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Lack of Association Between *PTPN22 1858 C>T* Gene Polymorphism and Susceptibility to Generalized Vitiligo in a Iraqi Population

Jalal A. Al-Tuama[1] , [1]Amina Al-Thwani1, [2]Husam. Al-Hraishawi[2]

[1] Institute of Genetic Engineering and Biotechnology-Baghdad University
[2] College of medical, Misan University, Misan, Iraq, husam.mcm@uomisan.edu.iq

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Abstract: The protein tyrosine phosphatase nonreceptor 22 (PTPN22) is associated with susceptibility to group of autoimmune diseases. The functional polymorphism in PTPN22 at 1857 It is considered a risk factor for vitiligo susceptibility in European; however, controversy exits in other populations. Present study aimed to study The exon 14 region of the PTPN22,and determine whether the PTPN22 C1857T polymorphism confers susceptibility to vitiligo in Iraqi patients. Genomic DNA was extracted and amplified using polymerase chain reaction and sanger sequencing method. The frequency of the *CC* and *CT* genotypes were 97.30% (36) and 2.70% (1) in the generalized vitiligo patients, respectively, and 93.88% (46) and 6.12% (3) in the healthy controls, respectively. There was no statistically significant difference between the generalized vitiligo patients and healthy controls.The homozygotes genotype (*TT)* was absent in both the generalized vitiligo patients and healthy controls. there was no significant difference between two groups. The frequencies of the polymorphic T allele were 1.28% and 3.06% in the patient and the control groups. The genotype and allele frequency of the rs2476601 did not show any statistical significance ($p > 0.05$) in its association with generalized vitiligo.

Keywords: PTPN22, SNPs, GV.

Corresponding author: (Email: jalal.ali18@yahoo.com).

Introduction

Vitiligo is a depigmented disorder characterized by white patches on the skin. Patients suffering from Vitiligo are typically depressed and have difficulty socializing. According to the World Health Organization, Vitiligo affects between 0.5 and 2 percent of the world's population (1).

It is still unclear what causes Vitiligo, but it appears to be associated with autoimmune diseases such as Hashimoto's thyroiditis (2), high levels of CD8+ cytotoxic lymphocytes in lesions, as well as autoantibodies, have been found in patients with Vitiligo (3).

The protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) gene is a key predisposing gene for autoimmune diseases in humans, according to recent research. The mutations in *PTPN*22 make a person more susceptible to developing autoimmune diseases, which can include multiple sclerosis and lupus (4; 5). A large number of single nucleotide polymorphisms (SNPs) have been discovered in the *PTPN22* gene, but only one non-synonymous SNP has been studied extensively in the context

of autoimmune disorders. Exon 14 of the *PTPN22* gene contains the C1858T (rs2476601) variant, which has been linked to many autoimmune diseases and is considered a risk factor due to the significant production autoantibodies caused by this variant (4; 5).

Although there is a lot of information out there about the *PTPN22* C1858T polymorphism and autoimmune diseases, there are some discrepancies and ethnic variations in the results (6).

The objective of this study was to determine a possible association between the *PTPN22* 1858 C>T gene polymorphism and generalized vitiligo susceptibility in a Iraqi population.

Materials and Methods

1-Sample Collect

A blood samples (5ml) were collected from (50) GV patients who were referred to Dermatology clinics at various hospitals in Iraq. The genomic study population (50 GV patients) was compared to 50 DNA sequences from the control group (local). (7). Individuals from the 1000 Genomes Project declared themselves to be healthy at the time they took part in the project study.

Whole genomic DNA was extracted from blood sample for NLRP1 patients using a Promega genomic DNA extraction kit. Polymerase Chain Reaction (PCR) was used to amplify the NLRP1 gene promoter region from each sample's. The genomic region (569bp) was amplified, and DNA sequencing method was done to detect each SNP genotype from the GV amplified product.

2-Primers

Three primers were used *http://bioinfo.ut.ee/primer3-0.4.0/primer3/*). *Forward primer* (Cccagccctacttttgagc*)* and a *reverse primer* (*ccaccatccaaatagttggga*) were designed around region that contains all 12 SNPs (rs925595, rs925596, rs925597, rs925598, rs8072203, rs2670642, rs1156989, rs2716936, rs1156990, rs79376273) . The genomic region was amplified at 56 9base pair.

3-PCR condation

PCR amplications were generated using the following primers: forward 5'-CCCAGCCCTACTTTTGAGC-3'and a reverse primer 5'-CCACCATCCAAATAGTTGGGA-3' were designed around region that contains 12 SNPs (rs554195846, rs138223016, rs765535869, rs759881801, rs76427534, rs2476601, rs201811041, rs74163660, rs768160390, rs368086285, rs775140391. and rs569454620) (product of 569 bp). PCR was performed using DNA as a template under the following conditions: Initial Denaturation 95 ℃ for 5 minutes, then 95 ℃ for 30 sec. Denaturation and Annealing temperature 60 ℃ for 30 sec. and 72 ℃ for 30 sec.Extension and Final Extension 72 ℃ for 7 mins.

4-Statistical Analysis

The GV - (*allele, genotype, and haplotype*) association were estimated using Fisher's exact test described by Wang, *et al.,* (2019)(8). The *R* statistical package (*https://www.r-project.org/*) was used to calculate odds ratio and confidence intervals for each selected variant and their haplotypes combinations.

