



TLR genetic variation is associated with Rotavirus-specific IgA seroconversion in South African Black infants after two doses of Rotarix vaccine



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ABSTRACT

Live oral rotavirus vaccines have significantly reduced rotavirus-related diarrheal morbidity and mortality globally, but low efficacy of these vaccines is observed in low-income countries where disease burden is highest. The biological basis of rotavirus vaccine failure remains unknown but likely includes both microbial and host factors. We investigated associations between 19 candidate SNPs in the *TLR3*, *TLR7*, *TLR8*, *DDX58* and *IFIH1* genes that play a role in innate immunity, and seroconversion in Black South African infants after vaccination with Rotarix at 6 and 14 weeks of age.

Rotavirus-specific IgA antibody titre was measured by ELISA before each vaccine dose and four weeks after the second dose, and seroconversion was defined as a four-fold or greater increase in IgA antibody titre at 18 weeks of age when compared to pre-vaccine titres. A total of 95/138 individuals seroconverted (68.8%) and seroconversion was significantly affected by birthweight ($P = 0.010$), pre-vaccine IgA and IgG titres ($P = 0.0002$ and $P = 0.007$ respectively).

rs2159377 SNP in *TLR8* was significantly associated with seroconversion in a univariate allelic model ($P = 0.015$) and was borderline significant in a multivariable logistic regression adjusted for birthweight and pre-vaccine titres ($P = 0.071$), although these values did not remain significant after Bonferroni correction. A haplotype of six SNPs on the X chromosome across *TLR7* and *TLR8*, including rs179008 and rs5935438 minor alleles, was significantly associated with seroconversion in a univariate model ($P = 0.042$), but not in a multivariable model or after Bonferroni correction. Epistatic interaction between rs5743305 in *TLR3* and rs55789327 in *DDX58* was significantly associated with seroconversion ($P = 0.034$) but a genetic risk score constructed from all 19 minor alleles was not.

Our results suggest that TLR variants may influence IgA antibody production and seroconversion to Rotarix vaccine in South Africans. Host genetic variation contributes to the varying immunogenicity of live oral rotavirus vaccines.

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1. Introduction

Rotaviruses are non-enveloped, double-stranded RNA viruses of the *Reoviridae* family, which infect the enterocytes of the small intestinal villi. Rotavirus infection causes acute gastroenteritis characterized by vomiting and watery diarrhoea, leading to dehydration which may be fatal in severe cases without sufficient rehydration. Four live oral rotavirus vaccines are currently in use, of

which two are licensed globally: Rotarix (GlaxoSmithKline Biologicals) and Rotateq (Merck & Co Inc) [1]. The introduction of live oral rotavirus vaccines has resulted in the reduction of rotavirus-induced diarrheal morbidity and mortality [2]. However, lower efficacy of Rotarix and Rotateq vaccines has been observed in low-income regions such as sub-Saharan Africa and Asia [1].

Variable immune responses to rotavirus vaccination may be influenced by inter-individual variation in the innate or adaptive immune system. In particular, innate immune responses are a critical first line of immune response that induce interferon production and shape subsequent adaptive immune responses [3]. The major rotavirus component recognised by host innate immunity

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is the viral RNA [3], which binds to endosomal toll-like receptors (TLR) or cytosolic retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), such as RIG-I, or MDA5. Viral dsRNA is recognised by TLR3, RIG-1 and MDA5, while viral ssRNA is recognised by TLR7, TLR8 and RIG-1. We hypothesized that variation in genes encoding these viral RNA sensors could associate with antibody response to Rotarix vaccination. We investigated associations between 19 candidate SNPs in the *TLR3*, *TLR7*, *TLR8*, *DDX58* (encoding RIG-1) and *IFIH1* (encoding MDA5) genes and rotavirus-specific IgA seroconversion one month after receipt of two doses of Rotarix vaccine in Black South African infants. Our results suggest that TLR variants may influence IgA antibody production and seroconversion to Rotarix vaccine in South Africans.

