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Genetic polymorphisms in host antiviral genes: Associations with humoral and cellular immunity to measles vaccine

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ABSTRACT

Host antiviral genes are important regulators of antiviral immunity and plausible genetic determinants of immune response heterogeneity after vaccination. We genotyped and analyzed 307 common candidate tagSNPs from 12 antiviral genes in a cohort of 745 schoolchildren immunized with two doses of measles–mumps–rubella (MMR) vaccine. Associations between SNPs/haplotypes and measles virus-specific immune outcomes were assessed using linear regression methodologies in Caucasians and African-Americans.

Genetic variants within the *DDX58*/RIG-I gene, including a coding polymorphism (rs3205166/Val800Val), were associated as single-SNPs ($p \leq 0.017$; although these SNPs did not remain significant after correction for false discovery rate/FDR) and in haplotype-level analysis, with measles-specific antibody variations in Caucasians (haplotype allele p-value = 0.021; haplotype global p-value = 0.076). Four *DDX58* polymorphisms, in high LD, demonstrated also associations (after correction for FDR) with variations in both measles-specific IFN-γ and IL-2 secretion in Caucasians ($p \leq 0.001$, $q = 0.193$). Two intronic *OAS1* polymorphisms, including the functional *OAS1* SNP rs10774671 ($p = 0.003$), demonstrated evidence of association with a significant allele-dose-related increase in neutralizing antibody levels in African-Americans. Genotype and haplotype-level associations demonstrated the role of *ADAR* genetic variants, including a non-synonymous SNP (rs2229857/Arg384Lys; $p = 0.01$), in regulating measles virus-specific IFN-γ Elispot responses in Caucasians (haplotype global p-value = 0.017). After correction for FDR, 15 single-SNP associations (11 SNPs in Caucasians and 4 SNPs in African-Americans) still remained significant at the q-value < 0.20.

In conclusion, our findings strongly point to genetic variants/genes, involved in antiviral sensing and antiviral control, as critical determinants, differentially modulating the adaptive immune responses to live attenuated measles vaccine in Caucasians and African-Americans.

1. Introduction

The attenuated measles vaccine offered the opportunity to considerably reduce measles-related morbidity and mortality. However, measles is still a public health issue with approximately 164,000 deaths in 2008 worldwide [1] and with a relative increase in reported cases in several European countries and in the United States, including cases in individuals after two doses of MMR vaccine [2–6]. Therefore, establishing effective measles immunity across the population is crucial and requires providing both immunization coverage and surveillance to detect new cases and outbreaks [7]. Despite measles control (but not eradication), questions still remain regarding the documented 2–10% primary failure rate after vaccination, and the immunogenetic causes of vaccine response heterogeneity and/or vaccine failure. Deeper understanding of the observed inter-individual differences in immune response to measles vaccine is needed to provide new insights for improving vaccine-induced humoral, cellular, and innate immune responses, and for designing better vaccines [8,9].

The first line of defense, innate immunity, orchestrates host responses to prevent or reduce viral replication and spread until the adaptive immune system is switched on to eliminate the specific invading pathogen. Cellular viral sensors, type I and III interferons (IFNs, IFNα/β, IFN λ1, IFN λ2, IFN λ3) and IFN-triggered

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antiviral proteins have long been recognized as crucial mediators of innate antiviral defense with important effects on the magnitude and quality of innate and adaptive immune responses. Important antiviral factors and pathways, such as the retinoic acid-inducible gene I protein (*RIG-I*/*DDX58*), melanoma differentiation associated protein-5 (*MDA-5*/*IFIH1*), mitochondrial antiviral signaling protein (*MAVS*/*VISA*/*IPS1*/*CARDIF*), dsRNA-activated protein kinase R (*PKR*/*EIF2AK2*), the 2′–5′-oligoadenylate-synthetase (*OAS*) – ribonuclease L (*RNASEL*) pathway, Mx protein GTPases, adenosine deaminase, RNA-specific 1(*ADAR*), the *ISG15* ubiquitin-like pathway and others play a role in viral sensing, control, pathogenesis and outcome of viral infections [10–13]. Polymorphisms in IFN-stimulated antiviral effector genes and cellular viral sensors are potential genetic determinants of immune response heterogeneity that may influence the immune responses to viral vaccines by altering the functionality and antiviral effects of the corresponding proteins. Genetic variants in these genes have been implicated as important regulators of immunity and host response to infection [14–24].

Bearing in mind the multifaceted interactions between viruses and factors of the innate immune system, our study sought to investigate for the first time the role of key antiviral effector proteins and cellular antiviral sensors as plausible contributors to immune response heterogeneity to live attenuated measles vaccine. For this reason, we performed a comprehensive candidate gene association study in a large racially diverse cohort of healthy schoolchildren after two doses of MMR vaccine. Our results suggest that multiple innate immunity genetic variants/genes are likely involved in modulating the adaptive immune responses to live attenuated measles vaccine in Caucasians and African-Americans.

2. Materials and methods

2.1. Study subjects and demographics

Our study cohort consisted of 2 independent age-stratified samples of randomly recruited healthy schoolchildren and young adults from Olmsted County, Minnesota, as described previously [25,26]. Briefly, cohort 1, enrolled in 2006–2007, comprised a sample of 440 subjects (age 11–19 years), from which 388 were eligible for the current study [14–16]. Cohort 2, enrolled in 2008–2009 year, consisted of 376 eligible subjects (age 11–22 years). All study participants resided in a community where no cases of measles infection had been reported during their lifetimes and all had received two doses of MMR-II vaccine (Merck, Whitehouse Station, N.J.), containing the Edmonston strain of measles virus ($TCID_{50} \geq 1000$). The Mayo Clinic Institutional Review Board granted approval for the study. Written, informed consent was obtained from parents/guardians as well as written assent from age-appropriate subjects at the time of enrollment in the study.

The demographic and clinical variables of the study cohort have been described previously [25,26]. Briefly, the study cohort ($n = 745$ subjects) used in our genetic association study, consisted of 417 males (55.97%), and 328 females (44.03%). The two most prevalent racial groups used were Caucasians (598, 80.27%) and African-Americans (89, 11.95%). Most of the study subjects were non-Hispanics (723, 97.05%). The median age at enrollment was 15 years (inter-quartile range/IQR 13; 17) and the median age at first and second immunization was 15 months (IQR 15; 16) and 5 years (IQR 4; 11), respectively. The median time from second immunization to enrollment and immunity measurements was 7.4 years (IQR 5.6; 9.2).

