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RESEARCH ARTICLE (CLINICAL STUDY)

The 1858T PTPN22 Gene Variant Confers Sex-Biased Susceptibility to Type 1 Diabetes; an Evaluation in Children and Adolescents of Egyptian origin

Ahmed M. Abd El-Mohsin Akabawy[1*], Hatem K. A. Amin[1], Fathia Z. El-Sharkawi[1], Mohamed H. El-Hefnawy[2] and Zeinab A. Hassan[1]

1. Biochemistry and Molecular Biology Department, Faculty of Pharmacy – Helwan University – Cairo – Egypt.
2. Pediatric Department, National Institute for Diabetes and Endocrinology (NIDE) – Cairo – Egypt.

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Corresponding Author

Ahmed M. Abd El-Mohsin Akabawy
E-mail:
dr.ahmed_moh@pharm.helwan.edu.eg

Mobile: 002 01119174482

Abstract

BACKGROUND AND OBJECTIVES: Type 1 diabetes "T1D" is a multigenic autoimmune disease with over 60 susceptibility loci identified. A PTPN22 C1858T single nucleotide polymorphism "SNP" has been implicated as a major contributor in the predisposition of T1D in different populations. Our aim was to evaluate the impact of such genetic variability in the pathogenesis of T1D in Egyptian patients.

RESEARCH DESIGN: In a retrospective study design and after full clinical and laboratory investigations for our subjects, DNA was extracted and genotyped for PTPN22 +1858 C>T (rs2476601) SNP analysis in 85-clinically diagnosed unrelated patients with T1D and 65- age, sex and BMI matched healthy controls using a real-time PCR TaqMan® sequence-specific probe-based 5`allelic discrimination assay.

RESULTS: Logistic regression analysis confirms the association of the PTPN22 C1858T polymorphic site with T1D [P-value = 0.0012; OR = 4.58 at 95% CI = 1.76 – 11.91]. Additionally, the minor 1858T allele is related to the disease in a female sex-specific manner (P-value = 0.001) with an increase in the OR = 8.12 at 95%CI = 2.14 - 30.78.

CONCLUSION: The PTPN22 1858T variant might play a pivotal role in the pathogenesis of T1D in Egyptian population by a gender-dependent mechanism that contributes to unique predisposition in females. Furthermore, neither age at disease onset nor susceptibility to diabetic nephropathy "DN" is conferred by the high-risk mutant allele.

INTRODUCTION

Type 1 Diabetes Mellitus "T1D" is a chronic multifactorial metabolic disorder resulting from selective destruction of the insulin-producing pancreatic β-cells. It satisfies many of the criteria for an autoimmune disease **(Harrison et al., 2008).** Although the exact causes remain unclear, the susceptibility to the disease is inherited and increased risk is associated with being a first-degree relative to a person with a diabetic proband. Together with an environmental contributor, this implies a strong genetic component to the risk for the disease incidence. The major genetic region associated with predisposition to the disease is the one that encodes genes for the highly polymorphic human leukocyte antigens **(Mark et al., 2012).** However, over 60 other loci have been proposed as contributing from 50 to 70% of the total genetic susceptibility. Among them, polymorphisms in the insulin and protein tyrosine

phosphatase, non-receptor 22 "PTPN22" genes are associated with highest odds ratios and hence, mostly contribute to T1D risk **(Mark et al., 2012; Bakay et al., 2013).**

The PTPN22 gene is located on chromosome 1p13.3-13.1 and encodes an 807-amino acid residue protein referred to as lymphoid-specific protein tyrosine phosphatase "LYP", an important inhibitor of the activation and proliferation of T lymphocytes. LYP is involved in preventing spontaneous T-cell activation by dephosphorylation and inactivation of T-cell receptor "TCR"-associated kinases and their substrates **(Mustelin et al., 2003).** LYP is specifically expressed in lymphocytes **(Cohen et al., 1999)** and through formation of a complex with C-terminal Src tyrosine kinase "Csk" suppresses the downstream mediators of TCR signaling **(Gjorloff-Wingren et al., 1999; Elhoseiny et al., 2012).** A functional single nucleotide polymorphism "SNP" at position 1858 (rs2476601) is located in the N-terminal proline-rich motif of the encoding sequence of PTPN22 gene. PTPN22 1858 C>T SNP involves the substitution of cytosine (C) by thymine (T) which results in the substitution of arginine amino acid (R) with tryptophan (W) at codon 620 of LYP (R620W) **(Giza et al., 2013).** The disease-associated PTPN22 1858T variant prevents the interaction of LYP with Csk. Consequently, the TCR-associated kinases might exhibit an uncontrolled T-cell induction and this may increase the overall reactivity of the immune system, thus, predisposing an individual to various autoimmune diseases **(Siminovitch, 2004; Giza et al., 2013).**