2. Materials and methods

2.1. Study population and demographics

The study cohort included samples from healthy Black South African infants previously collected as described by Groome et al. [4] and Moon et al. [5]. Briefly, infants born to HIV-negative mothers were recruited from 02 December 2009 to 09 April 2010 during their routine immunization visits at Diepkloof Primary Health Clinic, Soweto, South Africa. Infants were enrolled from five to eight weeks of age and were followed over a period of time until five years of age. Informed consent forms were signed by the parent/guardian of the participants. The original study and the current study using stored samples were approved by the Human Research Ethics Committee (HREC) (Medical) of the University of the Witwatersrand, South Africa, ethics reference number M090824. An oral live Rotavirus vaccine (Rotarix, GlaxoSmithKline) was administered at 6 and 14 weeks of age according to the standard schedule of the expanded program on immunization in South Africa and other vaccines were provided according to the standard schedule of the expanded program on immunization in South Africa. Serum and saliva samples were obtained from the infants before the first (6 weeks) and second (14 weeks) doses of RV vaccine and one month after the second RV dose (at 18 weeks of age). Serum and saliva samples were stored at -70°C until further use in ELISA or in DNA extraction. Characteristics of the cohort including gender, age and birthweight are shown in Table 1.

2.2. ELISA assay for Rotavirus-specific IgA and IgG titres

Oral vaccines are potent inducers of IgA antibody responses and rotavirus-specific IgA titre is a correlate of RV vaccine efficacy [6]. Rotavirus-specific IgA and IgG titres were measured in serum samples from the cohort by ELISA assay as previously described [4,5].

IgA titres were measured immediately prior to the first and second doses of RV vaccine and one month after the second RV dose (at 18 weeks of age) and were reported as reciprocals of the highest sample dilution factor with a positive result in the ELISA assay. IgG titres were measured prior to the first dose of RV vaccine and were reported as reciprocals of the highest sample dilution factor with a positive result in the ELISA assay. Seroconversion was defined as a four-fold or greater increase in serum rotavirus-specific IgA antibody titre when compared to the IgA titre measured before the first dose of Rotarix vaccine.

2.3. DNA extraction from serum or saliva

DNA was extracted from frozen stored serum using Macherey–Nagel Nucleospin kits (Germany) and from frozen stored saliva using Norgen Biotek (Canada) saliva isolation kits following the manufacturers' instructions. DNA quantity and purity (A260/280 and A260/230) were assessed using a NanoDrop® ND-1000 spectrophotometer (ThermoFisher, Wilmington DE, USA) and samples with DNA concentration above 20 ng/μl were used for genotyping. DNA extraction was successful for 141 samples.

2.4. Candidate gene and SNP selection

Genes encoding innate sensors of viral RNA were targeted in this study, including *TLR3*, *TLR7*, *TLR8*, the *DDX58* gene encoding RIG-1, and the *IFIH1* gene encoding MDA5. Genetic variation of interest within these candidate genes was identified by literature review, searching for SNPs that had functional effects on gene expression, SNPs that were reported to be associated with immune responses to other vaccines, SNPs that influenced immunity to other pathogens, or SNPs that were significantly associated with immune disorders. The resulting SNP list was then filtered according to minor allele frequency (MAF) of greater than 5% in a proxy population, the Luhya from Kenya (LWK) from the 1000 Genomes dataset. From this list, we prioritised tagSNPs. Finally a total of 19 SNPs that could be multiplexed for use in a MassArray genotyping assay were selected.

2.5. SNP genotyping

MassARRAY® (Agena Bioscience) genotyping of the 19 selected SNPs in 141 samples was performed at Inqaba Biotec, Pretoria. This assay uses initial PCR amplification of each locus of interest, followed by multiplexed PCR-based single base extension using mass-modified dideoxynucleotides and a single primer which anneals immediately upstream of each polymorphism of interest. Using MALDI-TOF mass spectrometry, the distinct mass of the

Table 1
Comparison between seroconverters and non-seroconverters in 138 Black South African infants vaccinated with Rotarix.

