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The genetic basis for interindividual immune response variation to measles vaccine: new understanding and new vaccine approaches

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Abstract

The live-attenuated measles vaccine is effective, but measles outbreaks still occur in vaccinated populations. This warrants elucidation of the determinants of measles vaccine-induced protective immunity. Interindividual variability in markers of measles vaccine-induced immunity, including neutralizing antibody levels, is regulated in part by host genetic factor variations. This review summarizes recent advances in our understanding of measles vaccine immunogenetics relative to the perspective of developing better measles vaccines. Important genetic regulators of measles vaccine-induced immunity, such as *HLA* class I and *HLA* class II genotypes, single nucleotide polymorphisms in cytokine/cytokine receptor genes (*IL12B*, *IL12RB1*, *IL2*, *IL10*) and the cell surface measles virus receptor *CD46* gene, have been identified and independently replicated. New technologies present many opportunities for identification of novel genetic signatures and genetic architectures. These findings help explain a variety of immune response-related phenotypes and promote a new paradigm of ‘vaccinomics’ for novel vaccine development.

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Keywords

adaptive immunity; genetic association studies; human leukocyte antigens; immunogenetics; measles vaccine; single nucleotide polymorphisms

Re-emergence of measles, current vaccine limitations & next-generation vaccines

Measles is the most highly contagious human disease and is one of the leading causes of death in children under 5 years of age worldwide. More than 20 million measles infections occur each year, with 139,300 deaths in 2010 alone [1,201]. The last 10 years have shown that the increasing immunization coverage with the current live-attenuated measles vaccine, the two-dose schedule and the implementation of appropriate measles control measures decreased global measles mortality by 74% from the 535,000 deaths that occurred in 2000 [1,201]. The vast majority of measles-related deaths still occur in Africa and Asia due to failure to vaccinate and a lack of appropriate healthcare [1,201]. Despite widespread availability of measles vaccine, middle- and high-income countries are currently reporting numerous and sizeable measles outbreaks [2,202]. In 2008, the Health Protection Agency of England and Wales announced that measles was once again endemic. In both 2010 and 2011, the 29 countries that form the EU and the European Economic Area reported approximately 30,000 cases each year. This was more than four times the number of cases in 2008 and 2009. Five of the 29 countries reported 90% of the cases; they include France, Italy, Romania, Spain and Germany. All but Iceland and Cyprus had measles cases in 2011. In the USA, a total of 222 cases occurred in 2011 involving 31 states [3]. Two hundred of these cases were related to the importation of measles from other countries, and approximately half of the importations were from Europe [3]. Of the 17 outbreaks reported to the CDC in 2011 – including 112 of the 222 cases – the median outbreak involved six cases, and the median length of an outbreak lasted 18 days [3]. The cost of containing measles outbreaks is high; an outbreak in Arizona in 2008 resulted in 14 cases and cost nearly US\$800,000 [4]. In addition to the morbidity and the expense of these outbreaks, recent concerns have been raised regarding the use of measles as an agent of bioterrorism owing to its extraordinary transmissibility [5].

Although effective, the current live-attenuated measles vaccines have limitations. Vaccine failure (i.e., the failure for the individual to either mount or sustain a protective immune response) occurs despite the receipt of two doses of vaccine [6–13,201]. Measles elimination has failed primarily due to failure to vaccinate, but also in part due to vaccine failure, allowing the accumulation of susceptible individuals and the occurrence of outbreaks when exposure occurs [9–11,14,15]. As a result, even in highly vaccinated populations, substantial proportions of those infected in an outbreak will have been previously vaccinated [9,12–14,16]. The 1989–1991 US measles outbreaks clearly demonstrate this; 20–40% of the reported cases were previously immunized with one or two doses of vaccine [11], and this phenomenon continues (Table 1). In October 2011, a prolonged measles outbreak in Quebec resulted in more than 700 cases; one high school had 98 reported measles cases, of which 53% had received two doses of a measles-containing vaccine [13].

Estimates of the immunogenicity of the current, live-attenuated measles vaccines vary substantially. Our laboratory found only 81% to be seropositive in a sample of 1490 school-aged children all having had a single dose of measles-containing vaccine received at 12 months or later in life; of the remaining 19% who were seronegative, one in five who received a second dose remained seronegative [11]. Various studies estimate anywhere from 2 to 10% of those vaccinated with two doses fail to develop protective humoral immunity

[11,14,17,18], and those antibody levels wane over time, which can result in infection [17,19–21]. In our evaluation of 763 healthy school-aged children in Rochester (MN, USA), all previously vaccinated with two doses of MMR, only 91.1% demonstrated protective humoral immunity [17]. Our recent measles vaccine study also revealed a wide range of interindividual variations in humoral and cell-mediated immune markers. For example, the median neutralizing antibody titer for our cohort was 844 mIU/ml (interquartile range: 418–1752; minimal value: 45; maximal value: 7723) 7.4 years after two doses of MMR vaccine, and the median IFN- γ ELISPOT response was 36 (interquartile range: 13–69; minimal value: –17; maximal value: 208) IFN- γ -positive spot-forming units (SFUs) per 200,000 cells, suggesting substantial biological variability, waning immunity and potential susceptibility to measles [17,22]. Our research team [23] and others [24–26,203] have called for maintenance of and/or progress toward measles elimination, recognizing the related challenges, including the need for research on determinants of measles vaccine response, and the development of improved measles vaccines.

Furthermore, the WHO and others have also repeatedly called for the worldwide eradication of measles [7,204]. However, measles eradication is unlikely as population immunity of 96–98% is required to prevent persisting measles endemicity [7,8,27,201]. In a recent study of measles-vaccine efficacy from 1960 to 2010, median efficacy was only 94% [28]. Thus, approaches to eradicating measles will likely require consideration of new measles vaccines and vaccination strategies that overcome the many barriers inherent in the current measles vaccines [6,29–32].

