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Implication of Genomic Alterations of Programmed Death Ligand 1 (PD-L1) in the progression of Type 1 Diabetes.

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ABSTRACT:

Objectives:Type 1 diabetes is a global health challenge, elucidating the underlying mechanisms of the disease might help identify novel early screening biomarkers and new therapeutic options for the disease. Recently, a growing body of research showed that immune checkpoint inhibitors such as Programmed death ligand 1 (PDL-1) are implicated in the development of the disease. Genomic alterations such as single nucleotide polymorphisms (SNPs) are tightly associated with susceptibility to various diseases.

Methods: 50 patients with type 1 diabetes and 25 healthy volunteers were enrolled in this case-control study. Genomic DNA was extracted for sequencing the selected SNPs (*rs822336 (-1813) GC, rs73641615 TC, rs73641616(-1491) GA, and rs822337(-1349) TA*) SNPs in the promoter region of *PD-L1* gene.

Results: SNP analysis revealed the absence of any association between the SNPs investigated in the study and Type 1 diabetes, however, haplotype computational analysis using 1000 genome data suggested that (rs73641615) SNP might be a risk factor associated with the disease progression. On the contrary, our results suggested that A allele of SNP (rs73641616) might be not a risk factor in the disease, however, this result should be validated with further studies including a larger number of participants.

Conclusions: Although several previous studies reported genomic alterations of Programmed death ligand one as a risk factor in the development of Type 1 diabetes, our study revealed that some alleles might be not associated with disease progression. However, further studies are highly recommended.

Keywords: Type 1 Diabetes Mellitus, Immune checkpoint inhibitors, PD-L1, 1000 genome

INTRODUCTION

The rising incidence and prevalence of type 1 diabetes is a global challenge; T1D incidence and prevalence differ ultimately globally, with higher incidence rates and mortality in lower and middle-income countries [1]. A better understanding of the underlying pathogenetic pathways and the disease's autoimmune nature is mandatory. Early identification and screening for T1D have entered a new phase after the availability of possible tools to prevent T1D [2]. The availability of disease-modifying agents that can delay the need for insulin and preserve the pancreatic beta cells will elevate the daily clinical challenge [3-5].

Immunological checkpoint inhibitors (ICI) are crucial mediators in the immunopathogenesis of T1D [6]; one of the important ICIs, is programmed cell death 1 (PD-1), which is implicated in controlling the T cell activation and maintaining peripheral tolerance [7]. Evidence has evolved regarding a possible link between the PD-1/PD-L1 pathway and autoimmune disorders, including T1D [8, 9]. Moreover, decreased PD-1 levels might stimulate the proliferation and activation of T cells, leading to the destruction of pancreatic beta cells, providing a new underlying mechanism for T1D [10, 11].

Single nucleotide polymorphisms (SNPs), particularly those in the promoter region of *PD-1*/*PD-L1*, have a crucial role in gene transcription and translation and are tightly associated with the onset and progression of various diseases including T1D.

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Therefore, several studies have directed great attention toward the association between SNPs and T1D risk and highlighted a possible association between *PD-1*/*PD-L1* SNPs and susceptibility to developing T1D among multiple populations [12, 13].

Therefore, the main objective of the present work was to investigate the association of T1D susceptibility and four SNPs in the *PD-L1* gene promoter sequence. In addition, we attempted to figure out all the possible haplotypes for the four SNPs from the database of the 1000 Genomes project and the T1D subjects. The occurrence frequency and the possible association with T1D risk were investigated for all haplotype patterns of the four SNPs

2. Methods

2.1. Study design and subjects:

This case-control study enrolled 50 patients with T1D and 25 gender and age-matched healthy subjects with no history of autoimmune diseases. Patients were recruited from the Pediatric Clinic at the Faculty of Medicine, Alexandria University (Alexandria - Egypt). T1D Patients were under 18 years old and diagnosed according to the criteria of the American Diabetes Association (ADA) [14]. All sampling and procedures implicated in this study were reviewed and approved by the Ethics Committee of the Faculty of Medicine at Alexandria University, the study was explained in a simple way to all participants, and then after getting their approval, an informed written consent was obtained from each participant's parent or caregiver.

2.2. Materials and methods of bioinformatics:

2.2.1 Thorough history-taking

A detailed history was collected, focusing on age, gender, duration of diabetes, and family history of diabetes.