Characteristic	Seroconverters N = 95	non-seroconverters N = 43	P value	total cohort N = 138
sample size (%)	95 (68.8%)	43 (31.2%)	–	138
number of males (%)	55 (57.9%)	22 (51.2%)	P = 0.467 ^b	77 (55.8%)
number of females (%)	40 (42.1%)	21 (48.8%)		61 (44.2%)
mean birth weight (kg) (±SD) ^a	3.09 ± 0.51	2.85 ± 0.52	P = 0.010 ^d	3.02 ± 0.53
median (range) infant age in days at vaccine dose 1	43 (42–54)	43 (42–50)	P = 0.083 ^c	43 (41–54)
infant median (range) IgA titre pre-vaccine dose 1, at 6 weeks of age	20 (1–320)	40 (1–1 280)	P = 0.0002 ^c	20 (1–1 280)
infant median (range) IgA titre post-vaccine dose 1, at 14 weeks of age	80 (1–10 240)	40 (1–1 280)	P = 0.133 ^c	40 (1–10 240)
infant median (range) IgA titre post-vaccine dose 2, at 18 weeks of age	320 (20–20 480)	80 (1–640)	P < 0.0001 ^c	160 (1–20 480)
infant median (range) IgG titre pre-vaccine dose 1, at 6 weeks of age	1 280 (80–10 240)	2 560 (80–20 480)	P = 0.007 ^c	1 280 (80–20 480)

^aStandard deviation; ^bFisher's exact test; ^cMann-Whitney test; ^dt-test.

extended primer identifies the SNP alleles present in each sample. The assay is further described in Gabriel et al. [7].

2.6. Statistical analyses

Characteristics of seroconverters and non-seroconverters were compared using univariate statistical tests in GraphPad Prism v6.07. Normally distributed data were compared by *t*-test, data that were not normally distributed were compared by Mann-Whitney test and categorical data were compared using Fisher's exact test. Pre-vaccination variables that were significantly different between seroconverters and non-seroconverters ($P < 0.05$) were used as covariates in the multivariable analysis.

SNP data were analysed using gPLINK v2.05 [8,9]. Samples or SNPs with more than 20% missing data were excluded, resulting in 138 individuals with data. Deviation from Hardy-Weinberg equilibrium was examined using $P < 0.001$ as the cut-off value. Minor allele frequencies (MAF) in the cohort were computed and compared by chi-squared test to MAF in two other African populations from the 1000Genomes project, the LWK from Kenya and the YRI from Nigeria using Ensembl as a data source (<https://www.ensembl.org>). SNPs within each gene were phased in gPLINK v2.05 [8,9] to compute haplotype frequencies in the cohort.

Univariate analyses were performed for each locus in gPLINK v2.05 [8,9] to test the associations between alleles, genotypes and haplotypes, and seroconversion status. Since seroconversion is a categorical variable, Chi-square test (χ^2) or Fisher's exact test were used. Three models of allelic dominance were examined: recessive, dominant and codominant (genotypic). Genotype models for SNPs on the X chromosome were calculated for females only. Odds Ratio (OR) values in this study were calculated with $OR > 1$ indicating a higher chance of finding the minor allele in seroconverters than in non-seroconverters. Bonferroni correction was used to adjust significance values to correct for multiple SNP testing. Multivariable analysis using logistic regression was conducted in gPLINK v2.05 [8,9] to analyse the associations of genetic data with seroconversion status while including birthweight and baseline IgA titres as covariates in the analyses. P values < 0.05 were considered statistically significant.

The association between multi-locus variation and seroconversion was considered using an additive model by means of a genetic risk score (GRS) with minor alleles weighted as 1 and divided by the total number of alleles genotyped in each sample (as this was affected by gender). We also analyzed association between multi-locus variation and seroconversion using a SNP by SNP epistatic model implemented in gPLINK v2.05, although PLINK does not calculate epistasis using SNPs on the X chromosome. Bonferroni correction was used to adjust significance values to correct for multiple SNP testing. In all analyses, P values less than 0.05 were considered statistically significant.

3. Results

3.1. Non-genetic factors influence seroconversion to Rotarix vaccine

Rotavirus-specific IgA titres in the cohort were low prior to vaccination (median titre 20, range 1–1280, Table 1). A total of 95/138 individuals seroconverted (68.8%). We observed significant differences in birthweight ($P = 0.010$), pre-vaccine IgA titres ($P = 0.0002$) and pre-vaccine IgG titres ($P = 0.007$) in seroconverters vs non-seroconverters (Table 1). Seroconverters had higher birthweight and higher pre-existing serum IgA and IgG titres than non-seroconverters and these variables were used as covariates in the multivariable analyses. Gender and age (in days) at vaccine dose 1 were not significantly associated with seroconversion.

