The Role of Protein Tyrosine Phosphatase Nonreceptor Type 22 Gene polymorphism in Patients With Type 1 Diabetes Mellitus.

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الخلاصة:

مرض السكر النوع الاول(T1DM) هو مرض المناعة الذاتية الذي يتميز با لتدمير التدريجي لخلايا بيتا في البنكرياس بسبب عوامل وراثية وبيئية مما يؤدي الى الاعتماد المطلق على الانسولين للبقاء على قيد الحياة والمحافظة على الصحة . هناك جين جديد نسبيا من مرض السكر هو(*PTPN2*) الذي يشفر الى بروتين التايروسين فوسفاتيز اللمفاوي (LYP) .الهدف من هذه الدراسة هو التحقيق فيما اذا كان التطور المظهري (C1858T)،(T1858T) متضمنا في امراضية السكري النوع الاول. أجرتْ هذه الدراسةِ على (64) مريض (37 ذكور،27 أناث) مصاب بمرض الداء السكري النوع الاول ، تتراوح اعمارهم من (1-18) سَنَة، مع (25) شخص معافى (13 أنثى و12 ذكورٍ) كمجموعة تحكم، تم مشاهدتها في مستشفى الاطفال في كربلاء من كانون الأولِ 2012 إلى كانون الثّاني 2013. عينات الدم جمعت من كلتا المجموعتين، الحمض النووي استخرج من الكريات البيض للكشف عن وجود أي ارتباطات بين تعدد الإشكال الجينية ل *PTPN22* واستعداد الإصابة بمرض السكري النوع الاول بواسطة تفاعلات البلمرة المتسلسله وتقنية الأجزاء المتكسرة المتعدد باستخدام إنزيم Rsa1 والتي تعطي أجزاء ذات حجوم جزيئيه مختلفة تعبر عن تراكيب جينية معينة. أظهرت نتائج الدراسة ان (36%) من المرضى هم ضمن الفئة العمرية (11-15) سنة، وكان 32.8% من المرضى ضمن الفئة العمرية (6-10) سنة ،21.8% من المرضى كانوا في الفئة العمرية اقل اويساوي 16 سنة، ومشاهدة 9.4% في الفئة العمرية اقل من 6 سنوات .كذلك كشفت النتائج ان 57.81 % من المرضى الذكور ولم يلاحظ ارتباط ذو اهمية احصائية مع الجنس والعمربين المرضى والاصحاء (قيمة (p) =0.170، 0.274 على التوالي).

ABSTRACT

Background: Type 1 diabetes mellitus (T1DM) is an autoimmune multifactorial disease characterized by progressive destruction of pancreatic beta cells by genetic and environmental factors which leads to an absolute dependence of insulin for survival and maintenance of health. **Materials and methods:** One polymorphic sites of *PTPN22* gene was genotyped in 64 patients with T1DM, and 25 healthy controls. Genotypes were determined by the polymerase chain restriction fragment length polymorphism (PCR-RFLP) method. **Results:** The single nucleotide polymorphisms T/T, of the *PTPN22* gene may be participate in the susceptibility of T1DM in the Iraqi population. **Conclusion:** Although *PTPN22*-T/T polymorphism not associated with T1DM in many population, our study confirmed significant correlation between *PTPN22* and T1DM.

Keywords: T1DM; Genetic; Protein tyrosine phosphatase nonreceptor type 22; Polymorphism; Genotype; Allele.

Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by the targeted destruction of the insulin secreting β-cells within the pancreatic islet . **(1).** It is due to pancreatic islet B-lymphocyte cell (*β*-cell) destruction and thus "insulin is required for survival, to prevent the development of ketoacidosis, coma and death **(2)**. This form of diabetes, accounts for only 5–10% of those with diabetes. Previously, it was encompassed by the terms insulin-dependent diabetes, type I diabetes, or juvenile-onset diabetes **(3).** Although the exact pathogenesis of T1DM is unknown, several gene loci involved in disease outbreak have been identified. Among these, genes of human leukocyte antigen (*HLA*) class II, insulin (*INS*), cytotoxic T lymphocyte antigen-4 (*CTLA-4*), and protein tyrosine phosphatase nonreceptor type 22 (*PTPN22*) play a key role **(4).** Protein tyrosine phosphatise non receptor 22 *(PTPN22)* is located on chromosome 1p13.3–13.1 and encodes a 807-amino acid residue protein referred to as the lymphoid tyrosine phosphatise (LYP).