Traditionally, vaccine development has followed an empiric ‘Isolate → Inactivate/Attenuate → Inject’ paradigm, and this is the paradigm under which the current measles vaccines were developed 50 years ago [33]. We have proposed an alternative course of research to support a directed vaccinomics paradigm of ‘Discover → Replicate → Validate → Apply’ – a novel paradigm that utilizes genome-based vaccine development [33–42], and that provides insights into the immunogenetic basis of immune responses to measles and other vaccines, ultimately leading to the next generation of measles vaccine candidates. In addition, this new vaccinomics paradigm utilizes novel integrative systems biology approaches for deep immune profiling of vaccine responses using comprehensive transcriptomics, proteomics and immunophenotyping arrays (rather than traditional labor-intensive immunologic assays), functional analyses and computational modeling to create and identify immune profiles and immune signatures that can discriminate among vaccine-induced phenotypes. As we and others have outlined in the literature, the knowledge gained will aid vaccine development by devising informed solutions to overcome immunologic and genetic restrictions to protective immunity after vaccination [33,35,43–47].

Replicated associations of *HLA* allelic variants with measles vaccine immune responses

Of the various genes linked to vaccine-induced immune responses, *HLA* genes have been intensely studied and perhaps have the highest impact on immunity. Antigen processing and presentation by *HLA* class I and class II molecules contributes to antigen-specific immune response [48]. Hence, polymorphisms in the *HLA* and other genes are a possible explanation for interindividual variations in vaccine-induced immune outcomes. In this regard, biologic insights into population-based associations with vaccine-induced adaptive immune responses, including measles, through genetic studies is essential for designing novel vaccines.

Our population-based studies have found specific *HLA* allelic associations, including haplotypes and supertypes, with measles vaccine-induced adaptive humoral and cellular

immune responses after one or two doses of vaccine [49–56]. For example, following a single dose of measles vaccine, lower vaccine antibody responses were found to be associated with *B*8*, *B*13*, *B*44*, *DRB1*03* and *DQA1*0201* alleles [49,50]. In contrast, class I *B*7*, and class II *DQA1*0104* and *DPA1*0202* alleles were associated with elevated measles vaccine antibody responses [57]. Healthy children homozygous for certain *HLA* alleles (class I B and class II *DQA1*) had lower measles vaccine IgG antibody levels than heterozygous children [58]. Furthermore, decreases in antibody response after a single dose of measles vaccine were associated with *HLA* homozygosity [58]. The interpretation of such population-based genetic association studies is influenced by several factors, such as sample size, ethnicity, allele frequency and genotyping methods. A major limitation of many genetic association studies is the lack of replication of discovery findings in subsequent independent studies [59,60]. Therefore, the current gold standard for identification of biologically relevant *HLA* associations is replication studies in independent cohorts.

For this reason, we examined two separate groups of healthy children (cohort 1: 346, 94% Caucasian; cohort 2: 388, 89% Caucasian) who were immunized with two doses of measles vaccine [61]. Consistent associations were found between *B*3503* (first cohort: $p = 0.01$; second cohort: $p = 0.07$), *DQA1*0201* (cohort 1: $p = 0.03$; cohort 2: $p = 0.03$) and *DRB1*0701* (cohort 1: $p = 0.03$; cohort 2: $p = 0.07$) allelic variants and measles virus-specific antibody levels. In both of the groups, the *B*0801* and *DRB1*0301* alleles, *C*0802* and *DPA1*0202* alleles and *DRB1*1303* alleles exhibited concordant associations with a spectrum of secreted cytokines, such as IFN- γ , IL-2 and IL-10 (Table 2). In cohort 1, we also examined relationships between *HLA* haplotypes, *HLA* supertypes and measles-induced antibody levels and found that the *DRB1*15/16-DQB1*06-DPB1*03* haplotype ($p = 0.09$), and *B7* supertype ($p = 0.01$) were correlated with higher measles vaccine antibodies [54,55]. Analysis of the *DRB1*15/16-DQB1*06-DPB1*03* ($p = 0.07$) haplotype and *B7* ($p = 0.08$) supertype associations in cohort 2 demonstrated the same trend as observed in cohort 1 [61]. Additional studies are needed to further refine these findings and determine the molecular basis for these associations. When associations are replicated, the next step is to conduct functional studies and elucidate the immune mechanisms that regulate the observed phenotypes. Such studies are now in progress in our laboratory to discover the molecular pathways involved in regulating measles vaccine-induced immunity.

In addition to humoral immunity (neutralizing antibody levels), a widely accepted vaccine-induced correlate of protection, measles-specific cellular immunity may also contribute to protection. In cohort 1, we studied associations between *HLA* genotypes and IFN- γ secretion levels and found that *A*3001* ($p = 0.09$) and *DQB1*0402* ($p = 0.08$) allelic variants were marginally correlated with variations in measles-specific IFN- γ secretion in cell culture supernatants [51,52]. However, in cohort 2, cell-mediated immune responses to measles virus were assessed using IFN- γ ELISPOT assay. This measured the frequencies of measles-specific IFN- γ -secreting cells in peripheral blood mononuclear cells. In the replication study (cohort 2), we found significant associations between *A*3001* ($p = 0.07$) and *DQB1*0402* ($p = 0.002$) alleles and IFN- γ ELISPOT responses. In spite of the differences in methods of cytokine detection, consistent associations (and in the same direction) were found between the *A*3001* and *DQB1*0402* alleles and measures of measles vaccine cellular immunity. These associations may potentially be explained by mapping measles virus T-cell epitopes to specific *HLA* molecules using tetramers. Future measles vaccine design for use in a heterogeneous outbred population must take into account the properties of specific *HLA* alleles that contribute to protective immune responses.