2.2. Immune measures

2.2.1. Plaque Reduction Microneutralization Assay (PRMN)

We measured neutralizing antibodies to measles virus using a high throughput fluorescence-based PRMN, as previously described [27], with the following modifications in the readout. We used an automated Olympus IX71 Fluorescent microscope system with Image-Pro Plus Software Version 6.3 (MediaCybernetics) to scan and count GFP-positive plaques [25]. Calculations of 50% end point titer (Neutralizing Dose, ND_{50}) were performed using Karber's formula and ND_{50} values were transformed into mIU/mL values using the 3rd WHO international anti-measles antibody standard (NIBSC code no. 97/648) as previously described [27].

2.2.2. Elispot

Human total IFN-γ Elispot kits (R&D Systems, Minneapolis, MN) were used to measure the number of IFNγ-producing cells, as previously described [28] following the manufacturer's protocol. Briefly, we stimulated subjects' peripheral blood mononuclear cells/PBMCs (or alternatively left them unstimulated), in triplicate, with the Edmonston strain of measles virus (multiplicity of infection, MOI = 0.5) and developed the reaction after 42 h incubation at 37 °C, in 5% CO_2. Phytohaemagglutinin/PHA (5 μg/mL) was used as a positive control. All plates were scanned and analyzed using the same counting parameters on an ImmunoSpot® S4 Pro Analyzer (Cellular Technology Ltd., Cleveland, OH, USA) using ImmunoSpot® version 4.0 software (Cellular Technology Ltd.).

2.2.3. ELISA

Secreted cytokines (Th1: IL-2, IFN-γ; Th2: IL-10; and innate/inflammatory: IL-6, TNF-α, IFN-α, IFN-λ1) were quantified in PBMC cultures after in vitro stimulation with live measles virus (stimulated and unstimulated in five replicate measurements), using pre-optimized conditions for MOI and incubation time for each cytokine, as previously described [29,30]. The different cytokines are measured using the following conditions: IFN-α and TNF-α MOI = 1.0, 24 h; IL-2 and IL-10 MOI = 0.5, 48 h; IL-6, IFN-γ and IFN-λ1 MOI = 1.0, 72 h. Secreted cytokine levels were measured with commercial kits according to manufacturers' recommendations (R&D Systems, Minneapolis, MN, for IFN-λ1; Mabtech, Cincinnati, OH, for IFNα, and BD Biosciences Pharmingen, San Diego, CA for the rest of the cytokines).

2.3. SNP selection and genotyping of SNPs in antiviral genes

The SNP selection approach and genotyping methodology have been published for our previous genetic association studies [14–16,26]. Briefly, we used the tagSNP selection approach based on linkage disequilibrium (LD) [31] to select SNPs from each of 12 antiviral genes, including the regions 5 kb upstream and downstream for each gene, from the source with the greatest number of SNPs from among the Hapmap Phase II (http://www.hapmap.org), Seattle SNPs (http://pga.mbt.washington.edu/) and NIEHS SNPs (http://egp.gs.washington.edu/). We binned SNPs with a minor allele frequency (MAF) ≥ 0.05, a pairwise LD threshold of $r^2 \geq 0.90$, and successful Illumina predictive genotyping scores, for the Caucasian sample and African-American sample separately, using the ldSelect program [31]. To accommodate a set of Illumina platform constraints, the SNP list was further post-processed and refined using the SNPPicker program. We also added a set of functional "obligate" SNPs (coding: nonsynonymous, synonymous, 5′ or 3′ untranslated regions) to the final list. A total of 348 SNPs across 12 antiviral genes were selected based on this approach. The nomenclature used for the description of the polymorphisms follows that described by den Dunnen and Antonarakis [32].

We genotyped the selected 348 SNPs using two custom Illumina GoldenGate SNP panels (Illumina Inc., San Diego, CA) for 1536 and 768 SNPs, which were used for genotyping all candidate SNPs from a large population-based measles vaccine study. All DNA samples ($n = 764$) were genotyped following the manufacturer's protocol and as previously described [14].

Seven hundred and sixty four ($n = 764$) subjects were initially genotyped for 348 known SNPs in 12 antiviral genes with a documented role in antiviral immunity (*DDX58/RIG-I*, *MAVS/VISA*, *IFIH1/MDA5*, *MX1*, *MX2*, *OAS1*, *OAS2*, *OAS3*, *PKR/EIF2AK2*, *RNASEL*, *ADAR* and *ISG15*). The genotyping locus success rate was 94.55%, sample success rate was 98.75% (including replicate samples), and reproducibility was 100%. Due to insufficient/inadequte DNA quality, genotyping failure or low call rates, nineteen DNA samples failed, leaving 745 subjects for further analysis. Of the 348 SNPs included for genotyping, eighteen SNPs failed the initial quality control because of failure to amplify, poor clustering or multiple replicate errors. 330 SNPs (95.12%) yielded genotyping data. Additional SNPs were also excluded from analysis based on low MAF < 0.05 ($n = 20$) or based on being monomorphic ($n = 3$). A total of 307 SNPs were used for the final race-specific analysis in 598 Caucasians and 89 African-Americans.

2.4. Statistical methods

The statistical methods described herein are similar to those published for our previous genetic association studies [14–16,26]. Data were descriptively summarized using frequencies and percentages for categorical variables, and medians and inter-quartile ranges for continuous variables. Assessments of cytokine secretion and cell-mediated immunity (CMI) measures resulted in multiple recorded values per outcome both prior to and after stimulation with measles virus. For descriptive purposes, a single response measurement per individual was obtained for each of these outcomes by subtracting the median of the unstimulated values from the median of the stimulated values. Assessments of antibody levels resulted in only one recorded value per individual.

Observed genotypes were used to estimate allele frequencies for each SNP and departures from Hardy-Weinberg equilibrium (HWE) were assessed using either a Pearson goodness-of-fit test or, for SNPs with a minor allele frequency (MAF) of less than 5%, a Fisher exact test [33]. Estimates of pair-wise linkage disequilibrium (LD) based on the r-squared statistic were obtained using Haploview software, version 3.32 [34].