Results and Discussion

1-Genomic DNA extraction

In order to study genetic polymorphism in Iraqi patients with GV and healthy controls, genomic DNA was extracted from blood samples of those subjects under study by using the Promega DNA Extraction Kit (Promega, USA). Results in (Figure 1) showed high molecular weight DNA bands typical of genomic DNA were obtained after electrophoresis of extracted DNA from healthy controls and pateints with GV on an agarose gel (1%).

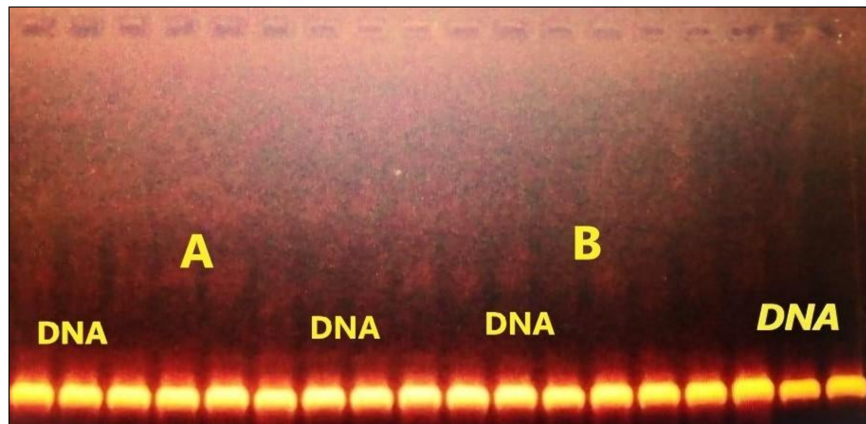

Figure (1): Quality of Genomic DNA extracted from GV patients (A) and healthy controls (B). Gel electrophoresis of DNA on agarose gel (1%) for 70 volt/cm2 for 30 mints, then exposed to UV light and photographed.

2-2 Genetic polymorphism of PTPN22

The amplification of PTPN22 gene fragment of DNA samples for patient and control by PCR were done by using forward and reverse primers which were designed for Exon14. The result showed clear bands appeared by gel electrophoresis in specific location with product size (569 bp) compared to the ladder (figure 2; figure 3).

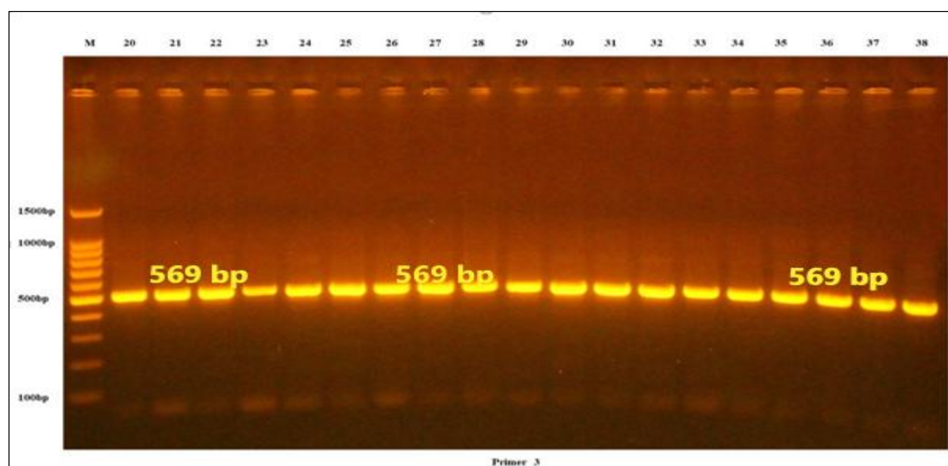

Figure (2): Amplification of PTPN22 gene of Exon 14. Gel electrophoresis for PCR products run on an agarose gel (2%) for 70 volt/cm2 for 90 mints in the presence of 1 kb DNA Ladder marker. PCR products for DNA extracted from blood samples of patients GV.