2.2.2 Genomic DNA extraction

Each participant in the study was instructed to come in the morning after 8 hours of overnight fasting; a blood sample was collected into EDTA-coated tubes. According to the manufacturer's instructions, the genomic DNA was extracted using Invitrogen Pure Link Genomic DNA Mini Kit (Thermo Fischer Scientific, USA). The concentration of the obtained DNA ranged between 100-200 ng/µl, with a purity range of 1.7 – 1.9. This DNA was stored at -20˚C till it was used in further genetic analysis, such as PCR and sequencing [15].

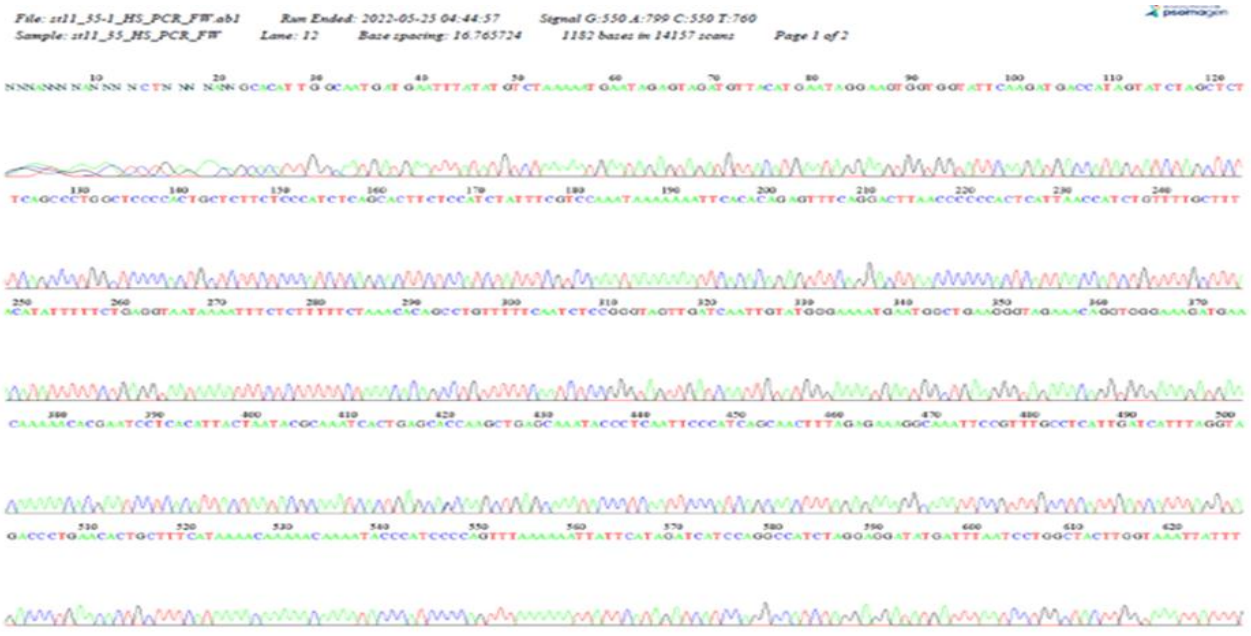
2.2.3 Position of the studied SNPs.

The position of (*rs822336* (-1813), *rs73641615*, *rs73641616*(-1491), and *rs822337*(-1349)) SNPs in the promoter of PD-L1 gene in chromosome 9 is illustrated in the Supplementary file (Figure 1). The primers used for amplification were determined using snapgene software (**www.snapgene.com**); and Supplementary files (Figures 2&3). Forward and reverse primers were designed around the region that contains all four SNPs (*rs822336*, *rs73641615*, *rs73641616*, and *rs822337*) in the promoter region of the PD-L1 gene, the fragment size amplified flanking the 4 SNPs was 1009 bp.

2.2.4 Genotyping

Genomic DNA was amplified by PCR using Applied Biosystems Thermocycler. The reaction was carried out in a final volume of 25 µl consisting of 5 µl reaction Buffer (5X), 0.5 µl dNTP (10 mM), 1.25 µl forward/reverse primers (10 µM), and 0.25 µl high-fidelity DNA polymerase (Thermo Fischer Scientific, USA), 100 ng DNA template. The thermal profile was performed using Psomagen's proprietary protocol (Psomagen, Maryland, USA).

