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Association of Auto Immune Regulator Gene Variant with The Susceptibility and Severity of Vitiligo

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ABSTRACT

Vitiligo is an acquired pigmentary anomaly of the skin, manifested by circumscribed depigmented macules and patches due to the disappearance of pigment cells from the epidermis. The prevalence of vitiligo is increasing and involves up to 1–4% of the world population of all races. The objectives of this study were to compare allele frequencies between vitiligo patients and normal controls, to determine the risk allele for vitiligo in an Egyptian population and to explore the association between the AIRE SNP and the occurrence and severity of vitiligo. **Methods:** This was non-interventional comparative case-control study, a total of 186 individuals were enrolled in this study that were recruited from the Dermatology outpatient clinic, Suez Canal University Hospital, Ismailia. Genetic and biochemical analyses were performed at the Medical Genetics Unit and Center of Molecular and Cellular Medicine of the Faculty of Medicine, SCU, and the *rs2075876 SNP of AIRE* gene was examined in all study participants. **Results:** Carriers for GG variants were less likely to develop vitiligo than their counterparts under homozygote model [crude odds ratio (OR) (95% CI): 13.8 (3.15-60.6], dominant model [OR (95% CI): 13.8 (3.15-60.5), and allelic model [OR (95% CI): 6.7(3.14-1.60.6). **Conclusion:** The AIRE gene has an important role in the pathogenesis of Vitiligo. Taking advantage of the great advances that genetics has brought into the studies of vitiligo have paved the road for a new era of clinical translation of scientific research.

Keywords: Auto immune regulator gene variant, Susceptibility, Severity, Vitiligo

1. Introduction

Vitiligo is a common autoimmune disease that destroys melanocytes in the skin, resulting in the appearance of patchy depigmentation. This disfiguring can be psychologically devastating. A view into the disease pathogenesis offer a better understanding of the natural history of the disease, its associations, and potential for future treatments (Scherschun *et al.,* 2001).

Vitiligo affects 0.5% to 2% of the world's population, without clear preference for race or gender, although women may be more likely to present for treatment (Kruger and Schallreuter, 2012).

Studies have shown that vitiligo, or a susceptibility to the disease, is inherited based on that familial aggregation that is often seen. Epidemiologic studies have shown one fourth to one third of patients with vitiligo have family members with the disease (Ongenae *et al.,* 2005).

Patients with vitiligo were shown to have higher levels of organ-specific antibodies and circulating autoantibodies to melanocyte antigen (Kemp *et al.,* 1997). Several candidate genes were found to be related to susceptibility to vitiligo. One of the elite genes is Autoimmune Regulator (AIRE), located on chromosome 21q22.3, whose gene mutations were associated with several

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autoimmune disorders. AIRE mRNA is expressed in several tissue types and is 10 times more expressed in thymus (Tazi-Ahnini *et al.,* 2008). It acts as a transcription regulator which controls the expression of many peripheral antigens in the thymus (Tazi-Ahnini *et al.,* 2008). It controls the differentiation of medullary thymic epithelial cells and thymocyte adhesions (Passos *et al.,* 2017).

AIRE gene is highly polymorphic. A single nucleotide polymorphism rs2075876 (c.653-387G>A) is located in intron 5. Strong association has been reported between these loci and rheumatoid arthritis in Japanese and Chinese populations (Li *et al.,* 2016).

The objectives of this study were to compare allele frequencies between vitiligo patients and normal control subjects. To determine the risk allele for vitiligo in an Egyptian population. To explore the association between the AIRE SNP and the occurrence and severity of vitiligo.

2. Patients and Methods

Research Design

Non-interventional comparative case-control study.

Research Setting

Subjects enrolled in the study were recruited from the Dermatology outpatient clinic, Suez Canal University (SCU) Hospital, Ismailia. Genetic and biochemical analyses were performed at the Medical Genetics Unit and Center of Molecular and Cellular Medicine of the Faculty of Medicine, SCU.

Study Population

Target Population

Study population included hundred patients diagnosed with vitiligo and compared to hundred control subjects.

Criteria of selection

A. For group I: cases

Inclusion criteria: Egyptian patients. Either gender. Diagnosed with vitiligo.
Exclusion criteria: Chronic dermatological diseases.

B. For group II: controls

Inclusion Criteria: Egyptian patients. Either gender.
Exclusion Criteria: *Having other autoimmune disease. Family history of vitiligo. Having vitiligo.*

3. Methodology

Data Collection Tools

Clinical assessment

Physicians can use a Wood's lamp, which shines ultraviolet (UV) light onto the white patches of the vitiligo to help differentiate from other skin conditions (Singh *et al.,* 2018).

Scoring

Vitiligo Area Severity Index (VASI)

The percentage of vitiligo involvement is calculated in terms of hand units. One hand unit is approximately equivalent to 1% of the total body surface area (Hamzavi *et al.,* 2004).

