BioGecko

Vol 12 Issue 03 2023
ISSN NO: 2230-5807

Gene polymorphisms of some toll like receptors associated with cytomegalovirus infection in pregnant women

[1]Adyan Haider Bander and [2]Ghaidaa Jihadi Mohammed
Department of Biology, College of Science , University of Al-Qadisiyah , Al- Diwaniayh , Iraq.

Abstract

Background: Human cytomegalovirus is the most common reason of intrauterine infections globally.Particularly, TLR2,TLR3 have been associated in antiviral immunity. So, the function of single nucleotide polymorphisms in TLR2, TLR3 genes in the emergence of human cytomegalovirus infection in pregnant women is very important.This research was done to determinea gene polymorphisms of some toll like receptors associated with cytomegalovirus infection in pregnant women.

Methods : The research was carried out for 112 pregnant women, including 68 pregnant women infected with Cytomegalovirus , and 44non infected pregnant women as control . Genotypes in TLR2 A > G and TLR3 C > T SNPs were identified using self-designed nested T-ARMS PCR tests. Sequencing validated randomly chosen PCR results that represented unique genotypes in TLR SNPs.

Results: TLR2 frequency ranges of (rs1898830) AA,AG,GG were 47.1%,33.1%,19.1% in the patients group, and 61.4%,34.1%,4.5% in the control group, respectively. There was non-significant variationamong patients and control group.Whereas, for TLR3 genotypes ,There was a significant variation in the patients and the control group. Regarding co-dominant mode, there was a significant variation in the frequency of genotypes between patientsand control ($p = 0.001$). Risk analysis revealed that the homozygous TT genotype was a significant risk factor (OR=10.18), also heterozygous C/T genotype was a significant risk-factor with (OR =7.21).

Conclusion:From this study ,It has been concluded TLR3rs377591C>T SNP might be linked to human cytomegalovirusinfection in women who have pregnancyandthe homozygous TT genotype was a significant risk factor ,also heterozygous C/T genotype was a significant risk-factor.

KEYWORDS: Human cytomegalovirus (HCMV); Toll-like receptors (TLRs); Single nucleotide polymorphism (SNP); Pregnant women, Antigen-Presenting Cells (APCs).

Introduction

Cytomegalovirus (CMV) is a member of the Herpesviridaegroup and the Betaherpesvirinae subfamily; CMV infection is extremely common in pregnant women (Auriti et al.,2021). CMV is identified during infection by a difference of pathogen recognition receptors, including toll-like receptors (TLRs), which trigger the first line of host defense,resulting in the production of inflammatory cytokines (Frascaroli et al.,2020).

TLRs are crucial pieces of the innate immune system because they identify a wide range of pathogens, including viruses. However, certain pregnancy complications have been associated to TLR-dependent antiviral responses (Olmos-Ortiz et al.,2019).The TLR protein family has been associated to both normal and abnormal pregnancy processes, including as premature labor, preeclampsia, and IUGR (Eldar-Yedidia.,2017).TLR2 is a kind of I integral membrane glycoprotein that identifies the most pathogen-associated molecular patterns (PAMPs) in innate immunity of any pattern recognition receptor (PRRs) (Sameerand Nissar,2021). TLR2 is encoded by 5 exons and introns. The SNP Rs1898830 is an A/G mutation in TLR2 intron1 region (Zhu et al.,2020

during the Human Cytomegalovirus replication, TLR3 detects viral double-stranded RNA (dsRNA) self-RNAs produced by damaged cells (Sghaier et al.,2019). TLR3 induces an alternative pathway (TRIF) that activates IRF3, NF-B, as a result of which type I IFN and inflammatory cytokine genes are activated (Kawasaki and Kawai.,2014).The SNP rs3775291 is one of the most important SNPs in the TLR3 gene (

chen et al.,2015), rs3775291 (C1234T) is the major gene polymorphism site: On exon 4, T mutated from C, causing the leucine to be replaced by phenylalanine (Barkhash et al.,2013).So, this study aimed to detect gene polymorphisms of some toll like receptors associated with cytomegalovirus infection in pregnant women.