Moreover, biochemical studies with primary human T cells, including T1D patient cells, as well as with Jurkat T leukaemia cells, have shown that the PTPN22 1858T is a gain of a function variant **(Vang et al., 2005).** In this regard and as illustrated in figure (1), the PTPN22*620W variant dephosphorylates and hence suppresses T cell signaling proteins much more efficiently than the PTPN22*620R variant and thus leads to a failure in apoptosis of autoreactive T cells and to an insufficient activity of regulatory T cells. Subsequently, it allows for more autoreactive T cells to survive and escape into circulation predisposing the patient to various autoimmune diseases **(Bottini et al., 2006).**

Unprecedentedly, **Bottini et al. (2004)** reported that PTPN22 1858T allele was observed more frequently in patients with T1D compared to healthy individuals from North America and Sardinia. Further studies on various populations confirmed the original findings and this disease-predisposing allele has been reported to be associated with T1D in more than 18 different populations principally localized in Europe region **(Giza et al., 2013).** Additionally, it was reported that this high-risk allele confers genetic susceptibility to many other autoimmune diseases such as rheumatoid arthritis "RA" **(Totaro et al., 2011)**, systemic lupus erythematosus "SLE" **(Lea and Lee, 2011)**, generalized vitiligo **(Canton et al., 2005)**, juvenile idiopathic arthritis **(Lee et al., 2012a)**, multiple sclerosis **(Lee et al., 2012b)**, autoimmune thyroid diseases **(Luo et al., 2012)**, psoriasis **(Li et al., 2009)**, Graves' disease **(Velaga et al., 2004)** and Addison disease **(Roycroft et al., 2009).** Recently, several studies have been conducted on populations from Egypt with different autoimmune diseases. However, there is no enough data about the association of PTPN22 gene and T1D in Egyptian population.

Given the fact that the frequency of the minor allele varies between populations and thus ongoing studies are important to confirm previous associations, the main objective of this study was to assess the role of PTPN22 C1858T polymorphism in type 1 diabetic Egyptian children and adolescents.

SUBJECTS AND METHODS

The study was conducted during the period from March, 2013 till July, 2014.

Study Subjects:

In a retrospective design, a total of one hundred and fifty subjects were enrolled into this study; 85 clinically-diagnosed patients with autoimmune T1D (42 ♂, 43♀) and 65 healthy volunteers (34 ♂, 31♀) selected as controls with matched age, gender, BMI, ethnicity and socioeconomic status. Patients were recruited from the pediatric inpatient-department of the National Institute for Diabetes and Endocrinology "NIDE" fulfilling the diagnostic criteria defined by the **ADA, (2007).** Patients who were receiving any medications other than insulin or those complaining from another chronic or acute disease were excluded from the study.

Ethical aspects:

The study protocol was approved by the local Research Ethics Committee of the General Organization for Teaching Hospitals and Institutes (Approval No. IDE00177, Date 4/3/2013). All participants and/or their parents gave an informed written consent for participation in the study. With respect to patients' confidentiality, patients

were represented in the study by code numbers and not by their names with all personal data concealed. The study was carried out in accordance with the ethical regulations and recommendations of the 2008 Declaration of Helsinki **(World Medical Association, 2013).**

Sample collection, preparation and storage:

Overnight fasting blood samples as well as fresh morning urine specimens were collected from each subject.

Blood samples were divided into four aliquots; the first two were used for immediate determinations of glycated heamoglobin "A1C" and fasting blood glucose level "FBGL". The third blood aliquot was prepared for serum separation which was used for immediate determination of different biochemical parameters. The last aliquot was stored at - 80 ^{0}C until used for DNA extraction. Urine samples were collected into special sterile cups and used for immediate determination of urinary biomarkers (Urinary albumin and creatinine).

Laboratory assessments:

Spectrophotometrically, FBGL was determined according to the method of **Kunst et al., (1983)** using Dimension® RxL MAX Integrated chemistry system utilizing a suitable kit (Dad Behring instruments inc. USA). A1C was determined by ion exchange HPLC technique according to **Jeppsson et al., (1986)** using BIO-RAD® D-10 Hemoglobin testing system utilizing a specialized kit (United States, BIO-RAD laboratories, Inc., Hercules, CA94547 France, Bio-Rad, Mames-la-Coquette).

Colorimetrically, amount of creatinine in urine samples was quantitatively measured according to the method approved by **Vasiliades, (1976).** Utilizing an immunoturbidimetric assay technique reported by **Gentilini et al., (2005)**, urinary albumin was quantitatively and automatically determined by the aid of ADVIA® 1650 clinical chemistry system (Bayer, Germany). This was followed by automatic estimation of Urinary Albumin-to-Creatinine Ratio "UACR"

Kits were provided by the manufacturing companies and purchased through their local distributers in Egypt. During determinations, all procedures were done according to the manufacturer's protocols.

Molecular analysis:

DNA extraction:

Genomic DNA was extracted and purified from peripheral blood of all study subjects according to standard spin-column protocol provided with PureLink® Genomic DNA extraction Kit (Catalog Number K1820-01, Invitrogen by life technologies™, USA). DNA samples were diluted and adjusted to concentrations 10-20 ng/µL using NanoDrop (Quawell® Q5000 UV-VIS SPECTROPHOTOMETER).