Vitiligo Disease Activity Score (VIDA)

VIDA score based on patients' opinion divided in 6 stages:

- +4: Activity of 6 weeks or less period.
- +3: Activity of 6 weeks to 3 months.
- +2: Activity of 3 to 6 months.
- +1: Activity of 6 to12 months.
- 0: Stable at least for 1 year.
- −1: Stable at least for 1 year with spontaneous re-pigmentation (Bhatnagar *et al.,* 2007).

Dermatology index quality of life (DLQI):

The DLQI contains 10 questions related to patients' symptoms and feelings, daily activities, leisure, work or school, personal relationships, and treatment over the previous 1 week, and each question has four possible answers scored from 0 to 3 (Chen *et al.,* 2019).

Genetic Analysis

The *rs2075876 of AIRE* gene was examined in all study participants.

A- Blood Sample Collection

Three milliliters of fresh venous blood were drawn in vacutainer tubes containing ethylene diamine tetra-acetic acid (EDTA) anticoagulant. Samples will be centrifuged for 20-min at the speed of 5000 rpm to extract the buffy coat.

B- DNA Extraction

DNA was extracted from the fresh blood samples using the ABIO-pure ™ TOTAL DNA (version 2.0) (Catalog no. M501DP100, Alliance-Bio, USA) per manufacturer's instructions.

Genotyping of the *rs2075876* SNP

Real-time polymerase chain reaction (RT-PCR) was performed to genotype the rs231775 SNP via the TaqMan® SNP genotyping assay (assay ID C- 2415786_20, Applied Biosystems, Waltham, USA). The missense mutation at rs231775 (c.49A>G) could be either A (the wild-type allele) or G (the minor allele). The genotyping assay (also known as the allelic discrimination assay) detects the presence of the A allele only (i.e., homozygous AA), or the G allele only (i.e., homozygous GG), or both alleles simultaneously (i.e., heterozygous A/G).

Preparation of the PCR Reaction Mix

The components of the PCR reaction mix to which the template DNA was added are shown in Table (1).

Table 1: Components of the PCR reaction mix

Reaction Components	Volume/Well
Taqman® Universal PCR Master Mix, No AmpErase UNG (2x)	12.5 µL
20 x TaqMan® SNP Genotyping Assay Mix	1.25 µL
RNase-free water	6.25 µL
Total	**20 µL**

Genotyping

It was done using StepOne™ Real-Time PCR System (Applied Biosystems Inc., 850 Lincoln Centre Drive, Foster City, CA 94404, USA). The PCR reaction consisted of a pre-read run, an amplification run, and a post-read run. Two NTCs were added to each PCR reaction to measure contamination that might give a false positive signal.

A) Pre-read run

This was done to determine the baseline fluorescence produced by the probes before amplification.

B) Amplification run

Default temperatures were used for the amplification run, as shown in Table (2).

Table 2: Thermal cycling conditions

Steps	Temperature	Time	No. of cycle
Initial step	95°C	10 min.	One
Denaturation	95°C	15 sec.	50 cycles
Annealing/ Extension	60°C	1 min.	

C) Post-read run

The baseline fluorescence detected during the pre-read run was subtracted from the total fluorescence. Results were plotted on a scatter plot where the X-axis represents allele G, and the Y-axis represents allele A. Manual calls were made for each sample according to its position on the graph. Points lying on the horizontal axis were designated as G/G genotype, those lying on the vertical axis were designated as A/A genotype, and those on the diagonal axis were designated as A/G genotype.

Sample Size

The following equation was used to calculate the sample size:

$$n = \left[\frac{Z_{\alpha/2} + Z_{\beta}}{P_1 - P_2}\right]^2 (p_1 q_1 + p_2 q_2)$$

Where n= sample size **Z α/2** = 1.96 (The critical value that divides the central 95% of the Z distribution from the 5% in the tail) **Zβ** = 0.84 (The critical value that separates the lower 20% of the Z distribution from the upper 80%) **p**1 = Prevalence/proportion of gene in study group = 91 % **p**2 = Prevalence/proportion of gene in control group = 74 % **q** = 1-P
So, n (per group) = 74
And 10% drop out percentage was calculated, so the final number of participants per group = 81
We investigated 100 patients and 100 controls.
TriolaMF. Elementary statistics. 9th ed .Addision-Wsley product; 2004. P .317-318. Recent insights into the role and molecular mechanisms of the autoimmune regulator (AIRE) gene in autoimmunity.

Statistical Analysis

Data were analyzed using the R software, version 3.3.1, Statistical Package for the Social Sciences (SPSS) version 20.0 software and GraphPad Prism version 7.0. Quantitative data were expressed as means ± standard deviation, while qualitative data were expressed as numbers and percentages. Two-sided Chi-square, Student-t, and ANOVA tests were used for parametric data, while Mann-Whitney U and Kruskal Wallis tests were employed for non-parametric variables. A *p* value of <0.05 was considered statistically significant. Analysis of *allele frequencies* (number of copies of a specific allele divided by the total number of alleles in the group) and *carriage rates* (number of individuals with at least one copy of the A allele divided by the total number of individuals within the group) were carried out. Genotype frequencies were assessed for deviation from the Hardy-Weinberg equation by the online program (http://www.oege.org/software/hwe-mr-calc.shtml). The relationship between allele frequencies and the presence of AA was determined under different genetic association models (reference) using odds ratio with multiple logistic regression analysis after adjustment for AA risk factors. Pearson's correlation test was performed for genotype-phenotype correlation. Multivariate analysis will be executed for data exploration and hierarchical clustering using PCORD version 5.

