NIH Public Access
Author Manuscript
Vaccine. Author manuscript; available in PMC 2009 March 25.

Published in final edited form as:
Vaccine. 2008 March 25; 26(14): 1731–1736.

Associations Between SNPs in Toll-like Receptors and Related Intracellular Signaling Molecules and Immune Responses to Measles Vaccine: Preliminary Results

Neelam Dhiman, PhD[*], **Inna G. Ovsyannikova, PhD**[*], **Robert A. Vierkant, MS**[§], **Jenna E. Ryan, MS**[*], **V. Shane Pankratz, PhD**[§], **Robert M. Jacobson, MD**[*,‡], and **Gregory A. Poland, MD**[*,||]

[*]*Mayo Vaccine Research Group, Mayo Clinic, Rochester, MN 55905 USA*

§ *Division of Biostatistics, Mayo Clinic, Rochester, MN 55905 USA*

‡ *Department of Pediatric and Adolescent Medicine, Mayo Clinic, Rochester, MN 55905 USA*

|| *Program in Translational Immunovirology and Biodefense, Mayo Clinic, Rochester, MN 55905 USA*

Abstract

Toll-like receptors (TLRs) represent the critical “bridge” between innate and adaptive immunity to viral pathogens. We hypothesized that single nucleotide polymorphisms (SNPs) that potentially influence the expression/function of TLRs and their associated intracellular signaling molecules contribute to variations in humoral and cellular immunity to measles vaccine. We genotyped 190 randomly selected subjects (12–18 years old), previously vaccinated with two doses of measles, for known SNPs in TLR 2, 3, 4, 5, 6, 7, 8 and 9, and their associated intracellular signaling genes. Specific SNPs in the TLR 2, 3, 4, 5, 6, MyD88 and MD2 genes were associated with measles-specific humoral and cellular immunity. Heterozygous variants for rs3775291 (Phe412Leu) and rs5743305 (−926bp in promoter region) of the TLR3 gene were associated with low antibody and lymphoproliferative responses ($p \leq 0.02$) to measles vaccination. Heterozygous variants for rs4986790 (Gly299Asp) and rs4986791 (Ile399Thr) in the TLR4 gene demonstrated higher levels of ($p \leq 0.02$) IL-4 secretion. Heterozygous variants for SNPs in TLR5 (rs5744174) and TLR6 (rs5743818) were associated with higher levels of ($p \leq 0.02$) IFN-γ secretion. In addition, SNPs in MyD88 and MD2, intracellular molecules that associate with TLRs, also demonstrated associations with variations in antibody and IL-10 production ($p \leq 0.03$). Thus, we identified specific SNP associations between TLRs and their associated signaling molecules that have a known role in viral immunity and variations in both humoral and cellular immunity following measles vaccination. These data contribute to understanding the immunogenetic mechanisms underlying variations in the immune response to measles vaccine.

Address correspondence to: Gregory A. Poland, M.D., Director, Mayo Vaccine Research Group, Mayo Clinic, Guggenheim 611C, 200 First Street SW, Rochester, Minnesota 55905, Phone: (507) 284-4968, Fax: (507) 266-4716, Email: poland.gregory@mayo.edu.

[1]The authors do not have a commercial association that might pose a conflict of interest.

[3]This work was partially presented at the 44th Meeting of Infectious Diseases Society of America. Toronto, Ontario, Canada. October 12–15, 2006. Abstract#1085

[4]Requests for reprints should be directed to Dr. Gregory A. Poland via e-mail at poland.gregory@mayo.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Keywords

Polymorphisms; Toll-like receptors; Measles; Vaccine; Cytokines

Introduction

Toll-like receptors (TLRs) have emerged as important sensors for viral recognition that act as "bridging molecules" between innate and adaptive immunity [1–4]. TLRs are able to discriminate between different viruses and bacteria by pathogen-associated molecular patterns (PAMPs) and thereby direct a pathogen specific immune response [2,5]. Upon stimulation, TLRs trigger a cascade of adaptor and intracellular signaling molecules that lead to induction of innate and adaptive immunity [3,6].