SNP associations with immune response outcomes were evaluated using regression models. Simple linear regression was used for measles antibody levels, for which we had only one measured value per individual. Repeated measures approaches were implemented for the cytokine secretion and IFN-γ Elispot variables, simultaneously modeling the multiple observed measurements and using an unstructured within-person variance–covariance matrix to account for within-subject correlations. This was achieved by including the genotype variable in the regression model, together with a variable representing stimulation status. The resulting genotype-by-stimulation status interaction was then tested for statistical significance. Tests of association assumed an ordinal (log-additive) SNP effect using simple tests for trend. Because of the lack of seronegative subjects (PRMN titer < 1:8) in our cohort and the low number of subjects (68) with antibody concentrations less than the protective threshold of 210 mIU/mL (corresponding to PRMN titer of 120) [25], our statistical approach rationale was to assess associations between SNPs and quantitative humoral/cellular immune outcomes across the entire cohort using linear regression methodologies rather than assess the distribution of SNPs across extremes of humoral/cellular immune responses (such as seronegative subjects and/or subjects with antibody concentrations less than the protective titer).

To further explore genomic regions containing statistically significant single-SNP effects, we performed post hoc haplotype analyses. Posterior probabilities of all possible haplotypes for an individual, conditional on the observed genotypes, were estimated using an expectation–maximization (EM) algorithm [35]. This information was used to define haplotype design variables that estimated the number of each of the haplotypes carried by an individual. Analyses were performed on all resulting common haplotypes (those with an estimated frequency of >1%) using simple least squares linear regression approach for antibody levels and repeated measures approaches for the CMI variables. Differences in immune response among common haplotypes were first assessed globally and simultaneously tested for statistical significance using a multiple degree-of-freedom test. Following these global tests, we examined individual haplotype effects. Each haplotype was included in a separate regression analysis, effectively comparing immune response levels for the haplotype of interest against all others combined. Due to phase ambiguity, haplotype-specific medians and inter-quartile ranges could not be calculated. Thus, descriptive summaries were represented using the corresponding t-statistics.

All of the association analyses described above were separately performed within the two most prevalent racial groups (Caucasians and African-Americans). Each analysis also adjusted for gender, age at first measles vaccination, age at second measles vaccination, age at enrollment (thus accounting for the time since last vaccination to enrollment/blood draw and immunity measurements), and cohort status (cohort 1 vs. cohort 2). We used an inverse normal transformation for all cytokine secretion and IFN-γ Elispot outcome variables, and a log transformation for the antibody response measure, in all formal tests to account for the skewed nature of the data. Because of the large number of statistical tests, we computed q-values for each SNP to estimate the corresponding false discovery rates (FDR) as per Storey and Tibshirani [36,37]. We considered SNPs as being significant if their q-value was less than 0.2 [38]. However, we also reported single-SNP associations as being of interest for further follow-up if the q-value was less than 0.5. This corresponded to single-SNP p-values that were roughly ≤ 0.01. All statistical tests were two-sided and, unless otherwise indicated, all analyses were carried out using the SAS software system (SAS Institute, Inc., Cary, N.C.).

3. Results

3.1. Immune responses for the study cohort

The immune characteristics of the study cohort are summarized in Table 1. The median level of measles-specific neutralizing antibodies (humoral immunity) for the study cohort was 846 mIU/mL (inter-quartile range/IQR: 418, 1772) and the median IFN-γ Elispot response (cellular immunity) was 36 IFN-γ-positive spots per 2×10^5 cells (IQR: 12, 69). The median measles-specific IFN-γ secretion level for the cohort was 67 pg/mL (IQR: 35, 120), the median measles-specific IL-2 secretion level was 38 pg/mL (IQR: 21, 64), and the median measles-specific IL-10 secretion level was 18 pg/mL (IQR: 11, 28) [26].

3.2. Genetic associations

3.2.1. Associations between SNPs in antiviral genes and humoral immune responses after measles vaccination

Race-specific analysis in the Caucasian group identified two significant associations between SNPs located in antiviral genes and neutralizing antibody levels after measles vaccination ($p \leq 0.01$), as well as one suggestive SNP association with $p = 0.017$ (Table 2). An intronic SNP in the *DDX58* (RIG-I) gene (rs11795343) was associated with an allele dose-related increase (19%, calculated as the ratio, minus one, of the immune outcome observed for the homozygous minor allele genotype to the immune outcome observed for the homozygous major allele genotype, multiplied

Table 1
Immune characteristics of the study cohort.

Response type	Cytokine	No. of subjects	Median response (IQR)[a]	No. subjects positive (%)[b]
Neutralizing antibody	–	744	846 (418; 1 772)	686 (92.2)
Th1	IL-2	739	38 (21; 64)	724 (98.0)
	IFNγ	737	67 (35; 120)	716 (97.2)
Th2	IL-10	740	18 (11; 28)	726 (98.1)
Innate/inflammatory	IL-6	737	355 (248; 461)	726 (98.5)
	TNFα	732	14 (9; 19)	722 (98.6)
	IFNα	734	551 (273; 1 025)	729 (99.3)
	IFNλ1	738	34 (14; 73)	666 (90.2)
Elispot	IFNγ	707	36 (12; 69)	676 (95.6)

[a] Humoral response is defined as the measles-specific mIU/mL titer in PRMN assay. Cytokine response is defined as the subject-specific median measles-stimulated response (measured in five replicates) minus the median unstimulated response (also measured in five replicates). Elispot response is defined as the subject-specific median measles-stimulated response (measured in triplicate) minus the median unstimulated response (also measured in triplicate). Values reported are in mIU/mL for antibody responses, pg/mL for secreted cytokines, and cytokine-positive spots per 2×10^5 cells for Elispot responses ± IQR, inter-quartile range.

[b] For antibody responses subjects reported as positive are with positive protective titers higher than the protective threshold of 210 mIU/mL (corresponding to ND_{50} 120). Subjects are considered to have a positive cytokine response if the median of the stimulated cells is larger than the median of the unstimulated cells.

by 100%) in antibody levels ($p = 0.01$). This intronic SNP was found to be in LD ($r^2 = 0.64$) with a synonymous *DDX58* SNP (rs3205166/Val800Val), which also demonstrated an allele dose relationship with higher neutralizing antibody levels (approximately 40% increase in antibodies), although this association did not reach statistical significance at $p \leq 0.01$ ($p = 0.017$, Table 2). In addition, eight more *DDX58* SNPs displayed suggestive associations with antibody levels in this racial group ($p < 0.05$, data not shown). An intronic genetic variant within the *VISA* gene demonstrated an association with antibody levels in the Caucasian group ($p = 0.003$).