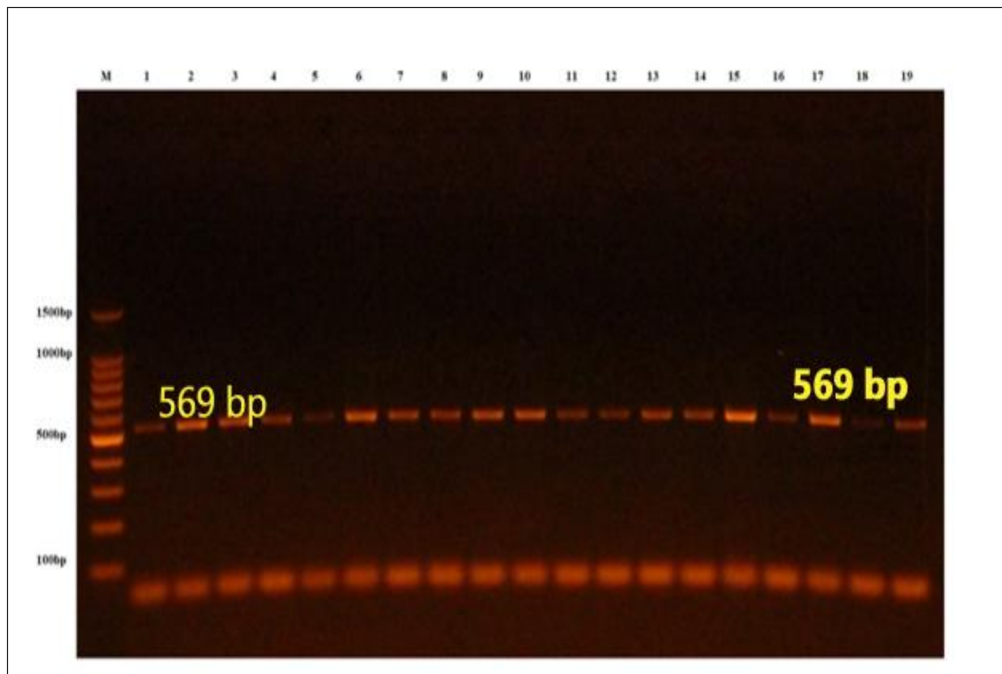

Figure (3): Amplification of PTPN22 gene of Exon 14. Gel electrophoresis for PCR products run on an agarose gel (2%) for 70 volt/cm2 for 90 mints in the presence of 1 kb DNA Ladder marker. PCR products for DNA extracted from blood samples of healthy controls.

T-cell activation via antigen stimulation is governed by a complex signaling architecture involving TCR subunits and tyrosine kinases such as LCK and ZAP70. Phosphatases tightly regulate this process to control the duration and extent of the immune response. PTPN22 is a key negative regulator of TCR signaling that functions by dephosphorylating ZAP70 and LCK (9).

The PTPN22 activating mutation, C1858T, results in a hyperactive variant (R620W) that has been linked to a variety of autoimmune diseases. This hyperactive variant, contrary to popular belief, has been shown to increase IL-2 accumulation and T cell proliferation. PTPN22 substrates have previously been identified (10).

The exon 14 region of the PTPN22 was targeted using a primer that amplifies 569 basepairs, which covers the 84 base pairs of the axon 14 of the PTPN22. 12 SNPs were identified in this region, as shown in Figure (4). Also the study aims to validate a SNP (rs2476601) which has been determined to be associated with lasusceptibility to autoimmune diseases (11; 12), and its potential association with vitiligo.

The PTPN22 1858T variant lead to change arginine to tryptophan substitution, which disrupts the interaction of Lyp and Csk protein tyrosine kinases, inhibiting T-cell activation and possibly increasing susceptibility to autoimmune disease (13;11). Type 1 diabetes, rheumatoid arthritis, systemic lupus erythematosus, Graves' disease (14; 13; 15; 16), and vitiligo have all been linked to the PTPN22 1858T variant (17). In contrast, two family-based studies of patients with psoriasis and multiple sclerosis found no association with the PTPN22 risk allele, implying that PTPN22 may not be truly associated with these diseases (15).

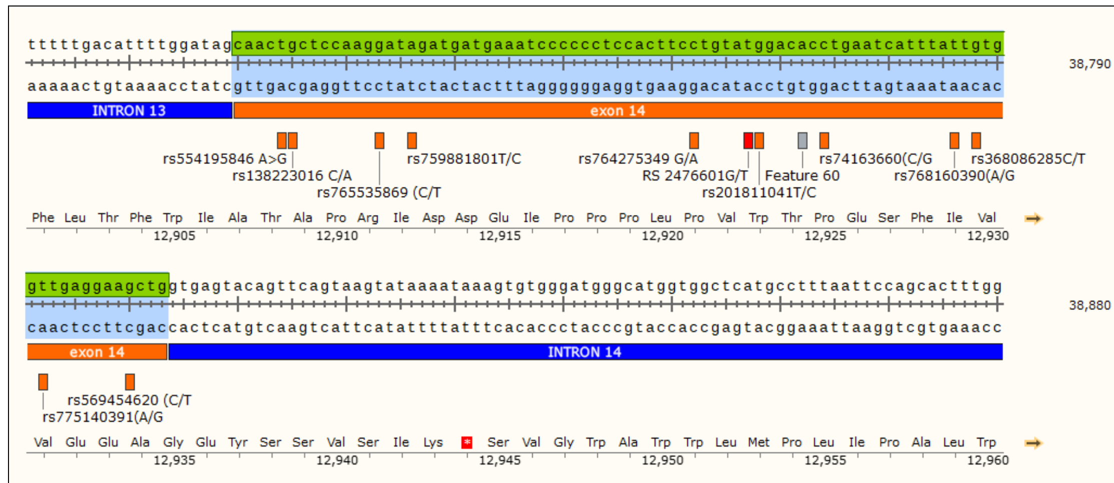

Figure (1): Shows the hypothetical changes that occur in the many polymorphisms recorded on exon 14, (snap gene).

The 12 SNPs' frequency distribution in exon 14 of the PTPN22 gene was determined using the slicer tool (http://useast.ensembl.org/Homo_sapiens/Tools/DataSlicer) and the available data from the 1000 genomes project Table (1). Six of the SNPs in Table (1) (rs554195846, rs138223016, RS2476601, rs201811041, rs74163660 and rs569454620) had genotype date available, yet, Six other SNPs is suspected of having a variant call bug that prevented making a genotype call for them; therefore, they were called missing (./.) and were not reported in the .vcf file of chromosome 1were the gene is located; so they were excluded from the genotype frequency calculation. All of the genotype frequencies of SNPs called missing (./.) were reported as NA (Not Applicable) in Table (1). All SNPs with genotype calls were not frequent, and all of them had a very high ~ 99-100% genotype frequency as a homozygous reference, except for rs2476601, which had a ~97% homozygous alternative alleles in its genotype. The same pattern is seen in the vitiligo patients, Vitiligo Iraqi control group, and the called genotypes for SNPs in individuals from the 1K genomes project Table (1).