In recent years, it has been suggested that signaling through TLRs may play a role in measles virus pathogenicity and immunogenicity. Wild-type, but not vaccine strains of measles virus, can activate TLR2 expressing human T cells, which leads to subsequent secretion of proinflammatory cytokines, such as IL-6, and up-regulated surface expression of signaling lymphocyte activation molecule (SLAM) [7]. Wild-type measles virus is also known to suppress TLR4 mediated IL-12 induction in dendritic cells [8]. In contrast, the attenuated strains of measles virus are known to induce the expression of TLR3 via an interferon-dependent mechanism triggered as a part of the host response [9]. Measles virus recognition and specificity by TLRs can be influenced by genetic variations in the interaction domains between virus and host receptors. For example, a single amino acid mutation of asparagine at position 481 to tyrosine in the measles virus hemagglutinin (H) protein abolishes the ability of wild-type measles virus to activate via TLR2 [7]. We hypothesize that genetic variations in the TLRs and their associated signaling molecules, that play an important role in measles virus recognition, could result in variable immune responses to measles vaccination.

Materials and Methods

Study Cohort and Immune Characterization

Our study subjects (n = 190) were randomly sampled from a previously well-characterized study cohort [10,11]. Measles virus-specific IgG levels were measured in sera using commercially available Enzygnost anti-masern-virus/IgG enzyme immunoassay (Dade Behring Marburg, Germany) following the manufacturer's instructions [12]. IFN-γ, IL-2, IL-4, IL-10 and IL-12p40 secretion in response to measles virus stimulation was determined in PBMC cultures by ELISA as previously described [13,14]. Measles virus-specific lymphoproliferative responses were measured using a 3[H]-thymidine incorporation assay as described previously [12]. Briefly, all assays were performed in triplicates at a pre-optimized time point (72 hrs) and stimulation dose (75 plaque forming units/well). Results were expressed as antigen-specific stimulation indices (SI), defined as the ratio between the median counts per minute (cpm) of measles vaccine-stimulated wells and the median cpm of control wells. Stimulation indices of three or higher were considered to be an indicator of positive lymphoproliferative response. PHA (5μg/ml) was used as a positive control.

SNP Selection and Genotyping

A panel of 96 known SNPs (located in the coding, intronic and regulatory regions 10kb upstream and downstream for each gene) from TLRs (TLR 2–9) and their associated intracellular signaling molecules (NF-κB, MyD88, IRAK, TRAF6, IkK, CD14, MAD3 and MD2) were selected from a literature review and public databases. We tested only those SNPs that had a reported minor allele frequency >5% in the Caucasian population. The nomenclature

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

used for the description of the variants follows that described by den Dunnen and Antonarakis [15].

Genotyping Methods

Genomic DNA was extracted from frozen clotted blood using the Puregene® extraction kit (Qiagen Inc., Valencia, CA). Multiplex PCR and SNP analyses were carried out using the GenomeLab SNPstream platform (Beckman Coulter Inc., Fullerton, CA) as described previously [16].

Statistical Methods

The statistical methods used to determine associations between SNPs in TLR and associated intracellular signaling molecules and immune measures after measles vaccination have been previously described in detail [17]. Briefly, all data were descriptively summarized using frequencies and percentages for all categorical variables, and medians and inter-quartile ranges for all continuous variables. TLRs and associated signaling molecule SNPs were examined on a genotypic level, creating three levels for each SNP: homozygous major, heterozygous and homozygous minor alleles. A cut-off at p-value ≤0.03 for associations between SNPs and immune responses to measles was selected prior to examining data results. We tested for SNP-specific deviation from Hardy-Weinberg Equilibrium (HWE) using chi-square goodness-of-fit tests and excluded all SNPs out of HWE (p-value <0.001) [18]. Associations of SNPs in TLR and associated intracellular signaling molecules with measles virus-induced immune responses were carried out using analysis of covariance (ANCOVA) methods. SNPs were modeled assuming a general (co-dominant) genotypic effect. To better assess the effects of multiple testing, we supplemented the p-values from the ANCOVA models with their associated q-values [19,20]. Briefly, the q-values are based on the concept of false discovery rates and can be interpreted as the expected proportion of false positive results among all features at least as extreme as the observed result. All analyses were adjusted for the potential confounding effects of age, gender, race, age at first MMR vaccination and age at 2nd measles-mumps-rubella (MMR) vaccination. Due to data skewness, p-values were calculated based on log-transformed values for IgG and lymphoproliferative measures, and rank-transformed values for cytokine secretion measures. All statistical tests were two-sided, and all analyses were carried out using the SAS system (SAS Institute Inc., Cary, NC).

