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IL-2RA rs2104286 gene Polymorphismamong Egyptian population with Multiple Sclerosis: a case control study

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- T-ARMS-PCR
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Abstract

Background: Multiple Sclerosis (MS) is a multifocal autoimmunedisabling disease of the central nervous system. While several autoimmune diseases have been linked to IL-2RA gene polymorphisms,only a limited number of studies have investigated the association between the IL-2RA rs2104286 polymorphism and the risk of multiple sclerosis, with conflicting results. **Purpose:** The aim of this study was to explore the possible association between the IL-2RA gene rs2104286 polymorphism and the risk of MS in the Egyptian population. **Methods:** Two hundred Egyptians were included in this study; 100 patients diagnosed with MS,and 100 age-matched healthy volunteers were enrolled as controls. One ml of blood was withdrawn from all subjectsand delivered to EDTA-containing tubes for genotyping of IL-2RA gene(rs2104286)polymorphism by T-ARMS-PCR method. **Results:** Our resultsshowed anon-significant difference in IL-2RA (rs2104286) genotypes or allele distribution between MS patients and controls. Furthermore, there was no statistically significant difference between the studied SNP and other clinic-demographic characteristics of MS including disease onset, disease duration, EDSS score, or number of relapses, except for the AG genotypewhich was significantly correlated with a higher median age of the disease onset. **Conclusion:**The lack of associationobserved between the IL-2RA (rs2104286) polymorphism and the risk of MS, as well as most clinical and demographic parameters,suggest that the IL-2RA gene (rs2104286) may not serve as a reliable biomarker for MS susceptibility in the Egyptians. These findings highlight the need for further research in larger and more diverse populations to validate these observations.

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1. Introduction

Multiple sclerosis (MS) is an autoimmune chronic neurological disease characterized by inflammatory progressivedemyelination in the central nervous system (CNS)that mediated by T cells.Nearly 2.3 million individuals globally have been diagnosed with MS, with North America, Australia andWestern Europereporting the highest prevalence**[1, 2].**

While Middle Eastern and North African countries were previously recognized as regions with a low to moderate risk of multiple sclerosis (MS), recent findings indicate that the prevalence of MS is rising in this area**[3, 4].**In Egypt, approximately 59,671 individuals have been diagnosed with MS, reflecting a prevalence rate of 59 cases per 100,000 people**[5].**MS typically begins in early adulthood, usually between the ages of 20 and 50 **[6, 7],** with a higher incidence among females compared to males (3:1 ratio)**[8, 9]**.

Multiple sclerosis is a multifactorial disease arising from a complex interaction of both genetic and environmental factors**[10, 11]**.Genome-wide association studies (GWAS) have identified IL2RA as a genetic risk factor for MS **[12].** The interleukin 2 alpha-receptor (IL-2RA) gene is located on the short arm of chromosome 10 (p15.1) **[13].** This gene encodes the IL-2RA subunit, which is critical for the proliferation and differentiation of T cells by its role in amplifying IL-2 signal **[14, 15].**

Expression of IL-2RA has been associated with the development of various autoimmune and inflammatory diseases, such as Graves' disease **[16],** systemic lupus erythematosus (SLE) **[17],** rheumatoid arthritis (RA) **[18],** generalized vitiligo**[19],** inflammatory bowel disease (IBD) **[20]**, intermediate uveitis **[21]**, type 1 diabetes (T1D) **[22]**, MS**[23]** and alopecia areata **[24].** Additionally, it was shown that the single nucleotide polymorphisms (SNPs) in or near the IL-2RA gene could be a risk factor for several autoimmune diseases including MS **[23,25, 26, 27, 28, 29].**

The rs2104286 is a polymorphism within the first intron of the IL-2RA gene **[30, 31].** Conflicting results were reported as regard the correlation of this SNP with the risk of MS. While the association between MS susceptibility and the IL-2RA SNP has been found among the population of some countries **[32,33, 34, 35],**other studies found insignificant associations **[36,37, 38].**The aim of the current study is to assess the possible association of the (rs2104286)SNP within the IL2RA gene among Egyptian population with the development of MS.

