ISSN NO. 2320-5407

Journal homepage: *http://www.journalijar.com*

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

The association of lymphoid protein tyrosine phosphatase non-receptor 22 (PTPN22) gene polymorphism with Egyptian type 1 diabetes risk

Dr Dalia Saber Morgan[1*], Dr Hanan Mohamed Farhan[2], Dr Gamal Al-Din Taha[1], Dr Hanan Taha[1]

1. Department of Pediatrics, Faculty of Medicine, Beni-Suef University.

2. Department of Clinical and Chemical Pathology, Faculty of Medicine, Beni-Suef University.

Manuscript Info

Manuscript History:

Received: 14 October 2015
Final Accepted: 22 November 2015
Published Online: December 2015

Key words:

pediatric patients, T1D, *PTPN22* 1858C>T, PCR-RFLP, diabetic complications

Corresponding Author

Dr Dalia Saber Morgan

Abstract

Background: Type 1 diabetes (T1D) is a T-cell mediated autoimmune disease. Protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) gene is a powerful inhibitor of T-cell activation. The single nucleotide polymorphism (SNP) of PTPN22 1858C>T is a gain-of-function increasing reactivity of immune system and risk of autoimmune diseases. Our aim was to evaluate PTPN22 1858C>T gene polymorphism in T1D and diabetic complications risk in Egyptian pediatric patients.

Methods: PTPN22 1858C>T gene polymorphism was analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay in 50 T1D pediatric patients and 100 healthy control subjects matched on age and sex.

Results: The frequency of wild genotype CC was statistically higher among controls (92%) versus T1D patients (74%), while the mutant CT/TT genotypes were statistically higher among patients (26%) versus controls (8%) (p=0.003). Higher mutant T allele frequency in patients (17%) versus controls (4%) was found, while C allele showed higher frequency among controls (96%) versus patients (83%) (P < 0.001).

Mutant genotypes CT/TT showed statistically significant higher frequencies among T1D patients with hypoglycaemic attacks, fundus abnormalities and peripheral neuropathy (p=0.029, p=0.001 and p=0.002), respectively.

Conclusion: Our study revealed the role of PTPN22 1858C>T gene polymorphism in T1D; thus, it may be concidered as a genetic risk factor in T1D and diabetic complications in Egyptian pediatric patients.

INTRODUCTION

Type 1 diabetes (T1D) is a T-cell mediated autoimmune disease leading to autoimmune destruction of insulin-producing β-cells in the pancreas and loss of insulin secretion resulting in severe hyperglycaemia. T1D results from a complex interaction between environmental and genetic factors [(1)]. The increased incidence rates for T1D observed in monozygotic twins and other relatives of patients suggest that some fraction of the disease risk is inherited [(2)].

Several gene polymorphisms have been identified as pathogenic risk factors for the development and progression of T1D [3-6].

Family of protein tyrosine phosphatases (PTPs) are involved in preventing spontaneous T-cell activation by dephosphorylating and inactivating T-cell receptor-associated kinases and their substrates [7]. PTPs act in a coordinated manner with protein tyrosine kinases to control cell signaling thereby regulating various physiological processes through formation of a complex with C-terminal Src Kinase (CSK) and suppression of the downstream mediators of T-cell receptor (TCR) signalling [8,9]. Malfunctioning of PTPs has major pathological implications [10]. Protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) gene is localized on chromosome 1p13.3-13.1 encoding for an 807-amino acid residue protein referred to as lymphoid tyrosine phosphatase (LYP), a powerful inhibitor of T-cell activation. In conjunction with protein tyrosine kinases, LYP restricts the response to antigens by disrupting protein tyrosine phosphorylation events that control cell activation and differentiation. Subsequently, this negative control mechanism prevents spontaneous T-cell activation and reverts activated T-blasts to a resting phenotype [11].

The single nucleotide polymorphism (SNP) of PTPN22 1858C>T is the change of cytosine to thymidine at nucleotide 1858 in exon 14. PTPN22 1858C>T SNP leads to the substitution of arginine (R) with tryptophan (W) at codon 620 in LYP (R620W) located in the N-terminal proline-rich motif that binds LYP to the SH3 domain in CSK leading to a gain-of-function form of the protein[12].*In vitro* experiments showed that only LYP with arginine 620 (allele 1858C) forms a complex with T-cell receptor-associated kinases whereas LYP with tryptophan 620 (allele 1858T) binds less efficiently[13].Accordingly, the PTPN22 1858T allele raises the potential for a 'hyper-reactive' pathogenetic T-cell response where the TCR associated kinases might exhibit an uncontrolled Tcell induction, and this may increase the overall reactivity of the immune system thus predisposing an individual to autoimmune disease [12].