Results

Demographic and Immunological Variables of Study Cohort

The study cohort was primarily Caucasian (94.2%) with a median age of 15 years and had near even gender representation (males = 54%). Median ages at first and second MMR were 15.6 months and 12 years, respectively. The median (interquartile range, IQR) measles-specific IgG response was 1430 (646, 2482) IU/L and for proliferation response as measured in stimulation indices (SI) was 3.5 (2.0, 6.1). The median (IQR) for secreted levels of signature Th1 cytokines (IL-12p40 and IFN-γ) was 7.3 (2.7, 20.0) pg/mL and 66.5 (13.1, 198.1) pg/mL, respectively. The median (IQR) for secreted levels of signature Th2 cytokines (IL-4 and IL-10) was 11.5 (4.5, 24.3) pg/mL and 30 (11.5, 75.5) pg/mL, respectively.

SNP Associations between TLRs/Associated Intracellular Molecules and Immune Responses following Measles Vaccination

A total of 29 significant SNP associations ($p \leq 0.03$) were identified between TLRs and their associated intracellular signaling molecules and measures of humoral and cellular immunity following measles vaccination (Table 1 and 2). Out of these, 11 SNPs were located in the coding and the regulatory regions of the genes corresponding to TLRs and their associated

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

intracellular signaling molecules (Table 1). Minor SNP allele variant GG for rs6853 located in the 3′ UTR in MyD88 was associated with a lower antibody level (272 IU/L vs. 1430 IU/L; $p = 0.001$; $q = 0.33$) as compared to the major allele variant (Table 1). The heterozygous variants for two SNPs (rs3775291; non-synonymous, Phe412Leu and rs5743305;-976bp in promoter region) in the TLR3 gene resulted in significantly lower ($p \leq 0.02$; $q \leq 0.46$) measles-specific antibodies as compared to the homozygous variants (Table 1).

The heterozygous variants for two non-synonymous SNPs (rs4986790; Gly299Asp and rs4986791; Ile399Thr) in the TLR4 gene were associated with significantly higher ($p \leq 0.02$; $q \leq 0.44$) secretion levels of the prototypic Th2 cytokine, IL-4, as compared to the corresponding major allele variants (Table 1). However, no allele dose-related response could be seen for this SNP, as we did not have any minor allele variant in our study population. The heterozygous genotype TC for a non-synonymous SNP (rs11466004; Ser157Pro) in the MD2 intracellular signaling molecule gene was associated with lower levels of IL-10 secretion (4 pg/mL vs. 31pg/mL; $p = 0.03$; $q = 0.46$) as compared to the major SNP allele variant CC. Again, due to a lack of minor SNP allele variant TT, no allele dose-related response could be determined (Table 1). Genotype TT for a synonymous SNP rs3804100 (Ser450Ser) in the TLR2 gene resulted in decreased levels of measles-specific IL-12p40 secretion as compared to the minor SNP allele variant CC (8 pg/mL vs. 133pg/mL; $p = 0.01$; $q = 0.35$).

For the prototypic Th1 cytokine, IFN-γ, heterozygous SNP variant GT for a SNP located in the 5′UTR (rs3775296) of the TLR3 gene was associated with significantly higher levels of ($p = 0.03$;$q = 0.46$) IFN-γ secretion as compared to homozygous alleles (Table 1). Similarly, heterozygous variants for a synonymous SNP (rs5743818; Ala644Ala) in the TLR6 gene and a non-synonymous SNP (rs5744174; Leu616Phe) in the TLR5 gene were associated with significantly higher ($p \leq 0.02$; $q \leq 0.46$) levels of secreted IFN-γ as compared to the homozygous variants.