2. Subjects and Methods

2. 1. Study Participants

This case-control observational study was conducted in the “Medical Biochemistry and Molecular Biology Department” in Mansoura Faculty of Medicine, Mansoura University, between March 2022 and October 2023. It included 200 Egyptian participants: 100 patients diagnosed with multiple sclerosis (MS), aged between 20 and 52 years, and 100 age-matched healthy subjects as a control group. Multiple sclerosis patients were selected from the “Neurology Department”, Mansoura University Hospitals, between March 2022 and January 2023.Control subjects in the study had nofamily history or symptoms of MS, and were free from other autoimmune diseases, cancers, or serious

infections. Written informed permission was given by each participant, and the Institutional Review Board (IRB) of Mansoura Faculty of Medicineauthorized the study**(IRB code MS.22.01.1824).**

The patients enrolled in the study were diagnosed with MSaccording to the 2017 McDonald Criteria **[39,40].**Individuals were excluded if they had any of the following conditions: rheumatoid arthritis (RA), autoimmune thyroiditis, SLE, scleroderma,hepatitis B virus (HBV) infection, HIV infection, or any serious infections, cancer, or if they were pregnant or breastfeeding.

A full medical history was taken for all patients, emphasizing the age at the disease onset, disease duration, and number of relapses. A full neurological examination was performed. Cerebrospinal fluid (CSF) analysis,Magnetic Resonance Imaging (MRI), and Evoked Potential test,measuring the electrical activity in areas of the brain and spinal cord inresponse to stimulation, were conducted, and the Expanded Disability Status Scale (EDSS) score was measured. Noteworthy, the EDSS is used to assess the level of disability in MS and to track its progression over time. It is a scoring system that ranges from 0, indicating normal neurological function, to 10, representing death caused by MS, with scores increasing in 0.5-point increments**[41].**

2. 2. Methods:

2. 2. 1. Blood Collection and Genomic DNA Extraction

Each participant had one milliliter (1 ml) of peripheral blood drawn by venipuncture, which was then placed in tubes with ethylene diamine tetra-acetic acid (EDTA) to inhibitblood clotting. After being appropriately labeled, the samples were kept for further molecular analysis at -20°C.GenomicDNA was isolated using the GeneJET Whole Blood spin column Genomic DNA extraction kits (Thermo Scientific, USA, Cat. no. #K0781) and preserved at −20°C until further use. Using a NanoDrop 2000c spectrophotometer,concentration and purity of DNA were measured, while DNA quality was assessed through ethidium bromide-stained agarose gel electrophoresis.

2. 2. 2. Genotyping of IL-2RA gene (rs2104286) polymorphism:

IL-2RA (rs2104286) gene polymorphism was analyzed by allele-specific tetra-primer amplification refractory mutation system polymerase chain reaction (T-ARMS-PCR).The tetra oligonucleotide primers used in this study are presented in **Table 1**and were purchased from Invitrogen (ThermoFisher Scientific).For each sample, a total PCR reaction of 25 µL mixture volume was prepared, consisting of 12.5 µL of PCR master mix (2X), 5 µL of template DNA (20 ng/µL), 1 µL (10 pmol/µL) of each tetra primer (forward and reverse), and 3.5 µL of Nuclease free water.

The following amplification program was used for programming the PCR thermal cycler (Applied Biosystems, model 2720): Initial Denaturation at 95 ˚C for 3 minutes; followed by 35 repeated cycles of: Denaturation (95 ˚C for 30 seconds), Annealing (62.5 ˚C for 30 seconds)and Extension (72 ˚C for 30 seconds);then Final Extension at 72 ˚C for 5 minutes. For separation of the PCR products, agarose gel (2.5%)(Thermo Scientific TopVision Agarose, USA, Cat. no #R0491) was used, utilizing a 50 bp DNA ladder

(GeneRuler 50 bp DNA Ladder, ready-to-use by Thermo Scientific, USA, Cat. no #SM0373) as a molecular weight marker. According to the sequence of the used primers, outer (G) forward and inner (G) reverse primers generated a 164 bp fragment when the G allele was present. Inner (A) forward and outer (A) reverse primers generated a 242 bp fragment when the A allele was present. An internal control fragment of 349 bp was generated using outer primers, which was independent of the presence of either the A or G allele **[38]** (**Figure 1**).

