaccine antigens within a single study are not without precedent. Lastly, limited studies have examined the effect of (DR9)-*DQA1*03-DQB1*03:03* on vaccine responses in Caucasian populations. Nevertheless, a study with Mongolian cohort reported higher neutralizing antibody titers associated with *DRB1*09:01* following Japanese encephalitis vaccination [39]. Conversely, the *DQA1*03:01* allele was associated with anti-HBsAg seronegativity [34], and the *DQB1*03:03* allele was linked to reduced vaccine responses to influenza and measles in Italians and Americans [11]. A meta-analysis further indicated that individuals carrying *DQB1*03:03* had 3.31 times higher odds of decreased antibody responses to hepatitis B, influenza, and MMR vaccines [40,41].

We also identified possible associations between (DR8)-*DQB1*04* and lower PTNA, as well as between *DRB1*04:01-DQA1*03-DQB1*03:02* and higher anti-PT and -Prn IgG following aP vaccination. There are few studies on the association of these haplotypes or alleles with vaccine responses in Caucasians, and the findings are inconsistent. For instance, *HLA-DRB1*08:03* was positively associated with the hepatitis B vaccine in Japanese and Taiwanese cohorts [11] but was negatively correlated with hepatitis B vaccine responses in Korean infants [42]. Meanwhile, a study carried out in the Inner Mongolia Autonomous Region revealed that *DQB1*04:02* was related to low neutralizing antibodies after receiving an inactivated Japanese encephalitis vaccine [39], and *DQB1*04:01* was associated with non-responsiveness after hepatitis B vaccine in a Japanese cohort [43]. For *DRB1*04:01* specifically, published vaccine immunogenicity data are sparse. A meta-analysis of 15 hepatitis B vaccine studies found that the broader *DRB1*04* allele group was associated with lower antibody responses, though this analysis did not resolve individual alleles within the group and cannot be

directly attributed to *DRB1*04:01* specifically [44]. Finally, a small Turkish study of 38 hepatitis B vaccine non-responders among 944 vaccinated hospital staff found *DRB1*04:01*-containing haplotypes to be more frequent among non-responders than responders, though the very small and unbalanced comparison groups limit the interpretability of this finding [45].

Without further studies, the mechanistic explanation for the positive and negative associations between specific HLA haplotypes and antibody concentrations after aP booster remains unknown. The HLA binding groove consists of multiple pockets that can bind peptides of varying lengths and conformations, depending on whether they contain suitable motifs that interact with amino acids lining the pockets, thereby giving rise to variability in immune responses [46]. These pockets are highly polymorphic, affecting the amino acids within them and allowing different alleles to exhibit distinct binding specificities and affinities [12]. Liu et al. showed that specific HLA alleles exhibited varying peptide-binding affinities for various pathogens, including *Bp* and *Corynebacterium diphtheriae* [47]. Another study has demonstrated that SARS-CoV-2 omicron variant spike protein mutations resulted in population-specific variations in binding affinity for different HLA class II alleles and not HLA class I in British, Russian, and Taiwanese populations [48]. This might explain not only the positive associations but also the opposite associations observed with (DR13)-*DQB1*06:03*, (DR15)-*DQA1*01-DQB1*06*, (DR9)-*DQA1*03-DQB1*03:03*, (DR8)-DQB1*04, and *DRB1*04:01-DQA1*03-DQB1*03:02* in this study, compared with the published literature on the other vaccines mentioned above. Moreover, polymorphisms can affect T-cell receptor recognition of the HLA class II-peptide complex through conformational changes, thereby disrupting these interactions [13]. Thus, it can be hypothesized that haplotypes or alleles negatively or positively associated with aP antibody concentrations may reflect impaired or enhanced recognition and processing of vaccine antigens, leading to a possible decrease or increase in T cell function, respectively. It is well known that adequate CD4+ T cell responses are required for the development of germinal centers for effective immunological memory; hence, impairment may lead to decreased antibody concentrations [49]. However, these associations could also be attributed to a different HLA allele in strong linkage disequilibrium with the reported haplotypes.

Sex-based differences in humoral immunity are phylogenetically conserved and contribute to variation in vaccine-induced antibody responses, with females typically producing higher antibody levels than males following vaccination [50]. In our primary model, sex was a significant covariate for anti-TT concentrations across all haplotype models, consistent with this established dimorphism. The secondary sex × carrier interaction analysis revealed that all five qualifying interactions showed crossover patterns where the carrier effect was in opposite directions in males and females, independent of differential haplotype frequencies between sexes. Mechanistically, estrogen increases MHC class II expression on dendritic cells, whereas testosterone exerts immunosuppressive effects. Sex-specific differences in HLA-DR-mediated antigen presentation have been demonstrated, with females showing higher immune responses to HLA-DR-presented antigens in humanized mouse models [51]. This provides a plausible biological basis for the observed interactions. However, we observed antigen-specificity in the crossover patterns, most notably the opposite DR15 carrier effects for anti-DT in males and for PTNA in females, and the lower anti-TT in female DR13 carriers versus higher in male carriers. This suggests that sex hormones do not globally amplify or suppress HLA-DR-restricted responses but may interact differentially with antigen-specific T helper pathways. For (DR11/12/13)-*DQA1*05-DQB1*03:01* and anti-Prn IgG, and (DR1/10)-*DQB1*05:01* and PTNA, crossover patterns were observed, but neither reached significance in either sex, suggesting that the cohort may have been underpowered to detect these effects at the individual-sex level. Whether these crossover patterns reflect a generalized principle of sex-hormone modulation of HLA-DR-restricted pertussis vaccine responses or represent chance findings in a small cohort requires replication in large sex-stratified studies.