Showed table (2). The frequency of the *CC* and *CT* genotypes were 97.30% (36) and 2.70% (1) in the generalized vitiligo patients, respectively, and 93.88% (46) and 6.12% (3) in the healthy controls, respectively. There was no statistically significant difference between the generalized vitiligo patients and healthy controls according to the frequency of the heterozygote genotype (*odds ratio* [OR]: 0.4259, 95% confidence interval [CI]: 0.0425 - 4.2689, $p=0.4680$).

The homozygotes genotype (*TT)* was absent in both the generalized vitiligo patients and healthy controls. In addition, there was no significant difference between the patient and control groups with respect to allele frequencies (*C* or *T*). The frequencies of the polymorphic T allele were 1.28% and 3.06% in the patient and the control groups, respectively (*OR:0.43*, 95% CI: 0.0442 - 4.2567, $P = 0.47$). The genotype and allele frequency of the rs2476601 did not show any statistical significance (p > 0.05) in its association

with GV in Table (2). In light of the autoimmune nature of vitiligo, Controversy surrounds reports on the association between vitiligo and the PTPN22+1858 C>T polymorphism in different ethnic groups. Three separate studies conducted in English, Romanian, and English-North American populations determined an association between generalized vitiligo and the PTPN22 C>T single nucleotide polymorphism (17; 18).

Table (1): Frequencies of 12 SNPs in PTPN22 gene in Iraqi vitiligo patients, controls, and individuals from the 1K genomes project.

SNP ID (ref/alt)	Position on Chr1 (GRCh38)	Vitiligo Patients (n=37) freq %			Control (n=49) freq %			1K genome (n=2504) freq %		
		Ref Homo	Hetero	Alt Homo	Ref Homo	hetero	Alt Homo	Ref Homo	hetero	Alt Homo
rs554195846 (A/G)	113834989	100	0	0	100	0	0	99.92	0.03	0
rs138223016 (C/A)	113834988	100	0	0	100	0	0	99.92	0.03	0
rs765535869 (C/G,T)	113834980	100	0	0	100	0	0	NA	NA	NA
rs759881801 (T/C)	113834977	100	0	0	100	0	0	NA	NA	NA
rs764275349 (G/A)	113834951	100	0	0	100	0	0	NA	NA	NA
rs2476601 (A/G,T)	113834946	0	2.70	97.29	0	6.12	93.87	5.229	0.11	94.61
rs201811041 (C/T)	113834945	100	0	0	100	0	0	99.92	0.039	0
rs74163660 (G/C)	113834939	100	0	0	100	0	0	99.92	0.039	0
rs768160390 (A/G)	113834927	100	0	0	100	0	0	NA	NA	NA
rs368086285 (C/T)	113834925	100	0	0	100	0	0	NA	NA	NA
rs775140391 (A/G)	113834921	100	0	0	100	0	0	NA	NA	NA
rs569454620 (C/T)	113834913	100	0	0	100	0	0	99.92	0.039	0

Table (2): Genotype and allele association of rs2476601 with GV

SNP	Genotype or allele	GV n (%)	Control n (%)	Odds Ratio (CI)	P value
rs2476601 (T>C)	CC	36(97.30)	46(93.88)	2.3478 (0.2343 -23.5316)	0.4680
	CT	1(2.70)	3(6.12)	0.4259 (0.0425 - 4.2689)	0.4680
	TT	0	0	1.274 (0.0247 - 65.7612)	0.9042
	C	73(93.59)	95(96.94)	2.30 (0.2349 to 22.6209)	0.4735
	T	1(1.28)	3(3.06)	0.43 (0.0442 to 4.2567)	0.4735

However, Laddha, *et al.* (19) did a study in 126 Gujarat Indian patients with generalized vitiligo and 140 healthy controls. Found no significant association between the PTPN22 1858 C>T polymorphism and generalized vitiligo. also, Alkhateeb, et al. (2010)(20) that there is no ignificant correlation between the PTPN22 1858 C>T polymorphism and vitiligo in a Jordanian population consisting of 55 patients with generalized vitiligo and 85 healthy controls.

Another study results suggest that this single-nucleotide polymorphism is not associated with generalized vitiligo in Turkish patients(21).

The *PTPN22* 620W allele associated with autoimmune disorders, highlighting the significance of a genetically mediated autoimmune mechanism in the pathogenesis of vitiligo. Evidence suggests that the *PTPN22* 1858C/T variants contribute to the risk of GV in European Caucasian and Mexican populations (18, 22), but not in Jordanian or Turkish generalized-vitiligo patients (21).

Many studies showed the association between *PTPN22* 1858 C/T polymorphisms and vitiligo which discovered that the *PTPN22* C1858T polymorphism is associated with vitiligo susceptibility in the European population (18). Variants of *PTPN22* have also been linked to a variety of Autoimmune diseases, including RA (23) and SLE (24).