The heterozygous variant for rs5743305 located in the 5′ region of the TLR3 gene was associated with a significantly lower ($p = 0.003$; $q = 0.35$) lymphoproliferative response as compared to the homozygous variants (Table 1). We also identified significant associations ($p \leq 0.03$) between 18 intronic SNPs in the TLR (3, 4 and 5) and associated signaling genes (IkK, NFκB and TRAF6) and variations in cellular immune response to measles vaccination (Table 2).

Discussion

The genetic mechanisms underlying measles virus modulation of host immunity in response to vaccination are poorly defined and are under active research. In the present study, we identified significant associations between SNPs in TLRs 3, 4, 5 and 6 and the downstream intracellular signaling molecules, MyD88 and MD2, with variations in both antibody and cellular responses following measles vaccination. The associations between TLR3 and measles vaccine immunity are particularly intriguing as TLR3 has been previously identified as a prime target for laboratory adapted, but not wild-type measles virus strains in the generation of host immunity [9]. Tanabe *et al* [9] reported that laboratory adapted and vaccine strains of measles virus, including Edmonston, up-regulate the expression of TLR3 in human dendritic cells via enhanced IFN-β secretion. The 500bp region upstream of exon 1 is characterized as a measles virus-responsive segment in the TLR3 gene. This region contains the NF-κB and STAT (family of eukaryotic transcription factors that mediate the response to a large number of cytokines and growth factors) binding sites, and an interferon-stimulated response element (ISRE) located 30bp upstream of exon 1 in the promoter region of the TLR3 gene, which has also been identified as an IFN-β induction site [9]. We identified a SNP in the 3′ UTR of TLR3 (rs5743305 at −976bp of TLR promoter) that demonstrated an association between heterozygous variant

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

AT and low antibody and low lymphoproliferative responses. Specific deletion mutations in the TLR3 promoter region, specifically in the ISRE and STAT binding sites, are known to dramatically decrease promoter activity and hence TLR expression [9]. Since rs5743305 is located in the promoter region within 1 kb upstream of exon 1 in the TLR3 gene in close proximity to the regulatory regions, it can be postulated that it might modulate or influence the promoter activity of TLR3. The GA variant of a non-synonymous SNP also in the TLR3 gene was associated with lower antibody production. Non-synonymous SNPs can directly alter protein expression by aberrant trafficking to cell surface [21], and function by changing protein structure and conformation.

TLR2 and TLR4 are known to stimulate innate and adaptive immune responses to wild-type measles viruses [7,22] [8]. Wild-type measles virus strains activate cells specifically via TLR2, induce SLAM expression and activate a TLR-responsive cytokine profile such as IL-6, IL-12p40 and IL-1α/β [7]. Vaccine strains of measles virus lack the ability to activate TLR2 due to a single amino acid mutation in the hemaglutinin protein of measles virus [7]. In addition, wild-type measles virus infected dendritic cells are rendered defective in IL-12 production in response to TLR4 stimulation [8]. We found heterozygous variants for two non-synonymous SNPs (Gly299Asp and Ile399Thr) in the TLR4 gene to be associated with higher IL-4 secretion to the measles vaccine strain of measles virus. These two SNPs have been studied extensively in association with septic shock after infection with gram negative bacteria, premature birth, myocardial infarction and allograft rejection [23]. Since only wild-type and not vaccine strains of measles viruses and bacteria are known to signal through TLR4, the biological relevance of the associations between SNPs in TLR4 and measles vaccine-induced immunity needs further validation. It is also possible that the associations observed between TLR4 SNPs and measles vaccine immunity may be spurious and not in agreement with the current literature for two reasons. First, very low level IL-4 responses were detected in response to measles stimulation using a modified IL-4 receptor blocking assay [14] and the differences in the magnitude of IL-4 secretion, though statistically significant, were small across genotypes. Second, there were no subjects carrying a homozygous minor allele to confirm the additive effect of the minor allele on IL-4 secretion. Similarly, we had a very small representation of a minor allele variant for a synonymous SNP in TLR2 that may have skewed the IL-12 response. Hence, these associations need verification and validation in a larger cohort to understand their biological significance.