Ethical considerations

- Before the beginning of the study and in accordance with the local regulation followed, the protocol and all corresponding documents were declared for Ethical and Research approval by Faculty of Medicine, Suez Canal University.
- The purpose of the study was explained to each participant.
- Informed consent was obtained from all individuals willing to participate in the study.
- No pressure will be made on anyone to take part in the research.
- All personal information will be regarded as confidential and patients' samples will be coded.
- The patients will have the right to withdraw from the study at any time with neither jeopardizing the right of the patient to be efficiently treated nor affecting the patient-doctor relationship.
- The patients have the right to refuse to participate in the study.
- Ensuring confidentiality of data, patient's data will not be used outside this study purpose.
- Leftover samples will be handled according to safety measures to all health care field and researchers and will be discarded in safety box, will be used in this research only.

- Management of possible risks will be done accurately by the researcher and the participant will not pay any costs.

4. Results

There were non- statistical significance difference between cases and control regarding their age or gender as shown in Table (3).

Table 3: Demographic characteristics of the study population

Variables	Categories	Controls	Vitiligo	P value	OR (95%CI)
Age (years)	Mean ± SD	36.3 ± 15.7	33.2 ± 17.6	0.194	
	≤ 20 y	24 (24.0)	35 (35.0)	0.316	*Reference*
	≤ 40 y	39 (39.0)	29 (29.0)		
	≤ 60 y	29 (29.0)	28 (28.0)		
	> 60 y	8 (8.0)	8 (8.0)		
Sex	Females	64 (64.0)	63 (63.0)	0.883	*Reference*
	Males	36 (36.0)	37 (37.0)		1.04 (0.58-1.85)
Residence	Rural	21 (21.0)	49 (49.0)	**<0.001**	*Reference*
	Urban	79 (79.0)	51 (51.0)		0.27 (0.14-0.51)
Obesity	Negative	62 (62.0)	57 (570)	0.670	*Reference*
	Overweight	26 (26.0)	27 (27.0)		
	Obese	12 (12.0)	16 (16.0)		
Smoking	Negative	100 (100)	93 (93.0)	**0.014**	*Reference*
	Positive	0 (0.0)	7 (7.0)		0.48 (0.41-0.55)

Data is shown as number (percentage) or mean ± standard deviation (SD). Chi-square and Fisher's Exact tests were used for qualitative variables and student-t test was used for quantitative variables. P value < 0.05 was considered as statistically significant. OR (95CI): odds ratio and confidence interval.

The clinical Features of Vitiligo Patients were shown in Table (4)

Among patient group, shows that positive family history was in 25% of cases, and family history of autoimmune disease was present in 1 % of cases. Regarding risk factors stress was in51% of cases and 19% in control group, and sun burn in was present in 5 % of cases and 21% in control with statistically significant difference also stress increased risk by 4.43% than in control group and sun burn increased risk by 19% than in control group. Regarding type of autoimmune disease, 4 (4 %) had thyroid disease,1 (1%) had alopecia, 1 (1%) had Halo nevi. Also, percentage of Rheumatoid arthritis was 16(16%). Assessment of clinical illness 12% had hypertension in case group and 1% in control with statistically significant difference also increased risk by 13.5% than in control group, 1% had CAD in case group and 4% in control with no statistically significant difference. 13% had T2DM in case group and 9% in control with no statistically significant difference also increased risk by 1.5% than in control group. Regarding drug history 9% had anti hypertension in case group and 1% in control with statistically significant difference also increased risk by 9.7% than in control group. Also 6% had anti rheumatoid drugs in case group and 0% in control with statistically significant difference also increased risk by 13.8% than in control group**.**

The Disease characteristics of vitiligo patients were shown in Figure (1)

The duration of disease is 60% of patient from (1 -10) years and 18% of patient from (10-30) years. Regarding distribution of cases according to skin type: 1% of cases were type I, 33% were type II, 41% were type III and 25% were type IV. Most common initial site of lesion was face 30% followed by trunk and upper limb 20%. Most common affected body segment was trunk and arm 50% followed by face and legs and feet. Also 53% of patients had premature graying of hair. The mean duration of vitiligo at diagnosis was 1.5 years; ranged from 0.18 – 2.0 years in localized lesion and the mean was 6.5 years; ranged from 2.5 – 10.0 years in generalized lesion group.

Table 4: Clinical characteristics and drug history of the study population.

