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Original Article

Evaluation of Protein Tyrosine Phosphatase Nonreceptor Type 22 gene polymorphism in alopecia areata

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Abstract

Background: Alopecia areata is a skin disease that causes partial or complete hair loss from the scalp and other parts of the body. **Objectives:** The current study aimed to determine genetic polymorphisms in PTPN22 in alopecia areata. **Methods**: This study is a case-control study and randomized controlled clinical trial included (86) subjects who were categorized into two groups; group (1) with 43 healthy people acted as the control group. Group (2) with 43 patients diagnosed with alopecia areata. The polymerase chain reaction restriction fragment length polymorphism (PCR-RELP) analysis technique is used to determine the C1858T polymorphism in PTPN22 gene. **Results:** The present study revealed that There was no remarkable variation between alopecia patients and healthy subjects regarding to age and gender. There was no significant difference between different PTPN22 C1858T genotypes among alopecia patients regarding to demographic data and clinical findings except family history. Concerning PTPN22 genotyping; CT, TT and CT+TT genotypes were significantly higher in cases compared to controls. Our study showed significant difference in dominant model CT + TT versus CC while showed an insignificant difference in recessive TT versus CC + CT between alopecia and healthy groups for PTPN22 C1858T polymorphism. there was statistically significant difference between cases and controls regarding to T allele distribution. **Conclusion:** Protein tyrosine phosphatase non receptor type 22 gene polymorphism is not excluded as a genetic cause of AA and showed good relation with family history.

Keywords: PTPN22, Alopecia areata, gene polymorphism.

Introduction

Alopecia is a disorder that causes loss of hair in one or more areas of the scalp or body. After androgenic alopecia, alopecia areata (AA) is the second most frequent form of non-scarring alopecia. In the United States, the prevalence rate is 2.1 percent, with a global incidence risk rate of 2% **[1].** In Egypt, the prevalence of alopecia areata is ~1% of population and is ~0.6% among children in Al Sharqia Governorate **[2].** The disease can affect any area of the body, including the scalp, eyebrows, and eyelashes, and its severity can range from a single point with a few millimeters to widespread hair loss. In other situations, nail dystrophy has also been identified. Patients' quality of life suffers significantly as a result of the disease **[3].**

Stress, genetic, dietary issues, and thyroid functional abnormalities, as well as psychological, and immunological disorders, have all been blamed for disease onset. T lymphocytes and antibodies are aggregated towards hair follicle compartments, making it an autoimmune illness. Perifollicular lymphocytic infiltrates are frequent on histological examination of the skin**,** and this infiltration primarily affects follicles in the anagen phase [4]. Because AA affects first-degree relatives and monozygotic twins, genetic factors affect disease's development **[5].**

Immune cells produce the protein tyrosine phosphatase nonreceptor type 22 (PTPN22) gene, which has been identified as a significant risk factor for several autoimmune disorders, including psoriasis, thyroid disorders, vitiligo, and diabetes mellitus **[6].** Its C1858T mutation in codon 620 results in the substitution of arginine to the amino acid tryptophan, which causes rapid breakdown of the phosphatase and T-cells and dendritic cells increased response capacity **[7].** The development of autoimmune disorders and the production of autoantibodies may be triggered by the hyperresponsive B cells' impaired ability to function as PTPN22 in T cells **[6].**

The current study aimed to determine genetic polymorphisms PTPN22 in alopecia areata.

Methods

This study was done in the departments of Biochemistry (clinical chemistry and stem cell lab) and Dermatology, Faculty of Medicine, Zagazig University. Approval for the study was obtained from the Institutional Review Board (IRB) Faculty of Medicine , Zagazig Univesity (reference number is 9056/7-11-2021). The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving human.