Sequencing of the PCR product using the Sanger method on 3730xl Genetic Analyzer, ABI Systems, and the reaction was performed using BigDye® v3.1 (Life Technologies, Applied Biosystems) as per the manufacturer's protocol. Signal Detection was done using 3730 Data collection software and sequencing analysis software v5.0. Figure (1)



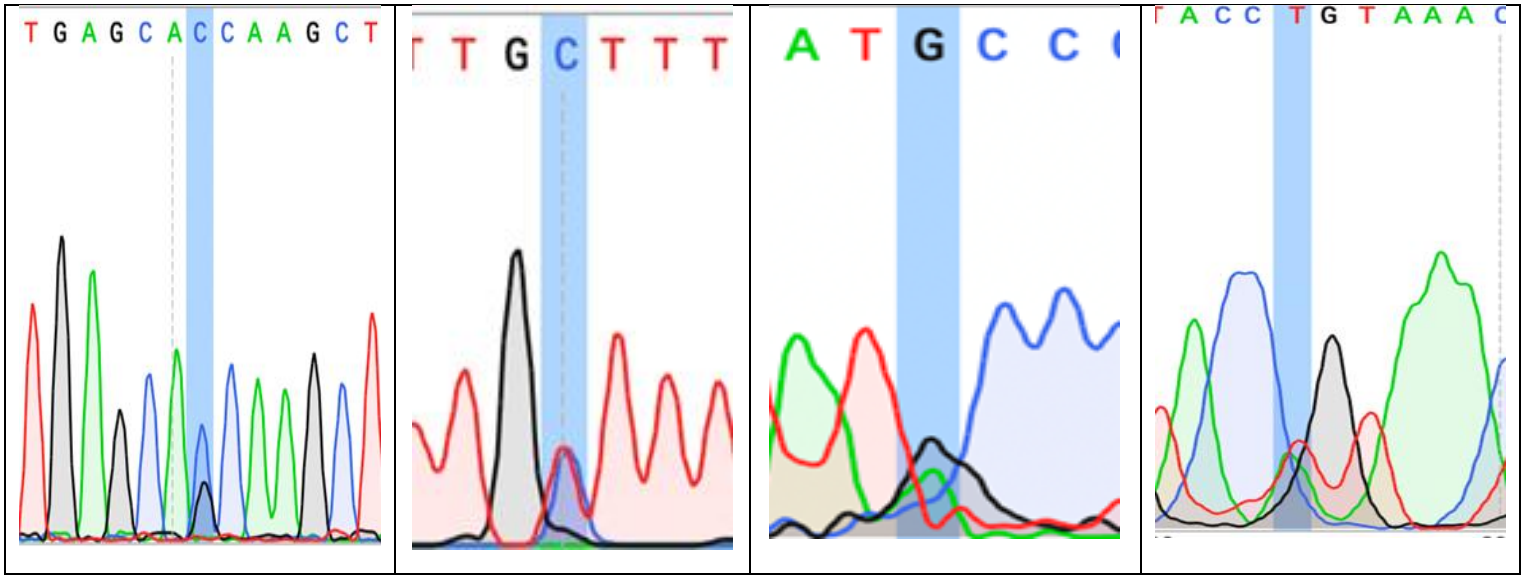


Fig. (1) (a) Chromatogram for the Sequence analysis of PCR product of the promoter region of *PD-L1* containing the four SNPs; (b) rs822336 (-1813) at position 149, (c) rs73641615 (-1547) at position 415, (d) rs73641616 (-1491) at position 471, and (e) rs822337 (-1349) at position 613

2.3. Statistical analysis

Hardy Weinberg equation (HWE) was applied to compare the observed frequencies to expected genotypes in healthy subjects and T1D patients. The association between allele, genotype, and haplotype with T1D was estimated using Fisher's exact test described by (Wang et al.,2016). [16] The R statistical package (https://www.r-project.org/) was used to calculate odds ratio (OR) at confidence intervals (CI) of 95% for each selected variant and their haplotype combinations; a P value < 0.05 was considered statistically significant.

3. Results:

3.1. Patients Characteristics

This case-control study enrolled 50 patients with T1D, and 25 age and sex-matched healthy control subjects. The T1D group included 29 males and 21 females (58.0 % vs 42.0% respectively). The mean age of the study subjects was 11.89 ± 2.46 years. The mean duration of diabetes was 6.98 ± 2.10 years with a mean HbA1c of 10.53 ±2.31.

Regarding family history, 10 % of the study participants had a family history of type 1 diabetes, while a positive family history of type 2 diabetes was reported in 30 % of the study participants. Among the study subjects, three participants were siblings.

3.2. Distribution of genotypes and alleles frequencies of *rs822336* SNP:

The genotype distribution **of *rs822336*** SNP did not deviate from those expected by HWE in patients and healthy subjects (p=0.143, p=0.997, respectively). The genotypes' and alleles' frequency of *rs822336* are represented in Table (1). Our results revealed that the CC and GC genotypes frequencies did not show any significant difference in T1D patients compared to healthy subjects (P =0.64 and P = 0.73) respectively. Additionally, our results showed that the OR for CC and GC genotype OR were (0.56 (0.09-3.44 and 1.41 (0.38-5.20)) respectively. In Addition, the allele C was found in 40.7% of patients and 43.75% of healthy individuals. It has been found that *rs822336* SNP has no significant association with patients with T1D (P=0.7649).

