Vaccine 30 (2012) 2159–2167

Contents lists available at SciVerse ScienceDirect

Vaccine

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

Multigenic control of measles vaccine immunity mediated by polymorphisms in measles receptor, innate pathway, and cytokine genes

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ARTICLE INFO

Article history:
Received 16 August 2011
Received in revised form
30 November 2011
Accepted 8 January 2012
Available online 20 January 2012

Keywords:
Measles vaccine
Immunogenetics
Vaccine response
Multigenic SNP association
Interferon response
Cytokines
Toll-like receptors

ABSTRACT

Measles infection and vaccine response are complex biological processes that involve both viral and host genetic factors. We have previously investigated the influence of genetic polymorphisms on vaccine immune response, including measles vaccines, and have shown that polymorphisms in HLA, cytokine, cytokine receptor, and innate immune response genes are associated with variation in vaccine response but do not account for all of the inter-individual variance seen in vaccinated populations. In the current study we report the findings of a multigenic analysis of measles vaccine immunity, indicating a role for the measles virus receptor CD46, innate pattern-recognition receptors (DDX58, TLR2, 4, 5, 7 and 8) and intracellular signaling intermediates (MAP3K7, NFKBIA), and key antiviral molecules (VISA, OAS2, MX1, PKR) as well as cytokines (IFNA1, IL4, IL6, IL8, IL12B) and cytokine receptor genes (IL2RB, IL6R, IL8RA) in the genetic control of both humoral and cellular immune responses. This multivariate approach provided additional insights into the genetic control of measles vaccine responses over and above the information gained by our previous univariate SNP association analyses.

1. Introduction

Measles is an extremely infectious viral infection resulting in hundreds of thousands of deaths worldwide every year. The introduction of effective measles vaccines has drastically reduced both disease cases and mortality [1]. Nevertheless, 2008 saw the largest number of measles cases in Europe and the US in a decade, and the disease has once again been declared endemic in the UK [2]. The WHO has reported outbreaks and rising case numbers throughout Europe in the past few years, with over 30,000 cases reported in 2010 [3]. In the US, the largest number of measles cases since 1996 had already occurred by May of 2011 [4].

Measles infection and vaccine response are complex biological processes that involve both viral and host genetic factors. Our laboratory has investigated the role of genetic polymorphisms in vaccine response, including measles vaccines, and demonstrated that polymorphisms in HLA, cytokine, cytokine receptor, and innate immune response genes are associated with inter-individual variations in vaccine response but do not account for all of the variance seen in vaccinated populations [5–17].

Given the multifactorial nature of diseases and the complexity of biological responses, it is highly likely that multigenic interactions affect vaccine response and immune outcomes, a process that is better explained by the immune response network theory than by a dominant allele mechanism [18]. Increasingly sophisticated approaches are utilized to unravel multigenic associations with disease and treatment outcomes [19,20] and serve as valuable complements to single SNP analyses in furthering our understanding of the complex genetic control of vaccine response. Accordingly, we took advantage of a wealth of existing genetic and immunologic data on healthy young recipients of two doses of MMR-II to examine potential multigenic interactions associated with robust or ineffective vaccine responses.

2. Materials and methods

2.1. Subjects

Our study population consists of 821 subjects recruited from two previously reported, independent, age-stratified, random

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doi:10.1016/j.vaccine.2012.01.025

cohorts of children and young adults (11–22 years of age) in Rochester, MN [14,17]. There was no known wild-type measles virus exposure in the community since the birth of the oldest subject. Each participant had medically documented receipt of exactly two doses of measles–mumps–rubella (MMR-II, Merck) vaccine. The parents of each participant provided written informed consent, while written assent was obtained from age-appropriate children. The Mayo Clinic Institutional Review Board (IRB) approved all study procedures. A single blood draw was obtained from each subject and DNA, serum, and PBMC samples were obtained as previously described [21].

2.2. TagSNP selection

One hundred twenty-six genetic loci encoding cytokines and cytokine receptors, Toll-like receptors and signaling molecules, antiviral proteins, measles virus receptors, vitamin A receptors, and other innate immunity-related proteins were identified as genes of interest from our data, literature searches and public databases [7–9,14,17,22,23]. All SNPs within each gene as well as those 5 kb upstream and downstream for each gene were included in our LD tagSNP selection algorithm [24]. The ldSelect program was used to bin SNPs with a minor allele frequency (MAF) $\geq$0.05 and a pairwise linkage disequilibrium (LD) threshold of $R^2 \geq 0.90$ among HapMap CEPH unrelated samples as well as successful Illumina predictive genotyping scores. The resulting selection of tagSNPs was further refined with SNPPicker [25]. Additionally, a list of putative functional "obligate" SNPs (including coding nonsynonymous, synonymous) SNPs and SNPs in either the 5′ or 3′ untranslated regions with a MAF $\geq$ 0.05 was added to the final list for a total of 2304 SNPs. We followed the nomenclature defined by den Dunnen and Antonarakis [26].

