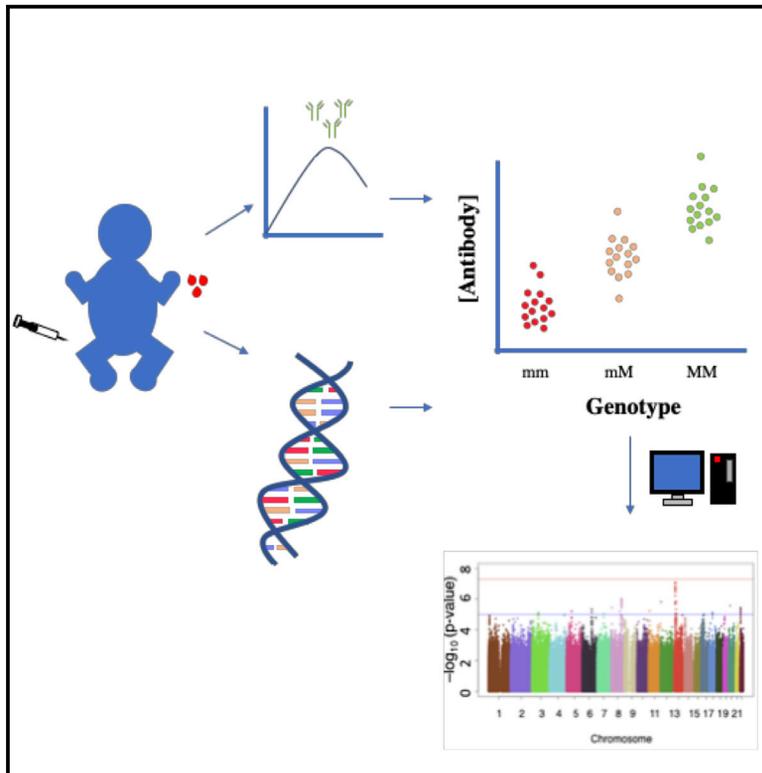# Common Genetic Variations Associated with the Persistence of Immunity following Childhood Immunization

Graphical Abstract

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In Brief

Genetic variants impact immune responses to antigenic stimuli. O'Connor et al. show that variants within the human leukocyte antigen (HLA) and signal-regulatory proteins loci are associated with persistence of immunity following immunization, suggesting these genes are involved in regulating immune responses to routine childhood immunization.

Highlights

- Signal-regulatory proteins locus associated with the persistence of MenC immunity

- SNPs within the HLA locus associated with persistence of TT-specific immunity

- Classical HLA alleles associated with TT-specific immunity, independently of lead SNPs

CellPress

Article

Common Genetic Variations Associated with the Persistence of Immunity following Childhood Immunization

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SUMMARY

Vaccines have revolutionized public health, preventing millions of deaths each year, particularly in childhood. Yet, there is considerable variability in the magnitude and persistence of vaccine-induced immunity. Maintenance of specific antibody is essential for continuity of vaccine-induced serological protection. We conducted a genome-wide association study into the persistence of immunity to three childhood vaccines: capsular group C meningococcal (MenC), *Haemophilus influenzae* type b, and tetanus toxoid (TT) vaccines. We detail associations between variants in a locus containing a family of signal-regulatory proteins and the persistence MenC immunity. We postulate a regulatory role for the lead SNP, with supporting epigenetic and expression quantitative trait loci data. Furthermore, we define associations between SNPs in the human leukocyte antigen (HLA) locus and the persistence of TT-specific immunity. Moreover, we describe four classical HLA alleles, HLA DRB1*0301, HLA DQB1*0201, HLA DQB1*0602, and HLA DRB1*1501, associated with TT-specific immunity, independent of the lead SNP association.

INTRODUCTION

Vaccination will prevent an estimated 23.3 million deaths this decade (2011–2020) (Lee et al., 2013). Globally, polysaccharide-encapsulated organisms are the leading cause of bacterial meningitis and pneumonia in children. *Haemophilus influenzae* type b (Hib) and *Neisseria meningitidis* are estimated to cause 8.13 million and 500,000 serious illnesses worldwide each year, respectively (Watt et al., 2009; Wilder-Smith and Memish,

2003). Over 150,000 Hib and 50,000 meningococcal cases result in death each year (GBD 2013 Mortality and Causes of Death Collaborators, 2015). Tetanus is an acute and life-threatening disease, caused by the bacterium *Clostridium tetani*. An estimated 61,000 deaths resulted from neonatal tetanus in the year 2011 alone (World Health Organization, 2017).

Unlike plain polysaccharide vaccines, conjugate vaccines (i.e., polysaccharides chemically conjugated to carrier proteins such as tetanus toxoid [TT]) are T cell-dependent antigens that are immunogenic from early infancy (Pollard et al., 2009; Black et al., 1991). In the United Kingdom, introduction of national immunization programs with the Hib conjugate vaccine (1992—) and the capsular group C meningococcal (MenC) conjugate vaccine (1999—) resulted in the virtual disappearance of invasive disease caused by these organisms (Miller et al., 2001; Salisbury et al., 2014). However, persistence of serum antibody to encapsulated bacteria such as Hib and MenC is essential for direct protection against these rapidly invading pathogens (McVernon et al., 2003; Snape et al., 2006). During a resurgence of Hib disease in the United Kingdom, Hib was found to be still colonizing older healthy children, and invasive disease was observed in some preschool children despite demonstrable immunological memory, indicating the failure of B cell memory to confer direct protection or block transmission (McVernon et al., 2003; Oh et al., 2008).

Unfortunately, following immunization with Hib conjugate and MenC conjugate vaccines in early infancy, antibody levels rapidly wane. By 1 year of age, >25% and >50% of children have antibody levels below the putative threshold of protection for Hib conjugate and MenC organisms, respectively (Trotter et al., 2003; Pace et al., 2015). In the United Kingdom, booster doses of Hib and MenC vaccines were introduced for all children at 1 year of age in 2003 and 2006, respectively (McVernon et al., 2003). Yet, the majority of older children were found to lack protective antibodies levels after the 12-month booster dose of MenC vaccine, prompting the recent introduction of an

adolescent dose of MenC (in the form of a quadrivalent Men-ACWY vaccine) to induce herd immunity and prevent transmission to younger children (Perrett et al., 2010; Pace et al., 2015; Public Health England, 2014). The mechanisms underlying the persistence of antibody are of major interest, because effectiveness and acceptability of vaccines would be improved if protection were sustained after infant immunization without the need for repeated boosting through childhood.

A number of factors have been implicated in determining vaccine responses and the persistence of immunity: age, sex, ethnicity, microbiota, nutritional status, and infectious diseases (Siegrist and Aspinall, 2009; Klein et al., 2015; Kollmann, 2013; Savy et al., 2009; Brodin et al., 2015; Nguyen et al., 2016). Importantly, twin studies have also shown vaccine-induced immunity to be highly heritable, and recent studies have started to unpick the genetic components underlying this complex trait (Newport et al., 2004; O'Connor and Pollard, 2013; Mentzer et al., 2015; O'Connor et al., 2014; Davila et al., 2010; Png et al., 2011). The genome-wide association study (GWAS) approach has already provided considerable insight into the genetic basis of a number of complex immune-related diseases (Parkes et al., 2013; Visscher et al., 2017). Here we used a two-stage GWAS approach to evaluate the genetic determinants of the persistence of immunity to three routine childhood immunizations: MenC conjugate vaccine, Hib conjugate vaccine, and tetanus vaccine.

RESULTS

Participants and Study Design

DNA was available from 3,602 children from nine vaccine studies conducted in Oxford, United Kingdom, and one vaccine study conducted in Bilthoven, the Netherlands (Blanchard-Rohner et al., 2013; de Voer et al., 2010; Khatami et al., 2011, 2014; Pace et al., 2007; Snape et al., 2006, 2008a, 2008b, 2010; Waddington et al., 2010). Median age at last vaccination was 1,966 days (interquartile range [IQR], 125–3,701.5), and median time since vaccination was 1,478 days (IQR, 287–1,769). Participant demographics are shown in Table S1. The two-stage GWAS design is illustrated in Figure 1. The discovery cohort (n = 2,061) was genotyped using the Illumina Omniexpress-12v1 or Omniexpress-12v1.1 microarray. Genotypes were further imputed using the 1000 Genomes Phase I integrated variant set release (March 2012) as the variant reference set (1000 Genomes Project Consortium et al., 2012). Approximately 6.7 million SNPs were included in quantitative trait association analyses of four $\log_{10}$ normalized vaccine-induced immunological measures: MenC-specific immunoglobulin G (IgG) concentrations, MenC-specific serum bactericidal antibody (SBA) titers (functional antibody), Hib polyribosylribitol phosphate (PRP)-specific IgG concentrations, and TT-specific IgG concentrations.

The genomic control (λ_{GC}) inflation factors for MenC-specific IgG, MenC-specific SBA, PRP-specific IgG, and TT-specific IgG were 1.013, 1.010, 1.002, and 1.019, respectively (Figures S1A–S1D). The percentage of overall variance, in each of the immunological measures, explained by genetic information captured on the genotyping microarray (i.e., heritability) varied

from 14% to 81% (Table 1). On the basis of the discovery stage, we selected 276 SNPs for replication-stage genotyping (Table S2). Replication SNPs were genotyped using either Sequenom or TaqMan assays on both the discovery (n = 2,061) and replication (n = 1,541) cohorts. A genome-wide significance threshold of $p < 5 \times 10^{-8}$ was set, and two independent loci were found to surpass this threshold in combined (discovery + replication) analysis (Tables 2 and 3).

MenC Conjugate Vaccine

Manhattan plots of the results of the discovery stage association analyses are displayed in Figure 2. Twenty-four SNPs had suggestive statistical associations ($p < 1 \times 10^{-5}$) with the persistence of MenC-specific IgG concentrations, 13 of which were within six genes: SENP2, NBEA, LRFN5, MCTP2, GALR1, and GGT5 (Table S3). Seventy-four SNPs had suggestive association with the persistence of MenC-specific SBA titers, of which 50 resided within nine genes: ALK, CNTN6, FAM124B, LINC00265, NPAS3, PDLIM1, PTPRD, SIRPG, and SLC1A3 (Table S3).

A subset of SNPs from the discovery stage were selected for genotyping in the replication cohort; these were selected to capture the most credible association signals in a parsimonious manner (as described in the STAR Methods). A total of 69 and 57 SNPs were selected to capture the association signals of MenC-specific IgG concentrations and SBA titers, respectively. Nine and 12 of these SNPs failed Sequenom iPLEX design, leaving 60 and 45 SNPs for MenC-specific IgG and SBA replication analyses, respectively (Table S2).

A single locus was found to contain SNPs that were significantly ($p < 5 \times 10^{-8}$) associated with the persistence of MenC-specific SBA titers in combined analysis of discovery and replication data (Table 2; Table S4). The SNP most significantly associated with MenC-specific SBA titers was rs6135736 ($p = 6.0 \times 10^{-9}$, dominant model), which lies within LOC105369219 (gene of unknown function) and <50 kb from the transcription start site of SIRPG. The meta-analysis of summary data from discovery and replication cohorts is shown in Table S5. Results of the combined analysis (i.e., mega-analysis) and meta-analysis were highly correlated (Pearson's correlation coefficient > 0.98; Figures S1E and S1F).