Several studies showed that PTPN22 1858C>T gene polymorphism is associated with multiple autoimmune diseases and T1D [11, 14-18]. Numerous studies revealed the correlation between 1858C>T SNP with rheumatoid artheritis (RA) [7, 13, 19-22], systemic lupus erythematosis (SLE) [8, 23, 24], T1D [11, 25, 26], Graves' disease [9, 25, 27], Myasthenia Gravis [26] and Wagners' granulomatosis [28]. However, this association may be questionable for some of these diseases, such as SLE and RA, as a number of studies failed to demonstrate it [29, 30]. At the same time, no association was found with multiple sclerosis [31, 32], Crohn's disease [33-35], ulcerative colitis [35], psoriasis and psoriatic arthritis [32].

Given the growing evidence that PTPN22 1858C>T gene polymorphism is associated with autoimmunity in general and T1D in specific as patients produce autoimmune antibodies against their own β-cells of pancreas, we analyzed this polymorphism in Egyptian pediatric patients with T1D and its association with diabetic complications . To our knowledge diabetic complications were not investigated with PTPN22 1858C>T gene polymorphism so far.

Materials and methods

Materials

The present study included 50 pediatric patients with T1D together with 100 healthy age and sex matched controls. Both patients and controls were recruited from Pediatric Diabetes Endocrinology Outpatients Clinic of Beni-Suef University Hospital. The study design was approved by the Scientific Research Committee, Faculty of Medicine, Beni-Suef University. Data confidentiality was preserved according to the Revised Helsinki Declaration of Bioethics (2008) [36]. Informed consent was obtained from all the parents of children who agreed and participated in this study.

Patients were subjected to [1] full history taking including age ,duration of disease, attacks of diabetic ketoacidosis (DKA) and attacks of hypoglycaemia, [2] full clinical examination including weight (Wt), height (Ht), body mass index (BMI) and peripheral neuropathy (p. neuropathy), [3] clinical investigations including fundus examination and [4] laboratory investigations including random blood sugar (RBS) and glycated haemoglobin (HbA_{1c}). The diagnosis of T1D was based on the 2010 Clinical Practice Recommendations of the American Diabetes Association (ADA) [37]. Exclusion criteria were [1] suspected non-type 1 diabetes (type 2 diabetes, maturity-onset diabetes of the young (MODY) or secondary diabetes), [2] recent manifestations of active infection, [3] decline of enrolment into the study by patients or parents and [4] presence of other autoimmune disease.

Methods

DNA extraction

Peripheral blood samples of the patients were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) for DNA isolation. The genomic DNA was extracted from each blood sample using QIAamp DNA mini Blood kit (cat. no.51304) {Qiagen, Germany} according to the manufacturer's instructions.

PTPN22 1858C>T genotype analysis

PTPN22 1858C>T polymorphism was determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The PCR primers were: 5′-GAT AAT GTT GCT TCA ACG GAA TTT A-3′ (forward) and 5′-TCA CCA GCT TCC TCA ACC ACA-3′ (reverse) [38].

PCR assay was performed for each sample in a final reaction volume of 25 µL, using 5 µL DNA, 12.5 µL universal master mix, 1 µL PTPN22 1858C>T forward primer, 1 µL PTPN22 1858C>T reversed primer, together with 5.5 µL distilled water (DW).

The PCR conditions were as follows: 35 cycles of amplification consisting of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min [38].All reactions were done using the thermal cycler Applied Biosystems (Perkin-Elmer 9600, USA).

After amplification, the PCR products were treated with the restriction endonuclease XcmI (Fermentas Life Sciences, Cat. No. R0533S,) at 37°C overnight [38].The products were then resolved on 2% agarose gel electrophoresis containing ethidium bromide, then visualized using UV transilluminator. DNA molecular weight marker (QIAGEN GelPilot 50 bp Ladder (100) {cat no. 239025}) was used to assess the size of PCR-RFLP products. After digestion of the PCR product with XcmI, the amplicon with homozygous wild genotype CC remains uncut yielding a 218-bp band, whereas the amplicon with homozygous mutant genotype TT of PTPN22 is cleaved by XcmI yielding 174 and 44-bp fragments, while the amplicon with heterozygous mutant genotype CT yields three fragments of 218, 174 and 44-bp length (Figure 1).

Statistical methods

Data were analyzed using SPSS win statistical package version 17. Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann– Whitney test (non-parametric t-test). Odds ratio (OR) with its 95% confidence interval (CI) was used for risk estimation. A p-value < 0.05 was considered significant [39].