Replicated associations of single nucleotide polymorphisms with measles vaccine immune responses

The literature, and biological plausibility, points to the role of multiple genetic polymorphisms in measles vaccine immune response heterogeneity [33,38,39,49–56,61–77]. Our previous twin study estimated the heritability for antibody response to measles vaccine to be approximately 89%. Single nucleotide polymorphisms (SNPs) in candidate immune response genes, together with *HLA* alleles, explain approximately 30% of the interindividual variability in measles-specific humoral immune response, suggesting tight genetic control over immunity induced by measles vaccine, as well as the involvement of additional genetic variants modulating the immune response to measles virus [78].

We and others have previously demonstrated significant associations between SNPs in a variety of immune response genes and measles vaccine-induced antibody and cellular immunity measures. These genes include cell surface viral receptors and related molecules (*SLAM*, *CD46*, *CD209/DC-SIGN*), cytokine and cytokine receptor genes (*IL2*, *IL10*, *IL12B*, *IL4RA*, *IL12RB1*, *IL7R*, *IL6*, *TNFA*), antiviral effector and innate immunity genes (*TLR2*, *TLR3*, *TLR4*, *TLR5*, *TLR6*, *TLR7*, *TLR8*, *MyD88*, *MD2*, *MAP3K7*, *IKBKE*, *TICAM1*, *NFKBIA*, *IRAK2*, *TRIM5*, *DDX58/RIG-I*, *OAS1*, *ADAR*, *MX2*, *OAS3*, *VISA*), vitamin A (*RARA*, *RARB*, *RARG*) and vitamin D (*RXRA*) receptor genes and *HLA* genes [33,38,39,56,61–77].

All of these findings are evidence that measles vaccine immune response is subject to regulation by multiple inter-related genes, genetic variants and gene networks, as is expected for complex biological processes such as infectious disease and immune response. Analytic approaches, such as multigenic assessment of SNP associations, gene set analysis, gene pathway and gene network analysis, rather than single SNP analysis, may prove useful in revealing the underlying biology of involved genetic variants/genes and their relation to immune function [62,79,80].

In subsequent independent studies, we have discovered and replicated important associations between genetic variants in immune response genes (such as cell surface receptors, cytokine and cytokine receptor genes) and measles vaccine immune response variations. For example, the immune response to measles is initiated through binding and virus entry into susceptible cells. The measles virus recognizes three distinct cellular receptors: *CD46*, *SLAM* and the recently discovered adherens junction *PVRL4* [81–85]. The role of *CD46* and *SLAM* receptors in measles infection led to an examination of the genes that encode for these receptors. In a population-based discovery study of 346 healthy subjects (ages: 12–18 years; 94% Caucasian) after two doses of measles-containing vaccine, we discovered several SNPs in the *CD46* and *SLAM* genes that exhibited an allele dose-dependent relationship with variations in measles virus antibody level [65]. In a replication study of an independent cohort of 745 subjects, after two doses of vaccine (ages: 11–22 years; 80% Caucasians, 12% African-Americans), DNA samples were genotyped for 43 SNPs in the *CD46* and *SLAM* genes using the Illumina genotyping platform. An earlier association of the *CD46* SNP rs2724384 with measles-induced antibody level was successfully replicated (Table 3). In particular, representation of the minor allele G for an intronic *CD46* SNP was associated with an allele dose-dependent reduction in measles antibody levels (median value: 978 mIU/ml for AA vs 522 mIU/ml for GG, $p = 0.0007$) (Table 3). Interestingly, the same *CD46* variant rs2724384 was reported to be associated ($p = 0.018$) with measles-specific IgG levels in a cohort of 137 Australian infants (ages: 12–14 months) after one dose of measles-containing vaccine [75]. In addition, this specific SNP (rs2724384) demonstrated associations with measles-specific IL-6, IFN- α and TNF- α secretion levels in a group of 745 subjects after two doses of measles vaccination [67]. Thus, this *CD46* intronic

polymorphism may be involved in the regulation of both antibody and cytokine responses to measles vaccination or may be tagging a different causative SNP. A coding SNP rs164288 in the *SLAM* gene that was associated with measles antibody levels in our previous study [65] was also found to be associated with measles cellular immune responses in a replication study (Table 3) [67]. Specifically, the minor allele G for this synonymous genetic variant (rs164288, Thr210Thr) was associated with variations in the frequencies of IFN- γ ELISPOT positive cells ($p = 0.04$) (Table 3). Associations were also discovered between two *CD46* haplotypes, AACGGAATGGAAAG ($p = 0.009$) and GGCCGAGAGGAGAG ($p < 0.001$), and measles virus-specific TNF- α secretion in Caucasians; however, these findings require replication and evidence for functional association [67]. These data suggest that *CD46* and *SLAM* gene receptor polymorphisms are likely to explain some of the individual differences in response to measles vaccination. The importance of host genetic variations in *PVRL4* (nectin-4) has yet to be determined.