Sample size statistics:

Assuming the CC genotype was 53.3% vs 83.3% in cases vs control. At 80% power and 95% CI,the estimated sample will be 86 subject, 43 subject in each group. Open epi

This study is a case-control study and randomized controlled clinical trial included (86) subjects who were categorized into two groups; **Group (1):** included 43 healthy individuals. **Group (2):** included 43 patients diagnosed as alopecia areata. The study was approved by the ethical committee of Faculty of Medicine, Zagazig University. Verbal and written consent were collected from all cases after giving information about the procedure and medical research.

All cases of the study were subjected to the following: full history taking, complete general examination, dermoscopic examination, blood tests to determine autoimmune conditions, and scalp swab.

Inclusion criteria:

Patients diagnosed alopecia areata and with age >20.

Exclusion criteria:

Patients on hormonal therapy, presence of other disease that may interfere with the study parameters, patient refuses to give consent and lack of cooperation, and Alopecia areata due to infection or wound

Sampling*:*

3ml of patient's blood was collected in EDTA tubes under complete aseptic condition then samples were stored at (-20°) C until the DNA isolation process.

DNA extraction:

The analytical PCR material used in the reagents was quite pure. To avoid contamination, all of the pipettes, tips, and tubes used for DNA extraction were DNase and RNase-free tubes bought from Gentra (Minneapolis, USA). Using the gSYNC™ DNA Extraction Kit and EDTA anticoagulated peripheral blood leukocytes, genome DNA was extracted as directed by the manufacturer (Geneaid Biotech Ltd).

Quantification and purity of DNA:

This was performed to determine the concentration of DNA and to assess the purity of DNA. This was achieved by calculating the A260/A280 ratio. This ratio was determined to be between 1.7 and 1.9 for pure double-stranded DNA. The process involved adding 20 µl of each isolated DNA sample to 1 ml of deionized water, then using the Milton Roy Spectronic 3000 Array to detect absorbance at 260 and 280 nm wavelengths.

Genotyping of (PTPN22) C1858T gene polymorphism by PCR-RFLP:

a PCR-RFLP test was performed to evaluate specific C1858T using a specific forward primer: 5′-TCACCAGCTTCCTCAACCACA-3′ and reverse primer : 5′-GATAATGTTGCTTCAACGGAATTTA-3′.

The PCR was performed in a final volume of 20µl including 10 µl of 2x i-Taq™ PCR Master Mix (Geneaid Biotech Ltd), 1 ul of each primer (Biolegio, Nijmegen, Netherland), 5 µl of genomic DNA, and 3 ul of deionized water.

The amplification was performed using DNA thermal cycler 480, PERKIN ELMER (Norwalk, CT 06856, USA), Serial No. P16462. The initial denaturation was performed as a single cycle (95°C, 1 min), followed by 30 cycles (95°C, 30 sec), then annealing (58°C, 40 sec), extension (72°C, 30 sec), and ended by final extension (72°C, 10 min). A negative control with PCR reactants with water instead of DNA.

The two percent agarose gel electrophoresis of the PCR products was followed by ethidium bromide staining and UV transilluminator visualization.

Statistical analysis

Data were collected and analyzed using SPSS software (IBM, Version 20.0). For characterization of quantitative data (IQR), mean, and standard deviation were used. Fisher's exact test was utilized. All tests were two-sided. p-value < 0.05 was considered statistically significant, p-value ≥ 0.05 was considered statistically insignificant.

Results

The present study revealed that 43 cases with AA were enrolled in the study with mean age of 32.2 years, 79% were females while 21% were males. While the control group included 43 cases with mean age of 34.5%, 72% were females, and 28% were males. There was no remarkable difference between alopecia cases and healthy subjects regarding to age and gender **(Table 1)**

Table (1): Demographic data of the studied groups.

