

Association of CD46 Cellular Receptor Gene SNP in Measles Vaccine Response

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Abstract

Background Measles is a highly contagious viral disease. It remains an important cause of death among young children globally, despite the availability of a safe and effective vaccine. Measles transmitted via droplets from the nose, mouth or throat of infected persons.

Objective To evaluate the immune response to measles infection among immunized school aged children, and to detect the cluster of differentiation 46 single nucleotide polymorphism (CD46 SNP) in association with the immune response.

Methods The current study is a cross sectional study including 158 hospitalized patients were previously vaccinated with two doses of measles-mumps-rubella vaccine. The ages of patients were 5-10 years school aged children. All samples were collected from blood collection unit in Al-Imamein Al-Kadhimein Medical city during the period from December 2018 to April 2019. The detection was based on the presence of IgG Antibody to measles. The positive results were considered according to enzyme linked immunosorbant assay and conventional polymerase chain reaction to detect CD46 SNP.

Results Among those 158 subjects' male children count for 62% and female children were 38%. Forty-one (41) immunized children (with mean age 7.98±1.92 years) were low immune response to measles vaccine (MV). On the other hand, only two cases (with mean age 5.50±0.71 years) were negative to measles virus vaccine.

Conclusion Ensuring two doses of MV give protective immune response to MV may declines with aging. CD46 cellular receptor gene SNP (rs7144) may play role in reduction in immune response to MV.

Keywords Measles, MMR, CD46, SNP, Iraq

Citation Al-Gburi SAJ, Kadhim HS, Ghazi HF. Association of CD46 Cellular Receptor Gene SNP in measles vaccine response. *Iraqi JMS*. 2020; 18(1): 39-46. doi: 10.22578/IJMS.18.1.6

List of abbreviations: CD46 = Cluster of differentiation 46, ELISA = Enzyme linked immunosorbant assay, MMR = Measles, mumps rubella vaccine; MV = Measles vaccine, PCR = Polymerase chain reaction, SNP = Single nucleotide polymorphism

Introduction

Measles is a highly infectious disease that results from infection with the measles virus. Measles was one of the top causes of childhood morbidity and mortality and responsible for over 2 million childhood deaths each year before the

introduction of measles vaccines and the increase in global measles vaccine coverage resulting from the Expanded Program on Immunization (EPI) that started in the 1980s⁽¹⁾. Measles incidence and mortality have declined substantially in the last 20 years due to the improvement in socioeconomic status, better nutrition, and the increasing use of live attenuated measles vaccines administered through routine childhood immunization programs and mass vaccination campaigns⁽²⁾.

The World Health Organization (WHO) reports annually the number of reported measles cases, the estimated number of deaths, as well as on national measles vaccination coverage. Reported measles cases decreased worldwide from 850,000 to 250,000 between 2000 and 2015 ⁽³⁾. During the same time period, estimated measles deaths, derived from a model based on reported cases, vaccine coverage, and age-specific fatality ratios ⁽⁴⁾, decreased by almost 80% ⁽²⁾. Global measles vaccine coverage with the first dose of measles vaccine (MV) increased from 72 to 85% from 2000 to 2010, but since then has plateaued at about 85% ⁽³⁾. In 2012, the World Health Assembly endorsed the Global Vaccine Action Plan with the objective of eliminating measles ⁽⁵⁾. The encouraging reductions in measles incidence and mortality have led to five out of six WHO regions adopting the Global Vaccine Action Plan with targets to eliminate measles by 2020 ⁽⁵⁾. Studies had shown that measles vaccine immune response declines due to genetic variants in CD46 cellular receptor that is responsible for the introduction and enhancement of immune response to MV ^(6,7). This study aimed to evaluate the immune response to measles infection among immunized school aged children, and to detect the CD46 SNP in association with the immune response.

Methods

The current study is a cross sectional study including 158 hospitalized patients were previously vaccinated with measles, mumps and rubella (MMR) vaccine.