The SNPs associated with the persistence of MenC-specific SBA titers were within a genomic region containing a family of signal-regulatory proteins: SIRPA, SIRPB, and SIRPG. Using the densely genotyped discovery dataset, the association signal appears to be confined to a region incorporating SIRPG, but separated from the other two signal-regulatory proteins (SIRPs) by recombination hotspots (Figure S2). The HaploReg tool (http://www.broadinstitute.org/mammals/haploreg) was used to systematically mine the Roadmap Epigenomics Consortium dataset, finding several variants to reside within putative promoter and enhancer regions, in a number of tissues including blood cells, thymus, and spleen (Romanoski et al., 2015; Ward and Kellis, 2012). Moreover, the lead SNP at this locus (i.e., most statistically associated with MenC SBA titers), rs6135736, was predicted to substantially alter the motifs of four TFs: serum response factor (SRF), zinc-finger protein 410 (ZNF410), retinoic acid receptor gamma (RARγ), and RAR-related orphan receptor alpha (RORα)
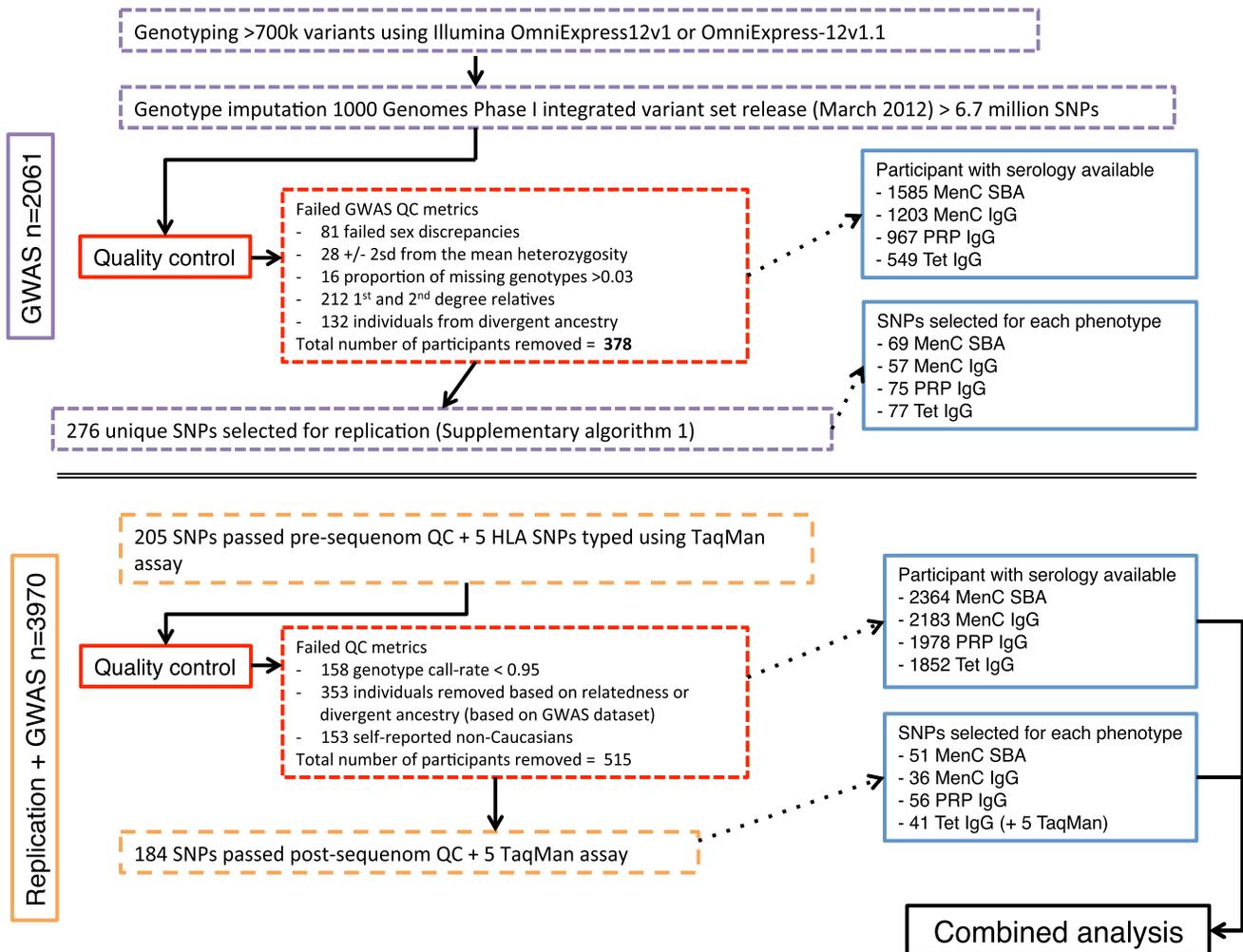
Figure 1. Study Design

(Figure S1I). Moreover, this SNP is an expression quantitative trait locus (eQTL) for *SIRPG* and *SIRPB1* (Figures S1J and S1K).

Hib Conjugate Vaccine

A total of 122 SNPs had regression p values suggestive of association ($p < 1 \times 10^{-5}$) with the persistence of PRP-specific IgG concentrations (Figure 2C). These SNPs were within five annotated genes: *FOXP1*, *PDSS2*, *MLXIPL*, *CSMD3*, and *IGL* (immunoglobulin lambda locus). Seventy-five variants were selected for replication analysis following the SNP pruning algorithm (https://github.com/dan-scholar/LD_pruning). Six of these SNPs failed Sequenom iPLEX design; none of the remaining 69 SNPs surpassed the level of genome-wide significance in the combined analysis of discovery and replication cohorts (Table S4).

TT Vaccine

In the discovery stage, 111 SNPs in eight genes surpassed the level of suggestive association with TT-specific IgG concentrations: *CDH9*, *MICB*, *HLA-DQA1*, *DOCK4*, *DENND1A*, *LHPP*,

TMEM135, and *RBFOX3* (Figure 2D). Furthermore, five SNPs in an intergenic region of chromosome 10 surpassed the level of genome-wide significance ($p < 5 \times 10^{-8}$); the closest gene (<250 kb) to these associations was *TCERG1L*. Seventy-seven SNPs were selected for replication. Twenty-two of these SNPs failed Sequenom iPLEX design, leaving 55 SNPs for genotyping. As anticipated, the replication SNPs selected within the human leukocyte antigen (HLA) locus failed Sequenom iPLEX design; due to the polymorphic nature of this locus, iPLEX primer design is problematic. Therefore, conditional linear regression analysis was used to select five HLA SNPs for genotyping using the TaqMan SNP genotyping methodology.

None of the Sequenom genotyped SNPs surpassed the level of genome-wide significance in combined analysis (Table S4). The lead SNP (rs75727401) in the intergenic region of chromosome 10 that reached the genome-wide significance threshold in the discovery stage was not assessed because it failed Sequenom quality control (QC). However, this locus was tagged by rs79697632 (r^2 of 0.88 with rs75727401), which was not found to substantiate the initial GWAS finding. A SNP in HLA

Table 1. The Overall Variance in Vaccine Responses, Calculated Using the GREML Method

Vaccine Measure	n	V_G (SE)	V_E (SE)	V_P (SE)	V_G/V_P (SE)
MenC SBA	1,585	0.21 (0.15)	0.54 (0.15)	0.75 (0.03)	0.28 (0.2)
MenC IgG	1,203	0.22 (0.12)	0.24 (0.12)	0.46 (0.02)	0.48 (0.27)
Hib IgG	964	0.05 (0.12)	0.3 (0.12)	0.35 (0.02)	0.14 (0.33)
TT IgG	547	0.21 (0.11)	0.05 (0.11)	0.26 (0.02)	0.81 (0.44)

GREML, genome-based restricted maximum likelihood; V_E, residual variance; V_G, genetic variance; V_P, phenotypic variance.

(rs2523650), typed on the TaqMan platform, did surpass the level of genome-wide significance in the combined analysis and meta-analysis (Table 3; Table S6). This variant resides within MICB-DT (gene of uncertain function) and is annotated as an eQTL for *HLA-DRB1* and *HLA-DRB5* on the HaploReg database (http://www.broadinstitute.org/mammals/haploreg).

Classical Human Leukocyte Antigen Alleles

The discovery GWAS dataset was used to impute 168 classical HLA alleles (Table S7). Four alleles, HLA-DQB1*0201, HLA-DRB1*0301, HLA DQB1*0602, and HLA DRB1*1501, were significantly associated with TT-specific IgG concentrations, after correction for multiple testing (i.e., by number of HLA alleles tested; p < 0.05/168) (Table 4). *In silico* epitope mapping predicted DRB1*0301 to have the fewest strong and weak binding affinity to TT epitopes of the panel of *HLA-DRB1* alleles assessed (Figure S3). Conditional analysis, by including HLA DRB1*0301, HLA DQB1*0201, HLA DQB1*0602, or HLA DRB1*1501 as covariates in the linear regression model, implied the lead SNP association (rs2523650) was independent of these classical alleles (Table S8). Results of the classical HLA association analyses conditioning on HLA DRB1*0301, HLA DQB1*0201, HLA DQB1*0602, or HLA DRB1*1501 are shown in Table S9.

Pleiotropic Loci Associated with Immunity to Childhood Immunizations

A total of 301 genes contained SNPs with a regression (additive model) $p < 1 \times 10^{-4}$, with one or more vaccine-induced serological measures (Figure 3). Nine of these genes were shared with more than one vaccine-induced immunological measure (Figure 3). Two genes, *SRSF7* and *RP11-115H18.1*, shared the same SNPs between these serological measures and were therefore correlated and concordant (Table S10).

DISCUSSION

Evoking robust and sustained vaccine-induced immunity from early life is a crucial component of global health initiatives to combat the humanitarian burden of infectious disease. Here we provide a GWAS of persistence of immunity following three routine childhood vaccines: MenC conjugate vaccine, Hib conjugate vaccine, and TT vaccine. We describe two genetic loci associated ($p < 5 \times 10^{-8}$) with the persistence of vaccine-induced immunity following childhood immunization.

First, we present a statistical association between SNPs within a genomic region containing a family of signal-regulatory pro-

teins (SIRPs, *SIRPA*, *SIRPB*, and *SIRPG*) and the persistence of MenC-specific SBA titers. SIRPs are part of the immunoglobulin superfamily, structurally similar to the antigen receptors but non-rearranging (van den Berg et al., 2004; Barclay and Brown, 2006). SIRP genes are termed "paired receptors" because they have similar extracellular regions but differing signaling potentials (Barclay and Brown, 2006). SIRPα has an inhibitory cytoplasmic motif, SIRPβ associates with an activating motif, and SIRPγ has a short cytoplasmic tail that is not believed to mediate signal transduction (Barclay and Brown, 2006; Nettleship et al., 2013). Whereas SIRPα and SIRPγ bind CD47, the ligand for SIRPβ has yet to be identified (Seiffert et al., 2001; Brooke et al., 2004). SIRPα is expressed mainly by myeloid cells and has an important role in innate immune recognition and regulation (Dai et al., 2017). However, SIRPγ is expressed by T cells and a subset of B cells, and engagement of SIRPγ on T cells by CD47 on antigen-presenting cells has been shown to enhance antigen-specific T cell proliferation (Piccio et al., 2005; Barclay and Brown, 2006). Upregulation of the *SIRPG* gene transcript has been observed in peripheral blood samples taken 7 days after vaccination with trivalent inactivated influenza or meningococcal (ACWY) vaccine, supporting a role for this gene in vaccine responses (Nakaya et al., 2011; O'Connor et al., 2017).

The SNPs most significantly associated with the persistence of MenC-specific antibody were intronic and intergenic. It is possible that low-frequency nonsynonymous coding variant(s) functionally underpins this association. However, it is now widely understood that regulatory, rather than protein-coding, variants underlie the majority of association signals arising from GWASs of complex phenotypes (Maurano et al., 2012). To further explore the theory of an underlying regulatory variant, we leveraged epigenetic and transcription factor motif data to assess the candidacy of the associated variants. Epigenetic data show the lead SNP (rs6135736) to be delineated by histone modifications characteristic of an active enhancer, in mitogen-stimulated T helper cells. Also, this SNP was predicted to severely alter the motifs of four transcription factors. Furthermore, this SNP is associated with the expression of two proximal genes (*SIRPG* and *SIRPB1*) in whole blood.