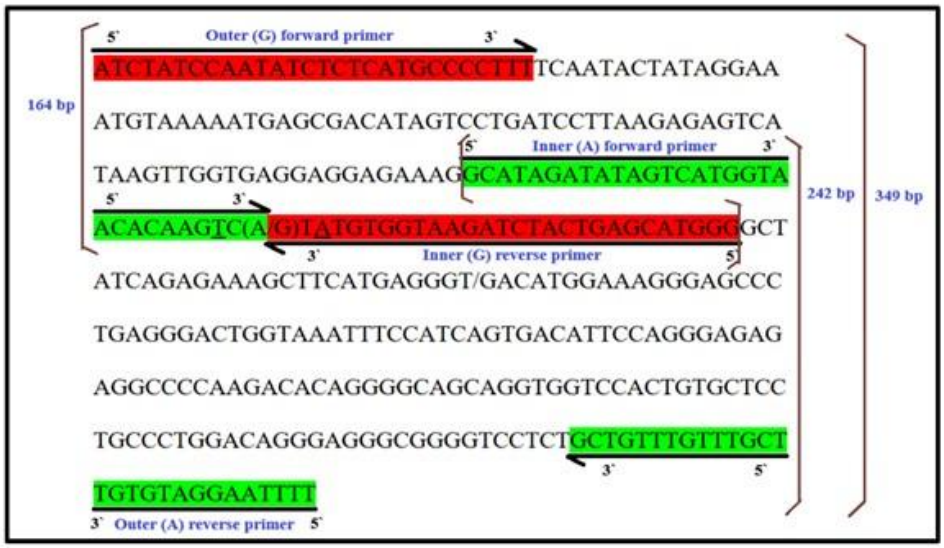

Figure1: TheIL-2RA gene(rs2104286)polymorphismdemonstratedtheprimers used (G>>red, A >>green).

Table (1): Primersused in thestudyfor amplification of IL-2RA gene (rs2104286) polymorphism:

Primer name	**Sequence '5->3'**
Outer (G) forwardprimer	ATCTATCCAATATCTCTCATGCCCCTTT
Outer (A) reverse primer	AAAATTCCTACACAAGCAAACAAACAGC
Inner (A) forward primer	GCATAGATATAGTCATGGTAACACAAGGCA
Inner (G) reverse primer	CCCATGCTCAGTAGATCTTACCACAGAC

The genotype of the sample would be identified by the appearance of the following bands: Homozygous wild genotype (A/A): two bands (349 bp, 242 bp); Homozygous (G/G)mutant genotype: Two bands (349 bp and 164 bp). Heterozygous (A/G)mutant genotype: Three bands (349 bp, 242 bp and 164 bp)**[38].** In our study, the homozygous (G/G) genotype could not be detected**(Figure 2).**

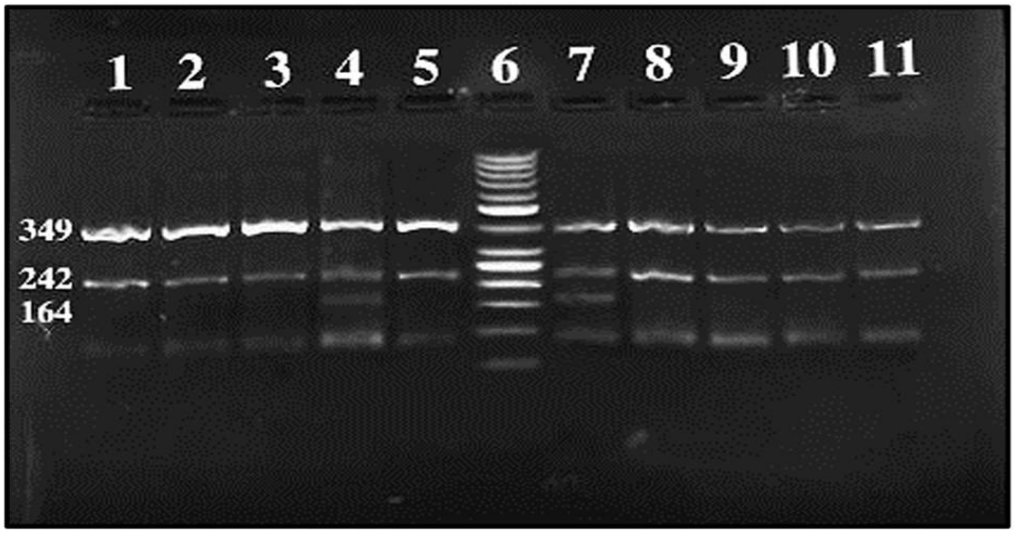

Figure2: IL-2RA rs2104286 PCR product. Lane (6) contains a 50 bp ladder. Lanes(1, 2, 3, 5, 8, 9, 10, 11) contain 349 and 242 bp bands (A/A genotype). Lanes (4, 7) contain 349, 242, and 164 bp bands (A/G genotype).