This study under the agreement of the ethical approval of the Institutional Board Review (IRB) in the College of Medicine, Al-Nahrain University under the No. 165 dated 17/12/2018.

The ages of patient 5-10 years school aged children. From all selected patients, 5 ml of whole blood were collected. Two ml of blood were collected in EDTA tubes for DNA extraction and conventional PCR and store at (-20 °C). DNA extraction was done according to DNA extraction protocol (Geneaid Kit, Taiwan)

The residual 3ml of blood were separated into serum for enzyme linked immunosorbant assay (ELISA) detects IgG Ab level to measles.

Conventional polymerase chain reaction (PCR) was used to detect the presence of SNP in CD46 cellular receptor gene that expected to affect the IgG antibody level in patient who is previously vaccinated with MMR vaccine. Primers were designed in online version of primer design software by Integrated DNA Technologies website and synthesized using Oligo Synthesis Service in Acculigo® Bioneer, Korea. as showed in table (1) and prepared for PCR reaction according to manufacture instructions. All primers that used in this experiment are demonstrate in table (1).

Table 1. The primers used in this study

Gene		Primer sequence (5'-----3')	Product size (bp)	Reference
CD46 rs2724384	F	CAAGTCCATTTCTCCACTG	228	(Clifford 2010) ⁽⁷⁾
	R	GGTTTACCAATGAGCTCCATA		
CD46 rs7144	F	AAGTGAACACTGTAGTCTTGTT	272	(Clifford 2010) ⁽⁷⁾
	R	TCTGCCTTTTAGGAGATGAG		

Results

This study included 158 hospitalized school age children were previously vaccinated with measles virus vaccine (MV). Forty-one children

were low immune response to MV with mean age 7.98±1.92 years. Two cases were negative to measles virus vaccine with mean age 5.50±0.71 years. Eighty-nine cases were



moderate immune response to MV with mean age 6.81 ± 1.10 years. There was a significant statistical difference in the means of ages among the study groups as shown in table (2).

Table 2. The mean and standard deviation of vaccinated school age children

Age	Vaccine response				Total (158)
	Negative (n=2)	Low (n=41)	Moderate (n=89)	High (n=26)	
Mean	5.50	7.98	7.98	6.81	7.75
Standard Deviation	0.71	1.92	1.57	1.10	1.66
Median	5.50	8.00	8.00	7.00	8.00
Percentile 25	5.00	6.00	7.00	6.00	6.00
Percentile 75	6.00	10.00	10.00	8.00	9.00
Minimum	5.00	5.00	5.00	5.00	5.00
Maximum	6.00	10.00	10.00	9.00	10.00
P value	0.002				

The study also classified patients according to their age groups, the results shows low immune response 12.9% in age group 7 years. While in age 10 years was 36.3%. Moreover, the moderate immune response was 41.9% in age range from (5-6) years. While in age groups 7 and 10 years was around 64%. High immune

response was measured in (5-6) years age group and it was 27.9%. While no one had high immune response, in age group 10 years (0.0%). There were statistically significant differences ($P=0.032$) between age groups as shown in table (3).

Table 3. Patients' categories according to age groups

Vaccine Immune Response	Age group (year)				
	5-6 years	7 years	8 years	9 years	10 years
Negative	2	0	0	0	0
%	4.7	0.0	0.0	0.0	0.0
Low	11	4	6	7	13
%	25.6	12.9	22.2	33.3	36.1
Moderate	18	20	16	12	23
%	41.9	64.5	59.3	57.1	63.9
High	12	7	5	2	0
%	27.9	22.6	18.5	9.5	0.0
Total	43	31	27	21	36
P value	0.032*				

p value < 0.05

On the other hand, the study shows variation in immune response to MV vaccine according to number of vaccine doses; 60.8% of cases

were immunized by three doses of MV vaccine with moderate immune response. In addition to that, 47.9% cases were immunized by 2

doses of MV vaccine with moderate immune response. There were statistically significant differences (P=0.008) between age groups as shown in table (4).

