Vaccine 32 (2014) 3424–3430

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Exonic single nucleotide polymorphisms within *TLR3* associated with infant responses to serogroup C meningococcal conjugate vaccine

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ARTICLE INFO

Article history:
Received 24 October 2013
Received in revised form 19 March 2014
Accepted 1 April 2014
Available online 21 April 2014

Keywords:
Meningococcal vaccine
Immunogenetics
Toll-like receptors

ABSTRACT

The introduction of the serogroup C meningococcal (MenC) conjugate vaccination has successfully controlled the burden of disease associated with this serogroup in many countries. However, considerable inter-individual variation is observed in immune responses to MenC vaccine, and little is understood of the determinants of this variability. Previously, we reported an association between single nucleotide polymorphisms (SNPs) in *TLR3* and *CD44* and the persistence of MenC vaccine immunity. Here we further examine polymorphisms within these two candidate genes and immune responses to MenC vaccine. MenC-specific IgG concentrations and serum bactericidal assay (SBA) titres were measured one month after a primary course of MenC vaccination in 318 human infants. Tagging SNPs (TagSNPs) within *TLR3* and *CD44* were genotyped and regional imputations carried out to screen these genes for variations associated with immunological responses to MenC vaccine. This study reports an association between an exonic variant (rs3775290, $P = 0.025$) in *TLR3* and MenC IgG concentrations, as well as an association between three SNPs in *CD44* (rs3794109, $P = 0.021$; rs3794110, $P = 0.022$; rs112762, $P = 0.049$) and MenC SBA titres. These data support our previous findings of an association between SNPs in *TLR3* and *CD44*, and present novel findings implicating exonic variants in these genes with MenC vaccine responses.

1. Introduction

Neisseria meningitidis is a Gram-negative diplococcus and a major cause of invasive bacterial infection worldwide, with peak disease incidence occurring in children under two years of age [1,2]. Twelve serogroups of meningococci have been described based on distinct capsular antigenicities; however, the majority of invasive disease worldwide is attributable to serogroups: A, B, C, W, X, and Y [3]. Prior to the introduction of the MenC conjugate vaccine, serogroups B and C were the major cause of invasive meningococcal disease in Europe and the Americas [4]. Following the introduction of the MenC conjugate vaccine a drastic decline in serogroup C disease was observed [5–7]. However, the magnitude and persistence of immunity following MenC vaccination varies considerably among individuals, a phenomenon that is characteristic of vaccine responses, but is not well understood [8,9]. This heterogeneity is particularly noteworthy in the young where responses wane rapidly, such that most older children's sera loses bactericidal activity following infant MenC vaccination and breakthrough disease has been demonstrated even when immune memory has been induced [9,10]. Consequently, the United Kingdom has recently introduced an adolescent booster dose of MenC vaccination to provide protection into early adulthood [11].

A number of factors including: age, sex, nutrition, smoking, ultraviolet light exposure, and infectious disease have been implicated in determining quantitative measures of vaccine-induced immunity [12]. Importantly, twin studies have shown vaccine responses to be highly heritable (36–89%), highlighting the importance of genetic variation in shaping vaccine immunity [13–17]. Despite the evident heritability of vaccine responses the particular factors involved remain largely uncharacterised. We previously reported SNPs within *TLR3* and *CD44* that associated with the persistence of MenC IgG concentrations in 905 vaccinated teenagers

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http://dx.doi.org/10.1016/j.vaccine.2014.04.009
0264-410X/

and 351 infants/children [18]. Here we further assess the influence of variations within *TLR3* and *CD44* on the magnitude of responses to MenC conjugate vaccine in infancy.

2. Materials and methods

2.1. Vaccine participants

Informed consent was obtained from parents/guardians of 355 infants enrolled in three vaccine trials conducted by one centre in Oxford, United Kingdom [19–22]. All participants received two or three doses of MenC conjugate vaccine at two and four or two, three and four months of age; these vaccines contained the MenC antigen alone (monovalent) or in combination with *Haemophilus influenzae* type b (Hib-MenC) or meningococcal serogroups A, C, W and Y (MenACWY). MenC-specific IgG concentrations and serum bactericidal titres (SBA) were measured at five months of age. Exclusion criteria are described elsewhere [19–22]. These studies were approved by the Oxfordshire research ethics committees (B07/Q1605/41, AO4/Q1604/28 and B07/Q1605/34).

2.1.1. Immunological measurements

SBAs to detect functional antibody against serogroup C-expressing meningococci were performed using either human complement (hSBA) at Novartis Vaccines, Germany; or rabbit complement (rSBA) at GlaxoSmithKline Biologicals, Belgium. In brief, twofold dilutions of heat-inactivated sera were incubated with suspensions of *N. meningitidis* serogroup C strain C11 and freshly thawed exogenous, human or rabbit, complement. SBA titres are the interpolated serum end point dilution that corresponds to ≥50% reduction in bacterial colonies. Titres are expressed as the reciprocal dilution. Serogroup C meningococcal capsular polysaccharide specific IgG concentrations were measured using an enzyme-linked immunosorbent assay (ELISA). Specific human anti-polysaccharide antibodies bound to the coated plates were detected using a peroxidase system and quantitated based on an in-house meningococcal standard calibrated against reference serum CDC1992 [23]. The serological results are published elsewhere [19–22].

