Associations Between Genetic Variation and Antibody Responses to the Measles Vaccine in South African Children

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Master of Science in Medicine in the field of Vaccinology

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AFRICAN LEADERSHIP IN VACCINOLOGY EXPERTISE

DECLARATION

I declare that this research report is my own work and that it has not been previously submitted for any academic purpose, degree, or examination in any other institution. It is being submitted as partial requirement for the degree of Master of Science in Medicine in the field of Vaccinology at the University of the Witwatersrand. All material which is not my own has been cited and acknowledged accordingly.

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ABSTRACT

This study aimed to investigate the associations between genetic variation of a selection of candidate SNPs and the IgG antibody responses to the measles vaccine MeasBio (Biovac) in a cohort of Black South African children (n=125). Of the 125 children, 17 were classified as seronegative (IgG titre <330 mIU/mL) and 108 were classified as seropositive (IgG titre ≥330 mIU/mL) using measles IgG antibody data gathered at 18 months of age, six months after completing the two-dose schedule of the MeasBio vaccine. Demographic variables such as sex, birthweight, and weight at 18 months were found to not be significantly associated with serostatus.

We formulated a candidate SNP panel from the literature comprised of 77 SNPs related to innate immune responses and vaccine responses. DNA was extracted from buccal swabs gathered from the children in a previous study, and MassARRAY technology was used to genotype the samples. PLINK (version 1.07) was used to conduct univariate and haplotype analyses, and Microsoft Excel (version 2112) was used to analyse demographic variables.

We generated novel MAF and haplotype data for the Black South African population and found six SNPs with significant associations to measles antibody levels through allele and genotype models (P<0.05): rs10774671 (*OAS1*), rs1801275 (*IL-4RA*), rs1805015 (*IL-4RA*), rs2243248 (*IL-4*), rs2546893 (*IL-12B*), rs2834160 (*IFNAR2*). Odds ratios showed that the rs1805015, rs2546893, and rs2834160 SNPs were associated with lower measles IgG levels (seronegativity) post vaccination, while rs10774671, rs1801275, and rs2243248 SNPs were associated with higher measles IgG levels (protective against seronegativity). Three haplotypes on chromosomes 5, 16, and 20 were significantly associated with measles serostatus post vaccination. While the *IFNAR2* and *OAS1* SNPs relate to the interferon type I pathway, the SNPs in *IL-4*, *IL-4RA*, and *IL-12B* relate to the interferon type II (IFN-II) pathway, suggesting that genetic variation in interferon production plays a critical role in measles vaccine immunity.

Within the IFN-II pathway, SNPs with significant associations were found in both the IL-12 and IL-4 pathways which stimulate CD4 T helper type 1 (Th1) vs CD4 T helper type 2 (Th2) immunity respectively. We did not detect significant associations between measles vaccine immunity and candidate SNPs in toll-like receptors (TLRs), cytoplasmic sensors, or the vitamin D pathway.

This study is an initial study and forms a basis for future investigations into measles vaccine responses within the Black South African population.

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1,25-(OH)2D3	1,25-dihydroxyvitamin D 3
APCs	Antigen presenting cells
bp	Base pairs
C and V	Virulence factors
CD46	Cluster of differentiation 46
CI	Confidence interval
DCs	Dendritic cells
ddNTP	Dideoxynucleotide triphosphate
DF	Degrees of Freedom
DI RNA	Defective interfering RNA
EPI	Expanded Programme on Immunisation
F	Membrane fusion protein
GAS	IFN-γ-activated site
H	Hemagglutinin
HLAs	Human leukocyte antigens
HLA-DP	Human leukocyte antigen, DP
HLA-DPB1	Human leukocyte antigen, DP beta chain 1
HREC	Human Research Ethics Committee
HWE	Hardy-Weinberg Equilibrium
IFNAR	Type I IFN-α/β receptor
IFNLR1	IFN-γ receptor 1
IFNs	Interferons
IKKε	IκB kinase-ε
IL	Interleukins
IL-10R2	Interleukin-10 receptor 2

IRF	Interferon regulatory factor
ISGF3	Interferon-stimulated gene factor 3
ISGs	Interferon-stimulated genes
ISRE	Interferon stimulated response element
JAK	Janus kinase
kb	Kilobase
L	RNA dependent RNA polymerase
LD	Linkage disequilibrium
LWK	Luhya population in Webuye, Kenya
M	Matrix protein
MAF	Minor allele frequency
MALDI-TOF	Matrix-assisted laser desorption/ionization—time of flight
MAVS	Mitochondrial antiviral-signalling proteins
MDA5	Melanoma differentiation-associated gene 5
mIU/mL	Milli-international units per millilitre
MMR	Measles, mumps, and rubella
mRNA	Messenger RNA
MV	Measles virus
NF-κb	Nuclear factor kappa-light-chain-enhancer of activated B cells
ng/µl	Nanograms per microlitre
NICD	National Institute for Communicable Diseases
NK	Natural killer cells
NP	Nucleocapsid protein
NSPs	Non-structural proteins
OR	Odds ratio
PAMPs	Pathogen-associated molecular patterns

PCR	Polymerase chain reaction
PCV	Pneumococcal Conjugate vaccine
Ph	Phosphoprotein
PRRs	Pattern recognition receptors
QC	Quality control
RA	Retinoic acid
RIG-I	Retinoic acid-inducible gene I
RLR	RIG-like receptor
RNA	Ribonucleic acid
RXRA	Retinoid X receptor alpha
SLAMF1 (CD150)	Signalling lymphocyte activation molecule family member 1
SNPs	Single nucleotide polymorphisms
STAT	Signal transducer and activator of transcription
TBK1	TANK-binding kinase 1
Th1	CD4 T helper type 1
Th2	CD4 T helper type 2
TIRAP	TIR-associated protein
TLR	Toll-like receptor
TRAM	TRIF-related adaptor molecule
Tregs	T regulatory cells
TRIF	TIR-domain-containing adaptor protein-inducing IFN-β
VDR	Vitamin D receptor

1. INTRODUCTION

The universal approach to childhood vaccination programmes has been to administer a standard set of vaccines to all eligible children, except those with contraindications. This approach has several advantages and disadvantages: primarily, it allows for a more streamlined and simple infrastructure through which vaccines can be distributed and administered, but it assumes that everyone who receives a vaccine will react in the same manner and therefore it disregards the possibility of individual variability in vaccine response (1). The advancing field of immunogenetics is uncovering the genetic mechanisms of immune responses to antigens and showing that genetic variation can affect an individual's immune response to vaccines (1). Therefore, genetic variation has become a key factor to consider when assessing a vaccine's efficacy or immunogenicity in a particular population (1).

This study aimed to investigate the associations between genetic variation of a selection of candidate genes and the IgG antibody responses to MeasBio (Biovac), the measles vaccine used in the South African Expanded Programme on Immunisation (EPI) schedule in a cohort of Black South African children. Several prior studies in other ethnicities have found associations between genetic variation and immune responses to measles vaccination in high-income countries, however there are no studies examining this matter in South African populations (1–4). There have been studies in the South African population examining the rates of seroconversion after receiving the measles vaccine, so there is available data on the measles antibody profile in the population, but these studies did not examine the associations of genetic variation on the vaccine responses (5).

The measles vaccine is also of interest because in multiple studies it has been shown to significantly reduce overall infant mortality rates, including infant mortality rates caused by pathogens other than measles (6,7). This suggests that the measles vaccine could induce heterologous immunity, meaning that it may offer cross-protection against a variety of other

pathogens and conditions aside from measles virus (MV) (6,7). For example, live vaccines such as the measles vaccine may train the innate immune system through epigenetic reprogramming or may influence inflammation profiles that impact other immune responses (7).

It has also recently been discovered that measles infection can induce a phenomenon called "immune amnesia", where existing immunological memory is damaged (8). This is because one of the key receptors for MV, signalling lymphocyte activation molecule family member 1 (SLAMF1), is located on immune cells including memory T and B lymphocytes (9). Consequently, as the immune system clears infected cells, it systematically erases its own memory – which is thought to take up to three years to be re-established (10). Therefore, in the wake of a measles infection, the individual becomes susceptible to subsequent infection with pathogens they are no longer protected from. The prevention of measles infection, and therefore the prevention of potential immune amnesia, through vaccination could also explain why measles vaccination results in such a significant reduction in all-cause childhood mortality. For this reason, it is important to understand the factors influencing measles vaccine efficacy at a population level as well as at an individual level.

The factors influencing the immune response to measles vaccination are therefore of considerable interest. This study can be leveraged to further understand mechanisms underlying vaccine failure in general and provide information that could inform future rational vaccine design.

1.1 MEASLES

Measles, or rubeola, is a highly contagious, febrile, respiratory disease caused by infection with the MV (11). The MV is spread through airborne droplets dispersed through coughing and sneezing, as well as directly through close contact with infected persons (12). With a basic reproduction number (R_0) of 9-18, measles is rated as possibly the most contagious disease recorded (11).

Most cases of measles recover after several days of illness with symptoms including fever, malaise, coryza, conjunctivitis, cough, and a maculopapular rash (11). Around 30% of cases in children under the age of five years can develop more severe complications including hearing loss, blindness, pneumonia, encephalitis, and diarrhoea (13,14). In cases of persistent MV infection, infected infants are also at risk of developing acute sclerosing panencephalitis, which is a degenerative and fatal neurological disease which can remain latent for many years after the original MV infection (15).

Measles causes significant morbidity and epidemics can have high mortality rates (16). Prior to the introduction of the measles vaccine in 1969, two to three million people died globally each year from measles (16). Despite extensive vaccination efforts and the significant decline in the mortality rate of measles, it remains a leading cause of vaccine-preventable deaths (11). In 2019, over 207,500 people died due to measles (17). Most of the deaths caused by acute measles are due to opportunistic, secondary infections as a result of the immunosuppression caused by MV (15). A significant outbreak in a partially vaccinated Kenyan population (vaccine coverage of 58%) had an overall case fatality rate of 12.6% for children below five years of age, although this increased to 16.2% for unvaccinated children of the same ages (18). In terms of the epidemiology of MV in South Africa specifically, the National Institute for Communicable Diseases (NICD) reported that of the 4,608 febrile rash cases noted in the country in 2019, 65 were laboratory-confirmed to be MV only and 44 were laboratory-

confirmed to be MV and rubella combinations (19). In the years 2015 to 2020, South Africa had only had an MV incidence rate of more than one per million in 2017, when using an strict case definition that excluded positive rubella cases (14).

Unfortunately, a combination of factors including vaccine hesitancy and complacency have led to a resurgence of MV cases worldwide (16). As a result of this, many countries are failing to control MV effectively, and it has regained endemic status in numerous high-income countries that had previously eliminated it (15,16). In 2019, over 500 000 cases of measles were reported globally, with the majority occurring in Africa (8).

1.2 VIROLOGY

The MV is part of the *Morbillivirus* genus which belongs to the *Paramyxoviridae* family, and is most probably descendant from the eliminated rinderpest virus which infected cattle, however MV is not known to infect any animal species (11,15). Its 16 kilobase (kb) genome is contained in non-segmented, single-stranded negative-sense RNA and encodes six structural and two non-structural proteins (NSPs) (11). The six structural proteins are the nucleocapsid protein (NP), phosphoprotein (Ph), matrix protein (M), membrane fusion protein (F), hemagglutinin (H), and an RNA dependent RNA polymerase (L), while the two NSPs are the virulence factors (C and V) (11).

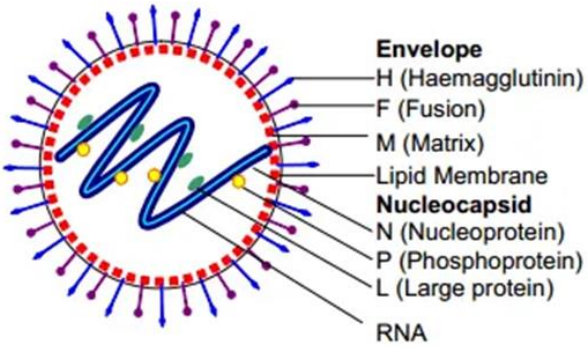

Figure 1: The structure of the measles virus showing the position of the six structural proteins; hemagglutinin (H), membrane fusion protein (F), matrix protein (M), nucleocapsid protein (NP), phosphoprotein (Ph), and the RNA dependent RNA polymerase (L) (20).

There are currently 24 genotypes of the MV recognised by the WHO, separated into eight clades, A to G (21). The endemic strain in South Africa is the B3 strain, although various other genotypes have been identified in South Africa after being imported by travellers, such as the B2 genotype which was imported from Angola (21). Importantly, MV is an antigenically monotypic virus despite the genotypic diversity of MV and high mutation rate associated with RNA viruses (11). This means that the surface proteins of the MV that are responsible for stimulating the immune response of the host retain their antigenic structure and MV has a single serotype (22). This is clearly advantageous from an immunisation perspective because it means that a single measles vaccine can induce protection worldwide (22).

1.3 Measles virus infection and replication

Measles virus infection is initiated in the respiratory tract before being transported to the regional lymph nodes by immature pulmonary dendritic cells (DCs) or alveolar macrophages where it can infect SLAMF1-expressing lymphocytes and facilitate primary viremia, which is the spread of the infection (15). Genetic variations in the genes encoding the cellular receptors used by MV could introduce alterations to the protein structure which could enhance or dampen the infectious capabilities of MV.

Infection begins with the attachment of the virus to the host cell receptors, controlled by the viral H glycoprotein, and then the fusion of the virus to the host cell membrane, controlled by the viral F glycoprotein (11). The H protein of the MV can attach to three human cell receptors: SLAMF1 (also known as CD150), cluster of differentiation 46 (CD46), or nectin-4 (9). The CD46 receptor is a complement regulatory protein and is found on all nucleated cells in the body (9). CD46 also induces the proliferation and differentiation of regulatory T cells and thus is an important regulatory protein of the adaptive immune system as well (15). SLAMF1, or CD150, is expressed in activated immune cells, and thus can be found on lymphocytes, dendritic cells, and macrophages (9,15). Nectin-4 is an immunoglobulin adherens junction

protein expressed in most epithelial cells, including those lining the respiratory tract (9). Both the wildtype and vaccine strains of MV can interact with the SLAMF1 and nectin-4 receptors, but wildtype MV strains preferentially use the SLAMF1 receptor as they do not use the CD46 receptors efficiently (23). While the SLAMF1/CD150, CD46, and nectin-4 receptors are the main receptors used in the initiation of MV infection, there are possibly additional receptors used in later stages of MV infection (15). This is supported by the fact that endothelial cells are also infected in acute MV infections in addition to epithelial and immune cells, and in persistent MV infections neurons and glial cells become targets for infection (15).

Replication of MV occurs predominantly in cells expressing SLAMF1, such as naive and memory B cells, although memory T cells are the primary sites of replication. Measles virus infection can result in lymphocyte cell death, and leukopenia regularly occurs with the viremia and systemic spread of MV (23).

The interaction of the H glycoprotein with one of the host cell receptors causes a chronological cascade of conformational changes in first the H and then the F glycoprotein which causes the virus and host cell membrane to merge and create fusion pores through which the viral RNA is injected into the host cell to induce viral replication (Figure 2) (24). The MV's L protein transcribes the negative-sense viral RNA into mRNA so that translation can occur (15). Replication takes place in the cytoplasm of the host cell. The P protein regulates transcription, replication, and the efficiency with which the N assembles into nucleocapsids (25). The M protein links ribonucleoproteins with envelope proteins during virion assembly, after which fully assembled virions exit the cell via exocytosis (15). Infected cells produce the viral glycoproteins, H and F, and express them on their own surface. The newly formed virions then pick up these glycoproteins when they bud from the host cell's plasma membrane (Figure 2). Infected cells that express these viral glycoproteins may also fuse with neighbouring uninfected

cells to produce multinucleated giant cells that are able to produce and disseminate infectious viral particles (26).