Methods

Collection of Blood Samples

Blood samples were taken from 112 pregnant women,including 68 pregnant women infected with Cytomegalovirus , and 44 non infected pregnant women as control groupin Maternity and Children's Teaching Hospital in Al-Diwaniyah city. The collection period extended from August 2022 to November 2022.A 2 ml of venous blood were collected in aseptic condition technique,it were put in ethylenediaminetetraacetic acid (EDTA) tube to usefor DNA exaction.

Genomic DNA Extraction

Genomic DNA from blood-samples were taken by using gSYAN DNA kit (extraction kit)from Frozen Blood that provided by Geneaid, Taiwanand done byfollowing company instructions, and the collected blood genomic DNA was tested using a Nanodrop spectrophotometer (Techne ,UK), It measured the concentration of DNA (ng/µL) and checked the purity of the DNA by reading the absorbance at (260 /280 nm).

Tetra- ARMS-PCR Method

The ARMS-PCR reaction (tetra-primer amplification refractory mutation system-polymerase chain) is a easy and inexpensive technique for genotyping single nucleotide polymorphisms.It employs four primers in a single PCR and is followed only by gel electrophoresis. nevertheless, the optimization stage might be time consuming and labor intensive. Two SNPs were chosen with varied amplification conditions. DNA extraction techniques, annealing temperatures,PCR cycle procedures, chemicals, and primer concentrations were all investigated. The use of T-ARMS-PCR for SNPs in cytosine and guanine-rich DNA regions. The melting temperature was deemed to be the most interfering factor. Small Changes in reagent concentration, particularly MgCl2, have a significant influence on the PCR. The inner primers band must be adjusted as well. So, in order to balance the inner primers band, Find the owner of the weaker band and enhance it by increasing its concentration.

T-ARMS-PCR method was performed for detection and genotyping of TLR2 rs1898830 A/G and TLR3 rs3775291 C/T gene polymorphism in Pregnant women with CMV and control samples. Tetra-ARMS-PCR Primers were This study was created utilizing NCBI-SNP data base and PRIMER1design for tetra-primer ARMS-PCR tool design online. These primers were provided from (Scientific Researcher . Co. Ltd. Iraq) as following tables:

Table (1): The T-ARMS-PCR Primers for TLR2 rs1898830 A/G gene polymorphism with their sequence and product size.

Primer	Sequence (5'-3')	Product size(bp)
Forward inner primer (G allele).	AGTAAAATAAATCCAGAGAAAGCG	153
Reverse inner primer (A allele).	TCTTATATTATTATTTCCCCTGTGCT	109
Forward outer primer	AAATGAAGAGTGACGAAAAATGA	212
Reverse outer primer.	AGCTTTTATTGTCTTGCCAGAG	212

Table (2): The Tetra-ARMS-PCR Primers for TLR3 rs3775291 T/C gene polymorphism with their sequence and product size.

Primer	Sequence (5'-3')	Product size(bp)
Forward inner primer (T allele).	CTCATTCTCCCTTACACAGAT	195
Reverse inner primer (C allele).	AGATTTTATTCTTGGTTAGGTTTAG	270
Forward outer primer.	AACGGTCTTTTACTAAACAAAGT	419
Reverse outer primer.	CAGGTACTTGTTGTAGGAAAGA	419

Statistical analysis :

Data were collected, summarized, analyzed and presented using a social sciences statistical software (SPSS) version 26and Microsoft Office Excel 2010. Numeric data were presented as mean, standard deviation, range. Independent sample the t-test was used to compare mean differences between any two groups as long as the variable was regularly distributed. Chi-square test was used to investigate the relationship between any two category variables. The P-value of significance was set at 0.05 or less, while the level of significance was set at 0.01 or less.

Results:

Detection of *TLR2 rs1898830A/G SNP* in pregnant women with CMV

The TLR2 distribution of rs1898830 A/G Polymorphism was detected by T-ARMS-PCR technique. figure (1).

