Polymorphism of Genes in Iraqi Females with Type 2 Diabetes Mellitus

Asmaa M. Salih Almohaidi[1], Rana Hadi Saeed[2], Seemaa Fahad Mirza[1], Walaa A. Mohammed[3] and Ibtisam Badday Hassan[4]

[1]*Department of Biology, College of Science for Women, University of Baghdad, 10011 Baghdad, Iraq*
[2]*Department of Biology, College of Education for Pure Science, University of Baghdad, 10011 Baghdad, Iraq*
[3]*Department of Microbiology, College of Science, Al-Karkh University of Science, 10011 Baghdad, Iraq*
[4]*Department of Biology, College of Education for Pure Science, University of Diyala, 32001 Baqubah, Diyala, Iraq*
asmaams_bio@csw.uobaghdad.edu.iq

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Abstract: Diabetes Mellitus Type 2 (T2DM) is a global medical challenge, with cytokines playing a significant role in its developing. This study evaluates the frequency of polymorphisms in Interleukin-1α (IL-1α) at position $_{-889}$ C/T (rs1800587), Interleukin-1β (IL-1β) at positions $_{-511}$ C/T (rs16944) and $_{+3962}$ C/T (rs1143634) and compared to healthy controls, diabetic patients exhibited an increased risk associated with the T allele of IL-1RL1$_{-27609}$. In the study, which was conducted by Al-Yarmouk Teaching Hospital, 720 newly diagnosed Iraqi females with T2DM, aged 20-55 years, were included along with 240 age- and gender-matched healthy controls recruited from their own hospital. Genotyping was performed by using 49 patients and 40 normal after excluded others diseases and conducted the PCR-SSP assay, and results were validated by sequencing. Allele and genotype frequencies were analysed, and significant associations were identified. The findings revealed that the T allele and TT genotype of IL-1α $_{(-889}$ C/T) significantly increased the risk of T2DM, while the C allele and CC genotype were protective. For IL-1β$_{-511}$ C/T, the T allele acted as a protective factor, whereas the C allele elevated the risk of diabetes. No significant associations were observed for IL-1β $_{+3962}$ C/T. Interestingly, polymorphisms in the IL-1RL1 receptor $_{-27609}$ T/C showed that the T allele was related with increased susceptibility to diabetes, whereas the C allele was protective. Polymorphisms in IL-1α$_{-889}$ C/T, IL-1β $_{-511}$ C/T, and IL-1RL1 $_{-27609}$ T/C appear to contribute to the genetic susceptibility to T2DM in Iraqi females. These findings highlight the potential role of interleukin signalling pathways in diabetes development. Further studies with larger, more diverse populations are recommended to confirm these results and explore their clinical implications.

1 INTRODUCTION

Diabetes mellitus represents a worldwide health disorder characterized by chronic hyperglycemia because of one or more disturbances in insulin secretion or action-or even both. Further on, this metabolic disease may be followed by other complications like cardiovascular diseases, neuropathy, nephropathy, or even retinopathy. In the view of modern studies, inflammation has become underlined to play a key role during the development and progression of T2DM, in which cytokines seem to play an important role in the mechanism of inflammation. These are small proteins that modulate immune responses and transmit information among immune cells. In particular, pro-inflammatory cytokines, including IL-1, have been implicated in the development of insulin resistance and β-cell dysfunction, hallmarks of T2DM. [1]

Members of the IL-1 family are all important; however, IL-1α and IL-1β are unique in taking part in the processes of inflammation, immune activation, and regulation of glucose metabolism. Both IL-1α and IL-1β are encoded by different genes on the long arm of the human chromosome 2 at 2q14.2. These genes encode proteins constituting a gene cluster that is important for the regulation of both innate and adaptive immune responses, particularly in response to microbial infection or tissue damage. Elevated levels of IL-1 cytokines have been observed in various

chronic inflammatory diseases, including rheumatoid arthritis, autoimmune disorders, and polycystic ovary syndrome, suggesting that dysregulation of IL-1 production could contribute to the pathogenesis of these conditions, including diabetes [2], [3].