The analysis restricted to the African-American subjects revealed three significant intronic SNP associations with measles-specific humoral immune response ($p \leq 0.01$, Table 2). Importantly, this analysis identified an association for a functional genetic variant rs10774671 located in intron 5 of *OAS1*, at the splice-acceptor site [39]. The minor alleles of the two *OAS1* intronic SNPs, rs10774671 and rs10744785, were associated with a significant increase (more than a 3-fold increase for rs10774671 and 4.9-fold increase for rs10744785) in measles neutralizing antibodies in an allele dose-related manner ($p \leq 0.003$, Table 2).

However, after adjusting for false discovery rate (FDR), none of the identified SNPs listed in Table 2 remained significant at the q-value = 0.20 [38], although all reported SNP associations demonstrated q-values < 0.5 and two SNP associations demonstrated q-values < 0.25.

3.2.2. Associations between SNPs in antiviral genes and cellular immune responses (INF-γ Elispot) after measles vaccination

Race-specific analysis in the Caucasian group revealed six significant associations between genetic variants in two antiviral genes (*OAS3* and *ADAR*) and measles-specific IFN-γ Elispot responses ($p \leq 0.01$, Table 3). Four *ADAR* SNPs in high LD ($r^2 \geq 0.96$), including a non-synonymous *ADAR* SNP (rs2229857/Lys384Arg), demonstrated an allele dose-related increase (29% for rs2229857) in IFN-γ Elispot responses in this racial group with the representation of the minor allele ($p \leq 0.01$, Table 3). Furthermore, we observed three additional *ADAR* SNPs, which demonstrated suggestive associations

Table 2
SNPs in antiviral genes associated with measles virus-specific neutralizing antibody responses.

Gene	SNP ID	Location/function	Geno type[a]	*N* (%)[a]	Median Ab level mIU/mL (IQR)[b]	*p*-Value[c]	*q*-Value[d]
Analysis in Caucasians							
VISA	rs6037678	Intron	CC	558 (93)	804 (409; 1 718)	0.003	0.23
			CG	40 (7)	1 419 (880; 2 144)		
			GG	0 (0)	(–)		
DDX58	**rs11795343**	Intron	AA	203 (34)	**759** (389; 1 528)	0.01	0.408
			AG	305 (51)	**898** (455; 1 806)		
			GG	89 (15)	**904** (497; 1 968)		
DDX58	**rs3205166**	Coding	AA	234 (39)	**797** (410; 1 726)	0.017	0.438
		Val800Val	AC	276 (46)	**839** (410; 1 711)		
			CC	87 (15)	**1 109** (551; 2 096)		
Analysis in African-Americans							
OAS1	**rs10744785**	Intron	GG	24 (27)	**398** (194; 637)	0.001	0.248
			GC	46 (52)	**857** (329; 2 042)		
			CC	19 (21)	**1 967** (918; 3 564)		
OAS1	**rs10774671**	Intron	GG	36 (40)	**492** (221; 1 138)	0.003	0.403
			GA	38 (43)	**857** (410; 2 228)		
			AA	15 (17)	**1 690** (918; 2 981)		
MX2	rs2838034	Intron	GG	60 (67)	681 (265; 1 948)	0.01	0.470
			GA	24 (27)	565 (283; 2 030)		
			AA	5 (6)	2 811 (2 042; 4 172)		

Bolded SNPs are associated with an allele dose-related increase (with the representation of the minor allele) in antibody levels.

[a] Values are presented as homozygous major allele/heterozygous/homozygous minor allele. *N* indicates the number of subjects with the specific genotype, % indicates genotype counts in percentages.

[b] Results are presented as median neutralizing antibody (Ab) levels in mIU/mL ± IQR (inter-quartile range), as measured by the fluorescence-based measles virus-specific PRMN assay.

[c] Ordinal *p*-value from the linear regression analysis adjusting for age at enrollment, gender, age at first and second immunization, and cohort status (cohort 1 vs. cohort 2).

[d] Corresponding *q*-values, adjusting for FDR.

Table 3
SNPs in antiviral genes associated with measles virus-specific IFN-γ Elispot responses.

Gene	SNP ID	Location/function	Geno type[a]	*N* (%)[a]	Median IFNγ Elispot (IQR)[b]	*p*-Value[c]	*q*-Value[d]
Analysis in Caucasians							
OAS3	**rs2072133**	3′UTR	AA	373 (66)	**36** (16; 70)		
			AG	170 (30)	**37** (11; 71)	0.007	0.328
			GG	19 (3)	**40** (10; 85)		
ADAR	rs1127317	3′UTR	AA	294 (52)	36 (12; 67)		
			AC	217 (39)	36 (16; 73)	0.009	0.387
			CC	52 (9)	45 (24; 89)		
ADAR	rs1127326	3′UTR	GG	293 (52)	36 (12; 67)		
			GA	217 (39)	36 (16; 73)	0.01	0.4
			AA	53 (9)	45 (22; 87)		
OAS3	rs12818640	3′ intergenic	AA	370 (66)	36 (16; 70)		
			AG	170 (30)	36 (11; 71)	0.01	0.408
			GG	18 (3)	33 (10; 85)		
ADAR	**rs2229857**	Coding	GG	291 (52)	**35** (11; 68)		
		Lys384Arg	GA	219 (39)	**36** (16; 73)	0.01	0.408
			AA	52 (9)	**45** (21; 87)		
ADAR	**rs3738029**	Intron	GG	292 (52)	**35** (11; 68)		
						0.01	0.408
			GA	219 (39)	**37** (16; 73)		
			AA	52 (9)	**45** (21; 87)		
Analysis in African-Americans							
EIF2AK2	**rs12712526**	Intron	AA	23 (26)	**30** (12; 61)		
			AG	43 (49)	**29** (5; 47)	0.001	0.248
			GG	21 (24)	**10** (2; 39)		
DDX58	rs7022323	Intron	AA	32 (37)	12 (3; 29)		
			AG	40 (46)	35 (5; 71)	0.004	0.447
			GG	15 (17)	33 (3; 49)		
MX2	**rs466092**	Intron	GG	39 (45)	**11** (2; 43)		
			GA	35 (40)	**27** (10; 51)	0.006	0.447
			AA	13 (15)	**30** (12; 43)		
OAS3	rs2072136	Coding	GG	71 (82)	26 (4; 49)		
		Ser567Ser	GA	15 (17)	10 (2; 47)	0.007	0.447
			AA	1 (1)	29 (29; 29)		
OAS2	rs11513733	Intron	AA	84 (97)	27 (4; 48)		
			AT	3 (3)	9 (0; 11)	0.009	0.454
			TT	0 (0)	(–)		

Bolded SNPs are associated with an allele dose-related increase/decrease in median IFN-γ Elispot responses.
[a] Values are presented as homozygous major allele/heterozygous/homozygous minor allele. *N* indicates the number of subjects with the specific genotype, % indicates genotype counts in percentages.
[b] Results are presented as median IFNγ-positive spots per 2×10^5 cells ± IQR (inter-quartile range), as measured by the total IFNγ Elispot assay.
[c] Ordinal *p*-value from the repeated measures linear regression analysis adjusting for age at enrollment, gender, age at first and second immunization, and cohort status.
[d] Corresponding *q*-values, adjusting for FDR.

with the immune outcome in the Caucasian group ($p < 0.05$, data not shown).