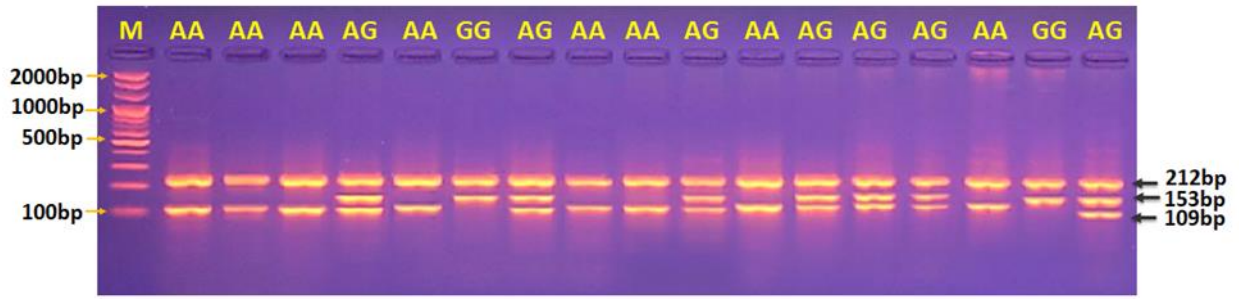

Figure (1): Agarose gel electrophoresis image that showed the T-ARMS-PCR product analysis of for TLR2 (rs1898830) A/G gene polymorphism . Where M: marker (3000-100bp). The lane (AA) wild type homozygote were showed only A allele at 109bp T-ARMS-PCR product. The lane (GG) mutant type homozygote were showed only G allele at 153bp T-ARMS-PCR product , whereas the (AG) heterozygote were showed as both A and G allele at 109bp and 153bp T-ARMS-PCR product . The outer internal control were observed at 212bp T-ARMS-PCR product.

The genotypes and allele frequencies for TLR2 rs1898830 A/G SNP were compared between patients and the control group was shown in table (3).In all research groups, the genotype distribution did not deviate from Hardy-Weinberg equilibrium. Regarding co-dominant mode, There was no significant difference in genotype distribution of frequencies between patients and controls (p = 0.072). Risk analysis revealed that the homozygous GG genotype was a non-significant risk factor (OR= 5.48), also heterozygous A/G genotype was a non-significant risk factor with an OR of (1.29), which means that patients with heterozygous GG genotype are approximately five time more liable to develop infection in comparison with patients with other genotypes. Regarding the dominant mode analysis there was a non-significant

difference between patients and control group (p= 0.138). But regarding the recessive mode analysis, There was a significantly different outcome between the patient and control groups (p<0.05). Also regarding the allele analysis, there was a significant-difference between patients and control (p< 0.05).

Table (3): *TLR2 rs1898830 A/G* poly genotype frequency in patients and control group

Mode	*TLR2 rs1898830*	Patient *n* = 68	Control *n* = 44	*P*	OR	95% CI
Co-dominant	GG	13 (19.1%)	2 (4.5%)	0.072 ¥ NS	5.48	1.13-26.5
	A/G	23 (33.1%)	15 (34.1 %)		1.29	0.56 -2.96
	AA	32 (47.1 %)	27 (61.4 %)		Reference	
Dominant	GG+A/G	36 (52.9 %)	17 (38.6 %)	0.138 ¥ NS	Reference	
	AA	32 (47.1 %)	27 (61.4 %)		0.559	0.258-1.21
Recessive	GG	13 (19.1%)	2 (4.5%)	0.027 ¥ S	4.96	1.06-23.2
	A/G+AA	55 (80.9%)	42 (95.4 %)		Reference	
Alleles	G	49 (36.0%)	19 (21.6 %)	0.021 ¥ S	2.04	1.14-3.79
	A	87 (64.0%)	69 (78.4 %)		Reference	

¥: Chi-square test;NS:not significant at *P*> 0.05;S: significant at P ≤ 0.05.