Genotyping of rs2476601:

Genotyping of the PTPN22 C1858T SNP was made by TaqMan® real-time PCR technique using the Rotor-Gene Q 5 Plex Platform (QIAGEN, Valencia, CA, USA). Commercially available (C_16021387_20) TaqMan® 5`-allelic discrimination SNP Genotyping on-demand Assay for rs2476601 (Applied Biosystems, Foster City, CA, USA) was used. As provided by the manufacturer, the TaqMan minor groove binder probes were labeled with the fluorescent dyes VIC® and FAM® which were used to detect different possible genotypes (CC, CT and TT).

The assay was performed with TaqMan® Universal Master Mix (Applied Biosystems), with 10 - 20 ng of DNA per reaction. PCR was carried out in a total volume of 25 µL using the conditions recommended by the manufacturer [Initial holding (95 ^{0}C, 10 min.) followed by 50 PCR cycles, each was composed of a denaturation (92 ^{0}C, 15 seconds) and an anneal/extend step (60 ^{0}C, 1.5 min.)]. Analysis of data was performed and the genotype of each sample was automatically attributed by measuring the allele-specific fluorescence using the Rotor gene Q specialized built-in integrated software for allele discrimination (QIAGEN).

AccuGENE® (Lonza, Belgium) DNase-, RNase-free molecular biology water was used when required during all molecular analysis steps.

Statistical analysis:

The statistical tools for genotype analysis of SNPs (Allele and genotype distributions as well as association tests, odds ratios "OR", confidence intervals "CI" and p-values) were assed using SPSS program (SPSS package version 22 for Windows, Chicago, IL, USA © 2013). Graphs were plotted using GraphPad Prism 5 (For Windows,

© 1992- 2007 Graphpad software Inc., V 5.01, USA). The following statistical measures were used for analysis of data:

- Genotype frequencies of the SNP were tested for being expressed in Hardy–Weinberg equilibrium "HWE" using X^2 test.
- Unpaired student t-test univariate analysis was used to test the significance of results of quantitative variables where data were expressed as M±SEM.
- Fisher's exact test was used to test for significance among qualitative variables and the corresponding OR with 95% CI was calculated.
- Adjusted OR with 95% CI was also calculated by multivariate binary logistic regression analysis when appropriate.
- All P-values were two-tailed and the significance of the results was set at the 5% level of significance **(Norman and Streiner, 2000).**

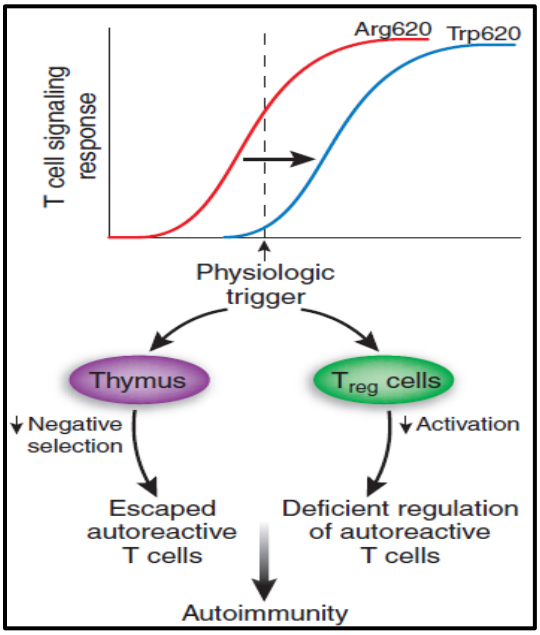

Fig. (1): The PTPN22 R620W substitution results in a gain of enzymatic function that is predicted to increase the threshold for TCR signaling. In the thymus, this shift in signaling threshold could result in the positive selection of thymocytes that would otherwise be deleted, resulting in the appearance of potentially autoreactive T cells in the periphery. A second mechanism could involve reduced signaling in Treg cells, with a resulting deficiency in regulation of autoreactive T cells. Either or both of these mechanisms could lead to a state of susceptibility for autoimmune disorders [Adapted from **Gregersen (2005)**].

RESULTS

Some demographic measures for patients and controls are listed in table (1). Concerning gender, BMI and age, in-significant variations were verified when diabetic and healthy control cohorts were compared ($p > 0.5$).

When PTPN22 1858 C/T genotypes were tested for being expressed in HWE, neither controls ($P = 0.70$) nor T1D patients ($P = 0.08$) showed any deviation. The frequency of the mutant T allele was 4.62% and 15.88% in control and T1D groups respectively, while that of C allele was 95.38% and 84.12% respectively.

Figure (2) demonstrates a representative example of our results showing different amplifications. Circle-labeled amplifications represent VIC® dye fluorescence indicating presence of the mutant T allele. Non-labeled amplifications represent FAM® dye fluorescence indicating presence of the wild-Type C allele.