2. 3. Statistical Analysis

Statistical data were analyzed using the Statistical Package for Social Science **(IBM SPSS Statistics for Windows, Version 25.0; IBM Corp., Armonk, NY, released 2017).** Categorical variables were presented as frequencies and percentages.The "Chi-Square test "was used to compare qualitative data for two groups. Quantitative data were initially tested for normality using "Kolmogorov-Smirnov" to determine parametric or non-parametric distribution.The data were presented as "median and interquartile range"as they were nonparametrically distributedand the "Mann-Whitney U test" was employed to assess the significance of differences between two groups.Additionally, odds ratios (ORs) were calculated to evaluate the strength of association between exposure and outcome, 95% confidence interval (95%CI) to estimate the precision of the OR, and p value to measure the probability of the observed data. The SNPs were tested for Hardy–Weinberg equilibrium (HWE) to ensure that the selected study groups were representative of the Egyptianpopulation. If p value was ≤ 0.05 for any of the tests used, results were considered statistically significant.

3. Results:

3.1. Demographic characteristics of the study groups:

Table (2): Demographic features of the MS and control groups:

Characteristics	Control N=100	MS N=100	Test	*P*
	N (%)	N (%)		
Sex: **Male** **Female**	 41 (41.0%) 59 (59.0%)	 28 (28.0%) 72 (72.0%)	X^2=3.739	0.053
	Median (IQR)	**Median (IQR)**		
Age (years)	36 (21-55)	35 (20-52)	U=4794	0.614

IQR: Interquartile range, X^2: Chi-Square test, U: Mann Whitney test, p: probability.

Age and sex distributions are shown in **Table 2**. The percentage of males in the control group in comparison to the MS group was 41.0% vs. 28.0% while the female'spercentage in the control group compared to the MS group was 59.0% vs. 72.0%. Regarding the sex, nostatistically significant difference was found between the patient and the control groups (p = 0.053).The median age was 35 years among the MS group whereas the median age of the controls was 36 years. There was also no significant difference between the MS and the control groups regarding age; (p =0.614).

Table (3): Clinical characteristics of the MS group:

Characteristics of MS Group	MS (N = 100) Median (IQR)
Age of onset (years)	30.5 (19-51)
Disease duration (years)	2.0 (1-17)
Number of relapses	2.0 (1-10)
EDSS score	1.0 (0.5-5)

EDSS: Expanded Disability Status Scale; IQR: Interquartile range.

3.2. Clinical characteristics of the MS group:

Table 3 shows the age of the MS onset, duration of the disease, number of relapses, and EDSS score for the MS group. The median age of onset of MS is 30.5 (19.0–51.0) years. The median disease duration is 2 (1.0–17.0) years. The median number of relapses is 2 (1–10), and the median EDSS score is 1 (0.5–5).

3.3. Distribution of IL-2RA gene rs2104286 polymorphism in the two study groups:

Table(4): Genotype and Allele frequency of IL-2RA gene rs2104286 polymorphism in MS vs. control group:

IL-2RA rs2104286	Control N (%)	MS N (%)	*P*	X^2	OR (95%CI)	P value for HWE (control group)
Genotype: AA	85 (85.0%)	86 (86.0%)	0.841	0.040	1.05(0.64-1.72)	0.417
AG	15 (15.0%)	14 (14.0%)	0.841	0.040	0.95(0.58-1.55)	
GG	0 (0.0%)	0 (0.0%)	-	-	-	
Alleles: A	185(92.5%)	186(93.0%)	0.847	0.037	1.05(0.65- 1.68)	
G	15(7.5%)	14(7.0%)			0.95(0.59- 1.53)	

X2: Chi-square test, OR: odds ratio, CI: confidence interval, p: probability.