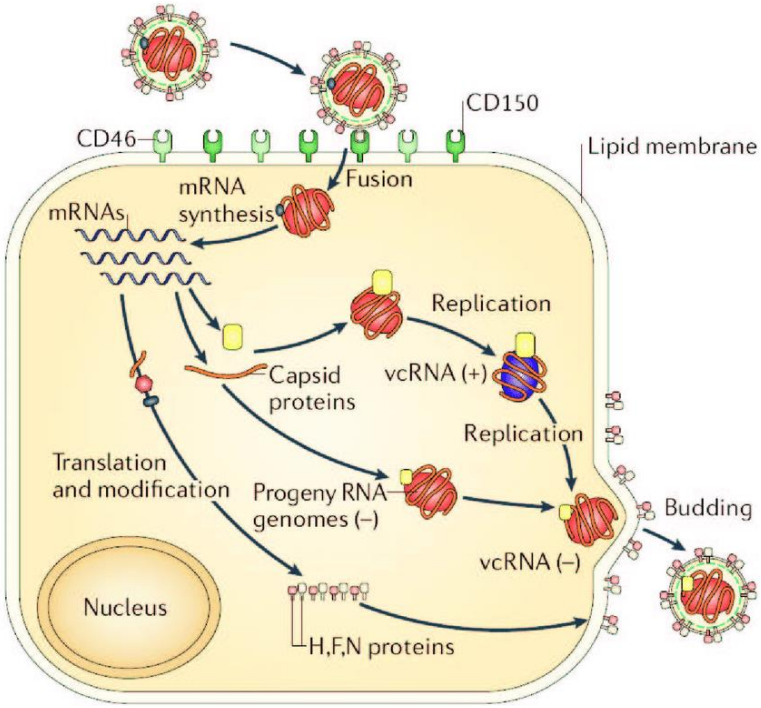

Figure 2: The viral replication cycle of MV (15)**.** Once the H protein has interacted with one of the available cell-surface receptors and the virion has fused with the cellular membrane, the viral RNA is injected into the cytoplasm of the cell where it is replicated. New virions bud from the cell. Note that vcRNA stands for viral complimentary RNA (15).

1.4 MEASBIO VACCINE

South Africa has included measles vaccination as part of its public immunisation programme since 1983 (5). Before 2016, the measles vaccine platform was the monovalent, live attenuated vaccine using the Schwartz strain, which was developed from the earlier Edmonston strain, and was administered at 9 and 18 months of age (5,27). However, since 2016, South Africa has used the MeasBio vaccine developed in 2015, which is also a monovalent, live attenuated vaccine but uses the CAM-70 strain, which was derived from a Japanese wild-type strain of MV (5). Since MV is monotypic, the fact that the Japanese wild-type strain and South Africa's endemic strains are of different genotypes is not an obstacle.

Since 2016 in South Africa, the MeasBio vaccine is administered at 6 and 12 months of age. The age of vaccination was lowered due to the high incidence rate of measles in infants less than 9 months of age as well as the fact that MeasBio cannot be administered with other vaccines (28). Therefore, lowering the age of administration to 6 and 12 months of age protected younger infants while simultaneously slotting into gaps in the EPI schedule between other routine immunisations.

Lowering the administration age of vaccines can be problematic, as neonates possess maternal antibodies that offer some protection from infectious disease whilst their adaptive immune systems develop (29). The presence of maternal antibodies can interfere with the generation of the infant's own antibodies in response to vaccination (29). The reason that this is a problem is because the maternal antibodies wane over the first year of life and do not have any form of immune memory, so they cannot be regenerated should the infant be infected with the target pathogen. The infant's own adaptive immune response to vaccination would generate immunological memory in the form of memory B and T cells, hence protecting the child for a long period of time. In the case of measles vaccination, it has been shown that maternal antibodies can inhibit the production of antibodies in the infant, although it has also been shown that the specific vaccine used can mitigate this (29). Serological studies have shown that MeasBio has a high seroconversion rate, indicating that the early age of administration of 6 months is effective (5).

According to the NICD factsheet on MeasBio, the vaccine efficacy of the full two dose schedule of the vaccine is 93-99%, although a recent retrospective report showed that between 2015 and 2020, vaccine effectiveness amongst one to four year old children was as low as 80% (14,30). Vaccine efficacy is calculated under controlled conditions during a clinical trial and refers to the reduction of disease between the vaccinated and placebo arms of the study. Vaccine effectiveness, however, is a measure of how well a vaccine performs in the real world, outside

of clinical trial conditions. Vaccine effectiveness is a much more accurate measure of the degree to which a vaccine is doing its job under real world conditions. The result of the 2020 retrospective report shows that MeasBio is underperforming in the whole population compared to its results in the clinical trials which yielded the efficacy results reported by the NICD.

At present in South Africa, the MeasBio vaccine is provided in the public sector, whereas combination vaccination against measles, mumps, and rubella (MMR) is available in the private sector at a cost. The strains of MV used in the MeasBio and MMR vaccines are genetically identical (30). The MeasBio vaccine has a good safety profile, with common adverse events such as pain at the injection site, fever, and morbilliform rash occurring in less than 1 in 20 people. The severe adverse events, while extremely rare, include encephalitis (1 in 2 000 000), anaphylaxis (1 in 1 000 000), thrombocytopenia (1 in 30 000), and febrile seizures (1 in 3000) (30).

While measles vaccines generally have a high efficacy and immunogenicity, various studies have revealed that 2%–10% of healthy individuals who receive the measles vaccine do not produce protective levels of antibodies against the virus (31). It is plausible that this phenomenon might be due to genetic variation in candidate genes affecting the immune response to the measles vaccines. Studies in populations based in Minnesota (USA), northern Newfoundland (Canada), and Israel have reported conflicting findings regarding the role of demographic variables such as sex and ethnicity in measles immune responses, with some finding these variables to be significantly associated and others stating that they were insignificant (32–34).

1.5 THE IMMUNE RESPONSE TO MEASLES AND MEASLES VACCINATION

1.5.1 VIRAL DETECTION AND INNATE IMMUNE SYSTEM ACTIVATION

Viral infections are often first recognised by pattern recognition receptors (PRRs) that recognise viral genetic material and proteins as pathogen-associated molecular patterns (PAMPs) (35). The subsequent activation of PRRs in response to binding viral PAMPs results in a cell signalling cascade that produces numerous cytokines which influence the host's immune response, especially pro-inflammatory cytokines and interferons (IFNs) (35). Genetic variation in the genes encoding PRRs could affect the effectiveness, magnitude, and nature of the resulting immune response.

Toll-like receptors (TLRs) are a subfamily of trans-membrane pattern recognition receptors (PRRs) that play a vital role in determining the host's immune response to both natural MV infection and measles vaccination (36). The TLR pathway ultimately leads to the activation of T cells and the innate anti-viral immune response (36). MV infection is detected predominantly by TLR2, TLR3, and TLR7 (36). However, measles vaccine strains do not stimulate TLR2 (37).

TLR2 is a cell-surface membrane-bound receptor that recognises viral surface proteins, while TLR3 and TLR7 are internal membrane-bound receptors that recognise viral RNA in the endosome (35). There are four main adapter proteins that are recruited upon TLR activation: MyD88, TIR-domain-containing adaptor protein-inducing IFN-β (TRIF), TIR-associated protein (TIRAP), and TRIF-related adaptor molecule (TRAM) (38). The TLR/MyD88 pathway upregulates expression of SLAM receptors and stimulates the transcription of proinflammatory cytokines such as interleukins (IL)-6 and IL-1β by producing the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (23). The TLR/TRIF pathway mainly produces type I IFNs (IFN-I) in macrophages, DCs, and epithelial cells (Figure 3)

(35,39). Alterations in the TLRs due to genetic variation could potentially affect the profile of the immune response by skewing cytokine production.

Wildtype MV strains activate TLR2 through its interaction with the viral H glycoprotein, which result in the upregulation of SLAMF1 expression as well as the activation of the inflammasome (35,40). This means that the production of these proinflammatory cytokines simultaneously speeds up the spread of MV by upregulating one of its key receptors and stimulates the immune response to MV infection. Vaccine strains of MV do not activate TLR2 as there is a structural modification within the H glycoprotein due to virus attenuation, so the vaccine strains of MV rely on the activation of TLR3-9 to produce the proinflammatory cytokines and initiate the immune response to vaccination (35)

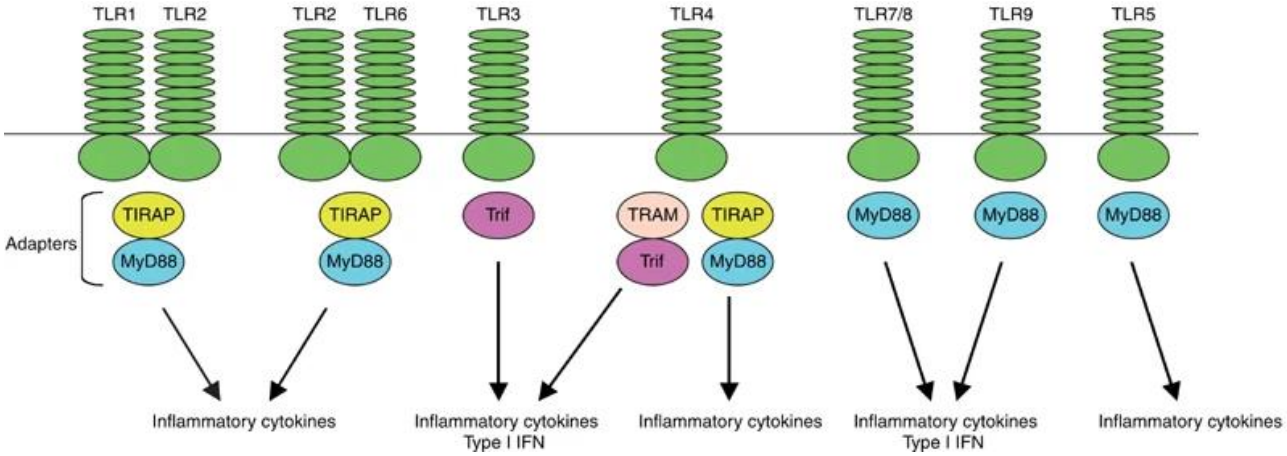

Figure 3: Summary of the TLRs and their cell signalling adapters, and the downstream expressed products of TLR activation. TLR1/26 use both TIRAP and MyD88 as adapters. TLR3 only uses TRIF and TLR5/7/8/9 only use MyD88 as essential adapters. Only TLR4 utilises all four adapters. (38)**.**

In addition to the membrane bound PRRs, there are also cytoplasmic PRRs that can detect MV once it has penetrated the cell and is no longer detectable by TLRs (35). These include the RIG-like receptors (RLRs), such as retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5), which detect viral RNA and induce IFN-I

production, although it is believed that RIG-I plays a much greater role in cytoplasmic MV detection than MDA5 (41). IFN-1 production induced through cytoplasmic PRRs occurs in multiple cell types in addition to those stimulated by TLR activation (35). The RIG-I and MDA5 pathways converge to result in the production of IFN-I (Figure 4) (42). RIG-I and MDA5 activation causes the activation of mitochondrial antiviral-signalling proteins (MAVS). MAVS relays the activation signal to TANK-binding kinase 1 (TBK1) and IκB kinase-ε (IKKε), which then activate interferon regulatory factor (IRF) 3 and IRF7, which can induce IFN-I production in the presence of NF-κB (42).

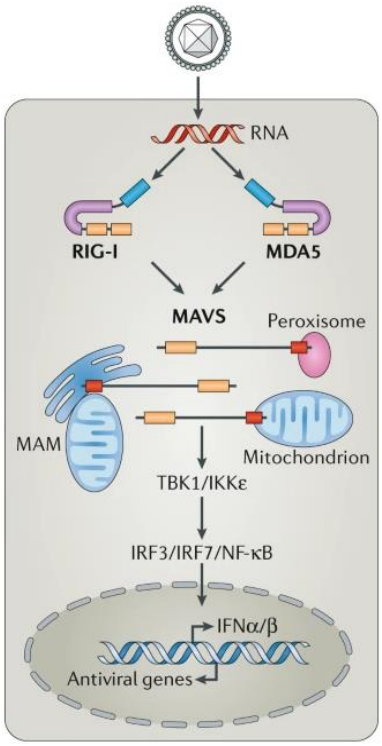

Figure 4: The RIG-I and MDA5 signalling pathways resulting in the production of IFN-I types IFN-α and IFN-β and the transcription of antiviral genes (42)**.**

1.5.2 Interferon response in MV and measles vaccination

There are three types of IFNs:, type I (IFN-I), type II (IFN-II) and type III (IFN-III) which collectively play an important role in antiviral immunity, and critically orchestrates antiviral immune responses through various mechanisms (43). IFN-I directly inhibits viral replication in infected cells in the early innate phase of the immune response, initiates and drives antigen

presentation, as well as directly and indirectly acting on B and T lymphocytes to begin the adaptive phase of the immune response (43).

Innate signalling by TLRs or RLRs generally stimulates the production of IFN-I or IFN-III. IFN-I includes 12 subtypes of IFN, of which the primary IFN-I subtypes are IFN-α and IFN-β (35). IFN-I bind to type I IFN-α/β receptors (IFNAR) 1 and 2 on both infected and uninfected neighbouring cells causes the transcription of a multitude of interferon-stimulated genes (ISGs) which trigger a robust antiviral response (44). There are hundreds of proteins produced by the many ISGs, but a few of the most prominent ones include protein kinase R (PKR) which inhibits viral RNA translation, 2′,5′-oligoadenylate synthetase (OAS1) which cleaves viral RNA, and Mx1 which sequesters viral nucleocapsid proteins to inhibit encapsulation of the viral RNA (35). The IFN-III type, consisting of IFN-λ, is the most recently discovered variety of IFNs. There are two IFN-III receptors: the interleukin-10 receptor 2 (IL-10R2) and the IFN-γ receptor 1 (IFNLR1), which seem to elicit similar immunological responses to IFN-I, and also promote Th1 proliferation over Th2 proliferation (45).

The IFN type II response is induced by IL-12 cytokine and is not induced directly by innate immune responses. The IFN-II type is represented by IFN-γ, which is mainly produced by T and natural killer (NK) cells and also promotes anti-viral immunity although through pathways independent of IFN-I (46). IFN-II binds to the IFN-γ receptors (IFNGR) 1 and 2 which activates the transcription of ISGs in the IFN-γ-activated site (GAS) (47). The presence of IFN-γ promotes CD4 T helper type 1 (Th1) proliferation and suppresses Th2 proliferation (48).

There are many similarities between the signalling pathways of IFN-I, IFN-II, and IFN-III, as they all converge into the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway, which activates the transcription of ISGs (Figure 5) (35,47).

