International Research Journal of Medicine and Medical Sciences
Vol. 3(2), pp. 30-34, May 2015
ISSN: 2354-211X
Full Length Research Paper

PTPN22 gene rs2476601 SNP is associated with type 1 diabetes and not anti-Gad autoantibodies in Egyptian children

Dalia A. El Aal[1], Marianne F. Morgan[1], Heba N. Baz[1*], Mona H. Hafez[2] and Hazem E. Abo Youssef[1]

[1]Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Egypt.
[2]Department of Pediatrics, Faculty of Medicine, Cairo University, Egypt.

Accepted 27 April, 2015

ABSTRACT

PTPN22 gene encodes lymphoid protein tyrosine phosphates (Lyp), which is a critical negative regulator of TCR signal transduction. A variant of PTPN22 (1858T) has been reportedly associated with the development and progress of T1DM. Since auto-reactive cytotoxic lymphocytes appear to be the main effector cells in T1DM, this might explain the reason of the aforementioned association. This study was conducted on 150 subjects; 100 T1DM patients and 50 controls; all were subjected to DNA analysis of PTPN22 C1858T polymorphism using PCR-RFLP technique, along with HbA1c%, Glutamic acid decarboxylase (GAD65) autoantibodies determinations. This study showed that 83 patients (83%) were homozygous for PTPN22 (CC), 15 patients (15%) were heterozygous for (CT) and 2 patients (2%) were homozygous for (TT). Statistical comparisons for the distribution of genotypes and allele frequency for the PTPN22 C1858T polymorphism between T1DM patients and controls showed that 17% of T1DM patients were TT/CT compared to 0% of controls (P = 0.002) and odds ratio [1.602 (1.404 to 1.828)]. Also, 181 alleles of T1DM patients were C (90.5%) compared to 100 alleles (100%) of controls with P value = 0.001. No significant associations between PTPN22 genotypes and other factors such as age, gender, age at onset of T1DM, duration of T1DM, DKA, hypoglycemia, nephropathy, neuropathy, retinopathy, macro-vascular complications, arthropathy, consanguinity, HbA1C or GAD65 autoantibodies. In conclusion, PTPN22 C1858T gene polymorphism is associated with development of T1DM in Egyptian pediatric population.

Keywords: Protein tyrosine phosphatase non-receptor type 22, T1DM, Lyp protein, Egyptian population.

*Corresponding author. E-mail: hebabaz@kasralainy.edu.eg. Tel: 002-23640501.

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is an autoimmune disease which results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas (American Diabetes Association, 2013). The PTPN22 gene codes for the protein tyrosine phosphatase, non-receptor 22, is also known as the lymphoid-specific phosphatase (Lyp), and maps to human chromosome 1p13.3-p13.1. Lyp is an important negative regulator of T-cell activation (Cohen et al., 1999). A single nucleotide polymorphism (SNP) 1858C>T in PTPN22 rs2476601 was implicated in disrupting the mechanism of T-cell deactivation and was found to be associated with T1DM in US (Steck et al., 2006) and Italian (Saccucci et al., 2008) populations. However, it was not encountered in African American (Begovich et al., 2004), Asian (Ikegami et al., 2006) or Japanese (Mori et al., 2005) populations likely due to low frequency of the T-allele in these three populations. Hence, the present study aimed at investigating the association of PTPN22 (1858C>T) variant with T1DM in the Egyptian population.