The etiology of vitiligo is polygenic and multifactorial, the result of a complex interaction of environmental, immunological, and genetic factors. Recently, polymorphisms in a number of genes implicated in the disease have been discovered (25).

The protein tyrosine phosphatase nonreceptor 22 gene encodes LYP, which is known to be involved in the control of T-cell activation. Under normal conditions, the LYP enzyme functions as a 'negative regulator' of T-cell activation and prevents over activity of immune cells by interacting with CSK (21). Which is involved in signal transduction of T-cell activation. The *PTPN22 1858T* variant causes in substitution of arginine with tryptophan, which has been shown to reduce the binding of LYP to CSK in vitro (14).

Reduced interaction between LYP and CSK has been suggested to inhibit the down-regulation of T-cell activation. Thus, T-cells without the LYP-CSK complex are more prone to be overactive and, consequently, more ready to evoke an autoimmune response. As a result, the *PTPN22 1858 C>T* polymorphism is thought to play a role in the pathogenesis of autoimmune diseases (26).

These studies showed that the frequencies of the polymorphic (*T*) allele changed from 10.8% to 14.5% in vitiligo patients and from 4.1% to 8.6% in healthy controls in Caucasians. However, the results of studies of Asian populations found that the *PTPN22 1858 C>T* gene polymorphism is not associated with vitiligo.

In this study, the allelic frequency of the mutant (*T*) allele in patients with vitiligo and healthy controls (1.28% and 3.06%, respectively) was lower than that in Caucasian populations. Similar to our results, in Gujarat Indian, Jordanian and turkey populations, the frequencies of the mutant (*T*) allele (0.79% , 2.14% and 2% in vitiligo patients and 2.14% , 2.9% and 3%in healthy controls, respectively) were lower than those of Caucasian populations (21).

Furthermore, the homozygote (*TT*) genotype was absent in both generalized vitiligo patients and healthy controls in our study, as well as in the studies of Akbas, *et al.* (21); Laddha, *et al.* (19) and Alkhateeb, *et al.* (20). These different findings on the frequencies of this polymorphism among Asian and Caucasian populations are attributed to ethnic differences(21). *PTPN22* may act via different pathways to evoke autoimmunity. Identification of novel genes that are associated with

autoimmunity in patients with vitiligo and their exact in the development of autoimmunity would be of great benef it. Genotyping of vitiligo patients will help identify those at higher risk of autoimmune disorders at an earlier stage of the disease. Furthermore, this would help to determine appropriate therapeutic and prophylactic approaches. Additional molecular studies with a large number of individuals from different ethnical populations are required for a better understanding of the relationship between autoimmunity and vitiligo.

The rs2476601 also showed slight differences when filtered using global and sub-population preferences. Three different databases were utilized to filter the allele frequency of the rs2476601 according to the population ancestor. Table (3), Table (4), and the Supplementary Table (5). categorized the global and sub-populations from the 1K genome project, and the National Library of Medicine data (https://www.ncbi.nlm.nih.gov/snp/rs2476601/download/frequency); the data shows slight (insignificant) variations in the allele/genotype frequency of the rs2476601among the different ancestor's populations. The European ancestors showed slightly lower genotype/allele frequency (0.90% in Table 4) of the mutant allele of the rs2476601(G) compared to other ancestors that had a gradually higher frequency that reached 100%, such as in the East Asian ancestor (Table 4). A detailed and wider population pool of ancestors were reported by other databases, such as the National Library of Medicine database, and is included in this study as a Supplementary Table (5).

Table (3): Genotype frequencies of the rs2476601 in 2504 individuals from 1K genomes filtered by ancestry

Population description (code)	Genotype frequency %			Super population
	AA	AG	GG	
African Caribbean in Barbados (ACB)	0	1.03	98.96	African Ancestry
African Ancestry in Southwest US (ASW)	0	3.27	96.72	African Ancestry
Esan in Nigeria (ESN)	0	0	100	African Ancestry
Gambian in Western Division, The Gambia - Mandinka (GWD)	0	0.88	99.11	African Ancestry
Luhya in Webuye, Kenya (LWK)	0	0	100	African Ancestry
Mende in Sierra Leone (MSL)	0	0	100	African Ancestry
Yoruba in Ibadan, Nigeria (YRI)	0	0	100	African Ancestry
Colombian in Medellin, Colombia (CLM)	0	7.44	92.55	American Ancestry
Peruvian in Lima, Peru (PEL)	0	1.17	98.82	American Ancestry
Puerto Rican in Puerto Rico (PUR)	0	11.53	88.46	American Ancestry
Mexican ancestry in Los Angeles (MXL)	1.562	4.68	93.75	American Ancestry
Chinese Dai in Xishuangbanna, China (CDX)	0	0	100	East Asian Ancestry
Han Chinese in Beijing, China (CHB)	0	0	100	East Asian Ancestry
Han Chinese South (CHS)	0	0	100	East Asian Ancestry
Japanese in Tokyo, Japan (JPT)	0	0	100	East Asian Ancestry
Kinh in Ho Chi Minh City, Vietnam (KHV)	0	0	100	East Asian Ancestry
Utah residents (CEPH) with Northern and Western European ancestry (CEU)	1.01	21.21	77.77	European Ancestry
Finnish in Finland (FIN)	0	26.26	73.73	European Ancestry
British in England and Scotland (GBR)	1.11	15.55	83.33	European Ancestry
Iberian populations in Spain (IBS)	0	15.88	84.11	European Ancestry
Toscani in Italy (TSI)	0	12.15	87.85	European Ancestry