3.3. Distribution of genotypes and alleles frequencies of *rs73641615* SNP:

The genotype distribution of ***rs73641615* SNP** in the healthy subjects did not differ significantly from those predicted by HWE (p=0.411); however, in patients, the distribution deviated from that expected by HWE (p=0.001). The distribution of genotypes and allele frequency of *rs73641615 SNP* is presented in Table (1). Our data revealed that there was no significant difference in the frequencies of CC and TC genotypes in T1D patients as compared to healthy subjects (P =0.58, P = 0.18) respectively. In addition, the OR results were 0.42 (0.06-2.85). The allele C was present in 11.62% of patients and 25% of healthy individuals. It has been found that *rs73641615* SNP has no significant association with T1D (P=0. 0789).

3.4. Distribution of genotypes and alleles frequencies of *rs73641616* SNP:

The genotype distribution of **rs73641616 SNP** in the healthy and patients with T1D showed no significant difference compared to those predicted by HWE (p=0.345 and p=0.943, respectively). Table (1) shows the genotypes' and alleles' frequency of *rs73641616* SNP. It was found that there was no significant in the frequencies of AA and GA genotypes in T1D patients in comparison with healthy subjects (P=0.4, P = 0.1) respectively. Our OR results were 0.27 (0.02-4.71). On the other hand, the distribution of the A allele was found in 8.13% of patients whereas in 21.87% of healthy individuals.

The OR of the A allele 0.31, (0.10- 0.98) with (P=0. 0479*), indicates that the A allele of the *rs73641616* SNP on the *PD-L1* gene might be not a risk factor for the development of T1D.

Table (1): Distribution of rs822336 (-1813), rs73641615, rs73641616, and rs822337(-1349) genotypes and alleles frequency in type 1 diabetes patients and healthy controls

Genotype/Allele	Patients (n)	Controls (n)	Odds Ratio (OR)	p-value
rs822336(-1813)				
GG	12(27.9%)	5(31.3%)	(reference)	
GC	27(62.8%)	8(50%)	1.41 (0.38-5.20)	0.73
CC	4(9.3%)	3(18.8%)	0.56 (0.09-3.44)	0.64
Alleles				
G	51(59.3%)	18(56.25%)	(reference)	
C	35(40.7%)	14(43.75%)	0.88(0.38- 2.00)	0.7649
rs73641615				
TT	36(83.72%)	10(62.5%)	(reference)	
TC	4(9.30%)	4(25%)	0.28(0.06-1.31)	0.18
CC	3(6.97%)	2(12.5%)	0.42(0.06-2.85)	0.58
Alleles				
T	76(88.37%)	24(75%)	(reference)	
C	10(11.62%)	8(25%)	0.39(0.14- 1.11)	0.0789
rs73641616(-1491)				
GG	37(86.04%)	10(62.5%)	(reference)	
GA	5(11.62%)	5(31.25%)	0.27(0.07-1.12)	0.1
AA	1(2.32%)	1(6.25%)	0.27(0.02-4.71)	0.4
Alleles				
G	79(91.86%)	25(78.12%)	(reference)	
A	7(8.13%)	7(21.87%)	0.31(0.10- 0.98)	**0.0479***
rs822337(-1349)				
TT	8(18.60%)	3(18.75%)	(reference)	
TA	17(39.53%)	5(31.25%)	1.28(0.24-6.70)	1
AA	18(41.86%)	8(50%)	0.84(0.18-4.04)	1
Alleles				
T	33(38.37%)	11(34.37%)	(reference)	
A	53(61.62%)	21(65.63%)	0.84(0.35- 1.96)	0.6899

3.5. Distribution of genotypes and alleles frequencies of *rs822337* SNP:

The distribution of ***rs822337*** genotypes in the healthy and patients with T1D showed no significant difference from those predicted by HWE (p=0.384, p=0.469, respectively). Table (1) reveals the genotype and allele frequencies of *rs822337* SNP. Our results indicate the absence of any significant difference in the frequencies of AA and TA genotypes in T1D patients compared to healthy subjects (P= 1, P=1) respectively. Moreover, our OR results were 0.84 (0.18-4.04). In addition, allele A was present in 61.62% of patients and 65.63% of healthy individuals. It has been found that *rs822337* SNP has no significant association with T1D (P=0.6899).