Mechanistically, there is evidence for the involvement of cytokines in T2DM pathogenesis by demonstration of IL-1 participation in insulin sensitivity and pancreatic β-cell function. To that respect, IL-1β induces the appearance of other pro-inflammatory cytokines associated with the development of a systemic inflammatory state that impairs insulin signaling and glucose metabolism [4]. Empirical studies have established that IL-1β, both directly and indirectly, contributes to the destruction of pancreatic β-cells via the activation of immune cells and induction of apoptosis within β-cells [5]. Moreover, IL-1β impacts insulin exocytosis; high levels of IL-1β may eventually reduce the capacity of β-cells to secrete insulin and thus worsen the hyperglycemic conditions of patients with diabetes [6].

IL-1α similarly play an important role in inflammation process and immune activation. It is produced mainly by tissue macrophages, but similar to the case for IL-1β, its effects are both local at the injury/infection site and systemic. The in vivo responses induced by IL-1α are the same as those triggered by IL-1β, including the activation of innate immunity, recruitment of immune cells into sites of inflammation, and induction of local tissue injury. In the setting of T2DM, increased levels of IL-1α have been detected in peripheral blood and pancreatic tissue from patients, thus indicating that it can play a role in β-cell inflammation and dysfunction [7].

The genetic polymorphisms can alter both IL-1α and IL-1β and thus may determine the level of their expression and the individual's susceptibility to diseases. A number of polymorphisms were identified within the IL-1 gene cluster including the IL-1α -889 C/T (rs1800587) and the IL-1β -511 C/T (rs16944). Some of these SNPs have been so far associated with variability in cytokine production and, hence, are likely to modulate susceptibility of an individual to develop T2DM or other inflammatory diseases. For instance, the IL-1α -889 C/T polymorphism was found to modulate the expression of IL-1α; it showed that the T allele indeed associates with increased levels of the cytokine and escalates the risk of inflammatory diseases [8], [9]. Similarly, the IL-1β -511 C/T polymorphism has been linked to increased IL-1β production, contributing to the chronic inflammation seen in T2DM patients [10].

Moreover, other genetic variants of IL-1β, such as rs1143634 occurring within exon 5, might influence the function of IL-1β itself, thus altering insulin sensitivity and β-cell function in diabetic patients [10]. These genetic variations point toward the possibility that IL-1 genetic polymorphisms may influence the individual's immune response and the pathogenesis of diabetes. Associations of these polymorphisms with susceptibility to T2DM have been evidenced in several populations, suggesting that genetic factors could modulate the inflammatory response and influence disease outcomes.

The aims of this work are to determine the frequency of gene polymorphisms concerning IL-1α and IL-1β in the case of Iraqi women with Type 2 Diabetes Mellitus. The respective study will particularly target the following polymorphisms: IL-1α -889 C/T (rs1800587), IL-1β -511 C/T (rs16944), and IL-1β +3954 C/T (rs1143634). By studying the frequency of such genetic variants in diabetic subjects, the investigation aims to establish whether they could be associated with the development of diabetes in the same subjects. The result could be highly instructive with regard to the genetic factors determining the inflammatory component of the disease and might contribute to identify those individuals who, due to their genetic background, are at particularly high risk for the disease.

2 MATERIALS AND METHODS

2.1 Sample Collection and DNA Extraction

In this study, 720 Iraqi females, ages ranging from 20 to 55 years old, attending NDC for checking and found to be newly diagnosed as having type 2 diabetes at AL-Yarmouk Teaching Hospital.. Two hundred forty Iraqi Arab females control subjects who were apparently normal in term non diabetic, non-hypertension and age, gender matched with the studied group were selected to be the control group. After that, from them Choosing 49 as patients and 40 as healthy control, who were free of acute illness and infection, had no history of hyperlipidemia, hypertension, renal disease, heart disease, smoking, alcohol use, or any other form of hyperlipidemia or hypertension.

1) Genotyping was conducted through experimental methods. Two milliliters of fasting peripheral cubical vein blood were collected and stored in an EDTA anticoagulant tube. Genomic DNA was extracted using the TIANGEN Biochemical Technology Co. kit, LTD (Beijing). Primers used in this study are listed in Table 1.

2) After extracting the DNA samples, they were stored at 20ºC and then amplification and purification were carried out. The purified PCR products were then sent to Macrogen Company in Korea for DNA sequencing, and their obtained sequences were aligned using (Mega-6) software [9]. Moreover, the nucleotide sequences were compared with the information on the NCBI website databases for SNPs and for any other differences in the PCR product segment by utilizing the BLAST search tool.