Results

Fifty T1D patients including 19 males (38%) and 31 females (62%) with mean age 10.8 ± 4.6 years were enrolled in our study .The control group comprised 100 healthy subjects including 39 males (39%) and 61 females (61%) with mean age 11 ± 4.2 years who were sex and age matched with patients group (P =0.906 and p= 0.823), respectively. As regards RBS (mg/dl) and HbA_{1c} (%), the mean values were significantly higher in the patients group versus the control group 228.3±81.7 mg/dl (12.7± 4.5 mmol/L)versus 104.8±9.7 mg/dl (5.8±0.5 mmol/L) and 9.8±2.7 % versus 5.5±0.3 %, respectively (p < 0.001).

The frequency of wild genotype CC of PTPN22 1858C>T gene polymorphism was statistically higher among control group (92%) versus T1D patients group (74%), while the mutant heterozygous CT and homozygous TT genotypes were found to have statistically higher frequency among T1D patients group (26%) versus control group (8%) (OR=4.041, 95%CI=1.547-10.551, p=0.003). Higher mutant T allele frequency in patients group (17%) was observed when compared with healthy control (4%), while C allele showed higher frequency among control group (96%) versus T1D patients group (83%) (OR=4.916, 95%CI=2.041-11.838, p < 0.001) (table1).

When evaluating RBS and HbA_{1c} at different cut off levels with PTPN22 genotype frequency among patients group, no statistically significant differences were found (p > 0.05) (table 2).

As regards age, duration of disease, number of DKA attacks , number of hypoglycaemic attacks ,weight , height and BMI among patients group, no statistically significant differences between mutant and wild genotypes were found (p > 0.05) (table 3). Concerning the same variables, only statistically significant difference between T and C alleles was found as regards number of DKA attacks (p= 0.018) (table 4).

When comparing PTPN22 1858C>T genotype and allele frequencies as regards diabetic complications, we found that mutant genotypes CT/TT showed statistically significant higher frequencies among T1D patients with hypoglycaemic attacks, fundus abnormalities and peripheral neuropathy (OR=8.182, 95%CI=1.000-69.747, p=0.029) (OR=18.500, 95%CI=1.817-188.389, p=0.001) and (OR=8.267, 95%CI=2.001-34.15, p=0.002), respectively (table 5). Mutant T allele showed statistically significant higher frequencies among T1D patients with fundus abnormalities and peripheral neuropathy (OR=18.667, 95%CI=4.149-83.977, p < 0.001) and (OR=7.118, 95%CI=2.303-22.002, p < 0.001), respectively (table 6).

Table 1: Frequency of PTPN22 genotypes and alleles among studied groups

Genotype	T1D Freq (%)	Control Freq (%)	OR	95% CI	P-value

Mutant TT/CT	13(26.0%)	8(8.0%)	4.041	1.547-10.551	0.003
Wild CC	37(74.0%)	92(92.0%)			
T	17.0%	4.0%	4.916	2.041-11.838	<0.001
C	83.0%	96.0%			

Table 2: PTPN22 genotypes among patients group at RBS and HbA_{1c} cut off levels

Variable	Genotype Freq (%)		OR	95% CI	P-value
	Mutant TT/CT	Wild CC			
RBS (mg/ dl)					
< 140(< 7.8 mmol/L)	2(20.0 %)	8(80 %)	1	-	-
140-< 200 vs < 140 (7.8- <11.1 vs< 7.8 mmol/L)	4(44.4 %)	5(55.6 %)	3.200	0.419-24.417	0.262
≥ 200 vs < 140 (≥ 11.1 vs < 7.8 mmol/L)	7(22.6 %)	24(77.4 %)	1.167	0.200-6.805	0.864
HbA_{1c} (%)					
< 7	1(12.5 %)	7(87.5 %)	1	-	-
≥ 7	12(28.6%)	30(71.4 %)	0.357	0.040-3.222	0.342

Table 3: statistical comparison between PTPN22 genotypes among patients group as regards different studied variables

Variable	Genotype		P-value
	Mutant TT/CT	Wild CC	
Age (years)			
Mean± SD	10.5± 5	10.9±4.5	0.859
Median	10 (2-18)	11 (2-18)	
Duration (months)			
Mean± SD	57.1± 63.5	28.5± 21.9	0.334
Median	36 (1-192)	24 (1-84)	
No of DKA attacks			
Mean± SD	1.5± 0.9	1.2± 1.2	0.106
Median	1 (0-3)	1 (0-6)	
No of hypoglycaemic attacks			
Mean± SD	2.1± 1.5	1.5± 1.5	0.220
Median	2 (0-5)	2 (0-5)	
Wt (kg)			
Mean± SD	37.8± 15.4	35.5± 15.2	0.506
Median	36 (13-58)	33 (11-67)	
Ht (cm)			
Mean± SD	135.4± 26	136.1± 24.4	0.912

BMI (kg/m^2) Mean± SD	19.9± 4.2	18.4 ± 2.9	0.314

Table 4: statistical comparison between PTPN22 alleles among patients group as regards different studied variables

