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Short Communication: Immunoglobulin GM and KM Genes and Measles Vaccine-Induced Humoral Immunity

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Abstract

Identifying genetic polymorphisms that explain variations in humoral immunity to live measles virus vaccine is of great interest. Immunoglobulin GM (heavy chain) and KM (light chain) allotypes are genetic markers known to be associated with susceptibility to several infectious diseases. We assessed associations between GM and KM genotypes and measles vaccine humoral immunity (neutralizing antibody titers) in a combined cohort (n=1,796) of racially diverse healthy individuals (age 18–41 years). We did not discover any significant associations between GM and/or KM genotypes and measles vaccine-induced neutralizing antibody titers. African-American subjects had higher neutralizing antibody titers than Caucasians (1,260 mIU/mL vs. 740 mIU/mL, $p=7.10 \times 10^{-13}$), and those titers remained statistically significant ($p=1.68 \times 10^{-09}$) after adjusting for age at enrollment and time since last vaccination. There were no statistically significant sex-specific differences in measles-induced neutralizing antibody titers in our study ($p=0.375$). Our data indicate a surprising lack of evidence for an association between GM and KM genotypes and measles-specific neutralizing antibody titers, despite the importance of these immune response genes.

Keywords

Measles; Measles Vaccine; Measles virus; Measles-Mumps-Rubella Vaccine; Immunoglobulins; Genetic Association Studies; Antibodies; Neutralizing; Immunoglobulin Allotypes; Immunoglobulin Gm Allotypes; Immunoglobulin Km Allotypes

1. Introduction

Humoral immune responses [neutralizing antibodies, e.g., virus-specific immunoglobulin G (IgG) isotype antibodies] to measles vaccine play a protective role against wild virus

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Conflict of Interest statement

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., Avianax, Dynavax, Novartis Vaccines and Therapeutics, Adjuvance Technologies, Seqirus, and Protein Sciences. Drs. Poland and Ovsyannikova hold three patents related to measles and vaccinia peptide research. 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.

disease; however, the host genetic determinants of humoral immune responses are not well known. In our study of 100 sets of twins, we determined that the heritability of measles vaccine-specific humoral immunity was 88.5% [1]. Gene polymorphisms of the HLA, cytokine, innate and viral receptor, and other gene SNPs account for ~30% of the inter-individual variation in measles vaccine-specific humoral immunity [2], suggesting that additional genetic determinants influence humoral immunity to measles vaccine.

Immunoglobulin GM and KM allotypes are genetic elements of IgG heavy (3) an333-type light chains on chromosomes 14 and 2, respectively. GM and KM frequencies vary between different ethnic groups and may be involved in the pathogenesis of human immunodeficiency virus (HIV), hepatitis C virus (HCV), *Haemophilus influenzae* type b (Hib), malaria, and *Neisseria meningitidis* [3–6]. These highly polymorphic genetic markers have been demonstrated to be linked with immune responses to a variety of self and non-self antigens, including polysaccharide vaccines [7]. A study by Pandey *et al.* demonstrated that GM allotypes influence serum concentrations of IgG subclasses (IgG1,2,3,4) in Caucasian and African-American cohorts [8, 9].

The purpose of this study was to investigate whether GM and KM allotypes play a role in antibody responses to measles virus (MV) vaccine, and whether GM and/or KM alleles are significantly associated with MV neutralizing antibody titers after measles vaccination, across both race and gender.

2. Material and methods

Study Subjects

Two independent cohorts of study subjects, previously described in detail [10, 11], were used in this study. In total, 1,796 healthy individuals (with proven measles vaccine-induced immunity), ranging in age from 18 to 41 years, from two cohorts (San Diego cohort, n=844; US cohort, n=952) were included in this study (Table 1). There were 320 (17.8%) females and 1,476 (82.2%) males. Most subjects were Caucasian (85.3%). The average age at enrollment was 25 years, and the average time from last vaccination to enrollment was 1.8 years. Written informed consent was obtained from each subject. The Institutional Review Board of the Mayo Clinic (Rochester, MN) approved the study and informed consent was obtained from all subjects.