To evaluate the impact of the allelic variants at 1858 polymorphic site of the PTPN22 gene in T1D, the present study showed that the distribution of the PTPN22 C1858T genotypes ($P = 0.0012$) and alleles ($P = 0.0024$) was

significantly different between patients and controls (Table 2). Although, a lower frequency of the wild-type CC genotype was observed in T1D patients when compared with the control group (68.2% vs. 90.8%), the heterozygous CT genotype was observed more often in T1D patients (31.8% vs. 9.2%, P = 0.0012; OR = 4.58, 95% CI [1.76–11.91]). The homozygous mutant genotype (TT) of this polymorphic site was not detected among our patients or controls. To confirm the result, the impact of the causative 1858T allele was further evaluated using binary logistic regression analysis adjusted for age and gender (Table 2). The difference between the CC genotype and the CT genotype, corresponding to the dominant effect of the T allele, was analyzed and showed a statistically significant association with the disease incidence (P = 0.002; OR = 4.68, 95% CI = [1.73–12.67]).

Figure (3) illustrates an estimation of the risk for T1D susceptibility conferred by abnormal allele and genotype after rs2476601 SNP analysis in Egyptian patients with T1D.

The sex-stratified analysis for different cohorts of this study revealed an interesting observation with respect to genotype distribution (Table 3); the frequency of PTPN22 1858T allele is significantly increased in diabetic females as compared to female controls (46.5% vs. 9.7%, P = 0.001; OR = 8.12, 95% CI [2.14–30.78]), whereas no significant difference is detected when diabetic and control males were compared (16.7% vs. 8.8%, P = 0.49).

In order to evaluate the impact of genotype distribution on the disease onset-age in the patient cohort, patients were divided into two groups, setting the median age at disease onset in our cohort (6 years) as the cut-off value. The distribution of the 1858T allele is non-significantly different between both groups: carriers of 1858T allele in younger patients vs. patients older than 6 years (13/50 vs. 14/35, P = 0.24; OR = 1.90, 95% CI [0.75 – 4.79]) (Fig. 4). Also insignificant mutant variant distributions were found when we considered both age at onset and gender stratifications simultaneously in our diabetic cohort.

After stratifying our patients according to UACR results, 41.18% of them were found to be albuminuric while the rest were normoalbuminuric. The association between the inherited genetic polymorphism at 1858C/T in the PTPN22 and UACR values within the diabetic cohort was investigated in order to study the impact of genotype distributions of our polymorphic site on the susceptibility for DN incidence. After adjustment of all independent metabolic parameters that may influence glomerular filtration, binary logistic regression analysis reveals in-significant association (P = 0.33, OR = 1.8, 95% CI = [0.56-5.82]) (Table 4).

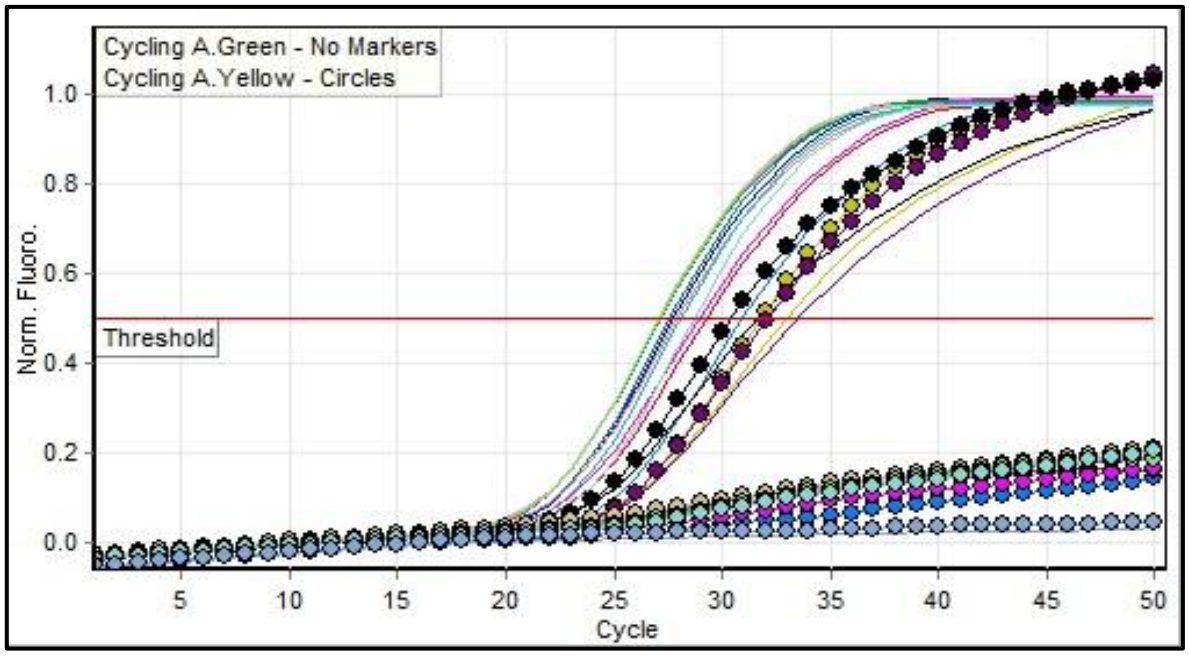

Fig. (2): Allelic discrimination results for genotyping of 15 diabetic and 1 No Template Control samples as given by allelic discrimination analysis software of Rotor Gene Q real-time PCR system.