Variable	Allele		P-value
	T	C	
Age (years) Mean± SD Median	 10.6± 5.1 10 (2-18)	 10.9 ± 4.5 11 (2-18)	0.883
Duration (months) Mean± SD Median	 54.3± 58.7 36 (1-192)	 32.1± 32.2 24 (1-192)	0.302
No of DKA attacks Mean± SD Median	 1.6± 0.9 2 (0-3)	 1.3± 1.1 1 (0-6)	0.018
No of hypoglycemic attacks Mean± SD Median	 2.3± 1.7 2 (0-5)	 1.5± 1.5 2 (0-5)	0.089
Wt (kg) Mean± SD Median	 38.6± 16 36 (13-58)	 35.6± 14.9 36 (11-67)	0.349
Ht (cm) Mean± SD	 135.2± 26.6	 136.1 ± 24.2	0.847
BMI (kg/m^2) Mean± SD	 20.4± 4.7	 18.5 ± 2.9	0.149

Table 5: PTPN22 genotypes among patients group as regards diabetic complications

Variable	Genotype freq(%)		OR	95% CI	P-value
	Mutant TT/CT	Wild CC			
DKA YES NO	 12(27.9 %) 1(14.3 %)	 31(72.1%) 6(85.7 %)	2.323	0.252-21.372	0.446
Hypoglycaemia YES NO	 12(35.3 %) 1(6.3 %)	 22(64.7 %) 15(93.7 %)	8.182	1-69.747	0.029
Fundus abnormalities YES NO	 5(100.0 %) 8(17.8 %)	 0(0.0 %) 37(82.2 %)	18.500	1.817-188.389	0.001
P.neuropathy YES NO	 8(57.1 %) 5(13.9 %)	 6(42.9 %) 31(86.1 %)	8.267	2.001-34.155	0.002

Table 6: PTPN22 alleles among the patients group as regards diabetic complications

Variable	Allele %		OR	95% CI	P-value
	T	C			
DKA YES NO	 18.6 % 7.1 %	 81.4 % 92.1 %	2.971	0.362-24.392	0.290
Hypoglycaemia YES NO	 22.1 % 6.3 %	 77.9 % 93.7 %	4.245	0.908-19.840	0.050
Fundus abnormalities YES NO	 70.0 % 11.1 %	 30.0 % 88.9 %	18.667	4.149-83.977	<0.001

P.neuropathy YES NO	 39.3 % 8.3 %	 60.7 % 91.7 %	7.118	2.303-22.002	<0.001

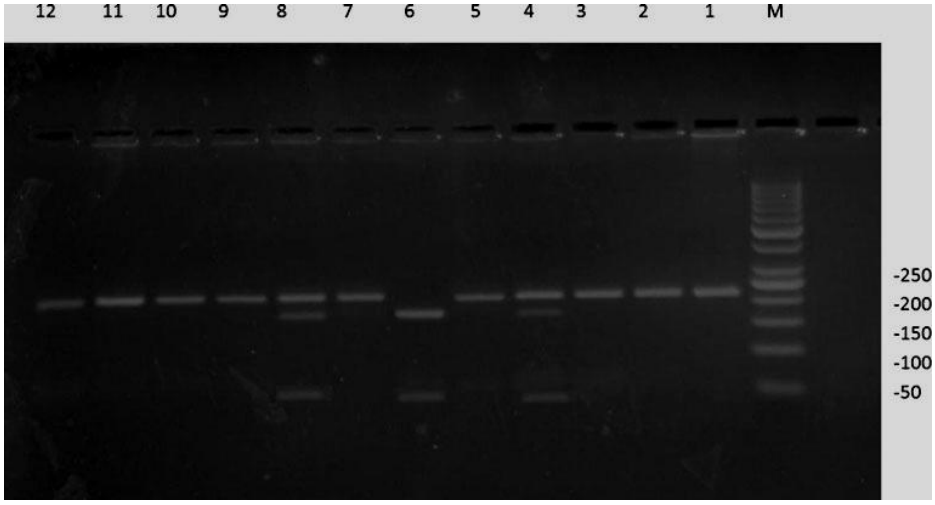

Figure 1: PCR-RFLP analysis of PTPN22 (1858C>T) gene polymorphism using XcmI restriction enzyme analysis by agarose gel electrophoresis:
M: DNA molecular weight marker (50–500 bp)
Lanes 1, 2, 3, 5, 7, 9, 10, 11, 12: homozygous wild genotype CC represented by one band at 218 bp.
Lanes 4 and 8: heterozygous mutant genotype CT represented by three bands at 218, 174 and 44 bp.
Lane 6: homozygous mutant genotype TT showing two bands at 174 and 44 bp.