3.2. Genetic variation in innate immune response genes in a Black South African cohort

Minor allele frequencies for the 19 loci in the study cohort are shown in Table 2. For SNP rs10930046 in *IFIH1*, the T allele is considered to be wild type and is most frequent in most populations, therefore the C is listed as the minor allele, however the C allele frequency in the SA cohort exceeded 0.5. A similar frequency of 0.556 was observed in the Yoruba from 1000 Genomes data (Table 2). Allele frequencies for some of the SNPs under investigation were significantly different in our SA cohort when compared to two other African populations (Chi-2 test; $P < 0.05$), including rs669260 in *DDX58* and four SNPs in *TLR7* and *TLR8* on the X chromosome (Table 2).

Haplotypes of SNPs in each gene are shown in Tables 3a–d. The three missense mutations examined in *IFIH1* were not linked whereas the intronic mutation rs984971 was inherited in combination with any of these three missense mutations (Table 3a). Wild-type *IFIH1* haplotypes were not observed, and two different haplotypes containing missense mutations were frequent in the cohort (haplotype 1 in 0.480 of chromosomes and haplotype 2 in 0.391 of chromosomes, Table 3a). The three SNPs studied in *DDX58* were not found to be linked and the wild-type haplotype was the most common (0.463, Table 3b). Of the ten haplotypes observed in *TLR3* (Table 3c), the wild-type haplotype was the most common (0.417) and the SNPs were not in strong linkage with each other. Since *TLR7* and *TLR8* genes are in close proximity on the X chromosome (approximately 10 kb apart), haplotypes were constructed using SNPs from both of these genes (Table 3d). A total of fifteen haplotypes were observed and three haplotypes were present at intermediate frequency (0.175–0.205, Table 3d); no strong linkage patterns were observed.

3.3. Associations between genetic variation in innate immunity genes and seroconversion to Rotarix vaccine

One significant association between SNPs located in antiviral genes and IgA seroconversion after Rotarix vaccination was identified in an allelic univariate model of analysis. The minor allele (T) of rs2159377 in *TLR8* was significantly increased in seroconverters compared with non-seroconverters ($P = 0.015$, $OR = 5.35$) in the current study (Table 4). When adjusted using Bonferroni correction, the P value was not significant ($P = 0.285$). When birthweight and baseline IgA titre were considered as covariates in a multivariable logistic regression, a trend to significant association of this allele with seroconversion was still observed ($P = 0.07$, $OR = 4.14$, Table 4). Genotypes of this SNP or of the other SNPs in this study were not significantly associated with seroconversion in dominant, recessive or genotypic models ($P > 0.05$).

When haplotypes within each gene were considered, one six-SNP haplotype on the X chromosome, TGACACC (haplotype 12 in Table 3d) was significantly associated with seroconversion ($P = 0.042$, $OR = 0.104$, Table 4). This haplotype was more frequent in the non-seroconverters than in the seroconverters. This haplotype did not contain the *TLR8* rs2159377 minor allele that was independently associated with seroconversion in an allelic model; instead, this haplotype included the *TLR7* minor allele T for rs179008, a missense mutation and the *TLR7* minor allele C for rs5935438, affecting a transcription factor binding site. After Bonferroni correction, the adjusted P value was not significant ($P > 0.05$). When birthweight and baseline IgA titre were considered as covariates in a multivariable logistic regression, no significant association of this haplotype with seroconversion was observed ($P > 0.05$, Table 4).

Genetic risk scores were calculated for each sample according to the proportion of minor alleles present. The absolute number of

Table 2

Minor allele frequencies (MAF) of 19 SNPs related to innate immune function in a South African Black cohort, compared to MAF in two other sub-Saharan African populations represented in the 1000 Genomes dataset.