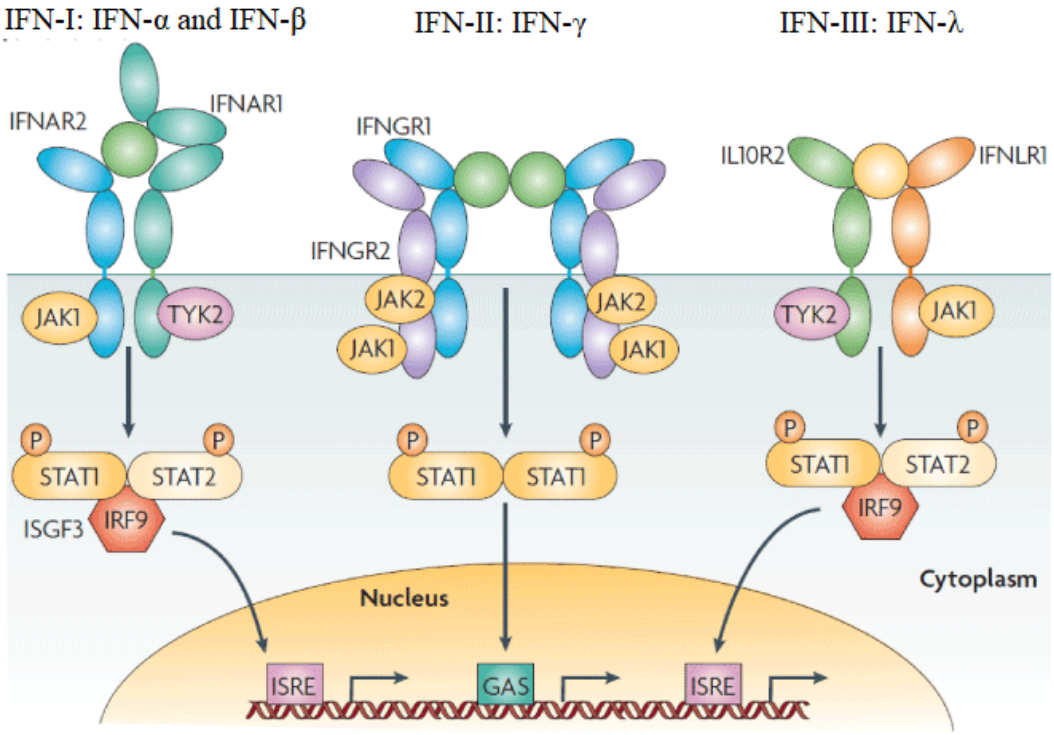

Figure 5: IFN signalling pathways. IFN-I interact with INFAR1 and IFNAR2, IFN-II interact with IFNGR1 and IFNGR2, and IFN-III interact with IL10R2 and IFNLR1. The receptor complexes are then associated with JAK kinases, which activate STAT transcription factors, in turn initiating transcription of the interferon-stimulated genes (47).

However, the interferon immune response to MV infection is atypical for infection with an RNA virus (40). There is little to no IFN-I production following MV infection (35,40). MV is capable of effectively inhibiting the synthesis and signalling pathways of IFN-I through the combined effects of its Ph, V, and C proteins (40). The C-terminal of the V protein blocks the synthesis of IFN through inhibition of both the TLR/MyD88 pathway and the MDA5 pathway (15). Even if any IFN production occurs through the RIG-I pathway, the C-terminal of the V protein is also capable of inhibiting STAT2 activation and the N-terminals of the P and V proteins can inhibit STAT1 activation, so the ISGF3 transcription factor complex cannot be formed (15). MV also stimulates IL-10 production which also inhibits IFN type I production (49).

It is due to the inhibition of the IFN-I pathway that the MV is able to replicate, establish persistent infection, and spread so efficiently during the 10-14 day latent period after infection (15,40). However, production of IFN-γ is not inhibited by MV, and both IFN-I and IFN-II are produced during measles vaccination (50).

Although the IFN-I pathways are effectively inhibited during MV infection, the production of proinflammatory cytokines can still take place. During acute MV infection, cytokines IL-1, IL-6 and IL-12 are produced, contributing to immune activation and to the start of the adaptive immune response (37). The presence of IL-12 is crucial as this cytokine drives Th1 cell proliferation, while the presence of IL-4 selects for Th2 cell proliferation (51,52).

DCs are effective cells in initiating the immune response to MV as they can contribute to the innate and adaptive responses and connect these processes by driving the production of proinflammatory cytokines and acting as antigen presenting cells (APCs) (35). The interaction of MV with DC-specific intercellular adhesion molecule-3 grabbing non-integrin (DC-SIGN) in APCs induces the expression of stress-response genes whilst inhibiting the IFN pathways.

1.5.3 The adaptive immune response to MV and measles vaccination

Presentation of MV peptides by the innate APCs activates T lymphocytes, where naïve $CD4^+$ T lymphocytes are differentiated into either Th1, Th2, or T regulatory cells (Tregs) (35). Genetic variation of any genes encoding key proteins within the immune signalling cascade such as the PRRs could influence the cytokine profile generated and consequently direct the characteristics of the adaptive immune response. Different cytokines initiate different cells of the adaptive immune system. The presence of IL-12 in wildtype MV infection means that Th1 lymphocytes are the dominant T lymphocyte type during the early adaptive immune response (35). This is supported by the fact that during the early phase of the adaptive immune response,

IFN-γ and IL-12 are detected, both of which are produced by Th1 cells (23). $CD8^+$ T cells also produce IFN-γ during acute MV infection. This phase coincides with the onset of the characteristic maculopapular rash (15). The burst of IFN-γ production and its subsequent effects are associated with the clearance of live MV and measles symptoms approximately a week after the appearance of the rash. IFN-γ ELISPOT assays measuring $CD4^+$ and/or $CD8^+$ memory response to measles antigen *in vitro* are commonly used as indicators of measles-specific cellular immunity (53).

The involvement of both Th1 and Th2 cells in the immune response to natural MV infection is thought to be a driving force in the development and maintenance of life-long immunity (52). However, due to the lack of TLR2 activation after measles vaccination because of the modified H glycoprotein, much lower levels of IFN-γ and IL-12 are detected but significantly higher levels of IL-4 occur at all time points (54,55). The Th2 state occurs for a prolonged period of time after MV infection and may also predominate after measles vaccination as has been observed in some studies, although others report a more balanced Th1/Th2 state after measles vaccination (4,55). It is thought that a Th2 dominated response to measles vaccination may not establish as long-lived immunity as the more balanced response (52).

Once the rash subsides, cytokine levels of IL-4, IL-10, and IL-13 increase, which indicates a shift toward more Th2, and Treg cell differentiation (35,56). IL-4 suppresses IFN-γ production and Th1/cell-mediated immunity, and instead stimulates Th2 immunity, B cell development, humoral immunity, and antibody class switching.

The humoral component of the measles immune response begins with immunoglobulin M (IgM) production which also coincides with the maculopapular (23). The presence of measles specific IgM antibodies is one of the first diagnostic tests conducted to identify MV infection at the time of presentation of the rash (23). The IgM antibodies persist for the first 8 weeks post-infection, before the mature B lymphocytes undergo class switching and begin to produce

measles-specific immunoglobin G (IgG) and immunoglobin A (IgA) antibodies, marking the maturation of the humoral response and the development of protective immunity (35).

There has been change over time in the literature regarding the IgG titre required to reach the threshold of protection; that is, what is considered to be the typical measles correlate of immunity (57). Minimum IgG titres for protection have been reported as low as 120 milli-international units per millilitre (mIU/mL), while many studies use 200 mIU/mL as the threshold of protection (54,58). A recent study in the South African population using the same cohort as this study defined the threshold IgG titre as 330 mIU/mL in order to ensure protection (5). Therefore, this threshold has been accepted for the current study.

The cellular component of the adaptive immune system, the $CD4^+$ and $CD8^+$ T lymphocytes, rather than the humoral component, is more responsible for the clearance of MV. This is supported by the evidence that children with agammaglobulinemia are able to recover whilst children with deficiencies in cellular immunity tend to develop progressive, fatal disease (15). However, it is the presence of measles specific IgG antibodies that ensures protection from future MV infection, and therefore IgG titres are an appropriate correlate of protection against MV (23). Although the live, infectious virus is typically cleared within a week of the onset of the rash, MV RNA can remain in the body for up to several months, therefore sustaining the adaptive immune response. This may be a key role in the development of life-long immunity following MV infection (23).

The immune response to MV is an interesting juxtaposition because while life-long immunity to MV is generated, immunosuppression with regards to other pathogens occurs, leaving the individual susceptible to secondary infections (59). Since memory T and B lymphocytes express SLAMF1, they are targets of MV infection. Therefore, when the immune system clears the virus and destroys infected cells through the action of $CD8^+$ cytotoxic T cells, the infected

memory lymphocytes are killed, depleting the host's immunological memory (60). This is the cause of the immune amnesia associated with MV infection.

1.5.4 Role of human leukocyte antigens in measles immunity

The human leukocyte antigen proteins (HLAs), also known as the major histocompatibility complex (MHC), play a vital role in adaptive immune responses, as these proteins on APCs display and present antigens to T cells and NK cells (51).

There are two classes of HLAs; class I HLAs are found on almost all cells and are used to indicate the infection status of that cell; the cell can present a pathogenic antigen on an HLA class I glycoprotein to indicate to immune cells such as NK cells or $CD8^+$ T cells that that cell has been infected and needs to be destroyed. Class II HLAs are only expressed on certain immune cells. Class II HLAs on macrophages or APC present foreign peptides to $CD4^+$ naïve T cells in order to stimulate their development to Th1 or Th2, whereas class II HLAs on B cells interacts with cognate activated Th2 helper cells to drive humoral immune responses to the pathogen (51). It has been shown that subtypes of class II HLAs have a significant effect on the host's immune response to MV infection and vaccination (61). T cell-generated IFN-γ and IL-4 cytokine responses to measles may be genetically restricted in part by class I or class II HLA genetic variation, which in turn can restrict the cellular immune response to measles vaccination (3,61).

1.5.5 THE VITAMIN A AND VITAMIN D PATHWAYS IN MEASLES IMMUNITY

Other factors that can significantly affect the immune response to MV infection and vaccination are the vitamin A and vitamin D pathways (62).

Vitamin D is an important immune regulator as its derivatives have been shown to play a significant role in both the innate and adaptive immune response (63). Vitamin D is absorbed through the skin and nutrition and is converted into 1,25-dihydroxyvitamin D 3 (1,25-$(OH)_2$D3) through a multistage process. 1,25-$(OH)_2$D3 is the ligand for the nuclear vitamin D receptor (VDR) and controls the downstream transcriptional effects of VDR activation (63). The presence of 1,25-$(OH)_2$D3 has been shown to suppress Th1 cell production and promote Th2 responses, with variants in the vitamin D pathway being associated with variable responses to measles vaccination (62,63). It has also been demonstrated that an inversely proportional relationship exists between serum vitamin D levels and the measles-specific antibody titre generated (64).

One of the main vitamin A receptors, retinoid X receptor alpha (RXRA), forms a heterodimer with VDR, facilitating intracellular signalling (63). Vitamin A has been shown to reduce morbidity and mortality in MV infection and vitamin A deficiency has been classified as a risk factor for severe measles by the World Health Organization (WHO) (65,66).

1.6 CANDIDATE GENES AFFECTING THE IMMUNE RESPONSE TO THE MEASBIO VACCINE

Genetic variation is a well-known influence in immune responses to both natural infection and vaccination; variation in genes encoding pathogen receptors, immune signalling molecules and their receptors, or immune regulatory proteins can all affect how efficiently a pathogen can infect a host and how efficiently the host can respond (67).

With regards to MV infection and vaccination, the effects of genetic variation have been studied extensively in populations outside of South Africa, and a variety of significant single nucleotide polymorphisms (SNPs) have been identified in various populations. Both candidate gene studies and genome-wide association studies (GWAS) have identified significant SNPs associated with host immune responses to MV infection or vaccination in multiple genes as reviewed below (53,68). While the functional consequences of these SNPs might not all be known or detailed, there is evidence that their presence has phenotypic associations with serostatus following measles vaccination.

With regards to SNPs in the genes coding for the cell receptors that facilitate MV infection, the *SLAMF1* and *CD46* genes, four SNPs were identified (53,69). Two SNPs in the *SLAMF1* gene, rs164288 and rs3796504, and two SNPs in the *CD46* gene, rs2724384 and rs11118580, were shown to be associated with a significant reduction in measles-specific IgG levels as well as variations in cell mediated immunity (2,4,70–73).

The innate immune system is the body's first immunological response to infection and in turn controls the activation of the adaptive immune system (74). This has been specifically shown in the innate immune reaction to measles, where the innate immune system induces the release of key initiators of the adaptive immune response (40). Therefore, variations in the genes of the

innate immune system that could alter its function can affect the adaptive immune response, which is measured by the antibody response to the vaccine.

Thirteen SNPs in the genes coding for proteins involved in pathogen detection and innate immune system activation were identified; 11 SNPs in the *TLR* genes, one SNP in each of the *MyD88* and *TRIM5* genes which encode signal transduction proteins, and one SNP in the *DDX58* gene, which encodes a RLR used for cytoplasmic viral detection. SNPs in the *TLR2* gene (rs3804100 and rs1816702), *TLR3* gene (rs5743305 and rs3775291), *TLR4* gene (rs5030710, rs11536897, rs4986790, rs4986791, and rs2770150), *TLR5* gene (rs851178), *TLR7* gene (rs864058), and *DDX58* gene (rs669260) were associated with decreased neutralising anti-measles IgG antibody titres post-vaccination with the MMR vaccine (36,53,69,75–78).

The next step where SNPs can make an impact is in the signalling cascade following the activation of the viral detection proteins. SNPs in the *MyD88* gene (rs6853) and *TRIM5* gene (rs7122620), as well as the *IKBKE* SNP rs1539241 and the *MAP3K7* SNP rs791062, have also been associated with decreased neutralising anti-measles IgG antibody titres post-vaccination (53,77). Multiple cytokine SNPs have been found to be associated with the immune response to measles vaccination and MV. The SNPs rs2243248 and rs2069824, in genes *IL-4* and *IL-6* respectively, are associated with a lowered measles-specific IgG antibody titre (53). However, the *IL-2* SNP rs2069763 has been associated with an increased measles-specific cell mediated immune response (31). The *IL-12B* SNPs rs3790567 and rs2546893 have been associated with decreased humoral and cell mediated immunity and increased levels of IL-6 production after MV infection (31,79). Cytokine receptor SNPs in the *IL-12RB1*, *IL-10RB,* and *IFNAR2* genes have also been identified as being significantly associated with measles immune responses. The minor allele of the *IL-10RB* SNP rs2284552 (T) has been associated with increased IL-6 production following MV infection (79). The *IL-12RB1* SNP rs372889 has been shown to produce increased Th1 responses, promote IFN-γ production, and increase cell mediated

immunity (2). Increasing copies of the minor alleles of the *IFNAR2* SNPs rs17860160 and rs2834160 have been associated with decreased IFN- γ secretion after measles vaccination (79).

Variation in the *HLA class I* and *class II* genes are well known to affect immune responses, and thus are the best-known candidate genes affecting most vaccine responses, including to the measles vaccine (2,3). Numerous *HLA class II* alleles in the *DRB1*, *DRP1*, and *DQB1* loci have been significantly associated with various aspects of the immune response to MV infection and measles vaccination (61). For example, the DRB1*0301, DQB1*0201, and DQB1*0201 alleles were significantly associated with IFN-γ production and a strong Th1 response, while the DRB1*0103 and DPB1*0501 alleles were significantly associated with IL-4 production and Th2 responses (61). However, due to the extent of *HLA* variation and the number of *HLA* SNPs required to perform *HLA* allele calling and limitations to the number of SNPs that can be selected for this investigation because of resource limitations, no *HLA* SNPs were selected for this candidate SNP panel.

Several polymorphisms in the vitamins A and D vitamin A and vitamin D receptor genes have been shown to impact measles vaccine responses as are key regulators of immune responses (62). The RXRA protein is an important Vitamin A receptor that forms a heterodimer with the VDR, hence influencing the vitamin D pathway as well as the vitamin A pathway (63). The rs3118523 SNP in the *RXRA* gene was found to be significantly associated with lowered IFN-γ levels in Caucasians (62). A second important receptor in the vitamin A pathway, the nuclear retinoic acid receptor beta (RARB), has been found to contain significant SNPs, including the rs6800566 SNP which has been associated with variations in measles-specific IgG levels and cytokine levels of IL-10 and IFN-α (62).