	Alopecia patients (N=43)	Healthy Subjects (N=43)	t/ X^2	P
Age	32.9 ± 9.8	34.5 ± 9.2	t= 0.75	0.45
Gender			X^2= 0.56	0.45
Female	9 (21%)	12 (28%)		
Male	34 (79%)	31 (72%)		

Data are represented as mean ± SD or number (%). Data are analyzed using Chi square (X^2) or independent student t test

Concerning PTPN22 genotyping; the CC genotype was detected in 22 (51%) cases; CT and TT were in 15 (46.7%) and 6 (14%) respectively, CT+TT was in 21 (49%). In the healthy group, CC was found in 36 (84%) individuals, CT and TT were in 6 (14%) and 1 (2%) controls and CT+TT were in 7 (16%). CT, TT and CT+TT genotypes were significantly higher in cases compared to controls. the analysis of dominant and recessive genetic models showed significant difference in dominant model CT + TT versus CC while showed an insignificant difference in recessive TT versus CC + CT between alopecia and healthy groups for PTPN22 C1858T polymorphism Regarding PTPN22 allele distribution, there was statistically significant difference between cases and controls regarding to T allele distribution. C allele was found in 59 (69%) cases and T allele in 27 (31%) cases, while in the healthy group, C allele was detected in 78 (91%) individuals and T allele in 8 (9%) individuals**. (Table 2) (Figure 1,2).**

Table (2): Genotype and allele frequency distribution of PTPN22 C1858T in Alopecia patients and control subjects.

PTPN22 C1858T	Alopecia patients (N=43)	Healthy Subjects (N=43)	OR (95%CI)	P- value
CC	22 (51%)	36 (84%)	Ref (1)	
CT	15 (35%)	6 (14%)	3.9 (1.2-11.2)	0.01*
TT	6 (14%)	1 (2%)	9.8 (1.1-87.1)	0.04*
CT+ TT	21 (49%)	7 (16%)	4.9 (1.7-13.4)	0.001*
Dominant CT+ TT Versus CC	-	-	4.9 (1.7-13.4)	0.001*
Recessive TT versus CC + CT	-	-	6.8 (0.78-59.2)	0.08
***C* allele**	59 (69%)	78 (91%)	Ref (1)	
***T* allele**	27 (31%)	8 (9%)	4.4 (1.8-10.5)	0.006*

Data are represented as number (%). Data are analyzed using odd ratio.

Table (3) showed that there was no remarkable variation between different PTPN22 C1858T genotypes among alopecia patients regarding to demographic data and clinical findings except family history.

Table (3): Demographic data and Clinical findings of different PTPN22 C1858T genotypes among Alopecia patients.

	CC (N=22)	CT (N=15)	TT (N=16)	F/ X^2	P
Age	33.6 ± 11.1	33.1 ± 8.1	30.1 ±8.3	F=0.28	0.75
Gender **Female** **Male**	 5 (23%) 17 (77%)	 3 (20%) 12 (80%)	 1 (6%) 5 (94%)	X^2=0.11	0.9
Duration	13.5 ± 9.3	15.4 ± 10.4	11.5 ± 12	F=0.35	0.7
Onset **Acute** **Gradual**	 20 (91%) 2 (9%)	 12 (80%) 3 (20%)	 6 (100%) 0	**Fischer exact** 0.42	
Course **Progressive** **Stationery**	 4 (18%) 18 (82%)	 5 (33%) 10 (67%)	 1 (6%) 5 (31%)	X^2=1.3	0.51
Family History	0	1 (7%)	2 (13%)	**Fischer exact 0.02***	

Data are represented as mean ± SD or number (%). Data are analyzed using Chi square (X^2) or Fischer exact or One way ANOVA followed by Tukey s test