We found that the SIRP locus association was limited to MenC-specific SBA titers and was not identified in the analysis of total MenC-specific IgG concentrations. Bactericidal antibody (measured by SBA) is contingent on antibody subclass and binding affinity, and therefore mechanistically links this association to the production of antibody with the functional ability to induce complement-mediated cytolysis (Michaelsen et al., 1991; Naess et al., 1999). T cells are essential for the maintenance of germinal centers (GCs), where B cells undergo affinity maturation, and also influence B cell IgG subclass selection (Mayumi et al., 1983; Vinuesa et al., 2010; Mongini et al., 1981). T follicular helper cells provide help to B cells within the GC, which differentiate into short-lived plasma cells, longer-lived plasma cells (LLPCs), or memory B cells. Only a fraction of GC-derived plasma cells migrate to the bone marrow, where there is a limited number of long-term survival niches and competition for residency (Radbruch et al., 2006). The properties pertaining to long-term persistence of plasma cell clones are unclear, but considerable

Table 2. Top 10 SNPs, Ranked by p Value, Associated with the Persistence of MenC-Specific SBA Titers following Vaccination, in Combined Analysis (Discovery + Replication Cohort)

CHR	SNP	Gene Symbol	Cohort	N	Additive Model β	Additive Model p	Dominant Model β	Dominant Model p
20	rs6135736	LOC105369219	discovery	1,436	0.19 (0.12, 0.26)	3.2×10^{-7}	0.24 (0.15, 0.33)	1.2×10^{-7}
			replication	915	0.08 (−0.01, 0.17)	0.09	0.12 (0.01, 0.22)	0.03
			combined[a]	2,351	0.16 (0.10, 0.22)	6.4×10^{-8}	0.20 (0.14, 0.28)	6.0×10^{-9}[b]
20	rs4814511		discovery	1,443	0.19 (0.11, 0.26)	3.5×10^{-7}	0.24 (0.15, 0.33)	1.7×10^{-7}
			replication	920	0.05 (−0.04, 0.13)	0.27	0.09 (−0.01, 0.20)	0.08
			combined[a]	2,363	0.14 (0.09, 0.20)	3.7×10^{-7}	0.20 (0.12, 0.27)	1.8×10^{-8}[b]
20	rs3818168	SIRPG	discovery	1,441	0.19 (0.12, 0.27)	2.7×10^{-7}	0.23 (0.14, 0.32)	4.1×10^{-7}
			replication	919	0.06 (−0.03, 0.14)	0.20	0.11 (0.01, 0.22)	0.04
			combined[a]	2,360	0.15 (0.09, 0.20)	5.9E−7	0.19 (0.12, 0.26)	6.2×10^{-8}
20	rs17770331	SIRPG-AS1, SIRPG	discovery	1,443	0.19 (0.11, 0.26)	5.6×10^{-7}	0.22 (0.13, 0.31)	1.8×10^{-6}
			replication	919	0.05 (−0.03, 0.14)	0.21	0.09 (−0.01, 0.20)	0.07
			combined[a]	2,362	0.14 (0.09, 0.20)	7.3×10^{-7}	0.17 (0.11, 0.25)	4.9×10^{-7}
4	rs10517505		discovery	1,444	−0.33 (−0.49, −0.17)	7.2×10^{-5}	−0.36 (−0.53, −0.20)	2.3×10^{-5}
			replication	920	−0.2359 (−0.42, −0.05)	0.01	−0.24 (−0.43, −0.05)	0.01
			combined[a]	2,364	−0.30 (−0.42, −0.17)	3.4×10^{-6}	−0.32 (−0.44, −0.19)	1.2×10^{-6}
20	rs4813191	SIRPG-AS1, SIRPG	discovery	1,444	0.15 (0.08, 0.22)	1.7×10^{-5}	0.17 (0.09, 0.27)	7.3×10^{-5}
			replication	918	0.06 (−0.02, 0.14)	0.17	0.10 (0.00, 0.21)	0.04
			combined[a]	2,362	0.12 (0.07, 0.17)	7.8×10^{-6}	0.16 (0.09, 0.228)	4.9×10^{-6}
2	rs1467180		discovery	1,431	−0.30 (−0.48, −0.14)	4.8×10^{-4}	−0.32 (−0.50, −0.15)	3.5×10^{-4}
			replication	920	−0.18 (−0.40, 0.05)	0.13	−0.18 (−0.40, 0.05)	0.13
			combined[a]	2,351	−0.27 (−0.41, −0.13)	1.5×10^{-4}	−0.27 (−0.42, −0.14)	1.2×10^{-4}
6	rs551216	ADGRB3	discovery	1,429	−0.12 (−0.19, −0.05)	2.6×10^{-4}	−0.16 (−0.25, −0.07)	3.3×10^{-4}
			replication	920	−0.03 (−0.11, 0.05)	0.48	−0.10 (−0.21, −0.00)	0.05
			combined[a]	2,349	−0.08 (−0.13, −0.03)	2.7×10^{-3}	−0.13 (−0.20, −0.06)	2.0×10^{-4}
12	rs1358274		discovery	1,444	−0.17 (−0.26, −0.09)	9.2×10^{-5}	−0.18 (−0.28, −0.09)	1.8×10^{-4}
			replication	919	−0.04 (−0.15, 0.06)	0.43	−0.07 (−0.18, 0.05)	0.28
			combined[a]	2,363	−0.13 (−0.20, −0.06)	2.3×10^{-4}	−0.14 (−0.22, −0.07)	2.2×10^{-4}
2	rs2042086		discovery	1,444	−0.15 (−0.23, −0.07)	1.7×10^{-4}	−0.18 (−0.28, −0.09)	9.6×10^{-5}
			replication	918	−0.03 (−0.13, 0.05)	0.43	−0.04 (−0.16, 0.06)	0.4
			combined[a]	2,362	−0.11 (−0.17, −0.04)	6.8×10^{-4}	−0.13 (−0.21, −0.06)	2.4×10^{-4}

CHR, chromosome; N, number of participants included in the analysis.
[a]Results from combining all individual-level data into a single analysis.
[b]p values $<5 \times 10^{-8}$ are genome-wide significant.

differences between the half-life of serum antibody to different vaccines (e.g., >200 years for measles and mumps compared with ~10 years for TT) suggest these features are programmed into plasma cells during their maturation in the GC (Amanna et al., 2007). We postulate that the lead SNP described here is causally involved in the generation of competent and competitive (i.e., for long-term survival niches) high-affinity LLPCs, via an altered TF-binding motif within a hypothetical enhancer region in antigen-stimulated T cells.

We also identified a region within the HLA gene complex that contained SNPs associated with the persistence of TT-specific IgG. HLA molecules present peptides to T cells, which in turn induce B cells to produce antibodies (Murphy et al., 2008). Given their role, HLA genes have frequently been candidates in studies exploring the genetic determinants of vaccine responses (O'Connor and Pollard, 2013; Mentzer et al., 2015). Moreover,

a GWAS of hepatitis B surface antigen (HBsAg) vaccine-induced immunity found independent signals across the HLA region that were associated with HBsAg-specific IgG concentrations following vaccination (Png et al., 2011). Furthermore, variants with the HLA-DPB1 locus have been linked to rubella neutralization antibody titers following measles-mumps-rubella vaccine, albeit the association did not reach the customary threshold of genome-wide significance (lead SNP p = 8.62×10^{-8}) (Lambert et al., 2015). Interestingly, HLA has been estimated to account for only a minority of the total heritability associated with humoral immunity to TT, rubella, and HBV. Yet, to date, no genes outside the HLA region have been implicated, at the level of genome-wide significance, with responses to these vaccines. One explanation for this could be a modest individual effect size for these putative non-HLA loci. Previous studies of immune-related diseases have shown a relationship between the number of loci

Table 3. The Results of Combined Analysis for the Five HLA SNPs Selected for Replication Analysis with TT-Specific IgG Concentrations

CHR	SNP	Cohort	N	Additive Model β (95% CI)	Additive Model p	Dominant Model β (95% CI)	Dominant Model p
6	rs2523650	discovery	638	0.12 (0.06, 0.18)	4.1×10^{-5}	0.15 (0.07, 0.22)	9.9×10^{-5}
		replication	1,151	0.10 (0.05, 0.14)	2.1×10^{-5}	0.12 (0.05, 0.16)	0.0003
		combined[a]	1,789	0.12 (0.08, 0.15)	2.6×10^{-10b}	0.14 (0.09, 0.18)	6.5×10^{-9b}
6	rs6903608	discovery	625	0.12 (0.06, 0.18)	4.7×10^{-5}	0.18 (0.10, 0.25)	5.4×10^{-6}
		replication	1,096	0.03 (−0.02, 0.07)	0.21	0.03 (−0.02, 0.09)	0.24
		combined[a]	1,721	0.06 (0.03, 0.10)	2.4×10^{-4}	0.09 (0.04, 0.14)	1.3×10^{-4}
6	rs3094188	discovery	621	0.11 (0.05, 0.16)	2.1×10^{-4}	0.12 (0.04, 0.19)	0.0031
		replication	1,108	0.03 (−0.01, 0.08)	0.11	0.07 (0.01, 0.13)	0.01
		combined[a]	1,729	0.06 (0.03, 0.10)	4.6×10^{-4}	0.08 (0.04, 0.14)	2.6×10^{-4}
6	rs9268877	discovery	602	0.10 (0.05, 0.16)	1.1×10^{-4}	0.13 (0.05, 0.22)	6.2×10^{-4}
		replication	1,094	0.03 (−0.01, 0.07)	0.17	0.04 (−0.02, 0.10)	0.17
		combined[a]	1,696	0.06 (0.02, 0.09)	0.0012	0.07 (0.02, 0.12)	0.0043
6	rs2523663	discovery	593	0.06 (0.00, 0.12)	0.03	0.08 (0.00, 0.16)	0.04
		replication	1,065	0.02 (−0.01, 0.07)	0.19	0.01 (−0.05, 0.07)	0.67
		combined[a]	1,658	0.04 (0.01, 0.08)	0.0151	0.04 (−0.01, 0.09)	0.1178

CHR, chromosome; CI, confidence interval; N, number of participants in the analysis.
[a]Results from combining all of the data into a single analysis.
[b]$p < 5 \times 10^{-8}$ are genome-wide significant.

reaching genome-wide significance and sample size, and it is likely that the study of larger vaccinee cohorts will yield more loci associated with immunity to vaccine antigens (Parkes et al., 2013). In our study, we estimated the heritability of vaccine-induced immunity to vary from 14% to 81%; TT immunity was estimated to be the most heritable of these measures. Interestingly, analysis of TT immunity required 10 principal components (PCs) to regress out genomic inflation, compared with 3 PCs for the other vaccine responses. There are a number of potential explanations for this finding, including trait heritability, sample size, linkage disequilibrium structure, and number of causal variants, which have all been shown to influence genomic inflation (Yang et al., 2011b).

The identification of causal variants within the HLA region is intrinsically difficult due to its complex and long-range linkage disequilibrium structure. The association signals may originate from differing abilities of classical HLA alleles to bind peptide. However, it may also be the case that cis-regulatory elements underlie these associations. To explore this further, we imputed classical HLA alleles using the discovery stage GWAS dataset (Dilthey et al., 2011). This analysis suggested HLA-DQB1*0201, HLA-DRB1*0301, HLA DQB1*0602, and HLA DRB1*1501 were associated with TT-IgG concentrations. Intriguingly, HLA-DRB1*0301 is distinct from the other major HLA-DRB1 alleles both genetically and immunologically, recognizing a peptide motif that is dissimilar from that recognized by the other major HLA-DRB1 alleles (Sidney et al., 1992). This allele has also been associated with increased susceptibility to several autoimmune and allergic phenotypes, as well as lower HBsAg-specific IgG concentrations following hepatitis B vaccine, supporting a generalizable difference in the peptide-binding preferences (Alper et al., 1989; Li et al., 2009; Handunnetthi et al., 2010). The data presented here lend some support to the idea of het-

erogeneous T cell responses to different class II alleles contributing to the quantitative differences in humoral responses to TT.

The index HLA SNP (rs2523650) remained significantly associated with TT-IgG concentrations after conditioning for HLA DRB1*0301, HLA DQB1*0201, HLA DQB1*0602, or HLA DRB1*1501, suggesting this association is independent of these classical alleles. Moreover, rs2523650 has been annotated as an expression-trait quantitative locus for HLA-DRB1 and HLA-DRB5 (Zeller et al., 2010). Therefore, these data support the mechanism underlying this association to be regulatory, rather than classical, HLA allele dependent.

Previous studies have shown considerable correlation in immune responses to a number of childhood vaccines (Newport et al., 2004; Finn et al., 2000; Goldblatt et al., 1999). There was some thought that this could be largely because of age-dependent immunological maturation. However, disparities in the correlations between vaccine antigens contradict a simple "responsiveness" trait (Finn et al., 2000). Nevertheless, it is not known whether there are pleiotropic loci that are involved in "vaccine responsiveness" in a generic sense. Here, analysis for pleiotropic loci identified nine genes, at a liberal p value cutoff of 10^{-4}, which were shared between two or more measures of vaccine-induced immunity. Intriguingly, PTPRD was associated with both MenC-specific SBA titers and PRP-specific IgG concentrations, and has previously been associated with rubella-specific IFN-γ responses following measles, mumps, and rubella (MMR) vaccination (Kennedy et al., 2014). Interestingly, we did not find any overlap in genes containing the most statistically associated ($p < 1 \times 10^{-5}$) SNPs between the vaccine-induced serological measures. This finding is consistent with data from the large study into pleiotropic loci among immune-related diseases, which suggested SNPs with the largest effect sizes tended to be phenotype specific (Parkes et al., 2013).