3.6. Linkage Disequilibrium (LD) Mapping of *PD-L1* Promoter SNPs:

A group of identifiers for the four most abundant SNPs located in the *PD-L1* promoter sequence was fed into the LD matrix tool (*https://ldlink.nci.nih.gov/?tab=ldmatrix*) to investigate the likelihood of their inheritance together in the form of haplotype. The obtained graphical LD heat map detected the possibility of the four SNPs being inherited together as a haplotype.

Link linkage disequilibrium (LD) variables are more likely to be inherited on the same DNA fragment. The LD tool is applied to create a correlation heat map figure to identify the SNPs of higher linkage disequilibrium using the 1000 Genomes database.

The investigation of the four most frequent SNPs in the *PD-L1* promoter region revealed the highest correlation among the four SNPs is between *rs73641615* and *rs73641616*. The solid red colour of the crossed SNPs represents the high correlation between these two SNPs, whereas a lower correlation is seen among the remaining variants, characterized by the faded red colouration, Figure (2 a&b)

3.7. In silico study to predict the modulation of transcription factor's binding sites.

In silico study was performed using Jaspar software (https://jaspar.genereg.net), to predict the modulation of transcription factors that may be changed due to different SNPs, Table (2).

Our results revealed that *rs822336* SNP modified the type and number of transcription factors that can bind to the promotor region, the presence of the C allele in the promotor region decreased the transcriptional activity, where two factors (E2F1 and PAX5) were replaced by another different one (NHLH1), Table (2).

Regarding *rs73641615* SNP, the presence of the C allele in the promotor region decreased the number and altered the types of transcription factors that can bind to the promotor region, where the G allele leads to the binding of 15 transcription factors, the C allele may allow only nine including three new different transcription factors which are not putative factors predicted for the G allele including (Sox 2, Sox17, and Foxa2), Table (2).

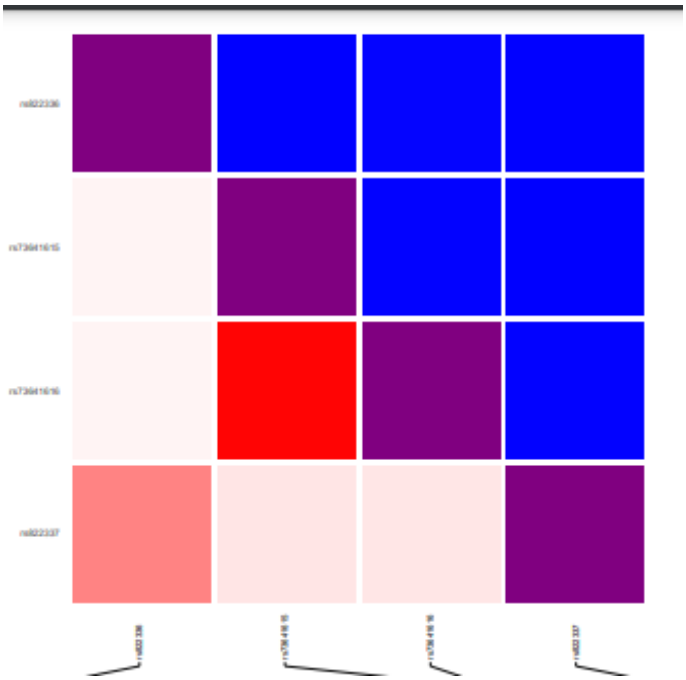


Fig. (2a) Heatmap matrix for the pairwise linkage disequilibrium of the four studied SNPs in the *PD-L1 gene* (https://ldlink.nci.nih.gov/?tab=ldmatrix)

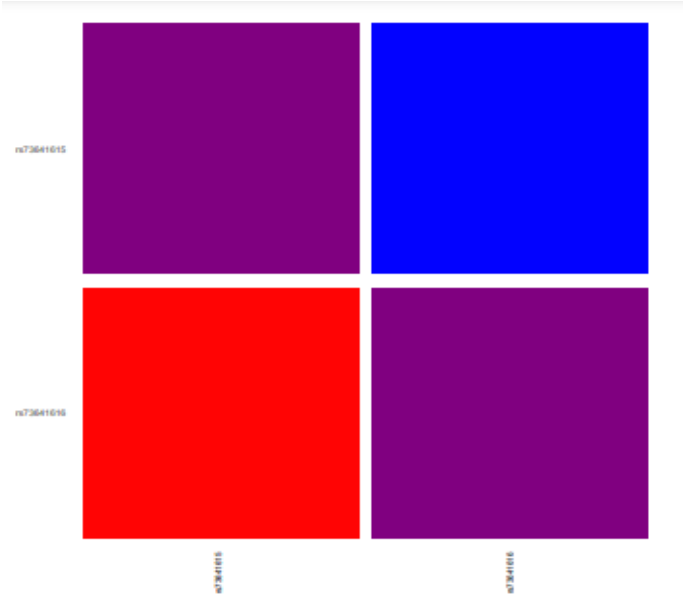


Fig. (2b) Heatmap matrix for the pairwise linkage disequilibrium of two SNPs in the *PD-L1* gene. (https://ldlink.nci.nih.gov/?tab=ldmatrix)