The PCR reaction was performed for the final 25 µl reaction volume by using 5 L of 2X Go Taq® bioneer master mix, 2 µl of 10M of each primer (forward, reverse), 5 µl of genomic DNA, and the volume was increased to 20 µl with nuclease-free distil water. As stated in Table 2, the Gene Amp® PCR System thermo cycler was used to carry out the reaction.

To conduct the present study, the University Clinic Heidelberg 3-Cytokine CTS-PCR-SSP Tray Kit was paired with PCR primers to identify alleles, genotypes, and some haplotypes in 22 positions of the promoter region of different types of interleukin. The Department of Transplantation Immunology at the University Clinic Heidelberg, Germany, designed these primers based on the WHO international nomenclature committee for cytokines. Each tray in the kit had primers that were ready for use for each allele, and they were composed of 48 PCR-dried lyophilized primer mixes that were placed in 96 wells to carry out cytokine genotyping for two individuals, with 48 wells per sample. Each well of the 96 well tray was identified by a combination of numbers and letters from H1 to A12. Numeric names were given to the PCR mix positions for each cytokine, which corresponded to locus specificities on the tray.

The kit included the master mix (CYT) that included ammonium sulfate, Tris buffer, magnesium chloride, glycerol (glycerin), cresol Red, and dNTPs.

2.2 Statistical Analysis

The direct gene counting method was utilized to calculate allele frequencies, and a significant deviation from Hardy-Weinberg (H-W) equilibrium was estimated using an H-W calculator for two alleles, which can be obtained for free on the website [1].

The WINPEPI computer programs for epidemiologists were used to calculate significant differences between alleles and genotypes displayed as percentage frequencies, with free online access at website [2].

3 RESULTS

The current study test the polymorphism for IL-1α $_{-889}$ C/T; rs1800587 and two SNPs including IL-1β$_{-511}$ C/T; rs16944 in promoter and IL-1 β $_{+3962}$ C/T; rs1143634 in encoding region exon 5 [8].

According to the presented results as shown in Table 3, the genetic polymorphism analysis of IL-1α-889 which was determined in promoter region -889 position, the result displayed a higher frequency of homozygous TT genotype in the patients' group than in the control group (40.82%vs.27.50%), that showed statistical significance. Conversely, the patients' group had a lower frequency of heterozygous TC genotype than the control group (26.53% vs. 32.50%), and the genotype frequency for both patients and the control group was higher than what HWE anticipated. This difference was statistically significant. However, the allele effect study, which included both genotypes carrying the T allele, revealed no significant association between the Diabetic group and the control group (Table 4).

While receptor gene SNP' -1970 and receptor alpha msp111000 showed significant devation from HWE with odd ration more than one for homozygous CC genotypes for both SNPs.

Table 1: Primer present researcher Design for IL1.

Primer	Sequence 5 -3	length	Tm Cº	Product length	References
Forward primer	GTTGCGCCATAGACCTGTTG	20	60	785bp	3ed Researcher design
Reverse primer	TCCAAAGTCACGTGGTGCTA	20	60		

[1] http://www.had2know.com/academics/Hardy-Weinberg-equilibrium-calculator-

[2] http://www.brixtonhealth.com.

Table 2: PCR reaction cycle.

Steps of reaction	Temperature C°	Time	Number of cycles
Initial denaturation	95	4min	1
Denaturation	95	45sec	40
Annealing	60	1m.	
Extension	72	1m.	
Final extension	72	5m.	1
Hold	4	α	α

Table 3: Genotyping frequency of present study Interleukins.