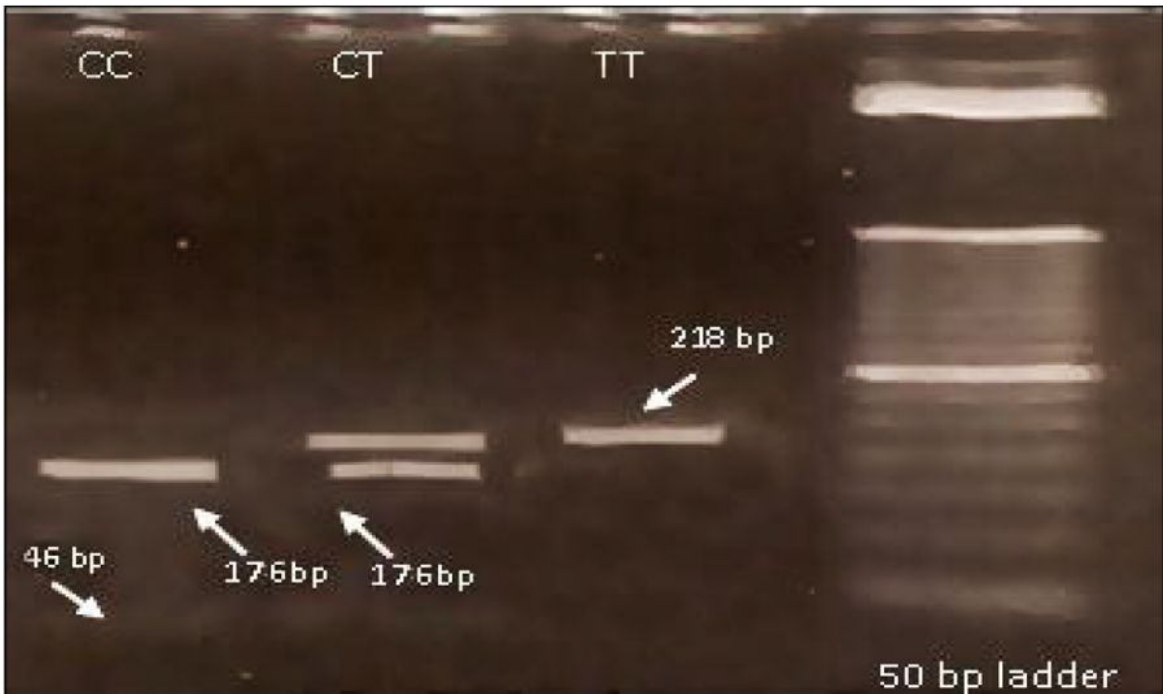

Figure 1. Showing identified bands after transillumination by UV: The 46 bp & 176 bp bands indicate the C allele. The 218 bp indicates the T allele. The 46 bp, 176 bp, and 218 bp bands indicate the CT genotype.

SUBJECTS AND METHODS

Subjects

This randomized case-controlled study was conducted on 150 subjects divided into two groups: Group I: composed of 100 previously diagnosed T1DM patients attending the Diabetic Endocrine and Metabolic Pediatric Unit (DEMPU) clinic of the new Children's Hospital, Cairo University. Informed consent was obtained from the parents of the participants included in the study. For Group I: the mean age at sample collection was 7.52 ± 2.55 years and the group composed of 51 males (51%) and 49 females (49%). Group II: composed of 50 healthy age and sex matched control subjects recruited from a health checkup program of school children, with a mean age of 7.23 ± 2.37 years: 26 males (52%) and 24 females (48%) with no family history of T1DM or any other types of autoimmune disease.

Methods

Glycosylated hemoglobin (HbA1c)

The turbidimetric inhibition immunoassay (TINIA) principle was carried out on dimension clinical chemistry system using kits supplied by Siemens (Bunn et al., 1978).

Glutamic acid decarboxylase (Anti-GAD) autoantibodies

Determination was based on Enzyme Linked Immunosorbant assay (ELISA) method (Wkea Med Supplies corp, China) previously described (Baekkeskov et al., 1990). The manufacturer's defined reference interval was used as a cut-off to define positive cases for anti-Gad.

Genotyping the (PTPN22) polymorphism

Extraction of genomic DNA from peripheral blood leukocytes employing Thermoscientific Genomic DNA Purification Kit for total DNA purification from human whole blood (Fisher Scientific - USA, 300 Industry Drive, Pittsburgh PA, United States).