Detection of *TLR3* rs3775291SNPin pregnant women with CMV

The TLR3 distribution of rs3775291 C/T Polymorphism was detected by T-ARMS-PCR technique. figure (2).

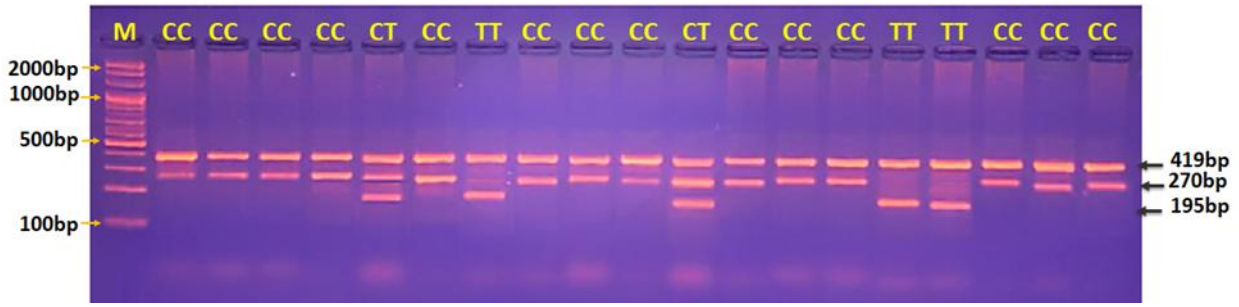

Figure (2): Agarose gel electrophoresis image that showed the T-ARMS-PCR product analysis of for TLR3 (rs3775291) C/T gene polymorphism. Where M: marker (3000-100bp). The lane (CC) wild type homozygote were showed only C allele at 270bp T-ARMS-PCR product. The lane (TT) mutant type homozygote were showed only T allele at 195bp T-ARMS-PCR product , whereas the (CT) heterozygote were showed as both C and T allele at 270bp and 195bp T-ARMS-PCR product . The outer internal control were observed at 419bp T-ARMS-PCR product.

Thecomparison of genotypes and allele frequencies concerning *TLR3 rs3775291 C/T* SNP between patients and controlgroup was appear in table (4). Regarding co-dominant mode, there was significant difference in the frequency distribution of genotypes between patients and control group (p = 0.001). Risk analysis revealed that the homozygous TT genotype was a significant risk factor (OR= 10.18) which means that patients with homozygous TT genotype are approximately ten time more liable to develop infection in comparison with patients with other genotypes. , also heterozygous C/T genotype was a

significant risk factor with an OR of 7.21. Regarding the dominant mode and recessive mode analysis, The patient and control groups had significantly different outcomes (p= 0.001). Also regarding the allele analysis, there There was significant variation between the patients and the controls (p< 0.05).

Table (4): *TLR3 rs3775291 C/T* Poly genotype frequency in patients and control

Mode	***TLR3 rs3775291***	**patient *n* = 68**	**Control *n* = 44**	***P***	**OR**	**95% CI**
Co-dominant	TT	40 (58.8%)	10 (22.8%)	0.001 ¥ S	10.18	3.81-27.2
	C/T	17 (25.0%)	6 (13.6 %)		7.21	2.25 -23.07
	CC	11 (16.2 %)	28 (63.6 %)		Reference	
Dominant	TT+C/T	57 (83.8%)	16 (36.4 %)	0.001 ¥ S	Reference	
	CC	11 (16.2 %)	28 (63.6 %)		0.110	0.04-0.268
Recessive	TT	40 (58.8%)	10 (22.8%)	0.001 ¥ S	7.55	3.07-18.55
	C/T+CC	18 (41.2%)	34 (77.2 %)		Reference	
Alleles	T	97 (71.3%)	26 (29.5 %)	0.001 ¥ S	5.93	3.29-10.7
	C	39 (28.7%)	62 (70.5 %)		Reference	

¥: Chi-square test, S: significant at P ≤ 0.05.