The C1858T polymorphism in *PTPN22*(rs2476601) has been associated

with the risk of T1DM as well as the risk of multiple other autoimmune diseases, including autoimmune thyroid disease, juvenile idiopathic arthritis, rheumatoid arthritis, and systemic lupus erythematosus (SLE). **(5).**The mechanism by which the risk variant of *PTPN22* predisposes to autoimmunity is unknown. Asingle nucleotide polymorphism (SNP) at position 1858 (rs2476601) of the encoding sequence of *PTPN22* gene, consisting of the substitution of cytosine by thymine, results in mutation of arginine to tryptophan at codon 620 of Lyp protein, and work involving lymphocytes suggests that the mutation results in decreased T cell and B cell responsiveness as well as alterations in cytokine production in lymphocytes in vitro**(6).** The same allelic variant mediates risk in several other autoimmune diseases, suggesting the involvement of a crucial signaling axis **(7).** Indeed, the LYP protein is an important negative regulator of T-lymphocyte cell (T-cell) receptor signaling by way of de- phosphorylation of Src family kinases Lck and Fyn, ITAMs of the TCR_/CD3 complex, as well as ZAP-70, Vav, valosin containing protein, and other key signaling molecules **(8).**Explanations for the mechanism are contradicting. A loss-of function mutation can cause a lower threshold for autoreactive T-cell activation in the periphery. In contrast, a gain-of-function mutation that suppresses TCR signaling during thymic development can allow autoreactive T cells to escape negative selection **(9).** An association between type 1diabetes mellitusand a single nucleotide polymorphism in the *PTPN22* gene (C1858T) has been described by *Bottini* and his co-workers. Lymphoid-specific phosphatase encoded by *PTPN22* is known to be one of the strongest inhibitors of T-cell activation, and a mutation has been shown to reduce the binding affinity of the molecule to its substrate and thereby weaken the inhibitory effect on T-cell activation, possibly conferring susceptibility to T1DM **(10)** .

Materials and methods

Subjects. The current study was conducted on 64 patients (37 males, 27 females) were seen in Al-Diwaniya Teaching Hospital and Maternity and Child Teaching Hospital from December 2012 to January 2013.The patients were diagnosed clinically by physician as having T1DM.Patients were interviewed directly by using an anonymous questionnaire from which covered age, sex. Another group consist of 25 apparently healthy individuals (12 male and 13 female) without any history of systemic disease were clinically considered as healthy also included in this study as a control group.

DNA Extraction and Genotyping. Genomic DNA was extracted according to the manufacturer's protocol from 5 ml of frozen whole blood using a DNA Extraction Kit (Geneaid / USA). The polymorphic region was amplified by PCR. Amplification reaction were preformed in 0.2 ml tube of Accu Power PCR Premix tube according to the SolGent corporation then the thermocycling condition for this reaction carried out and products analyzed by 1% agarose gel electrophoresis. PCR products were digested overnight with restriction enzymes (Rsa1), according to the manufacturer's protocol, and analyzed by 2% agarose gel electrophoresis.

Ras1 recognized its target sequence only when the *PTPN22* 1858c allele is present. The 1858T allele is not digested and yields one fragment of 218bp, while 1858 C allele 1s digested and yields two fragments of 176bp and 46bp**.(11)**

Statistical analysis. Statistical analyses were done using SPSS version 20 computer software (Statistical Package for Social Sciences) in association with Microsoft Excel 2010. Odd ratio was used to measure the strength of association between 2 categorical variables and the statistical significance of the measured odd ratio is assessed by a special χ^2 formula. Deviations from Hardy-Weinberg equilibrium were investigated for all polymorphisms using the $\chi 2$ statistic, with expected frequencies derived from allele frequencies. An estimate was considered

statistically significant if its P value was less than an α level of significance of 0.05.

Results

Twenty five control subjects and 64 individuals suffering from T1DM were recruited and genotyped for *PTPN22-C1858T* polymorphism. Tables 1 and 2 show the case-control difference in mean of age and gender distribution. Genotyping analyses revealed (CT-TT) SNP in the 3′ region of *PTPN22*, figure (1), being distributed in Hardy-Weinberg equilibrium.