Discussion

An increasing amount of investigations has associated the presence of the 1858C>T polymorphism in the PTPN22 gene to the onset of several autoimmune diseases including T1D. Kyogoku et al. (2004) [23] proposed that the PTPN22 1858C>T gene polymorphism may predispose individuals to autoimmune diseases by facilitating the generation of certain disease-associated autoantibodies, thereby contributing to disease progression. Hermann et al. (2006) [40] showed evidence that the PTPN22 1858C>T variant regulates T1D specific autoimmunity and strongly affects the progression from preclinical to clinical diabetes.

In our study, PTPN22 1858C>T gene polymorphism was compared among 50 Egyptian children with T1D versus 100 normal age and sex matched healthy controls. We found that the frequency of CC wild genotype and C allele were statistically higher among control group versus T1D patients group, while the CT/TT mutant genotypes and T allele were found to have statistically higher frequency among T1D patients group versus control group.

These findings are in agreement with those found by *Bottini et al. (2004)* [11], *Maier and Wicker (2005)* [41], Kahles et al. (2005) [42], Zheng and She (2005) [43], Onengut-Gumuscu et al. (2006) [2], Chelala et al. (2007) [44], Santiago et al. (2007) [45], Zoledziewska et al. (2008) [46], D'Silva et al. (2008) [47] and Zhebrun et al. (2011) [9]. Giza et al. 2013 [48] reported that mutant CT/TT genotypes as well as T allele were found more frequently in T1D patients than in healthy individuals but at non statistically significant level. Almasi et al. (2014) [49] reported that T allele frequency

was significantly increased in T1D patients with Hashimoto's thyroiditis compared with T1D only indicating that T1D carriers of the T1858 allele could be at enhanced risk for other comorbid autoimmune disorders.

The present study revealed absence of association between PTPN22 1858C>T gene polymorphism and glycaemic control as assessed by HbA_{1c}. Bottini and co-workers (2006) [50] suggested the use of *PTPN22* SNP as a prognostic factor for disease severity and variability in autoimmune diseases, but also requested further studies taking this point under investigation. Petrone et al. (2008) [6] found an association between the T allele of PTPN22 1858C>T and low fasting C-peptide (as a surrogate marker of residual beta-cell mass) and poorer glycemic control (HbA_{1c}). Nielsen et al. (2011) [51] revealed no significant *PTPN22* genotype differences in clinical and demographic data at diabetic disease onset. The authors did not find any statistically significant difference between carriers of the *PTPN22* variant and glycaemic control (HbA_{1c}).

Although our study provides another replication of the association of the PTPN22 1858C>T gene polymorphism with T1D, to our knowledge this studied genetic polymorphism was not investigated with diabetic complications so far. Our study analyzed PTPN22 1858C>T gene polymorphism with T1D and its association with diabetic complications in Egyptian pediatric patients. Statistically significant difference between T and C alleles was found as regards number of DKA attacks. Mutant genotypes CT/TT showed statistically significant higher frequencies among T1D patients with hypoglycaemic attacks, fundus abnormalities and peripheral neuropathy. Mutant T allele showed statistically significant higher frequency among T1D patients with fundus abnormalities and peripheral neuropathy.

Several academic laboratories have begun small molecule inhibitors of LYP as a treatment of autoimmunity by reverting the gain-of-function of LYP-W620 and restoring normal levels of TCR signalling in autoimmune patients carrying the 1858T allele [50].Gianchecchi et al. (2013) [52] highlighted the pathogenic significance of the 1858C>T PTPN22 polymorphism in human autoimmunity with special reference to T1D. The authers reported that the genetic variation in PTPN22 was shown to induce altered function of T and B-lymphocytes and speculated on the possible development of novel therapeutic treatments in human autoimmunity aiming to selectively target the variant LYP protein in autoreactive T and B lymphocytes.

In conclusion, our study revealed that PTPN22 1858C>T gene polymorphism may be considered as a genetic risk factor in T1D and diabetic complications in Egyptian paediatric patients.

Further studies investigating PTPN22 1858C>T gene polymorphism with diabetic complications on larger populations are recommended. Also, further studies with sufficient information as regards the effect of the studied genetic polymorphism in the response to therapy are recommended.

Conflicts of interest: The authors have no conflicts of interest to declare. The authors alone are responsible for the content and writing of the paper. The authors did not receive any funds from any source.

Ethical considerations: All patients and parents of children patients or healthy controls included in this study gave their informed consent and approval upon participating in the study. Neither patients' names nor photos were included in this study.

References

1. Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes K, Plagnol V, Bailey R, Nejentsev S. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat Genet 2007; 39:857-864.