Discussion

The mechanisms through which Cytomegalovirus causes inflammation are still understandable. During illness, Cytomegalovirus is identified through a variety of pathogen recognition receptors, including toll-like receptors (TLRs), which stimulate the first defensive line of host defense, resulting in the release of inflammatory cytokines and, in most cases, type I IFN. TLR2 identified CMV envelope components such as glycoproteins expressed on the cell plasma membrane gB and gH (Frascaroli*et al*.,2020). The genetic associations with cytomegalovirus infections risk were evaluated using T-ARMS-PCR analysis to identify if genetic variants in TLR2 could be a potentially genetic marker to predict the susceptibility of Cytomegalovirus infections. Frequencies of gene and allele (rs1898830 Polymorphism) were measured in 68 pregnant women infected by Cytomegalovirus and 44 pregnant women uninfected with Cytomegalovirusand statistically there was non-significant variations found in the distribution of the genotype frequencies of TLR2 (rs1898830) among the patient and the control groups (P= 0.072).

The frequency ranges of the GG, AG, and AA genotypes were 13, 23, 32 and 2, 15, 27, among patient and the controls, respectively. The homozygous genotype GG was more common in the patients group in comparison to the control group, 13 versus 2, respectively, and it was considered as a non significant risk factor with an OR of 5.48, this study agreement with Taniguchi *et al* (2013) who showed the homozygous genotype GG was greater in people who have congenital CMV infection than in general population. Also this study in line with Kim *et al* (2013) who found the GG genotype is more frequency in patient (23.8%) than control (19.9%). The heterozygous AG genotype was also non significant risk factor (OR= 1.29) but it's low frequency in patient group than control group, this study agree with Huang *et al*. (2015) who found the heterozygous AG is more common in control group than in patient group.Further this current results was disagree with Chen *et al* (2010) that showed the AG genotype is more frequent in patient (54.9%) than control (49.5%) .

TLR3 is a part of the TLR family that mediates type I interferon transcriptional stimulation(IFNs), proinflammatory cytokines, and chemokines, resulting in an antiviral host response (Yujuan*et al*.,2021). TLR3 detects viral dsRNA as well as double-stranded RNA (dsRNA) analogs that can be produced as an intermediary during the replication cycle of single-stranded RNA (ssRNA) or DNA viruses (Studzińska*et al*.,2017).TLR3 is located on the 4q35 chromosome and encodes 904 amino acids; the key gene polymorphism site is rs3775291: T evolved from C, resulting in the conversion of 421 leucine to phenylalanine (Jiménez-Sousa *et al*.,2015).

The current study's findings shown high frequency of (TT) genotype among patients when compared to healthy controls, 40 versus 10, respectively, and it was considered as significant risk factor with an OR of 10.18, this result agreement with Rong*et al*. (2013) who found the genotype (TT) more frequency in patients group than in control group.

The heterozygous CT genotype was also a significant risk factor (OR= 7.21). It more frequency in patients than control group, this current results was consistent with results of Redondo *et al*. (2022) who was finding the rate of CT genotype is high in kidney transplant recipients infected with CMV than people who are non infected.

The T allele was detected in (71.3%) of the patients, and in (29.5 %) of the healthy controls, and this results agree with results of Habibabadi*et al*.(2020), which found that T allele frequency in patients (30.0%) higher than controls (29.7%). Also the result of current study agree with result of Singh *et al*. (2021) , reported that T allele frequency in patients (26.5%) higher than controls (14.2%).

Conclusion: From this study , It has been concluded TLR3 rs377591 C > T SNP might be linked to HCMV illness in pregnant women and the homozygous TT genotype was a significant risk factor ,also heterozygous C/T genotype was a significant risk factor.

Reference

Auriti, C., De Rose, D.U., Santisi, A., Martini, L., Piersigilli, F., Bersani, I., Ronchetti, M.P. and Caforio, L., 2021. Pregnancy and viral infections: Mechanisms of fetal damage, diagnosis and prevention of neonatal adverse outcomes from cytomegalovirus to SARS-CoV-2 and Zika virus. Biochimica et BiophysicaActa (BBA)-Molecular Basis of Disease, 1867(10), p.166198.