IL-s types	Genotype	No: Patients 49 control 40	Genotype Frequency (Observed% / Expected %)		HWE Patients control	OR (CI: 95%) Patients vs. Control	Etiological or prevention fraction	Fisher's exact proba-bility
			Patients	Control				
IL-1α-889 rs1800587	TT	20:11	40.82/29.25	27.50/19.14	*0.001**	1.82(0.74-4.56)	0.45	0.225
	TC	13:13	26.53/49.67	32.50/49.22		0.75(0.31-1.91)	0.25	0.564
	CC	16:16	32.65/21.08	40.0/ 31.64	*0.03**	0.73(0.30-1.76)	0.27	0.443
IL-Rα-msp111000	TT	14:18	28.57/21.08	45.0/42.25	*0.005**	0.49(0.20-1.19)	0.511	0.100
	TC	17:16	34.69/49.67	40.0/45.5		0.81(0.33-1.92)	0.20	0.586
	CC	8:6	36.73/29.25	15.0/12.25	*0.03**	1.11(0.34-3.72)	0.96	0.887
IL-1R-1970	TT	23/10	46.94/50.0	50.0/42.25	*0.005**	0.88(0.38-2.06)	0.115	0.833
	TC	14/12	28.57/47.48	30.0/45.50		093(0.37-2.38)	0.67	0.909
	CC	12/8	24.49/15.04	20.0/12.25	*0.03**	1.30(0.47-3.71)	0.229	0.710
IL-1β-511 (rs16944)	TT	12:6	24.49/28.15	15.00/14.06	NS	1.84(0.62-5.80)	0.456	0.243
	TC	28:18	57.14/49.81	45.00/46.88		7.56(2.69-22.50)	0.868	4.3E-5
	CC	9/16	18.37/22.03	40.0/39.06	NS	0.34(0.13-0.89)	0.663	0.025*
IL-1β+3962 rs1143634	TT	18/18	36.73/34.78	40.82/48.39	NS	0.71(0.30-1.68)	0.290	0.453
	TC	20/15	40.82/48.39	37.50/46.22		1.15(0.48- 2.75)	0.130	0.747
	CC,	11/7	2245/16.83	17.50/13.14	NS	1.36(0.47- 4.12	0.267	0.517
IL-1RL1 -27609	TT	15/8	30.61/25.84	20.00/55.06	Ns	1.76(0.66-4.92)	0.433	0.281
	TC	24/ 14	48.9 / 42.7	35.00/55.06		1.78(0.75-4.26)	0.439	0.167
	CC	10/18	20.41/31.46	45.00/55.06	*0.013**	0.31(0.12-0.81)	0.687	*0.017**

*P≤0.05 significant , **P<0.001 high significant

Table 4: Allele frequency of present study.

ILs type	Allele type	Number patients No.: control No.	Allele frequency ratio		Odds ratio	Etiology fraction	Prevention fraction	Fisher's exact proba-bility	CI (95%)
			Patients	Control					
IL-1α-889 rs1800587	T	53:35	54.08	43.75	1.51	0.45	0.25	0.155	0.87-2.75
	C	45:45	45.92	56.25	0.66			0.155	0.36-1.20
IL-Rα-msp111000	T	45:52	45.92	65.00	1.67	0.4	0.57	0.083	0.94-2.99
	C	53:28	54.08	35.00	0.94			0.854	0.50-1.77
IL-1R-1970	T	60:52	61.22	65.00	0.45	1.15	0.55	0.11	0.64-2.18
	C	38:28	38.78	35.00	1.18			0.588	0.64-2.18
IL-1β-511 (rs16944)	T	52:30	53.06	37.50	0.188	0.469	0.469	0.049*	1.03-3.45
	C	46:50	46.94	62.50	0.58			0.049*	0.29-0.97
IL-1β+3962 (rs1143634)	T	56:51	57.14	63.75	0.76	0.242	0.25	0.401	0.41-1.39
	C	42:29	42.85	36.25	1.32			0.40	0.72-2.43
IL-1RL1 -27609	T	54:30	55.10	12.50	8.59	0.88	0.019	0.0001*	4.0-19.2
	A	44:50	44.90	62.50	0.49			0.020*	0.27-0.93

*P≤0.05 significant , **P<0.001 high significant

There was no discernible difference between the observed and expected frequencies of the three genotypes in the current study of the IL-1β$_{-511}$ gene polymorphisms, which were identified in the promoter region at the $_{-511}$ position gene in patients with diabetes mellitus (a good agreement with Hardy-Weinberg equilibrium; HWE) (Table 3).

Whereas, the two alleles' frequency (T, C) revealed some significant difference (Table 4). Nevertheless, comparing patients to controls some differences were revealed in homozygous TT genotype for patients and control (24.49 vs 15.00), and the T allele was significantly increased in patients (53.06%) compared to controls (37.50%) respectively (Tables 3 and 4)

Patients with the heterozygous TC genotype did not differ significantly from control. However, compared to control (40.0 and 62.50%), patients' CC genotype and c allele frequencies were significantly lower (18.37 vs. 46.94%).