Table 4. Top 10 Imputed Human Leukocyte Antigen Alleles, Based upon p Values, in Association Analysis with TT-Specific IgG Concentrations in Discovery Stage

CHR	SNP	N	Additive Model β (95% CI)	Additive Model p	Dominant Model β (95% CI)	Dominant Model p
6	HLA_DRB1_03	535	−0.19 (−0.28, −0.11)	1.42×10^{-5a}	−0.18 (−0.28, −0.09)	1.75×10^{-4a}
6	HLA_DRB1_0301	535	−0.19 (−0.28, −0.11)	1.42×10^{-5a}	−0.18 (−0.28, −0.09)	1.75×10^{-4a}
6	HLA_DQB1_0201	535	−0.18 (−0.27, −0.09)	5.46×10^{-5a}	−0.17 (−0.27, −0.07)	6.35×10^{-4}
6	HLA_DQB1_02	535	−0.14 (−0.21, −0.07)	5.91×10^{-5a}	−0.17 (−0.26, −0.08)	1.10×10^{-4a}
6	HLA_DRB1_1501	535	0.15 (0.07, 0.24)	5.31×10^{-4}	0.20 (0.10, 0.30)	9.02×10^{-5a}
6	HLA_DRB1_15	535	0.14 (0.06, 0.23)	8.87×10^{-4}	0.19 (0.10, 0.29)	1.08×10^{-4a}
6	HLA_DQB1_0602	535	0.15 (0.06, 0.23)	1.08×10^{-3}	0.18 (0.08, 0.28)	2.88×10^{-4a}
6	HLA_A_0201	535	−0.11 (−0.18, −0.04)	1.26×10^{-3}	−0.13 (−0.21, −0.043)	3.32×10^{-3}
6	HLA_DQA1_01	535	0.10 (0.04, 0.16)	1.58×10^{-3}	0.10 (0.01, 0.19)	2.50×10^{-2}
6	HLA_A_24	535	0.19 (0.07, 0.32)	2.32×10^{-3}	0.18 (0.04, 0.31)	8.88×10^{-3}

CHR, chromosome; CI, confidence interval; N, number of participants included in the analysis.
[a]$p < 3 \times 10^{-4}$ (0.05/168).

In this study, participants were included from 10 sub-studies, which differed in terms of age of vaccination, time since vaccination, immunological assay protocols, vaccine schedules and compositions, and concomitant vaccines administered. Although these factors were included as covariates in the linear models, this heterogeneity will have been detrimental to statistical power (Ioannidis et al., 2009). Of note, there was considerable heterogeneity, between the discovery and replication cohorts, in the effect size estimates for the SNPs with the most statistical evidence for association with persistence of MenC-specific SBA titers. Here we report two SNPs associated with MenC-specific SBA titers, at the level of genome-wide significance, based on combining individual-level data from all cohorts; although this results in increased power (compared with meta-analysis), the caveat is that this approach is more susceptible to inflated error rates or loss of power when there is heterogeneity between cohorts (Skol et al., 2006). Nevertheless, the SNP with the most statistical evidence for association with the persistence of MenC-specific SBA titers showed evidence of replication (p value < 0.05) in the replication cohort and a meta-analysis p value of 5.1×10^{-8}.

No SNPs were found to be associated with PRP-specific IgG concentrations at the level of genome-wide significance, an observation that could have a number of possible explanations. The statistical power to identify SNPs associated with quantitative traits is influenced by variant effect size, the number and frequency of causal variants, sample size, as well as the robustness of the phenotypic measure (Manchia et al., 2013; Shin and Lee, 2015). Of note, the PRP-specific IgG assessments described here were made in several laboratories, and problems with inter-assay standardization of PRP-specific IgG quantification have been described (Kelly et al., 2004). Moreover, although Hib carriage rates have been extremely low in the United Kingdom for the last decade among preschool children, older participants, in particular, may have been exposed to Hib colonization in the pre-vaccine era, when carriage rates in young children were up to 8% (McVernon et al., 2004). Equally, polysaccharides cross-reactive with the Hib polysaccharide, PRP, are present on several bacterial species, such that the rate of immunizing exposures (Hib or cross-reactive bacteria) is estimated to be 1.2 per year per child, even in the post-vaccination era (Leino et al., 2002). Phenotypical variance introduced by measurement variability and immunological boosting, because of natural exposure, may have severely diminished statistical power to detect genetic variants associated with the persistence of PRP-specific IgG.

Because these data were limited to Caucasians from the United Kingdom and the Netherlands, it is unclear how these would relate to other ethnic populations, especially considering the allelic frequency of the lead SNP proximal to *SIRPG* varies drastically between HapMap populations: European ancestry (CEU) minor allele frequency (MAF) ~20% and Sub-Saharan Africans (YRI) MAF ~1%. Conversely, the future use of trans-ethnic vaccine cohorts, such as those described by Mentzer et al. (2015), may leverage different linkage equilibrium patterns to help fine-map causal variants. Even though GWAS captures common variation adequately, it does not capture rare variants efficiently (i.e., MAF < 1%). This may be particularly relevant because rare variants of large effect sizes may conceivably contribute to the "extremes" of vaccine responsiveness (e.g., vaccine failure). Next-generation sequencing approaches are more conducive to the description of rare variants; however, the cost of these approaches often precludes their use. In this study, we described genetic variants associated with the persistence of immunity following immunization. However, the absence of data from the acute vaccine response (peaking at about 1 month post-vaccination) meant we could not differentiate individuals who responded poorly ("primary vaccine failures") from those who failed to maintain serological immunity ("secondary vaccine failures").

Currently, we are not aware of any implemented healthcare system to identify children whose vaccine-induced immunity has waned and would benefit from additional immunizations. In fact, the additional healthcare provision required for such a system might make it unfeasible in many settings. Conversely, we envisage genetic markers of vaccine responses could soon prove their clinical utility. Although the cost of whole-genome sequencing is constantly decreasing, our understanding of these data and their clinical utility has been rapidly rising (Khera et al., 2018). It is realistic to conceive that neonatal screening
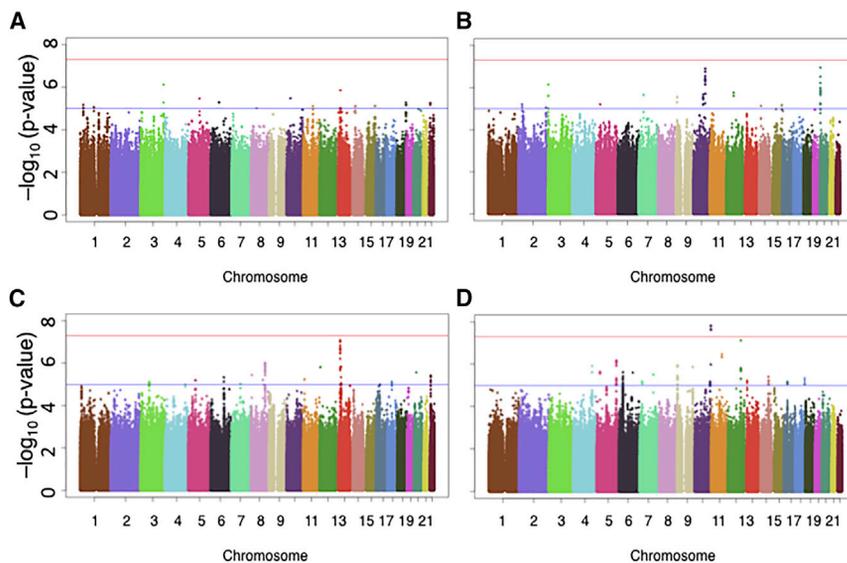
Figure 2. Manhattan Plots of p Values from Regression Analyses between SNPs and $\log_{10}$-Transformed Vaccine-Induced Immunological Measures

(A–D) Manhattan plots of p values from regression analyses between SNPs and $\log_{10}$-transformed vaccine-induced immunological measure: (A) MenC-specific IgG concentrations (n = 1,203), (B) MenC-specific SBA titers (n = 1,585), (C) PRP-specific IgG concentrations (n = 964), and (D) TT-specific IgG concentrations (n = 547). The horizontal blue line represents the level of suggestive statistical association ($p < 1 \times 10^{-5}$), and the horizontal red line denotes the level of genome-wide statistical significance ($p < 5 \times 10^{-8}$).

approaches will soon incorporate a number of genetic risk factors (potentially derived the whole-genome sequencing at birth) (Howard et al., 2015). Therefore, it is feasible that in the near future that the persistence of immunity could be predicted and vaccine regimens personalized to maximize vaccine effectiveness.

The modest effect size of the genetic associations described in this study are consistent with the hypothesis that the genetic variants most strongly associated with complex phenotypes typically account for only a small fraction of the genetic variance; many additional causal loci, with small effect sizes, explain much of the trait heritability (Manolio et al., 2009; Yang et al., 2013). Also, a number of other host factors such as age, nutritional status, microbiota, and infectious diseases have been shown to influence immune responses (Kampmann and Jones, 2015). For example, some acute viral infections have been shown to have profound immunomodulatory effects, long after symptomatic recovery (Mina et al., 2015; Huang and Hong, 1973). Naturally acquired immunity, and immunological boosting through natural exposure, may also strongly influence immunity to organisms against which children are vaccinated (Andrews et al., 2015; Wilson et al., 2017; Voysey et al., 2017). However, this is not likely to have played a major role in immunity to the vaccines studied here; during the time frame of this study, carriage rates for Hib and MenC were very low in young children, and tetanus is non-communicable (McVernon et al., 2004; Maiden et al., 2008). Age at vaccination is known to influence vaccine responses; although we included this in linear regression analysis, heterogeneity in age of vaccination will have been detrimental to study power (Snape et al., 2008a; Tang et al., 2015). Moreover, twin-twin variability in a number of immune system parameters has been shown to increase with age, suggesting GWAS studies focused on vaccine responses early in life may have the greatest statistical power (Brodin et al., 2015). Here we assessed genetics factors with generalizable effects on vaccine responses, notwithstanding there may also be sex-dependent factors that influence immune responses to vaccines (Klein et al., 2015).

In conclusion, we describe a GWAS of the persistence of immunity to three routine childhood vaccines. We detail two loci that were associated with the persistence of vaccine-induced immunity, at the level of genome-wide significance. Although these variants inevitably account for only a small portion of the total genetically determined contribution to persistence of vaccine-induced immunity, this study demonstrates the utility of applying this "hypothesis-free" approach in generating candidates for future functional and mechanistic investigations, the description of which may ultimately influence vaccine development and implementation.

STAR★METHODS

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

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SUPPLEMENTAL INFORMATION

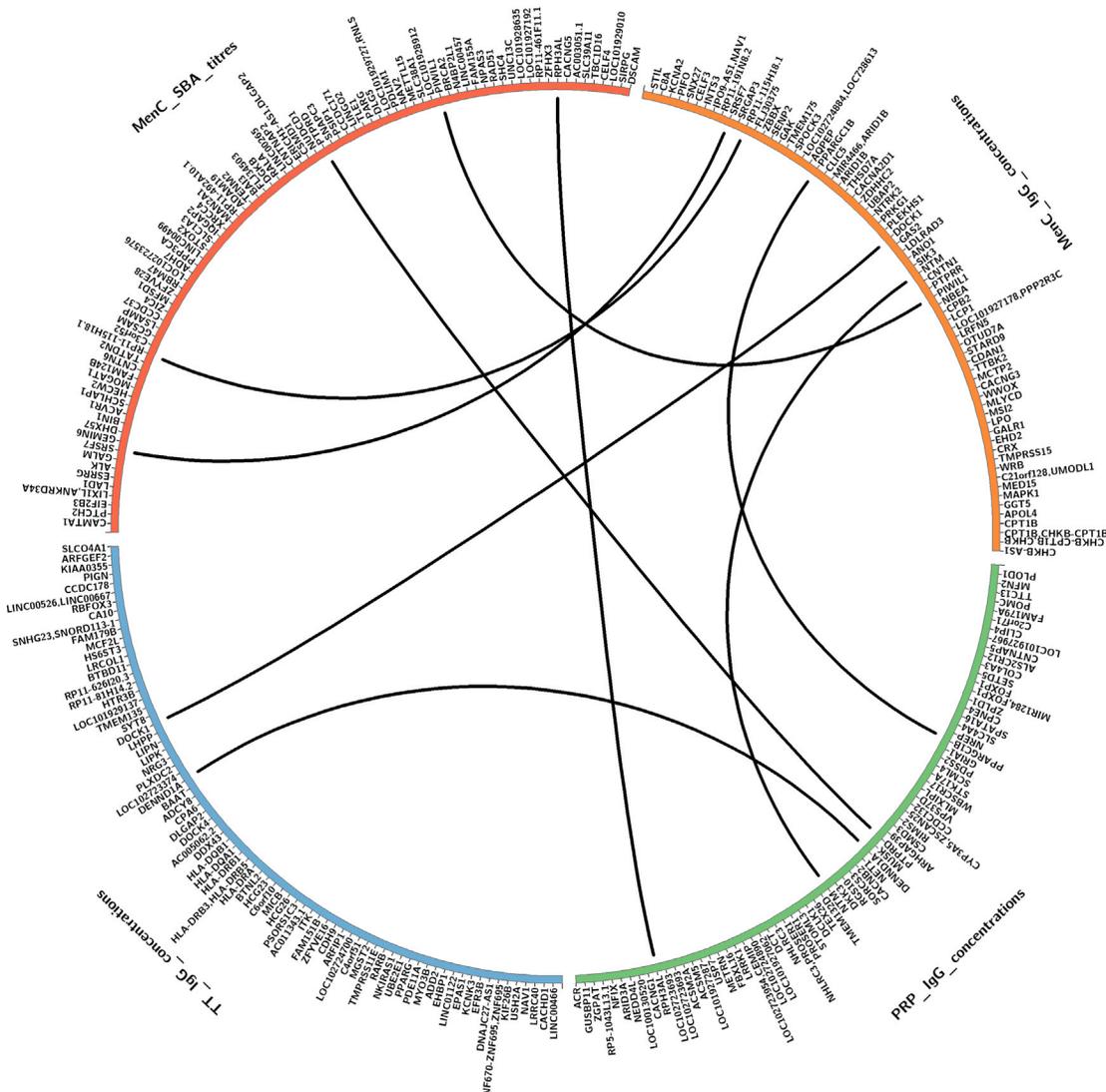Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2019.05.053.

