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# Heritability of Vaccine-induced Measles Neutralizing Antibody Titers

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## Abstract

Understanding how genetics influences inter-individual variation of antibody titers in response to measles vaccination is vital to understanding possible sources of vaccine failure as well as improved vaccine development. Although it is recognized that both the human leukocyte antigen (HLA) genes and the immunoglobulin allotype genes play significant roles in immune response, there is significant variation in antibody titers that is not explained by these genes. To obtain a more complete estimate of the role of the entire genome, we used a large panel of single nucleotide polymorphisms to estimate the heritability of antibody response to measles vaccine. Based on 935 subjects with European ancestry, we estimated the heritability to be 49% (standard error 0.17). We also estimated the heritability attributable to each chromosome, and found a large range in chromosome-specific heritabilities. Notably, chromosome 1 had the largest estimate (28%), while chromosome 6, which harbors HLA, had an estimated heritability of 13%. Compared with a prior study of twins in the same community, which resulted in a heritability estimate of 88.5%, our study suggests there are either many rare genetic variants, or many common genetic variants of small effect sizes that contribute to variations of antibody titers in response to measles vaccine.

### Keywords

Heritability; Genetic Variation; Genome-Wide Association Study; Measles; Measles Vaccine; Immunity; Humoral; Immunity; Cellular; Polymorphism; Single Nucleotide; Genetic Variation; Adult

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**Data Availability**

The study data are all available, without restriction, at ImmPort. https://immport.niaid.nih.gov Study #:SDY839

**Competing Interests:** Dr. Poland is the chair of a Safety Evaluation Committee for novel investigational vaccine trials being conducted by Merck Research Laboratories. Dr. Poland offers consultative advice on vaccine development to Merck & Co. Inc., CSL Biotherapies, Avianax, Dynavax, Novartis Vaccines and Therapeutics, Emergent Biosolutions, Adjuvance, Microdermis, Seqirus, NewLink, Protein Sciences, GSK Vaccines, and Sanofi Pasteur. Drs. Poland and Ovsyannikova hold three patents related to measles and vaccinia peptide research. Dr. Kennedy has received funding from Merck Research Laboratories to study waning immunity to measles and mumps after immunization with MMR-II®. These activities have been reviewed by the Mayo Clinic Conflict of Interest Review Board and are conducted in compliance with Mayo Clinic Conflict of Interest policies. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic Conflict of Interest policies.

## Introduction

The heritability of a trait is often used to summarize the total genetic influence on the variation of a trait. Heritability in the narrow sense is the proportion of phenotypic variance due to additive genetic variance, which can be measured in twin, family, or population-based studies. Estimates of heritability have been used to evaluate how much genetic variation from current genome-wide association studies (GWAS) explains trait variation. Although the focus of this paper is the heritability of immune response to measles vaccination, lessons on heritability can be learned from the study of human height, which has approximately 80% heritability based on twin studies [1, 2]. An alternative way to estimate heritability with population-based data is to use genetic markers, such as single nucleotide polymorphisms (SNPs), to measure genetic similarity between pairs of subjects. This similarity can be viewed as an average, over all SNPs, of how much a pair of subjects is genetically related. These estimated relationships, computed for all pairs of subjects, can then be used as a genetic relationship matrix in a variance component analysis of a trait, essentially determining how much similarity of genetic relationships corresponds to similarity of traits[3].

When applying this method to human height, it was found that restricting to about 50 SNPs that had genome-wide statistically significant associations with height resulted in a heritability estimate of about 5%, yet when using all measured 294,831 SNPs simultaneously, the estimated heritability was 45% [3]. This suggested that most of the undetected heritability was due to individual SNP effects that were too small to pass the genome-wide level of statistical significance. The authors also concluded that the remaining heritability (the difference between 80% based on twin studies and 45% based on GWAS data) is likely due to incomplete linkage disequilibrium between the causal variants and the measured SNPs, compounded by causal variants with low minor allele frequency, which are difficult to capture with the common SNPs used for most GWAS SNP arrays.