Gene	Chromosome	SNP	mutation location or type	major allele	minor allele	MAF in SA	MAF in LWK ^d	MAF in YRI ^e	P-value ^f
<i>IFIH1</i>	2	rs1990760	missense	C	T	0.062	0.101	0.06	0.190
<i>IFIH1</i>	2	rs3747517	missense	C	T	0.400	0.465	0.384	0.234
<i>IFIH1</i>	2	rs10930046	missense	T	C ^c	0.537	0.424	0.556	0.015
<i>IFIH1</i>	2	rs984971	intron	G	A	0.118	0.202	0.194	0.001
<i>TLR3</i>	4	rs5743305	5' TF binding site ^a	T	A	0.303	0.318	0.278	0.660
<i>TLR3</i>	4	rs13126816	intron	C	A	0.069	0.071	0.097	0.456
<i>TLR3</i>	4	rs3775296	splice region	G	A	0.127	0.131	0.125	0.964
<i>TLR3</i>	4	rs5743312	intron	C	T	0.091	0.081	0.079	0.939
<i>TLR3</i>	4	rs3775292	non-coding exon	G	C	0.091	0.091	0.130	0.260
<i>TLR3</i>	4	rs10025405	3' UTR ^b	A	G	0.202	0.278	0.194	0.087
<i>DDX58</i>	9	rs55789327	missense	G	A	0.083	0.121	0.097	0.393
<i>DDX58</i>	9	rs669260	intron	T	C	0.234	0.187	0.125	0.009
<i>DDX58</i>	9	rs10813831	missense	G	A	0.221	0.162	0.194	0.281
<i>TLR7</i>	X	rs179008	missense	A	T	0.096	0.091	0.165	0.066
<i>TLR7</i>	X	rs864058	synonymous	G	A	0.384	0.279	0.213	0.002
<i>TLR7</i>	X	rs3853839	3' UTR ^b	C	G	0.107	0.214	0.183	0.019
<i>TLR7</i>	X	rs179007	intergenic	A	G	0.250	0.104	0.152	0.001
<i>TLR7</i>	X	rs5935438	TF binding site ^a	G	C	0.318	0.416	0.415	0.088
<i>TLR8</i>	X	rs2159377	synonymous	C	T	0.112	0.286	0.165	<0.001

^aTF, transcription factor; ^bUTR, untranslated region; ^cC is the minor allele in most populations but MAF in SA and YRI was > 0.5. T allele is considered to be wild type. ^dLuhya from Kenya; ^eYoruba from Nigeria; ^fChi² test.

minor alleles present per sample ranged from 2 to 15, and proportion ranged from 0.07 to 0.39. GRS was not significantly associated with seroconversion status ($P > 0.05$). Epistasis assessed in a SNP by SNP interaction model indicated one significant association, between presence of SNP pair rs5743305 in *TLR3* and rs55789327 in *DDX58*, and seroconversion ($P = 0.034$, OR = 0.09, Table 4). When this P value was adjusted for the total number of possible SNP pairwise combinations, using Bonferroni correction, the P value was 1 (Table 4).

4. Discussion

In this study we hypothesized that genetic variation in genes encoding viral sensors could influence immunogenicity to live oral rotavirus vaccine (Rotarix) in South Africans. We examined 19 selected variants in genes encoding RIG-1, MDA5- LTR3, *TLR7* and *TLR8* in a cohort of 138 Black South African infants. Minor allele frequencies and haplotype frequencies for these SNPs were reported and contribute to the knowledge of genetic diversity related to immune function in South Africans. Comparison of the minor allele frequencies in this cohort to frequencies in two other sub-Saharan African populations demonstrated that data from these 1000 Genomes populations cannot always be used as proxies for South African allele frequencies, particularly for *TLR7/TLR8* loci on the X chromosome. This is dissimilar to data from selected other *TLR* loci [10]. This might be due to small cohort size, but it is well known that South African populations have a unique demographic history with contributions from Khoisan-speaking populations, with concomitant genetic diversity patterns that are somewhat different to other African populations [11]. The different frequencies of mutations in these *TLR* genes in different African populations could also indicate different evolutionary events such as genetic drift or natural selection. The *TLR7* and *TLR8* loci have been shown to be under strong purifying selection and to display a relative lack of genetic diversity when compared to other *TLR* genes [12], however genes on the X chromosome are more susceptible to the effects of genetic drift than autosomes, and patterns of *TLR7* and *TLR8* diversity in African populations should be further studied.