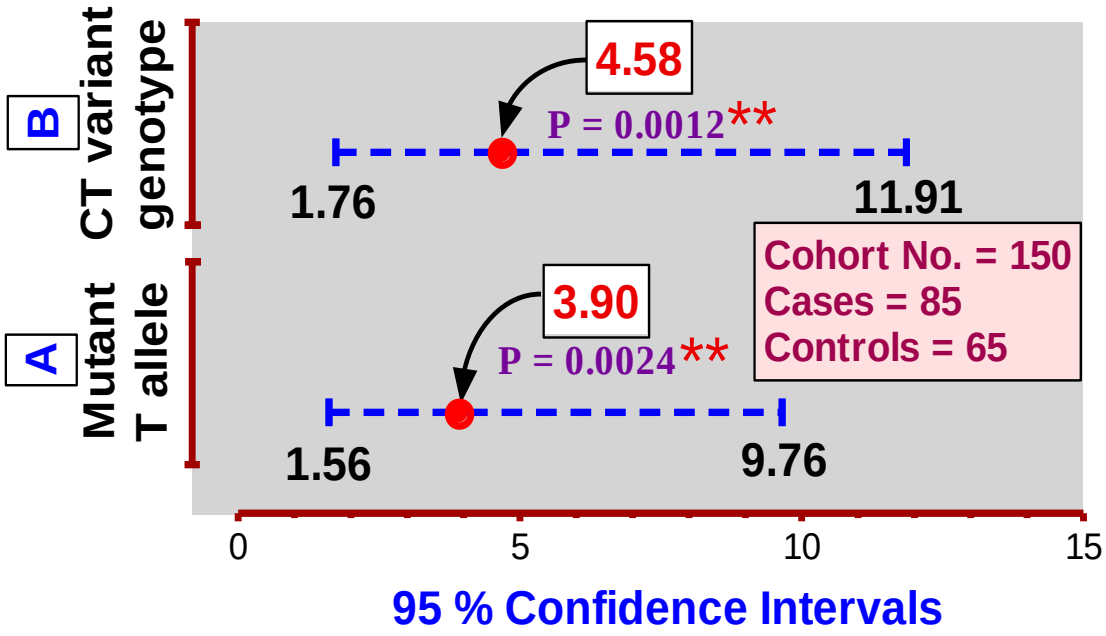

Fig. (3): Risk estimation calculated as Odds ratio "OR" (●) with 95 % Confidence Intervals (dotted lines) for rs2476601 SNP analysis in Egyptian patients with T1D.

A: OR for T allele; B: OR for CT genotype. **: P < 0.01 compared to Healthy Controls using Fisher's Exact test.

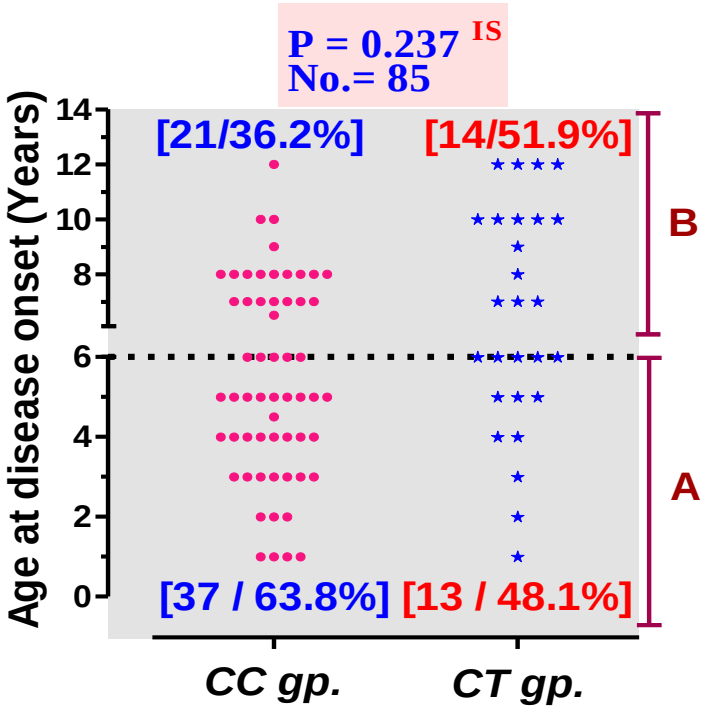

Fig. (4): Age at disease onset (years) of wild-type "CC gp." and mutant "CT gp." genotypes of PTPN22 C1858T gene polymorphism for Diabetic patients represented as scattered dot plot diagram. Data were categorized into A: disease onset ≤ 6 years and B: disease onset > 6 years.
Frequency and distribution (%) for each data group are shown between brackets and separated by (/). IS: Insignificant association "P > 0.05" compared to CC gp. using Fisher's Exact test and No.: Number of diabetic cohort.