Immune response to measles vaccination is controlled by many genes, such as *HLA* class I and *HLA* class II genes [4], SNPs in cytokine/cytokine receptor genes (*IL12B*, *IL12RB1*, *IL2*, *IL10*) [5], innate immune response genes (*TLR, TRIM*) [6, 7], vitamin A (*RARA, RARB, and RARG*) and vitamin D (*RXRA*) receptors [8], host antiviral [9], and the cell surface measles virus receptor (*CD46, SLAM)* genes [10]. Because many genes are involved, and natural selection is likely to have whittled the effects of genes on response to be relatively small, we might anticipate immune phenotypes in response to vaccination to have genetic complexities that parallel those of height.

We recently completed a GWAS of immune response to measles vaccine, and found two distinct regions on chromosome-1 that achieved genome-wide statistical significance for their association with vaccine-induced measles neutralizing antibody titers. The 1q32 region contained 20 significant SNPs in the vicinity of the measles virus receptor-encoding *CD46* gene, including the intronic rs2724384 (p-value = $2.64\times10^{-09}$) and rs2724374 (p-value = $3.16\times10^{-09}$) SNPs. The 1q31.1 region contained nine significant SNPs in the vicinity of the *IFI44L gene*, including the intronic rs1333973 (p-value = $1.41\times10^{-10}$) and the missense

rs273259 (His73Arg, p-value = $2.87\times10^{-10}$) [11]. In contrast, earlier we estimated the heritability of vaccine-specific IgG levels for measles to be 88.5%, based on 100 twin pairs [12]. Based on this strikingly high level of heritability, it was somewhat surprising that only two regions had genome-wide significant results. To further understand the potential scale of genetic influence on immune response to measles vaccination, we used the complete genetic information from a GWAS to estimate heritability of vaccine-induced measles neutralizing antibody titers.

## Methods

### Study Subjects

The cohort comprised 1,062 individuals enrolled into three age-stratified cohorts of healthy, school-age children and young adults from all socioeconomic strata in Rochester, MN, recruited between 2001–2009, as described previously [4, 7, 13, 14]. Parental consent was obtained for all participants and each subject had written records of having received two doses of MMR vaccine. Of the 1,062 individuals for this study, 935 (88%) were successfully genotyped and assayed for measles-specific neutralizing antibodies. The Institutional Review Board of the Mayo Clinic (Rochester, MN) approved the study, and written informed consent was obtained from each subject, from the parents of all children who participated in the study, as well as written assent from age-appropriate participants.

### Neutralizing Antibody Assay

Measles-specific neutralizing antibody titers were quantified using a high-throughput, fluorescence-based plaque reduction microneutralization assay (PRMN), using a recombinant GFP-expressing measles virus, as previously described [10, 13–15]. The plates were scanned and counted on an automated Olympus IX71 Fluorescent microscope using the Image-Pro Plus Software Version 6.3 (MediaCybernetics; Rockville, MD). The 50% end-point titer (Neutralizing Doze, $ND_{50}$) was calculated automatically using Karber's formula, and transformed into mIU/mL (using the 3$^{rd}$ WHO international measles antibody standard), as described previously [10, 13–15]. The variability of the PRMN assay, calculated as a coefficient of variation (CV) based on the log-transformed $ND_{50}$ values of the third WHO standard, was 5.7% [10, 13–15].

### Genotype Quality Control and Imputation

Subjects were genotyped with the Omni 1M-Quad array. SNPs on the Y chromosome and mitochondria were removed, and SNPs were eliminated if they were monomorphic or had a missing rate of 1% or more. Subjects were eliminated if they were missing more than 1% of the SNPs. The 1000 Genomes cosmopolitan samples (African, AFR; AMR; Asian, ASN; European, EUR) were used as a reference for imputation and were based on Build 37. SNPs that could not be converted to Build 37, or mapped to more than one position, or could not have their alleles verified for the forward strand, were eliminated. The reference genome was then filtered to include only those SNPs with a minor allele frequency (MAF) greater than 0.005. The data were phased using SHAPEIT [16] and imputed via IMPUTE2 [17]. SNPs with an imputation dosage allele $r^2$ of at least 0.3 and a MAF of at least 0.01 were retained for analyses, resulting in 5,611,233 SNPs.