Table (1): The case-control difference in mean age

	Healthy controls	Cases (T1DM)	P
Age (years)			**0.573^{NS}**
Range	**(2.5-18)**	**(1.5-18)**	
Mean	**12.24**	**11.61**	
SD	**4.19**	**4.38**	
SE	**0.84**	**0.55**	
N	**25**	**64**	

❖ **NS= No Significant, SD= Standard Deviation, SE= Standard Error, N= Number**

Table (2) : The case-control difference in gender distribution.

		Case-control comparison					
		Healthy controls (n=25)		**Cases (T1DM) (n=64)**			
Gender type		N	%	N	%	Total	P
	Female	13	52	27	42.19	40	0.274 [NS
	Male	12	48	37	57.81	49	
Total		25	100	64	100	89	

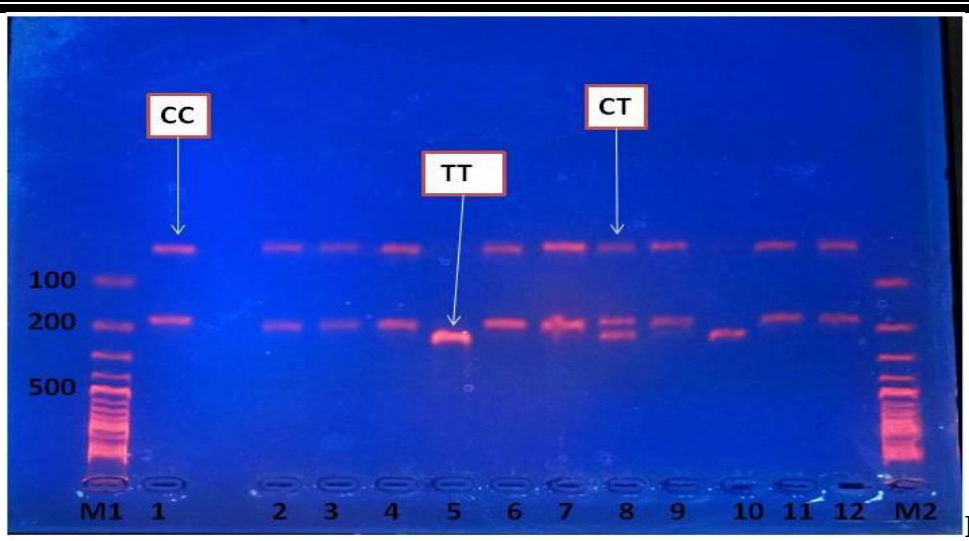

Figure (1):Ethidium bromide-stained agarose gel of PCR – RFLP amplified 218bp of PTPN22 gene for study groups .Lane (M1,M2): DNA molecular size marker (KAPA Universal Ladder) , Lane 1,2,3,4,6,7,9,11,12= CC genotype(176/46 bp), Lane 5,10 =TT genotype (218 bp), Lane 8 =CT genotype (176/46/218 bp).

Distributions of genotypes and alleles in cases and controls groups.

Table (3) showed The genotype distribution of the *PTPN22* C1858T variant among the 64 T1DM patients from Iraq population were as follow; CC 42 (65.62%), CT 8(12.50%) & TT 14 (221.88%) was in Hardy-Weinberg equilibrium and the genotype distribution of PTPN22 variant among the 25 healthy control were CC 17 (68%), CT 7 (28%) & TT 1 (4%) ,table (4-6).

Table (3):**The Case-Control Difference in Relative Frequency (Rate of Positive) of PTPN22 Genotype .**

	Case-control comparison						
	Healthy controls (n=25)		Cases (DM1) (n=64)				
PTPN 22 gene	N	%	N	%	P	OR	95% CI OR
CC	17	68	42	65.62	0.516^{NS}	0.898	(0.335-2.408)
CT	7	28	8	12.5	0.079^{NS}	0.367	(0.117-1.154)
TT	1	4	14	21.88	0.036*	6.72	(0.834-54.131)

❖ **OR=odd ratio, p= p value**

The 1858T allele was detected more frequently in patients (28.18%) compared to controls (18%) but with no statistically significant level (*P value* 0.113, odds ratio, OR= 1.783, and CI = (0.787-4.039) , table (4).

Table (4): The Case-Control Difference in Relative Frequency (Rate of Positive) of PTPN22 Allele .