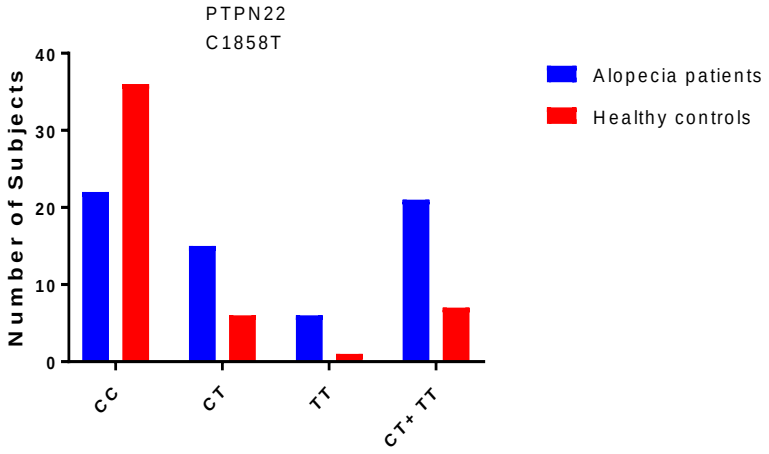


Figure 1: genotypes among different groups

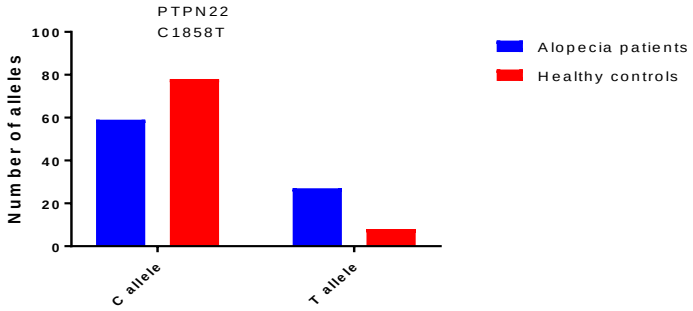
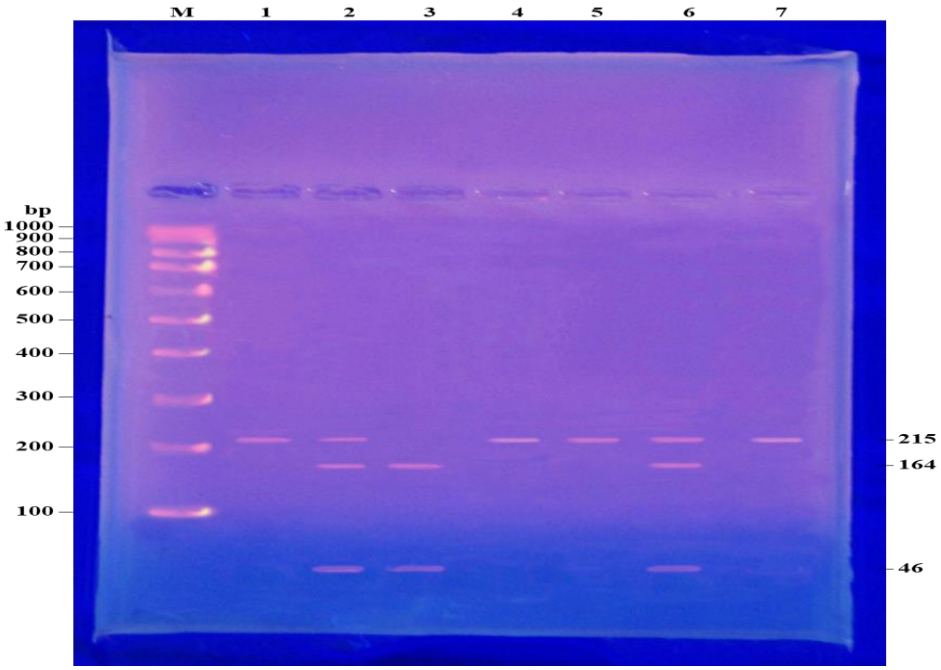


Figure 2: Alleles among different groups



Discussion

The skin condition known as alopecia areata causes hair loss from some or all sections of the body, usually from specific regions of the scalp. Although AA can manifest at any age, young people seem to experience the illness more frequently **[8].**

So, we aimed in the current study, to evaluate genetic polymorphisms in PTPN22 gene in alopecia areata.

Our results demonstrated that there was no obvious variation between alopecia patients and healthy subjects respecting age and gender. In agreement with our results in relation to age.