### Genetic Ancestry

Genetic data were used to assign ancestry groups (African, Caucasian, or Asian) for individuals using the STRUCTURE software [18], and using the 1000 Genomes data as a reference. As suggested by others [19], the SNPs used for STRUCTURE were selected by LD pruning from an initial pool consisting of all autosomal SNPs with the following filters: SNPs with a minor allele frequency (MAF) < 5% were excluded; influential SNPs were removed (according to the following chromosome regions: chromosome 8 [bp 1–12700000]; chromosome 2 [bp 129900001–136800000, 5700000–33500000]; chromosome 4 [bp 0900001–44900000]); correlation ($r^2$) pruning was used to subset to uncorrelated SNPs. SNPs passing these selection criteria were input to STRUCTURE [18] to make ancestry "triangle" plots that depict the admixture proportions of ancestry groups for each subject. Subjects were classified into major ancestry groups based on the largest estimated STRUCTURE ancestry proportion. Because ancestry can confound estimates of heritability, and the majority of subjects reported themselves to have Caucasian ancestry, we restricted our analyses to subjects that were estimated to have European ancestry, based on their major ancestry group estimated by STRUCTURE.

### Neutralizing Antibody Trait

The trait used for heritability estimates was the log of the neutralizing antibody titers. To account for the effects of confounding factors, we evaluated the following potential covariates to determine if they were associated with the trait: ages and dates of birth and study enrollment, ages at first and second (or most recent) vaccination, time from vaccination to enrollment, sex, enrollment cohort, and assay characteristics (laboratory operator, plate position, plate, and run variable). Variables that were marginally associated with the trait with p-value < 0.1 were included in backwards selection with a p-value threshold of 0.1. This somewhat liberal threshold achieves the goal of controlling for potential confounding covariates. Only operator, run number, and plate were selected as adjusting covariates. In addition, we accounted for potential population stratification by using the first 10 principal components estimated from the GWAS SNPs. These covariates were used to create residuals (adjusted traits) that were then used for the heritability analyses.

### Heritability Estimation

The software GCTA[20] was used to estimate genetic relationship matrices based on the imputed SNPs and to estimate heritability, based on variance components models that used the adjusted traits. Principal components were estimated by the GCTA software to control for potential population stratification.

## Results

A total of 935 subjects were estimated to have European ancestry, and hence were included in the analyses. Summary information is presented in Table 1. Females represented 45% of study subjects, and 94% reported themselves to be of White racial heritage. The average age at enrollment was 15 years. The average age at second vaccination was 8 years, and the average time from second vaccination to enrollment was 6.6 years.

We estimated heritability attributed to each chromosome, as well as the heritability based on the totality of all SNPs across all chromosomes. Results in Table 2 illustrate that the genome-wide heritability was estimated to be 49% (standard error, 17%). The heritability attributed to each chromosome varied from 0% on chromosomes 8, 11, 13, and X, to 28% on chromosome 1. The heritability ranged 10–14% on chromosomes 2, 5, 6, 9, 12, 16, and 18. It is important to examine chromosomes 2, 14, and 22, because these chromosomes harbor loci that encode immunoglobulin (Ig). Ig is comprised of two heavy chains and two light chains — each chain has a variable domain and a constant domain. The immunoglobulin gene loci reside on these chromosomes: the heavy chain locus on chromosome 14, kappa light chain locus on chromosome 2, and lambda light chain locus on chromosome 22[21]. These loci are highly variable in terms of alleles and copy number variation. The heritabilities for chromosomes 2, 14, and 22 were only 10%, 2%, and 2%, respectively. It is also worthy to note that a heritability of 13% was attributed to chromosome 6, which harbors the HLA region. Because there are many genes related to immune response, particularly the generation and persistence of humoral immunity, it is not surprising that the genes responsible for the titers of measles-specific neutralizing antibody do not seem to be concentrated on any single chromosome. We considered whether the higher 28% heritability for chromosome-1 could be driven by the two regions on this chromosome that we found to harbor genome-wide significant SNP associations: the 1q32 region in the vicinity of the measles virus receptor-encoding *CD46* gene, and the 1q31.1 region in the vicinity of the gene *IFI44L* [22]. To evaluate the impact of these two regions, we re-estimated heritability after excluding 310 SNPs that had correlations ($r^2$>0.1) with the genome-wide significant SNPs (leaving 432,329 SNPs). However, after excluding these SNPs, the heritability did not budge from 28%. Interestingly, the other two known measles virus receptors are also encoded by genes (*SLAMF1* and *PVRL4)* on chromosome 1, as are other key innate antiviral effectors (e.g., *ADAR, RNASEL, ISG15*) and immune factors (e.g., *IL10, IL6R, IL28RA, BCL6*, tumor necrosis factor superfamily members, receptors for the Fc region of immunoglobulins). Finally, it was interesting to find that the heritability attributed to the X chromosome was 0%, despite the fact that the X chromosome has a large number of genes directly or indirectly involved in immunity, and implicated as genetic mediators of sex-based differences in immune response following vaccination [23, 24].