The fifth position in the promoter region +3962 (rs1143634) of IL-1β gene, for diabetic patients and control showed that the homozygous TT genotype was detected in (36.73 vs 40.82%), heterozygous TC genotype was detected in (40.82 vs 37.50 %). Homozygous CC genotype was detected in (22.45 vs 17.50 %) Table (3). The T allele frequency was (57.14 vs 63.75%) and the C allele frequency was 42.85 vs 36.25%). (Table 4).

There were no discernible variations in the genotype distribution or allele frequency between our study groups, and the above-observed genotype frequencies matched those anticipated by the HWE.

IL-1RL1 receptor genotype polymorphism (-27609 T/C), the genotype distribution, the frequency of the TT genotype was significantly higher in diabetic patients, 30.61%, compared with controls, 20.00%, thus showing strong association with increased risk for T2DM, On the contrary, CC genotype was highly expressed among the control group, 45.00%, compared with diabetic patients, 20.41%, indicating a protective role against the disease.

In the case of allele frequencies, the T allele was strongly associated with an increased risk of T2DM, with an odds ratio of 8.59. In contrast, the C allele showed a protective effect, as evidenced by the significant prevention fraction. These contrasting roles underline the differential impact of receptor allele variants on diabetes risk within the studied population.

In addition, the genotype frequencies of the IL-1RL1 receptor -27609 T/C followed HWE expectations, showing a stable genetic distribution in this cohort. This may indicate that the observed associations are not related to population stratification or sampling bias.

4 DISCUSSION

In the Iraqi population, IL-1 polymorphisms have been linked to the etiology of metabolic diseases, diabetes, and inflammatory disorders [5], [12] and until now, no local studies have illustrated the association between IL-1α -889, IL-1β -511, and IL-1β +3962 genotypes polymorphisms with diabetes risk.

In light of these findings, the current genetic analysis provides a more precise assessment of the relationship between the genotypes IL-1α -889, IL-1β -511, and IL-1β +3962 with the risk of diabetes in female Iraqi patients.

First of all, comparison of the results of IL-1α -889 polymorphism shows that the homozygous TT genotype and the T allele may be considered as risk factors for diabetes in Iraqi females that make them more susceptible to developing T2DM by modifying immune response for most inflammatory diseases [10], [11]; while TC, CC genotypes with C allele significantly decreased the risk of diabetes which can be considered as a protective factor from T2DM. The outcome is consistent with other research that demonstrated the risk allele has a larger role in the pathophysiology of specific diseases [12], with mutant allele C being mostly derived from the ancestral allele A [13].

Neutral progressive mutations are most likely the source of late-onset diseases. These mutations can result in the total replacement of ancestral alleles, creating a situation where the common (derived) allele is the dangerous one and the rare (ancestral) allele is the protective one [14], [15].

Moreover, current findings along with previous local maintained studies results showed that the IL-1α -889 is a strong indicator for the incidence of T1DM in the Iraqi population [12], [16], as well as in an Indian study supporting a positive association between aggressive periodontitis and the presence of the IL-1α -889 with two allele polymorphisms [17], [18].

The present study disagrees with a study on older people in Brazil, where allele T of IL-1α -889 protects against the occurrence and intensity of tinnitus in the elderly, while allele C has a role in the pathophysiology of the inflammatory response, making older people more tinnitus susceptibility

associated with a history of workplace noise exposure [19].

The current findings conclude that IL-1α -889 SNP might have a role in the developing mechanism of diabetes in Iraqi female patients.

The second SNP under investigation is associated with Interleukin-1β, IL-1β -511. SNP rs16944 has been linked to immune-mediated diseases like diabetes mellitus because it plays a vital role in controlling inflammatory and immunological responses [20]. The current results suggest that; although the present expected is compatible with observed according to HWE, the IL-1β -511 polymorphism for both alleles acts significantly as protected barriers against diabetes. The present findings contradict a prior study conducted in the Indian population that demonstrated a correlation between a higher risk of diabetes and the IL-1β TT genotype and T allele [21].

In contrary, an Egyptian study finds that there is a strong significant association of C allele with the diabetic mellitus risk. Environmental factors, genetic background, and ethnic variance could all be responsible for this disparity in risk allele [22]. This finding is consistent with a systemic assessment published by the Jiao team, which discovered a link between a lower risk of diabetes and the T allele of IL-1β -511 [23].