Also, **Albalat and Ebrahim,** demonstrated that eighty AA patients (68 males and 12 females) were subjected to their study. Their mean age was 34.29±9.226 years, patients were mostly males **[9].**

In disagreement with our study, **Salinas-Santander et al.** reported that the majority of patients were female, showing that 289 people in total were included. These subjects were split into two groups based on whether they had AA or not (AA group; 26 males and 38 females); or whether they did not (control group; 94 meles and 131 females). The mean age of healthy cases was 24.58 years, whereas the patients' mean age was 30.73 years at the time of consultation **[10].**

In the present study, the analysis of dominant and recessive genetic models showed significant difference in dominant CT + TT versus CC while showed an insignificant difference in recessive TT versus CC + CT between alopecia and healthy groups for PTPN22 C1858T polymorphism. There was statistically significant difference between cases and controls regarding to T allele distribution.

In agreement with our study, **Shehata et al.** found that respecting PTPN22 genotyping in the examined groups, Hardy-Weinberg equilibrium was observed with the CC genotype detected in 53.3% of cases and the CT and TT genotypes in 46.7%. In the control group, 16.7% of controls had the CT or TT genotype, while 83.3% of controls had the CC genotype. Patients' CC genotypes were considerably lower than healthy individuals. Concerning the PTPN22 alleles distribution, the C allele was found in 70% of the cases under study, while the T allele was found in 30% of the cases. In contrast, the C allele was found in 90% of the healthy individuals, while the T allele was found in 10% of healthy individuals. In comparison to controls, the sick group's T allele was statistically higher **[11].**

El-Zawahry et al. also assumed that according to a statistical analysis of the frequency of PTPN22 gene polymorphism in controls and AA patients, AA patients had considerably more of the PTPN22-CT and TT mutant genotypes than controls, which enhanced their chance of developing AA **[12].**

on the other hand, in **Bhanusali et al.** study although they found that the PTPN22 C1858T genotype frequency was not substantially higher in cases compared to controls, despite the association between the C1858T and 1858T/T genotypes and decreased immune response. Additionally, they

discovered no statistically remarkable variation between the frequency of the C1858T genotype in non-Caucasian and Caucasian cases compared to ethnically matched healthy individuals; In comparison to all AA cases, the CC genotype was more commonly detected in the controls **[13].**

Contrarily, those with patchy or complete AA were more likely to have the CT genotype. When the patchy AA group was compared to controls, in the same patients, a statistically significant connection between the T allele (P=0.040) and the CT genotype was recorded (P=0.038) **[11].**

Kemp et al. showed in their results that the Hardy-Weinberg equilibrium was not significantly disturbed. Between the control cohort and the group of AA patients, there was no obvious variation in the frequency of the 1858T allele. Of the 392 alleles associated with alopecia areata, 10.5% encoded the W620 variation, compared to 8.5% control alleles. Compared to the control group, the subgroup of patients with severe AA (alopecia totalis/universalis) had considerably more instances of the 1858T allele: out of 168 alleles, 14.9% encoded W620 (P = 0.0127). The frequency of the genotypes C/C, C/T, and T/T between the control and alopecia patient groups did not significantly differ, with the exception of the cohort with severe alopecia areata. Compared to 15.6% of controls, 29.8% of patients had the C/T genotype (P = 0.0061) **[14].**

We revealed that there was no remarkable variation between different PTPN22 C1858T genotypes among alopecia patients regarding to demographic data and clinical findings except family history and hair regrowth.

In agreement with our study, **Shehata et al.** demonstrated that there was a statistically remarkable variation between the 3 genotypes respect to family history when PTPN22 genotypes and clinical data of the examined cases were compared (P = 0.02) **[12].**

Although, **Kemp et al. (2006)** showed that There was no evidence to suggest a connection between the prevalence of the 1858T allele and the onset of alopecia areata **[14].**

Conclusion

Protein tyrosine phosphatase non receptor type 22 gene polymorphism is not excluded as a genetic cause of AA and showed good relation with family history.

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