	Case-control comparison						
	Healthy controls (n=25)		Cases (DM1) (n=64)				
PTPN 22 gene	N	%	N	%	P	OR	95% CI OR
					0.113		
C allele	41	82	92	71.82		0.640	(0.333-1.230)
T allele	9	18	36	28.18		1.783	(0.787-4.039)

Genotypes Distribution of TIDM and Control Subjects according to Gender

There was a trend towards higher frequency of C1858T and T1858T genotypes in males were (13.51%, 24.32%) respectively versus (11.11%, 18.52%) respectively in females in patient group , while in the control group the frequency of C1858T was (30.77%) in male versus (25%) in female and the frequency of T1858T was (8.33%) in male versus (0%) in female. However, the distribution of C1858T and T1858T genotypes carrying the predisposing allele did not differ significantly between males and females in the patient group when compared with the control groups, table (4-8).

Table (5):Genotypic and Allelic Distribution of PTPN 22 among Study Groups according to the Gender

PTPN 22 Genotype	Gender type			
	Female		Male	
	Control	Patients	Control	Patients
CC	9 (69.23%)	19 (70.37%)	8 (66.67%)	23 (62.16%)
CT	4 (30.77%)	3 (11.11%)	3 (25.00%)	5 (13.51%)
TT	0 (0%)	5 (18.52%)	1 (8.33%)	9 (24.32%)
p value	0.114 NS		0.387 NS	
PTPN22 Allele frequency				
C	22 (91.67%)	41 (75.93%)	19 (79.17%)	51 (68.92%)
T	4 (16.67%)	13 (24.07%)	5 (20.83%)	23 (31.08)
P value	0.374 NS		0.334 NS	

Discussion

In this case–control study, we found (tables 1 and 2) the highest frequency of T1DM among young patients particularly from age (11-15) years old (36%), followed by the age group of (6-10)years old (32.8%) The variation in the age incidence reflects the interaction of both genetic and environmental factors in different social, racial, and geographical areas in the world as well as hormonal changes during adolescence **(12).** The higher prevalence rate of T1DM among males (57.81%) than females (42.19%) within the patient's group but without significant differeance Prevalence rate of T1DM among males than females could be related to Major Histo- compatibility Complex (MHC) genes as there are two main T1DM susceptibility haplotypes, HLA-DR3 and -DR4, the bias in male incidence is largely restricted to the DR3/X category of patients, (X ≠DR4) compared with a lesser ratio in the DR4/Y category, (Y ≠DR3) **(13) .** Table (3) showed the significant association of T1858T SNP of *PTPN22* gene with the patient T1DM in our population. The study found that the homozygous mutant genotype(TT) frequencies of SNP had significant association with T1DM, this may act for increase the risk of T1DM**(14; 15; 16; 17; 18; 19).** In the present study population, although increased 1858T allele frequency was confirmed in patients with T1DM, the result was not statistically significant. However, OR of 1858T versus 1858C allele in patients compared to controls was similar to that of other studies **(20; 21).** Our observations suggest that the susceptibility to the T1DM is increased in presence of T allele of *PTPN22* could be related to failure to delete auto reactive T cell during intra-thymic selection **(22)**, or due to the *1858T* variant may contribute to autoimmunity via B cells. The B cell from these subjects carry the *1858T* variant have a decreased ability to respond to stimulation via the BCR. Although B cell differentiation and proliferation are influenced by T cell function, the association of the *1858T* variant with a diminished response to BCR stimulation indicates that B cell intrinsic processes are directly altered by the *1858T* variant. A diminution of the BCR signal could result in the escape of autoreactive B cells into the periphery and, in addition, the relative decrease in memory B cells seen in subjects with the *1858T* variant may be the result of a shunting of B cell into the plasma cell pool. In each case, these changes could enhance the subsequent development of autoantibodies and is notable in light of the fact that the diseases associated with this

variant have the production of autoantibodies as a common characteristic **(23).**

Table (5) observed a trend towards higher frequency of C1858T and T1858T genotypes in males were (13.51%, 24.32%) respectively versus (11.11%, 18.52%) respectively in females in patient group , while in the control group the frequency of C1858T was (30.77%) in male versus (25%) in female and the frequency of T1858T was (8.33%) in male versus (0%) in female. but did not differ significantly between males and females in the patient group when compared with the control group**.(24)** evaluated the distribution of 1858 C/T genotypes on the basis of sex in 1600 T1D subjects and about 2000 families ,absolutely no effect of patient sex was observed in the large family-based cohort from Great Britain, Northern Ireland, USA and Romania In regard to the relationship of PTPN22 C1858T polymorphism with T1DM, some studies have suggested a gender differentiation in prevalence in favour of females **(25; 26)**, and another in favour of males **(27).** While a few studies revealed a statistically significant association of the PTPN22 C1858T polymorphism with gender, a recently published meta-analysis suggests that males who carried the 1858T allele were more susceptible to T1DM than females **(20)** . As a result, the interaction of the PTPN22 C1858T polymorphism with gender in TD1M requires further investigation.