A GWAS study was conducted in 2017 that identified clusters of 20 SNPs around the *CD46* gene region and nine SNPs around the *IFI44L* gene on chromosome 1 that were significantly

associated to vaccine-induced measles-specific IgG antibody titres, including the previously described rs2724384 SNP in the *CD46* gene (80).

After a literature review, 31 candidate SNPs in 22 genes were identified for possible use in the current study (Table 1). All candidate SNPs were identified in multiple studies, which gives weight to their reported associations.

Most of these studies were completed in Caucasian or ethnically European populations in Europe or North America, with very few being conducted in African populations. South African populations have a great amount of genetic variation and could be genetically distant from the populations studied in previous immunogenetic studies related to measles vaccination (81–83).

The minor allele frequencies (MAFs) of each candidate SNP in Table 1 were checked in the Luhya population in Webuye, Kenya (LWK). It is necessary to check the MAFs of each SNP because a very low MAF (below 5%) means that that SNP is unlikely to be found in a small cohort and is less likely to be associated with more common immunological phenomena in the population. A MAF of zero means that that SNP has not been identified in that population at all, so there would be no point in including it in an explorative candidate SNP study. The LWK population has been used as a proxy population for Southern African populations because the majority of the Southern African populations are classified as South-eastern Bantu-speaking populations, descendant from migrations of Bantu-speaking people from East Africa between 2000 and 1000 years ago (83,84). However, due to the admixture of migratory Bantu-speaking populations with existing Southern African Khoisan-speaking populations and the extended period of geographical isolation from the original East African population, the Southern African population is not genetically identical to the LWK population (85). Therefore, it is becoming increasingly important to generate population genetics data for Southern African populations.

This study aimed to create these immunogenetic data in the Black South African population and analyse the associations between the candidate SNPs and the IgG antibody response to measles vaccination within this population. This study formed a part of a larger study looking at the role of candidate genetic variation in immune responses in Black South African children against multiple routine childhood vaccinations.

Table 1: Candidate SNPs with associations with MV infection or measles vaccination immune response from the literature and MAFs from the LWK[1] population.

Gene	SNP	Allele 1 (minor allele)	Allele 2	MAF (LWK)
SLAMF1	rs164288	A	G	0.056
SLAMF1	rs3796504	T	G	0
CD46	rs2724384	G	A	0.121
CD46	rs11118580	C	T	0
TLR2	rs3804100	C	T	0.061
TLR2	rs1816702	T	C	0.429
TLR3	rs5743305	A	T	0.318
TLR3	rs3775291	T	C	0.035
TLR4	rs5030710	C	T	0.136
TLR4	rs11536897	A	G	0.106
TLR4	rs4986790	G	A	0.096
TLR4	rs4986791	T	C	0
TLR4	rs2770150	G	A	0.136
TLR5	rs851178	G	A	0.157
TLR7	rs864058	A	G	0.279
DDX58	rs669260	C	T	0.187
MyD88	rs6853	G	A	0.298
TRIM5	rs7122620	A	G	0.404
IKBKE	rs1539241	A	G	0.202
MAP3K7	rs791062	C	T	0.141
IL-4	rs2243248	G	T	0.202
IL-6	rs2069824	C	T	0.141
IL-2	rs2069763	A	C	0.126
IL-12B	rs3790567	G	A	0.217
IL-12B	rs2546893	A	G	0.121
IL-10RB	rs2284552	T	G	0.146
IL-12RB1	rs372889	C	T	0.424
IFNAR2	rs17860160	T	G	0
IFNAR2	rs2834160	C	T	0.146
RXRA	rs3118523	G	A	0.394
RARB	rs6800566	A	G	0.187

[1] Luhya population in Webuye Kenya

2. AIMS AND OBJECTIVES

The aim of this study was to characterise the associations between variation in selected candidate genes and the antibody response to the MeasBio vaccine in a cohort of healthy South African children.

Objectives:

- Objective 1: To investigate the association between demographic variables such as weight and gender, and measles vaccine IgG responses measured at 18 months of age.
- Objective 2: To genotype selected SNPs in the cohort by MassARRAY technology, to subsequently characterise frequencies and haplotypes of selected genetic variation in candidate genes in a South African cohort.
- Objective 3: To investigate the association between selected genetic polymorphisms and measles vaccine IgG responses measured at 18 months of age.

3. METHODS

3.1. STUDY COHORT

The study cohort included 283 South African infants presenting for routine immunisations in Soweto, Gauteng, enrolled as part of the Nutrivac study (HREC M180519, clinical trials registration NCT02943902) which assessed the impact of nutritional status, metabolic markers and microbiota on vaccine-induced humoral and cellular immunity in South African children (5). The enrolment criteria stipulated that the infants were 6-18 weeks of age at the time of enrolment, more than 37 weeks gestation at birth and had a birthweight of more than 2499 grams (5). These infants received the MeasBio vaccine at 6 and 12 months of age, as per the standard South African EPI schedule (34). Serum and buccal swap samples were collected by the study team at visit 10 of the study, when the children were 18 months of age, and stored for further testing. This was a cross-sectional study as the samples used to obtain the antibody data were captured at a single point in time, when the participants were 18 months of age.

The sample size was limited by the number of available samples that had complete measles antibody data recorded. This relatively small sample size was also convenient as the study was also limited by resource and funding availability, so a larger cohort might not have been possible. While the sample size is a clear limitation, the study stands as a proof-of-concept investigation and can be used as a viable justification for a larger scale study.

The informed consent and ethics approval for the Nutrivac study included the use of the samples for future genetic studies. The current genetics study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, South Africa (HREC reference number M2111129). See Appendix 2 for the HREC approval certificate for this study, and Appendix 3 for the HREC approval certificate for the Nutrivac study.

3.2. SAMPLE SELECTION

There were 198 buccal swab samples available from the Nutrivac study for use in the current study. DNA was extracted from all available samples. The final sample selection was based on the DNA concentration of the extracted DNA. Only samples with DNA concentrations equal to or greater than 10 ng/µl were selected.

3.3. DNA EXTRACTION AND QUANTIFICATION

DNA extraction from the buccal swabs was performed using the QIAamp Buccal Swab DNA Extraction kit (QIAGEN). The extracted DNA was quantified using a NanoDrop Spectrophotometer. The minimum volume and DNA concentration requirements for the MassARRAY protocol were 10 µl DNA at a concentration of 10 ng/µl. The DNA extraction process yielded 100 µl of eluate, so 10 µl was aliquoted to be sent to Inqaba Biotec. Aliquots from samples with DNA concentrations greater than 25 ng/µl were diluted with distilled water to a concentration of 25 ng/µl.

3.4. MEASLES VACCINE ANTIBODY TITRE DATA

The IgG antibody titres were obtained by enzyme-linked immunosorbent assay (ELISA) performed during the original Nutrivac study (5). Blood samples taken from the participants were centrifuged to extract serum, which was stored at −70ºC at the Respiratory and Meningeal Pathogens Research Unit laboratory (RMPRU), University of the Witwatersrand. This unit has since been renamed as the Vaccines and Infectious Diseases Analytics (VIDA) unit. Measles-specific IgG antibody levels were obtained using a commercial ELISA kit (Enzygnost; Dade Behring), following the manufacturer's instructions. Optical density values were converted to mIU/mL (5). Measles IgG titres were classified as either seronegative (<330 mIU/mL) or seropositive (≥330 mIU/mL) (5). These tests had already been conducted and the data were

provided in a Microsoft Office Excel spreadsheet. For the purposes of this study, cases were defined as seronegative, and controls were defined as seropositive.

3.5. MASSARRAY SNP PANEL DESIGN

In the literature review, a total of 31 SNPs were identified in candidate genes that have been shown to be associated with measles vaccine responses and the immune response to MV in previous studies in other populations (Table 1, section 1.6). Since this study was part of a larger study assessing immunogenetic variation related to multiple vaccines in the School of Molecular and Cell Biology, Faculty of Science, the measles-specific 31 SNP candidate panel was then complied with other candidate panels relevant to other vaccines. Only 95 SNPs in total were chosen for the final panel design due to cost constraints, including only some of the SNPs identified in the measles-specific literature review. Appendix 4 shows the final SNPs analysed in the current study.

The SNPs in the candidate genes selected for this study were chosen using the following process:

A literature review of SNPs in candidate genes related to multiple childhood vaccines was performed, including any SNPs that are known to change the function or activity of selected candidate genes, or were reported to be associated with vaccine or immune related phenotypes in the literature. Candidate genes and SNPs that appeared on more than one candidate list (so SNPs that potentially played a role in immunity to multiple vaccines) were prioritised. For example, certain cytokine and interferon genes were common across multiple candidate panels for different vaccines, particularly since most of the vaccines under consideration were against viral diseases. It is important to note that the prioritisation of the SNPs was conducted considering all the studies involved, therefore some of the SNPs proposed for this current measles vaccine study were excluded from the final panel.

We checked the MAFs of the SNPs in a proxy population closely related to the South African Black population, because South African genetic data were not easily available. Therefore, we used data from the 1000 Genomes Project from the LWK population, available through https://www.ensembl.org/index.html. SNPs with MAFs of greater than 0.05 in the LWK were prioritised for use in the current study.

The linkage disequilibrium (LD) pattern of selected candidate SNPs was analysed in the LWK population using 1000 Genomes data and the LDlink tool at https://ldlink.nci.nih.gov/?tab=ldmatrix (86). R^2 was considered as a more sensitive measure of LD than D′. SNPs that were not in LD with other SNPs in the candidate lists (i.e., not usually inherited together in a haplotype, or low R^2 pairwise values) were prioritised (R^2<0.2).

The SNP MassARRAY software was used to check if SNPs could be included in a multiplex panel; for example, in cases where two SNPs were in close enough proximity so that the extension primers could interfere with each other, the less important SNP was excluded. This step was performed by Inqaba Biotec, who performed the MassARRAY genotyping assays.

3.6. DNA GENOTYPING BY MASSARRAY

The DNA extracted from the samples was sent to Inqaba Biotec (Pretoria) for genotyping using a MassARRAY SNP panel. This method was chosen according to project budget. The assay is cost-effective since up to 30 SNPs can be multiplexed for genotyping in a single well. Before genotyping the samples for this study, test runs were executed using DNA with known genotypes to test primer concentrations and accuracy. This validation step was performed by Inqaba Biotec. The genotypic data were returned in a Microsoft Office Excel spreadsheet.

The Sequenom MassARRAY iPLEX assay used a 3-stage process to genotype samples: firstly a polymerase chain reaction (PCR) step was used to amplify the target locus, and then an extension PCR was run on the SNPs, and finally matrix-assisted laser desorption/ionization—

time of flight (MALDI-TOF) mass spectrometry was used to genotype the SNPs (Figure 3) (87,88).

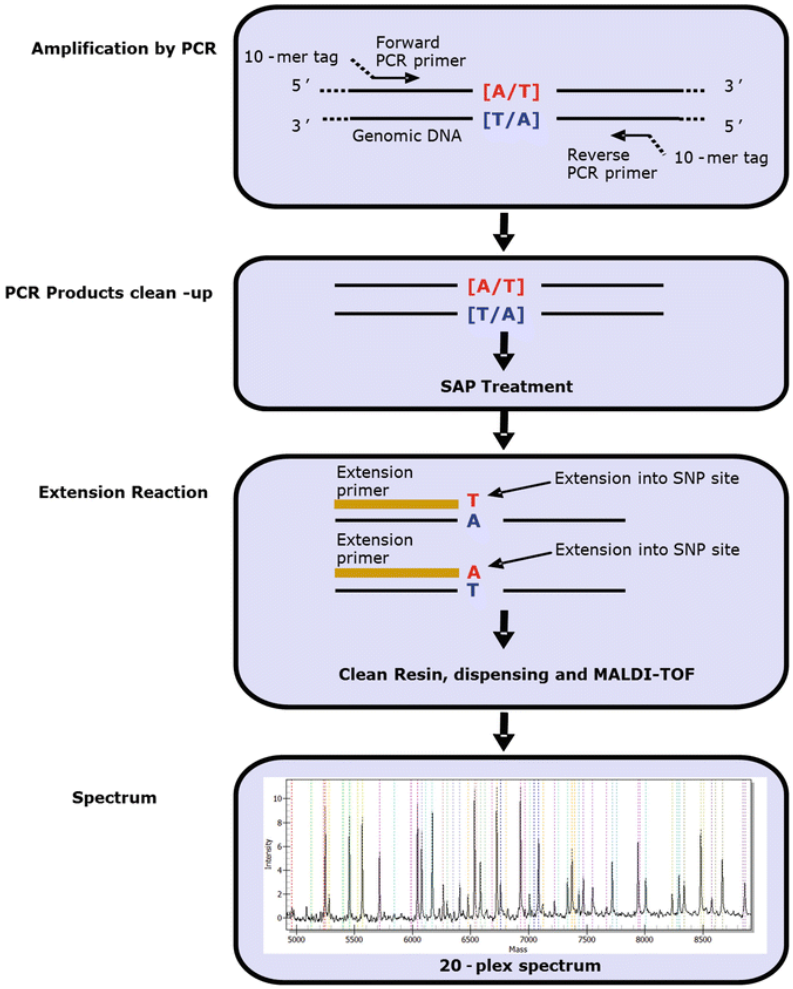

Figure 6: Overview of the steps involved in MassARRAY genotyping (87). The cleaning step between the initial PCR and the extension PCR uses shrimp alkaline phosphatase (SAP) to neutralise unbound nucleotides.

In the extension PCR step, an extension primer was annealed that ended one base upstream of the target SNP, and then in a single PCR cycle, a single complimentary, mass-modified dideoxynucleotide triphosphate (ddNTP) was added onto the polymorphic base (89). The ddNTPs do not have a 3′ hydroxyl group, so additional bases cannot be added to them; hence they are known as terminator bases (87).

After the extension PCR step, the multiplex analyte was dispensed onto a metal plate called a SpectroCHIP Array, that has matrix material to protect the nucleic acids from damage during

the desorption and ionisation step of the MALDI-TOF assay (87). Nucleic acids can experience degradation under the desorption and ionisation conditions employed by standard mass spectrometry, but the MassARRAY uses a soft ionisation technique in which the nucleic acids are embedded in a matrix of 3-hydroxypicolinic acid that absorbs excess energy from the ultra-violet laser applied to the sample and thus protects the nucleic acids from damage (90). After the desorption and ionisation process, a strong electrostatic field was activated, which propelled the ionised and mass-modified DNA molecules up a vacuum tube; the lighter molecules hit the top first and thus a detector at the top could genotype the SNPs by evaluating which ddNTP was attached to the primer using the time of flight of the molecule (87). The resulting data were captured in a Microsoft Office Excel spreadsheet as SNP calls per sample.

3.7. DATA ANALYSIS

The measles IgG antibody data (de-identified) and demographic data were provided by the RMPRU/VIDA in Microsoft Office Excel spreadsheet. IgG titres at 18 months of age were used to classify participants as seronegative (cases) or seropositive (controls). We analysed demographic data such as sex, ethnicity, birthweight, and weight at 18 months of age to identify any significant differences between the case and controls. Statistical tests including the Fisher's exact test, Mann-Whitney U test, and Jarque-Bera normality test were performed, as appropriate, using Microsoft Excel (version 2112). The ethnicity and sex of the participants was recorded in the data provided from the Nutrivac study and were not confirmed with genotypic evidence in the current study. While this is a limitation of the study, the scope of the current study was limited by time and resources. Therefore, it was not possible to include Y chromosome SNPs to check sex, and the genotype results supplied by Inqaba Biotec could not be used to infer sex from the X chromosome genotypes, although PLINK would have returned a "haploid heterozygosity" error if any male participants had a heterozygous genotype for an X chromosome SNP. The ethnicities reported in the Nutrivac study data were assumed to be

accurate, and it was not possible to confirm the ethnicity nor investigate the cryptic relatedness or divergent ancestry of the participants in the timeframe available for the study.