The analysis in the African-American group revealed five associations for SNPs belonging to five antiviral genes with measles-specific cellular immunity ($p \leq 0.009$, Table 3). A synonymous *OAS3* polymorphism (rs2072136/Ser567Ser) was found to be associated with IFN-γ Elispot responses in this racial group ($p = 0.007$, Table 3). The minor alleles of two intronic SNPs, *EIF2AK2* SNP rs12712526 ($p = 0.001$) and *MX2* SNP rs466092 ($p = 0.006$), demonstrated significant allele dose-related variations (decrease and increase, respectively) in the immune outcome (Table 3).

After adjusting for false discovery rate (FDR), none of the identified SNPs in Table 3 remained significant at the *q*-value = 0.20 [38], although all reported SNP associations demonstrated *q*-values < 0.5 and one SNP association demonstrated *q*-values < 0.25.

3.2.3. Associations between DDX58 and ADAR haplotypes and measles vaccine-induced immunity in Caucasians

To further explore genomic regions enriched with statistically significant ($p \leq 0.01$) and suggestive ($p < 0.05$, data not shown) single-SNP associations, we performed haplotype analyses for the *DDX58* and *ADAR* gene regions.

The haplotype level analysis revealed that genetic variants/haplotypes within the *DDX58* and *ADAR* genes (Haploview output presented in Fig. 1) were associated with measles-specific humoral and cellular immunity (IFN-γ Elispot), respectively, in Caucasians, but not in African-Americans (*DDX58* haplotype allele *p*-value = 0.021; *ADAR* global *p*-value = 0.017, Table 4). Particularly, the *DDX58* haplotype GGAAAAAAAA was associated with decreased antibody levels (haplotype *t*-statistic = −2.31; allele *p*-value = 0.021) compared to all other *DDX58* haplotypes, while the *ADAR* haplotype ATAATCGGGAAAGGAA was associated with increased IFN-γ Elispot response (haplotype *t*-statistic = 2.82; allele *p*-value = 0.005) in the Caucasian group (Table 4).

3.2.4. Associations between SNPs in antiviral genes and secreted cytokines after measles vaccination

We found 58 and 47 significant associations ($p \leq 0.01$) in the Caucasian and African-American group, respectively, between genetic variants in antiviral effector genes and measles-specific secretion of Th1, Th2 and innate/inflammatory cytokines, however most of the SNP associations (with cytokine secretion immune outcomes) did not meet the FDR threshold of 0.20 [38]. Analysis in the Caucasian group revealed seven significant SNP associations with measles-specific IFN-γ secretion, which remained significant at

Table 4
DDX58 and *ADAR* haplotype associations with measles-specific immune responses in Caucasians.

Haplotype	Frequency	Test statistic (haplotype *t*-statistic)[c]	Allele *p*-value[d]	Global *p*-value[d]
DDX58 haplotype associations with antibody levels				
ACAGGGAAAA[a]	0.295	−1.21	0.229	
ACAGGGAAAC[a]	0.016	0.41	0.680	
GGAAAAAAAA[a]	0.208	−2.31	**0.021**	0.076
GGAGGGAGGA[a]	0.092	0.51	0.609	
GGAGGGGAAC[a]	0.038	1.04	0.297	
GGGGGGGGGC[a]	0.305	1.59	0.113	
ADAR haplotype associations with IFN-γ Elispot responses				
ATAATCGGGAAAGGAA[b]	0.072	2.82	**0.005**	
GAAGAAAAGGGCAAAA[b]	0.031	1.00	0.320	
GAAGAAAAGGGCAAAG[b]	0.247	−0.97	0.331	
GAAGTAGAGGGAGGCA[b]	0.183	−1.43	0.153	**0.017**
GAGGTAAAGGGCAAAG[b]	0.210	0.04	0.971	
GTAATCGGAAAAGGAA[b]	0.203	1.36	0.173	
GTGGTAAGGGGAGGAA[b]	0.013	−1.62	0.106	

[a] *DDX58* genetic variants from left to right: rs7042042, rs10970985, rs10970986, rs12006123, rs12555727, rs17289116, rs7865082, rs10738889, rs10738890, and rs3205166 (all genotyped in the current study).
[b] *ADAR* genetic variants from left to right: rs3811450, rs6656743, rs9427092, rs1127326, rs9616, rs1127317, rs1127311, rs2131902, rs3738032, rs2229857, rs3738029, rs3766922, rs12125166, rs9427100, rs2335230, and rs884618 (all genotyped in the current study).
[c] Haplotype effects (for haplotypes with estimated frequencies ≥0.01) are calculated using the haplotype *t*-statistic, which shows the direction and magnitude of the estimated haplotypic effect on the immune measure.
[d] Allele *p*-values compare individual haplotypes to all other haplotypes combined; global *p*-values reflect differences in immune response among common haplotypes as assessed globally and tested for statistical significance using a multiple degree-of-freedom test. Statistically significant *p*-values for haplotype-level associations ($p < 0.05$) are highlighted in bold. Analyses are adjusted for age at enrollment, gender, age at first and second MMR immunization and cohort status (cohort 1 or cohort 2).

the *q*-value = 0.20 [38] after adjusting for FDR ($p \leq 0.001$, $q = 0.193$; Table 5). Two *MX2* intronic SNPs demonstrated an allele–dose relationship with lower IFN-γ secretion levels ($p \leq 0.001$). In addition, the minor alleles of four *DDX58* SNPs (in high LD; for rs12555727, 12006123 and rs17289116, $r^2 = 1$) were associated with a significant allele dose-related decrease (approximately 30%) in secreted IFN-γ in the Caucasian group (Table 5). Interestingly, the same four *DDX58* SNPs demonstrated a more than 2-fold reduction in measles-specific IL-2 secretion levels (with the representation of the minor allele) in the Caucasian group and still remained significant after adjusting for FDR ($p \leq 0.001$, $q = 0.193$, Table 6). Of note, three of the *DDX58* SNPs (rs12555727, 12006123 and rs17289116, $r^2 = 1$) were also found to be associated at the haplotype level with variations in measles-specific humoral immunity in Caucasians (Table 4).