Measles Virus Neutralizing Antibody Testing

Methodology used for MV antibody testing is identical to what we have previously published [12]. MV-induced neutralizing antibodies were measured by a fluorescence-based plaque reduction microneutralization assay (PRMN) using a recombinant, GFP-expressing MV, as previously described [12]. The variability of the assay, as measured by its coefficient of variation that is built on log-transformed ND50 (neutralizing dose 50%) measurements of the third WHO international anti-measles standard, was 5.7%.

GM and KM Typing

DNA samples were genotyped for several immunoglobulin GM (18 testable GM specificities) and KM (three KM specificities) alleles by TaqMan, direct DNA sequencing, and by using PCR-RFLP methods that have been previously described [4, 13].

Analysis Strategy

We conducted analyses with the data combined across San Diego and US cohorts to increase power. Ancestry was determined by clustering subjects based on their SNPs from a prior genome wide association study (GWAS) [14]. We analyzed the association of GM/KM alleles with antibody titers in the combined pool of both ancestries, as well as within subsets of ancestries. To facilitate this process, we first adjusted for the effects of potential confounders and population-stratification eigenvectors within cohort-race strata, by using linear regression, as previously described; [15] this produced adjusted traits that were then used as our new endpoints. To analyze the Caucasian and African-American aggregate, we constructed linear regression models that adjusted for cohort indicator, ancestry indicator, dose of minor allele, and interaction of ancestry with dose of minor allele. These full models were compared with reduced models that included a cohort indicator and ancestry indicator. The full and reduced models were used to create likelihood ratio tests for the effect of an allele, allowing for the possible interaction of the allele with ancestry. Subset analyses for Caucasians and African-Americans were analyzed in a similar fashion; however, their full and reduced models differed only by an adjustment for cohort. To explore differences in KM/GM alleles by race, ordinal logistic regression was used with count of the minor allele as the endpoint. These models were adjusted for cohort.

3. Results

There were 264 African-Americans and 1,532 Caucasians used for analyses in the combined US and San Diego cohorts (Table 1). We found evidence for racial differences in MV-specific neutralizing antibody titers following measles vaccine. African-American subjects had significantly higher neutralizing antibody titers than Caucasian subjects (1,260 [578; 2,649] mIU/mL vs. 740 [347; 1,485] mIU/mL; $p=7.10 \times 10^{-13}$), and those titers remained statistically significant ($p=1.68 \times 10^{-9}$) after correcting this analysis for age at enrollment and time since last vaccination. Table 2 demonstrates the distribution of GM and KM allotypes between African-American and Caucasian subjects and provides p-values that test differences in allele frequencies by race. The homozygous GM5 (74.2%) and GM17 (71.6%) alleles were most common in African-American subjects, while the vast majority of Caucasian subjects carried the homozygous KM3 (76.7%) allotype. There were no significant differences in MV neutralizing antibody titers between males and females in our study cohort ($p=0.375$).

The association of each GM and KM genotype with MV neutralizing antibody titers is summarized in Table 3. This table illustrates that the GM and KM genotypes show no statistical evidence of association with MV neutralizing antibody titers.

4. Discussion

The current lack of knowledge regarding genetic determinants of vaccine-induced humoral immunity is a critical barrier to understanding vaccine responses in the population, and inhibits the discovery of new correlates of vaccine immunogenicity (vaccine response biomarkers). Our previous population-based studies have demonstrated significant associations between genetic variants and inter-individual variations in humoral (antibody) responses to measles vaccination [16, 17]. Given the function of GM and KM genes in genetic control of IgG isotypes, and the association of GM and KM genotypes with susceptibility to diverse infectious diseases, the aim of this study was to reveal whether GM and KM genotypes are associated with antibody responses following measles vaccination.

We found some evidence for racial differences in MV-specific neutralizing antibody titers. In our study, African-American subjects had a higher frequency of the individual GM (5 and 17) allotypes and exhibited significantly higher (1.7-fold) measles antibody titers than Caucasians. The causes for these racial differences are not well understood. This may be linked to higher IgG concentrations in African-Americans compared to Caucasians, which have been observed previously [18]. Differences in vaccine-induced antibody responses have also been described between Caucasians and African-Americans for other vaccines [19, 20].