Barkhash, A.V., Voevoda, M.I. and Romaschenko, A.G., 2013. Association of single nucleotide polymorphism rs3775291 in the coding region of the TLR3 gene with predisposition to tick-borne encephalitis in a Russian population. Antiviral research, 99(2), pp.136-138.

Chen, D.N., Song, C.G., Yu, K.D., Jiang, Y.Z., Ye, F.G. and Shao, Z.M., 2015. A prospective evaluation of the association between a single nucleotide polymorphism rs3775291 in toll-like receptor 3 and breast cancer relapse. PLoS One, 10(7), p.e0133184

Chen, Y. C., Hsiao, C. C., Chen, C. J., Chin, C. H., Liu, S. F., Wu, C. C., ... & Lin, M. C. (2010). Toll-like receptor 2 gene polymorphisms, pulmonary tuberculosis, and natural killer cell counts. *BMC medical genetics*, *11*(1), 1-10.

Eldar-Yedidia, Y., Hillel, M., Cohen, A., Bar-Meir, M., Freier-Dror, Y. and Schlesinger, Y., 2017. Association of toll-like receptors polymorphism and intrauterine transmission of cytomegalovirus. Plos one, 12(12), p.e0189921.

Frascaroli, G., Rossini, G., Maltoni, V., Bartoletti, M., Ortolani, P., Gredmark-Russ, S., Gelsomino, F., Moroni, A., Silenzi, S., Castellani, G. and Sambri, V., 2020. Genetic and functional characterization of Toll-like receptor responses in immunocompetent patients with CMV mononucleosis. Frontiers in Cellular and Infection Microbiology, 10, p.386.

Frascaroli, G., Rossini, G., Maltoni, V., Bartoletti, M., Ortolani, P., Gredmark-Russ, S., Gelsomino, F., Moroni, A., Silenzi, S., Castellani, G. and Sambri, V., 2020. Genetic and functional characterization of Toll-like receptor responses in immunocompetent patients with CMV mononucleosis. Frontiers in Cellular and Infection Microbiology, 10, p.386.