Table 4. Vaccine categories according to vaccine doses

Vaccine Response Category	Number of vaccines Doses			Total
	1.00	2.00	3.00	
Negative	1	1	0	2
%	12.5	2.1	0.0	1.3
Low	2	19	20	41
%	25.0	39.6	19.6	25.9
Moderate	4	23	62	89
%	50.0	47.9	60.8	56.3
High	1	5	20	26
%	12.5	10.4	19.6	16.5
Total	8	48	102	158
%	100.0	100.0	100.0	100.0
P value	0.008*			

P value < 0.01 highly significant

The study also showed the frequency of heterozygous mutant (CT) 25% was slightly more in age group 10 years, a marked rise in homozygous mutant genotype among the same groups (22.2%). Moreover, there were significant elevated in homozygous wild genotype (TT) 80.6% in age group 7 years.

Likewise, the frequency of minor allele (CT) 34.7% in age group 10 years. There were elevated in minor allele (TT) among age group 5-6 years. There were statistically significant differences among age groups as shown in table (5).

Table 5. Vaccine categories according to vaccine doses

Age Group	Genotype			Total
	Homozygous mutant	Heterozygous	Homozygous wild	
5-6 years	1	7	33	41
%	2.4	17.1	80.5	100
7 years	4	2	25	31
%	12.9	6.5	80.6	100
8 years	0	6	21	27
%	0.0	22.2	77.8	100
9 years	3	3	15	21
%	14.3	14.3	71.4	100
10 years	8	9	19	36
%	22.2	25.0	52.8	100
Total	16	27	113	156
%	10.3	17.3	72.4	100
P value	0.001*			

P value < 0.01 highly significant

In addition to that, this study showed vaccine response level according to the polymorphism in CD46 gene there were significant elevated in heterozygous genotype (CT) 36.4% in subjects with low immune response to Measles vaccine.

While there were elevated in homozygous wild genotype (TT) 85.5%. Moreover, there were no significant differences in heterozygous and homozygous mutant genotypes except in high immune response as shown in table 6.

Table 6. The association of vaccine response level according to the polymorphism in CD46 gene

Age Group	Genotype			Total
	Homozygous mutant	Heterozygous	Homozygous wild	
Low	26	40	44	110
%	23.6	36.4	40.0	100
Moderate	6	14	118	138
%	4.3	10.1	85.5	100
High	0	0	64	64
%	0.0	0.0	100.0	100
Total	32	54	226	312
%	10.3	17.3	72.4	100
P value	<0.001*			

P value < 0.01 highly significant

Based on allele specific technique (Figure 1), this polymorphism had only one genotypes in vaccinated school age children which were CC. The results showed the frequency of homozygous mutant genotype of CD46 receptor gene CC polymorphism was found in patient with lower level of IgG antibody response.

Figure 2 shows the polymorphism had only two genotypes in patient that immunized with measles vaccine which were TT and CT. It showed the frequency of genotype and allele of CD46 receptors gene polymorphism in immunized children. Heterozygous genotype CT was higher in patient with intermediate immune response to measles mumps vaccine.

Discussion

There are several reasons for an outbreak of measles among children, like variability in MV response between children. Poor immunogenicity and vaccine failure in young children contribute to the ongoing burden of measles. This study aimed to identify whether genetic variation in the MV receptor CD46 gene

contributed toward measles-specific antibody responses in an Iraqi cohort of children following their contact with measles vaccine. This study also aimed to investigate whether CD46 polymorphisms were associated with functional effects on the CD46 receptor in order to determine a possible mechanism through which the genetic variant acts on antibody responses. Three CD46 gene polymorphisms were associated with measles IgG levels (rs7144, rs11118580, and rs2724384), supporting the hypothesis that CD46 gene polymorphism plays an important role in how a child responds to measles vaccine (6). There is significant association between rs7144 CT and rs7144 CC and lowering the IgG Ab level particularly in children with aged between 9-10 years, there were significant decrease in IgG Ab due to the heterozygous, homozygous mutant allele CC, CT respectively in compared to TT homozygous wild type allele that have been association with elevated IgG Ab level, this is agreed with Clifford et al. (2012) (7).