Variables	Controls	Vitiligo	P value	OR (95%CI)
Family history				
Vitiligo	0 (0.0)	25 (25.0)		
Autoimmune disease	0.0) 0(	1 (1.0)		
Risk factors				
Stress	19 (19.0)	51 (51.0)	**<0.001**	**4.43 (2.35-8.37)**
Sunburn	21 (21.0)	5 (5.0)	**0.001**	**0.19 (0.07-0.54)**
Pregnancy	30 (30.0)	29 (29.0)	0.877	0.95 (.0.51-1.75)
Meningitis	0 (0.0)	1 (1.0)	0.316	0.49 (0.43-0.57)
Autoimmune disease				
Thyroid disease	0 (0.0)	4 (4.0)	**0.008**	**0.21 (0.07-0.68)**
Alopecia	0 (0.0)	1 (1.0)	0.316	0.49 (0.43-0.57)
Halo nevi	0 (0.0)	1 (1.0)	0.316	0.49 (0.43-0.57)
Rheumatoid arthritis	0 ((0.0)	16 (16.0)	**<0.001**	**39.2 (2.31-664)**
Clinical illness				
Hypertension	1 (1.0)	12 (12.0)	**0.002**	**13.5 (1.72-105)**
Coronary artery disease	4 (4.0)	1 (1.0)	0.369	0.24 (00.02-2.20)
T2DM	9 (9.0)	13 (13.0)	0.499	1.51 (0.61-3.71)
Drug history				
Anti-hypertensive drugs	1 (1.0)	9 (9.0)	**0.018**	**9.7 (1.21-78.8)**
Cholesterol-modifying drugs	8 (8.0)	3 (3.0)	0.213	0.35 (0.09-1.38)
Anti-diabetic (oral/insulin)	9 (9.0)	12 (12.0)	0.488	1.37 (0.55-3.43)
Anti-rheumatic drugs	0 (0.0)	6 (6.0)	**0.029**	13.8 (0.76-248)
Allergic drugs	6 (6.0)	1 (1.0)	0.118	0.15 (0.01-1.33)
Anti-thyroid drugs	2 (2.0)	1 (1.0)	0.561	0.49 (0.04-5.54)
Oral contraceptive drugs	2 (2.0)	0 (0.0)	0.497	0.49 (0.43-0.57)

Data is shown as number (percentage) or mean ± SD. Chi-square and Fisher's Exact tests were used for qualitative variables and student-t test was used for quantitative variables. P value < 0.05 was considered as statistically significant. T2DM: type II diabetes mellitus; OR (95CI): odds ratio and confidence interval.