Sex-specific differences in the genetic susceptibility to type 1 diabetes have been reported for HLA DQ locus and early disease onset or its seasonality for males **(28).** Furthermore, a male/female bias has been observed for HLA DR3/non DR4 patients**(14)** that can only be explained by an X-chromosomal linked inheritance of an additional interacting susceptibility factor **(28).**

Conclusion

i. Males appear to be effected more and earlier than females by autoimmune diabetes(T1DM).
ii. There is a significantly higher prevalence of SNP of PTPN22 T1858T and C1858T among patients with type 1 diabetes in comparison to non diabetic control group.
iii. Increased frequency of the 1858T allele patients with T1DM compared to healthy subjects.

Recommendations

Further studies with larger samples size are required to cover the Iraqi population to better understanding the effect of *PTPN22* polymorphism on type 1diabetes mellitus .

References

1. **Baumann B, Salem HH and Boehm BO.(2012).** Anti-inflammatory therapy in type 1 diabetes. Curr Diab Rep. 2012; 12:499-509. | Article| PubMed
2. **WHO (1999).** Definition, Diagnosis and Classification of DM & it's Complication, Report of WHO Consultation. part1: Diagnosis & Classification, p. 17.
3. **ADA (2009).** Diagnosis and Classification of Diabetes Mellitus. Diabet care, 32(suppl.1):562-67.
4. **Rich S.S., P. Concannon, H. Erlich et al.,(2006)** "The type 1 diabetes genetics consortium," Annals of the New York Academy of Sciences, vol. 1079, pp. 1–8, 2006.
5. **Cohen S, Dadi H, Shaoul E, Sharfe N & Roifman CM(2013).** Cloning and characterization of a lymphoid-specific, inducible human protein tyrosine phosphatase. Lyp. Blood 1999 93 2013–2024.
6. **Lee YH, Rho YH, Choi SJ, Ji JD, Song GG, Nath SK, et al.,(2007).** The PTPN22 C1858T functional polymorphism and autoimmune diseases: a meta-analysis. Rheumatology (Oxford) 2007;46:49–56.
7. **Rieck M, Arechiga A, Onengut-Gumuscu S, Greenbaum C, Concannon P, Buckner JH.(2007).** Genetic variation in PTPN22 corresponds to altered function of T and B lymphocytes. J Immunol2007;179:4704–10.
8. **Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M,MacMurray J, Meloni GF, Lucarelli P, Pellecchia M, Eisenbarth GS,Comings D, Mustelin T:(2004).** A functional variant of lymphoid tyrosine phosphatise is associated with type I diabetes. Nat Genet 36:337–338.
9. **Smyth D., J. D. Cooper, J. E. Collins et al.,(2004).** "Replication of an association between the lymphoid tyrosine phosphatase locus(LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus," Diabetes, vol. 53, no. 11, pp.3020–3023.
10. **Bottini N, Vang T, Cucca F, Mustelin T.(2006)**

Role of PTPN22 in type1 diabetes and other autoimmune diseases. Semin Immunol 18:207–213.