The analysis in the African-American group revealed four significant SNP associations with measles-specific IL-10 secretion, which remained significant at the *q*-value = 0.20 [38] after adjusting for FDR ($p \leq 0.0003$, $q \leq 0.192$; Supplementary Table 1). In particular, the minor alleles of two SNPs in the 3′ intergenic region of the *MX1*

Table 5
SNPs in antiviral genes associated with measles virus-specific IFN-γ secretion in Caucasians.

Gene	SNP ID	Location/function	Geno type[a]	*N* (%)[a]	Median IFN-γ pg/mL (IQR)[b]	*p*-Value[c]	*q*-Value[d]
MX2	**rs442014**	Intron	AA	456 (77)	**71** (38; 126)		
			AG	131 (22)	**67** (29; 109)	0.0004	0.193
			GG	5 (1)	**14** (11; 38)		
DDX58	**rs12555727**	3′UTR	GG	363 (62)	**71** (37; 139)		
			GA	199 (34)	**70** (36; 107)	0.001	0.193
			AA	28 (5)	**50** (26; 96)		
DDX58	**rs12006123**	3′UTR	GG	364 (62)	**71** (37; 139)		
			GA	199 (34)	**70** (36; 107)	0.001	0.193
			AA	28 (5)	**50** (26; 96)		
MX2	**rs441437**	Intron	AA	432 (73)	**72** (38; 126)		
			AG	153 (26)	**61** (30; 109)	0.001	0.193
			GG	7 (1)	**38** (11; 161)		
DDX58	**rs17288914**	3′ Intergenic	AA	364 (61)	**71** (37; 139)		
			AG	199 (34)	**70** (36; 107)	0.001	0.193
			GG	29 (5)	**54** (27; 95)		
DDX58	**rs17289116**	Intron	GG	364 (61)	**71** (37; 139)		
			GA	199 (34)	**70** (36; 107)	0.001	0.193
			AA	29 (5)	**54** (27; 95)		
OAS1	rs4766662	Intron	CC	331 (56)	72 (39; 126)		
			CA	229 (39)	61 (32; 116)	0.001	0.193
			AA	31 (5)	88 (35; 144)		

Bolded SNPs are associated with an allele dose-related decrease in IFN-γ secretion levels. Only polymorphisms with $p \leq 0.01$ and $q < 0.2$ are presented in the table.
[a] Values are presented as homozygous major allele/heterozygous/homozygous minor allele. *N* indicates the number of subjects with the specific genotype, % indicates genotype counts in percentages.
[b] IQR, inter-quartile range, values are median levels of the cytokine in pg/mL as measured by ELISA.
[c] Ordinal *p*-value from the repeated measures linear regression analysis adjusting for age at enrollment, gender, age at first and second MMR immunization, and cohort status (cohort 1 or cohort 2).
[d] Corresponding *q*-values, adjusting for FDR.

Table 6
SNPs in antiviral genes associated with measles virus-specific IL-2 secretion in Caucasians.

Gene	SNP ID	Location/Function	Geno type[a]	*N* (%)[a]	Median IL-2 pg/mL (IQR)[b]	*p*-Value[c]	*q*-Value[d]
DDX58	**rs12555727**	3′UTR	GG	364 (61)	**44** (25; 71)		
			GA	200 (34)	**37** (20; 63)	0.0003	0.193
			AA	28 (5)	**21** (12; 42)		
DDX58	**rs12006123**	3′UTR	GG	365 (61)	**44** (25; 70)		
			GA	200 (34)	**37** (20; 63)	0.0003	0.193
			AA	28 (5)	**21** (12; 42)		
DDX58	**rs17288914**	3′ Intergenic	AA	365 (61)	**44** (25; 70)		
			AG	200 (34)	**37** (20; 63)	0.001	0.193
			GG	29 (5)	**21** (14; 43)		
DDX58	**rs17289116**	Intron	GG	365 (61)	**44** (25; 70)		
			GA	200 (34)	**37** (20; 63)	0.001	0.193
			AA	29 (5)	**21** (14; 43)		

Bolded SNPs are associated with an allele dose-related decrease in IL-2 secretion levels. Only polymorphisms with $p \leq 0.01$ and $q < 0.2$ are presented in the table.

[a] Values are presented as homozygous major allele/heterozygous/homozygous minor allele. *N* indicates the number of subjects with the specific genotype, % indicates genotype counts in percentages.

[b] IQR, inter-quartile range, values are median levels of the cytokine in pg/mL as measured by ELISA.

[c] Ordinal *p*-value from the repeated measures linear regression analysis adjusting for age at enrollment, gender, age at first and second MMR immunization, and cohort status (cohort 1 or cohort 2).

[d] Corresponding *q*-values, adjusting for FDR.