The genotype distribution and allele frequency of the IL-1β +3962 SNP (rs1143634) DNA genotyping results showed no discernible differences between the diabetic patients and control groups. The observed genotype frequencies were in line with the Hardy-Weinberg Equilibrium, indicating that no evolutionary mechanisms were performed on this locus in the female population of Iraq. Even though the T allele's relative risk (OR) was 0.76, suggesting that it might be a protective factor, and the C allele's was 1.32, suggesting that it might be a risk allele, the above variation was not statistically significant. Since the three genotypes (CC, CT, and TT) of the IL-1β +3962 SNP appear to have the same picture and there is no discernible difference in their frequencies between patients and the control group, these current results are consistent with local research regarding Iraqi patients with multiple sclerosis (MS). This suggests that IL-1β gene polymorphisms may not be relevant to susceptibility to MS in Iraqi patients [4]. Its functionality is still debatable, although IL-1β, rs1143634, is situated in a coding area on the fifth exon of the IL-1β gene and has a synonymous codon as a functional consequence. While some studies do not indicate any difference in IL-1β secretion, others indicate that the T allele is linked to greater IL-1β production [24]. An Arabian study concluded that T allele for both IL-1β SNPs can be used as a strong marker for susceptibility to develop chronic hepatitis B in the Tunisian population [25], [26].

Another article identified independent association of common IL-1β SNPs polymorphic with HBV-related hepatocarcinogenesis in a Caucasian population [27].

The results confirm that the IL-1RL1 receptor (-27609 T/C) polymorphism is significantly associated with susceptibility to T2DM in Iraqi females, with evidence from results for the importance of genotype variations in modulating susceptibility to diabetes. At the same time, the T allele was found to be a risk allele, whereas the C allele seemed to be protective, underlining the importance of interleukin receptor pathways in the genetic predisposition to T2DM.

This is likely different across populations for a variety of reasons, including sample size limitations, which are crucial for SNP studies, racial and ethnic diversity, which causes SNPs to correlate with gene pools in each population, the relevance of this gene's haplotype with other cytokine-encoding gene haplotypes, and environmental factors that disrupt genetics and may cause the population's selection machinery to evolve.

Lastly, for the cluster of IL-1 and receptor genes polymorphism more research is needed to shed light on this relationship so that researchers may assess genetic vulnerability to prognostic and diagnostic diseases. SNPs may play crucial roles when coupled with one another and have an impact on protein production.

5 CONCLUSIONS

The analysis of IL-1 genetic polymorphisms indicates that variations, particularly in the IL-1α $_{-889\ C/T}$ and IL-1β$_{-511\ C/T}$ genotypes and ILR$_{-1970}$, may contribute to the susceptibility to T2DM among Iraqi females. The findings suggest that the T allele of IL-1α$_{-889}$ is associated with an increased risk of T2DM, while the C allele demonstrates a protective effect. Similarly, the T allele of IL-1β$_{-511}$ acts as a protective females who carry it from T2DM, whereas the C allele increases the risk of developing diabetes.

In this population, there was Fisher's ratio analysis showed that the genotypes of both alleles for IL-1β$_{-511}$and IL-1RL1 were significant, making it important to follow up with T2DM

These results emphasize the necessity for additional research with advanced methodologies

such as haplotype analysis, and Real-Time PCR techniques to validate and expand upon these results.

6 LIMITATIONS AND FUTURE DIRECTIONS

This study provides valuable insights into the genetic factors influencing T2DM risk in Iraqi females, even that there are several limitations that need to be addressed in future research. First, the sample size of 49 patients and 40 controls may limit the generalizability of the findings. Larger cohort studies are necessary to confirm the association between IL-1 polymorphisms and T2DM risk. Additionally, exploring the role of gene-environment interactions, including factors such as diet, physical activity, and obesity, is crucial in T2DM pathogenesis as epigenetic factors.

Furthermore, functional studies examining the impact of these polymorphisms on IL-1α and IL-1β expression levels, as well as their role in immune modulation, could help elucidate the underlying mechanisms linking these polymorphisms to T2DM.

REFERENCES

[1] Y.-L. Shih, K.-T. Ho, C.-H. Tsao, Y.-H. Chang, M.-Y. Shiau, C.-N. Huang, and S.-C. Yang, "Role of cytokines in metabolism and type 2 diabetes mellitus," International Journal of Biomedical Laboratory Science, vol. 2, no. 1, pp. 1–6, 2013.