Bengali in Bangladesh (BEB)	0	5.81	94.18	South Asian Ancestry
Gujarati Indians in Houston, TX (GIH)	0	1.94	98.05	South Asian Ancestry
Indian Telugu in the UK (ITU)	0	2.94	97.05	South Asian Ancestry
Punjabi in Lahore, Pakistan (PJL)	0	2.08	97.91	South Asian Ancestry
Sri Lankan Tamil in the UK (STU)	0	0.98	99.02	South Asian Ancestry

Table (4): Allele frequency of rs2476601 in global and sub ancestor populations

Population	Group	Sample Size	Ref Allele	Alt Allele
Total	Global	318168	A=0.085819	G=0.914181
European	Sub	266830	A=0.095068	G=0.904932
African	Sub	11378	A=0.01740	G=0.98260
African Others	Sub	392	A=0.003	G=0.997
African American	Sub	10986	A=0.01793	G=0.98207
Asian	Sub	6816	A=0.0006	G=0.9994
East Asian	Sub	4914	A=0.0000	G=1.0000
Other Asian	Sub	1902	A=0.0021	G=0.9979
Latin American 1	Sub	1346	A=0.0379	G=0.9621
L atin American 2	Sub	5984	A=0.0363	G=0.9637
South Asian	Sub	5220	A=0.0130	G=0.9870
Other	Sub	20594	A=0.06798	G=0.93202
*National Library of Medicine data (https://www.ncbi.nlm.nih.gov/snp/rs2476601)				

#URL https://www.ncbi.nlm.nih.gov/snp/rs2476601/download/frequency
#Organism Homo sapiens
#Position chr1:113834946 (GRCh38.p13)
#Alleles A>G / A>T
#Variation Type SNV (Single Nucleotide Variation)

Table (5): Supplementary table 3. Frequency table NCBI reference SNP (rs2476601)

#Study	Population	Group	Sample size	Ref Allele	Alt Allele
TopMed	Global	Study-wide	264690	A=0.05	G=0.94
gnomAD - Exomes	Global	Study-wide	219224	A=0.07	G=0.9
gnomAD - Exomes	European	Sub	124770	A=0.10	G=0.89
gnomAD - Exomes	Asian	Sub	40198	A=0.00	G=0.99
gnomAD - Exomes	American	Sub	26114	A=0.03	G=0.96
gnomAD - Exomes	African	Sub	13748	A=0.01	G=0.98
gnomAD - Exomes	Ashkenazi Jewish	Sub	9222	A=0.05	G=0.94
gnomAD - Exomes	Other	Sub	5172	A=0.07	G=0.92
gnomAD - Genomes	Global	Study-wide	140144	A=0.06	G=0.93
gnomAD - Genomes	European	Sub	75902	A=0.10	G=0.89
gnomAD - Genomes	African	Sub	42006	A=0.01	G=0.98
gnomAD - Genomes	American	Sub	13634	A=0.03	G=0.96
gnomAD - Genomes	Ashkenazi Jewish	Sub	3324	A=0.05	G=0.94
gnomAD - Genomes	East Asian	Sub	3128	A=0.00	G=1.00
gnomAD - Genomes	Other	Sub	2150	A=0.05	G=0.94
ExAC	Global	Study-wide	121404	A=0.06	G=0.93
ExAC	Europe	Sub	73348	A=0.10	G=0.89
ExAC	Asian	Sub	25166	A=0.00	G=0.99
ExAC	American	Sub	11578	A=0.02	G=0.97
ExAC	African	Sub	10404	A=0.01	G=0.98