We did not find evidence that antibody responses to measles vaccine are sex-dependent. Further investigation with a larger sample size may be warranted to explore this topic. None of the GM and KM allotypes were associated with MV-specific neutralizing antibody titers. This may be related to, in part, our measuring of functional neutralizing antibody titers (that protect against measles), but not circulating IgG isotypes in human sera. Given the relevance of GM and KM allotypes in immune responses to several pathogens and their potential role in humoral immunity [3–6], it is surprising we could not find an association between GM and KM genotypes and measles-specific neutralizing antibodies. Moreover, GWAS data with all imputed SNPs were also available on these study subjects; however, we found no genome-wide significant associations between immunoglobulin GM and KM allotypes with measles neutralizing antibody titers. The reasons for the limited effects of the GM and KM allotypes on humoral immune response to MV are not clear. Additional studies including GM and KM allotypes and vaccine-induced immunity are warranted. This is the first study to examine the potential role of GM and KM gene polymorphisms and humoral immune responses to measles vaccine in healthy individuals. This study has several strengths: we use a well-characterized vaccine (MMR, Merck); we have well-defined study cohorts (total n=1,796 individuals); we have access to demographic information; and we use a large sample size. Limitations include an underrepresentation of African-American and female subjects, which limits our ability to learn about these subgroups. Further studies may be warranted given the paucity of information regarding the interplay between GM/KM allotypes and haplotypes, and measles-induced immune responses in various ancestral groups. Information such as this is important to the science as it informs other hypotheses regarding genetic regulation of immune responses, shares such information with other scientists, and adds to the knowledge base of science.

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Table 1

Demographic and immune characteristics of the study subjects.

	African- American (N ^d =264)	Caucasian (N ^d =1,532)	Total (N ^d =1,796)	P-Value
Neutralizing Antibody Titer (mIU/mL)^a				<0.001
Mean (SD) ^b	2,111 (2,933)	1,243 (1,851)	1,371 (2,068)	
Q1, Q3 ^c	578; 2,649	347; 1,485	373; 1,613	
Median	1,260	740	811	
Range	66 – 26,929	18 – 39,153	18 – 39,153	
Sex				0.297
Female (n=320)	53 (20.1%)	267 (17.4%)	320 (17.8%)	
Male (n=1,476)	211 (79.9%)	1,265 (82.6%)	1,476 (82.2%)	
Cohort				<0.001
San Diego	159 (60.2%)	685 (44.7%)	844 (47.0%)	
US	105 (39.8%)	847 (55.3%)	952 (53.0%)	
Ethnicity				<0.001
Hispanic/Latino	9 (3.41%)	345 (22.5%)	354 (19.7%)	
Not Hispanic/Latino	237 (89.8%)	1,163 (75.9%)	1,400 (78.0%)	
Unknown	18 (6.82%)	24 (1.57%)	42 (2.34%)	
Age at Enrollment (years)				0.051
Number missing	48	188	236	
Mean (SD) ^b	26 (4.98)	25.1 (4.21)	25.2 (4.33)	
Q1, Q3 ^c	22; 28	22; 27	22; 27	
Median	24	24	24	
Range	20 – 41	19 – 41	19 – 41	
Time From Last Vaccination to Enrollment (years)				<0.001
Number missing	102	546	648	
Mean (SD) ^b	2.49 (2.38)	1.7 (2.04)	1.81 (2.11)	
Q1, Q3 ^c	0.025; 3.6	0.011; 3.03	0.011; 3.11	
Median	2.16	0.557	1.3	
Range	0.003 – 8.24	0 – 8.64	0 – 8.64	

^aNeutralizing antibody titer (mIU/mL), measured by measles virus plaque reduction microneutralization assays (PRMN);^bStandard deviation;^cQ1, first quartile, Q3, third quartile;^dN, number of subjects/assays.