IgA seroconversion to Rotarix vaccine occurred in 68.8% of the Black South African cohort in this study, a percentage that is similar to that reported in the larger parent cohort (61%, [4]) and that

reported in another South African study of Rotarix immunogenicity (57.1%, [13]). These values were much higher than the seroconversion rate of only 20% reported in a rural Zimbabwean cohort [14] and slightly higher than the seroconversion rate of 52.9% reported in a Malawian study of Rotarix vaccine immunogenicity [15]. The IgA assays and definition of seroconversion varied between the current study [and 4, 5] and the GlaxoSmithKline-sponsored studies [13–15], which may also contribute to the variation in reports of seroconversion rates.

We observed both genetic and non-genetic factors influencing seroconversion to Rotarix vaccination in our study. Non-genetic factors included birthweight and pre-vaccination rotavirus-specific IgA and IgG titres. Birthweight was significantly higher in seroconverters than non-seroconverters in this cohort. Similar results were seen in the Zimbabwean study where higher birthweight was also associated with increased IgA titres after Rotarix vaccination [14], and in a study in Ghana where underweight babies experienced decreased Rotateq vaccine efficacy compared to normal weight babies [16]. The mechanisms relating birthweight to oral/parenteral vaccine immunogenicity are not clear but may relate to gut mucosal integrity and mucosal immunity. The influence of birthweight on immunogenicity of other vaccines is variable, for example, lower birthweight has also been shown to significantly reduce immunogenicity of hepatitis B vaccine [17] and polio vaccine [18] but not influenza vaccination [19]. Innate genetic variation and function might influence birthweight and related outcomes such as preterm birth; for example, Singh et al. (2013) [20] showed that *TLR* expression levels influence birthweight. Therefore innate genetic variation might therefore both directly and indirectly influence vaccine seroconversion (by influencing immune responses to vaccine directly, and by influencing birthweight, which in turn influences seroconversion).

The inhibitory effect of higher pre-existing serum IgA and IgG titres on seroconversion in this cohort was previously discussed in [4,5]. Pre-vaccine IgA or IgG titres in the children were not significantly correlated with IgA or IgG titres in maternal breastmilk pre-vaccination, but were significantly correlated with IgA and IgG titres in maternal serum samples [4,5]; it was therefore suggested that the high pre-vaccine antibody titres were acquired by infants trans-placentally [4,5]. Pre-vaccine IgA in infant serum at 6 weeks of age could be from exposure to natural rotavirus, but RV infection was not tested in these infants. Delayed breastfeeding

Table 3

(a–d) Haplotypes of variation at five loci influencing innate immunity in a Black South African cohort, n = 138.

(a) Haplotypes in IFIH1 on chromosome 2 (encodes MDA5).							
SNP	rs1990760	rs3747517	rs10930046	rs984971			
reference allele	C	C	T	G			
minor allele	T	T	C*	A			
mutation type	missense	missense	missense	intron	frequency		
ht1	.	.	C	.	0.480		
ht2	.	T	.	.	0.391		
ht3	.	.	C	A	0.057		
ht4	T	.	.	A	0.050		
ht5	T	.	.	.	0.012		
ht6	.	T	.	A	0.010		
*C is the minor allele in most populations but MAF in SA was > 0.5. T allele is considered to be wild type.							
Dots indicate identity to reference allele.							
(b) Haplotypes in DDX58 on chromosome 9 (encodes RIG-1).							
SNP	rs55789327	rs669260	rs10813831				
reference allele	G	T	G				
minor allele	A	C	A				
mutation type	missense	intron	Missense	frequency			
ht1	.	.	.	0.463			
ht2	.	C	.	0.234			
ht3	.	.	A	0.220			
ht4	A	.	.	0.083			
Dots indicate identity to reference allele.							
(c) Haplotypes in TLR3 on chromosome 4.							
SNP	rs5743305	rs13126816	rs3775296	rs5743312	rs3775292	rs10025405	
reference allele	T	G	C	C	G	A	
minor allele	A	A	A	T	C	G	
mutation type	5' TF binding site	intron	splice region	intron	non-coding exon	3' UTR	frequency
ht1	.	.	.	.	.	.	0.417
ht2	A	.	.	.	.	.	0.179
ht3	.	.	.	.	.	G	0.116
ht4	.	.	.	.	C	.	0.068
ht5	.	.	A	T	.	G	0.056
ht6	.	A	.	.	.	.	0.032
ht7	.	.	A	T	.	.	0.029
ht8	.	.	A	.	.	.	0.028
ht9	A	.	.	.	.	G	0.025
ht10	A	A	.	.	.	.	0.020
Dots indicate identity to reference allele.							
(d) Haplotypes of SNPs in TLR7 and TLR8 on chromosome X.							
Gene	TLR7	TLR7	TLR7	TLR7	TLR7	TLR8	
SNP	rs179008	rs864058	rs3853839	rs179007	rs5935438	rs2159377	
reference allele	A	G	C	A	G	C	
minor allele	T	A	G	G	C	T	
mutation type	missense	synonymous	3' UTR	intergenic	TF binding site	synonymous	frequency
ht1	.	A	.	G	.	.	0.205
ht2	.	.	.	.	C	.	0.188
ht3	.	.	.	.	.	.	0.175
ht4	.	.	.	.	.	T	0.064
ht5	.	.	G	.	.	.	0.063
ht6	.	A	.	.	C	.	0.062
ht7	.	A	.	.	.	.	0.057
ht8	T	.	.	.	.	.	0.034
ht9	.	.	.	G	.	.	0.029
ht10	.	A	.	.	C	T	0.026
ht11	.	.	G	.	.	T	0.017
ht12	T	.	.	.	C	.	0.013
ht13	T	A	.	.	.	.	0.011
ht14	.	A	.	G	C	.	0.012
ht15	T	.	.	G	.	.	0.010