Age group was a significant covariate for most antibodies in the primary model, reflecting the combined influence of primary vaccination history and age-related immune changes on antibody concentrations across the cohort. Acellular and whole-cell pertussis vaccines induce qualitatively distinct immunological memory, with aP priming associated with Th2-dominant responses and wP priming associated with Th1/Th17 memory, differences that persist into adolescence and adulthood and shape the magnitude and quality of responses to subsequent booster vaccination [52,53]. In addition, the older adult age group in our cohort who received wP primary vaccination may be subject to immunosenescence, which adds a further layer of complexity. Older adults who were vaccinated with tetanus, diphtheria, and pertussis antigens have lower antibody levels than younger adults, and a booster dose at age 60 may still fail to elicit protective antibody levels, reflecting the well-documented age-related decline in humoral immunity [54].

The exploratory age-group × carrier interactions observed in this study may reflect a complex interplay between HLA haplotype, vaccination history-dependent immune memory, and age-related immune decline that cannot be separated in this cohort, given the collinearity between age group and vaccination history.

Despite its exploratory nature, this study contributes additional data on the relationship between HLA haplotypes and antigen-specific antibody concentrations after aP booster vaccination in the Finnish population. The haplotype frequencies observed in the present study are consistent with those derived from the FDPR and is considered representative of the general Finnish population [29]. Taken together, the findings presented here provide preliminary observations that may inform future studies aimed at characterizing HLA-associated variability in immune responses to pertussis vaccination.

Several limitations of this study warrant consideration. The small sample size of the BERT cohort limits the statistical power for subgroup analyses, particularly for haplotypes with low carrier frequencies, where individual age or sex subgroups may contain fewer than five carriers, precluding reliable estimation of subgroup-specific effects. Critically, haplotype groups at or near the minimum carrier frequency threshold, specifically (DR13) – *DQB1*06:04, (DR7) - DQA1*02:01 - DQB1*03:03*, and (DR9)-*DQA1*03-DQB1*03:03*, are too small to yield statistically reliable associations. Findings from groups of this size must be regarded as hypothesis-generating signals only and cannot be interpreted as confirmed associations without independent replication in substantially larger cohorts. Despite this, haplotype frequencies did not differ significantly between age groups or between sexes for any haplotype. The sex distribution in adult age groups was also uneven, with a higher proportion of female participants, especially in the young and older adult groups. This imbalance was mainly due to the exclusion of individuals who had received a pertussis-containing booster vaccine within the past five years [6]. In Finland, males aged 18 and older entering military service typically receive Tdap booster vaccinations. Given the known sex differences in innate and adaptive immunity, a potential impact of this bias on the results cannot be ruled out [35], though sex was included as a covariate in all primary and secondary models. The age group × carrier interaction model is further limited by the perfect collinearity between age group and primary vaccination type in this cohort, as discussed above, such that the interaction cannot be attributed to age and is treated as entirely exploratory. Despite these limitations, sensitivity analyses using outlier exclusion confirmed that the two FDR-significant associations, (DR7)-*DQA1*02:01-DQB1*02* × anti-PT IgG and (DR15)-*DQB1*06:02* × anti-FHA IgG, were robust, remaining FDR-significant after removal of flagged observations, lending greater confidence to these specific findings. In contrast, the nominally significant association between (DR13)-*DQB1*06:03* and anti-DT was attenuated after outlier exclusion. Finally, as an exploratory study with a modest sample size, all nominally significant associations, and particularly those that do not survive FDR correction, require independent replication in larger

cohorts with defined individual vaccination histories before firm conclusions can be drawn.

In conclusion, our findings further support a possible association of HLA class II antigens with antibody concentrations after aP booster vaccination. These findings emphasize the importance of further research to clarify how HLA class II haplotypes may influence aP vaccine responses in various populations.

CRediT authorship contribution statement

Denise Anabe: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Jorma Ilonen:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Alex-Mikael Barkoff:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Johanna T. Teräsjärvi:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Jussi Mertsola:** Writing – review & editing, Project administration, Investigation, Formal analysis. **Pieter van Gageldonk:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Annemarie Buisman:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Minna Kiviniemi:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Johanna Lempainen:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Qiushui He:** Writing – review & editing, Writing – original draft, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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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 data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2026.128697.

Data availability

Data described in the article will be availabe on request.

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