Table (2): In silico study to predict the putative transcription factors for different alleles of the four selected SNPs using Jaspar software

Rs number	Allele	Putative transcription Factor	Score
rs822336 (-1813)			
	Wild type (G)	E2F1	4.143386
		PAX5	7.653228
	Mutant type (C)	NHLH1	9.065701
rs73641615			
	Wild type (T)	SRY	8.913894
		FOXI1	9.778044
		Foxd3	10.13454
		Foxd3	8.835987
		Foxd3	8.743531
		FOXC1	6.074068
		FOXA1	7.010849
		FOXA1	7.087943
		Sox5	6.562933
		FOXI1	8.337788
		Foxd3	8.207851
		FOXD3	10.80253
		Foxd3	7.881491
		IRF1	10.54726
		SOX9	6.112851
	Mutant type (C)	Sox17	9.598514
		Sox5	8.055312
		FOXA1	10.32341
		FOXA1	10.24808
		Sox2	6.692991
		FOXA1	6.600159
		Foxa2	7.316248
		SOX2	7.327177
		SOX9	6.730874
rs73641616			
	Wild type (G)	TFAP2A	8.187411
		Foxq1	11.23805
		EBF1	7.973264
	Mutant type (A)	No putative transcription factors	
rs822337			
	Wild type (T)	ZEB1	6.8307343
		FOXA2	7.729797
		Foxq1	8.280023
		FOXA1	6.4319224
	Mutant type (A)	No putative transcription factors	

The wild alleles of *rs73641616* (G) and *rs822337* **(T)** were predicted to have three and four putative transcription factors, respectively. In contrast, inserting the A allele in the promotor region may reduce its transcriptional activity, as no putative transcription factors were predicted for this allele for both SNPs, Table (2).

3.8. Pairwise comparison of four SNPs about 1000 genome project data:

The haplotype computational analysis of the four studied SNPs revealed that only (*rs73641615*) were significantly associated with T1D when compared to haplotype frequency from 1000 genome subjects, Tables (3 a&b)

Table (3a): Pairwise comparison of the four SNPs in healthy control concerning 1000 genome project data.

SNP/ Genotype	Controls	1000 genome	Odds Ratio	P- Value
rs822336(-1813)				
GG	5(31.3%)	1085(43.3)	(reference)	
GC	8(50%)	1072(42.8)	1.62(0.53-4.97)	0.42
CC	3(18.8%)	347(13.85)	1.88(0.45-7.89)	0.41
G	18(56.25%)	3242(64.73)	(reference)	
C	14(43.75%)	1766(35.26)	0.70(0.3475 - 1.4115)	0.3192
rs73641615(-1547)				
TT	10(62.5%)	2119(84.62)	*(reference)*	
TC	4(25%)	354(14.13)	*2.39(0.75-7.68)*	*0.13*
CC	2(12.5%)	31(1.23)	*13.67(2.88-65.00)*	**0.01**
T	24(75%)	4592 (91.69)	*(reference)*	
C	8(25%)	416 (8.30)	0.27(0.12- 0.60)	**P = 0.0015**
rs73641616(-1491)				
GG	10(62.5%)	2121(84.70)	(reference)	
GA	5(31.25%)	350(13.97)	3.03(1.03-8.92)	0.05
AA	1(6.25%)	33(1.31)	6.43(0.80-51.66)	0.16
G	25(78.12%)	4592(91.69)	(reference)	
A	7(21.87%)	416(8.30)	0.32(0.13- 0.75)	**P = 0.0088**
rs822337(-1349)				
TT	3(18.75%)	595(23.76)	(reference)	
TA	5(31.25%)	1184(47.28)	0.84(0.20-3.52)	0.36
AA	8(50%)	725(28.95)	2.19(0.58-8.29)	0.36
T	11(34.37%)	2374(47.40)	(reference)	
A	21(65.63%)	2634(52.59)	0.58(0.27- 1.20)	P = 0.1460

Table (3b): Pairwise comparison of four SNPs in T1D patients concerning 1000 genome project data.