## Discussion

From a population-based sample of 935 children and young adults, we estimated a heritability of 49% for antibody titers in response to measles vaccination. This contrasts to an earlier estimate of 88.5% heritability of measles virus-specific IgG levels based on 100 twin pairs from the same community [12]. The age range of subjects from this twin study was 2–18 years, not appreciably different from the ages of subjects in our current study. This is important because we and others have shown that age strongly influences the heritability of immune parameters [25]. For example, a high heritability of 91% was observed for antibody to hepatitis B vaccine among infants less than one year old [26], and a study of 207 Gambian twin pairs with measured immune responses at 5 months showed high heritability for antibody responses to hepatitis B (77%), oral polio (60%), tetanus (44%) and diphtheria (49%) vaccines [27]. In contrast, immunologic parameters measured over wider age ranges

show much less heritability and stronger influences by age and environmental factors [28, 29].

The somewhat low heritability estimates for chromosomes 2, 14, and 22, which harbor loci that encode Ig, could result from several factors. First, the current SNP arrays do not adequately capture the genetic variation of the immunoglobulin gene loci [30]. Second, the amount of genetic variation in the immunoglobulin loci could be drowned out by larger random variation from the remaining SNPs on these chromosomes, when averaging the genetic sharing for the entire chromosome. Third, other immune response factors specific to measles vaccine response (one versus two doses regimen) could diminish the heritability attributed to variation in the immunoglobulin loci.

The lack of any measurable heritability on the X chromosome was unexpected, especially given the number of immune-related genes (e.g., *IL2RG*, *TLR7*, *CD40L*) present on the X chromosome, and the well-documented differences between men and women in immune response to vaccination and infection [31]. It is likely that sex-effects are driven by multiple complex biological processes and their interactions, rather than by a few major mechanisms. An early and easy suspect for sex-effects was sex hormone production. Although sex hormones are involved, sex-based differences also exist between pre-pubertal girls and boys and between post-menopausal women and similarly aged men, indicating that sex hormones are only a small part of the complete picture [32].

Our results indicate that the most significant hits from our GWAS study (SNPs in *CD46* and *IFI44L*), although involved, have small individual contributions to variation in humoral immunity to measles vaccine. This highlights one of the main limitations of GWAS on complex phenomenon such as immune response to vaccines – the need for extremely large cohorts to reliably identify extremely small effects.

It is important to recognize that heritability measures the percentage of the trait variation that can be attributed to additive effects of genes, so a change in the environment, without changing the genetics of a population, will change the heritability. Because the twin study that reported 88.5% heritability of measles vaccination response was from the same Rochester, MN, community as the population-based sample used in this study, large environmental differences are not likely to explain the difference in heritability estimates. It is known, however, that twin studies can give inflated estimates of heritability when twins share more environment than distantly related subjects [33]. This raises the question of whether the earlier twin study overestimated the heritability of response to measles vaccination. On the other hand, in the twin study, whole measles virus-specific circulating IgG levels were assessed by performing enzyme-linked immunosorbent assay (EIA) [12]. In contrast, our current study utilized a high-throughput, fluorescence-based PRMN assay that is thought to be the gold standard in quantifying neutralizing antibodies against the two measles virus surface glycoproteins (H and F). Dissimilarities between these two assays could also account for some of the differences in estimates of heritability between the two studies.