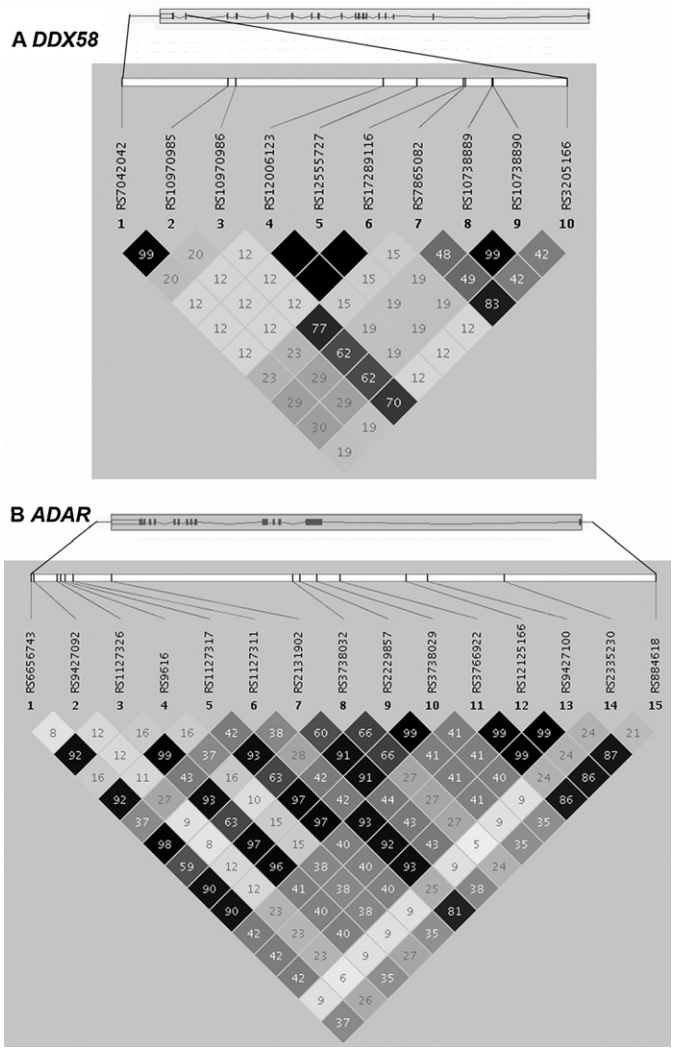

Fig. 1. Haplotype block structure of the *DDX58* and *ADAR* genetic variants in the study cohort (Caucasian sample). The schematic gene representation and LD block structure of (A) *DDX58* and (B) *ADAR* genetic variants is depicted. The LD block structure was analyzed using Haploview software, version 3.32. All SNPs presented in A and B were directly genotyped in the study. The r^2 color scheme is: white ($r^2 = 0$), shades of grey ($0 < r^2 < 1$), black ($r^2 = 1$). The numbers report the r^2 value multiplied by 100.

gene (in high LD) were associated with a significant increase in IL-10 secretion in an allele dose-related manner ($p = 0.0002$, $q = 0131$; Supplementary Table 1).

To summarize, we identified two *DDX58* and four *ADAR* genetic variants, as well as *DDX58* and *ADAR* haplotypes, associated with neutralizing antibody levels and measles-specific cellular immunity (IFN-γ Elispot), respectively, in our Caucasian group. Four *DDX58* polymorphisms (associated with neutralizing antibody variations at the haplotype level) demonstrated significant associations with variations in both IFN-γ and IL-2 secretion in Caucasians. Two *OAS1* genetic variants, including the functional *OAS1* SNP rs10774671, were associated with humoral immune response variations in our African-American group.

4. Discussion

Various genetic variants and genes have been implicated as important regulators of immunity to viral vaccines (including measles vaccine), such as cytokine and cytokine receptors, toll-like receptors (TLRs) and signaling molecules, viral receptors (such as CD46, SLAM and DC-SIGN for measles virus) and vitamin A and D receptors [15,16,26,38,40–47]. The objective of our population-based study was to comprehensively evaluate the influence of human genetic variation in key host antiviral sensor and antiviral effector genes on immune response heterogeneity after measles vaccination.

Genetic polymorphisms (SNPs) in genes related to innate immunity, such as the interferon β gene (*IFNB1*), type I IFN receptor genes *IFNAR1* and *IFNAR2*, the antiretroviral gene *TRIM5* and *TLRs* are known to be associated with variations in immune responses to measles and rubella vaccines [15,16,38,43,48]. We have previously demonstrated that specific variations in antiviral sensor genes (*DDX58*/*RIG-I* and *MAVS*/*VISA*) and key IFN-induced antiviral genes (particularly polymorphisms in the *OAS* gene cluster) were associated with differences in rubella vaccine-induced adaptive immunity [14–16], while little is known regarding the importance of these genes or variations in measles-specific immunity. Furthermore, multiple previous genetic association studies revealed that polymorphisms in the *MX1*, *OAS1*, *OASL* and *PKR* genes correlate with response to IFN therapy and/or susceptibility to viruses such as WNV, HCV, HBV, SARS-coV, and measles virus [17–24].

Our genetic data from single SNP and haplotype analyses point to the possible involvement of genetic variants within the *DDX58* (RIG-I) gene, including a coding polymorphism (rs3205166/Val800Val), in antibody variations following measles

vaccine in Caucasians (haplotype allele p-value = 0.021). Mitochondrial antiviral pathways involve the detection of viral RNA by cytosolic helicases belonging to the RIG-I-like helicase family (RIG-I/*DDX58* and MDA5/*IFIH1*), and downstream signaling through interaction with the IPS-1 (*VISA*/*MAVS*/*CARDIF*) adaptor protein [49,50]. In addition, vitamin A and its metabolites, which are known to affect measles replication and antibody responses after vitamin supplementation, are linked with RIG-I/DDX58 through RARα and IFN type I-dependent mechanism [51,52]. Genetic variants within these antiviral sensor genes were associated with different measles-specific immune outcomes in our study (including common *DDX58* genetic variants regulating both antibody responses, and measles-specific IFN-γ and IL-2 secretion in the Caucasian group), which is consistent with a recent report of the recognition of measles virus by RIG-I and MDA5 to instigate interferon-specific innate immune responses [53]. Interestingly, a common coding functional *DDX58* polymorphism (rs10813831/Arg7Cys), known to modulate *IFNB1* and *DDX58* transcription in dendritic cells [54], and suggested as a plausible regulator of rubella vaccine-induced humoral immunity [15], was associated with measles-specific IFN-α secretion in the African-American sample, although did not remain significant after adjusting for FDR ($p = 0.005$, $q = 0.447$, data not shown).

In addition, our analysis of genetic associations with measles-specific vaccine responses revealed enriched representation of genetic variants within the OAS cluster of genes: *OAS1*, *OAS2* and *OAS3* (Tables 2, 3 and 5, Supplementary Table 1). The interferon-induced OAS1 protein is known to catalyze the synthesis of 2′,5′-oligoadenylates, which activate RNase L, resulting in antiviral (viral RNA degradation, inhibition of viral replication) and pro-apoptotic activities, and genetic polymorphisms in the coding genes have been linked to various immunological and infectious outcomes [21–24]. The involvement of *OAS* genetic variants in regulating measles vaccine–immune responses is highly plausible bearing in mind that *OAS* genetic variations are likely involved in modulating the adaptive immune responses to live attenuated rubella vaccine [14]. Particularly convincing is the association of *OAS1* SNP rs10774671, a polymorphism with established functionality, with variations in measles-specific humoral immune responses (more than 3-fold increase associated with the homozygous minor allele genotype) in the African-American sample ($p = 0.003$). This genetic variant is located in intron 5, at the splice-acceptor site, and has been demonstrated to regulate splicing, resulting in isoforms with different enzyme activity [39,55]. Recent data from the literature suggests that genetic variation in *OAS1* (rs10774671) can alter viral replication in human lymphoid tissue and is a risk factor for infection with West Nile virus [39]. In addition, our previous work links this same polymorphism in addition to other *OAS* polymorphisms with variations in rubella vaccine-induced cytokine secretion [14].