We have also conducted a comprehensive candidate gene association study on 994 known SNPs in cytokine and cytokine receptors in 764 subjects following two doses of MMR vaccine, and replicated four genetic associations with measles virus-specific immune outcomes (Table 3) [63,64,69]. Of note, the replicated cytokine and cytokine receptor genetic associations involved primarily SNPs/genes (*IL2*, *IL12B*, *IL12RB1*) involved in cellular immunity and Th1 immune responses to measles virus. A previously identified functional genetic variant rs3212227 association ($p = 0.01$) (Table 3) [64], located in the 3' UTR region of the *IL12B* gene, was replicated ($p = 0.037$) (Table 3) [69]. This *IL12B* SNP, reported as the TaqI polymorphism, is known to alter gene expression and secretion of IL-12 [86] and has been repeatedly associated with inflammatory and immune conditions, infectious diseases and HBsAg-induced antibody levels after immunization [86–91]. An intronic polymorphism (rs372889) in the *IL12RB1* gene (a component of the IL-12 and IL-23 receptor complex that binds to IL-12 and IL-23) was also replicated in a study comparing measles virus-specific immunophenotypic extremes ($p = 0.03$) (Table 3) [63,64]. Although less investigated, intronic SNPs may modify gene function by altering gene splicing, transcription factor-binding activity, epigenetic gene regulation/DNA methylation and miRNA-binding specificity/activity [92,93]. Both replicated polymorphisms (*IL12B* rs3212227 and *IL12RB1* rs372889) are located in functionally closely related genes, impacting IL-12 and IL-23 binding, signaling pathways and functional consequences including Th1 immune response and IFN- γ production, as well as cell-mediated immunity. IL-23, a cytokine induced during inflammation, is also known to redirect memory T cells and stimulate Th17 differentiation with a plausible impact on vaccine-induced immunity [94]. Two promoter SNPs (*IL2* rs2069762 and *IL10* rs1800890), previously associated with measles-specific cellular immunity [64], were replicated in an independent study ($p = 0.034$ and $p = 0.025$, respectively) (Table 3) [69]. The *IL2* promoter minor allele variant C demonstrated an allele dose-dependent relationship with variations in IFN- γ ELISPOT responses (Table 3). Evidence from the literature confirms the functionality of *IL2* rs2069762 (–330 promoter *IL2* polymorphism) and its effect on IL-2 production [95]. Its reported associations with infections (upper respiratory infections and subacute sclerosing panencephalitis) and the response to hepatitis B vaccination [96–98], further enhance the confidence that this is a likely causal genetic variant with an impact on vaccine-induced immunity. Thus, we have identified and replicated genetic associations that uncover plausible genetic regulators of measles vaccine immune response.

Statistical/bioinformatics challenges of large genetic association vaccine studies

The existing knowledge base of confirmed genetic associations has been developed primarily using candidate gene approaches, where genes with known or suspected roles in

immune response have been selected for study in cohorts of immunized persons. These candidate gene studies present an array of challenges that have mostly been overcome. For *HLA* loci, the challenges were in identifying the full range of alleles at each of the candidate loci and performing analyses that appropriately accounted for the large number of alleles observed at the highly polymorphic *HLA* loci. For other loci, the identification of millions of SNPs in the HapMap project [99] made it possible to effectively describe the genetic variation within any given gene by careful selection of tagSNPs. These tagSNPs are selected such that all of the known SNPs within a gene are represented by at least one other SNP with which it shares a high correlation, as reflected in the observed degree of linkage disequilibrium. However, even with the intensive study of a large number of candidate immune-relevant genes, we are currently unable to fully explain all of the genetic control of response to measles vaccination.

Newer technologies make it possible to broaden the search for the genetic effects that may better identify the genetic contributors to variations in measles vaccine-induced immune responses. One of these technologies is the genome-wide SNP array. These genome-wide arrays have been designed to simultaneously assay the genome for hundreds of thousands to millions of SNPs from a single DNA sample. The availability of such genome-wide data has opened the door to a broader, and more agnostic, study of genetic effects on a range of relevant immune phenotypes. The first and most obvious use of genotype data from these arrays is testing for genetic associations point by point for each SNP along each of the chromosomes in the genome. This use of genome-wide data takes advantage of the comprehensive annotation of the SNP's location, both positionally on a chromosome as well as locationally and functionally within a gene, and requires careful collation of data to appropriately draw inferences from the observed associations. These considerations corresponding to genome-wide association studies of individual SNPs are now well understood, and the data analysis obtained from whole-genome arrays has led to the discovery of many genetic variants associated with many outcomes [100]. While these genome-wide studies have led to an increase of knowledge through the simple study of single-SNP associations, it is now clear that any individual SNP has, at best, only a modest contribution to explaining the full heritability of any given phenotype [101]. Therefore, it is important to apply a broader range of analysis techniques and approaches to these data to extract more genetic information from them, and to reach a complete understanding of which genetic features control differences in phenotypes, and how they do so.

As genome-wide SNP arrays are used in studies of vaccine response, a broader palette of options await our use. Clearly, examining individual SNPs across the genome in association with measles vaccine immune responses will lead to an expanded knowledge base concerning which genes actually do influence/control measles immune responses, even those which have not previously been implicated as being important in immune functions. However, even the simple identification of an individual SNP association necessitates further analyses that focus on replicating the association in independent samples, and others that focus on identifying which SNP out of all the correlated SNPs in the replicated region is most likely to be the causal variant. These fine mapping efforts often do not provide adequate representation of the full genetic diversity of the implicated genomic region. Therefore, it is necessary to apply other techniques to more deeply study the region of interest. The simplest of these methods involves using the SNP data that have been obtained from the whole genome array in concert with external data sources such as HapMap [99,102] and the 1000 Genomes Project [103] to impute the likely genotypes of all other known SNPs within the genotyped subjects. This makes it possible to make initial assessments of associations between the phenotype and SNPs that have not been directly genotyped. When these analyses identify specific genetic variants that are likely to drive the

original observed findings, additional genotyping experiments on a smaller number of SNPs are required to confirm the results from the imputation-based studies.