SNP/ Genotype	Patients	1000 genome	Odds Ratio	P- Value
rs822336(-1813)				
GG	12(27.9%)	1085(43.3)	(reference)	
GC	27(62.8%)	1072(42.8)	2.28(1.15-4.52)	0.22
CC	4(9.3%)	347(13.85)	1.04(0.33-3.25)	1
G	51(59.3%)	3242(64.73)	(reference)	
C	35(40.7%)	1766(35.26)	1.25(0.81- 1.94)	P = 0.2970
rs73641615(-1547)				
TT	36(83.72%)	2119(84.62)	(reference)	
TC	4(9.30%)	354(14.13)	0.67(0.24-1.88)	0.64
CC	3(6.97%)	31(1.23)	5.70(1.66-19.49)	**0.02**
T	76(88.37%)	4592 (91.69)	(reference)	
C	10(11.62%)	416 (8.30)	1.45(0.74- 2.82)	P = 0.2727
rs73641616(-1491)				
GG	37(86.04%)	2121(84.70)	(reference)	
GA	5(11.62%)	350(13.97)	0.82(0.32-2.10)	0.82
AA	1(2.32%)	33(1.31)	1.74(0.23-13.04)	0.45
G	79(91.86%)	4592(91.69)	(reference)	
A	7(8.13%)	416(8.30)	0.97(0.44-2.13)	P = 0.9556
rs822337(-1349)				
TT	8(18.60%)	595(23.76)	(reference)	
TA	17(39.53%)	1184(47.28)	1.07(0.46-2.49)	1
AA	18(41.86%)	725(28.95)	1.85(0.80-4.28)	1.66
T	33(38.37%)	2374(47.40)	(reference)	
A	53(61.62%)	2634(52.59)	1.44(0.93- 2.24)	P = 0.0980

4. Discussion:

Type 1 Diabetes Mellitus (T1D) is recognized by insulin deficiency caused by pancreatic cell destruction. It's a complex polygenic disorder with genetic and environmental factors implicated in the pathophysiology of the disease [17, 18].

The PD-1/PD-L1 axis is crucial in immunological homeostasis in different tissues[19]. Recently, emerging evidence has revealed that this axis is fundamental in maintaining immunological tolerance towards insulin-producing cells of the pancreas [11, 20]. The critical role of the PD-1/PD-L1 axis in maintaining pancreatic peripheral tolerance renders it worth studying. Moreover, a better understanding of the association between the programmed death 1 (PD-1)/PD-L1 axis and the constraint of T1D might provide novel therapeutic approaches.

Owing to the different genetic backgrounds of T1D among diverse populations, several studies have been directed to investigate the association between PD-L1 single nucleotide polymorphisms and T1D progression in different ethnicities. However, those studies involved only polymorphism inside the intronic or exonic regions of the PD-L1 gene [21]. Therefore, our study group selected the most abundant four variants, based on their high Major Allele Frequencies in the 1000 Genome project database, positioned in the 2kb promoter sequence of the PD-L1 gene and investigated their possible association with T1D susceptibility and to elucidate the impact of the selected SNPs on T1D risk in a sample of Egyptian patients with T1D.

Our SNP analysis results revealed for the first time that (*rs73641616)* SNP might have a protective role against T1D progression [OR (95% CI) = 0.31 (0.10- 0.98), $p < 0.05$)]; however, the other three SNPs did not show any significant association with the susceptibility of T1D.

Despite the lack of association between *(rs822337)* SNP with T1D in our study, this SNP has been investigated as a predictive prognostic marker to predict response to chemotherapy in patients with late-stages of non-small cell lung cancer (NSCLC) receiving paclitaxel-cisplatin combined therapy. The objective of this study was to predict response after therapy, which might affect the level of PD-L1 expression in the tumor, in addition to tumor-specific cytotoxic T cells expressing surface receptors for PD-1 [22].

In the same manner, *rs822336* SNP investigated in the present study didn't show any significant association with T1D, although it was previously documented that the *rs822336* carrier (CC homozygous as well as CG heterozygous) might be a risk factor for developing ankylosing spondylitis among the Chinese population [23], indicating that this SNP may be implicated in the pathogenesis of other autoimmune.

Despite the implication of PD-L1 SNPs in the vast majority of autoimmune diseases such as rheumatoid arthritis and ankylosing spondylitis [21], the absence of association between the studied SNPs and T1D susceptibility may be attributed to the different pathogenesis of T1D and other autoimmune disorders.