Table 1: Demographic profiles of T1D and control groups.

Parameter		Healthy controls (№ = 65)	Type 1 diabetes (№ = 85)
Age (years)	Range	6 - 18	7 - 18
	M ± SEM	13.14 ± 0.42	13.61 ± 0.33
BMI(Kg/m^2)	Range	14 - 39	14 - 34
	M ± SEM	20.59 ± 0.66	21.32 ± 0.46
Gender distribution (%)	Male	52.30	49.40
	Female	47.70	50.60

BMI: Body mass index; №: Number; M ± SEM: Mean ± standard error of mean; Data were approximated to the second decimal.

Table 2: PTPN22 1858 C/T polymorphism distribution and OR for T1D and control groups.

PTPN22 1858 C/T SNP		Study groups			
		Healthy Controls (№ = 65)		Type 1 Diabetes (№ = 85)	
		№	%	№	%
Genotypes	CC	59	90.8	58	68.2
	CT	6	9.2	27	31.8
	TT	--	--	--	--
	P(HWE)	0.697		0.0817	
	OR*[1] [95%CI], P[1]			4.58 [1.76-11.91], **0.0012**	
	Adjusted[a] OR[2] [95%CI], P[2]			4.68 [1.73-12.67], **0.002**	
Alleles	C	124	95.38	143	84.12
	T	6	4.62	27	15.88
	OR[#1] [95%CI], P[1]			3.90 [1.56-9.76], **0.0024**	

OR reference estimate settled for wild-type CC-genotype and C-allele = 1.

№: Number; P(HWE): Probability of deviation of the observed from the expected genotype frequencies by chance; P: Probability compared to healthy controls; OR: Odds ratio for: *: CT genotype, #: T allele; CI: Confidence interval; [a]: Adjusted for age and gender; [1]: Data of fisher's exact association test; [2]: Data of binary logistic regression analysis.

Table 3: PTPN22 1858 C/T SNP sex-related genotype distribution probabilities and OR estimates for T1D incidence in Egyptian population.

PTPN22 1858 C/T SNP genotypes	Male				Female			
	Healthy Controls (№ = 34)		Type 1 Diabetes (№ = 42)		Healthy Controls (№ = 31)		Type 1 Diabetes (№ = 43)	
	№	%	№	%	№	%	№	%
CC	31	91.2	35	83.3	28	90.3	23	53.5
CT	3	8.8	7	16.7	3	9.7	20	46.5
TT	--	--	--	--	--	--	--	--
P	0.497				**0.001**			
OR[95%CI]	2.07 [0.49-8.69]				8.12 [2.14-30.78]			

№: Number; P: Probability compared to healthy controls of corresponding gender type; OR: Odds ratio for CT genotype; CI: Confidence interval.

Table 4: PTPN22 1858 C/T polymorphism genotype distribution probabilities and OR estimates for DN development within Egyptian patients with T1D.

PTPN22 1858 C/T SNP genotypes	UACR-based grouping within diabetic cohort			
	Normoalbuminuria* (№ = 50)		Albuminuria# (№ = 35)	
	№	%	№	%
CC	39	78	19	54.3
CT	11	22	16	45.7
OR[1] [95%CI], P[1]			2.99 [1.16-7.67], **0.032**	
Adjusted[a] OR[2] [95%CI], P[2]			1.8 [0.56-5.82], 0.326	

OR reference estimate settled for wild-type CC-genotype = 1.

UACR: Urinary Albumin to creatinine ratio; №: Number; P: P-value compared to normoalbuminuric patients; OR: Odds ratio for CT genotype; CI: Confidence interval; [a:] Adjusted for Gender, diabetes duration, systolic blood pressure, fasting blood glucose, A1C, Total Cholesterol and Triacylglycerol; [1:] Data obtained from fisher's exact association test; [2:] Data obtained from logistic regression analysis; *:UACR < 30 mg/gm creatinine; #:UACR = ≥30 mg/gm creatinine.

DISCUSSION

In the present study, the role of the PTPN22 1858 C/T polymorphism in T1D was investigated in an attempt to corroborate the general role of PTPN22 as a susceptibility locus for this autoimmune disease in the Egyptian population. Notably, to avoid any clinical misclassification, patients with any other autoimmune disease were excluded in the present study from the T1D group.