We also found associations between SNPs in TLR5 and TLR6 genes and variations in IFN-γ secretion in response to measles virus stimulation. TLR5 and TLR6 (a co-receptor for TLR2) are known receptors for bacterial derivatives [5,6,24]. Hence, the significance of the associations between TLR5 and TLR6 and measles immunity is currently unknown.

The effects of TLRs are mediated by a complex network of intracellular molecules that trigger the signal from the cell surface to the nucleus for activation of antiviral response genes. Therefore, we also looked at associations between SNPs in genes of intracellular signaling molecules associated with TLRs and the immune response to measles. A minor allele variant for a SNP in the 3′ UTR of MyD88, an intracellular adaptor molecule that signals for most of the TLRs, was found to be associated with a lower antibody response to measles. The role of MyD88 signaling has been implicated in the generation of long-term antibody response to viral infection using a MyD88 knockout mice model [25]. MD2, another molecule that associates intra-cellularly with TLR4, demonstrated decreased IL-10 secretion in the presence of the heterozygous variant. Again, due to limited sample size, we did not observe any minor allele variant for this SNP. We also found several intronic SNPs in TLR and their associated intracellular molecule genes to be significantly associated with variations in cellular immune responses to measles vaccine. No direct relevance or mechanism of action of intronic SNPs on gene function has been reported. However, multiple indirect mechanisms have been proposed

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

by which intronic SNPs can influence gene function such as: i) intronic SNPs can prevent the correct splicing of introns and hence affect the intron splicing efficiency. ii) intronic SNPs may alter splicing and result in premature stop codons or exon deletion and hence generate aberrant or instable mRNA. iii) SNPs within introns may create cryptic splice sites that can affect the production of functional mRNA [26–29].

Overall, consistent with the limited literature on the role of TLRs in measles vaccine-induced immunity, we found specific SNPs in the coding and regulatory regions of TLRs, specifically TLR3, that were significantly associated with variations in antibody and cellular immune responses to measles vaccination. Ours is the first report suggestive of the possible influence of SNPs in TLR and their associated intracellular signaling genes in modulating the immune response to measles vaccine. These findings are consistent with the fact that multiple genetic variations in candidate immune response genes contribute to the complex architecture of the immune response, and are important in understanding the immunogenetics of vaccine response. We assessed associations between a large number of SNPs and several different measures of immune response and, therefore, multiple testing issues are a legitimate concern. However, we found a higher number of significant associations than we would expect by chance alone (29 vs. 20 significant results assuming independent hypothesis tests and a type I error rate of 0.03) indicating a possible genetic component to measles immune response. Furthermore, the q-values for a few of the more significant SNPs indicate the relatively low probability of a false positive result.[20] Note that the calculation of the q-values assumes independent tests of hypothesis, which we may not have due to linkage disequilibrium across SNPs. Thus, these results should be interpreted with a certain level of caution. Other limitations of our study are the small sample size and modest P-values due to the hypothesis-generating nature of this study. New guidelines for SNP association studies for candidate genes in humans suggest the use of lower p-values [30], hence the associations we identified need further validation in a larger cohort.

Acknowledgements

We thank the parents and children who participated in this study. We acknowledge the efforts of the research fellows, nurses and students from the Mayo Vaccine Research Group. We thank Yanhong Wu, Ph. D and Julie Cunningham, Ph.D for assistance with genotyping in the Mayo Advanced Genomic Technology Center. We thank Cheri Hart for editorial assistance.

This work was supported by NIH grants AI 33144, AI 48793 and was made possible by Grant Number 1 UL1 RR024150-01* from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and the NIH Roadmap for Medical Research. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH. Information on NCRR is available at http://www.ncrr.nih.gov/. Information on Reengineering the Clinical Research Enterprise can be obtained from http://nihroadmap.nih.gov/clinicalresearch/overviewtranslational.asp.