Table 4 shows that the genotypes of the studied SNP in the control groupwere in accordance with HWE (P=0.417). The wild homozygous genotype AA frequency was (86.0%) in MS patients compared to (85.0%) in healthy individuals. The frequency of the heterozygous genotype AG was (14.0%) in MS patients and (15.0%) in healthy controls.Furthermore, the A allele was (93.0%) in

MS patients and (92.5%) in healthy controls, and the G allele was (7.0%) in MS patients and (7.5%) in healthy controls. According to these results, no statistically significant difference wasexistingconcerningthe distribution of either(AA and AG) genotypesor (A and G alleles) between MS and healthy control groups (p=0.841 and p=0.841 andp=0.847) respectively.

3.4. Association between IL-2RA gene rs2104286 polymorphism with different demographic and clinical data:

Table (5): Association of IL-2RA gene rs2104286 polymorphism with gender among the studied groups:

		Control		MS	
Genotype	**Gender**	**N (%)**	**X^2 (*p*)**	**N (%)**	**X^2 (*p*)**
AA	**Male**	37(43.5%)	1.499 (0.221)	24(27.9%)	0.003 (1^)
	Female	48(56.5%)		62(72.1%)	
	P	0.278		**<0.001***	
AG	**Male**	4(26.7%)		4(28.6%)	
	Female	11(73.3%)		10(71.4%)	
	P	0.071		0.109	

X^2: chi-square test; ^: (FET) Fisher Exact test; p: probability, p<0.05 is considered significant.

The association between IL-2RA gene rs2104286 polymorphism and gender among the studied groups is analyzed in **Table 5**. In the MS group, the AA genotype was significantly more prevalent among females with MS than males (*p*<**0.001**).However, no significant association was found between AAgenotypeand gender among control group(*p*=0.278). Moreover, the distribution of genotypes AGdid not show any significant difference between males and females either in the MS or in the control group(*p*>0.05).

Table (6): Association of IL-2RA gene rs2104286 polymorphism with clinical data among patients with MS:

Characteristics of MS Group	**IL-2RA rs2104286**			
	AA	**AG**	**Test (U)**	***P***
	N=86	**N=14**		
Age at disease onset (years)	29(19-51)	40(29-42)	280.0	0.001*
Disease duration (years)	2 (1-17)	2 (1-10)	502.0	0.294
Number of relapses	2 (1-10)	2 (1-5)	596.0	0.950
EDSS score	1 (0.5-5)	2 (0.5-2.5)	478.0	0.200

EDSS: Expanded Disability Status Scale, U, Mann Whitney test

The association between IL-2RA gene rs2104286 polymorphism and clinical data among patients with MS is analyzed in **Table 6**. The median age of disease onset was higher for individuals with the AG genotype (40 years) compared to those with the AA genotype (29 years), with a significant *p* value (*p*=**0.001**). There were no significant associations between IL-2RA gene rs2104286 polymorphism and disease duration, EDSS scores, or number of relapses.

4. Discussion

Multiple sclerosis (MS) is a chronic autoimmune disorder characterized by inflammationand demyelination in the CNS. It is

the commonest cause of non-traumatic neurological impairment in young adults**[42, 43].** The incidence and prevalence of MS are increasing worldwide, along with the socioeconomic impact of the diseaseburden **[4]**.Numerous genetic factors are believed to contribute to MSrisk **[44].** GWAS have demonstrated a strong correlationbetween IL-2RA gene SNPs and theraised risk of autoimmune diseases, including MS**[35, 45, 46, 47].** To the best of the authors' knowledge, ourstudyisthefirsttoinvestigatethe possible association betweentheIL-2RA(rs2104286) SNP andMSinEgyptian population.

First, in our study, we attempted to explore the demographic characteristics of the study groups, where we found no significant difference in age or sex distribution between multiple sclerosis (MS) patients and the control group (p= 0.614 and 0.053, respectively).The predominance of females (72.0%) in the MS group aligns with global and regional epidemiological trends, particularly within the Middle East and North Africa (MENA) region, where the female-to-male ratio in MS cases is typically elevated with 2.57:1 ratio **[48].** This sex distribution is consistent with **Farghaly et al. [49], Khedr et al. [50] and El-Samahy et al. [51]**studies on MS in the Egyptian population. Similar observations have also been documented in international studies, such as those by **Xia et al.** among Han and Hui ethnic groups in China**[47],** and **Jahromi et al.** in the Iranian population **[52]**,where no significant associations were found between age or sex and MS status.In contrast, **Stefanović et al. [35]**studied the IL-2RA gene rs2104286 polymorphism and observed a significant difference between SerbianMSandcontrolgroupsinageandsexdistribution.Thisdifference may be due to large sample size and varied MS group subtypes included in their study.