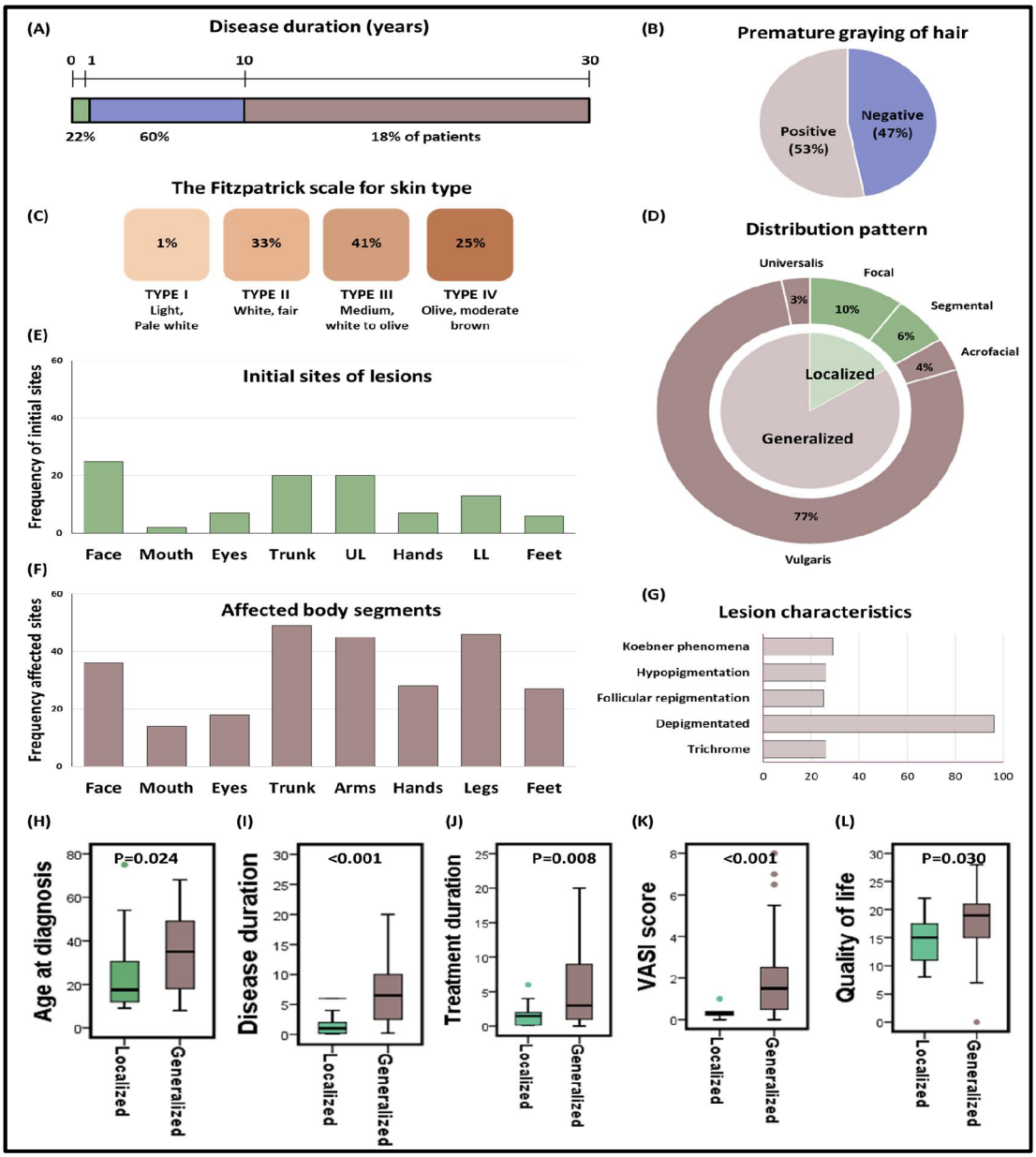

Fig. 1: (A) Frequency of patients (in percentage) with different duration of vitiligo disease. Disease duration is classified into three categories: ≤ 1, ≤ 10, and ≤ 30 years; (B) Proportion of associated premature graying of hair; (C) Frequency of patients according to their Fitzpatrick scale for skin type. Only four subtypes were found in the study population; (D) Categorization of patients according to distribution pattern of their lesions. Double donut chart shows classification of patients according to localized and generalized vitiligo, each is then divided into subtypes; (E-F) Distribution of patients according to locations of initial sites of lesions and the current affected body segments. UL: upper limb, LL: lower limb; (G) Frequency of patients with different lesion activity. (H-J) Comparison between patients with localized lesions compared to generalized vitiligo according to their age at presentation (years), duration of the disease (years), and treatment span (years); (K) Assessment of disease severity by vitiligo area severity index (VASI) score; (L) Dermatological Quality of Life assessment.

The assessment of disease severity was shown in Table (5)

Mean VASI score is (2.18 ± 3.1); the mean VASI of the patients in localized lesion was 0.3 ± 0.21 and in generalized lesion 2.54 ± 3.46 with statistically significant difference. Stable (0/-1) vitiligo was in 4 (25%) of cases and unstable in 75% of cases in localized lesion and Stable (0/-1) vitiligo was in 23 (27.1%) of cases and unstable in 72.9% in generalized lesion with no statistically significant difference.

Table 5: Assessment of disease severity (n=100).

Score		Total	Localized	Generalized	P value
VASI	Mean ± SD	2.18±3.28	0.30±0.21	2.54±3.46	
	Median (IQR)	1.25 (2.04)	0.30 (0.20)	1.50 (2.0)	**<0.001**
VIDA	-1	20 (20.0)	3 (18.8)	17 (20.0)	0.973
	0	7 (7.00	1 (6.2)	6 (7.1)	
	+1	9 (9.0)	1 (6.2)	8 (9.5)	
	+2	16 (16.0)	2 (12.5)	14 (16.7)	
	+3	13 (13.0)	3 (18.8)	10 (11.9)	
	+4	35 (35.0)	6 (37.5)	29 (34.5)	

The association between quality of life and disease severity was shown in Figure (2).

That there was non-significant negative correlation between DLQI score and duration of disease and VASI score. The mean DLQI score of the patients in localized lesion was 14.9 ±6.3 and 17.2 ±7.1 in generalized lesion with statistically significant difference between them.