The data from the genotyping for each sample were captured in an Excel spreadsheet and compared to the IgG antibody data using a unique participant identifier. The genetic differences between cases and controls were examined, including analyses of allele, genotype, and haplotypes associations. Statistical tests including the chi-squared test and Fisher's exact test were performed, as appropriate, in PLINK (version 1.07), which is a specialised software for analysing associations between genetic variation and either case-control phenotypes or quantitative phenotypes (91).

The genotype data were filtered through various quality control (QC) procedures. SNPs that failed genotyping in more than 15% of individuals were excluded. Individual samples with more than 10% missing genotype data were excluded. SNPs which failed the Hardy-Weinberg Equilibrium (HWE) test in cases only were also excluded ($P<0.005$).

Univariate analysis was conducted to examine each SNP against case-control status. Candidate SNPs were assessed for significant associations with case-control status using the chi-squared test. The SNPs were each tested using four models: allelic, recessive, dominant, and genotypic (codominant). Allelic association tested the MAF of each SNP between the cases and controls to establish whether any significant differences exist. The recessive, dominant, and genotypic models tested frequencies of genotypes for each SNP between cases and controls, where each genotype was tested three times using each of the models according to the models described in PLINK and shown below, if D is the minor allele (and d is the major allele):

Allelic:	D versus d
Dominant:	(DD, Dd) versus dd
Recessive:	DD versus (Dd, dd)
Genotypic:	DD versus Dd versus dd

The recessive and dominant models generated a 2x2 contingency table of phenotype-by-genotype with one degree of freedom (DF), and the Fisher's exact test or chi-squared test were used to evaluate significance. The genotypic, or codominant, model generated a 3x2 contingency table of phenotype-by-genotype with two DF, meaning that a greater chi-squared value is required to show significance. P values less than 0.05 were considered significant. The Bonferroni correction was used to adjust the P values in order to mitigate the risk of false positives that increases with multiple comparisons (92). Therefore, each P value was multiplied by the total number of SNPs tested after QC, with 0.05 still being the significance threshold.

PLINK supplied odds ratios (ORs) for the SNPs significantly associated through the allele model. ORs for significantly associated SNPs in the genotype models were manually calculated using Microsoft Excel. ORs whose 95% confidence intervals (CI) crossed 1 were not significant.

4. RESULTS

4.1. DNA EXTRACTION AND QUANTIFICATION

The extracted DNA concentration ranged from zero to 92.7 ng/µl with a mean of 13.02 ng/µl. Of the 198 available frozen buccal swab samples, 11 samples failed during the extraction optimisation process and yielded no DNA, and a further 62 samples yielded DNA with a concentration below 10 ng/µl and thus could not be used for MassARRAY genotyping. Therefore, DNA from 125 samples were aliquoted at concentrations of 10-25 ng/µl and sent to Inqaba Biotec for MassARRAY genotyping. The A260/A280 ratios for these samples ranged from 1.57 to 2.51 with an average of 1.93, therefore the extracted DNA could be considered pure and contaminant free.

4.2. MEASLES VACCINE IGG ANTIBODY TITRES AT 18 MONTHS OF AGE AND ASSOCIATION WITH DEMOGRAPHIC VARIABLES

The anti-measles IgG antibody titres at 18 months of age for each of the 125 participants (corresponding to six months after the second dose of the MeasBio vaccine) ranged from 75 mIU/mL to 9029.04 mIU/mL with a median of 1025.23 mIU/mL. The IgG antibody data were not normally distributed using normality tests including the Jarque-Bera test, Anderson Darling test, and Shapiro-Francia test.

When these were categorised as seronegative (IgG titres <330 mIU/mL) or seropositive (IgG titters ≥330 mIU/mL), 17 (13.6%) were seronegative and 108 (86.4%) were seropositive. Pre-vaccine IgG antibody data were not available for all participants.

The cases (seronegative) and controls (seropositive) were analysed for significant differences in demographic data such as sex, ethnicity, birthweight, and weight at 18 months of age (Table 2). The birthweights and weights at 18 months of age were also shown to be not normally distributed using the Jarque-Bera, Anderson Darling, and Shapiro-Francia normality tests. The

demographic variables of sex, ethnicity, birthweight, and weight at 18 months of age were not significantly associated with case-control status, and thus no multivariate analysis was required.

Table 2: Association between demographic variables and measles IgG antibody serostatus at 18 months of age.[1]

Variable		Controls (n=108, 86.4%)	Cases (n=17, 13.6%)	Statistical test used	P value
Sex	Female	55 (44.0%)	9 (7.2%)	Chi-squared test	0.877
	Male	53 (42.4%)	8 (6.4%)		
Ethnicity	Black	106 (84.8%)	17 (13.6%)	Fisher's exact test	1
	Mixed race	2 (1.6%)	0 (0%)		
Median Birthweight (g), (min-max)		3225 (2505-5400)	3195 (2715-4005)	Mann–Whitney U test	0.659
Median weight at 18 months (kg), (min-max)		10.9 (7.9-16)	10.2 (8.5-12.7)	Mann–Whitney U test	0.368

[1] Control are seropositive participants (IgG ⩾ 330 IU/ml), and Cases are seronegative participants (IgG < 330 IU/ml).

4.3. LD ANALYSIS AND SNP SELECTION

Four of the 31 measles-specific SNPs were excluded from the preliminary panel based on their MAF scores in the LWK population, leaving 27 SNPs in the measles-specific candidate SNP panel. The 27 measles-specific SNPs identified through literature review were analysed for LD in the LWK population. Heatmaps showing the pairwise R^2 and D′ values were drawn using the LDmatrix Tool available at https://ldlink.nci.nih.gov/?tab=ldmatrix. The heatmaps are shown in Appendix 3. When pairwise R^2 values were considered, no LD was shown between the measles-specific SNPs, and all candidate SNPs were therefore retained for further consideration for inclusion in the final panel.

These LD results were then combined with other similar results from the other vaccine specific candidate panels relevant to other studies using this cohort. Due to funding restrictions, all the candidate SNP panels for each study were curated and collated to be run as one panel. Since

SNPs that influenced multiple phenotypes were prioritised, the SNPS that only influenced measles vaccine (e.g., in CD46) were excluded in the final panel.

After considering all SNP prioritisation steps, the final panel for our research lab for multiple vaccines consisted of 95 SNPs genotyped in three multiplexes. However, the *ABO*, *FUT2*, and *FUT3* SNPs were excluded for analysis for this study as there is no literature evidence or plausible roles for the involvement of these candidate genes in immune response to MV vaccination. Therefore, a total of 77 SNPs involved in six immune pathways were analysed for this study (Table 3). A full list of the 77 candidate SNPs and their associations with immune and/or vaccine related phenotypes is included in Appendix 4.

Few *HLA* SNPs were included in the final panel as HLA typing was not an objective in this study. The *HLA* SNPs included were present in the panel due to their effect on HLA-DP expression levels. These SNPs were not associated with MV immune responses in the literature but were associated with Hepatitis B vaccine response. Since other HLA-DP genetic variation has been shown to influence MV immune responses, these SNPs affecting HLA-DP expression were retained in the panel and were used in the measles-specific analysis (61).

Table 3: Summary of the pathways and number of SNPs analysed in this study.

Pathway	Genes	Number of SNPs
HLA	*HLA-DP, DO*	8
Vitamin D	*VDBP, VDR, CYP21R, CYP24A1*	6
TLR	*TLR2, 3, 4, 7, 8, 9*	19
Cytoplasmic sensors	*IFIH1 (encodes MDA5) DDX58 (encodes RIG1) MAVS*	10
Signalling pathways	*MYD88, STING, IRF7*	5
IFN type I and type III pathways and cytokines	*IFNAR1, IFNAR2, IL-10, IL-28B, OAS1*	9
IFN type II pathway and cytokines	*IFNG, IFNGR1 IL-12A, IL-12B, IL-12RB1, IL-12RB2, IL-4, IL-4R IL1B*	20
Total		77

4.4. SNP PANEL TROUBLESHOOTING

Some difficulties were experienced during the final MassARRAY panel optimisation. The average PCR product size was increased to 200 basepairs (bp) – 250 bp to accommodate all the SNPs. Some SNPs in close proximity shared initial PCR products and then used different extension primers. For three SNPs, there were proximal SNPs in the primer regions for which we used the major alleles in primer design. Several SNPs required the design of new MassARRAY primer sets after they failed in initial experiments. The MassARRAY genotyping required multiple validation rounds but was well optimised. A few SNPs required manual calls where the MassARRAY system was not able to automatically call the genotype.

4.5. QUALITY CONTROL OUTCOMES OF THE GENETIC DATA

Four SNPs had low call-rates in the MassARRAY genotyping, with the *IL1B* SNP rs1143627 and the *IL-2* SNP rs2069763 failing in more than 15% of the 125 samples, and SNPs rs2770150 and rs5743836, in the *TLR4* and *TLR9* genes respectively, failing in all samples. Therefore, these SNPs were excluded due to SNP missingness. The *TLR3* SNP rs3775291 was excluded because it showed no variation in the cohort and thus had a MAF of zero, despite its MAF in LWK population was previously noted as 3.5%. No individuals were removed due to genotype missingness. Therefore, after QC, the final cohort size was 125 participants and with 72 SNPs being analysed.

4.6. MAFS AND HAPLOTYPES IN THE SOUTH AFRICAN TEST COHORT

The MAFs for the remaining 72 candidate SNPs were calculated using PLINK (Table 4). The MAFs ranged from 0.012 (rs3804100, *TLR2*) to 0.479 (rs2070874, *IL-4*).

The MAFs of the candidate SNPs in the South African population were compared with the data from the Luhya population in the 1000 Genomes Project (Table 4). A total of 24 of the 72 candidate SNPs were found to have MAFs in the South African population that were significantly different from the corresponding MAFs in the LWK population.

In order to identify the haplotypes present within the cohort and their frequencies, the SNPs were separated by chromosome and then every possible combination of alleles on each chromosome were calculated and counted using PLINK. The haplotypes formed by the major allele for every SNP on a given chromosome were labelled as being wildtype, and thus any haplotype containing at least one minor allele for any SNPs on that chromosome were not regarded as wildtype. Therefore, a wildtype haplotype was defined as being the haplotype containing no minor alleles of the SNPs located on that chromosome.

Table 4: MAFs for the 72 candidate SNPs retained after QC in the South African cohort compared to the MAF data from the LWK population. Significant P values are shown in bold[1].

Chr.	Gene	SNP	A1	A2	MAF (LWK)	MAF (SA)	P value
1	*IL-12RB2*	rs3790567	G	A	0.217	0.096	**0.001**
1	*IL-10*	rs1800871	A	G	0.389	0.352	0.421
1	*IL-10*	rs1800896	C	T	0.328	0.348	0.662
2	*IL1B*	rs1143634	A	G	0.111	0.12	0.771
2	*IFIH1*	rs1990761	A	G	0.384	0.368	0.731
2	*IFIH1*	rs1990760	T	C	0.101	0.08	0.439
2	*IFIH1*	rs3747517	T	C	0.465	0.339	**0.007**
2	*IFIH1*	rs10930046	C	T	0.424	0.42	**0.001**
3	*MYD88*	rs6853	G	A	0.298	0.44	**0.002**
3	*TLR9*	rs352139	T	C	0.384	0.356	0.544
3	*TLR9*	rs187084	G	A	0.328	0.367	0.395
3	*IL-12A*	rs568408	A	G	0.192	0.236	0.261
4	*GC (VDBP)*	rs2282679	G	T	0.056	0.057	0.951
4	*TLR2*	rs3804100	C	T	0.061	0.012	**0.005**
4	*TLR3*	rs5743305	A	T	0.318	0.236	0.052
4	*TLR3*	rs3775296	A	C	0.131	0.132	0.983
4	*TLR3*	rs10025405	G	A	0.278	0.176	**0.010**
5	*IL-4*	rs2243248	G	T	0.202	0.3	**0.018**
5	*IL-4*	rs2243250	C	T	0.192	0.286	**0.021**
5	*IL-4*	rs2070874	C	T	0.419	0.479	0.203
5	*IL-4*	rs2227284	G	T	0.02	0.032	0.442
5	*STING / TMEM173*	rs13181561	A	G	0.444	0.456	0.807
5	*STING / TMEM173*	rs7380824	T	C	0.338	0.264	0.089
5	*STING / TMEM173*	rs1131769	T	C	0.086	0.076	0.715
5	*IL-12B*	rs3212227	G	T	0.409	0.341	0.154
5	*IL-12B*	rs3213093	T	C	0.399	0.375	0.327
5	*IL-12B*	rs2546893	A	G	0.121	0.288	**0.001**
6	*HLA-DOB*	rs2857130	T	A	0.384	0.412	0.546
6	*HLA-DOB*	rs2857127	A	G	0.384	0.412	0.546
6	*HLA-DOB*	rs2857114	G	A	0.419	0.456	0.436
6	*HLA-DPB1*	rs7770370	A	G	0.505	0.372	**0.005**
6	*HLA-DPB1*	rs931	G	A	0.545	0.398	**0.002**
6	*HLA-DPB1*	rs9277533	C	T	0.48	0.411	0.148
6	*HLA-DPB1*	rs9277534	A	G	0.48	0.356	**0.008**
6	*HLA-DPB1*	rs9277536	C	T	0.48	0.364	**0.014**

Table 5: MAFs for the 72 candidate SNPs retained after QC in the South African cohort compared to the MAF data from the LWK population (continued)

6	*IFNGR1*	rs7749390	A	G	0.46	0.456	0.940
9	*DDX58*	rs55789327	A	G	0.121	0.107	0.651
9	*DDX58*	rs669260	C	T	0.187	0.252	0.100
9	*DDX58*	rs10813831	A	G	0.162	0.212	0.177
9	*TLR4*	rs5030710	C	T	0.136	0.272	**0.001**
9	*TLR4*	rs4986790	G	A	0.096	0.032	**0.005**
9	*TLR4*	rs11536897	A	G	0.106	0.076	0.268
11	*IRF7*	rs936470	G	C	0.207	0.108	**0.004**
11	*CYP2R1*	rs10741657	A	G	0.182	0.198	0.674
12	*VDR*	rs731236	G	A	0.268	0.224	0.285
12	*VDR*	rs2228570	A	G	0.172	0.169	0.947
12	*VDR*	rs11568820	C	T	0.141	0.173	0.359
12	*IFNG*	rs2069727	C	T	0.131	0.18	0.161
12	*IFNG*	rs1861493	G	A	0.091	0.032	**0.013**
12	*IFNG*	rs1861494	C	T	0.101	0.06	0.108
12	*OAS1*	rs10774671	A	G	0.389	0.412	0.620
16	*IL-4RA*	rs1805010	A	G	0.47	0.406	0.177
16	*IL-4RA*	rs1805015	T	C	0.49	0.444	0.333
16	*IL-4RA*	rs1801275	A	G	0.157	0.168	0.745
19	*IL-12RB1*	rs372889	C	T	0.424	0.471	0.329
19	*IL-28B*	rs8103142	T	C	0.424	0.404	0.666
19	*IL-28B*	rs8099917	G	T	0.076	0.048	0.220
20	*MAVS*	rs17857295	G	C	0.066	0.096	0.247
20	*MAVS*	rs7262903	A	C	0.177	0.136	**0.001**
20	*MAVS*	rs7269320	T	C	0.298	0.28	0.676
20	*CYP24A1*	rs6013897	A	T	0.288	0.244	0.295
21	*IFNAR2*	rs1051393	G	T	0.192	0.168	0.512
21	*IFNAR2*	rs2834160	C	T	0.146	0.152	0.870
21	*IFNAR1*	rs2843710	G	C	0.247	0.368	**0.006**
21	*IFNAR1*	rs2257167	C	G	0.096	0.198	**0.003**
X	*TLR7*	rs179008	T	A	0.091	0.102	0.764
X	*TLR7*	rs864058	A	G	0.279	0.423	**0.006**
X	*TLR7*	rs3853839	G	C	0.214	0.101	**0.003**
X	*TLR7*	rs5935438	C	G	0.416	0.339	0.143
X	*TLR8*	rs3764880	G	A	0.331	0.204	**0.008**
X	*TLR8*	rs5744077	G	A	0.117	0.169	0.171
X	*TLR8*	rs2159377	T	C	0.286	0.164	**0.007**

[1]A1 refers to the minor allele.