The IL-2RA gene rs2104286 polymorphism is an intronic variant polymorphism located in the 5- proximalintron1regionoftheIL-2RA geneandinvolvesasubstitutionof an A nucleotide with a G within the gene[**53,54,55**].Inthecurrent study, Genotype and allele analysis of the investigated IL-2RA gene rs2104286 polymorphism revealednon-significant association between the rs2104286 and the risk of MS (p>0.05).

The observed frequency of wild homozygous AA genotype was (86.0%) in MS patients and (85.0%) in controls (*p*=0.841). The frequency of the heterozygous genotype AG was (14.0%)intheMSpatientsand(15.0%)inhealthycontrols(p=0.841), while the GG genotype could not be detected. The absence of any detected association between our SNP and MS may be attributed to the small sample size used in this study. This limitation, likely due to the low prevalence of MS in Egypt, may have reduced the statistical power needed to identify significant differences.This result may also be supported by **Elghzaly et al. (2015**)who previously studied rs2104286polymorphism association withSLE, which is autoimmuneinnature, and found no significant association of thisSNP and SLE in theEgyptianpopulation**[56].**

Regarding the deficient GG genotype in our study results, and according to the **NCBI (2024)**,thefrequencyoftheGGgenotypein other African populations was0.0%whichwasconsistentwithourresult**[57].**Similarly,**Elghzalyetal.,**studiedthisSNPin the Egyptian

population and did not detect the GG genotype, which was consistent with our results**[56].**

In agreement with our study, **Ainiding et al.**,**Akkad et al.**and **D′Cunha et al.** studied the rs2104286 polymorphism in Japanese, German, and Indian populations, respectively, and found no significant association in all genotypes between the IL-2RA gene rs2104286 polymorphism and MS development (p>0.05)**[37, 58, 59].**

Contrary to our findings, **Xia et al. (2018)** studied this SNP in Han and Hui vicinities in China and showed that the GG and AG genotypes distribution washigher in the MS patients than in controls with a significant riskforMSdevelopment**[47].**Additionally,**Stefanović et al. (2020)** stated that the AG genotype, though it was less frequent in Serbian MS patients than in controls which is consistent with our results, but with a significant protective effect against MS development **[35]**.Thesedifferencesmightbeduetolargersample sizes, diverse dietary habits, sun exposure, infection susceptibilityand ethnic diversity. Also, **Xia et al.** study was done on different subtypes of MS, unlike our study**[47].**

Our findings indicated that there was no significant difference in the allele frequency of the rs2104286 polymorphism between individuals with MS and the control group. (p=0.847). This result matches with another Egyptian study by **Elghzaly etal.**Whostatedthatnosignificantassociationbetweentheallele frequency of this SNP and SLE was detected**[56].** This result is also in agreementwith**Matielloetal.,Akkadetal.**and**Traboulsee etal.**case-controlstudiesintheAmerican,GermanandCanadian populations**[30, 58, 60]**.

In contrast to our results,**Rubio et al.** found a significant association between the A allele and increasedsusceptibility to MS in an Australian population**[32],** as did **Weber et al.** in their study on French and German populations**[45].**Similarly,meta-analysis by**Wangetal. (2011)**included eightcase-control studiesfromAmericanandEuropeanpopulations,foundthattheAAgenotypeandtheAallele ofthers2104286polymorphismwere linked to a higher risk of MS **[61].** Another meta-analysis performed by **Wang and Chen,**includingeleven case-control studies, concluded that the A allele was associated with an elevatedrisk of MS in Caucasian and Asian populations**[31].**Onepotential explanation is the possible ethnic variation and the diversity in the haplotype structure of the IL-2RA gene **[62].**

Additionally, **Xia et al.** showed that the frequency ofG allele was higher in Chinese patients with MS than in control group with a significant risk association with MS development**[47].**On the other hand, a study of the IL-2RA rs2104286 SNP by **Cavanillas et al.**, in Madrid andAndalucia populations from Spain, revealed that the G allele had a significant protective effect against MS development**[33]**. Similarly, **Stefanović et al.** reportedthattheGallelewasprotectiveagainstMSonsetinSerbian patients**[35]**.This contradiction may be due to the diverse ethnic composition inSpainandSerbia.Also,theGallelefrequencyinourcontrolgroup (7.5%) was notably smaller than that observed in otherstudy groups.