Another very likely possibility is that our estimate of 49% heritability, based on population data, is an underestimate. This could result from the SNPs measured in the Omni 1M-Quad array, and those that we imputed, having low correlation with the underlying causal variants. The approximately one million SNPs on the Omni 1M-Quad array were chosen to have minor allele frequencies at least 5%, and chosen to tag common genetic variants. Furthermore, our imputation of approximately 5.6 million SNPs restricted to those that had reasonable imputation accuracy and minor allele frequency at least 1%. Causal genetic variants that are not common (e.g., minor allele frequencies less than 1%) are not likely to be in strong linkage disequilibrium with our measured or imputed SNPs. These types of variants would be missed in our estimate of heritability.

In conclusion, our estimate of 49% heritability of antibody response to measles vaccination, and its contrast to a prior estimate of 88.5% heritability based on a twin study in the same community, suggests that many genes of small effect are likely to be responsible for inter-individual variations in antibody response to measles vaccine. It might be that some of the causal variants are rare, or it could be that some are common, yet with effect sizes that are not statistically detectable with current sample sizes. Much larger studies, perhaps facilitated by meta-analyses, would be required to discover the missing genetic factors. The value of this finding is in part related to future immunogenetic studies seeking to determine heritability estimates of vaccine response, and in planning systems biology studies that consider genetic heritability estimates.

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## Table 1

Patient and Assay Characteristics (N = 935)

| | |
|---|---|
| **Neutralizing Antibody** | |
| Mean (SD) | 1,313 (1,263) |
| Median | 902 |
| Q1, Q3[(1)] | 435; 1,751 |
| **Sex** | |
| Female | 424 (45.3%) |
| Male | 511 (54.7%) |
| **Self-reported Race** | |
| American Indian, Alaska Native | 4 (0.43%) |
| Asian, Hawaiian, Pacific Islander | 7 (0.75%) |
| Black or African American | 13 (1.19%) |
| Multiple | 19 (2.03%) |
| Other | 5 (0.54%) |
| Unknown | 6 (0.64%) |
| White | 881 (94.20%) |
| **Ethnicity** | |
| Don't Know | 7 (0.75%) |
| Hispanic or Latino | 18 (1.93%) |
| Not Hispanic or Latino | 910 (97.30%) |
| **Age Enrolled (years)** | |
| Mean (SD) | 15 (2.19) |
| Median | 15 |
| Q1, Q3 | 13, 17 |
| **Age at 2nd Vaccination (years)** | |
| Mean (SD) | 8.43 (3.46) |
| Median | 10 |
| Q1, Q3 | 5, 12 |
| **Years from Vaccination to Enrollment** | |
| Mean (SD) | 6.58 (2.77) |
| Median | 6.4 |
| Q1, Q3 | 4.65, 8.5 |

[(1)] Q1 and Q3 are first and third quartiles, respectively

**Table 2**

Heritability according to chromosome and genome-wide

| Chromosome | Heritability | Standard Error |
|---|---|---|
| 1 | 0.28 | 0.10 |
| 2 | 0.10 | 0.09 |
| 3 | 0.07 | 0.08 |
| 4 | 0.05 | 0.08 |
| 5 | 0.14 | 0.08 |
| 6 | 0.13 | 0.07 |
| 7 | 0.08 | 0.08 |
| 8 | 0.00 | 0.07 |
| 9 | 0.12 | 0.08 |
| 10 | 0.02 | 0.07 |
| 11 | 0.00 | 0.06 |
| 12 | 0.13 | 0.08 |
| 13 | 0.00 | 0.07 |
| 14 | 0.02 | 0.06 |
| 15 | 0.02 | 0.06 |
| 16 | 0.10 | 0.07 |
| 17 | 0.01 | 0.06 |
| 18 | 0.15 | 0.07 |
| 19 | 0.02 | 0.05 |
| 20 | 0.02 | 0.06 |
| 21 | 0.06 | 0.05 |
| 22 | 0.02 | 0.05 |
| X | 0.00 | 0.04 |
| Genome-wide | 0.48 | 0.17 |