PCR was performed employing (2X PCR master mix kit (#K1081 fermentas), using the following primers: Forward primer: 5'ACTGATAATGTTGCTTCAACGG-3' and Reverse primer: 5'-TCACCAGCTTCCTCAACCAC-3'. The reaction was performed in Hybiad thermal cycler (Promega Corporation, USA), in the following steps: initial denaturation at 94°C for 5 min, then 30 cycles of denaturation (94°C for 30 s), annealing (62°C for 60 s) and extension (72°C for 60 s); then a final extension step for 5 min at 72 ºC. The amplification was then verified by running the products on 1.5% agarose gel electrophoresis (Promega gel electrophoresis apparatus). The amplicon size was 218 bp.

The amplified product was digested by RsaI restriction enzyme (#EN-129L, Fermentas), the 218 bp segment was digested for sixteen hours at 37°C and the digested products were separated by electrophoresis on agarose 3% gel and visualized using ethidium bromide. The wild type allele yielded 176 bp and 46 bp fragments, whereas the variant allele remained un-cleaved 218 bp fragment (Figure 1).

Statistical methods

Quantitative data are summarized as mean ± SD or median (25th to 75th percentile). Differences between groups are detected using Student's t test and/or Mann-Whitney test as appropriate. Qualitative data are presented as number (percent) and compared using Chi-Square (X2) or Fischer's Exact test as appropriate with risk estimate calculation. Tests are considered statistically significant at a P value < 0.05. Statistical analysis was run on SPSS

Table 1. Anthropometric measures of the included groups.

Parameter		T1DM patients (n = 100)	Controls (n = 50)	p
Gender	Male	51(51%)	26 (52%)	0.908
	Female	49(49%)	24 (48)	
Age	Min - Max	3 - 14	3 - 17	0.473
	Mean ± SD	7.52 ± 2.55	7.23 ± 2.37	

Table 2. Demographic, clinical and laboratory data of the patients.

Parameter		T1DM Patients (n = 100)
Age at diagnosis of diabetes (Years)		4.00 (3.00 – 5.00)
Duration of diabetes (Years)		3.00 (2.00 – 4.00)
HbA1C % last		7.00 (6.60 – 9.00)
DKA (No/year)	Once	29 (29%)
	Twice	14 (14%)
Hypoglycemia		84 (84%)
Nephropathy		20 (20%)
Neuropathy		0 (0%)
Retinopathy		0 (0%)
Macrovascular		0 (0%)
Arthropathy		0 (0%)
Failure to Thrive		0 (0%)
Hospital Admissions	Once	29 (29%)
	Twice	14 (14%)
Blood Transfusion		0 (0%)
Other Endocrine Autoimmune Diseases		0 (0%)
Non-endocrine Autoimmune Diseases		0 (0%)
Consanguinity		6 (6%)
GAD antibody	Positive	94 (94%)
	Negative	6 (6%)

Results are reported as mean ± SD, median with range or number of patients (%) as appropriate.

for mac, release 20.0 (SPSS Inc., Chicago, Ill, USA).

RESULTS

The anthropometric measures of the included groups along with the clinical and laboratory characteristics of the patient's group are represented in Tables 1 and 2.

Results of genetic analysis of PTPN22 1858C/T SNP

The genotype CC was the common genotype between the patients and control groups (83/100 and 50/50 respectively, with a significant statistical difference between both groups (p = 0.008). The CT/TT genotype was present in 17 patients and none of the control (P=0.002). Carriers of the genotype were more likely to develop T1DM OR = 1.602, (95% CI = 1.404-1.828) (Table 3).

There was a significant statistical difference between both groups as regards allele frequency. The T allele frequency in the studied population (patients and controls) was 0.063, compared to the Global Minor Allele Frequency (GMAF): 0.04224 (Table 4).

No statistically significant relationship was found between the PTPN22 genotypes and the frequency of DKA (p = 0.108), the frequency of hypoglycemic episodes (p = 0.212), the positive consanguinity (p = 0.253), the age of onset (p = 0.411), the duration of disease (p = 0.928), nephropathy (p = 0.690), hospital admission (p = 0.108), neuropathy and retinopathy (p = 0.649). The frequency of GAD antibodies positive cases were compared to the PTPN22 genotypes. Neither was there a

Table 3. Frequency distribution of PTPN22 genotypes between the two studied groups.