Figure 3. Circos Plot Illustrating Genes Containing SNPs Associated (p < 1 × 10⁻⁴) with One or More of Four Vaccine-Induced Serological Measures

Data are included from the discovery stage GWAS of persistence of MenC-specific IgG concentrations (n = 1,203), MenC-specific serum bactericidal titers (n = 1,585), PRP-specific IgG concentrations (n = 964), and TT-specific IgG concentrations (n = 547). Genes that are associated (p < 1 × 10⁻⁴) with more than the serological measures assessed are highlighted with links. This diagram was generated using Circos (http://circos.ca/).

AUTHOR CONTRIBUTIONS

Conceptualization, D.O., M.L.H., C.C.K., and A.J.P.; Methodology, D.O., E.P., C.C.K., and C.H.; Investigation, D.O. and E.P.; Resources, M.D.S., A.V.S.H., F.v.d.K., M.L.H., and A.J.P.; Writing – Original Draft, D.O.; Writing – Review & Editing, D.O., E.P., C.C.K., M.D.S., A.V.S.H., F.v.d.K., C.H., M.L., M.L.H., and A.J.P. Funding Acquisition, M.L., M.L.H., and A.J.P.; Supervision, A.V.S.H., M.L., M.L.H., and A.J.P.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1000 Genomes Project Consortium; Abecasis, G.R., Auton, A., Brooks, L.D., DePristo, M.A., Durbin, R.M., Handsaker, R.E., Kang, H.M., Marth, G.T., and McVean, G.A. (2012). An integrated map of genetic variation from 1,092 human genomes. Nature 491, 56–65.

Alper, C.A., Kruskall, M.S., Marcus-Bagley, D., Craven, D.E., Katz, A.J., Brink, S.J., Dienstag, J.L., Awdeh, Z., and Yunis, E.J. (1989). Genetic prediction of nonresponse to hepatitis B vaccine. N. Engl. J. Med. 321, 708–712.

Amanna, I.J., Carlson, N.E., and Slifka, M.K. (2007). Duration of humoral immunity to common viral and vaccine antigens. N. Engl. J. Med. 357, 1903–1915.

Anderson, C.A., Pettersson, F.H., Clarke, G.M., Cardon, L.R., Morris, A.P., and Zondervan, K.T. (2010). Data quality control in genetic case-control association studies. Nat. Protoc. 5, 1564–1573.

Andrews, S.F., Kaur, K., Pauli, N.T., Huang, M., Huang, Y., and Wilson, P.C. (2015). High preexisting serological antibody levels correlate with diversification of the influenza vaccine response. J. Virol. 89, 3308–3317.

Aulchenko, Y.S., Ripke, S., Isaacs, A., and van Duijn, C.M. (2007). GenABEL: an R library for genome-wide association analysis. Bioinformatics 23, 1294–1296.

Barclay, A.N., and Brown, M.H. (2006). The SIRP family of receptors and immune regulation. Nat. Rev. Immunol. 6, 457–464.

Black, S.B., Shinefield, H.R., Fireman, B., Hiatt, R., Polen, M., and Vittinghoff, E.; The Northern California Kaiser Permanente Vaccine Study Center Pediatrics Group (1991). Efficacy in infancy of oligosaccharide conjugate Haemophilus influenzae type b (HbOC) vaccine in a United States population of 61,080 children. Pediatr. Infect. Dis. J. 10, 97–104.

Blanchard-Rohner, G., Snape, M.D., Kelly, D.F., O'Connor, D., John, T., Kibwana, E., Parks, H., Ford, K., Dull, P.M., and Pollard, A.J. (2013). Seroprevalence and placental transmission of maternal antibodies specific for Neisseria meningitidis Serogroups A, C, Y and W135 and influence of maternal antibodies on the immune response to a primary course of MenACWY-CRM vaccine in the United Kingdom. Pediatr. Infect. Dis. J. 32, 768–776.

Booy, R., Taylor, S.A., Dobson, S.R., Isaacs, D., Sleight, G., Aitken, S., Griffiths, H., Chapel, H., Mayon-White, R.T., Macfarlane, J.A., et al. (1992). Immunogenicity and safety of PRP-T conjugate vaccine given according to the British accelerated immunisation schedule. Arch. Dis. Child. 67, 475–478.

Brodin, P., Jojic, V., Gao, T., Bhattacharya, S., Angel, C.J., Furman, D., Shen-Orr, S., Dekker, C.L., Swan, G.E., Butte, A.J., et al. (2015). Variation in the human immune system is largely driven by non-heritable influences. Cell 160, 37–47.

Brooke, G., Holbrook, J.D., Brown, M.H., and Barclay, A.N. (2004). Human lymphocytes interact directly with CD47 through a novel member of the signal regulatory protein (SIRP) family. J. Immunol. 173, 2562–2570.

Coetzee, S.G., Coetzee, G.A., and Hazelett, D.J. (2015). motifbreakR: an R/Bioconductor package for predicting variant effects at transcription factor binding sites. Bioinformatics 31, 3847–3849.

Collins, C.L., Salt, P., McCarthy, N., Chantler, T., Lane, L., Hemme, F., Diggle, L., Buttery, J., Kitchin, N.R., Moxon, E.R., and Pollard, A.J. (2004). Immunogenicity and safety of a low-dose diphtheria, tetanus and acellular pertussis combination vaccine with either inactivated or oral polio vaccine as a pre-school booster in UK children. Vaccine 22, 4262–4269.

Dai, H., Friday, A.J., Abou-Daya, K.I., Williams, A.L., Mortin-Toth, S., Nicotra, M.L., Rothstein, D.M., Shlomchik, W.D., Matozaki, T., Isenberg, J.S., et al. (2017). Donor SIRPα polymorphism modulates the innate immune response to allogeneic grafts. Sci. Immunol. 2, eaam6202.

Davila, S., Froeling, F.E., Tan, A., Bonnard, C., Boland, G.J., Snippe, H., Hibberd, M.L., and Seielstad, M. (2010). New genetic associations detected in a host response study to hepatitis B vaccine. Genes Immun. 11, 232–238.

de Voer, R.M., Schepp, R.M., Versteegh, F.G., van der Klis, F.R., and Berbers, G.A. (2009). Simultaneous detection of Haemophilus influenzae type b polysaccharide-specific antibodies and Neisseria meningitidis serogroup A, C, Y, and W-135 polysaccharide-specific antibodies in a fluorescent-bead-based multiplex immunoassay. Clin. Vaccine Immunol. 16, 433–436.

de Voer, R.M., Mollema, L., Schepp, R.M., de Greeff, S.C., van Gageldonk, P.G., de Melker, H.E., Sanders, E.A., Berbers, G.A., and van der Klis, F.R. (2010). Immunity against Neisseria meningitidis serogroup C in the Dutch population before and after introduction of the meningococcal c conjugate vaccine. PLoS ONE 5, e12144.

Delaneau, O., Zagury, J.F., and Marchini, J. (2013). Improved whole-chromosome phasing for disease and population genetic studies. Nat. Methods 10, 5–6.

Dilthey, A.T., Moutsianas, L., Leslie, S., and McVean, G. (2011). HLA*IMP—san integrated framework for imputing classical HLA alleles from SNP genotypes. Bioinformatics 27, 968–972.

Finn, A., Blondeau, C., and Bell, F. (2000). Haemophilus influenzae type B (Hib) antibody responses in children given diphtheria-tetanus-acellular pertussis-Hib combination vaccines. J. Infect. Dis. 181, 2117–2118.

GBD 2013 Mortality and Causes of Death Collaborators (2015). Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 385, 117–171.

Gheesling, L.L., Carlone, G.M., Pais, L.B., Holder, P.F., Maslanka, S.E., Plikaytis, B.D., Achtman, M., Densen, P., Frasch, C.E., Käyhty, H., et al. (1994). Multicenter comparison of Neisseria meningitidis serogroup C anti-capsular polysaccharide antibody levels measured by a standardized enzyme-linked immunosorbent assay. J. Clin. Microbiol. 32, 1475–1482.

Goldblatt, D., Richmond, P., Millard, E., Thornton, C., and Miller, E. (1999). The induction of immunologic memory after vaccination with Haemophilus influenzae type b conjugate and acellular pertussis-containing diphtheria, tetanus, and pertussis vaccine combination. J. Infect. Dis. 180, 538–541.

Handunnetthi, L., Ramagopalan, S.V., Ebers, G.C., and Knight, J.C. (2010). Regulation of major histocompatibility complex class II gene expression, genetic variation and disease. Genes Immun. 11, 99–112.

Howard, H.C., Knoppers, B.M., Cornel, M.C., Wright Clayton, E., Sénécal, K., and Borry, P.European Society of Human Genetics; P3G International Paediatric Platform; Human Genome Organisation; PHG Foundation (2015). Whole-genome sequencing in newborn screening? A statement on the continued importance of targeted approaches in newborn screening programmes. Eur. J. Hum. Genet. 23, 1593–1600.

Howie, B.N., Donnelly, P., and Marchini, J. (2009). A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet. 5, e1000529.

Huang, S.W., and Hong, R. (1973). Lymphopenia and multiple viral infections. JAMA 225, 1120–1121.

Ioannidis, J.P., Thomas, G., and Daly, M.J. (2009). Validating, augmenting and refining genome-wide association signals. Nat. Rev. Genet. 10, 318–329.

Jia, X., Han, B., Onengut-Gumuscu, S., Chen, W.-M., Concannon, P.J., Rich, S.S., Raychaudhuri, S., and de Bakker, P.I. (2013). Imputing amino acid polymorphisms in human leukocyte antigens. PLoS ONE 8, e64683.

Kampmann, B., and Jones, C.E. (2015). Factors influencing innate immunity and vaccine responses in infancy. Philos. Trans. R. Soc. Lond. B Biol. Sci. 370, 20140148.

Kelly, D.F., Moxon, E.R., and Pollard, A.J. (2004). Haemophilus influenzae type b conjugate vaccines. Immunology 113, 163–174.

Kennedy, R.B., Ovsyannikova, I.G., Haralambieva, I.H., Lambert, N.D., Pankratz, V.S., and Poland, G.A. (2014). Genome-wide SNP associations with rubella-specific cytokine responses in measles-mumps-rubella vaccine recipients. Immunogenetics 66, 493–499.

Khatami, A., Peters, A., Robinson, H., Williams, N., Thompson, A., Findlow, H., Pollard, A.J., and Snape, M.D. (2011). Maintenance of immune response

throughout childhood following serogroup C meningococcal conjugate vaccination in early childhood. Clin. Vaccine Immunol. 18, 2038–2042.

Khatami, A., Clutterbuck, E.A., Thompson, A.J., McKenna, J.A., Pace, D., Birks, J., Snape, M.D., and Pollard, A.J. (2014). Evaluation of the induction of immune memory following infant immunisation with serogroup C Neisseria meningitidis conjugate vaccines—exploratory analyses within a randomised controlled trial. PLoS ONE 9, e101672.

Khera, A.V., Chaffin, M., Aragam, K.G., Haas, M.E., Roselli, C., Choi, S.H., Natarajan, P., Lander, E.S., Lubitz, S.A., Ellinor, P.T., and Kathiresan, S. (2018). Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. Nat. Genet. 50, 1219–1224.

Klein, S.L., Marriott, I., and Fish, E.N. (2015). Sex-based differences in immune function and responses to vaccination. Trans. R. Soc. Trop. Med. Hyg. 109, 9–15.