ExAC	Other	Sub	908	A=0.05	G=0.945
The PAGE Study	Global	Study-wide	78702	A=0.02	G=0.97
The PAGE Study	AfricanAmerican	Sub	32516	A=0.01	G=0.98
The PAGE Study	Mexican	Sub	10810	A=0.03	G=0.96
The PAGE Study	Asian	Sub	8318	A=0.00	G=0.99
The PAGE Study	PuertoRican	Sub	7918	A=0.05	G=0.94
The PAGE Study	NativeHawaiian	Sub	4534	A=0.02	G=0.97
The PAGE Study	Cuban	Sub	4230	A=0.06	G=0.93
The PAGE Study	Dominican	Sub	3828	A=0.01	G=0.98
The PAGE Study	CentralAmerican	Sub	2450	A=0.02	G=0.97
The PAGE Study	SouthAmerican	Sub	1982	A=0.02	G=0.97
The PAGE Study	NativeAmerican	Sub	1260	A=0.06	G=0.93
The PAGE Study	SouthAsian	Sub	856	A=0.00	G=0.99
8.3KJPN	JAPANESE	Study-wide	16760	A=0.00	G=1.00
1000Genomes	Global	Study-wide	5008	A=0.02	G=0.97
1000Genomes	African	Sub	1322	A=0.00	G=0.99
1000Genomes	East Asian	Sub	1008	A=0.00	G=1.00
1000Genomes	Europe	Sub	1006	A=0.09	G=0.90
1000Genomes	South Asian	Sub	978	A=0.01	G=0.98
1000Genomes	American	Sub	694	A=0.03	G=0.96
Genetic variation in the Estonian population	Estonian	Study-wide	4480	A=0.14	G=0.85
The Avon Longitudinal Study of Parents and Children	PARENT AND CHILD COHORT	Study-wide	3854	A=0.10	G=0.89
UK 10K study – Twins	TWIN COHORT	Study-wide	3708	A=0.10	G=0.89
KOREAN population from KRGDB	KOREAN	Study-wide	2930	A=0.00	G=0.99
HGDP-CEPH-db Supplement 1	Global	Study-wide	2084	A=0.01	G=0.98
HGDP-CEPH-db Supplement 1	Est_Asia	Sub	470	A=0.00	G=0.99
HGDP-CEPH-db Supplement 1	Central_South_Asi a	Sub	414	A=0.01	G=0.98
HGDP-CEPH-db Supplement 1	Middle_Est	Sub	350	A=0.02	G=0.98
HGDP-CEPH-db Supplement 1	Europe	Sub	320	A=0.05	G=0.94
HGDP-CEPH-db Supplement 1	Africa	Sub	242	A=0.00	G=0.99
HGDP-CEPH-db Supplement 1	America	Sub	216	A=0.00	G=1.00
HGDP-CEPH-db Supplement 1	Oceania	Sub	72	A=0.01	G=0.99
HapMap	Global	Study-wide	1860	A=0.03	G=0.96
HapMap	American	Sub	756	A=0.04	G=0.95
HapMap	African	Sub	682	A=0.00	G=0.99
HapMap	Asian	Sub	250	A=0.01	G=0.98
HapMap	Europe	Sub	172	A=0.07	G=0.93
Korean Genome Project	KOREAN	Study-wide	1832	A=0.00	G=1.00
Genome-wide autozygosity in Daghestan	Global	Study-wide	1134	A=0.03	G=0.96
Genome-wide	Daghestan	Sub	626	A=0.03	G=0.96

autozygosity in Daghestan					
Genome-wide autozygosity in Daghestan	Near_East	Sub	144	A=0.01	G=0.99
Genome-wide autozygosity in Daghestan	Central Asia	Sub	122	A=0.05	G=0.94
Genome-wide autozygosity in Daghestan	Europe	Sub	108	A=0.07	G=0.92
Genome-wide autozygosity in Daghestan	South Asian	Sub	98	A=0.00	G=1.00
Genome-wide autozygosity in Daghestan	Caucasus	Sub	36	A=0.03	G=0.97
Genome of the Netherlands Release 5	Genome of the Netherlands	Study-wide	998	A=0.09	G=0.90
A Vietnamese Genetic Variation Database	Global	Study-wide	612	A=0.00	G=1.00
Northern Sweden	ACPOP	Study-wide	600	A=0.11	G=0.88
SGDP_PRJ	Global	Study-wide	558	A=0.01	G=0.98, T=0.00
Medical Genome Project healthy controls from Spanish population	Spanish controls	Study-wide	534	A=0.08	G=0.91
FINRISK	Finnish from FINRISK project	Study-wide	304	A=0.13	G=0.86
Qatari	Global	Study-wide	216	A=0.02	G=0.97
Ancient Sardinia genome-wide 1240k capture data generation and analysis	Global	Study-wide	86	A=0.07	G=0.93
Siberian	Global	Study-wide	56	A=0.05	G=0.95
The Danish reference pan genome	Danish	Study-wide	40	A=0.10	G=0.90

References

1. Bergqvist, C. and Ezzedine, K. (2020). Vitiligo: A Review. Dermatology,236,571–592.
2. Baldini, E.; Odorisio, T.; Sorrenti, S.; Catania, A.; Tartaglia, F.; Carbotta, G.; Pironi, D.; Rendina, R.; D'Armiento, E.; Persechino, S.; *et al.* (2017).Vitiligo and Autoimmune Thyroid Disorders. Front. Endocrinology.,8,290-293.
3. Rodrigues, M.; Ezzedine, K.; Hamzavi, I.; Pandya, A.G.; Harris, J.E.(2017).Vitiligo Working Group. New discoveries in the pathogenesis and classification of vitiligo. Journal of Americane Dermatology.,77(1),1–13.
4. Zheng, J.; Ibrahim, S.; Petersen, F.; Yu, X.(2012). Meta-analysis reveals an association of PTPN22 C1858T with autoimmune diseases, which depends on the localization of the affected tissue. Genes and Immunity.,13(8),641-652.
5. Tizaoui, K.; Kim, S.H.; Jeong, G.H. *et al.* (2019). .Association of PTPN22 1858C/T polymorphism with autoimmune diseases: A systematic review and Bayesian approach. Journal of Clinical Medicine. ,8(3),347.
6. Ghaleb ,Bin Huraib, ;Fahad, Al Harthi,; Misbahul, Arfin ; Abdulrahman, Al-Asmari.(2020). The Protein Tyrosine

Phosphatase Non-Receptor Type 22 (PTPN22) Gene Polymorphism and Susceptibility to Autoimmune Diseases.Submitted: October 7th, 2019Reviewed: D