Reference List

1. Boehme KW, Compton T. Innate sensing of viruses by toll-like receptors. J Virol 2004;78(15):7867–73. [PubMed: 15254159]
2. Bowie AG, Haga IR. The role of Toll-like receptors in the host response to viruses. Mol Immunol 2005;42(8):859–67. [PubMed: 15829275]
3. Rassa JC, Ross SR. Viruses and Toll-like receptors. Microbes Infect 2003;5(11):961–8. [PubMed: 12941388]
4. Bowie AG. Translational mini-review series on Toll-like receptors: recent advances in understanding the role of Toll-like receptors in anti-viral immunity. Clin Exp Immunol 2007;147(2):217–26. [PubMed: 17223961]
5. Kawai T, Akira S. Pathogen recognition with Toll-like receptors. Curr Opin Immunol 2005;17(4):338–44. [PubMed: 15950447]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

6. Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol 2003;21:335–76. [PubMed: 12524386]
7. Bieback K, Lien E, Klagge IM, et al. Hemagglutinin protein of wild-type measles virus activates toll-like receptor 2 signaling. J Virol 2002;76(17):8729–36. [PubMed: 12163593]
8. Hahm B, Cho JH, Oldstone MB. Measles virus-dendritic cell interaction via SLAM inhibits innate immunity: Selective signaling through TLR4 but not other TLRs mediates suppression of IL-12 synthesis. Virology 2006;358(2):251–7. [PubMed: 17070884]
9. Tanabe M, Kurita-Taniguchi M, Takeuchi K, et al. Mechanism of up-regulation of human Toll-like receptor 3 secondary to infection of measles virus-attenuated strains. Biochem Biophys Res Commun 2003;311(1):39–48. [PubMed: 14575692]
10. Ovsyannikova IG, Jacobson RM, Vierkant RA, Jacobsen SJ, Pankratz VS, Poland GA. The contribution of HLA class I antigens in immune status following two doses of rubella vaccination. Hum Immunol 2004;65:1506–15. [PubMed: 15603879]
11. St Sauver JL, Jacobson RM, Jacobsen SJ, et al. Assessing participation bias in a population-based study of measles-mumps-rubella vaccine immunity in children and adolescents. Paediatric and Perinatal Epidemiology 2007;21:376–84. [PubMed: 17564596]
12. Dhiman N, Ovsyannikova IG, Ryan JE, et al. Correlations among measles virus-specific antibody, lymphoproliferation and Th1/Th2 cytokine responses following MMR-II vaccination. Clinical & Experimental Immunology 2005;142(3):498–504. [PubMed: 16297162]
13. Ovsyannikova IG, Jacobson RM, Ryan JE, Vierkant RA, Pankratz VS, Poland GA. Human Leukocyte Antigen and Interleukin 2, 10 and 12p40 cytokine responses to measles: Is there evidence of the HLA effect? Cytokine 2006;36:173–9. [PubMed: 17234427]
14. Dhiman N, Ovsyannikova IG, Howe RC, Ryan JE, Jacobson RM, Poland GA. Interleukin-4 induced by measles virus and measles-derived peptides as measured by IL-4 receptor-blocking ELISA. J Immunol Methods 2004;287(1–2):217–25. [PubMed: 15099769]
15. den Dunnen JT, Antonarakis SE. Nomenclature for the description of human sequence variations. Hum Genet 2001;109(1):121–4. [PubMed: 11479744]
16. Dhiman N, Ovsyannikova IG, Kennedy RB, et al. Associations between measles vaccine immunity and single nucleotide polymorphisms in cytokine and cytokine receptor genes. J Infect Dis 2007;195:21–9. [PubMed: 17152005]
17. Dhiman N, Cunningham JM, Jacobson RM, et al. Variations in measles vaccine-specific humoral immunity by polymorphisms in SLAM and CD46 measles virus receptors. J Allergy Clin Immunol 2007;120(3):666–72. [PubMed: 17560639]
18. Emigh TH. A comparison of tests for Hardy-Weinberg equilibrium. Biometrics 1980;36:627–42.
19. Storey JD. A direct approach to false discovery rates. J R Statist Soc B 2002;64:479–98.
20. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A 2003;100(16):9440–5. [PubMed: 12883005]
21. Johnson CM, Lyle EA, Omueti KO, et al. Cutting edge: A common polymorphism impairs cell surface trafficking and functional responses of TLR1 but protects against leprosy. J Immunol 2007;178(12):7520–4. [PubMed: 17548585]
22. Hahm B, Cho JH, Oldstone MB. Measles virus-dendritic cell interaction via SLAM inhibits innate immunity: selective signaling through TLR4 but not other TLRs mediates suppression of IL-12 synthesis. Virology 2007;358(2):251–7. [PubMed: 17070884]
23. Schroder NW, Schumann RR. Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious disease. Lancet Infect Dis 2005;5(3):156–64. [PubMed: 15766650]
24. Trinchieri G, Sher A. Cooperation of Toll-like receptor signals in innate immune defence. Nat Rev Immunol 2007;7(3):179–90. [PubMed: 17318230]
25. Guay HM, Andreyeva TA, Garcea RL, Welsh RM, Szomolanyi-Tsuda E. MyD88 is required for the formation of long-term humoral immunity to virus infection. J Immunol 2007;178(8):5124–31. [PubMed: 17404295]
26. Silverman TA, Noguchi M, Safer B. Role of sequences within the first intron in the regulation of expression of eukaryotic initiation factor 2 alpha. J Biol Chem 1992;267(14):9738–42. [PubMed: 1374407]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