Kollmann, T.R. (2013). Variation between Populations in the Innate Immune Response to Vaccine Adjuvants. Front. Immunol. 4, 81.

Lambert, N.D., Haralambieva, I.H., Kennedy, R.B., Ovsyannikova, I.G., Pankratz, V.S., and Poland, G.A. (2015). Polymorphisms in HLA-DPB1 are associated with differences in rubella virus-specific humoral immunity after vaccination. J. Infect. Dis. 211, 898–905.

Lee, L.A., Franzel, L., Atwell, J., Datta, S.D., Friberg, I.K., Goldie, S.J., Reef, S.E., Schwalbe, N., Simons, E., Strebel, P.M., et al. (2013). The estimated mortality impact of vaccinations forecast to be administered during 2011-2020 in 73 countries supported by the GAVI Alliance. Vaccine 31 (Suppl 2), B61–B72.

Leino, T., Auranen, K., Mäkelä, P.H., Käyhty, H., Ramsay, M., Slack, M., and Takala, A.K. (2002). Haemophilus influenzae type b and cross-reactive antigens in natural Hib infection dynamics; modelling in two populations. Epidemiol. Infect. 129, 73–83.

Li, Y., Ni, R., Song, W., Shao, W., Shrestha, S., Ahmad, S., Cunningham, C.K., Flynn, P.M., Kapogiannis, B.G., Wilson, C.M., and Tang, J. (2009). Clear and independent associations of several HLA-DRB1 alleles with differential antibody responses to hepatitis B vaccination in youth. Hum. Genet. 126, 685–696.

Maiden, M.C., Ibarz-Pavón, A.B., Urwin, R., Gray, S.J., Andrews, N.J., Clarke, S.C., Walker, A.M., Evans, M.R., Kroll, J.S., Neal, K.R., et al. (2008). Impact of meningococcal serogroup C conjugate vaccines on carriage and herd immunity. J. Infect. Dis. 197, 737–743.

Manchia, M., Cullis, J., Turecki, G., Rouleau, G.A., Uher, R., and Alda, M. (2013). The impact of phenotypic and genetic heterogeneity on results of genome wide association studies of complex diseases. PLoS ONE 8, e76295.

Manolio, T.A., Collins, F.S., Cox, N.J., Goldstein, D.B., Hindorff, L.A., Hunter, D.J., McCarthy, M.I., Ramos, E.M., Cardon, L.R., Chakravarti, A., et al. (2009). Finding the missing heritability of complex diseases. Nature 461, 747–753.

Marchini, J., and Howie, B. (2010). Genotype imputation for genome-wide association studies. Nat. Rev. Genet. 11, 499–511.

Maslanka, S.E., Gheesling, L.L., Libutti, D.E., Donaldson, K.B., Harakeh, H.S., Dykes, J.K., Arhin, F.F., Devi, S.J., Frasch, C.E., Huang, J.C., et al.; The Multi-laboratory Study Group (1997). Standardization and a multilaboratory comparison of Neisseria meningitidis serogroup A and C serum bactericidal assays. Clin. Diagn. Lab. Immunol. 4, 156–167.

Maurano, M.T., Humbert, R., Rynes, E., Thurman, R.E., Haugen, E., Wang, H., Reynolds, A.P., Sandstrom, R., Qu, H., Brody, J., et al. (2012). Systematic localization of common disease-associated variation in regulatory DNA. Science 337, 1190–1195.

Mayumi, M., Kuritani, T., Kubagawa, H., and Cooper, M.D. (1983). IgG subclass expression by human B lymphocytes and plasma cells: B lymphocytes precommitted to IgG subclass can be preferentially induced by polyclonal mitogens with T cell help. J. Immunol. 130, 671–677.

McVernon, J., Johnson, P.D., Pollard, A.J., Slack, M.P., and Moxon, E.R. (2003). Immunologic memory in Haemophilus influenzae type b conjugate vaccine failure. Arch. Dis. Child. 88, 379–383.

McVernon, J., Howard, A.J., Slack, M.P., and Ramsay, M.E. (2004). Long-term impact of vaccination on Haemophilus influenzae type b (Hib) carriage in the United Kingdom. Epidemiol. Infect. 132, 765–767.

Mentzer, A.J., O'Connor, D., Pollard, A.J., and Hill, A.V. (2015). Searching for the human genetic factors standing in the way of universally effective vaccines. Philos. Trans. R. Soc. Lond. B Biol. Sci. 370, 20140341.

Michaelsen, T.E., Garred, P., and Aase, A. (1991). Human IgG subclass pattern of inducing complement-mediated cytolysis depends on antigen concentration and to a lesser extent on epitope patchiness, antibody affinity and complement concentration. Eur. J. Immunol. 21, 11–16.

Miller, E., Salisbury, D., and Ramsay, M. (2001). Planning, registration, and implementation of an immunisation campaign against meningococcal serogroup C disease in the UK: a success story. Vaccine 20 (Suppl 1), S58–S67.

Mina, M.J., Metcalf, C.J., de Swart, R.L., Osterhaus, A.D., and Grenfell, B.T. (2015). Long-term measles-induced immunomodulation increases overall childhood infectious disease mortality. Science 348, 694–699.

Mongini, P.K., Stein, K.E., and Paul, W.E. (1981). T cell regulation of IgG subclass antibody production in response to T-independent antigens. J. Exp. Med. 153, 1–12.

Murphy, K.P., Travers, P., Walport, M., Ehrenstein, M., and Janeway, C.A. (2008). Janeway's Immunobiology (Garland Science).

Naess, L.M., Aarvak, T., Aase, A., Oftung, F., Høiby, E.A., Sandin, R., and Michaelsen, T.E. (1999). Human IgG subclass responses in relation to serum bactericidal and opsonic activities after immunization with three doses of the Norwegian serogroup B meningococcal outer membrane vesicle vaccine. Vaccine 17, 754–764.

Nakaya, H.I., Wrammert, J., Lee, E.K., Racioppi, L., Marie-Kunze, S., Haining, W.N., Means, A.R., Kasturi, S.P., Khan, N., Li, G.M., et al. (2011). Systems biology of vaccination for seasonal influenza in humans. Nat. Immunol. 12, 786–795.

Nettleship, J.E., Ren, J., Scott, D.J., Rahman, N., Hatherley, D., Zhao, Y., Stuart, D.I., Barclay, A.N., and Owens, R.J. (2013). Crystal structure of signal regulatory protein gamma (SIRPγ) in complex with an antibody Fab fragment. BMC Struct. Biol. 13, 13.

Newport, M.J., Goetghebuer, T., Weiss, H.A., Whittle, H., Siegrist, C.A., and Marchant, A.; MRC Gambia Twin Study Group (2004). Genetic regulation of immune responses to vaccines in early life. Genes Immun. 5, 122–129.

Nguyen, Q.N., Himes, J.E., Martinez, D.R., and Permar, S.R. (2016). The Impact of the Gut Microbiota on Humoral Immunity to Pathogens and Vaccination in Early Infancy. PLoS Pathog. 12, e1005997.

O'Connor, D., and Pollard, A.J. (2013). Characterizing vaccine responses using host genomic and transcriptomic analysis. Clin. Infect. Dis. 57, 860–869.

O'Connor, D., Moore, C.E., Snape, M.D., John, T., Hill, A.V., and Pollard, A.J. (2014). Exonic single nucleotide polymorphisms within TLR3 associated with infant responses to serogroup C meningococcal conjugate vaccine. Vaccine 32, 3424–3430.

O'Connor, D., Clutterbuck, E.A., Thompson, A.J., Snape, M.D., Ramasamy, M.N., Kelly, D.F., and Pollard, A.J. (2017). High-dimensional assessment of B-cell responses to quadrivalent meningococcal conjugate and plain polysaccharide vaccine. Genome Med. 9, 11.

Oh, S.Y., Griffiths, D., John, T., Lee, Y.C., Yu, L.M., McCarthy, N., Heath, P.T., Crook, D., Ramsay, M., Moxon, E.R., and Pollard, A.J. (2008). School-aged children: a reservoir for continued circulation of Haemophilus influenzae type b in the United Kingdom. J. Infect. Dis. 197, 1275–1281.

Pace, D., Snape, M., Westcar, S., Hamaluba, M., Yu, L.M., Begg, N., Wysocki, J., Czajka, H., Maechler, G., Boutriau, D., and Pollard, A.J. (2007). A new combination haemophilus influenzae type B and Neisseria meningitidis serogroup C-tetanus toxoid conjugate vaccine for primary immunization of infants. Pediatr. Infect. Dis. J. 26, 1057–1059.

Pace, D., Khatami, A., McKenna, J., Campbell, D., Attard-Montalto, S., Birks, J., Voysey, M., White, C., Finn, A., Macloed, E., et al. (2015). Immunogenicity of reduced dose priming schedules of serogroup C meningococcal conjugate

vaccine followed by booster at 12 months in infants: open label randomised controlled trial. BMJ *350*, h1554.

Parkes, M., Cortes, A., van Heel, D.A., and Brown, M.A. (2013). Genetic insights into common pathways and complex relationships among immune-mediated diseases. Nat. Rev. Genet. *14*, 661–673.

Perrett, K.P., Winter, A.P., Kibwana, E., Jin, C., John, T.M., Yu, L.M., Borrow, R., Curtis, N., and Pollard, A.J. (2010). Antibody persistence after serogroup C meningococcal conjugate immunization of United Kingdom primary-school children in 1999-2000 and response to a booster: a phase 4 clinical trial. Clin. Infect. Dis. *50*, 1601–1610.

Piccio, L., Vermi, W., Boles, K.S., Fuchs, A., Strader, C.A., Facchetti, F., Cella, M., and Colonna, M. (2005). Adhesion of human T cells to antigen-presenting cells through SIRPbeta2-CD47 interaction costimulates T-cell proliferation. Blood *105*, 2421–2427.

Pickering, J.W., Martins, T.B., Schroder, M.C., and Hill, H.R. (2002). Comparison of a multiplex flow cytometric assay with enzyme-linked immunosorbent assay for auantitation of antibodies to tetanus, diphtheria, and Haemophilus influenzae Type b. Clin. Diagn. Lab. Immunol. *9*, 872–876.

Png, E., Thalamuthu, A., Ong, R.T., Snippe, H., Boland, G.J., and Seielstad, M. (2011). A genome-wide association study of hepatitis B vaccine response in an Indonesian population reveals multiple independent risk variants in the HLA region. Hum. Mol. Genet. *20*, 3893–3898.

Pollard, A.J., Perrett, K.P., and Beverley, P.C. (2009). Maintaining protection against invasive bacteria with protein-polysaccharide conjugate vaccines. Nat. Rev. Immunol. *9*, 213–220.

Price, A.L., Patterson, N.J., Plenge, R.M., Weinblatt, M.E., Shadick, N.A., and Reich, D. (2006). Principal components analysis corrects for stratification in genome-wide association studies. Nat. Genet. *38*, 904–909.

Public Health England (2014). Changes to the meningococcal C conjugate (MenC) vaccine schedule 2013-2015 (PHE publications), gateway no. 2013458. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/336171/MenC_information_for_health care_professionals_V7_.pdf.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., and Sham, P.C. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. *81*, 559–575.

Radbruch, A., Muehlinghaus, G., Luger, E.O., Inamine, A., Smith, K.G., Dörner, T., and Hiepe, F. (2006). Competence and competition: the challenge of becoming a long-lived plasma cell. Nat. Rev. Immunol. *6*, 741–750.

Romanoski, C.E., Glass, C.K., Stunnenberg, H.G., Wilson, L., and Almouzni, G. (2015). Epigenomics: Roadmap for regulation. Nature *518*, 314–316.

Salisbury, D., Ramsay, M., and Noakes, K. (2014). Immunisation Against Infectious Disease (The Green Book).

Savy, M., Edmond, K., Fine, P.E., Hall, A., Hennig, B.J., Moore, S.E., Mulholland, K., Schaible, U., and Prentice, A.M. (2009). Landscape analysis of interactions between nutrition and vaccine responses in children. J. Nutr. *139*, 2154S–2218S.

Seiffert, M., Brossart, P., Cant, C., Cella, M., Colonna, M., Brugger, W., Kanz, L., Ullrich, A., and Bühring, H.J. (2001). Signal-regulatory protein alpha (SIRPalpha) but not SIRPbeta is involved in T-cell activation, binds to CD47 with high affinity, and is expressed on immature CD34(+)CD38(-) hematopoietic cells. Blood *97*, 2741–2749.

Shin, J., and Lee, C. (2015). Statistical power for identifying nucleotide markers associated with quantitative traits in genome-wide association analysis using a mixed model. Genomics *105*, 1–4.