A total of 163 haplotypes were identified in the 14 chromosomes included in the panel (Table 5). For a full list of all 163 haplotypes identified, their frequencies, and the genes and SNPs they contain, see Appendix 5.

Table 6: Summary of the haplotype data found in the South African cohort.

Chr.	No. of haplotypes	SNPs	Genes	Wildtype haplotype	Frequency of wildtype haplotype
1	5	rs3790567, rs1800871, rs1800896	*IL-12RB2, IL-10*	AGT	0.298
2	9	rs1143634, rs1990761, rs1990760, rs3747517, rs10930046	*IL1B, IF1H1*	GGCCC	0.346
3	11	rs6853, rs352139, rs187084, rs568408	*MyD88, TLR9, IL-12A*	ACAG	0.123
4	9	rs2282679, rs3804100, rs5743305, rs3775296, rs10025405	*GC (VDBP), TLR2, TLR3*	TTTCA	0.533
5	31	rs2243248, rs2243250, rs2070874, rs2227284, rs13181561, rs7380824, rs1131769, rs3212227, rs3213093, rs2546893	*IL-4, STING/TMEM173, IL-12B*	TTTTGCCTCG	0.121
6	15	rs2857130, rs2857127, rs2857114, rs7770370, rs931, rs9277533, rs9277534, rs9277536, rs7749390	*HLA-DOB, HLA-DPB1, IFNGR1*	AGAGATGTG	0.175
9	12	rs55789327, rs669260, rs10813831, rs5030710, rs4986790, rs11536897	*DDX58, TLR4*	GTGTAG	0.333
11	4	rs936470, rs10741657	*IRF7, CYP21R*	CG	0.719
12	18	rs731236, rs2228570, rs11568820, rs2069727, rs1861493, rs1861494, rs10774671	*VDR, IFNG, OAS1*	AGTTATG	0.211
16	6	rs1805010, rs1805015, rs1801275	*IL-4RA*	GTG	0.206
19	6	rs372889, rs8103142, rs8099917	*IL-12RB1, IL-28B*	TCT	0.292
20	8	rs17857295, rs7262903, rs7269320, rs6013897	*MAVS, CYP24A1*	CCCT	0.492
21	10	rs1051393, rs2834160, rs2843710, rs2257167	*IFNAR1, IFNAR2*	TTCG	0.438
X	19	rs179008, rs864058, rs3853839, rs5935438, rs3764880, rs5744077, rs2159377	*TLR7, TLR8*	AGCGAAC	0.069

4.7. ASSOCIATIONS BETWEEN MEASLES VACCINE IGG ANTIBODY TITRES AND CANDIDATE SNPS

Six SNPs were found to be significantly associated with IgG seroresponse after measles vaccination (P<0.05) (Table 6).

In the allelic model, minor alleles of the SNPs *IL-4RA* rs1801275 (P=0.005), *IL-4RA* rs1805015 (P=0.028), and *IL-4* rs2243248 (P=0.036) showed significant differences between cases and controls. The minor alleles of the rs1801275 (A) and rs2243248 (G) SNPs were significantly more frequent in seropositive controls than in the seronegative cases, and therefore showed moderately protective associations against low measles-specific antibody titres with ORs of 0.060 (95% CI: 0.004 - 0.990) and 0.360 (95% CI: 0.134 - 0.969), respectively. However, the rs1805015 SNP had an OR of 2.262 (95% CI: 1.076 - 4.753), indicating that this SNP associated with susceptibility to measles seronegativity post vaccination.

In the genotype models, the minor A allele of the *IL-12B* SNP rs2546893 showed significant P value associations through both the recessive and genotypic (codominant) models. The OR for the recessive model of rs2546893 was 3.846 (95% CI: 1.015 - 14.573). Therefore, the recessive AA genotype was associated with low measles-specific antibody titres. The genotypic model showed ORs of 2.750 (95% CI: 0.694 – 10.892) for the minor allele homozygous genotype (AA), and 0.367 (95% CI: 0.095 – 1.413) for the heterozygous genotype (AG). The 95% CI for these ORs crossed 1, suggesting that the genotypic model for this SNP might not be statistically significant.

The minor C allele of the *IFNAR2* SNP rs2834160 was also associated with the antibody response to measles vaccination through both the recessive and genotypic models. The OR corresponding to the recessive model was 14.267 (95% CI: 1.218 - 167.084), indicating a very strong relationship between the C allele and seronegative status. The OR for the genotypic

models were still high, with OR=11.846 (95% CI: 1.001 – 140.246) for the minor allele homozygous genotype (CC) but was opposite in direction 0.395 (95% CI: 0.084 – 1.856) for the heterozygous CT genotype with a 95% CI that crossed 1. Together, this evidence supported the suggestion that the rs2834160 SNP has a strong association with seronegative status through recessive CC genotype.

SNP rs10774671 (*OAS1*) was shown to be significantly associated in both the dominant and genotypic (codominant) models. The dominant model of rs10774671 association returned an OR of 0.295 (95% CI: 0.103 - 0.843), indicating a protective relationship (i.e., more frequent in seropositive controls). The genotypic association of this SNP returned an OR of 0.217 (95% CI: 0.063 – 0.747) for the AG genotype (heterozygous), supporting a role for rs10774671 SNP (*OAS1*) with protective relationship against seronegative measles IgG titres. However, the 95% CI of the OR of 0.565 (95% CI: 0.137 – 2.332) for the AA genotype (minor allele homozygous) crossed 1 and therefore this model was not supported.

After applying the Bonferroni single-step adjustment to each p-value, where each p-value was multiplied by the number of SNPs tested, all the p-values were no longer below 0.05 and therefore lost significance.

Table 7: SNPs significantly associated in allelic or genotype models with Measles IgG seronegativity post-vaccination.

Chr.	SNP	Gene	A1	A2	Model	CHISQ	DF	P value	OR (95% CI[1])
12	rs10774671	*OAS1*	A	G	Dominant	5.611	1	0.018	0.295 (0.103 - 0.843)
12	rs10774671	*OAS1*	A	G	Genotypic[2]	6.578	2	0.037	AA: 0.565 (0.137 – 2.332) AG: 0.217 (0.063 – 0.747)
16	rs1801275	*IL-4RA*	A	G	Allelic	7.946	1	0.005	0.060 (0.004 - 0.990)
16	rs1805015	*IL-4RA*	C	T	Allelic	4.807	1	0.028	2.262 (1.076 - 4.753)
5	rs2243248	*IL-4*	G	T	Allelic	4.383	1	0.036	0.360 (0.134 - 0.969)
5	rs2546893	*IL-12B*	A	G	Recessive	4.399	1	0.036	3.846 (1.015 - 14.573)
5	rs2546893	*IL-12B*	A	G	Genotypic	6.36	2	0.042	AA: 2.750 (0.694 – 10.892) AG: 0.367 (0.095 – 1.413,)
21	rs2834160	*IFNAR2*	C	T	Recessive	7.367	1	0.007	14.267 (1.218 - 167.084)
21	rs2834160	*IFNAR2*	C	T	Genotypic	8.716	2	0.013	CC: 11.846 (1.001 – 140.246) CT: 0.395 (0.084 – 1.856)

[1]CI: confidence interval

[2]Note that for the genotypic models, the minor allele homozygous and heterozygous genotypes were compared to the major allele homozygous genotype to obtain the ORs

Haplotypes of SNPs on the same chromosome were also tested for significant associations using the chi-squared test. Three significant haplotypes were identified on chromosomes 5, 16, and 20, including ten, three, and four SNPs respectively (Table 7).

The significant haplotype in chromosome 5 (P=0.006) consisting of 10 SNPs contained the minor alleles of four SNPs: rs2243250 (*IL-4*), rs2070874 (*IL-4*), rs13181561 (*STING/TMEM173*), and rs2546893 (*IL-12B*). The minor A allele of the *IL-12B* SNP rs2546893 was also found to be associated with measles antibody responses through both the recessive and genotypic models. The *IL-4* minor alleles present in this haplotype were not the same as the *IL-4* minor allele that was significant in allele analysis

The significant haplotype in chromosome 16 (P=0.015) consisting of three SNPs contained the minor allele (A) of one SNP: the *IL-4RA* rs1801275 SNP, which was also associated with antibody titres in an allelic model.

The significant haplotype in chromosome 20 (P=0.014) consisting of four SNPS contained the minor alleles of two SNPs: the *MAVS* SNP rs17857295 and the *CYP24A1* SNP rs6013897, neither of which were identified as significant in the univariate allele/genotype SNP association tests. However, all P values lost significance after the Bonferroni adjustment was applied (all P values became greater than 0.05).

Table 8: Haplotypes significantly associated with Measles IgG seronegativity post-vaccination. Note that minor alleles within these haplotypes and their corresponding SNP names are shown in red.

Chr.	Haplotype	Genes	SNPs	Frequency (Cases)	Frequency (Controls)	CHISQ	DF	P value
5	TCCTACCTCA	*IL-4* *STING / TMEM173*	rs2243248 rs2243250 rs2070874 rs2227284 rs13181561 rs7380824 rs1131769 rs3212227 rs3213093 rs2546893	0.081	0.007	7.705	1	0.006
16	GTA	*IL-4RA*	rs1805010 rs1805015 rs1801275	0	0.151	5.881	1	0.015
20	GCCA	*MAVS* *CYP24A1*	rs17857295 rs7262903 rs7269320 rs6013897	0.124	0.031	6.034	1	0.014

5. DISCUSSION

In this study we explored patterns of genetic variation in selected genes related to immune function and characterised the associations between variation in selected candidate genes and the antibody response to the MeasBio vaccine in a cohort of healthy South African children. We genotyped a total of 77 SNPs in 125 samples, which included 17 seronegative individuals (defined as measles-specific IgG titre <330 mIU/mL) and 108 seropositive individuals (defined as measles-specific IgG titre ≥330 mIU/mL), although five SNPs were excluded after QC filters were applied to the genetic data.

The seropositivity rate of 86.4% observed in our cohort is similar to that noted in other populations (33,93,94). We employed a high threshold IgG titre (330 mIU/mL) to define serostatus: this was the threshold used in the Nutrivac study from which our cohort was sourced, and there has been much change over time regarding what an appropriate correlate of protection for measles is (5,57). Previous studies have used seroconversion thresholds ranging from 120 to 200 mIU/mL, although it has been acknowledged that there is little evidence to support a minimum of 120 mIU/mL as protective. Therefore, the decision was made to employ the same high threshold as the Nutrivac study.

In this study, no demographic variables were found to be significantly associated with seronegative measles-specific IgG titres, including sex, ethnicity, birthweight, or weight at 18 months of age. Previous studies have also reported no associations between demographic variables such as ethnicity or sex and serostatus following measles vaccination (34,94). However, other studies have reported significant associations between the demographic variables and measles immune responses (32,33). As the study is aimed at investigating genetic variations and measles antibody responses in Black South African children, the two participants identified as being mixed race should have been removed from the cohort. Further investigation into the relationship between these demographic factors and measles vaccine antibody

responses is required in a larger cohort of the South African population to characterise these relationships.

We investigated candidate SNPs in genes governing viral infection, viral detection, and various signalling pathways within the innate immune system. Even though the measure of measles vaccine success used in this study is solely the measles-specific IgG antibody titre, which is purely an adaptive immune response, all the candidate SNPs selected for investigation in this study were related to innate immune responses. This is appropriate since the outcome of the adaptive immune response is entirely dependent on the direction of the innate immune response that activates the adaptive immune system.

The MAFs and haplotype frequencies are of note as these contribute to the novel understanding of these in the South African population. The deviation of the MAFs in the South African compared to the LWK populations indicate that the LWK population is not, in fact, an ideal proxy population for the South African population and more population genetics data for the South African population must be generated for use in future studies. Various genetic studies in the South African population have highlighted the genetic diversity of the South African population and the fact that it is not appropriate to use proxy populations for accurate inferences regarding the South African population (83,95,96). For future studies, it would increase the rigour of the findings if the MAFs of the South African populations were compared to other populations beyond the LWK population.

The haplotype frequencies also provide insight into the immune profile of the South African population when one considers the genes and functional SNPs contained within the haplotypes and the high frequencies of polymorphic haplotypes that occurred in the cohort. Interestingly, the wildtype haplotypes on chromosomes 1, 3, 16, and X were not the most common haplotypes in those loci. Furthermore, the wildtype haplotypes on chromosomes 2, 5, 6, 9, 12, 19, 20, and

21 had frequencies below 50%, despite being the most common haplotypes, which also indicates that these loci were highly polymorphic.

We typed two SNPs in the promoter region of the *IL-10* gene on chromosome 1, rs1800896 and rs1800871, which are known to affect IL-10 expression. The rs1800896 C allele is associated with high IL-10 expression and the rs1800871 A allele is associated with low IL-10 expression (97). Haplotypes containing the rs1800896 C allele occurred in 34.8% of the samples, and haplotypes with rs1800871 A allele were found in 35.2% of the samples. This suggests that roughly one third of the Black South African population might have genetic susceptibility for low IL-10 expression levels.

On the X chromosome, we typed two SNPs in the *TLR7* gene that have known functional consequences affecting the expression and processing of TLR7. The T allele of the rs179008 SNP (MAF=10.2%) is a non-synonymous substitution that causes a structural change in the N-terminal of the TLR7 protein, which is thought to affect the way that this protein is processed by the endoplasmic reticulum after being translated, although the consequence of this altered processing is not clear (98). The G allele of the rs3853839 SNP has been shown to increase TLR7 expression (98). However, X chromosome haplotypes containing the G allele of the rs3853839 SNP only occurred in 9.6% of the samples genotyped, so this suggests that TLR7 expression could be increased in only roughly 10% of the Black South African population.

The haplotype on chromosome 12 contained numerous functionally consequential SNPs. The minor alleles of the rs10774671 (*OAS1*), rs731236 (*VDR*), rs2228570 (*VDR*), rs11568820 (*VDR*) all have functional effects on their respective genes. The minor A allele of the *OAS1* rs10774671 SNP results in decreased enzymatic activity of the OAS1 protein, which weakens the innate immune response to viral RNA (99). Haplotypes containing this allele occurred at a frequency of 39%, suggesting that a large percentage of the Black South African population could have decreased OAS1 activity. The minor alleles of the *VDR* SNPs rs731236 (G) and

rs11568820 (C) result in decreased expression of VDR, and rs2228570 (A) results in decreased affinity of VDR to its ligand (100,101). Haplotypes containing the minor A, G, and C alleles of the rs731236, rs11568820, and rs2228570 SNPs respectively occur at frequencies of 19.3%, 14.1%, and 14.8% respectively. This suggests that the VDR component of the vitamin D pathway is unaffected in the majority of Black South Africans.