Table 2

Distribution of GM and KM allotypes in study subjects.

Genotype	African-American (N ^b =264)	Caucasian (N ^b =1,532)	Total (N ^b =1,796)
G3M (IgG3 markers)			
N-Missing	0	8	8
GM5/5	196 (74.2%)	690 (45.3%)	886 (49.6%)
GM5/21	58 (22%)	637 (41.8%)	695 (38.9%)
GM21/21	10 (3.79%)	197 (12.9%)	207 (11.6%)
P-Value ^a			1.7×10^{-17}
G1M (IgG1 markers)			
GM3/3	13 (4.92%)	613 (40%)	626 (34.9%)
GM3/17	62 (23.5%)	673 (43.9%)	735 (40.9%)
GM17/17	189 (71.6%)	246 (16.1%)	435 (24.2%)
P-Value ^a			2.9×10^{-64}
G2M (IgG2 markers)^c			
N-Missing	0	1	1
GM--	213 (80.7%)	552 (36.1%)	765 (42.6%)
GM-/23	41 (15.5%)	665 (43.4%)	706 (39.3%)
GM23/23	10 (3.79%)	314 (20.5%)	324 (18.1%)
P-Value ^a			3.9×10^{-33}
KM			
N-Missing	0	2	2
KM3/3	140 (53%)	1,174 (76.7%)	1,314 (73.2%)
KM1/3	108 (40.9%)	315 (20.6%)	423 (23.6%)
KM1/1	16 (6.06%)	41 (2.68%)	57 (3.18%)
P-Value ^a			9.1×10^{-15}

^aP-values are from ordinal logistic regression of allotypes on race category adjusting for cohort membership.^bN, number of subjects/assays.^cAllotypes are either present (GM23) or not present (-).

Table 3

GM and KM allelic associations with measles virus-induced antibody titers in study subjects.

Genotypes	Median Neutralizing Antibody Titer (Q1, Q3), mIU/mL African-American (N ^b =264)	Median Neutralizing Antibody Titer (Q1, Q3), mIU/mL Caucasian (N ^b =1,532)	Median Neutralizing Antibody Titer (Q1, Q3), mIU/mL Overall (N ^b =1,796)
G3M (IgG3)			
GM5/5	1,282 (577.2; 2,811)	818.5 (362.2; 1,596)	898.5 (399.2; 1,769)
GM5/21	1,294 (670.2; 1,992)	690 (328; 1,395)	731 (340; 1,464)
GM21/21	1,031 (552.5; 1,767)	723 (371; 1,400)	728 (382.5; 1,408)
P-Value ^a	0.427	0.489	0.567
G1M (IgG1)			
GM3/3	714 (619; 3,159)	770 (349; 1,611)	766 (353.2; 1,624)
GM3/17	1,138 (554.2; 2,362)	701 (333; 1,417)	743 (344; 1,490)
GM17/17	1,330 (578; 2,767)	797.5 (375.2; 1,411)	967 (436; 1,808)
P-Value ^a	0.274	0.737	0.515
G2M (IgG2) ^c			
GM-/–	1,287 (580; 2,767)	753.5 (376; 1,378)	885 (414; 1,612)
GM-/23	1,164 (533; 2,396)	713 (340; 1,566)	750.5 (344.2; 1,612)
GM23/23	724.5 (454.8; 1,742)	727 (316.5; 1,632)	727 (319.5; 1,640)
P-Value ^a	0.317	0.855	0.585
KM			
KM3/3	1,173 (676.5; 2,690)	732.5 (347.2; 1,494)	810 (364; 1,598)
KM1/3	1,336 (558; 2,811)	751 (345; 1,400)	819 (385; 1,652)
KM1/1	723 (479; 2,022)	747 (401; 1,630)	747 (401; 1,695)
P-Value ^a	0.255	0.642	0.472

^aP-values from ordinal logistic regression of allotypes on race adjusted for cohort. Q1, first quartile, Q3, third quartile;

^bN, number of subjects/assays,

^cAllotypes are either present (GM23) or not present (-).