Sidney, J., Oseroff, C., Southwood, S., Wall, M., Ishioka, G., Koning, F., and Sette, A. (1992). DRB1*0301 molecules recognize a structural motif distinct from the one recognized by most DR beta 1 alleles. J. Immunol. *149*, 2634–2640.

Siegrist, C.A., and Aspinall, R. (2009). B-cell responses to vaccination at the extremes of age. Nat. Rev. Immunol. *9*, 185–194.

Skol, A.D., Scott, L.J., Abecasis, G.R., and Boehnke, M. (2006). Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat. Genet. *38*, 209–213.

Snape, M.D., Kelly, D.F., Salt, P., Green, S., Snowden, C., Diggle, L., Borkowski, A., Yu, L.M., Moxon, E.R., and Pollard, A.J. (2006). Serogroup C meningococcal glycoconjugate vaccine in adolescents: persistence of bactericidal antibodies and kinetics of the immune response to a booster vaccine more than 3 years after immunization. Clin. Infect. Dis. *43*, 1387–1394.

Snape, M.D., Kelly, D.F., Lewis, S., Banner, C., Kibwana, L., Moore, C.E., Diggle, L., John, T., Yu, L.M., Borrow, R., et al. (2008a). Seroprotection against serogroup C meningococcal disease in adolescents in the United Kingdom: observational study. BMJ *336*, 1487–1491.

Snape, M.D., Perrett, K.P., Ford, K.J., John, T.M., Pace, D., Yu, L.M., Langley, J.M., McNeil, S., Dull, P.M., Ceddia, F., et al. (2008b). Immunogenicity of a tetravalent meningococcal glycoconjugate vaccine in infants: a randomized controlled trial. JAMA *299*, 173–184.

Snape, M.D., Klinger, C.L., Daniels, E.D., John, T.M., Layton, H., Rollinson, L., Pestridge, S., Dymond, S., Galiza, E., Tansey, S., et al. (2010). Immunogenicity and reactogenicity of a 13-valent-pneumococcal conjugate vaccine administered at 2, 4, and 12 months of age: a double-blind randomized active-controlled trial. Pediatr. Infect. Dis. J. *29*, e80–e90.

Tang, Y., Plikaytis, B.D., Preziosi, M.P., and Borrow, R. (2015). Influence of Age on Antibody Response and Persistence Following Immunization With MenAfriVac. Clin. Infect. Dis. *61* (*Suppl 5*), S531–S539.

Trotter, C.L., McVernon, J., Andrews, N.J., Burrage, M., and Ramsay, M.E. (2003). Antibody to Haemophilus influenzae type b after routine and catch-up vaccination. Lancet *361*, 1523–1524.

van den Berg, T.K., Yoder, J.A., and Litman, G.W. (2004). On the origins of adaptive immunity: innate immune receptors join the tale. Trends Immunol. *25*, 11–16.

Vinuesa, C.G., Linterman, M.A., Goodnow, C.C., and Randall, K.L. (2010). T cells and follicular dendritic cells in germinal center B-cell formation and selection. Immunol. Rev. *237*, 72–89.

Visscher, P.M., Wray, N.R., Zhang, Q., Sklar, P., McCarthy, M.I., Brown, M.A., and Yang, J. (2017). 10 Years of GWAS Discovery: Biology, Function, and Translation. Am. J. Hum. Genet. *101*, 5–22.

Voysey, M., Kelly, D.F., Fanshawe, T.R., Sadarangani, M., O'Brien, K.L., Perera, R., and Pollard, A.J. (2017). The Influence of Maternally Derived Antibody and Infant Age at Vaccination on Infant Vaccine Responses: An Individual Participant Meta-analysis. JAMA Pediatr. *171*, 637–646.

Waddington, C.S., Walker, W.T., Oeser, C., Reiner, A., John, T., Wilkins, S., Casey, M., Eccleston, P.E., Allen, R.J., Okike, I., et al. (2010). Safety and immunogenicity of AS03B adjuvanted split virion versus non-adjuvanted whole virion H1N1 influenza vaccine in UK children aged 6 months-12 years: open label, randomised, parallel group, multicentre study. BMJ *340*, c2649.

Ward, L.D., and Kellis, M. (2012). HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res. *40*, D930–D934.

Watt, J.P., Wolfson, L.J., O'Brien, K.L., Henkle, E., Deloria-Knoll, M., McCall, N., Lee, E., Levine, O.S., Hajjeh, R., Mulholland, K., and Cherian, T.; Hib and Pneumococcal Global Burden of Disease Study Team (2009). Burden of disease caused by Haemophilus influenzae type b in children younger than 5 years: global estimates. Lancet *374*, 903–911.

Wellcome Trust Case Control Consortium (2007). Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature *447*, 661–678.

Wilder-Smith, A., and Memish, Z. (2003). Meningococcal disease and travel. Int. J. Antimicrob. Agents *21*, 102–106.

Wilson, R., Cohen, J.M., Reglinski, M., Jose, R.J., Chan, W.Y., Marshall, H., de Vogel, C., Gordon, S., Goldblatt, D., Petersen, F.C., et al. (2017). Naturally Acquired Human Immunity to Pneumococcus Is Dependent on Antibody to Protein Antigens. PLoS Pathog. *13*, e1006137.

World Health Organization (2017). Immunization, vaccines and biologicals: neonatal tetanus. https://www.who.int/immunization/monitoring_surveillance/burden/vpd/surveillance_type/active/neonatal_tetanus/en/.

Yang, J., Lee, S.H., Goddard, M.E., and Visscher, P.M. (2011a). GCTA: a tool for genome-wide complex trait analysis. Am. J. Hum. Genet. *88*, 76–82.

Yang, J., Weedon, M.N., Purcell, S., Lettre, G., Estrada, K., Willer, C.J., Smith, A.V., Ingelsson, E., O'Connell, J.R., Mangino, M., et al.; GIANT Consortium (2011b). Genomic inflation factors under polygenic inheritance. Eur. J. Hum. Genet. *19*, 807–812.

Yang, J., Lee, T., Kim, J., Cho, M.C., Han, B.G., Lee, J.Y., Lee, H.J., Cho, S., and Kim, H. (2013). Ubiquitous polygenicity of human complex traits: genome-wide analysis of 49 traits in Koreans. PLoS Genet. *9*, e1003355.

Zeller, T., Wild, P., Szymczak, S., Rotival, M., Schillert, A., Castagne, R., Maouche, S., Germain, M., Lackner, K., Rossmann, H., et al. (2010). Genetics and beyond—the transcriptome of human monocytes and disease suscepti-bility. PLoS ONE *5*, e10693.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
1000 Genomes variant reference set	http://www.internationalgenome.org/data#download	1000 Genomes Phase I integrated variant set release (March 2012) as the variant reference set
Type 1 Diabetes Genetics Consortium reference panel	Wellcome Centre for Human genetics	Consortium reference panel (5225 unrelated individuals)
Critical Commercial Assays		
Illumina OmniExpress12v1	Illumina®, California, United States	Cat#WG-311-1125
Illumina OmniExpress12v1.1	Illumina®, California, United States	Cat#WG-312-1120
Illumina® scanner with Ilumina®BeadStudio software	Illumina®, California, United States	Cat#SY-101-1001
Oligonucleotides		
Custom TaqMan SNP Genotyping Assays rs2523650	https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/snp-genotyping-taqman-assays.html	Cat#4331349
Custom TaqMan SNP Genotyping Assays rs6903608	https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/snp-genotyping-taqman-assays.html	Cat#4331349
Custom TaqMan SNP Genotyping Assays rs3094188	https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/snp-genotyping-taqman-assays.html	Cat#4331349
Custom TaqMan SNP Genotyping Assays rs9268877	https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/snp-genotyping-taqman-assays.html	Cat#4331349
Custom TaqMan SNP Genotyping Assays rs2523663	https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays/snp-genotyping-taqman-assays.html	Cat#4331349
Software and Algorithms		
PLINK whole genome association analysis toolset	http://zzz.bwh.harvard.edu/plink/	PLINK version 1.9
SMARTPCA software	https://www.hsph.harvard.edu/alkes-price/software/	EIGENSOFT version 4.2
Segmented HAPlotype Estimation and Imputation Tool	Delaneau et al., 2013	SHAPEIT version 2
IMPUTE	Howie et al., 2009	IMPUTE version 2
SNP2HLA	http://software.broadinstitute.org/mpg/snp2hla/	SNP2HLA version 1.0.1
NetMHCIIpan	http://www.cbs.dtu.dk/services/NetMHCIIpan/	NetMHCIIpan version 3.1
SNPTEST	https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html	SNPTEST version 2.4.0
Genome-wide SNPs for complex traits (the GREML method)	https://cnsgenomics.com/software/gcta/#Overview	GCTA version 1.91.7 (beta)
Deposited Data		
Raw genotyping data, meta-data and analysis script	https://data.mendeley.com	O'Connor, Daniel (2019), "Common genetic variations associated with the persistence of immunity following childhood immunisation," Mendeley Data, V1 (https://doi.org/null/d7y6jhc36b.1)
R code algorithm devised to select SNPs for stage 2	https://github.com	https://github.com/dan-scholar/LD_pruning

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and data should be directed to and will be fulfilled by the Lead Contact, Daniel O'Connor (daniel.oconnor@pediatrics.ox.ac.uk).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study participants and vaccines

Healthy children and adolescents were included from nine studies conducted in Oxford, United Kingdom and one study conducted in Bilthoven, Netherlands (1634 males and 1795 females; demographics are shown in Table S1). In accordance with national guidelines (UK and Netherlands) immunisations were not delayed for minor illnesses but were deferred during acute illness (with fever above 38°C) until recovery. Regional research ethics committees approved these studies and the genetic analysis presented in this work (CO2.328, 07/Q1605/34, 09/H0604/107, 10/H0604/07, 04/Q1604/28, 06/Q1604/67, Stichting Therapeutische Evaluatie Geneesmiddelen [R05-044], B07/Q1605/41,10/H0504/25).

In brief, cohorts 1 and 2 were studies to examine the persistence serological of immunity to MenC in adolescents aged 11-20 years; 3-5 years after immunisation with a single dose of MenC vaccine, as part of the UK MenC catch-up immunisation program (Snape et al., 2006, 2008a). Participants received one of three vaccines administered during the UK MenC vaccine campaign: two of which contained MenC capsular polysaccharide conjugated to CRM_{197} and the other was conjugated to TT. These vaccines were Menjugate (Novartis Vaccines and Diagnostics, Siena), Meningitec (Wyeth Vaccines, New York), and NeisVac-C (Baxter Vaccines, Maryland). While Hib vaccination status was recorded for these participants, the vaccine formation was not noted.

Cohort 3 was a group of infants from a study assessing the immunogenicity of the Hib-MenC conjugate vaccine (Pace et al., 2007). Study participants received 3-doses of Hib-MenC (Menitorix, GSK Biologicals), which is conjugated to TT, or Meningitec, at 2, 3, and 4 months of age. Persistence of immunity to MenC, Hib and TT was assessed at 1 year of age. Cohorts 5 and 7 were infants enrolled into studies evaluating the immunogenicity of immunisation with 2- or 3-doses of capsular group A, C, W and Y quadrivalent meningococcal CRM-conjugated vaccine Menveo (Novartis Vaccines and Diagnostics, Siena) at 2, 3, 4 months or 2 and 4 months of age (Blanchard-Rohner et al., 2013; Snape et al., 2008b). Participants also received their routine infant immunisation, in accordance with the UK schedule, of relevance Pediacel (DTaP-Hib-IPV, Sanofi-Pasteur-MSD, Maidenhead, United Kingdom) was administered at 2, 3, 4 months of age; persistence of MenC and Hib immunity was measured at 1 year of age. Cohort 4 comprised of infants recruited into a study evaluating immunological memory following infant MenC conjugate vaccine (Menjugate or NeisVac-C) in consistent or alternating limbs. Participants received either a single dose of Menjugate or NeisVac-C at 3 months of age or a 2-dose schedule of Menjugate at 3 and 4 months of age; administered concomitantly with routine immunisations including Pediacel at 2, 3, 4 months of age (Khatami et al., 2014). Persistence of immunity to MenC and Hib was measured at 1 year of age.