The major T allele of the rs1861494 SNP in the *IFNG* gene has been associated with increased IFN-γ expression (102). Haplotypes containing the T allele of this SNP occur at a cumulative frequency of 91% within our cohort, suggesting that increased IFN-γ production could occur in a very high proportion of the Black South African population. However, SNPs in each of the *IFNAR1* and *IFNAR2* genes on chromosome 21 have functional consequences that lower sensitivity to IFN signalling. The *IFNAR1* SNP rs2843710 results in decreased transcription and therefore decreased IFN-I signalling (103). Haplotypes containing this functionally consequential SNP were found in 36.8% of the cohort. The *IFNAR2* SNP rs2834160 has been associated with decreased IFN-γ production in a dose-dependent manner (79). Haplotypes containing this SNP were found in 15% of samples. These haplotype frequencies suggest that over a third of Black South Africans could have decreased sensitivity to IFN signalling, which would have considerable results on innate immune responses.

We next examined if any of the candidate SNPs were significantly associated with IgG immune response to measles vaccination in this cohort. This study showed that the minor alleles of the SNPs in the *OAS1* (rs10774671), *IL-4RA* (rs1801275 and rs1805015), *IL-4* (rs2243248), *IL-12B* (rs2546893), and *IFNAR2* (rs2834160) genes, and selected haplotypes in chromosome 5 (*IL-4* and *STING/TMEM173*), chromosome 16 (*IL-4RA*), and chromosome 20 (*MAVS* and *CYP24A1*) were significantly associated with measles-specific IgG titres in Black South African children.

These SNPs have varied effects and associations with vaccine responses previously reported in the literature. The minor G allele of the *IL-4* SNP rs2243248 has been associated with decreased measles neutralising antibody titres in a large study in the United States of America, whose cohort was predominantly Caucasian (104). However, in the current study this SNP had an OR of 0.360, which indicates association with increased measles-specific IgG titres – therefore showing an opposite effect in our cohort to that reported in the literature. The effect of this promoter SNP on IL-4 cytokine expression is not known. The C allele of the *IL-4RA* SNP rs1805015 was shown to be protective against hepatitis B vaccine non-response in a study China (105). However, in the current study the C allele of the rs1805015 SNP was found to increase risk of measles vaccine non-response, with an OR of 2.262. A second *IL-4RA* SNP, rs1801275, was found to be significantly associated with increased measles-specific antibody responses in the current study. The major allele (G) of this SNP is associated with enhanced IL-4 signal transduction which enhances the Th2 immune response, whereas the minor allele has been linked with decreased IL-4 signal transduction (106). Therefore, one might expect the minor allele to be associated with decreased antibody responses, given the critical role of IL-4 in antibody production, but in the current study this SNP was found to increase measles positivity post vaccination (OR=0.060). These findings are noteworthy as they diverge from the hypothesised associations of these SNPs previously reported.

The *IFNAR2* SNP rs2834160 was shown to be significantly associated to measles serostatus in the current study. The recessive CC genotype (minor allele homozygous genotype) was strongly associated with measles seronegativity, occurring more frequently in the seronegative cases than in the seropositive controls. The heterozygous CT genotype of this SNP was associated with measles seropositivity, occurring more in the controls than the cases. The rs2834160 SNP has been associated with decreased IFN-γ secretion and nonsense-mediated decay in measles and rubella vaccine responses (79).

The A allele of the *OAS1* SNP rs10774671 has been associated with decreased enzymatic activity of OAS1; the major allele G encodes the p46 isoform of the enzyme and the minor A allele encodes the p52 isoform (107,108). The p52 isoform of OAS1 has been shown to be less active and effective as the p46 isoform as p46 was able to inhibit hepatitis C replication, whereas the p52 isoform was unable to prevent replication of the virus (107,108). Given this evidence, the result in the current study is rather counterintuitive: the minor A allele of the OAS1 rs10774671 SNP was associated with increased measles IgG antibody levels: i.e., it showed a protective role against measles seronegativity. It is noteworthy that this SNP has also been associated with COVID-19 outcomes in people of African ancestry (108). Therefore, future studies of this nature could be used in understanding and shaping outbreak response to emerging diseases.

The minor allele (A) of the *IL12B* SNP rs2546893 has been associated with increased IL-6 secretion post-viral stimulation by MV, which promotes Th2 cell differentiation preferentially over Th1 cell differentiation (79). The rs2546893 SNP was found to be significantly associated with measles serostatus in the current study through both the recessive and genotypic models. The associations through recessive model and the AA homozygous genotype of the genotypic model were both in the direction of seronegative measles IgG titres; that is, these configurations were associated with increased risk of low measles antibody titres, which is unexpected given that the A allele of this SNP promotes Th2 cell proliferation. The heterozygous genotype of this SNP (AG) was associated with increased measles antibody titres, having occurred more frequently in the controls than in the cases.

Linkage patterns of functional variation in these genes should be investigated further in our cohort as it might help explain the observed associations in the current study.

The genes and SNPs contained within the significant haplotypes identified in the study were not all the same as those identified in the allele/genotype models. For the significant

chromosome 5 haplotype, the association might be driven by it containing the A allele of the *IL-12B* SNP rs2546893 that was also found to be associated with measles antibody responses through both the recessive and genotypic models. However this haplotype also contained two minor *IL-4* alleles which have previously been associated with Hepatitis B vaccine and PCV responses, the minor allele of the rs13181561 (*STING/TMEM173*) SNP which has been associated with decreased IFN-α production and increased susceptibility to viral infections, and the minor allele of the rs2546893 (*IL-12B*) SNP has been described as being associated with increased IL-6 production in measles vaccination (47,79,109–112).

The chromosome 16 haplotype contains the previously detailed *IL-4RA* rs1801275 SNP minor allele. The chromosome 20 haplotype contains the minor allele of the *MAVS* SNP rs17857295, associated with increased IFN-β production, and the minor allele of the *CYP24A1* SNP rs6013897, which has been associated with 1,25-$(OH)_2$D3 deficiency (113,114). The *STING/TMEM173*, *MAVS* and *CYP24A1* genes are involved in IFN-α signalling and vitamin D metabolism, both processes that have significant roles in the antiviral innate immune response stimulated by measles vaccination (40,62).

It is interesting that many of the minor alleles of the SNPs contained within the potentially significant haplotypes were not independently significantly associated with case-control status in the univariate allelic and genotypic association tests. That they are significant in these haplotypes could indicate that their individual effects are not great enough individually to disrupt the measles vaccine response, but together they have a significant compound effect on inhibiting the antibody response to measles vaccination. This could be further examined using polygenic risk scores.

The alleles of the SNPs that showed independent significant association to seronegative measles IgG titres before correction for multiple testing were all involved in the innate immune response to viral infection or vaccine stimulation. The *IFNAR2* and *OAS1* SNPs relate to the

IFN-I pathway, and the SNPs in *IL-4*, *IL-4RA*, and *IL-12B* relate to the IFN-II pathway. Within the IFN-II pathway, it was interesting to note that significant SNPs were found in both antagonistic pathways (IL-12 vs IL-4 stimulating Th1 vs Th2 pathways respectively). The IL-4 pathway preferentially stimulates Th2 proliferation and produces a robust antibody response in the adaptive immune system, while the IL-12 pathway preferentially stimulates Th1 cell proliferation and produces a robust cell-mediated immune response in the adaptive immune system. Considering that the phenotype the SNPs were being tested for association with was seronegative measles-specific IgG titres (<330 mIU/mL), one would expect that SNPs with functional consequences that suppress the Th2 response and heighten the Th1 response would be significant, and that those that suppress the Th1 response in favour of Th2 cell proliferation would not be associated with a poor antibody response. However, the results of the current study don't necessarily conform to these expectations; the C allele of the rs1801275 SNP is a good example of this. Again, this supports the requirement for further, broader investigations into this topic in the Black South African population.

Of the SNPs found to be independently associated with measles antibody titres in the univariate allele/genotype analysis, the A allele of rs10774671 (*OAS1*) and the C allele of rs1805015 (*IL-4RA*) had the highest MAFs in the cohort, occurring in 41.2% and 44.4% of samples respectively. This is important to note because the ORs of the minor alleles of these two SNPs indicate that they have antagonistic associations with measles antibody responses: the *OAS1* SNP's minor A allele has a protective relationship against seronegative IgG titres, while the *IL-4RA* SNP's minor C has a causal relationship with seronegative IgG titres.

These results must be interpreted cautiously since all the associations lost significance after the Bonferroni correction for multiple testing was applied. Although this is also contentious as the Bonferroni correction is often regarded as being too strict, especially in explorative studies (115). Therefore, we cannot definitively state whether these SNP and haplotype associations

are factually significant or not. This justifies future studies on a larger scale to determine the true nature of these findings.

There were several limitations to the study. The use of the Kenyan population as the reference population when checking SNP LD during SNP panel design may be a limitation: South African MAF data would be optimal to use instead of the MAFs from the Kenyan population. Unfortunately, access to population genetics data from the South African population is limited to the investigators, whereas the 1000 Genomes data is freely accessible.

The administration of other vaccines could interfere with the response to the measles vaccine. Most importantly, the participants in the Nutrivac study were given Pneumococcal Conjugate vaccine (PCV) at different dosing schedules: the infants either received one dose at 6 or 14 weeks plus a booster at nine months of age, or they received two doses at 6 and 14 weeks plus a booster at 9 months of age. This dose scheduling difference could influence the measles vaccine response.

Despite fact that the HLA genes are known to have great effects on immune responses, very few HLA gene SNPs were included in the SNP panel. Only HLA SNPs which affect HLA-DP expression levels were included due to their significance for other vaccines being investigated using this panel. It was not our intention to perform HLA typing and therefore no more HLA SNPs were included. This is due to the limited financial resources available, as the inclusion of the numerous HLA SNPs would make the genotyping too expensive. Furthermore, since the HLA SNPs are tightly clustered, including many HLA SNPs would not work in the multiplex MassARRAY genotyping system, so additional methods would be required.

The lack of baseline antibody data (i.e., pre-vaccination measles-specific IgG titres) meant that we could not classify participants as seroconverters or non-seroconverters. Furthermore, the

lack of data on maternal HIV exposure meant that this could not be used as a covariate with respect to antibody Reponses.

The most considerable limitation to the study is the limited statistical power of the study due to the small sample size and very limited number of SNPs examined. For this reason, we have only targeted SNPs with high frequencies and SNPs that could have associations with multiple vaccine responses because this current study formed part of a larger study investigating multiple vaccine responses. Therefore, some genes that might have been significant or interesting to investigate specifically for measles vaccination could not be selected due to resource limitations. For example, genes coding for MV receptors and proteins in the vitamin A pathway were excluded. Employing a GWAS would be an ideal method to increase the statistical power of the study and find all significant associations. Only one GWAS has been performed looking at measles vaccine responses, and it was not in an African population (80).

In summary, this study has provided evidence of potentially significant associations of the minor alleles of the SNPs in the IL-4, IL-12, and IFN-α pathways with seronegative measles-specific IgG antibody titres after vaccination with two doses of the MeasBio measles vaccine in the South African population. This is the first South African data in this regard, and the association results have mostly aligned with those reported in the literature and previous studies in non-African populations.

This is an initial study which can be grown in the future with the collection of further samples. Further investigations can be expanded to characterise the associations between genetic variations and cell-mediated immunity as well as antibody responses. Understanding the mechanisms underlying vaccine responses in the South African population is vital for future efforts to select appropriate vaccines and implement effective immunisation schedules, with the goal of reducing the burden of infectious disease and childhood morbidity and mortality in the country.

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APPENDIX 1: HREC APPROVAL FOR THIS STUDY

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Mr D Kapelus

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M2111169

NAME: (Principal Investigator)	Mr D Kapelus
DEPARTMENT:	School of Molecular and Cell Biology University
PROJECT TITLE:	*Associations between genetic variation and antibody responses to the measles vaccine in South African children*
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Drs D De Assis Rosa and M Groome
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2022/01/18

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

APPENDIX 2: HREC APPROVAL FOR THE NUTRIVAC (COHORT) STUDY

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Drs E Mutsaerts & MC Nunes & Prof SA Madhi

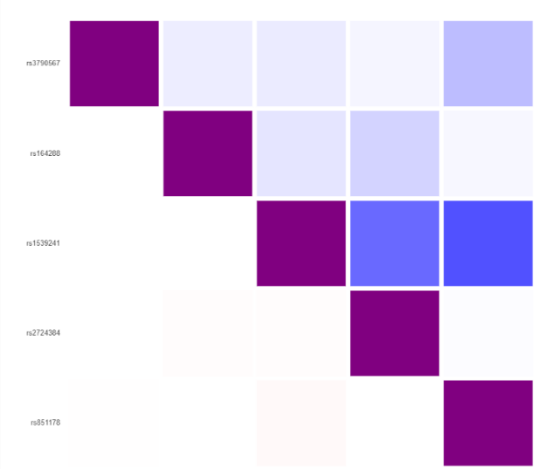
HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M180519

NAME: (Principal Investigator)	Drs E Mutsaerts & MC Nunes & Prof SA Madhi
DEPARTMENT:	School of Pathology Respiratory & Meningeal Pathogens Research Unit Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Impact of nutritional status, metabolic markers and microbiota on vaccine-induced humoral and cellular immunity in South African children
DATE CONSIDERED:	25/05/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Professor SA Madhi
APPROVED BY:	Professor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	01/08/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

APPENDIX 3: LD HEATMAPS FOR THE MEASLES-SPECIFIC CANDIDATE SNPS

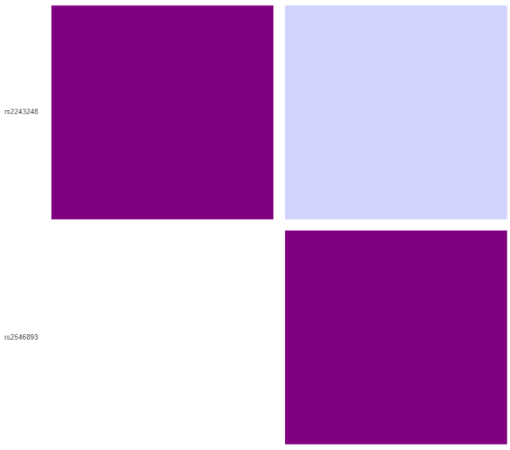
Chromosome 1 heatmap:



Chromosome 3 heatmap:

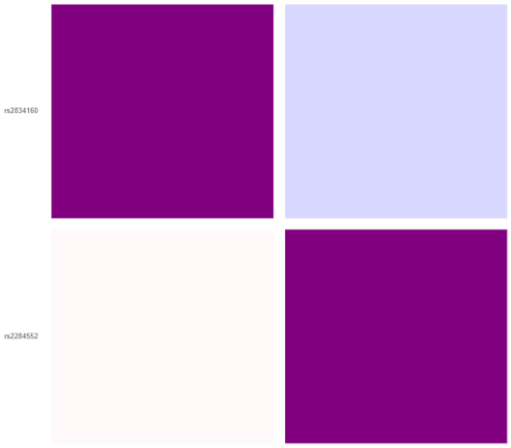
Chromosome 4 heatmap:

Chromosome 5 heatmap:



Chromosome 9 heatmap:

Chromosome 21 heatmap:



APPENDIX 4: 77 CANDIDATE SNPS AND THEIR ASSOCIATED IMMUNE/VACCINE-RELATED PHENOTYPES

Table 9: The associated immune/vaccine related phenotypes of each of the 77 candidate SNPs included in the final SNP panel (2,31,53,69,77,79,97–103,106,110–113,116–122)

Chr.	Gene	SNP	A1	A2	Associated immune phenotype
1	*IL-10*	rs1800871	A	G	Decreased IL-10 expression
1	*IL-10*	rs1800896	C	T	Increased IL-10 expression
1	*IL-12RB2*	rs3790567	G	A	Decreased measles antibody and CMI levels in response to measles vaccine
2	*IF1H1*	rs1990761	A	G	Associated with diabetes, arthritis, and multiple sclerosis
2	*IF1H1*	rs1990760	T	C	Decreased pro-inflammatory response
2	*IF1H1*	rs3747517	T	C	Associated with autoimmune disease, arthritis, diabetes
2	*IF1H1*	rs10930046	C	T	Associated with autoimmune disease, diabetes
2	*IL1B*	rs1143634	A	G	Associated with HBV vaccine response in SA
2	*IL1B*	rs1143627	A	G	Associated with HBV vaccine response
3	*IL-12A*	rs568408	A	G	Associated with non-response to HBV vaccine
3	*MYD88*	rs6853	G	A	Associated with decreased measles antibody titres.
3	*TLR9*	rs352139	T	C	Increased transcription
3	*TLR9*	rs187084	G	A	Decreased transcription
3	*TLR9*	rs5743836	G	A	Decreased transcription
4	*GC (VDBP)*	rs2282679	G	T	Associated with vitamin D serum levels in GWAS, several autoimmune diseases
4	*IL-2*	rs2069763	A	C	Associated with increased measles-specific cell mediated immunity.
4	*TLR2*	rs3804100	C	T	Increase in measles-specific antibodies in a dose-dependent manner
4	*TLR3*	rs5743305	A	T	Heterozygous genotype associated with lower measles-specific antibody titre, and lower lymphoproliferation to measles vaccine.
4	*TLR3*	rs3775296	A	C	Associated with HCV infection, HBV, Steven Johns syndrome
4	*TLR3*	rs10025405	G	A	Associated with HIV-1
4	*TLR3*	rs3775291	T	C	Minor allele associated with decreased IFN-γ production in response to TLR3 stimulation in a dose-dependent manner + heterozygous genotype associated with lower MV Ab titre
5	*IL-12B*	rs3212227	G	T	HBV vaccine response, measles vaccine response
5	*IL-12B*	rs3213093	T	C	Associated with measles and HBV vaccine responses
5	*IL-12B*	rs2546893	A	G	Minor allele associated with increased IL-6 secretion post-viral stimulation by MV
5	*IL-4*	rs2243248	G	T	Associated with decreased measles neutralizing antibody titres.
5	*IL-4*	rs2243250	C	T	Associated with PCV and HBV vaccine responses

5	*IL-4*	rs2070874	C	T	Associated with HBV vaccine response in SA
5	*IL-4*	rs2227284	G	T	T allele associated with lower IgG response to PCV serotypes 4 and 23F
5	*STING/ TMEM173*	rs13181561	A	G	Associated with decreased IFN-α production, increased susceptibility to viral infections
5	*STING/ TMEM173*	rs7380824	T	C	Associated with decreased IFN-α production, increased susceptibility to viral infections
5	*STING/ TMEM173*	rs1131769	T	C	Associated with decreased IFN-α production, increased susceptibility to viral infections
6	*HLA-DOB*	rs2857130	T	A	TT genotype associated with lower IgG response to PCV serotypes 4 and 9V
6	*HLA-DOB*	rs2857127	A	G	GG genotype associated with lower IgG response to PCV serotypes 4 and 9V
6	*HLA-DOB*	rs2857114	G	A	AA genotype associated with lower IgG response to PCV serotypes 4 and 9V
6	*HLA-DPB1*	rs7770370	A	G	Associated with HBV vaccine response in SA
6	*HLA-DPB1*	rs931	G	A	HBV vaccine response, HBV vaccine response in SA
6	*HLA-DPB1*	rs9277533	C	T	Associated with HBV infection outcome, HLA*DPB1 expression levels
6	*HLA-DPB1*	rs9277534	A	G	Associated with HBV infection outcome, HLA*DPB1 expression levels
6	*HLA-DPB1*	rs9277536	C	T	Associated with HBV infection outcome, HLA*DPB1 expression levels
6	*IFNGR1*	rs7749390	A	G	Associated with TB
9	*DDX58*	rs55789327	A	G	Associated with antiviral immune response
9	*DDX58*	rs669260	C	T	Minor allele associated with increased Ab levels (increased IFN-β and RIG-1 signalling) in a dose-dependent manner
9	*DDX58*	rs10813831	A	G	Minor allele associated with decreased Ab levels (decreased IFN-β and RIG-1 signalling) in a dose-dependent manner
9	*TLR4*	rs5030710	C	T	Decrease in measles-specific antibodies
9	*TLR4*	rs4986790	G	A	Heterozygous genotype associated with increased IL-4 production (Th2 skew)
9	*TLR4*	rs11536897	A	G	Associated with increased neutralizing antibody titres
9	*TLR4*	rs2770150			Minor allele associated with decreased secreted IFN-γ in a dose-dependent manner
11	*CYP2R1*	rs10741657	A	G	Major allele (G) associated with low serum 25(OH)D
11	*IRF7*	rs936470	G	C	Associated with TLR induced cytokine production (IFNG2). Heterozygous genotype associated with increased pro-inflammatory response (IP-10)
12	*IFNG*	rs2069727	C	T	Minor allele associated with increased IFN-γ expression and major allele is associated with decreased IFN-γ expression. Heterozygotes have intermediate expression.
12	*IFNG*	rs1861493	G	A	Reduced expression of IFN-γ, associated with several diseases
12	*IFNG*	rs1861494	C	T	T allele associated with increased IFN-γ expression
12	*OAS1*	rs10774671	A	G	Decreased enzymatic activity - weaker innate immune response to RNA viruses

12	*VDR*	rs731236	G	A	Homozygous CC (minor allele) associated with decreased VDR expression.
12	*VDR*	rs2228570	A	G	Minor allele associated with decreased affinity of VDR to vitamin D
12	*VDR*	rs11568820	C	T	Minor allele associated with up to 70% reduction in VDR transcription (decreases Vit D pathway and innate immune responses)
16	*IL-4RA*	rs1805010	A	G	G allele is associated with enhanced signal transduction, causing sustained phosphorylation and prolonged activation of downstream transcription
16	*IL-4RA*	rs1805015	T	C	Associated with HBV vaccine response
16	*IL-4RA*	rs1801275	A	G	G allele is associated with enhanced signal transduction, causing sustained phosphorylation and prolonged activation of downstream transcription
19	*IL-12RB1*	rs372889	C	T	Functional consequences including Th1 immune response, IFN-γ production, and cell-mediated immunity.
19	*IL-28B*	rs8103142	T	C	HBV viral loads and influenza vaccine response
19	*IL-28B*	rs8099917	G	T	HBV viral loads and influenza vaccine response
20	*CYP24A1*	rs6013897	A	T	Associated with 25(OH)D deficiency
20	*MAVS*	rs17857295	G	C	Increased IFN-β production
20	*MAVS*	rs7262903	A	C	Increased IFN-β production
20	*MAVS*	rs7269320	T	C	Decreased IFN-β production
21	*IFNAR1*	rs2843710	G	C	Decreases transcription: less IFN-I signalling
21	*IFNAR1*	rs2257167	C	G	Increased expression - potentially increasing antiviral immune response
21	*IFNAR2*	rs1051393	G	T	Associated with HCV
21	*IFNAR2*	rs2834160	C	T	Minor allele Associated with decreased secreted IFN-γ in a dose-dependent manner (decreased Th1 response?)
X	*TLR7*	rs179008	T	A	Truncates N-region of TLR7, affecting processing of TLR7
X	*TLR7*	rs864058	A	G	Associated with increased neutralizing antibody titres
X	*TLR7*	rs3853839	G	C	Associated with increased TLR7 transcription and expression and upregulation of downstream ISGs including IFN-responsive genes
X	*TLR7*	rs5935438	C	G	Located in transcription factor binding side, affecting transcription.
X	*TLR8*	rs3764880	G	A	Associated with HIV-1
X	*TLR8*	rs5744077	G	A	Associated with measles vaccine response
X	*TLR8*	rs2159377	T	C	Associated with seroconversion after rotavirus vaccination

APPENDIX 5: 163 HAPLOTYPES IDENTIFIED

Table 10: All haplotypes identified in the South African cohort with their frequencies.

Chr.	Haplotype	*Genes* (SNPs)	Frequency	Chr.	Haplotype	*Genes* (SNPs)	Frequency
1	GGC	*IL-12RB2* (rs3790567)	0.042	9	GTGTAA	*TLR4* (rs5030710, rs4986790, rs11536897)	0.025
1	AGC		0.306	9	GTACAG		0.08
1	GAT		0.052	9	GCGCAG		0.055
1	AAT	*IL-10* (rs1800871, rs1800896)	0.3	9	ATGCAG		0.03
1	AGT		0.298	9	GTGCAG	*DDX58* (rs55789327, rs669260, rs10813831)	0.089
2	GACTT	*IL1B* (rs1143634)	0.089	9	GTATAG		0.107
2	AGCTT		0.043	9	ACGTAG		0.017
2	GGCTT		0.208	9	GCGTAG		0.144
2	GATCT	*IFIH1* (rs1990761, rs1990760, rs3747517, rs10930046)	0.057	9	ATGTAG		0.045
2	GGTCT		0.019	9	GTGTAG		0.333
2	AACCC		0.057	11	GA	*IRF7* (rs936470) *CYP2R1* (rs10741657)	0.026
2	GACCC		0.161	11	CA		0.172
2	AGCCC		0.017	11	GG		0.083
2	GGCCC		0.346	11	CG		0.719
3	GCGA	*MYD88* (rs6853) *TLR9* (rs352139, rs187084) *IL-12A* (rs568408)	0.084	12	AGCCATG	*VDR* (rs731236, rs2228570, rs11568820) *IFNG* (rs2069727, rs1861493, rs1861494) *OAS1*	0.027
3	GTAA		0.025	12	AGTTATA		0.186
3	ATAA		0.039	12	AGCTATG		0.033
3	ACAA		0.069	12	AGTCATA		0.021
3	GTGG		0.012	12	AGTTATG		0.211
3	GCGG		0.095	12	AGCTATA		0.016
3	ACGG		0.169	12	AGTCATG		0.085
3	GTAG		0.129	12	AATTATG		0.064
3	ATAG		0.151	12	GGTTATG		0.055

3	GCAG		0.086	12	GGTCATG	(rs10774671)	0.024
3	ACAG		0.123	12	AATTATA		0.037
4	TTTAG	*GC (VDBP)* (rs2282679)	0.047	12	GGTTATA		0.085
4	TTACG		0.019	12	AACTATA		0.02
4	TTTCG	*TLR2* (rs3804100)	0.092	12	AGTTGCG		0.024
4	GTTAA		0.011	12	GGCTATG		0.019
4	TTTAA	*TLR3* (rs5743305,	0.056	12	AACTATG		0.016
4	GTACA	rs3775296, rs10025405)	0.019	12	AGTTACA		0.012
4	TTACA		0.176	12	GACCATA		0.01
4	GTTCA		0.018	16	ATA	*IL-4RA* (rs1805010, rs1805015, rs1801275)	0.038
4	TTTCA		0.533	16	GTA		0.13
5	TTTTGCCTCG	*IL-4* (rs2243248,	0.121	16	ACG		0.186
5	TTTTGTCGTG	rs2243250, rs2070874,	0.035	16	GCG		0.258
5	TTTTGCCGTG	rs2227284)	0.029	16	ATG		0.182
5	GCCTATCTCG		0.019	16	GTG		0.206
5	GCTTATCTCG	*STING/*	0.012	19	CCG	*IL-12RB1* (rs372889)	0.031
5	TTCTGCCTCG	*TMEM173* (rs13181561,	0.036	19	TCG		0.017
5	TTTTACCTCG	rs7380824, rs1131769)	0.022	19	CTT		0.183
5	GTTTGTCTCG		0.023	19	TTT	*IL-28B*	0.221
5	TCCTACCTCG	*IL-12B* (rs3212227,	0.033	19	CCT	(rs8103142,	0.257
5	GCCTGCCTCG	rs3213093, rs2546893)	0.017	19	TCT	rs8099917)	0.292
5	TTCTACCTCG		0.025	20	CATA	*MAVS* (rs17857295,	0.037
5	TTTTATCTCG		0.026	20	CCTA	rs7262903, rs7269320)	0.024
5	TCCTACCTCA		0.014	20	GCCA		0.043
5	TTTTGTCTCA		0.03	20	CCCA	*CYP24A1*	0.14
5	GTCTGCCTCA		0.013	20	CATT	(rs6013897)	0.099
5	GTCTGCCGTG		0.011	20	CCTT		0.113
5	GTCTACCGTG		0.036	20	GCCT		0.045
5	TCCTACCGTG		0.048	20	CCCT		0.492
5	GTTTACCTCA		0.022	21	TCGC		0.047
5	TTTTGCCTCA		0.017	21	GTGC	*IFNAR2*	0.02
5	TTCTACCGTG		0.017	21	TTGC	(rs1051393, rs2834160)	0.117

5	TTTTACCGTG		0.012	21	GTCC	*IFNAR1* (rs2843710, rs2257167)	0.014
5	TCCTGCCTCA		0.031	21	TCGG		0.034
5	TTTTATCGTG		0.035	21	GTGG		0.023
5	TTCTGTCGTG		0.012	21	TTGG		0.128
5	TTCTGCTGTG		0.018	21	TCCG		0.069
5	TTTTACCTCA		0.044	21	GTCG		0.109
5	GCCTGCCGTG		0.013	21	TTCG		0.438
5	TTCTGTCTCA		0.015	X	AGCCAAC	*TLR7* (rs179008, rs864058, rs3853839, rs5935438) *TLR8* (rs3764880, rs5744077, rs2159377)	0.164
5	GCTTATCGTG		0.011	X	AACCAAC		0.08
5	GCCTACCTCA		0.011	X	AGCGGAT		0.029
6	AGAAGCACA	*HLA-DOB* (rs2857130, rs2857127, rs2857114) *HLA-DPB1* (rs7770370, rs931, rs9277533, rs9277534, rs9277536) *IFNGR1* (rs7749390)	0.062	X	AACGAAC		0.184
6	AGAGATGTG		0.175	X	AGCCGAT		0.011
6	AGAAGCACG		0.07	X	AACCAAT		0.02
6	AGAGATGTA		0.151	X	AGCGGAC		0.058
6	AGAAATGTG		0.046	X	AACGAAT		0.013
6	AGAGGCACG		0.015	X	AGCGAAC		0.069
6	TAGGATGTG		0.109	X	TGCGAAC		0.018
6	TAGGATGTA		0.097	X	AGCGAGC		0.066
6	TAGAGCACA		0.115	X	AGGGGAT		0.042
6	TAGAGCACG		0.046	X	TACGAAC		0.014
6	AGAGGCGTG		0.015	X	AGGGGAC		0.016
6	TAGGGCACA		0.011	X	TGGGGAC		0.011
6	TAGGGCACG		0.016	X	AGCGAAT		0.029
6	TAGGGCGTG		0.01	X	TGGCAAC		0.027
6	AGGAGCACG		0.016	X	AACGAGC		0.082
9	GTATAA		0.019	X	TGCGGAC		0.011
9	GCGTAA		0.016				