Parameter	T1DM Patients (n = 100)	Controls (n = 50)	*P*	*OR (95% CI)*	
TT Genotype	2 (2%)	0 (0%)			
CT Genotype	15 (15%)	0 (0%)	0.008		
CC Genotype	83 (83%)	50 (100%)			
TT/CT Genotype*	17(17%)	0 (0%)	0.002	1.602	(1.404-1.828)

*Versus CC genotype.

Table 4. Frequency distribution of PTPN22 alleles between the two studied groups.

Parameter	T1DM patients (n = 200)	Controls (n = 100)	*P*
C Allele	181(90.5%)	100 (100%)	0.001
T Allele	19 (9.5%)	0 (0%)	

statistically significant correlation between the PTPN22 and the GAD antibody (p = 0.982), nor the assayed HbA1c (p= 0.786) of the patients.

DISCUSSION

This study aimed at exploring the association of protein tyrosine phosphates non-receptor type 22 (PTPN22) C1858T gene polymorphism with T1DM in patients of Egyptian population. Results of previous studies have been conflicting as regard the association between a PTPN22 C1858T SNP and T1DM in different ethnicities; an association has been found in US (Bottini et al., 2004; Onengut-Gumuscu et al., 2004; Smyth et al., 2006; Ladner et al., 2005; Zheng and She, 2005), German (Kahles et al., 2005), Danish (Nielsen et al., 2007), Estonian (Douroudis et al., 2008), Italian (Saccucci et al., 2008; Zoledziewsk et al., 2008), Croatian (Korolija et al., 2009) and Russian populations (Zhebrun et al., 2011). In contrast, this association was absent in studies carried out in African American (Begovich et al., 2004) and Asian Indian populations (Baniasadi and Das, 2008). The importance of this study lies in that it adds a new ethnic group with positive association between the PTPN22 gene C1858T SNP and T1DM. This study showed that patients exhibiting T genotype (whether homozygous TT or heterozygous CT) were 1.6 times more prone to have T1DM than a patient who is homozygous for CC (95th CI: 1.404 to 1.828).

It has been reported by Maziarz et al. (2010) that the association between PTPN22 (CT/TT) and T1DM is mainly due to the association between PTPN22 (CT/TT) and Anti-GAD-positive T1DM. This is understood within the following hypothesis; the mutant 1858T PTPN22 gene SNP produces a Lyp protein with reduced capabilities to negatively regulate the T-cell functions, with subsequent B-cell activation and production of autoantibodies among which is Anti-GAD. However, the results of this study showed no association between Anti-GAD autoantibodies and PTPN22 genotypes in the studied T1DM patients. These results are similar to a previous report shown in Finnish T1DM patients (Hermann et al., 2006). The observed contradiction can be explained by the presence of a third factor that modulate the risk, HLA is strongly suggested. Another explanation could be because the levels of Anti-GAD generally increase with age (Lohmann et al., 1997), however in this study, we recruited strictly pediatric T1DM patients (3 to 14 years), contrary to other studies (Maziarz et al., 2010).

Similar to previous reports (Saccucci et al., 2008; Gomez et al., 2005); this study showed no significant differences in genotypes or alleles as regard to sex. Other authors, however, found a statistically significant association between gender and the different PTPN22 genotypes only in females, not in males (Kahles et al., 2005; Nielsen et al., 2007; Fedetz et al., 2006). Further meta-analysis is required to resolve this conflict.

In this study, no association between PTPN22 genotypes and age at disease onset of T1DM patients was found (P = 0.411). This finding is in agreement with other studies (Kahles et al., 2005; Korolija et al., 2009; Gomez et al., 2005), while, other studies found a significant association between PTPN22 genotypes and age at disease onset. Moreover, the PTPN22 1858T allele frequency was higher in young patients with T1DM than in healthy controls and patients carrying the PTPN22 1858T allele were significantly younger at diabetes onset compared with patients without this allele (Fedetz et al., 2006; Santiago et al., 2007; Kordonouri et al., 2010). Similar to Maziarz et al. (2010), there was no significant association between age and the different PTPN22 genotypes among the studied groups (P = 0.742). In addition, we found no association between HbA1c and PTPN22 genotypes in T1DM patients, while Petrone et al. (2008) found a statistically significant association between PTPN22 genotypes and HbA1c

where subjects carrying the 1858T allele variant had significantly higher HbA1c compared to subjects homozygous for the C1858 allele.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

REFERENCES

American Diabetes Association, **2013**. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care, 36(1):8:1-8.

Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olesen H, De Camilli P, **1990**. Identification of the 64K autoantigen in insulin dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature, 347:151–156.

Baniasadi V, **Das** SN, **2008**. No evidence for association of PTPN22 R620W functional variant C1858T with type 1 diabetes in Asian Indians. J Cell Mol Med, 12(3):1061-1062.

Begovich A, Carlton V, Honigberg L, Schrodi S, Chokkalingam A, Alexander H, Ardlie K, Huang Q, Smith A, Spoerke J, Conn M, Chang M, Chang S, Saiki R, Catanese JLD, Garcia V, McAllister L, Jeffery D, Lee AA, **2004**. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet, 75:330-337.

Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, MacMurray J, Meloni G, Lucarelli P, Pellecchia M, Eisenbarth G, Comings D, Mustelin T, **2004**. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet; 36:337–338.

Bunn HF, Gabbay KH, Gallop PM, **1978**. The glycosylation of hemoglobin: Relevance to diabetes mellitus. Science, 200:21–27.

Cohen S, Dadi H, Shaoul E, Sharfe N, Roifman C, **1999**. Cloning and characterization of a lymphoid-specific, inducible human protein tyrosine phosphatase. Lyp Blood; 93:2013–2024.

Douroudis K, Prans E, Haller K, Nemvalts V, Rajasalu T, Tillmann V, Kisand K, Uibo R, **2008**. Protein tyrosine phosphatase non-receptor type 22 gene variants at position 1858 are associated with type 1 and type 2 diabetes in Estonian population. Tissue Antigens, 72(5):425-430.

Fedetz M, Matesanz F, Caro-Maldonado A, Fernández O, Tamayo JA, Guerrero M, Delgado C, López-Guerrero JA, Alcina A, **2006**. OAS1 gene haplotype confers susceptibility to multiple sclerosis. Tissue Antigens, 68:446-449.

Gomez L, Anaya J, Gonzalez C, Pineda-Tamayo R, Otero W, Arango A, **2005**. PTPN22 C1858T polymorphism in Colombian patients with autoimmune disease. Genes Immun, 6:628–631.

Hermann R, Lipponen K, Kiviniemi M, Kakko T, Veijola R, Simell O, **2006**. Lymphoid tyrosine phosphatase (LYP/PTPN22) Arg620Trp variant regulates insulin autoimmunity and Function of PTPN22 in T1D is modified by HLA and GADA progression to type 1 diabetes. Diabetologia; 49:1198–1208.

Ikegami H, Fujisawa T, Kawabata Y, Noso S, Ogihara T, **2006**. Genetics of type 1 diabetes: Similarities and differences between Asian and Caucasian populations. Ann N Y Acad Sci, 1079:51-59.

Kahles H, Ramos-Lopez E, Lange B, Zwermann O, Reincke M, Badenhoop K, **2005**. Sex-specific association of PTPN22 1858T with type 1 diabetes but not with Hashimoto's thyroiditis or Addison's disease in the German population. Eur J Endocrinol, 153(6):895-899.

Kordonouri O, Pankowska E, Rami B, Kapellen T, Coutant R, Hartmann R, Lange K, Knip M, Danne T, **2010**. Sensor-augmented pump therapy from the diagnosis of childhood type 1 diabetes: results of the Pediatric Onset Study (ONSET) after 12 months of treatment. Diabetologia, 53(12):2487-2495.

Korolija M, Renar IP, Hadzija M, Medvidovic EP, **2009**. Association of PTPN22 C1858T and CTLA-4 A49G polymorphisms with Type 1 Diabetes in Croatians. Diabetes Res Clin Pract, 86:e54-e57.