Another interesting antiviral gene potentially associated with measles virus-specific immune variations is *ADAR*, which is involved in RNA editing (that substitutes adenosines by inosines) of cellular mRNA and viral dsRNA targets. Our data show evidence for an association of a nonsynonymous SNP (rs2229857/Arg384Lys, in LD with other significant *ADAR* SNPs, $r^2 \geq 0.96$) with measles virus-specific cellular immunity (IFN-γ Elispot responses) in Caucasians ($p = 0.01$, Table 3). Consistent with the current findings, our previous rubella vaccine study demonstrated an increase in rubella-specific IFN-γ responses ($p = 0.021$) with the representation of the minor allele genotype for this *ADAR* polymorphism [14], which further suggests the involvement of this gene/genetic variant in immune response variations after vaccination. In addition, other regulatory and intronic SNPs within the same gene were associated at the genotype and haplotype level with variations in measles-specific cellular immunity, particularly in the Caucasian group (haplotype global p-value = 0.017, Table 4), implying cooperative effects of multiple SNPs in the genetic regulation of the immune outcome. This is in concert with the identification of ADAR as a critical determinant of local and systemic inflammatory responses through RNA editing and modulation of protein production [56]. It is important, however, to note that we did not find similarities between the genes/genetic variants associated with measles-specific IFN-γ Elispot responses and IFN-γ cytokine secretion in our analyses in Caucasians, and while the genetic associations with secreted IFN-γ remained significant after adjusting for FDR, none of the SNPs associated with IFN-γ Elispot responses remained significant after adjustment. Both our IFN-γ assays quantify IFN-γ responses, but as reported previously these assays do not correlate well with each other [57]. IFN-γ Elispot measures the number of cytokine-producing cells, while the IFN-γ ELISA measures the cytokine protein concentration released in the culture supernatant by virus-stimulated cells. We speculate that the lack of correlation (or weak correlation) between the two assays [57] is likely the main reason for the different genetic associations found in our analyses for these two immune outcomes.

Our findings point to the plausible role of several interrogated innate immunity genes in the observed inter-individual variations following measles vaccination, although some of the observations should be interpreted with caution since many of the single-SNP associations did not remain significant after adjusting for FDR at $q < 0.20$ [38].

The identified genes influencing vaccine-induced measles immunity are all interconnected components of the innate antiviral responses targeting viral nucleic acids. Viral nucleic acids stimulate the OAS-RNase L pathway resulting in RNA degradation. Viral RNA is also targeted by ADAR, which deaminates viral replication intermediates. In addition, viral RNA activates RNA helicase-like receptors, including RIG-I, to initiate signaling and transcription of IFNs (through IRFs), which feed back through their receptors to increase the expression of antiviral proteins, thus augmenting the antiviral response [10,58]. Hence, our findings imply that key genetic variants/genes involved in the initial antiviral response to viral nucleic acids are modulating immune responses after vaccination with a live viral vaccine, likely due to alterations in viral sensing and/or direct antiviral activity that restricts viral replication and antigen load/antigenic stimulation. Although some of the genes such as *DDX58*, *ADAR* and *OAS1* (and even genetic variants: *OAS1* rs10774671) were found to be associated with variations in both rubella vaccine [14–16] and measles vaccine-induced immune responses in our studies, most of the identified genetic variants and association patterns appear to be virus-specific, possibly due to differences between viruses with respect to their ability to induce and/or counter some of the involved proteins and antiviral pathways [11–13,58].

A major strength of our study is the use of a relatively large racially diverse cohort of healthy vaccines after two doses of MMR vaccine, with extensive demographic and clinical information available, and no known contact with wild type measles virus infection. This allowed us to probe for race-specific associations (in the two most prevalent racial groups, Caucasians and African-Americans) with vaccine-induced immunity outcomes in our study cohort. A novel aspect of the present study is the comprehensive list of genetic variants, genotyped across 12 key antiviral genes (never before studied in the context of measles vaccine response heterogeneity) and correlations with clinically relevant vaccine-induced immune outcomes, such as neutralizing antibody levels.

We recognize that there is a real possibility that some of the reported associations reflect false–positive results, particularly in the smaller sample sizes (and reduced power) available in our race-specific analysis. In our work we choose to report the findings for the two most prevalent racial groups (Caucasians and

African-Americans), separately, as the allele frequencies can vary considerably between racial and ethnic groups and thus can confound the results. Importantly, most of the genes/SNPs and genetic association patterns identified in our study were different between the Caucasian and African-American racial groups we studied, which is not surprising given the differences in allele frequencies for immune response genes reported for these two races [59], as well as the differences in allele/haplotype frequencies observed for the two groups in our study. It is plausible that different genetic polymorphisms would be important in other populations, such as African children, and our findings from a cohort of older children in Minnesota (an area with high vaccination coverage) may not apply to other areas of the world (with low vaccination coverage) where mainly younger children of different race/ethnicity contract the disease.

We also acknowledge the relatively large number of comparisons in our analyses and addressed the multiple testing issues by adjusting for FDR and supplementing the *p*-values with corresponding *q*-values. After correction for FDR, 15 SNPs (out of 32 reported single-SNP associations at the level $p \leq 0.01$; 11 SNPs in Caucasians and 4 SNPs in African-Americans) still remained significant at the *q*-value < 0.20 [38]. As this is the first study assessing polymorphisms in antiviral genes and their possible effect on measles vaccine-induced immunity in a race-specific manner, we chose to also use the less stringent *q*-value cutoff value of 0.5 (roughly corresponding to *p*-values ≤ 0.01 for single-SNP associations) believing that the risk of false negatives outweighs that of false positives.

Nevertheless, the high biological plausibility for some of the genetic variants, the haplotype-level associations, the observed allele dose-related relationships and cross-regulation of different immune outcomes by the same genetic variants, and considerable effect on immune outcome measures, increase our confidence in the reported findings. Follow-up replication and function-based studies are planned to validate and extend our results, and increase our understanding of the mechanisms, involved in immune response variations after measles vaccination. This will potentially inform the development of new and/or improved vaccines and support a novel paradigm of vaccine development, a concept we have called vaccinomics [9].

Taken together, the increasing evidence for the role of novel innate immunity genes (such as *DDX58*/*RIG-I*, *OAS* and *ADAR*), in the regulation of measles vaccine adaptive immunity will likely open new avenues for designing better vaccines and improved vaccination strategies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.vaccine.2011.09.043.

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