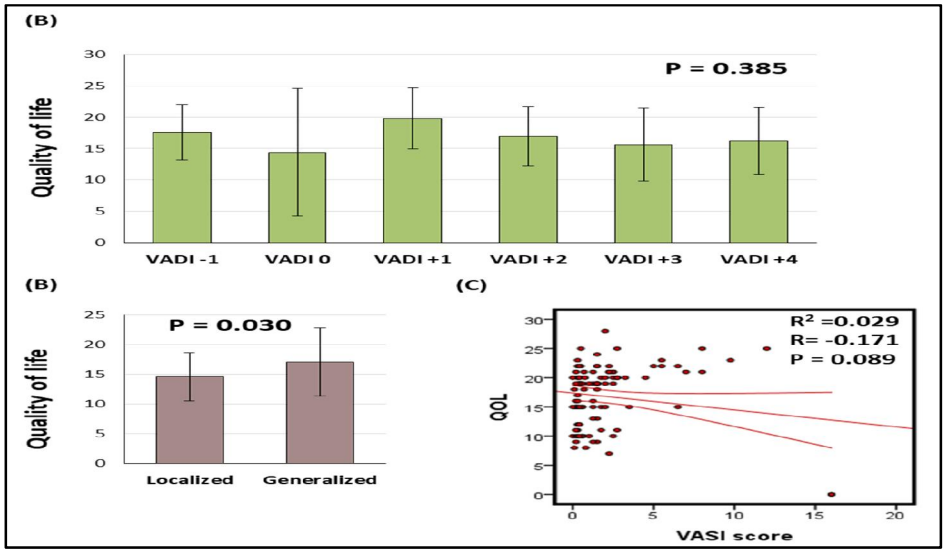

Fig. 2: Association between quality of life and disease severity. (A) QoL score in vitiligo patients with different Vitiligo Disease Activity score (VIDA) score. ANOVA test was used. (B) QoL score in vitiligo patients with localized and generalized distribution pattern. Student's t test was employed. (C) Correlation analysis between QoL and Vitiligo Area Severity Index (VASI) score. Pearson's correlation test was used. P value < 0.05 was considered as statistically significant.

The association of AIRE polymorphism variant with disease risk was shown in Table (6).

A total of 100 patients and 100 controls were genotyped. Genotype and allele frequencies of AIRE polymorphism showed that the frequencies of AA, AG, GG and AA+AG alleles in the whole study respectively. Genotyping of AIRE polymorphism detected A and G alleles. The distribution of AIRE genotype among patients and controls were found in accordance with those expected by the Hardy Weinberg equilibrium ($p < 0.001$).

Table 6: Genotype frequencies and carriage rates of *AIRE* polymorphism in vitiligo patients and controls.

Genotype frequency	Controls (n=100)		Patients (n=100)		χ^2	*P* value	OR (95% CI)
AA	22	(220)	2	(2.0)	18.9	**<0.001**	1.0
AG	4	(4.0)	5	(5.0)			13.7 (1.9-97.1)
GG	74	(74.0)	93	(93.0)			**13.8 (3.14-60.6)**
Allele frequency							
A	48	(24.0)	9	(4.5)	31.12	**<0.001**	1.0
G	152	(76.0)	191	(95.5)			**6.7 (3.1-14.0)**
Carriage rate							
A	26	(26.0)	7	(7.0)	13.1	**<0.001**	**0.21 (0.08-0.52)**
G	78	(78.0)	98	(980)	18.9	**<0.001**	**13.8 (3.15-60.5)**

Values are shown as number (%). Two-sided Chi square ($\chi 2$) test was used. OR (95% CI), odds ratio and confidence interval; calculated as present versus absence. The carriage rate was calculated as the number of individuals carrying at least one of the investigated alleles divided by the total number of individuals in each group. Bald values statistically significant at $p<0.05$.

On comparing between AA patients and controls which was shown in Table (7). AA+AG was significantly the most prevalent genotype among controls (7% in patients *versus* 26 % in controls), while GG genotype was predominant in the vitiligo group (93% in patients *versus* 74% in controls) (*P* = 0.022). Consistently increased risk by 13.8% than in control group**.**

Table 7: Hardy Weinberg equilibrium (n=200).

	A/A	A/G	G/G	A	G	P-value
All subjects	167	9	24	343	57	<0.001
Controls	74	4	22	152	48	<0.001
Patients	93	5	2	191	9	0.009

Carriers for GG variants, shown in Table (8) & Fig (3), were more likely to develop vitiligo than their counterparts under homozygote model [crude odds ratio (OR) (95% CI): 13.8 (3.15-60.6], dominant model [OR (95% CI): 13.8 (3.15-60.5), and allelic model [OR (95% CI): 6.7(3.14-1.60.6).

Characteristics of vitiligo according to AIRE genotypes are demonstrated in Fig. (4).

Multivariate analysis demonstrated that family history of vitiligo, distribution pattern, and disease duration are independent predictors for VASI score (Table 9). However, stepwise regression analysis did not reveal any selected variables for predicting the level of quality of life.

Data is shown as number (percentage), mean ± SD or median (quartiles). Chi-square and Mann-Whitney U tests were used. P value < 0.05 was considered as statistically significant. Localized vitiligo includes focal and segmental patterns, while generalized vitiligo includes acrofacial, vulgaris and universalis distribution.

Vitiligo Area Severity Index (VASI): body is divided into 5 regions (hands, upper limb, trunk, lower limb, feet). Each measured in hand unit. Residual depigmentation value is estimated within each region. The VASI is calculated as the sum of all body sites [Hand Units] × [Residual Depigmentation]. Vitiligo Disease Activity score (VIDA) is a six-point scale for assessing vitiligo stability over time. It depends on the patient's own reports of disease activity.

Table 8: Risk of vitiligo disease according to the genetic association models.

Association model	Variant comparison	Controls (n=100)	Patients (n=100)	OR (95% CI)
Allelic model	G *versus* A	152/48	191/9	**6.7 (3.1-14.0)**
Homozygote comparison	GG *versus* AA	74/22	93/2	**13.8 (3.14-60.6)**
Heterozygote comparison	AG *versus* AA	4/22	5/2	13.7 (1.9-97.1)
Dominant model	GG+AG *versus* AA	78/22	98/2	**13.8 (3.15-60.5)**
Recessive model	GG *versus* AG+AA	74/26	93/7	**4.6 (1.91-11.3)**

Two-sided Chi square (□2) test was used. OR (95% CI), odds ratio and confidence interval. Bald values statistically significant at $p<0.05$.