11. **Wu J.,Katrekar A., L. A. Honigberg, A. M. Smith, M. T. Conn, J. Tang,D. Jeffery, K. Mortara, J. Sampang, S. R. Williams, et al. (2006).** Identification of substrates of human protein-tyrosine phosphatase PTPN22. J. Biol. Chem. 281:11002–11010.
12. **Vang, T., M. Congia, M. D. Macis, L. Musumeci, V. Orru, P. Zavattari, K. Nika,L. Tautz, K. Tasken, F. Cucca, et al. (2005).** Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. Nat. Genet. 37: 1317–1319.
13. **Scott CR, Smith JM, Cradock MM, et al.,(1997).** Characteristics of youth-onset noninsulin-dependent diabetes mellitus and insulin-dependent diabetes mellitus at diagnosis. Pediatr, 100:84-91.
14. **Cucca F, Goy JV, Kawaguchi Y, Esposito L, Merriman ME, Wilson AJ, Cordell HJ, Bain SC & Todd JA.(1998).** A male-female bias in type 1 diabetes and linkage to chromosome Xp in MHC HLADR3- positive patients. Nature Genetics 1998 19 301–302.
15. **Peng H, Zhou M, Xu WD, Xu K, Zhai Y, Li R, et al.(2012).** Association of PTPN22 C1858T polymorphism and type 1 diabetes: a meta-analysis. Immunol Invest 2012; 41: 484-496.
16. **Tang S, Peng W, Wang C, Tang H, Zhang Q.(2012).** Association of the PTPN22 gene (+1858C/T,-1123G/C) polymorphisms with type 1 diabetes mellitus: a systematic review and meta-analysis. Diabetes Res Clin Pract 2012;97:446-452.
17. **LeeY.H. and Song G.G.,(2013)** "Meta-analysis of the family-basedassociation between the PTPN22 C1858T polymorphism and type 1 diabetes,"Molecular Biology Reports, vol. 40,no. 1, pp. 211–215.
18. **Zheng J., Ibrahim S., F. Petersen, and X. Yu,(2012)** "Meta-analysis reveals an association of PTPN22 C1858T with autoimmune diseases, which depends on the localization of the affected tissue," Genes & Immunity, vol. 13, no. 8, pp. 641–652.
19. **Wang X.F., Chen Z.X., Shao Y.C. et al.,(2013)** "Population-based and family-based studies on the protein tyrosine phosphatase nonreceptor 22 gene polymorphism and type 1 diabetes: a metaanalysis," Gene, vol. 517, no. 2, pp. 191–196.
20. **Xuan C., L. M. Lun, J. X. Zhao et al.,(2013).** "PTPN22 gene polymorphism (C1858T) is associated with susceptibility to type 1 diabetes: a meta-analysis of 19, 495 cases and 25, 341 controls,"Annals of Human Genetics, vol. 77, no. 3, pp. 191–203.
21. **Heinrich Kahles*, Elizabeth Ramos-Lopez*, Britta Lange, Oliver Zwermann1, Martin Reincke1 and Klaus Badenhoop,(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
22. **Peng H, Zhou M, Xu WD, Xu K, Zhai Y, Li R, et al.(2012).** Association of PTPN22 C1858T polymorphism and type 1 diabetes: a meta-analysis. Immunol Invest 2012; 41: 484-496.
23. **Gloria-Bottini1* F., P. Saccucci1 P., M. L. Manca-Bitti2, N. Rapini2, A. Neri1,L. Coppeta 1, G. Renzetti3, E. Bottini1 and A. Magrini1 (2013).**British Journal of Medicine & Medical Research3(4): 1240-1247, SCIENCEDOMAIN international www.sciencedomain.org Evidence of Interaction between PTPN22 andp53 codon 72 Polymorphisms on Susceptibility to Immune Related Diseases.
24. **Santiago J.L., A. Mart´ınez, H. de la Calle et al.,(2007).** "Susceptibility to type 1 diabetes conferred by the PTPN22 C1858T polymorphism in the Spanish population," BMC Medical Genetics, vol.8, article 54, .
25. **Nielsen C., D. Hansen, S. Husby, and S. T. Lillevang,(2007).** "Sexspecific association of the human PTPN22 1858T-allele with type 1 diabetes," International Journal of Immunogenetics, vol. 34, no. 6, pp. 469–473,.
26. **Zhernakova A., P. Eerligh, C. Wijmenga, P. Barrera, B. O. Roep, and B. P. C. Koeleman,(2005).** "Differential association of the PTPN22 coding variant with autoimmune diseases in a Dutch population," Genes and Immunity, vol. 6, no. 6, pp. 459–461,.
27. **Weets I, Kaufman L, Van der Auwera B, Crenier L, Rooman RP, De Block C, Casteels K, Weber E, Coeckelberghs M, Laron Z, Pipeleers DG & Gorus FK.(2004).** Belgian Diabetes Registry. Seasonality in clinical onset of type 1 diabetes in Belgian patients above the age of 10 is restricted to HLA-DQ2/DQ8-negative males, which explains the male-to-female excess in incidence. Diabetologia 2004 47 614–621.
28. **Contu D, Morelli L, Zavattari P, Lampis R, Angius E, Frongia P, Murru D, Maioli M, Francalacci P, Todd JA & Cucca F.(2002).** Sex-related bias and exclusion mapping of the nonrecombinant portion of chromosome Y in human type 1 diabetes in the isolated founder population of Sardinia. Diabetes 2002 51 3573–3576.