Cohort 6 entailed a study evaluating the immunogenicity of the 2009 pandemic H1N1 influenza vaccine, in children 6 months to 12 years of age; data were also collected on routine childhood immunisations and sera were assessed to determine immunity to MenC, Hib and TT vaccines (Waddington et al., 2010). Cohort 8 was a study assessing the immunogenicity of the 7- and 13-valent pneumococcal conjugate vaccine alongside routine infant immunisations. As part of this study participants received NeisVac-C at 2 and 4 months of age and Pediacel at 2, 3, and 4 months of age. Persistence of vaccine-induced immunity was measured at 1 year of age (Snape et al., 2010). Cohort 9 was a longitudinal study assessing kinetics of the decline in MenC-specific antibody following early childhood (1-4 years of age) MenC immunisation, as part of the UK MenC catch-up immunisation program (Khatami et al., 2011). All of these children received a single dose of a licensed MenC conjugate vaccine of Menjugate, Meningitec or NeisVac-C. Cohort 10 examined the persistence of serological immunity to MenC in adolescents aged 9-20 years; 3-5 years after immunisation with a single dose of NeisVac-C, as part of the Dutch national MenC catch-up immunisation program (de Voer et al., 2010). These participants received their last dose of Hib containing vaccine (Pediacel) at 11 months of age; and their last dose of TT containing vaccine (assuming only routine immunisation) at 9 years of age or as a carrier protein to the MenC vaccine in year 2002.

METHOD DETAILS

Serum bactericidal assays

The Vaccine Evaluation Unit (VEU), Public Health England (Manchester), performed MenC-specific SBAs for study cohorts 2, 8 and 9 using rabbit complement (rSBA), and using human complement (hSBA) for cohorts 4 and 6. Novartis Vaccines, Germany (Marburg), performed MenC-specific SBAs using human complement for cohorts 1, 5 and 7 (Snape et al., 2006). GSK Biologicals, Belgium, performed MenC-specific hSBA titers using human complement for cohort 3 (Pace et al., 2007). Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven, measured MenC-specific rSBA titers using rabbit complement for cohort 10 (Maslanka et al., 1997). The lower limit of quantification (LLQ) for the hSBA assays was a reciprocal titer of 4 and for the rSBA assay this was 8, sera without detectable bactericidal antibody were assigned an arbitrary value of two.

MenC-specific IgG concentrations

Novartis Vaccines, Germany (Marburg), performed MenC-specific IgG for Cohort 1, by a standardized enzyme-linked immunosorbent assay (ELISA) with a LLQ of 0.3 μg/ml (Snape et al., 2006). MenC-specific IgG concentrations were measured by ELISA in-house for cohorts 2 and 7, with a LLQ of 0.00167 μg/mL (Blanchard-Rohner et al., 2013). MenC-specific IgG concentrations were measured using ELISA at GlaxoSmithKline Biologicals, Belgium, for Cohort 3, with a LLQ of 0.3 μg/mL (32). MenC-specific IgG concentrations were measured at the Vaccine Evaluation Unit (VEU), Public Health England (Manchester) by ELISA for Cohort 4 and 6, with a LLQ of 0.12 μg/mL (Gheesling et al., 1994). MenC-specific IgG concentrations were measured at the RIVM, Bilthoven, by multiplexed immune assay (Bioplex) for Cohort 10, with a LLQ of 0.01 μg/mL (de Voer et al., 2009). For the purposes of analysis MenC-specific IgG concentrations below the LLQ were assigned of half the respective assay LLQ.

Hib-specific IgG concentrations

An in-house ELISA was performed to measure Hib-specific IgG concentrations for cohort 2, with a LLQ of 100ng/ml (Booy et al., 1992). Hib-specific IgG concentrations were measured at Public Health England (Manchester), by Bioplex PHE for Cohort 4 and 6, with a LLQ of 46 ng/ml (Pickering et al., 2002). GlaxoSmithKline Biologicals, Belgium, performed Hib-specific IgG quantification for Cohort 3, by ELISA with a LLQ of 150ng/ml (Pace et al., 2007). Hib-specific IgG concentrations for Cohort 10 were assessed at RIVM, Bilthoven, by multiplexed immune assay, with a LLQ of 0.01 μg /ml (Pickering et al., 2002).

TT-specific IgG concentrations

TT-specific IgG concentrations were measured using ELISA at GlaxoSmithKline Biologicals, Belgium, for Cohort 3, the LLQ for this assay was 0.1IU/ml (Pace et al., 2007). Vaccine Evaluation Unit (VEU), Public Health England (Manchester) performed TT-specific IgG quantification using Bioplex PHE for Cohort 4 and 6, with a LLQ of 2 (IU × 1000/ml) (Pickering et al., 2002). Aventis Pasteur, Toronto, measured TT-specific IgG Cohort 9, using an ELISA with an in-house reference standard, the LLQ for this assay was 0.1 IU/ml (Collins et al., 2004). TT-specific IgG concentrations were measured using ELISA at Novartis Vaccines, Germany (Marburg) for Cohort 5, with a LLQ of 0.1 IU/ml (Snape et al., 2008b). RIVM, Bilthoven, measured TT-specific IgG concentrations using multiplexed immune assay, for Cohort 10, with a LLQ of 0.001 IU/ml (Pickering et al., 2002).

DNA extraction and genotyping

DNA was extracted either extracted from whole blood collected in EDTA tubes or from clotted whole blood samples. In the case of clotted samples, 1ml of blood clots (remaining from serum separated peripheral blood) were placed in a Clotspin® basket (QIAGEN, Hilden, Germany) and centrifuged at 2000 × g_0 for 5 minutes to disperse the clot. Then, 5 mL of red blood cell lysis solution (16.52 g NH_4Cl, 2 g $KHCO_3$ and 0.074 g DNA in 2l of H2O) was added, samples were vortexed for 3 s, and shaken for 5 minutes on an orbital mixer at 120-200 rpm. Samples were vortexed for 3 s and centrifuged at 2000 × g_0 for 5 minutes. Supernatant was discarded and an additional 5 mL of red blood cell lysis solution was added, vortexed for 3 s, before repeating the orbital mixer step. Samples were centrifuged at 2000 × g_0, supernatant discarded and vortexed. Next, 1 mL of Cell Lysis solution (1 mL nuclei lysis solution [Promega, Wisconsin, United States]; 25 μL Proteinase K [QIAGEN, Hilden, Germany]; and 250 μL 0.5 M ethylenediamine-tetraacetic acid). Following this incubation, DNA was extracted according to the standard spin protocol for whole blood, using the Qiamp® midi kit (QIAGEN, Hilden, Germany). For blood samples collected directed into EDTA blood tubes (anticoagulant), the pre-extraction process was skipped and DNA was extracted according to the standard spin protocol for whole blood, using the Qiamp® midi kit (QIAGEN, Hilden, Germany).

Genotyping of the discovery cohort (n = 2,061) was performed using either Illumina OmniExpress12v1 or OmniExpress-12v1.1 genotyping microarrays (Illumina®, California, United States), at the Genome Institute of Singapore as according to manufacturers protocol. In brief, DNA samples were whole-genome amplified and enzymatically cleaved into 300-600bp fragments. Samples were then purified using isopropyl precipitation and re-suspended in hybridization buffer. Samples were hybridized to the microarray and an allele-specific enzymatic single-base extension step was performed, to add a biotin-labeled (guanine or cytosine) or dinitrophenol-labeled (adenine or thymine) base. Sequential rounds of staining were performed with green-fluorescent streptavidin and biotin-labeled antibodies, and red-fluorescent anti-dinitrophenol antibodies and dinitrophenol-labeled antibodies. Following staining, the arrays were read on an Illumina® scanner and genotypes were called using Illumina® BeadStudio software.

A number of quality control (QC) checks were undertaken on the genotyping data before statistical association testing, the QC pipeline utilized was similar to that described by Anderson et al., 2010, using the PLINK whole genome association analysis toolset (http://zzz.bwh.harvard.edu/plink/) (Anderson et al., 2010; Purcell et al., 2007). Participants with discrepancies between the sexes (n = 81) reported on participant information data and those deduced from the genotyping data were removed. Participants with genotype heterozygosity greater than two standard deviations from the cohort mean heterozygosity rate (n = 28), or with a missing genotyping proportion > 0.03 were removed (n = 16). First- and second-degree relatives were identified identity-by-descent (IBD) analysis, and all but the best-performing participant (in terms of genotype call-rate) were removed (n = 212). SMARTPCA software was used to identify ancestral outliers, and population outliers were removed (n = 132) using the metrics described by Anderson et al., 2010 (Anderson et al., 2010; Price et al., 2006). SNPs with minor allele frequencies (MAF) of < 0.01 (n = 77991), genotype call rate < 0.95 (n = 1984) and/or Hardy-Weinberg equilibrium (HWE) p values < 1 × 10^{-5} (n = 16305) were eliminated.

Imputation

The post quality filtered dataset from the GWAS array was used for genotype imputation, Segmented HAPlotype Estimation and Imputation Tool (SHAPEIT) version 2 was used to pre-phase each chromosome of the genotype data individually (Delaneau et al., 2013). Imputation was performed using IMPUTE version 2 on 5 Mb chunks of this pre-phased data, applying the 1000 Genomes Phase I integrated variant set release (March 2012) as the variant reference set (Howie et al., 2009). Imputed data were filtered for MAF > 0.02, HWE p values > 1×10^{-9} and information scores > 0.8.

Imputation of classical HLA alleles

The SNP2HLA framework was used to impute classical human leucocyte antigen alleles from the genotype data, using the Type 1 Diabetes Genetics Consortium reference panel (5225 unrelated individuals) (Jia et al., 2013; Wellcome Trust Case Control Consortium, 2007). Classical HLA alleles were annotated as present or absent, and a linear regression analysis was conducted using PLINK 1.9 (Purcell et al., 2007). Conditional analysis of the five replication HLA SNPs including HLA DRB1*0301, HLA DQB1*0201, HLA DQB1*0602 or HLA DRB1*1501, as covariate in the linear regression model was undertaken.

In silico epitope mapping

Clostridium tetani tetanus toxin protein sequence was retrieved from UniProt (https://www.uniprot.org/). Tetanus toxin 20-mer, over-lapping 19 amino acids, peptides were assessed against a panel of *HLA-DRB1* alleles, using NetMHCIIpan 3.1 (http://www.cbs.dtu.dk/services/NetMHCIIpan/), pan-specific MHC class II prediction tool.

QUANTIFICATION AND STATISTICAL ANALYSIS

Association analysis

A linear regression model was used to assess the relationship between SNP allele dosage and the persistence (> 120 days post-vaccination) of log10 vaccine-specific measures. Analysis was conducted using SNPTEST version 2.4.0: incorporating age at vaccination, time since vaccination, study enrolled in, complement source (for SBA analysis) and the first three principal components (PCs) as covariates (Marchini and Howie, 2010). Post-association QC was also conducted to check for inflation, lambda gc (λgc), of test-statistics, using the 'GenABEL' R package, under the assumption that the vast majority of the genetic markers assessed would not be associated with the phenotype (Aulchenko et al., 2007). Genomic inflation was observed for regression analysis of TT-specific IgG concentration (λgc = 2.30); however, this was ameliorated by the inclusion of the first 10 PCs into the regression model.

Heritability analysis

The GREML method was used to estimate the phenotypic variance explained by all SNPs, as described by Yang et al., 2011a (Yang et al., 2011a). Heritability analysis used only the genome-wide genotyping data generated from the discovery cohort.

Replication SNP selection

The R code for the algorithm devised to select SNPs for stage 2 is deposited on Github (https://github.com/dan-scholar/LD_pruning). In brief, genotyped SNPs from stage 1 with association p values < 1×10^{-4}, as well as imputed SNPs with p values < 1×10^{-5}, in the regression models of MenC-specific IgG concentrations or SBA titers were selected. A different statistical cut-off for genotyped and imputed SNPs was imposed as imputed SNPs have an inevitable reduced certainty. These SNPs were separated into 500kb chromosomal windows and windows with < 3 SNPs were eliminated as these are more likely to represent genotyping and/or imputation errors. Next, to avoid redundant genotyping of markers in high LD the SNP list was pruned by filtering SNPs with an $R^2 > 0.95$ with the lead SNP within each 500kb window. Then, the next most significant SNP remaining in each window was selected and LD filtering repeated iteratively, until all SNPs had been interrogated. We carried out combined analysis of the individual level discovery- and replication-stage data in a single analysis, for both additive and dominant genetic models, using PLINK 1.9 (Purcell et al., 2007). Meta-analysis of the discovery- and replication-stage cohorts was conducted by the inverse variance method, without adjusting for principal components, using PLINK 1.9 (Purcell et al., 2007).

Pleiotropic loci analysis

SNPs associated with the persistence of serological immunity to MenC, Hib or TT at a p < 1×10^{-4} were collapsed into genetic loci by gene boundaries. Genes linked with more than one measure of vaccine immunity were interpreted as pleiotropic.

Transcription factor motif prediction

Sequences surrounding SNPs were evaluated for potential transcription factor (TF) binding sites and the allelic variants were considered for their impact on TF motifs, using the R package 'motifbreakR' (Coetzee et al., 2015).