Habibabadi, H.M., Parsania, M., Pourfathollah, A.A., Haghighat, S. and Sharifi, Z., 2020. Association of TLR3 single nucleotide polymorphisms with susceptibility to HTLV-1 infection in Iranian asymptomatic blood donors. Revista da SociedadeBrasileira de Medicina Tropical, 53.
Huang, W.H., Nie, L.H., Zhang, L.J., Jing, L.P., Dong, F., Wang, M., Zhang, N., Liu, Y., Zhang, B.H., Chen, C. and Lin, H.S., 2015. Association of TLR2 and TLR4 non-missense single nucleotide polymorphisms with type 2 diabetes risk in a southern Chinese population: a case-control study. Genetics and Molecular Research, 14(3), pp.8694-8705.
Jiménez-Sousa, M.A., Rallón, N., Berenguer, J., Pineda-Tenor, D., López, J.C., Soriano, V., Guzmán-Fulgencio, M., Cosín, J., Retana, D., García-Álvarez, M. and Miralles, P., 2015. TLR3 polymorphisms are associated with virologic response to hepatitis C virus (HCV) treatment in HIV/HCV coinfected patients. Journal of Clinical Virology, 65, pp.62-67.
Kawasaki, T. and Kawai, T., 2014. Toll-like receptor signaling pathways. Frontiers in immunology, 5, p.461.
Kim, J., Cho, Y.A., Choi, I.J., Lee, Y.S., Kim, S.Y., Hwang, J.A., Cho, S.J., Kook, M.C., Kim, C.G. and Kim, Y.W., 2013. Effects of polymorphisms of innate immunity genes and environmental factors on the risk of noncardia gastric cancer. Cancer Research and Treatment: Official Journal of Korean Cancer Association, 45(4), pp.313-324.
Liu, J. and He, M., 2023. High expression of TLR2 influenced immune infiltration in pancreatic cancer.
Olmos-Ortiz, A., Flores-Espinosa, P., Mancilla-Herrera, I., Vega-Sánchez, R., Díaz, L. and Zaga-Clavellina, V., 2019. Innate immune cells and toll-like receptor–dependent responses at the maternal–fetal interface. International Journal of Molecular Sciences, 20(15), p.3654.
Redondo, N., Rodríguez-Goncer, I., Parra, P., Ruiz-Merlo, T., López-Medrano, F., González, E., Polanco, N., Trujillo, H., Hernández, A., San Juan, R. and Andrés, A., 2022. Influence of single-nucleotide polymorphisms in TLR3 (rs3775291) and TLR9 (rs352139) on the risk of CMV infection in kidney transplant recipients. Frontiers in immunology, 13.
Rong, Y., Song, H., You, S., Zhu, B., Zang, H., Zhao, Y., Li, Y., Wan, Z., Liu, H., Zhang, A. and Xiao, L., 2013. Association of Toll-like receptor 3 polymorphisms with chronic hepatitis B and hepatitis B-related acute-on-chronic liver failure. Inflammation, 36, pp.413-418.
Sameer, A.S. and Nissar, S., 2021. Toll-like receptors (TLRs): structure, functions, signaling, and role of their polymorphisms in colorectal cancer susceptibility. BioMed Research International, 2021.
Sghaier, I., Zidi, S., Mouelhi, L., Ghazoueni, E., Brochot, E., Almawi, W.Y. and Loueslati, B.Y., 2019. TLR3 and TLR4 SNP variants in the liver disease resulting from hepatitis B virus and hepatitis C virus infection. British journal of biomedical science, 76(1), pp.35-41.
Singh, A., Prakash, S., Garg, R.K., Jain, P., Kumar, R., Jain, A. and Singh, A.K., 2021. Study of single nucleotide polymorphisms in endosomal toll-like receptors-3, 7, and 9 genes in patients with dengue: A case-control study. Cureus Journal of Medical Science, 13(5).
Studzińska, M., Jabłońska, A., Wiśniewska-Ligier, M., Nowakowska, D., Gaj, Z., Leśnikowski, Z.J., Woźniakowska-Gęsicka, T., Wilczyński, J. and Paradowska, E., 2017. Association of TLR3 L412F polymorphism with cytomegalovirus infection in children. PLoS One, 12(1), p.e0169420.
Taniguchi, R., Koyano, S., Suzutani, T., Goishi, K., Ito, Y., Morioka, I., Oka, A., Nakamura, H., Yamada, H., Igarashi, T. and Inoue, N., 2013. Polymorphisms in TLR-2 are associated with congenital cytomegalovirus (CMV) infection but not with congenital CMV disease. International Journal of Infectious Diseases, 17(12), pp.e1092-e1097.
Yujuan, C. H. E. N., Junhong, L. I. N., Yao, Z. H. A. O., Xianping, M. A., &Huashan, Y. I. (2021). Toll-like receptor 3 (TLR3) regulation mechanisms and roles in antiviral innate immune responses. Journal of Zhejiang University. Science. B, 22(8), 609.
Yujuan, C.H.E.N., Junhong, L.I.N., Yao, Z.H.A.O., Xianping, M.A. and Huashan, Y.I., 2021. Toll-like receptor 3 (TLR3) regulation mechanisms and roles in antiviral innate immune responses. Journal of Zhejiang University. Science. B, 22(8), p.609.

Zheng, W., Xu, Q., Zhang, Y., Gao, W., Zhang, M., Zhai, W., Rajkumar, R.S. and Liu, Z., 2020. Toll-like receptor-mediated innate immunity against herpesviridae infection: A current perspective on viral infection signaling pathways. Virology Journal, 17(1), pp.1-15.
Zhu, Z., Liu, J.B., Liu, X. and Qian, L., 2020. Association of interleukin 10 rs1800896 polymorphism with susceptibility to breast cancer: a meta-analysis. Journal of International Medical Research, 48(4), p.0300060520904863.