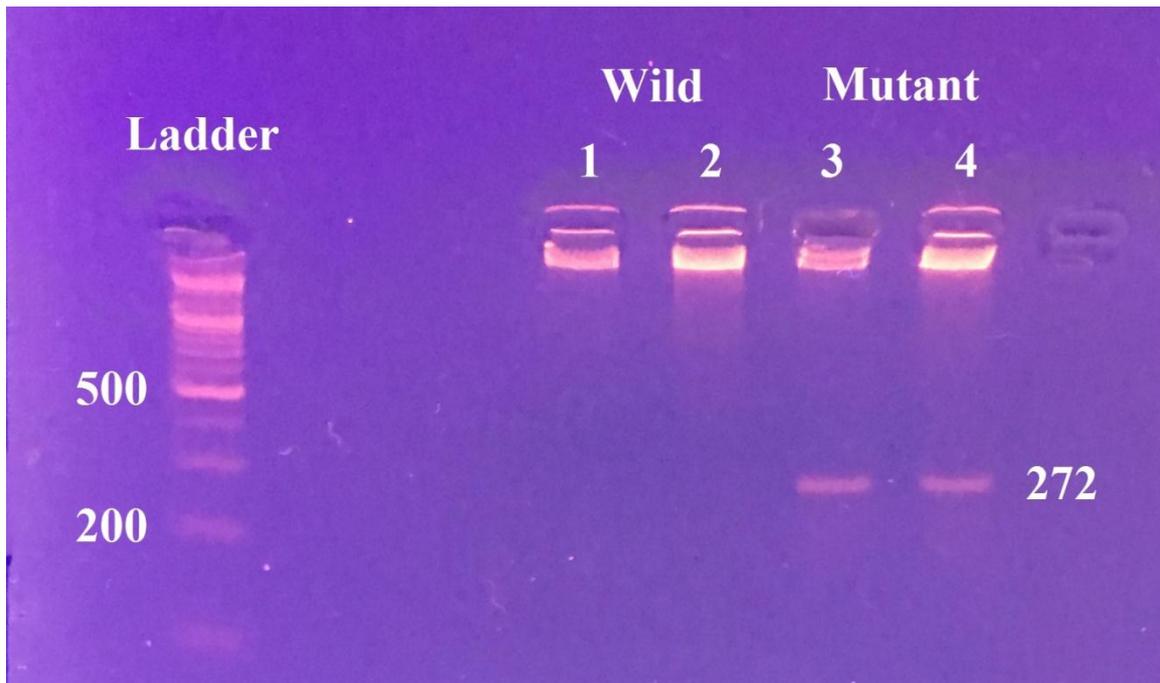


Figure 1. Gel electrophoresis for CD46 gene PCR products visualized U.V light after staining with ethidium bromide. Ladder:100-2000 bp; lanes 3,4; homozygous genotype CC, lanes 1,2; homozygous wild genotype TT