When these efforts do not provide evidence of a single SNP, or group of SNPs, that describes the replicated genetic associations from the initial genome-wide study, there are still other options. To study rare variants that may be unique to a specific disease, or to individuals from a particular genetic subpopulation, it is becoming more feasible to conduct higher throughput studies. These studies are based on the resequencing of the entire genome, or, to control costs, the subset of the genome that encodes proteins (the 'exome'). These 'NextGen' sequencing studies present tremendous opportunities to identify novel genetic architectures that might explain a variety of phenotypes, but they also present new challenges in genetic studies. NextGen sequencing studies generate extremely large amounts of data that must be annotated and collated before it can be usefully studied in association with any given phenotype. They also identify variants that are at a much lower frequency in the population, making study of the individual variants more challenging. In addition, NextGen technology can be applied to identify dynamic epigenome changes in DNA methylation and histone modification patterns as they occur upon antigenic challenge and development of adaptive immunity [104,105]. Such epigenetic alterations are known to characterize and control immune activation and T-helper cell differentiation that govern adaptive immunity [104,105]. Ongoing work will enable more efficient data use from NextGen sequencing studies to identify individual variants and genes that associate with, or even directly cause, variation in immune function.

The aforementioned methods focus on identifying individual variants that play a role in phenotype variability. However, genetic data, whether from candidate gene or genome-wide sources, offer a variety of options that allow for the identification of other genetic effects beyond those arising from individual alleles. From candidate gene panels, it is possible to search for combinations of variants that jointly contribute to variability in phenotype in a multigenic fashion [62,79,80]. Similarly, data from genome-wide studies can be used to perform a variety of pathway-based assessments of association with outcomes. The best understood of these is called gene-set enrichment analysis, where groups of genes in a predefined pathway or network are tested simultaneously to determine if they contribute significantly to the target phenotype [106]. Still other analytical options are available for use in the analysis of high-throughput genotyping data. For instance, if the genotypes were obtained in a set of subjects who belong to distinct racial subgroups, it is possible to perform admixture mapping analyses to identify candidate regions in the genome that appear to explain racial differences in immune response [107]. Also, the data available from common genome-wide SNP arrays can be used to search the genome to identify potential regions where variation in the number of copies may describe differences in the outcomes of interest [108].

We find ourselves in an exciting and challenging time – when we are able to search the entire genome for individual variants that are associated with a range of phenotypes, including multigenic and non-Mendelian effects on these outcomes. Although these data present challenges to analysts and to other researchers working to identify and explain these genetic associations and the biologic mechanisms behind them, they also present a tremendous opportunity that may make it possible to finally elucidate all of the genetic components that contribute to a wide variety of phenotypes, including vaccination-induced measures of immune responses.

Validation of genetic associations through functional studies

In considering the effect of genotype on phenotype, discovering the functional significance of polymorphisms is a major bottleneck in validating the results of genetic association studies. Confirmation of relevant causal genetic targets is not possible without assessment of the functional consequences of replicated and selected genetic variants that were found to be associated with interindividual variations in immune response. For vaccine-related genetic association studies, these genetic variants will typically come from *HLA* alleles and polymorphisms from candidate and/or genome-wide studies (interrogating immune and/or novel genes) that have been previously identified and replicated. The approach and detailed methodology for each functional study depends on the nature of the replicated SNP or *HLA* allele, and its associated gene and protein product. In general, the approach must include characterization and prioritization of replicated genetic variants and fine mapping efforts to identify likely causal variants, followed by the design of focused experiments that take into account the unique features of any given genetic variant (such as SNP type, location and putative effect) and measure its likely effect(s). To better illustrate the methodology for functional assessment of replicated genetic associations, we provide examples of experimental design using cell line model systems and advanced bioinformatics databases, proven powerful in conducting functional analyses [92,109,110].

Functional assessment for replicated HLA findings

Each *HLA* allele presents a different repertoire of epitopes to T cells, directly impacting cellular immune responses and indirectly (through T-helper cell activation) affecting humoral responses. We found that *DRB1*0701* and *DRB1*0201* are associated with significantly lower measles-specific antibody responses [61]. It is highly likely that *DRB1*0701* or *DRB1*0201* present quantitatively or qualitatively different sets of epitopes than other *DRB1* or *DQA1* alleles. This may elicit weaker measles-specific T-helper responses and therefore, less robust humoral immunity. The experimental approach for functional assessment of these associations includes PCR amplification of the corresponding *HLA* alleles of interest (e.g., *DRB1*0701* and a control allele not associated with variations in antibody response, such as *DRB1*0105*) from homozygous individuals and cloning of the alleles of interest into expression plasmids used to transfect *HLA* class II deficient B cell lines. The generated antigen-presenting cell lines expressing either *DRB1*0701* or a control allele/*DRB1*0105* (that are otherwise genetically identical) are used for stimulation of peripheral blood mononuclear cells from individuals possessing both *DRB1*0701* and *DRB1*0105* with subsequent quantification of the resulting B- and T-cell responses using a B-cell or IFN- γ ELISPOT assay, respectively. Both B- and T-cell responses could also be assessed by flow cytometry to measure cellular activation, costimulatory status (CD38, CD40, CD57, CD86 and CD154) and cytokine production (IFN- γ , TNF- α , IL-2, IL-10), to compare and contrast measles-specific T- and B-cell responses restricted by the two *HLA* alleles.

Functional assessment for replicated SNP associations

The general approach for replicated SNPs is illustrated and summarized in Table 4 and includes: identification and characterization of the genetic variant/variants most likely to be causal, including fine mapping of regions of interest; design of experiments appropriate for determining the functional consequences of the prioritized genetic variant and characterization of downstream consequences leading to variations in immune outcome. SNP associations discovered during genome-wide analyses are likely not the genetic polymorphisms causing the phenotypic change; rather, they are likely to be in linkage disequilibrium (LD) with the causal variant. Therefore, it is of the utmost importance to identify which variant is most likely driving the replicated association in each region of

interest. The genetic regions of interest can be interrogated using HapMap and the 1000 Genomes Project (or other databases) to identify all SNPs in the region that show LD. The LD regions surrounding the tagSNPs are typically fine mapped to test for the significance of each SNP within the fine mapping region and are then prioritized. The resulting list of SNPs is annotated using Go Ontology software and advanced bioinformatics algorithms and databases (i.e., SNP Function Portal, UCSC's phastCons, Transfac Public, BIOBASE and JASPER) to integrate other putative functional information (intergenic/evolutionary conserved regions, regulatory domains, promoter sequences) into the SNP annotation. Prioritization of genetic variants for functional studies depends on the likelihood of a functional effect and the ability to design rational approaches to identify functional effects using an integrated approach, such as Bayesian latent variable modeling. Bayesian latent variable modeling incorporates biological and statistical information (p-value, recombination hotspots, ECR, phastCons score) to create a quality score for SNPPrank [60,111–113] and identify the most likely causal SNP within the region that best captures the replicated genetic association of interest.