2. Onengut-Gumuscu S, Buckner J, Concannon P. A Haplotype-Based Analysis of thePTPN22 Locus in Type 1 Diabetes. Diabetes 2006*;* 55(10):2883-2889.

3. Nielsen LB, Mortensen HB, Chiarelli F, Holl R, Swift P, de Beaufort C, Pociot F, Hougaard P, Gammeltoft S, Knip M, Hansen L, Hvidoere Study Group. Impact of IDDM2 on disease pathogenesis and progression in children with newly diagnosed type 1 diabetes: reduced insulin antibody titres and preserved beta cell function. Diabetologia 2006; 49:71-74.

4. Pörksen S, Nielsen LB, Mortensen HB, Danne T, Kocova M, Castaño L, Pociot F, Hougaard P, Ekstrøm CT, Gammeltoft S, Knip M, Hansen L, Hvidøre Study Group on Childhood Diabetes. Variation within the PPARG gene is associated with residual beta-cell function and glycemic control in children and adolescents during the first year of clinical type 1 diabetes. Pediatr Diabetes 2008*;* 9:297-302.

5. Nielsen LB, Ploug KB, Swift P, Ørskov C, Jansen-Olesen I, Chiarelli F, Holst JJ, Hougaard P, Pörksen S, Holl R, Beaufort C, Gammeltoft S, Rorsman P, Mortensen HB, Hansen L, on behalf of the Hvidøre Study Group. Co-localization of the Kir6.2/SUR1 channel complex with glucagon-like peptide-1 and glucose-

dependent insulinotrophic polypeptide expression in human ileal cells and implications for glycaemic control in new onset type 1 diabetes. European Journal of Endocrinology 2007; 156:663-671.
6. Petrone A, Spoletini M, Zampetti S, Capizzi M, Zavarella S, Osborn J, Pozzilli P, Buzzetti R, Immunotherapy Diabetes (IMDIAB) Group. The PTPN22 1858T gene variant in type 1 diabetes is associated with reduced residual beta-cell function and worse metabolic control. Diabetes Care 2008; 31:1214-1218.
7. Briggs FB, Ramsay PP, Madden E. Supervised machine learning and logistic regression identifies novel epistatic risk factors with PTPN22 for rheumatoid arthritis. Genes Immun 2010; 11(3):199–208.
8. Lea WW, Lee YH. The association between the PTPN22 C1858T polymorphism and systemic lupus erythematosus: a meta-analysis update. Lupus 2011; 20(1):51–57.
9. Zhebrun D, Kudryashova Y, Babenko A, Maslyansky A, Kunitskaya N, Popcova D. Association of PTPN22 1858T/T genotype with type 1 diabetes, Graves' disease but not with rheumatoid arthritis in Russian population. Aging 2011; 3 (4):368-373.
10. Tonks NK. Protein tyrosine phosphatases: from genes, to function, to disease. Nat Rev Mol Cell Biol *2006*; 7:833-846.
11. Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet 2004; *36*:*337*–338.
12. Siminovitch KA. PTPN22 and autoimmune disease. Nature Genetics 2004; 36 1248–*1249*.
13. Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. American Journal of Human Genetics 2004*;* 75:330–337.
14. Criswell LA, Pfeiffer KA, Lum RF, Gonzales B, Novitzke J, Kern M, Moser KL, Begovich AB, Carlton VE, Li W, Lee AT, Ortmann W, Behrens TW, Gregersen PK. Analysis of families in the multiple autoimmune disease genetics consortium (MADGC) collection: the PTPN22 620W allele associates with multiple autoimmune phenotypes. Am J Hum Genet 2005; 76:561 –*571*.
15. Lee YH, Rho YH, Choi SJ, Ji JD, Ji JD, Song GG, Nath SK, Harley JB. The PTPN22 C1858T functional polymorphism and autoimmune diseases–a meta-analysis. Rheumatology 2006*; 7*:*7*.
16. Gregersen PK, Lee HS, Batliwalla F, Begovich AB. PTPN22: setting thresholds for autoimmunity. Semin Immunol 2006*;* 18*:* 214 –223.
17. Chung SA, Criswell LA. PTPN22: its role in SLE and autoimmunity. Autoimmunity 2007; 40:582-590.
18. Vang T, Miletic AV, Arimura Y, Tautz L, Rickert RC, Mustelin T. Protein tyrosine phosphatases in autoimmunity. Annu Rev Immunol 2008; 26:29-55.
19. Carlton VE, Hu X, Chokkalingam AP, Schrodi SJ, Brandon R, Alexander HC, Chang M, Catanese JJ, Leong DU, Ardlie KG, Kastner DL, Seldin MF, Criswell LA . PTPN22 genetic variation: evidence for multiple variants associated with rheumatoid arthritis. American journal of human genetics 2005; 77:567-81.
20. Viken MK, Amundsen SS, Kvien TK, Boberg KM, Gilboe IM, Lilleby V, Sollid LM, Forre OT, Thorsby E, Smerdel A, Lie BA. Association analysis of the 1858C>T polymorphism in the PTPN22 gene in juvenile idiopathic arthritis and other autoimmune diseases. Genes and immunity 2005; 6:271-3.
21. Pierer M, Kaltenhauser S, Arnold S, Wahle M, Baerwald C, Hantzschel H, Wagner U. Association of PTPN22 1858 single-nucleotide polymorphism with rheumatoid arthritis in a German cohort: higher frequency of the risk allele in male compared to female patients. Arthritis research & therapy 2006; 8: R75.
22. Wesoly J, Hu X, Thabet MM, Chang M, Uh H, Allaart CF, Toes RE, Houwing-Duistermaat JJ, Begovich AB, Huizinga TW. The 620W allele is the PTPN22 genetic variant conferring susceptibility to RA in a Dutch population. Rheumatology 2007; 46:617-21.
23. Kyogoku C, Langefeld CD, Ortmann WA, Lee A, Selby S, Carlton VE, Chang M, Ramos P, Baechler EC, Batliwalla FM, Novitzke J, Williams AH, Gillett C. Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. American journal of human genetics 2004; 75:504-7.
24. Pradhan V, Borse V, Ghosh K. PTPN22 gene polymorphisms in autoimmune diseases with special reference to systemic lupus erythematosus disease susceptibility. Journal of postgraduate medicine 2010; 56:239-42.
25. Smyth D, Cooper JD, Collins JE, Heward JM, Franklyn JA, Howson JM, Vella A, Nutland S, Rance HE, Maier L, Barratt BJ, Guja C, Ionescu-Tirgoviste C. Replication of an association between the lymphoid tyrosine phosphotase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. Diabetes 2004; 53:3020 –3023.