[2] A. M. S. Almohaidi, "Association among P-selectin, blood system ABO and family history in the sera of Iraqi Arab females patients with diabetes mellitus type II with hypertension," BAOJ Diabet., vol. 1, no. 003, 2015.

[3] A. Litmanovich, K. Khazim, and I. Cohen, "The role of interleukin-1 in the pathogenesis of cancer and its potential as a therapeutic target in clinical practice," Oncology and Therapy, vol. 6, pp. 109–127, 2018, doi: 10.1007/s40487-018-0089-z.

[4] E. D. Salman, "Genetic polymorphisms of interleukin-1 beta gene in association with multiple sclerosis in Iraqi patients," Iraqi Journal of Science, pp. 594–598, 2016.

[5] A. H. Ad'hiah, E. B. Hessan, and B. A. Shahab, "Interleukin-1 single nucleotide polymorphisms as risk factors for susceptibility of inflammatory bowel disease: an Iraqi Arab population-based study," Alexandria Journal of Medicine, vol. 55, no. 1, pp. 1–6, 2019, doi: 10.1080/20905068.2019.1585971.

[6] H. S. Saleh, I. A. AlRufaei, and E. S. Jawad, "Physiological change of interleukin-1 alpha (IL-1α) in Iraqi, Thi-Qar women with polycystic ovarian syndrome," University of Thi-Qar Journal of Science, vol. 10, no. 1, 2023.

[7] A. Nemetz, M. Toth, M. A. Garcia-Gonzalez, T. Zagoni, J. Feher, A. S. Pena, and Z. Tulassay, "Allelic variation at the interleukin 1β gene is associated with decreased bone mass in patients with inflammatory bowel diseases," Gut, vol. 49, no. 5, pp. 644–649, 2001, doi: 10.1136/gut.49.5.644.

[8] S. Kang, J. W. Kim, N. H. Park, Y. S. Song, S. Y. Park, S. B. Kang, and H. P. Lee, "Interleukin-1 beta-511 polymorphism and risk of cervical cancer," Journal of Korean Medical Science, vol. 22, no. 1, pp. 110–113, 2007, doi: 10.3346/jkms.2007.22.1.110.

[9] K. Tamura, G. Stecher, D. Peterson, A. Filipski, and S. Kumar, "MEGA6: Molecular evolutionary genetics analysis version 6.0," Molecular Biology and Evolution, vol. 30, no. 12, pp. 2725–2729, 2013, doi: 10.1093/molbev/mst197.

[10] M. G. Netea, B. J. Kullberg, and J. W. M. Van der Meer, "Circulating cytokines as mediators of fever," Clinical Infectious Diseases, vol. 31, suppl. 5, pp. S178–S184.

[11] Y. Liu, M. B. Costa, and C. Gerhardinger, "IL-1β is upregulated in the diabetic retina and retinal vessels: cell-specific effect of high glucose and IL-1β autostimulation," PLOS One, vol. 7, no. 5, p. e36949, 2012, doi: 10.1371/journal.pone.0036949.

[12] M. I. B. H. D. Maha and N. Falh, "A genetic polymorphism of interleukin-1a and interleukin 1 receptor antagonist gene with type 1 diabetes mellitus of Iraqi patients."

[13] O. Y. Gorlova, J. Ying, C. I. Amos, M. R. Spitz, B. Peng, and I. P. Gorlov, "Derived SNP alleles are used more frequently than ancestral alleles as risk-associated variants in common human diseases," Journal of Bioinformatics and Computational Biology, vol. 10, no. 2, p. 1241008, 2012, doi: 10.1142/S021972001241008X.

[14] J. Lachance, "Disease-associated alleles in genome-wide association studies are enriched for derived low frequency alleles relative to HapMap and neutral expectations," BMC Medical Genomics, vol. 3, no. 1, p. 57, 2010, doi: 10.1186/1755-8794-3-57.

[15] D. Fredman, S. L. Sawyer, L. Strömqvist, S. Mottagui-Tabar, K. K. Kidd, C. Wahlestedt, S. J. Chanock, and A. J. Brookes, "Nonsynonymous SNPs: validation characteristics, derived allele frequency patterns, and suggestive evidence for natural selection," Human Mutation, vol. 27, no. 2, pp. 173–186, 2006, doi: 10.1002/humu.20299.