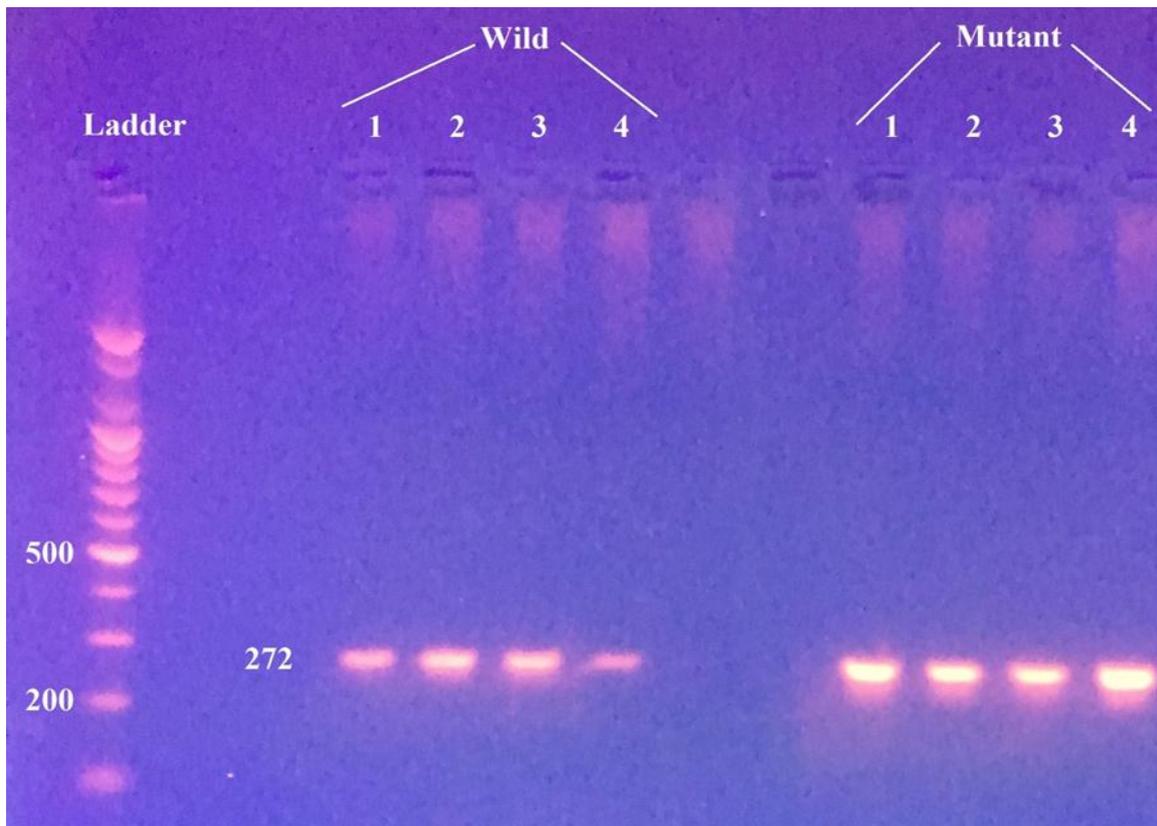


Figure 2. Gel electrophoresis for CD46 gene PCR products visualized U.V light after staining with ethidium bromide. Ladder: 100-2000 bp; the right lanes 1,2,3 and 4: homozygous mutant genotype CC, and the left lanes1,2,3and 4: heterozygous mutant genotype CT