For example, as discussed earlier, we have identified an intronic SNP (rs2724384) in the *CD46* gene found to be associated with a twofold allele dose-dependent decrease in measles antibody titer, as well as variations in secreted cytokine levels, including measles-virus specific IL-6, IFN- α and TNF- α [65,67]. Measles virus binding to its receptor, CD46 has been shown to enhance IFN- α/β production, thus illustrating that genetic variants may affect the ability of CD46 to trigger antiviral response pathways [114,115], which may in turn affect antibody production [116].

To logically follow this pathway, the two *CD46* variants that differ at rs2724384 can be PCR amplified and each allelic variant (A/G) expressed into a *CD46*^{-/-} macrophage cell line using plasmid transfection. The two cell lines would then be infected with GFP-expressing measles virus and the ability of the virus to infect the two cell lines would be compared. Surface expression of CD46 can be monitored by flow cytometry to detect differences in surface expression, while IFN- α/β gene expression or protein could be assessed as a measure of antiviral response. B cells from multiple individuals homozygous for each *CD46* variant could be stimulated with virus for analysis/quantification of measles-specific antibody production. This set of experiments would probe potential effects of rs2724384 variants on CD46 expression, signal transduction, B-cell proliferation and antibody production. Detailed studies of the downstream effects of a given genetic polymorphism and its effect on the immune system/immune outcome of interest would be facilitated by the use of high dimensional technologies, such as NextGen sequencing technology (transcriptomic profiling), genome-wide transcription factor binding analyses, proteomic arrays and others, allowing investigators to link genotype to phenotype pathways and determine which immune pathways are differentially activated by the genetic variants being studied.

How vaccinomics will inform future vaccine development

As discussed above, the new paradigm of vaccinomics and systems biology has allowed us to use novel approaches to advance the science of measles vaccinology. There is still a long way to go from assessment of candidate genetic determinants of measles vaccine responsiveness/unresponsiveness to rational design of better vaccines, including genome-wide association and replication studies, functional and mechanistic studies of host immune response and host–microbe interactions (at the single cell and systems biology level) and development and assessment of new vaccine candidates and formulations. Understanding the genetic control of measles vaccine-induced immune responses allows us to understand more holistically how the immune response is generated and understand how host genetic and epigenetic variations change and impact vaccine immune responses. It also furthers our

understanding of how pathogens interact with the immune system. In turn, such knowledge provides a method of ‘reverse engineering’ around genetic restrictions to create new vaccine candidates informed by immunogenetics (at the individual level) and immunogenomics (at the population level). Doing so requires the directed vaccinomics paradigm of ‘Discover → Replicate → Validate → Apply’ we have advocated [33–39,117,118].

It is important to emphasize that the vaccinomics approach is a very different approach to developing vaccines than the traditional approach and, in part, relates to the growing interest in individualized medicine. We predict, based on the type of work discussed herein, a time when it will be possible to determine what diseases an individual is at risk for, what vaccine(s) they should receive, at what doses and the chance that they may experience any significant adverse event. This is a very different experience than the current situation where essentially everyone gets every vaccine, given in the same manner, at the same dose and same number of doses – ignoring the reality that we are genetically preprogrammed to immunologically respond in predetermined differing patterns. Full realization of this level of personalization will require what we do not currently have – research informing us what genetic signatures predict what immune response phenotypes (the subject of our work) and large population-level genotype:phenotype databases. Such databases are being established on a research basis, but full implementation will require legal, legislative, public and ethical discussions to insure privacy and nondiscrimination. We believe these issues will be solved, as the medical and scientific value is significant to individuals and society as a whole.

It is also worth pointing out that in addition to the utility of the vaccinomics approach in developing new vaccine candidates, this approach can also be useful in at least three other settings: the ability to predetermine the likelihood of a significant adverse event to a vaccine (what we have labeled as ‘adversomics’) [118]; developing new study designs outside of population-based clinical trials in an effort to identify genetic subgroups who may benefit from a vaccine even though other subgroups do not; and serving as an early ‘go – no go’ decision node in the early clinical trials progression of a new vaccine. In regard to this latter point, currently vaccine candidates progress from Phase I through Phase III clinical trials. These are extremely expensive and, in the end, provide information about study end points at a population level, not at an individual level. Such costs could be avoided early in the process if it can be determined that a given vaccine antigen is unlikely to be highly immunogenic or is likely to cause a significant adverse event in persons carrying a specific gene (e.g., a particular *HLA* supertype).

Thus, the science and power of vaccinomics as a new paradigm from which to organize research and clinical development of novel vaccine candidates are multifold. To give a specific example, knowledge that a SNP in the gene coding for a viral receptor leads to inadequate binding of that virus to an immune processing cell, and hence leads to a lack of protective antibody response, is an important finding. This can be used to reverse engineer a new vaccine candidate; perhaps by inserting a plasmid coding for transcription of a receptor protein that allows high-affinity binding of virus to receptor and thereby allows the vaccine virus to be naturally processed, presented and translated into a protective immune response. Many other examples exist, but the point is that information about the genes and genetic pathways by which immunity to a vaccine antigen is regulated provides a wealth of information that can be useful in developing novel vaccine candidates in a directed manner.