Moreover, a meta-analysis performed by

Zhou et al. (2024), across different populations, indicated that the IL-2RA rs2104286 polymorphismwas identified as a susceptibility factor associated with higher MS risk in the Caucasian population, whereas no such association was observed in the Asian population.In agreement with our findings, they observed that carriers of AG genotype revealed a reduced risk of MSthanthose withofAA,butwithasignificantpvalue.However,they stated that their results need to be confirmed in future studies to find obvious evidence that this genetic variant is functionally significant to MS pathology**[63].**

Regarding the clinical criteria of the diseased patients, our study revealed that the median age at the onset of MS was 30.5 (19-51)years, median disease duration was 2 (1.0–17.0) years. Themediannumberofrelapseswas2(1–10),andthemedianEDSSscore was1(0.5-5),whichareconsidered lower than the results of**Zakaria et al. (2016)** in the Egyptian population, where the median disease duration in their study was 4.0 (2.0–8.0) years, the median number of relapseswas 3.0 (2.0–5.0), and the median EDSS score was 3.0(2.0–6.0). This is primarily attributed to delayed diagnosis—exceeding two years—in 28% of cases reported in the study by **Zakaria et al.** which may be explained by the fact that neurologists were not the primary physicians managing most of these patients**[64].**

Generally, inthecurrent study,nostatisticallysignificantdifference was found between the studied SNP and the disease duration, number of relapses, or EDSS score.Notably, however, the AG genotype was significantly linked to a higher median age at disease onset than those with the AA genotype (40 vs. 29 years) (p= 0.001). Our findings agreewith**Rubio et al.**, who studied the IL-2RA rs2104286 polymorphism in Australians and found no significant association between the IL-2RA gene rs2104286 and age at disease onset,gender or clinical course of the disease**[32].**

On the other hand, **Ainiding et al. [37],** who studied IL-2RA (rs2104286) in a Japanese population, reported that annualized relapse rates (AAR), a measure used to quantify relapse frequency in MS patients, the rs2104286-AA genotype, which in turn affected the number of relapses. Also, **Xia et al.** studied the IL-2RA gene polymorphism (rs2104286) in theHan and Hui progenies ofthe Chinese population and found that there was a significant difference regarding EDSS score, and a significant association of rs2104286 with MS risk,which was contradictory to our result **[47].**Variations between our results and those of previous studies may be explained by ethnic differences between Egyptians and Asiansunique to each population.

Conflicting results are common in genetic association studies owing to the complexity of disease, where multiple genes and different genetic and environmental backgrounds may contribute to MS development**[31, 65].**These different findings in our study and the others may also be due to the differences in the characteristics of the selected cases, differences in frequency of gene polymorphisms between different traces, and different study design may be due to the smaller sample size.Hence, further studies are required to gain a deeper understanding of the role these genetic variants play in MS susceptibility.

5. Conclusion:

To the best of our knowledge, this study the first to explore the possible association between the IL-2RA (rs2104286)and the risk of MS in the Egyptian population. The results of the current study showed no significant association between IL-2RA gene (rs2104286) polymorphism and the risk of MS.Furthermore, IL-2RA gene rs2104286 polymorphism appears to have no evident influence on disease clinical characteristicsexcept for the AG genotype of the IL-2RA rs2104286,which presented asignificantly highermedianageofdiseaseonset.These findings suggest that the IL-2RA gene (rs2104286) polymorphism may not serve as a reliable biomarker for MS susceptibility or progression and highlight the need to explore other genetic factors that may be associated with MS in the Egyptian population.

6. Limitations and Recommendations:

This study involves a relatively small sample from the Egyptian population. Therefore, further research including a larger and more ethnically diverse group of patients would be valuable to validate the significance of the IL-2RA gene (rs2104286) polymorphism in the MS development.Also,considering the complexity of MS, more research is needed to study the association between the studied SNPs and other subtypes of MS. Further research investigating the expression of the IL-2RA gene may help validate the findings of the present study.