26. Kawasaki E, Awata T, Ikegami H, Kobayashi T, Maruyama T, Nakanishi K, Shimada A, Uga M, Kurihara S, Kawabata Y, Tanaka S, Kanazawa Y, Lee I. Systematic search for single nucleotide polymorphisms in a lymphoid tyrosine phosphatase gene (PTPN22): association between a promoter polymorphism and type 1 diabetes in Asian populations. American journal of medical genetics 2006; 140:586-93.
27. Velaga MR, Wilson V, Jennings CE, Owen CJ, Herington S, Donaldson PT, Ball SG, James RA, Quinton R, Perros P, Pearce SH. The codon 620 tryptophan allele of the lymphoid tyrosine phosphatase (LYP) gene is a major determinant of Graves' disease. The Journal of clinical endocrinology and metabolism 2004; 89:5862-5.
28. Jagiello P, Aries P, Arning L, Wagenleiter SE, Csernok E, Hellmich B, Gross WL, Epplen JT. The PTPN22 620W allele is a risk factor for Wegener's granulomatosis. Arthritis and rheumatism 2005; 52:4039-43.
29. Wu H, Cantor RM, Graham DS, Lingren CM, Farwell L, Jager PL, Bottini N, Grossman JM, Wallace DJ, Hahn BH, Julkunen H, Hebert LA, Rovin BH. Association analysis of the R620W polymorphism of protein tyrosine phosphatase PTPN22 in systemic lupus erythematosus families: increased T allele frequency in systemic lupus erythematosus patients with autoimmune thyroid disease. Arthritis and rheumatism 2005; 52:2396-402.
30. Ikari K, Momohara S, Inoue E, Tomatsu T, Hara M, Yamanaka H, Kamatani N. Haplotype analysis revealed no association between the PTPN22 gene and RA in a Japanese population. Rheumatology 2006; 45: 1345-8.
31. Begovich AB, Caillier SJ, Alexander HC, Penko JM, Hauser SL, Barcellos LF, Oksenberg JR. The R620W polymorphism of the protein tyrosine phosphatase PTPN22 is not associated with multiple sclerosis. American journal of human genetics 2005; 76:184-7.
32. Hinks A, Barton A, John S, Bruce I, Hawkins C, Griffiths CE, Donn R, Thomson W, Silman A, Worthington J. Association between the PTPN22 gene and rheumatoid arthritis and juvenile idiopathic arthritis in a UK population: further support that PTPN22 is an autoimmunity gene. Arthritis and rheumatism 2005; 52:1694-9.
33. van Oene M, Wintle RF, Liu X, Yazdanpanah M, Gu X, Newman B, Kwan A, Johnson B, Owen J, Greer W, Mosher D, Maksymowych W, Keystone E. Association of the lymphoid tyrosine phosphatase R620W variant with rheumatoid arthritis, but not Crohn's disease, in Canadian populations. Arthritis and rheumatism 2005; 52:1993-8.
34. Wagenleiter SE, Klein W, Griga T, Schmiegel W, Epplen JT, Jagiello P. A case-control study of tyrosine phosphatase (PTPN22) confirms the lack of association with Crohn's disease. International journal of immunogenetics 2005; 32:323-4.
35. Martin MC, Oliver J, Urcelay E, Orozco G, Gomez-Garcia M, Lopez-Nevot MA, Pinero A, Brieva JA, de la Concha EG, Nieto A, Martin J. The functional genetic variation in the PTPN22 gene has a negligible effect on the susceptibility to develop inflammatory bowel disease. Tissue antigens 2005; 66:314-7.
36. World Medical Association. Declaration of Helsinki: ethical principles for medical research involving human subjects. The 59th WMA General Assembly, Seoul, South Korea; 2008.
37. American Diabetes Association (ADA). Diagnosis and Classification of Diabetes Mellitus, Diabetes Care. 33:S62–S69; 2010.
38. Canto'n I, Akhtar S, Gavalas NG. A single-nucleotide polymorphism in the gene encoding lymphoid protein tyrosine phosphatase (PTPN22) confers susceptibility to generalised vitiligo. Genes Immun 2005; 6:584–587.
39. Dawson B, Trapp RG. Basic and clinical biostatistics, 3rd edn. McGraw-Hill, New York; 2001.
40. Hermann R, Lipponen K, Kiviniemi M, Kakko T, Veijola R, Simell O, Knip M, Ilonen J. Lymphoid tyrosine phosphatase (LYP/PTPN22) Arg620Trp variant regulates insulin autoimmunity and progression to type 1 diabetes. Diabetologia 2006; 49:1198 –1208.
41. Maier LM, Wicker LS. Genetic susceptibility to type 1 diabetes, Curr Opin Immunol *2005;* 17:601 *–608.*
42. Kahles H, Ramos-Lopez E, Lange B, Zwermann O, Reincke M , Badenhoop K. 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 2005; 153:895-899 .
43. Zheng W, She J. Genetic Association Between a Lymphoid Tyrosine Phosphatase (PTPN22) and Type 1 Diabetes. Diabetes 2005; 54*(*3):906-908.
44. Chelala C, Duchatelet S, Joffret M, Chelala C, Duchatelet S, Joffret M,Bergholdt R, Dubois-Laforgue D, Ghandil P, Pociot F, Caillat-Zucman S, Timsit J and Julier C. PTPN22 R620W Functional Variant in Type 1 Diabetes and Autoimmunity Related Traits. Diabetes 2007; 56*(*2):522-526.
45. Santiago J, Martínez A, Fernández-Arquero M, Figueredo A, Concha E, Urcelay E. Susceptibility to type 1 diabetes conferred by the *PTPN22*C1858T polymorphism in the Spanish population. BMC Medical Genetics 2007; 8:54.
46. Zoledziewska M, Perra C, Orru`V , Moi L, Frongia P, Congia M, Bottini N, Cucca F. Further Evidence of a Primary, Causal Association of the PTPN22 620W Variant With Type 1 Diabetes. Diabetes 2008; 57:229–234.