Expert commentary

While the current measles vaccine used in the USA and many other countries is safe and effective, paradoxically in the unique case of measles, it appears to insufficiently induce herd immunity in the population. This relates to a combination of factors including: higher

than observed rates of primary and secondary vaccine failure in clinical practice versus that seen in clinical trials; the inability to guarantee a minimum of two doses of vaccine to every individual in the population; the need for two doses of vaccine and the inability to use the vaccine in significant subgroups of the population (immunocompromised persons, persons with significant contraindications or adverse reactions to the vaccine, and so on); cost; cold-chain requirements; and other factors including interindividual variations in immune responses due to genetic variations. Thus, we believe that a third-generation measles-vaccine candidate should be developed. Furthermore, such vaccine development should be informed by current science and be directed by a systems biology vaccinomics approach to overcome the current barriers that have prevented measles eradication. One such approach may be a peptide-based approach. It would be affordable, would not require a cold chain and, at least in Western countries, would overcome hesitancy to use a live measles vaccine due to a variety of fears (including the unsubstantiated fear of autism resulting from vaccination) and would be informed by population genetics. Other options such as inhaled vaccines should also be considered. Regardless, the current vaccine, for both scientific and cultural reasons, has not demonstrated the ability to eradicate measles, despite high rates of use over decades. The need for new approaches is apparent and pressing.

Five-year view

New and expanded knowledge on host genetic factors regulating immune responses elicited by vaccines, including live-attenuated measles vaccine, will advance our understanding of how the immune system works to ensure and maintain protection against important human pathogens. In the next 5 years, large population-based genome-wide genetic association studies, followed by replication studies, will comprehensively explore the human genome and identify/replicate immune response and novel genes, gene pathways and genetic networks that influence and possibly predict immune response variations to measles vaccine. Fine mapping studies of selected genetic intervals will allow for more precise localization of the associated genetic variants and enhance the identification of causal variants. Functional studies of the identified genetic elements and pathways will uncover the underlying mechanisms directing the observed immune phenotype. Novel cutting-edge technologies (such as next generation sequencing) and advanced bioinformatics/statistical approaches will provide new insights and integrated knowledge into genetic architecture, epigenetic regulation, gene expression/regulation, protein expression and function, thus elucidating in depth the immunogenetic factors regulating vaccine-induced immunity. Despite the progress in our knowledge, improved and novel next-generation measles vaccines will not be developed for routine clinical use within the next 5 years. However, the knowledge base described herein and imminent scientific contributions in immunogenetics will substantially advance the field of viral vaccines, and measles vaccine in particular, with important implications for future longer-term vaccine development, vaccination practices and measles eradication.

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Key issues

- Although vaccination with attenuated measles vaccine is effective, measles outbreaks still occur in developed countries, even in highly vaccinated populations, which necessitates research on determinants of measles vaccine-induced immunity and development of better vaccines and vaccination practices.
- Several important genetic determinants regulating measles vaccine-induced immunity, including *HLA* class I and *HLA* class II genotypes, and single nucleotide polymorphisms in cytokine/cytokine receptor genes and the membrane cofactor measles receptor *CD46*, have been identified and replicated in independent studies.
- Genome-wide association and replication studies, followed by fine mapping efforts and functional validation of the causal genetic variants, will further advance our understanding of vaccine-induced immunity and its regulation.
- Next-generation measles vaccines and improved vaccination practices, informed by current and forthcoming scientific discoveries and directed by a novel vaccinomics and systems biology approach, will overcome the current vaccine limitations and barriers that have prevented measles eradication.

Table 1

Measles-containing vaccine status in the recent measles cases in the USA.

Year	Total infected, n	No measles vaccine received, n (%)	Unknown measles vaccine status, n (%)	Received only 1 dose, n (%)	Received at least 1 dose, n (%)	Received at least 2 doses, n (%)
2005	66	50 (76)	8 (12)	7 (11)	8 (12)	1 (2%)
2006	55	25 (45)	15 (27)	12 (22)	15 (27)	3 (5)
2007	43	25 (58)	10 (23)	4 (9)	8 (19)	4 (9)
2008	140	108 (77)	21 (15)	6 (4)	11 (8)	5 (4)
2009	71	49 (69)	15 (21)	6 (8)	7 (10)	1 (1)
2010	63	37 (59)	20 (32)	2 (3)	6 (10)	4(6)
2011	222	145 (65)	47 (21)	17 (8)	30 (14)	13 (6)

Source: Division of Viral Diseases, Epidemiology Branch, National Center for Immunization and Respiratory Diseases, CDC, 10 May 2012. The 2011 data are preliminary and have not been finalized yet.

Table 2*HLA* allelic associations with measles-specific immune responses.

<i>HLA</i> allele [†]	Cohort 1 (n = 346) p-value	Cohort 2 (n = 388) p-value
<i>Measles-specific antibody response</i> [‡]		
<i>B*3503</i>	0.01	0.07
<i>DRB1*0701</i>	0.03	0.07
<i>DQA1*0201</i>	0.03	0.03
<i>B*7</i> supertype	0.01	0.08
<i>Measles-specific IFN-γ (pg/ml)</i>		
<i>B*0801</i>	0.04	0.03
<i>DRB1*0301</i>	0.05	0.09
<i>Measles-specific IL-2 (pg/ml)</i>		
<i>C*0802</i>	0.06	0.06
<i>DPA*0201</i>	0.09	0.06
<i>Measles-specific IL-10 (pg/ml)</i>		
<i>DRB1*1303</i>	0.06	0.04

[†] Linear regression analysis was used to assess genetic associations; p-values for antibody levels were based on log-transformed data, using simple linear regression; p-values for cytokine levels were based on rank-transformed data. Only alleles found to be statistically or marginally significant in cohorts 1 and 2 (p < 0.10) are shown. Analyses are adjusted for age at blood draw, gender, race, age at first measles and age at second measles immunization.