2.2. Genotyping and exon re-sequencing

TagSNPs within TLR3 and CD44 were selected from the HapMap version 3 release 27 dataset (http://www.hapmap.org/) using pairwise tagging, set for a correlation coefficient ≥0.8 and a minor allelic frequency (MAF) of ≥0.05. Genomic DNA was extracted using the Maxwell® 16 System (Promega, Wisconsin), following overnight incubation of a 1 ml blood clot in 1 ml of nuclei lysis solution (Promega, Wisconsin) and 25 μl of Proteinase K (Qiagen, Hilden, Germany) at 56 °C. Extracted DNA was quantified using the Quant-iT™ PicoGreen assay (Invitrogen, California). Genotyping was performed using either a PCR based tetra-primer amplification refractory mutation system (ARMS), as described by Little et al., or using MassARRAY iPLEX® (Sequenom, San Diego) system [24,25].

The five exons of *TLR3* were sequenced using the Sanger sequencing methodology [26]. Purified sequencing reactions were separated by capillary electrophoresis (ABI PRISM® 3700 Genetic Analyzer, Applied Biosystems, California). Sequence chromatograms were assembled and analysed using the Staden package version 1.6 (http://staden.sourceforge.net/). The mutation scanner module was used to compare sequence traces to a reference trace to detect mutation, as described in Bonfield et al. [27]. SNPs called by this algorithm were then manually inspected on the forward and reverse strand for verification. Exon re-sequencing was not conducted on CD44, as this gene has a number of isoforms that incorporate a varying number of the numerous exons (>20) annotated as this locus. Instead we relied on regional imputations to capture variation at this locus.

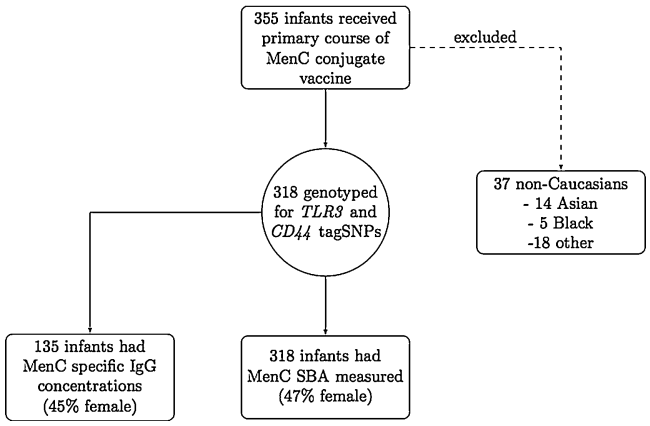

Fig. 1. Flow diagram of participants included in the study to assess the influence of polymorphisms within *TLR3* and *CD44* on infant responses to serogroup C meningococcal conjugate vaccine. SBA, serum bactericidal assay; tagSNP, tag single nucleotide polymorphisms.

3. Statistical analyses

3.1. Linear regression

Additive and genotypic linear regression models were used to test for associations between tagSNPs and MenC vaccine responses, using the statistical package 'Genetics' in R [28]. Reciprocal MenC SBA titres and MenC IgG concentrations were $\log_{10}$-transformed, and vaccine group was included as a covariate.

3.2. Regional imputations

We used Bayesian IMputation-Based Association Mapping (BIMBAM) software to infer additional untyped genotypes in the two candidate genes, using the HapMap Phase II–III release 28 dataset (forward strand) as a reference panel [29,30].

$$Y_i = \mu + aX_i + dI(X_i = 1) + \varepsilon_i$$

The Bayes factor (BF) for each SNP was computed under the linear model where Y_i denotes the residuals of the log-transformed reciprocal SBA titres or IgG concentrations regressed for covariates (vaccine group), X_i the genotype and ε_i denotes an error term. SNPs were filtered to exclude SNPs with ≥0.2 missingness and/or ≤0.05 MAF. SNP *P*-values were computed by assessing the proportion of permutations whose SNP BFs exceed that of the observed data.

3.3. Haplotype analysis

Haplotype based analysis was undertaken using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/) [31]. An expectation-maximisation algorithm was used to predict phase and: two, three, four and five SNP sliding-windows were examined.

4. Results

4.1. Summary of study participants

DNA samples were available from 355 infants enrolled in MenC vaccine trials, 37 non-Caucasians were excluded from analyses (see, Fig. 1). MenC SBA titres were available on 318/318 and MenC IgG concentrations on 135/318 participants. Serological results are presented elsewhere [19–22].