Dots indicate identity to reference allele.

around the time of vaccination did not improve Rotarix immunogenicity in this South African cohort [4]. Maternal immunity has also been shown to negatively influence rotavirus vaccine immunogenicity in Zambians [21] and other developing countries [22].

In addition to the above non-genetic factors, we obtained novel evidence that genetic variation in genes encoding viral sensors

may affect seroconversion to Rotarix vaccine in South Africans. Different models of inheritance suggested that variation in the TLR-encoding genes can influence Rotarix immunogenicity, including variation in *TLR3*, *TLR7* and *TLR8*. The rs2159377 allele in *TLR8* was significantly associated with seroconversion in the current study in allelic univariate and multivariable models. While the P value became non-significant when Bonferroni correction was

Table 4

Significant associations in candidate genes encoding innate immunity receptors, and seroconversion after Rotavirus vaccination in a South African cohort.

Gene	SNP	model of association	Univariate analyses			Multivariable analyses ^a	
			P value (uncorrected)	OR (95% CI)	P value (Bonferroni correction)	P value (uncorrected)	OR (95% CI)
TLR8	rs2159377 (T)	allelic	0.015	5.351 (1.21–23.66)	0.285	0.071	4.14 (0.88–19.30)
TLR7-TLR8	TGCACC (haplotype 12)	haplotype of 6 SNPs	0.042	0.104 (0.11–0.96)	0.630	0.433	0.021 (ND)
TLR3/DDX58	rs5743305 and rs55789327	epistatic	0.034	0.090 (ND)	1	ND	ND

OR odds ratio; CI confidence interval; ND not done.

^aUsing birthweight and baseline IgA titre as covariates.

applied, this correction is sometimes thought to be too stringent particularly for explorative studies [23]. The rs2159377 SNP is a synonymous mutation which does not offer any explanation to its functional role in vaccine immunogenicity. However, since tagSNPs were prioritized in this study, this SNP is likely to be in linkage with other causative SNPs in *TLR8* or even with other linked genes on the X chromosome. This SNP has previously reported to be part of a haplotype which was significantly associated with systemic lupus erythematosus (SLE) [24] and was significantly associated with HIV infection risk in African-Americans [25]. Interestingly this SNP was also reported to associate with type 1 diabetes risk in a European cohort [26]. Other genetic variants in *TLR8* have previously been shown to associate with measles vaccine-induced cytokine production [27] and with viral infection outcomes, including HIV infection [28] and hepatitis C infection [29].