27. Law AJ, Kleinman JE, Weinberger DR, Weickert CS. Disease-associated intronic variants in the ErbB4 gene are related to altered ErbB4 splice-variant expression in the brain in schizophrenia. Hum Mol Genet 2007;16(2):129–41. [PubMed: 17164265]

28. Aretz S, Uhlhaas S, Sun Y, et al. Familial adenomatous polyposis: aberrant splicing due to missense or silent mutations in the APC gene. Hum Mutat 2004;24(5):370–80. [PubMed: 15459959]

29. Yu Y, Panhuysen C, Kranzler HR, et al. Intronic variants in the dopa decarboxylase (DDC) gene are associated with smoking behavior in European-Americans and African-Americans. Hum Mol Genet 2006;15(14):2192–9. [PubMed: 16740595]

30. Freimer NB, Sabatti C. Guidelines for association studies in Human Molecular Genetics. Hum Mol Genet 2005;14(17):2481–3. [PubMed: 16037069]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 1

Associations between SNPs located in coding and regulatory regions in TLRs and associated intracellular signaling molecules and measles-specific immune responses

Gene	SNP ID	Location	Function	Genotype	N*	Median response	P- value#	Q- value†
						Ab (IU/L)		
MyD88	Rs6853	3′UTR	-	AA/AG/GG	149/38/3	1430/1583/272	0.001	0.33
TLR3	Rs3775291	Ex4 + 601C > T	Phe412Leu	GG/GA/AA	106/67/17	1602/1025/2133	0.02	0.46
TLR3	Rs5743305	−976T > A	-	AA/AT/TT	36/93/59	1412/1063/1670	0.004	0.35
						IL-4 (pg/mL)		
TLR4	Rs4986790	Ex4 + 636A > G	Gly299Asp	AA/AG/GG	169/21/0	11/23/-	0.02	0.44
TLR4	Rs4986791	Ex4 + 936C > T	Ile399Thr	CC/CT/TT	169/21/0	10/23/-	0.009	0.35
						IL-10 (pg/mL)		
MD2	Rs11466004	Ex5 + 85C > T	Ser157Pro	CC/CT/TT	177/6/0	31/4/-	0.03	0.46
						IL-12p40 (pg/mL)		
TLR2	Rs3804100	Ex3 + 1366T > C	Ser450Ser	TT/TC/CC	159/29/2	8/5/133	0.01	0.35
						IFN-γ (pg/mL)		
TLR3	Rs3775296	5′UTR	-	GG/GT/TT	134/50/6	44/112/101	0.03	0.46
TLR6	Rs5743818	Ex1 + 756G > T	Ala644Ala	TT/TG/GG	96/76/18	51/78/38	0.02	0.46
TLR5	Rs5744174	Ex4 + 936C > T	Leu616Phe	CC/CT/TT	42/87/61	72/82/37	0.002	0.33
						LPA (SI)		
TLR3	Rs5743305	−976T > A	-	AA/AT/TT	36/93/59	3.7/3.1/4.4	0.003	0.35