[‡] Antibodies were quantified as IgG antibody levels in mIU/ml by EIA (Dade Behring, Germany) for cohort 1 and neutralizing antibody levels in mIU/ml by the fluorescence-based plaque reduction microneutralization assay for cohort 2.

Replicated single nucleotide polymorphism associations with measles-specific immune responses.

Table 3

Immune measure	Gene	SNP (location)	Genotype [‡]	Median immune response [‡]	p-value ₁ [§]	p-value ₂ [§]
CMI	<i>IL2</i>	rs2069762 (promoter)	AA/AC/CC	35/38/39	0.04	0.034
CMI	<i>IL10</i>	rs1800890 (promoter)	TT/TA/AA	34/34/43	0.05	0.025
Antibody	<i>IL12B</i>	rs3212227 (3' UTR)	AA/AC/CC	837/807/951	0.01	0.037
Antibody	<i>CD46</i>	rs2724384 (intron)	AA/AG/GG	978/736/522	0.01	0.0007
Antibody and/or CMI	<i>SLAM</i>	rs164288 (coding)	GG/GA/AA	29/10/–	0.03	0.04
Antibody and/or CMI	<i>IL12RBI</i>	rs372889 (intron)	AA/AG/GG	–	0.05	0.03

[‡] Values are presented as homozygous major allele/heterozygous/homozygous minor allele.

[‡] Median immune response is presented for the replication only as median neutralizing antibody levels in mIU/ml ± interquartile range, as measured by the fluorescence-based plaque reduction microneutralization assay, or as median IFN-γ-positive spot-forming units per 200,000 cells ± interquartile range, as measured by the IFN-γ ELISPOT assay.

[§] p-value₁ stands for p-value from the discovery study assessed using analysis of covariance methods or χ^2 tests of significance that compared the distribution of SNPs across four quadrants, defined by the extremes of humoral (antibody) and cellular (lymphoproliferative) immune responses [64,65], while p-value₂ stands for ordinal p-value from the replication study, assessed using linear regression methodologies (for all presented SNPs, except rs372889, which was replicated using the discovery approach) and quantitative measures of immune outcomes for humoral and cellular immunity in the entire cohort [63,67,69].

CMI: Cell-mediated immunity; SNP: Single nucleotide polymorphism.

Table 4

Functional assessment of replicated single nucleotide polymorphisms.

Molecular biology assays to determine functional effects of replicated SNPs		
SNP location	Assay	Outcome
Promoter region	Reporter assay	Measure ability of promoter variants to drive gene expression
	qRT-PCR	Measure single gene expression when linked to promoter variants
	mRNA-Seq	Measure transcriptomic changes induced by variant promoters
Splice site	mRNA-Seq	Measure effect of SNP on splice isoform mRNA expression
CpG site	DNA methylation assay	Measure effect of SNP on methylation at specific site or genome-wide
TF binding site	Reporter assay	Quantitative measure of TF-driven gene expression
	ChIP, ChIP-Seq	Quantitative measure of TF binding to variant DNA sequences
	mRNA-Seq, qRT-PCR	Quantitative measure of TF-driven gene expression
miRNA site	miRNA assay	Measure ability of miRNA variants to bind to target sequence
	qRT-PCR	Measure ability of miRNA variants to suppress target gene
Coding sequence	Flow cytometry, ELISA	Measure effect of SNP on protein expression
	Reporter assay	Measure effect of SNP on protein function
<i>Target gene search techniques</i>		<i>Outcome</i>
Expression quantitative trait loci		Association between SNP and expression levels of selected genes
3C/5C (chromosomal conformation capture)		Long-distance interactions between SNPs and genes
Immune assays to elucidate downstream immunologic mechanisms		
<i>Process</i>	<i>Assay</i>	<i>Outcome</i>
Virus infection	RT-PCR	Quantitative measure of viral gene expression
	Flow cytometry	Measure percentage infected and cell type infected
Intracellular signaling pathways	RT-PCR	Quantitative measure of cellular gene expression
	NextGen sequencing	Transcriptomic measurement of gene expression
		Allele-specific gene expression Splice variant expression and identification
Cytokine/chemokine secretion	Multiplex ELISA	Quantitative measure of protein production
	Flow cytometry	Quantitative measure of cytokine production
		Frequency of cells producing given cytokine
Cell subsets	Flow cytometry	Frequencies of cellular phenotypes (percentage B cells)
	ELISPOT	Frequencies of responding T cells (percentage CD8 ⁺ , others)
Antibody production	ELISA	Quantitative measure of antibody titer
		Quantitative measure of antibody isotype

Molecular biology assays to determine functional effects of replicated SNPs		
SNP location	Assay	Outcome
T-cell effector function	Neutralization assay	Quantitative measure of neutralizing antibody titer
	Proteomic arrays	Measure protein-specific antibodies
	Cr ⁵¹ release	Measure of Ag-specific T/NK cell cytotoxicity
	MTS assay	Measure of Ag-specific T/NK cell cytotoxicity
T- and B-cell activation	Flow cytometry	Measure of Ag-specific T/NK cell cytotoxicity
		Frequency of cytolytically active T/NK cells
	LPA	Quantitative measure of lymphocyte proliferation
	Flow cytometry	Measurement of cellular proliferation
		Measurement of activated cell phenotype

Ag: Antigen; ChIP-Seq: Chromatin immunoprecipitation sequencing; LPA: Lymphocyte proliferation assay; mRNA-Seq: Messenger RNA sequencing; MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; qRT-PCR: Quantitative reverse transcription polymerase chain reaction; SNP: Single nucleotide polymorphism; TF: Transcription factor.