47. D'Silva KJ, Zamora MB, Gerlach J, Schwartz KA. PTPN22 mutation is present in an increased proportion of ITP patients. Blood 2008; 112.
48. Giza S, Goulas A, Gbandi E, Effraimidou S, Papadopoulou-Alataki E, Eboriadou M, Galli-Tsinopoulou A. The role of PTPN22 C1858T gene polymorphism in diabetes mellitus type 1: first evaluation in Greek children and adolescents. Biomed Res Int 2013; ID 721604, 6 pages.

49. Almasi S, Aliparasti MR, Yazdchi-Marandi L, Aliasgarzadeh A, Sioofy-Khojine A, Mesri A, Zamani F. Analysis of PTPN22 C1858T gene polymorphism in cases with type 1 diabetes of Azerbaijan, Northwest Iran. Cell Immunol 2014 ;292(1-2):14-8.
50. Bottini N, Vang T, Cucca F, Mustelin T. Role of PTPN22 in type 1 diabetes and other autoimmune diseases. Semin Immunol 2006; 18:207-213.
51. Nielsen L, Pörksen S, Andersen M, Fredheim S, Svensson J, Hougaard P, Vanelli M, Aman J, Mortensen H, Hansen L. The PTPN22 C1858T gene variant is associated with proinsulin in new-onset type 1 diabetes. BMC Medical Genetics 2011; 12:41.

52. Gianchecchi E, Palombi M, Fierabracci A. The putative role of the C1858T polymorphism of protein tyrosine phosphatase PTPN22 gene in autoimmunity. Autoimmun Rev. 2013; 12(7):717-25.