In the present study, we tried to use the 1000 genome project data for computational analysis to predict the possible association between the studied SNPs and T1D. Our results revealed that although Hardy Weinberg equilibrium (HWE) indicated that the genotype distribution of studied SNPs did not deviate from those predicted- except for *rs73641615* which showed significant deviation in patients with T1D- the results compared to 1000 genomes revealed that both *rs73641615* and *rs73641616* had a significant difference in healthy subjects. Therefore, we infer that reaching 1000 genome analysis should overcome the SNPs analysis; this can be attributed to the ability to construct all possible combinations of all promoter region variants from the subjects in the 1000 genomes project with a large sample size of 2504 individuals enrolled 1000 genomes, this polymorphism and haplotype pattern comparison might be of great importance in the prediction of T1D susceptibility.

Moreover, the SNP analysis showed that none of the four studied SNPs was associated significantly with T1D; however, the haplotype computational analysis for the four studied SNPs revealed that (*rs73641615*) is significantly associated with T1D as compared to haplotype frequency predicted for 1000 genome subjects. Hence, we infer that haplotype-based analysis might overwhelm the SNPs-based studies; this can be attributed to the ability to construct all possible combinations of all promoter region variants from the database of 1000 genomes enrolled in the population. Hence the actual haplotype pattern obtained from an accurate database such as 1000 genomes is a more prominent tool for the discrimination between patients and healthy individuals [24, 25].

Regarding the in-silico analysis, our study revealed that the mutant alleles of the four studied SNPs at the promotor region may modulate its transcriptional activity. The mutant C allele of *rs822336* and *rs73641615* SNPs reduced the number and altered the type of transcription factors. In contrast, the insertion of the mutant A allele of *rs73641616* and *rs822337* SNPs lacked the binding ability to any transcription factor.

The *rs822336*G>C and *rs822337*T>A SNPs are located near the transcription starting site at the promoter region. The *rs822336*(C) and *rs822337* (A) haplotypes were associated with significantly reducing promoter transcriptional activity. Moreover, *rs822336*C and *rs822337*A haplotypes resulted in the downregulation of PD-L1 expression at the protein level.[26] The two SNPs are situated at sites that play a crucial role in promoter activation; the region of *rs822337*T>A SNP is considered a significant binding site of NF-κB, the PD-L1 promoter region [27]. However, by abolishing their binding site, the A allele prevents the binding of other transcription factors, such as SPIB and FOXO3 [28].

This study had some limitations; including the small size of the study sample, enrolling only the Egyptian population so whether these findings can be applied to other ethnicities is still to be investigated. Although this study was single-centered, it was carried on in a tertiary center serving more than four Egyptian governments which is a point of strength in this research. We recommend building on the results of the current findings and further exploring the association of the studied SNPs with the progression of T1D through future studies involving a larger number of subjects. While the study identifies that one SNP (rs73641616), might be not a risk factor implicated in disease progression, further validation through replication studies in independent cohorts is necessary to confirm these findings robustly.

5. Conclusion:

Although previous studies have indicated the role of the four selected PDL1 SNPs in different diseases, this is the first study to explore their role in T1D susceptibility. Our study provides the first evidence that the A allele of *rs73641616* might be not considered as a risk factor T1D. Moreover, linkage disequilibrium between *rs73641615* and *rs73641616* polymorphisms is reported for the first time. However, these findings need to be validated with further studies including a larger number of participants and to be investigated through various ethnicities

6. Recommendation:

Although, our study involved a small sample size of patients, we suggest that the present study might be considered as a building block for further research for deeper understanding of the implication of PDL1 genomic alterations in the progression of T1D, and to extend the research to different diseases.

Conflict of interests:

All authors declare that they have no conflicts of interest.

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Availability of data and materials:

The datasets used and analysed during the current study are available from the corresponding author upon reasonable request.

Authors' contribution:

Fawziya A.R Ibrahim and Husam Raheem Al-hraishawi designed the study, wrote the protocol, and participated in data analysis and the interpretation of results. Shaymaa Elsayed Abdel Meguid Ahmed and Noha Gaber Amin provided the samples, participated in data analysis and interpretation of results, and wrote the manuscript. Azhar Mohamed Nomair and Hanan Mohamed Nomeir participated in the data analysis, results interpretation, and manuscript writing. All authors revised the manuscript, provided advice, and approved the final manuscript.

Ethics approval and consent to participate:

The research methodology in the present work was reviewed and approved by the Ethics Committee of the Faculty of Medicine at Alexandria University, and each participant provided written informed consent before enrollment in the study. Experimental procedures and sampling followed the international and national regulations following the Declaration of Helsinki. The IRB approval number is 00012098 dated 18/NOV/2021.

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