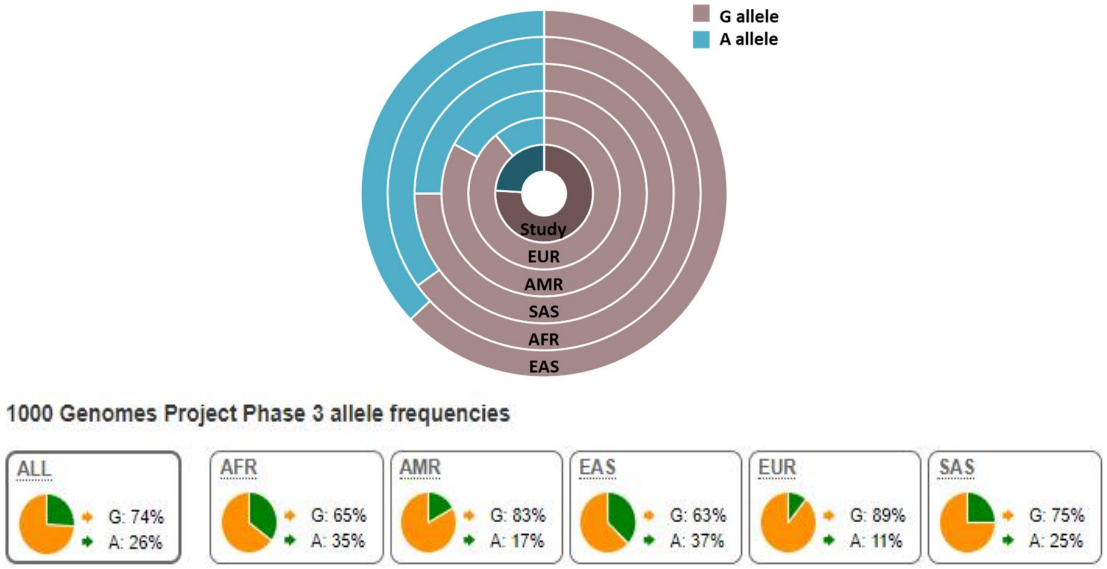

Fig. 3: Allele frequency of *AIRE* rs2075876 polymorphism in different populations. AFR: Africans; AMR: Americans; EAS: East Asians; EUR: European; SAS: South Asians. Data source (www.ensemble.org).