Ladner M, Bottini N, Valdes A, Noble J, **2005**. Association of the single nucleotide polymorphism C1858T of the PTPN22 gene with type 1 diabetes. Hum Immunol, 66:60–64.

Lohmann T, Sessler J, Verlohren H J, Schroder S, Rotger J, Dahn K, Morgenthaler N, Scherbaum WA, **1997**. Distinct genetic and immunological features in patients with onset of IDDM before and after age 40. Diabetes Care. 20(4):524-529.

Maziarz M, Janer M, Roach J, Hagopian W, Palmer JP, Deutsch K, Sanjeevi CB, Kockum I, Breslow N, Lernmark Å, **2010**. The association between the PTPN22 1858C>T variant and type 1 diabetes depends on HLA risk and GAD65 autoantibodies, Genes Immun, 11(5):406–415.

Mori M, Yamada R, Kobayashi K, Kawaida R, Yamamoto K, **2005**. Ethnic differences in allele frequency of autoimmune-disease-associated SNPs. 50(5):264-266.

Nielsen R, Hellmann I, Hubisz M, Bustamante C, Clark AG, **2007**. Recent and ongoing selection in the human genome. Nat Rev Genet, 8:857–868.

Onengut-Gumuscu S, Ewens K, Spielman R, Concannon P, **2004**. A functional polymorphism (1858C/T) in the PTPN22 gene is linked and associated with type I diabetes in multiplex families, Genes Immun, 5:678–680.

Petrone A, Suraci C, Capizzi M, Giaccari A, Bosi E, Tiberti C, Cossu E, Pozzilli P, Falorni A, Buzzetti R, the NIRAD Study Group, **2008**. The protein tyrosine phosphatase nonreceptor 22 (PTPN22) is associated with high GAD antibody titer in latent autoimmune diabetes in adults non-insulin requiring autoimmune diabetes (NIRAD) study 3. Diabetes Care, 31(3):534-538.

Saccucci P, Compagnone E, Verrotti A, Galasso C, Curatolo P, **2008**. Lack of association between MTHFR C677T and MTHFR A1298C genetic polymorphisms and mental retardation. Nutr Neurosci, 11(5):241-242.

Santiago J, Martinez A, de la Calle H, Fernandez-Arquero M, Figueredo M, de la Concha E, **2007**. Susceptibility to type 1 diabetes conferred by the PTPN22 C1858T polymorphism in the Spanish population. BMC Med Genet, 8:54.

Smyth D, Cooper J, Bailey R, Field S, Burren O, Smink L, Guja C, Ionescu- Tirgoviste C, Widmer B, Dunger D, Savage D, Walker N, Clayton D, Todd J, **2006**. A genome-wide association study of non-synonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (IFIH1) region. Nat Genet, 38:617-619.

Steck AK, Liu SY, McFann K, Barriga KJ, Babu SR, Eisenbarth GS, Rewers MJ, She JX, **2006**. Association of the PTPN22/LYP gene with type 1 diabetes. Pediatr Diabetes, 7:274-278.

Zhebrun D, Kudryashova Y, Babenko A, Maslyansky A, Kunitskaya N, Popcova D, Klushina A, Grineva E, Kostareva A, Shlyakhto E, **2011**. Association of PTPN22 1858T/T genotype with type 1 diabetes, Graves' disease but not with rheumatoid arthritis in Russian population. Aging, 3(4):368–373.

Zheng W, **She** JX, **2005**. Genetic association between a lymphoid tyrosine phosphatase (ptpn22) and type 1 diabetes. Diabetes, 54(3):906-908.

Zoledziewsk M, Perra C, Orrù V, Moi L, Frongia P, Congia M, Bottini N, Cucca F, **2008**. Further evidence of a primary, causal association of the PTPN22 620W variant with type 1 diabetes. Diabetes, 57(1):229-234.

Citation: El Aal DA, Morgan MF, Baz HN, Hafez MH, Abo Youssef HE, 2015. PTPN22 gene rs2476601 SNP is associated with type 1 diabetes and not anti-Gad autoantibodies in Egyptian children. Int Res J Med Med Sci, 3(2): 30-34.