The current study is concordant with several previous population-based association studies **(Kahles et al., 2005; Zheng and She, 2005; Fedetz et al., 2006; Hermann et al., 2006; Steck et al., 2006; Chelala et al., 2007; Nielsen et al., 2007; Santiago et al., 2007; Douroudis et al., 2008; Saccucci et al., 2008; Smyth et al., 2008; Korolija et al., 2009; Fichna et al., 2010; Zhebrun et al., 2011)** in that a statistically significant higher frequency of the 1858T causative allele as well as CT genotype were observed in TID patients when compared with healthy controls. This result may lead us to deduce that the minor allele 1858T variant might confer susceptibility to T1D in the Egyptian population, as already described in most other white Caucasian populations. Hence, this locus proves to be a predisposing factor in the pathogenesis of T1D in Egypt.

These findings are reinforced by recently published meta-analyses which suggest that PTPN22 C1858T SNP may contribute to the predisposition of the T1D especially in populations of Europe and America **(Peng et al., 2012; Tang et al., 2012; Lee and Song, 2013; Wang et al., 2013; Xuan et al., 2013).**

Many genetic studies carried out on different populations have shown also a geographic gradient in the frequency of the disease-associated PTPN22 1858T allele, with the highest value in Finland (15.5%), Sweden (12%) and the UK (8%), decreasing southward to around 6% in Spanish and to 2% in Italian populations and being lowest in African and Asian populations **(Douroudis et al., 2008).** Unfortunately, no enough data were provided concerning Middle East and Arab region.

Being a gain of a function variant, the disease-predisposing 1858T allele encodes for a significantly more active phosphatase, which in a dominant manner suppresses TCR signaling better than equal amounts of the more common PTPN22*620R variant. This could explain why heterozygous 1858CT carriers have an elevated risk of the disease **(Vang et al., 2005).** This hypothesis and observation are in concordance with the present study in that a significantly higher frequency of the 1858CT genotype was seen in T1D patients when compared with the control group.

Indeed, conflicting data exist concerning sex-specific distribution of 1858C/T genotypes among T1D patients. Despite the fact that T1D is an autoimmune disease which does not show sex-bias (gender distribution in our Egyptian diabetic population: 50.6% females vs. 49.4% males), **Kahles et al., (2005)** reported an unprecedented

observation of an association of PTPN22 1858T allele only in type 1 diabetic females of German population. In agreement with this novel characteristic, an interesting observation of worth mentioning in our results is that this polymorphic susceptibility factor proved to be also unique to the female sex. However no significant difference is detected between patients and controls of the male gender in our diabetic cohort.

Our finding came also in accordance with previously published data of Nielsen and his coworkers **(Nielsen et al., 2007)** who provide evidence of an association of the PTPN22 1858T allele with T1D to the female sex. On the other hand, a single study has shown a pronounced effect of PTPN22 on susceptibility to T1D in males **(Hermann et al., 2006).** Furthermore, **Smyth et al. (2004)** found no evidence of such phenomenon among T1D-affected offspring in a large family-based cohort consisting of T1D families of Caucasian origin from UK, USA and Romania. In the same study, a large case-control study of individuals solely from the UK suggested a sex-specific distribution of the 1858C/T genotypes among T1D patients, but no further details were given by the authors.

Controversial data are obtained from a study conducted on T1D patients of the Spanish population **(Santiago et al., 2007);** authors fail to find a significant difference between diabetic female and male patients and thus they concluded that this susceptibility factor is not exclusively associated with female patients, as it has been proposed. But, after stratifying patients on the basis of age at disease onset, whilst there is no evidence of sex-bias in patients older than 15 years of disease onset, the analysis of their pediatric-onset patients (< 15 years) supported a preferential association in T1D females as our Egyptian diabetic female patients do. Notably, our entire diabetic cohort could also be considered as pediatric-onset patients (< 12 years).

On the contrary, several studies did not show a gender-specific effect and authors postulated that the strength of the association was similar in males and females **(Gomez et al., 2005; Ladner et al., 2005; Steck et al., 2006).**

Given that allelic frequencies vary in each population, these discrepancies in results might be due to genetic heterogeneity of the populations studied given the variation in population frequency for the PTPN22 1858T allele **(Gregersen et al., 2006).** However, the disagreement between our results and some previously published data regarding gender-specific distribution of the minor allele may be attributable to the smaller study cohort and to the relatively shorter duration of disease (7.66 years on average). Nevertheless, we cannot exclude the possibility that sex-bias effect in our study may be due to some technical issues that could be handled later for obtaining more reliable conclusions. The motives for this sex-dissimilarity in pathogenesis of T1D remain unclear. However, it could be considered as a complex process suggesting the presence of a third-party interacting contributor yet to be identified that may influence PTPN22 locus; the effect of sex hormones or epigenetic alterations in DNA could elucidate this sex-biased association.