Phe-Phenylalanine, Leu- Leucine, Gly-Glycine, Asp-Aspartic acid, Ile-Isoleucine, Thr-Threonine, Ser-Serine, Pro-Proline, Ala-Alanine, Ex-Exon, A-Adenine, C-Cytosine, G-Guanine, T-Thymine, LPA- lymphoproliferative assay; SI- stimulation index

P-values ≤0.03 are shown

*Values presented as homozygous major allele/heterozygous/homozygous minor allele.

#Two degree-of-freedom p-value from analysis of covariance adjusting for age, gender, race, age at first MMR and age at second MMR

†Corresponding q-value, based on the concept of false discovery rates. Values can be interpreted as the expected proportion of false positive results among all features at least as extreme as the observed result.

Vaccine. Author manuscript; available in PMC 2009 March 25.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 2

Associations between SNPs located in the intronic regions in TLRs and associated intracellular signaling molecules and measles-specific immune responses

Gene	SNP ID	Genotype	N*	Median response	P-value#	Q-value†
				IL-4 (pg/mL)		
IkK	Rs5029748	CC/CA/AA	95/77/17	10/18/6	0.02	0.44
TLR4	Rs7864330	TT/TG/GG	165/20/0	11/23/-	0.02	0.46
				IL-10 (pg/mL)		
IkK	Rs3747811	TT/TA/AA	47/105/37	15/39/30	0.005	0.35
TLR3	Rs1879026	CC/CA/AA	144/36/4	32/16/79	0.01	0.35
				IL-12p40 (pg/mL)		
NFkB	Rs1801	CC/CG/GG	27/90/65	5/7/11	0.03	0.48
TLR5	Rs1773727	AA/CA/CC	66/83/42	5/9/11	0.004	0.35
				IFN-γ (pg/mL)		
TLR5	Rs1773726	GG/GA/AA	57/84/34	37/85/70	0.005	0.35
TLR5	Rs1773729	TT/TG/GG	56/84/44	38/81/72	0.01	0.35
TLR5	Rs1291584	TT/TG/GG	61/87/42	37/82/72	0.002	0.33
TLR5	Rs5744159	GG/CG/CC	67/81/40	37/82/72	0.007	0.35
TLR5	Rs851138	TT/TG/GG	54/87/42	22/82/72	0.001	0.33
TLR5	Rs851139	GG/GA/AA	67/86/37	37/77/73	0.009	0.35
TLR5	Rs851186	GG/TG/TT	60/88/42	37/81/72	0.005	0.35
NFkB	Rs230494	AA/GA/GG	61/94/35	66/94/21	0.01	0.35
NFkB	Rs230544	CC/TC/TT	56/96/37	66/73/38	0.02	0.46
NFkB	Rs230547	CC/TC/TT	170/19/1	58/116/18	0.007	0.35
NFkB	Rs1585213	GG/GA/AA	61/96/29	64/74/16	0.01	0.36
TRAF6	Rs5030419	CC/CG/GG	135/46/5	72/22/94	0.02	0.46

Phe-Phenylalanine, Leu- Leucine, Gly-Glycine, Asp-Aspartic acid, Ile-Isoleucine, Thr-Threonine, Ser-Serine, Pro-Proline, Ala-Alanine, Ex-Exon, A-Adenine, C-Cytosine, G-Guanine, T-Thymine

Values ≤0.03 are shown

*P-values presented as homozygous major allele/heterozygous/homozygous minor allele

#Two degree-of-freedom p-value from analysis of covariance adjusting for age, gender, race, age at first MMR and age at second MMR

†Corresponding q-value, based on the concept of false discovery rates. Values can be interpreted as the expected proportion of false positive results among all features at least as extreme as the observed result