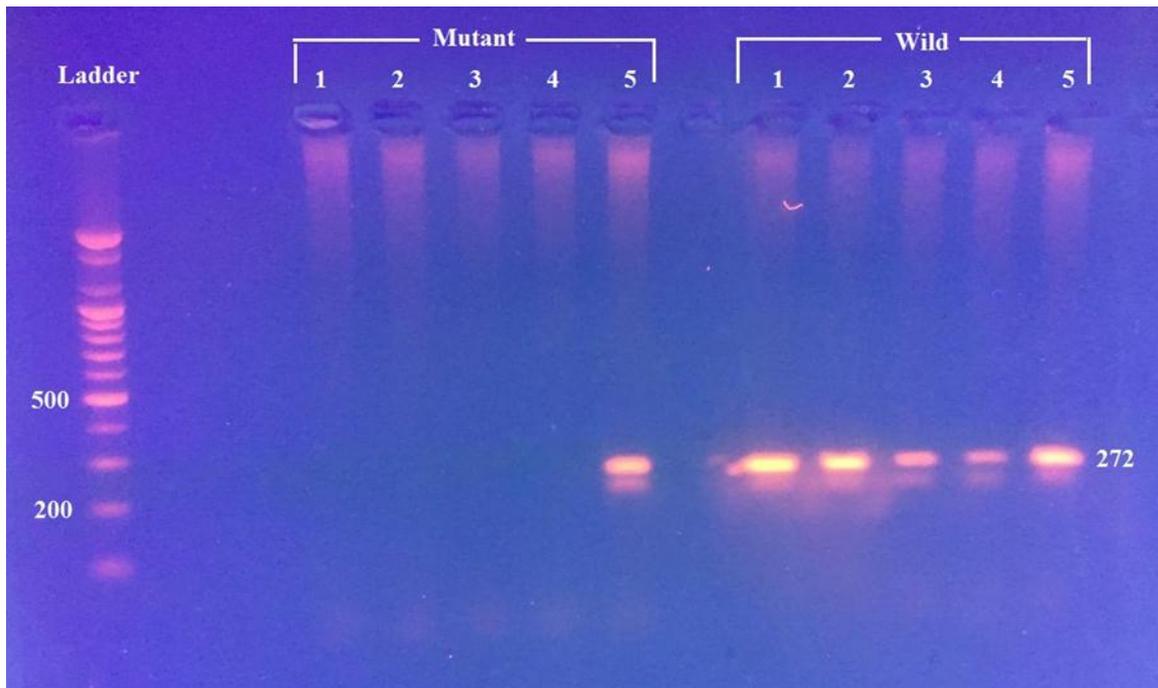


Figure 3. Gel electrophoresis for CD46 gene products visualized U.V light after staining with ethidium bromide. Ladder: 100-2000 bp;1,2,3 and 4 homozygous wild genotype TT. Lane 5; heterozygous CT

This study also showed the elevated proportion in number of subjects who were taken three doses of MMR vaccine, they showed moderate immune response (60.8%), in addition to those subjects with two doses of MMR vaccine they showed low immune response (47.9%), this could be due to the possibility of existence of heterozygous and homozygous genotype (CT, CC) (10.1%, 4.3%) respectively in subjects with moderate immune response to MV response, on the other hand, the frequency of minor allele (CT, CC) were showed in subject with low immune response (36.4%, 36.6%) respectively these results agreed with Dhiman et al. (2007) ⁽⁶⁾.

The study also showed that children unknown history of vaccination was about 12.5% with negative immune response to MMR vaccine, 25%, 50% subjects with low and moderate immune response respectively to MMR vaccine who had an unknown vaccination history or sub-optimal measles vaccine coverage. The majority of those with an unknown vaccination history were among the cases reported from those living outside the capital city of the province ⁽⁸⁾. A study showed the immune

response to MV was measured as low response 12.9% in age group 7 years. While in age 10 years was 36.3%. Moreover, the moderate immune response was 41.9% in age range from 5-6 years, while in age groups 7 and 10 years was around 64%. High immune response was measured in (5-6) years age group and it was 27.9%. While no one had high immune response, in age group 10 years (0.0%). This could be due to that immune response may decline with age ^(9,10).

In conclusions, this study showed that CD46 SNP may affect immune response to MV, in addition to the possible reduction in immune response to this vaccine with age.

Acknowledgement

Thanks to laboratory staff in the blood collection unit in Al-Imamein Al-Kadhimein Medical city and all the participants

Author contribution

Al-Gburi: did the sampling and lab works; Dr. Kadhim supervised the study; Dr. Ghazi prepared the manuscript and the statistical analysis.

Conflict of interest:

Authors declare no conflict of interest.

Funding:

Self-funded.

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Received Mar. 12th 2020

Accepted Jun. 14th 2020