The proposed disease-predisposing PTPN22 model that allows for more autoreactive T cells to survive and escape into circulation could also determine an earlier disease onset-age **(Santiago et al., 2007)** and in order to evaluate this hypothesis in our population, we divided our patients into two groups, setting the median age at disease onset in our cohort (6 years) as the cut-off value. We selected the median age at disease diagnosis, because it is the best central statistic for highly skewed distributions, as T1D age at diagnosis is. Additionally, it maximizes the statistical power. Our data revealed that the age at disease onset is not influenced by the presence of the mutant PTPN22*620W variant. This finding is consistent with that of **Santiago et al., (2007)** who reported no differences between T1D patients younger and older than 15-years old in Spanish population **(Santiago et al., 2007).** Our study also supports another one conducted on T1D patients of German population stratified by the presence of PTPN22 T allele; authors showed no statistically significant associations between genotype distributions and age at disease onset **(Kahles et al., 2005).** Similarly and in agreement with our result, previously published data did not reveal any evidence for an interaction between the PTPN22 genotypes and age at onset of diabetes and thus authors concluded that the strength of the association was similar in patients diagnosed before and after age of 10 years **(Steck et al., 2006).** More recently, a large prospective study aimed primarily at evaluating the prevalence of PTPN22 C1858T polymorphism in young T1D patients was conducted. Interestingly, authors deduced an opposite conclusion; PTPN22 1858T allele is associated with younger age at the onset of the T1D. They found that the 1858T-positive patients were significantly younger at diagnosis of diabetes than those without this allele **(Kordonouri et al., 2010).**

The contribution of the R620W polymorphism to susceptibility for different autoimmune disorders was evaluated on Egyptians. In the last few years, several studies have been conducted in populations from Egypt. Collectively, the minor allele was not associated with non-segmental vitiligo **(Elmongy et al., 2013)** while it was associated with alopecia areata **(El-Zawahry et al., 2013)** as well as RA **(Salama et al., 2014).** Additionally, association is confirmed regarding immune thrombocytopenic purpura **(Anis et al., 2011; Elhoseiny et al., 2012)** while controversial data were obtained with respect to SLE **(Moez and Soliman, 2012; Hamza et al., 2013).** There

is no sufficient data about PTPN22 on Egyptian T1D except for one study **(El-Kafoury et al., 2014)** in which authors reported that the SNP -1123 C > G (rs2488457) showed a significant difference between patients and controls. We mentioned the role of our polymorphic site in other autoimmune diseases in our country in order to provide or deduce an evidence for its role as a general autoimmunity locus in Egyptian population.

Being the first-line chronic microvascular complication of T1D, DN is the leading contributor to end-stage renal disease "ESRD" and both are the chief cause of morbidity and premature mortality amongst patients with T1D **(Pezzolesi et al., 2009).** It is important to emphasize that this complication is first manifested clinically as an increase in microalbuminuria, which progresses to overt albuminuria and then to ESRD **(Powers, 2008).** Overall, ESRD develops in about 20% of all patients with T1D **(Pezzolesi et al., 2009).** Urinary albumin excretion, as measured here by means of UACR, has emerged as one of the best biomarkers and most practical methods for detection of early DN **(Abd El-Maksoud et al., 2009; ADA, 2015).**

The pathogenesis of DN appears to be complex and multifactorial. Several genetic and environmental factors are likely to contribute to its development and progression, although the precise nature of the genetic burden involved in DN remains poorly understood **(Germain et al., 2015).** Despite evidence that genetic susceptibility plays a role in the development of DN in T1D, the identification of susceptibility genes and their variants has had limited success **(Pezzolesi et al., 2009).** Using unbiased genome-wide case-control association studies "GWAS", extensive worldwide efforts have been performed to identify the gene(s) involved in the development and progression of DN. However, to date only a small number of GWAS for DN have been performed **(Germain et al., 2015).**

In this study and in an attempt to identify whether PTPN22 could confer susceptibility to DN, we examined whether variants of PTPN22 C1858T are associated with urinary albumin excretion in Egyptian patients with T1D. Notably, no definitive data have yet emerged about such genetic susceptibility. Results of this study suggest that susceptibility to DN in T1D is not influenced by our genetic marker. This may be attributable, in part, to the small sample size that probably often inappropriate to identify any solid evidence to indicate a genetic susceptibly to the disease. Another challenge that adds a major failure to obtain a solid conclusion is possibly that multiple genetic factors are involved in DN in a complex manner and the influence of each individual factor is too weak to be identified **(Shimazaki et al., 2005).** Hence, there is an urgent need for approaches other than standard candidate-gene analysis to discover novel genetic loci conferring susceptibility to DN.

In summary, conducting GWAS using available gene-based SNPs as genetic markers is a powerful strategy for identifying genes associated with susceptibility to common diseases.

CONCLUSION

Based on improved knowledge of the immunopathogenesis of T1D, the main goal of our work is being a step on the way that holds promise for future interventions aimed at disease prevention and reversal.

We replicated the association between PTPN22 +1858 C/T polymorphism and T1D susceptibility risk in Egyptian population. This SNP seems to be an independent risk factor that may be involved in the pathogenesis of T1D by a sex-specific mechanism that contributes to susceptibility in females. Whether children carrying this high-risk genetic allele are also vulnerable to environmental factors precipitating the development of T1D needs further attention and evaluation in prospective studies.

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