[16] Z. A. Hlail and K. I. A. Mohammed, "Immunological detection of interleukin-1alpha (IL-1α) in Iraqi women with polycystic ovarian syndrome," SAR Journal of Medical Biochemistry, vol. 3, no. 3, pp. 39–42, 2022.

[17] K. Puri, M. Chhokra, V. Dodwad, and N. Puri, "Association of interleukin-1 α (–889) gene polymorphism in patients with generalized aggressive and chronic periodontitis," Dental Research Journal, vol. 12, no. 1, p. 76, 2015, doi: 10.4103/1735-3327.150322.

[18] L. Q. Abdulhameed and M. W. M. Alzubaidy, "Association between polymorphism of the CRP gene rs1130864 and hypertension among Iraqi hypertensive patients," Advances in Life Sciences, vol. 10, no. 2, pp. 157–160, 2023.

[19] L. L. de Moraes Marchiori et al., "Interleukin-1 alpha gene polymorphism (IL-1α) and susceptibility to tinnitus in the elderly," Noise & Health, vol. 21, no. 99, p. 77, 2019, doi: 10.4103/nah.NAH_41_18.

[20] R. Zhao, H. Zhou, and S. B. Su, "A critical role for interleukin-1β in the progression of autoimmune diseases," International Immunopharmacology, vol. 17, no. 3, pp. 658–669, 2013.

[21] A. K. Tripathi, S. Shukla, J. K. Tripathi, R. D. Saket, S. Kol, P. Mishra, U. K. Chauhan, and M. Indurkar, "Association of genetic polymorphism of inflammatory genes (IL-1β and IL-4) with diabetes type 2," Enliven: Journal of Genetic Molecular and Cellular Biology, vol. 1, no. 1, p. 004, 2015.

[22] S. I. Tayel, E. A. M. Fouda, E. I. Elshayeb, A. R. A. Eldakamawy, and S. M. El-kousy, "Biochemical and molecular study on interleukin-1β gene expression and relation of single nucleotide polymorphism in promoter region with type 2 diabetes mellitus," Journal of Cellular Biochemistry, vol. 119, no. 7, pp. 5343–5349, 2018.

[23] J. Jiao, Z. Wang, Y. Guo, J. Liu, X. Huang, X. Ni, D. Gao, L. Sun, X. Zhu, and Q. Zhou, "Association between IL-1β (-511)/IL-1RN (VNTR) polymorphisms and type 2 diabetes: a systematic review and meta-analysis," PeerJ, vol. 9, p. e12384, 2021, doi: 10.7717/peerj.12384.

[24] Z. M. Al-Maamouri and H. A. H. Al-Saadi, "Isolation and antimicrobial susceptibility patterns of bacterial pathogens causing respiratory tract infections in children," International Journal of Applied Sciences, vol. 1, no. 3, pp. 42–49, Dec. 2024, doi: 10.69923/p9tdez63.

[25] S. Abtahi, A. Farazmand, M. Mahmoudi, A. Ashraf-Ganjouei, A. Javinani, B. Nazari, H. Kavosi, A. A. Amirzargar, A. R. Jamshidi, and F. Gharibdoost, "IL-1A rs1800587, IL-1B rs1143634 and IL-1R1 rs2234650 polymorphisms in Iranian patients with systemic sclerosis," International Journal of Immunogenetics, vol. 42, no. 6, pp. 423–427, 2015, doi: 10.1111/iji.12200.

[26] I. Ben Dhifallah, K. Ayouni, G. Najjar, H. Chelbi, A. Sadraoui, W. Hammami, H. Touzi, and H. Triki, "Interleukin IL-1B gene polymorphism in Tunisian patients with chronic hepatitis B infection: association with replication levels," Microbiology and Immunology, vol. 64, no. 7, pp. 512–519, 2020, doi: 10.1111/1348-0421.12796.

[27] J. Fischer, S. Long, E. Koukoulioti, T. Müller, B. Fueloep, R. Heyne, M. Eslam, J. George, F. Finkelmeier, and O. Waidmann, "Association of common polymorphisms in the interleukin-1 beta gene with hepatocellular carcinoma in Caucasian patients with chronic hepatitis B," Pathogens, vol. 12, no. 1, p. 54, 2022, doi: 10.3390/pathogens12010054.