2.3. Genotyping methods

Genomic DNA was extracted using Puregene kits (Gentra Systems Inc., Minneapolis, MN) and quantified by Picogreen (Molecular Probes) as described [14,17]. We genotyped 2304 SNPs from 126 candidate genes. SNPs were divided into two Illumina GoldenGate SNP panels (Illumina Inc., San Diego, CA) of 1536 and 768 SNPs. Design scores for all were >0.4, and all genotyping was performed as per manufacturer's protocols [6]. Genotype calls were made using BeadStudio 2 software. Quality control relied on control genomic DNA samples (CEPH family trio and two other genomic DNAs) and both replicate and inheritance datasets to assess and refine clustering. After removal of the SNPs that failed quality-assurance measures (HWE, amplification failure, poor clustering, or multiple replicate errors), we obtained genotyping data on 1912 SNPs. The study sample genotyping success rate (including replicate samples) was 98%. After excluding samples due to insufficient DNA or inadequate DNA quality and removing genotyping failures as well subjects with low call rates, this multigenic analysis focused on the 568 remaining Caucasian subjects.

2.4. Neutralizing antibody assay

We assessed measles-specific neutralizing antibody levels using a fluorescent plaque reduction neutralization assay as previously described [27]. Briefly, 4-fold serum dilutions were incubated with GFP-expressing measles virus (MV) for 1 h before the addition to Vero cells for 43 h. Fluorescent syncytia were imaged and counted and Karber's formula was used to determine the 50% end-point titer (Neutralizing Dose, ND_{50}). The inclusion of a WHO measles antibody standard enabled the transformation of ND_{50} values into mIU/ml [27].

2.5. ELISPOT assay

IFNγ cytokine response was quantified by an ELISPOT assay (R&D Systems, Minneapolis, MN) as previously described [28]. PHA (5 μg/ml) served as a positive control, 'unstimulated' samples contained PBMCs and culture medium only, while "stimulated" samples also contained measles virus at a multiplicity of infection (MOI) of 0.5. Plates were incubated for 42 h and spots were developed and counted using optimized counting parameters (detection sensitivity, thresholds for minimum and maximum spot size, background hue, spot separation, removal of debris and/or overdeveloped regions) were created and maintained while counting all ELISPOT plates.

2.6. Statistical analyses

The analyses focused on attempting to identify multigenic contributions of SNPs identified from candidate immune response genes. Subject characteristics were first summarized and compared to observed measures of immunity to measles (i.e., neutralizing antibodies and IFNγ ELISPOT) as has been previously reported [14,17]. The median and interquartile ranges of each measurement were summarized overall for each category of the descriptive variables. The observed measurements were compared across the demographic categories with analysis of variance (ANOVA) techniques. Neutralizing antibody levels were log-transformed before analysis, and differences in IFNγ ELISPOT counts between stimulated and unstimulated cells were analyzed on an inverse normal scale to meet linear models assumptions. Median values across the replicated measurements were used as the outcome variables, and all analyses were adjusted for the following set of covariates potentially associated with measurements of response to measles vaccination: age at enrollment, gender, age at first measles vaccination, age at second measles vaccination, and cohort status.

A series of analyses was carried out to identify combinations of SNPs that might simultaneously influence measures of measles immune response. Our initial assessment was to individually examine the additional information gained from individual SNPs using a stepwise model-selection approach for the identification of groups of SNPs for each immune response measure. In this stepwise process, single ordinal SNP variables were added/removed one at a time, using p-values for entrance or removal of 0.01, until only SNPs contributing to the phenotypic association in question remained in the regression model. We applied randomization tests (repeated random re-assignment of phenotypes to SNP genotypes) to adjust for the fact that we identified associated SNPs in the same dataset in which the tests were performed [29]. These randomized data sets then provide estimates of the magnitude of effects that would be observed if no associations are present, and the observed model is then compared to this empirical distribution of results to obtain empirical p-values that are not sensitive to the model-selection approach. LD blocks were defined as described by Gabriel et al. [30]. All statistical tests were two-sided. SAS software (SAS Institute Inc., Cary, NC) was used for all analyses unless otherwise indicated.

3. Results

From the 2304 SNPs selected for the study, we excluded 392 due to monomorphic expression in our population, genotyping failures, call rates < 95%, or a minor allele frequency < 1%, leaving 1912 SNPs for analysis. While the original cohort was 19.7% non-white, the studies described here focus on the Caucasian subset due to statistical power issues and the relatively small number of other racial groups (ranging in size from $n = 2$ to $n = 89$). After excluding individuals who did not meet inclusion criteria and for whom we