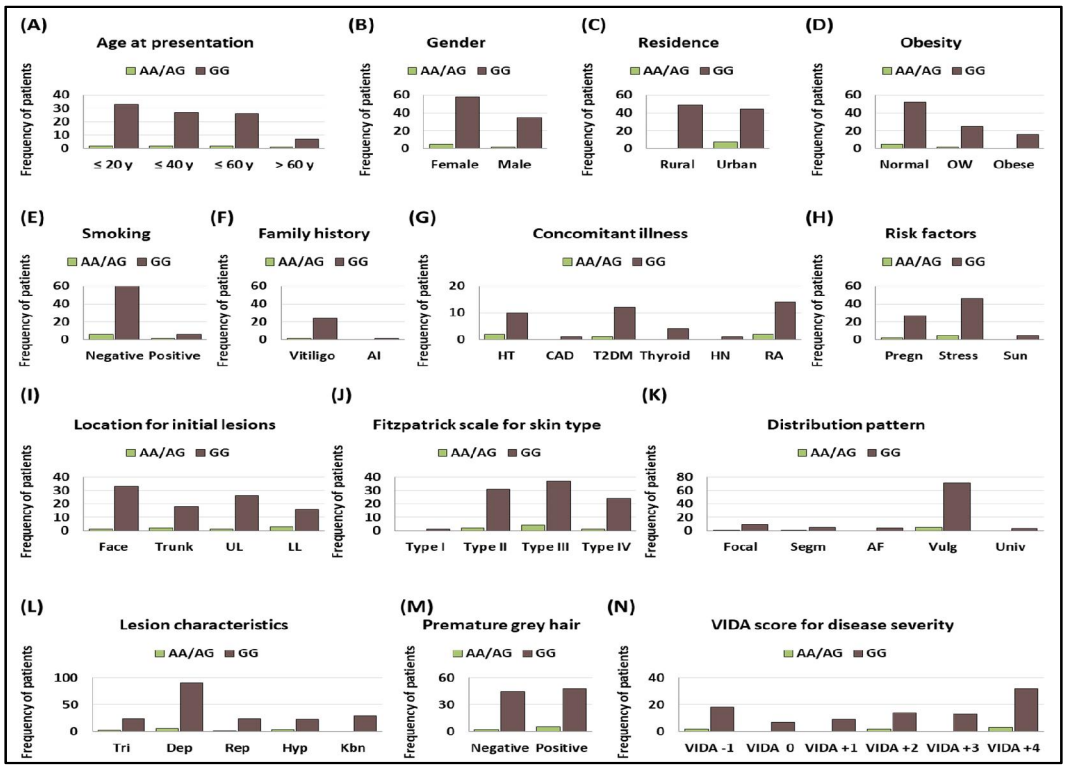

Fig. 4: Association of *AIRE* rs2075876 polymorphism with clinical features. Chi-square test was employed. All p values were non-significant (>0.05). OW: overweight, AI: autoimmune, HT: hypertension, CAD: coronary artery disease, T2DM: type 2 diabetes mellitus, HN: halo nevi, RA: rheumatoid arthritis, pregn: pregnancy, sun: sun exposure, UL: upper limb, LL: lower limb, Segm: segmental, AF: acrofacial, vulg: vulgaris, Uni: universalis, Tri: trichrome, Deep: depigmented, Rep: follicular re-pigmentation, Hyp: hypopigmentation, Kbn: Koener phenomenon

Table 9: Multivariate regression analysis.

Model summary				Statistics		Coefficients				
Model	R	Adjusted R^2	SE	R^2 Change	Sig. F change	Independent predictors	B	Beta	95% CI	Sig.
1	0.380	0.135	3.05	0.144	<0.001	(Constant)	-2.186			0.053
						Distribution	1224	0.380	0.626-1.822	<0.001
2	0.452	0.188	2.95	0.060	0.008	(Constant)	-2.537			0.022
						Distribution	1.193	0.370	0.613-1.773	<0.001
						FH	1.855	0.246	0.499-3.212	0.008
3	0.501	0.228	2.88	0.047	0.016	(Constant)	-2.358			0.029
						Distribution	0.908	0.282	0.297-1.519	0.004
						FH	1.895	0.251	0.572-3.218	0.005
						Duration	0.124	0.233	0.023-0.225	0.016

Dependent variable is VASI score. Independent variables in the linear regression model were age, gender, and family history of vitiligo, stresses, sun exposure, associated grey hair, disease duration, distribution pattern and AIRE genotype. Predictors in model 1: Distribution pattern, model 2: Distribution pattern and family history, while model 3: Distribution pattern, family history and disease duration.

4. Discussion

Vitiligo develops at any age, although half of patients have vitiligo before 20 years. No difference in prevalence exists according to sex, skin type, or race (Alikhan *et al.,* 2011).

The available patients recruited from the Dermatological Clinic during sampling were the mean age of the patients was 29.1 ±4.1 years. and most common age grouping was ≤ 40 y represent 29% in vitiligo group and in control group The mean age of the patients was 36.3 ±15.7 years, most common age grouping was ≤ 40 y represent (39%) in control which coincided with Kota *et al.* (2019).

On comparison between patients and control subjects, AA+AG was significantly the most prevalent genotype among controls (7% in patients *versus* 26 % in controls), while GG genotype was predominant in the vitiligo group (93% in patients *versus* 74% in controls). Consistently increased risk by 13.8% than in control group.

In contrast, carrying one minor allele (A/G genotype) renders protection against vitiligo under heterozygote and dominant genetic association models.

In agreement with our results, the minor G allele and GG genotype were associated with increased risk of developing Graves' disease in Moscovite and Chinese (Chen *et al.,* 2019) and SLE risk in Portuguese (Narita *et al.,* 2011) A recent experimental study described two distinct SNPs of AIRE is AIRE−230Y, and AIRE−655G. AIRE−230T haplotype transcriptionally modifies AIRE expression and influence negative selection, elevating the risk of autoimmunity (Tazi-Ahnini *et al.,* 2008). Various SNPs in the AIRE genetic sequence have garnered attention; however, only a minority of case-control studies have observed an association between gene polymorphism and susceptibility to diseases, including vitiligo (Tazi-Ahnini *et al.,* 2008). Strong association has been reported between a single nucleotide polymorphism (SNP) in exon 9 of CAT and vitiligo (Casp *et al.,* 2002; Tazi-Ahnini *et al.,* 2008). Ethical differences and phenotype heterogeneity might explain the variability of risky allele in AIRE gene polymorphism.

Our data suggest an important role of the AIRE gene in the pathogenesis of Vitiligo disorder. However, some limitations need to be addressed. First, the study population represented Suez Canal Area; studies involving other geographical regions in Egypt are needed prior to generalization of the results. Second, vitiligo patients enrolled in the study had clinical presentation and patterns, replication on cohorts with wider variety of disease phenotype is recommended. Moreover, functional consequence of the polymorphism was not studied in the lab, correlation analysis between the signal peptide variant and expression levels in patients would have added more value on the study outcomes.

5. Conclusion

The study concluded an important role of the AIRE gene in the pathogenesis of Vitiligo disorder. Also, Vitiligo is known to be mediated in part by heritable variants in co-signaling genes regulating immune responses, and AIRE gene seem to be a promising candidate gene. Taking

advantage of the great advances that genetics has brought into the studies of vitiligo have paved the road for a new era of clinical translation of scientific research.

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