7. ecember 12th, 2019Published: January 31st, 2020.
8. Auton, A.; Brooks, LD.; Durbin, R.M.; Garrison, E.P.; Kang, H.M.; Korbel, J.O.; *et al.* (2015).A global reference for human genetic variation. Nature.,526(7571),68-74.
9. Yi ,Wang; Yi, Li; Meng, Hao; Xiaoyu, Liu; Menghan, Zhang; Jiucun ;Wang; Momiao, Xiong; Yin, Yao, Shugart; Li Jin (2019).Robust Reference Powered Association Test of Genome-Wide Association Studies. Front Genetic., 10: 319.
10. Mustelin, *et al*. (2004).Protein tyrosine phosphatases in T cell physiology. Molecular. Immunology. 41, 687–700.
11. Wu, J. *et al*. (2006).Identification of Substrates of Human Protein-tyrosine Phosphatase PTPN22. Journal of Bioogical Chemistry, 281, 11002–11010 .
12. Brand, O.; Gough, S.; King, J. (2005). HLA, CTLA4 and PTPN22: the shared genetic master key to autoimmunity. *Expert* Reviews in Molecular Medicine, *7*, 1-15.
13. Gregersen, P.K.; Lee, H.S.; Batliwalla, F.; Begovich, A.B. (2006). PTPN22: Setting threshold for autoimmunity. Semin Immunology., 18, 214–223.
14. Siminovitch, K.A. (2004). ***PTPN22*** and autoimmune disease. Natural Genetic., 36,1248–1249.
15. Bottini, N.; Musumeci, L.; Alonso, A.; Rahmouni, S.; Nika, K.; Rostamkhani, M.; *et al*.(2004). A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Natural Genetic.,36,337-338.
16. Criswell, L.A.; Pfeiffer, K.A.; Lum, R.F.; Gonzales, B.; Novitzke, J.; Kern, M.; Moser, K.L.; Begovich, A.B.; Carlton, V.E.H.; Li, W.; Lee, A.T.; Ortmann, W.; Behrens, T.W.; Gregersen, P.K. (2005). Analysis of families in the Multiple Autoimmune Disease Genetics Consortium (MADGC) collection: the *PTPN22* 620W allele associates with multiple autoimmune phenotypes. Am., J., Human Genetic., 76,561–571.
17. Zhernakova A, Eerligh P, Wijmenga C, Barrera P, Roep BO, Koeleman BP (2005) Differential association of the PTPN22 variant with autoimmune diseases in a Dutch population. Genes Immunology.,6(6):459-61.
18. Cantón, I.; Akhtar, S.; Gavalas, N.G.; Gawkrodger, D.J.; Blomhoff, A.; Watson, P.F.; *et al.* (2005).A single-nucleotide polymorphism in the gene encoding lymphoid protein tyrosine phosphatase (PTPN22) confers susceptibility to generalised vitiligo. Genes Immunology.,6,584-587.
19. LaBerge, G.S.; Bennett, D.C.; Fain, P.R.; Spritz, R.A.(2008). PTPN22 is genetically associated with risk of generalized vitiligo, but CTLA4 is not. Journal Invest Dermatology.,128,1757-1762.
20. Laddha, N.C.; Dwivedi, M.; Shajil, E.M.; Prajapati, H.; Marfatia, Y.S.; Begum, R. (2008).Association of PTPN22 1858C/T polymorphism with vitiligo susceptibility in Gujarat population. Journal. Dermatological. Sciences ,49,260-262.
21. Alkhateeb A, Qarqaz F, Al-Sabah J, Al Rashaideh T. (2010). Clinical characteristics and PTPN22 1858C/T variant analysis in Jordanian Arab vitiligo patients. Molecular Diagnostic Therapy;14: 179-184.
22. Halit, Akbas; Selma ,Bakar Dertlioglu1; Fuat Dilmec; Ahmet, Engin Atay.(2014). Lack of Association between PTPN22 Gene +1858 C>T Polymorphism and Susceptibility to Generalized Vitiligo in a Turkish Population. Annals Dermatology. 26, 1, 14-20.
23. Garcia-Melendez, M.E.; Salinas-Santander, M.; Sanchez-Dominguez, C. *et al*.(2014). Protein tyrosine phosphatase PTPN22 +1858C/T polymorphism is associated with active vitiligo. ExprimentalTherapy Medicine.,8,1433–7.
24. Begovich, A. B.; *et al.* (2004). A Missense Single-Nucleotide Polymorphism in a Gene Encoding a Protein Tyrosine Phosphatase (PTPN22) Is Associated with Rheumatoid Arthritis. *Am.* Journal of Human. Genetic., 75, 330–337.
25. Kyogoku, C. *et al.* (2004). Genetic Association of the R620W Polymorphism of Protein Tyrosine Phosphatase PTPN22 with Human SLE. American Journal of Human Genetic. 75, 504–507 .
26. Pehlivan ,S.; Ozkinay, F.; Alper, S.; Onay, H.; Yuksel, E.; Pehlivan, M. *et al*. (2009). Association between IL4 (-590), ACE

(I)/(D), CCR5 (Delta32), CTLA4 (+49) and IL1-RN (VNTR in intron 2) gene polymorphisms and vitiligo. European Journal of Dermatology.,19,126- 128.

27. Eliopoulos, E.; Zervou, M.I.; Andreou, A.; Dimopoulou, K.; Cosmidis, N. Voloudakis, G. *et al*.(2011). Association of the PTPN22 R620W polymorphism with increased risk for SLE in the genetically homogeneous population of Crete. Lupus.,20,501-506.