Genetic variation in *TLR7* may also influence Rotarix immunogenicity. In our study, the *TLR7/8* haplotype TGACACC was significantly more frequent in the non-seroconverters than in the seroconverters. This haplotype did not contain the *TLR8* rs2159377 minor allele that was independently associated with seroconversion in the allelic models; instead, this haplotype included the *TLR7* minor allele T for rs179008, a missense mutation and the *TLR7* minor allele C for rs5935438, affecting a transcription factor binding site. This haplotype was not significantly associated with seroconversion after Bonferroni correction or in multivariable analyses. Genetic variation that is significant in univariate but not multivariable analyses can be associated with one of the covariates used in multivariable analyses. It would be interesting to assess the role of the SNPs in the current study in the mothers of the vaccinated infants, to determine if they are associated with maternal antibody titre and transplacental transmission. Elsewhere, rs179008 has been associated with hepatitis C infection clearance and disease progression [29] while rs5935438 was associated with allergic rhinitis [30]. Other genetic variants in *TLR7* have previously been shown to associate with measles vaccine-induced cytokine production [27] and with outcomes in cytomegalovirus infection [31] and vaccination [32].

An analysis of epistatic interactions between pairs of SNPs in this study revealed that an interaction between SNPs rs5743305 in *TLR3* and rs55789327 in *DDX58* significantly associated with seroconversion. Rs5743305 is a regulatory region variant in *TLR3* that causes reduced *TLR3* expression [33–34] and was associated with increased rotavirus-specific IgG antibody titres in IgA-deficient individuals but not in healthy individuals [33]. This SNP increased the risk of breast cancer [34] and was also associated with GM-CSF production after rubella vaccination [35]. Rs55789327 in *DDX58* is a missense mutation which inactivates a caspase recruitment domain repeat, and confers an overall inhibitory

RIG-1 function which inhibits antiviral signalling [36,37]. Several other SNPs in *TLR3* have been associated with immune responses to measles vaccine, affecting IL-6 and IL-10 production cytokine production [27] and some have also been described that influence diabetes type 1 in South Africans [38]. Several other SNPs in *DDX58* have been associated with immune responses to measles vaccine, including measles-specific antibody variation and IFN γ and IL-2 cytokine secretion [39].

Cellular signalling cascades via TLR and RLR genes converge on the interferon α/β production pathway. The overlap in SNPs that affect rotavirus infection or rotavirus vaccine outcomes and risk of diabetes type 1 may also be linked via changes in interferon production in these phenotypes. These observations may contribute to understanding of recent theories that vaccination against rotavirus may protect against diabetes type 1 [40,41].

In summary this is the first study to examine and demonstrate links between variation in genes encoding viral sensors and immunogenicity to live oral rotavirus vaccine (Rotarix) in South Africans. Our data contribute to the understanding of the role of host genetic variation in vaccine immune responses, a field where African genetic data are lacking. Our work has suggested that genetic variation in several TLR genes, but not independently in RLR genes, may influence immunogenicity of Rotarix in our cohort. These observations should be interpreted with caution since associations did not remain significant after adjusting for multiple testing.

The study was limited by the small sample size and results need to be replicated in larger South African cohorts. Many more SNPs and more candidate genes or whole genome analyses should be conducted. Many previous studies have demonstrated a role for *FUT2* and *FUT3* genetic variation in immunogenicity of rotavirus vaccine (reviewed by [42]) and these loci need to be studied in our cohort. Other loci such as HLA genes have been shown to have large effects on immune outcomes of many vaccines [43] and these also need to be examined. No GWAS study has been performed in any ethnicity related to outcomes of rotavirus vaccination and this is much needed. Analyses of associations of genetic variation with particular immunophenotypes after rotavirus vaccination, such as IFN or cytokine production, would be of interest to elucidate further the mechanisms of variation in immunogenicity of vaccine.

Studies of associations between host genetic variation and immune responses to vaccines can contribute to future vaccine design, and may also provide information regarding use of adjuvants during vaccination. In the case of the current study where genetic variation in the innate immunity pathway may affect response to a vaccine antigen, the addition of an adjuvant known to enhance signalling through that specific pathway might enhance immunogenicity and efficacy of the vaccine, overriding the effects of any polymorphisms in that innate pathway [43].

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CRediT authorship contribution statement

Thabiso V. Miya: Investigation, Formal analysis, Writing – original draft. **Michelle J. Groome:** Conceptualization, Resources, Funding acquisition, Supervision, Writing – review & editing. **Debra de Assis Rosa:** Conceptualization, Formal analysis, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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