7. References:

1. **GBD 2016 Multiple Sclerosis Collaborators.** Global, regional, and national burden of multiple sclerosis 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 2019;18(3):269–285.
2. **Mercadante S.** Palliative Care Aspects in Multiple Sclerosis. *J Pain Symptom Manage*. 2024;67(5):e425–437.
3. **Yamout BI, Assaad W, Tamim H, Mrabet S, Goueider R.** Epidemiology and phenotypes of multiple sclerosis in the Middle East North Africa (MENA) region. Mult Scler J Exp Transl Clin. 2020;6(1):205521731984188.
4. **Qian Z, Li Y, Guan Z, Guo P, Zheng K, Du Y, et al**. Global, regional, and national burden of multiple sclerosis from 1990 to 2019: Findings of global burden of disease study 2019. *Front Public Health*. 2023;11:1073278.
5. **The Multiple Sclerosis International Federation.** Atlas of MS, 3rd Edition. September 2020. Available from: https://www.atlasofms.org/map/united-kingdom/epidemiology/number-of-people-with-ms [Accessed 2024 Oct 22].
6. **Reich DS, Lucchinetti CF, Calabresi PA.** Multiple Sclerosis. *N Engl J Med*. 2018;378(2):169–80.
7. **Haki M, AL-Biati HA, Al-Tameemi ZS, Ali IS, Al-hussaniy HA**. Review of multiple sclerosis: Epidemiology, etiology, pathophysiology, and treatment. *Medicine* (Baltimore). 2024;103(8):e37297.
8. **Gold SM, Willing A, Leypoldt F, Paul F, Friese MA**. Sex differences in autoimmune disorders of the central nervous system. *Semin Immunopathol*. 2019;41(2):177–88.
9. **Abbasi M, Farokhnezhad M, Taheri A, Lotfi A, Arghavanfar H.** Effects of Aerobic Training on Sex Hormones in A Cuprizone Rat Model of

Multiple Sclerosis. *Int J Fertil Steril.* 2024;18(3):234–9.

10. **Biernacki T, Sandi D, Kincses ZT, Füvesi J, Rózsa C, Mátyás K, et al.** Contributing factors to health- related quality of life in multiple sclerosis. *Brain Behav.* 2019;9(12):e01466.
11. **Gouider R, Souissi A, Mrabet S, Gharbi A, Abida Y, Kacem I, et al**. Environmental factors related to multiple sclerosis progression. *J Neurol Sci.* 2024;464:123161.
12. **Babron MC, Perdry H, Handel AE, Ramagopalan SV, Damotte V, Fontaine B,et al.**Determination of the real effect of genes identified in GWAS: the example of IL2RA in multiple sclerosis. *Eur J Hum Genet.* 2012;20(3):321–5.
13. **Sawicka B, Borysewicz-Sańczyk H, Wawrusiewicz-Kurylonek N, Aversa T, Corica D,**Gościk J, et al. Analysis of Polymorphisms rs7093069-IL-2RA, rs7138803-FAIM2, and rs1748033-PADI4 in the Group of Adolescents with Autoimmune Thyroid Diseases. *Front Endocrinol (Lausanne).*2020;11:544658.
14. **Robb RJ, Greene WC, Rusk CM.** Low and high affinity cellular receptors for interleukin 2. Implications for the level of Tac antigen. *J Exp Med.* 1984;160(4):1126–46.
15. **Hernandez R, Põder J, LaPorte KM, Malek TR.** Engineering IL-2 for immunotherapy of autoimmunity and cancer. *Nat Rev Immunol.* 2022;22(10):614–28.
16. **Brand OJ, Lowe CE, Heward JM, Franklyn JA, Cooper JD, Todd JA, et al.** Association of the interleukin-2 receptor alpha (IL-2Rα)/CD25 gene region with Graves' disease using a multilocus test and tag SNPs. *Clin Endocrinol (Oxf).* 2007;66(4):508-512.
17. **Carr EJ, Clatworthy MR, Lowe CE, Todd JA, Wong A, Vyse TJ, et al.** Contrasting genetic association of IL2RA with SLE and ANCA–associated vasculitis. *BMC Med Genet*. 2009;10(1):22.
18. **Hinks A, Ke X, Barton A, Eyre S, Bowes J, Worthington J,et al.** Association of the IL2RA/CD25 gene with juvenile idiopathic arthritis. *Arthritis Rheum.* 2009;60(1):251-257.
19. **Jin Y, Birlea SA, Fain PR, GowanK, Riccardi S, HollandP J, et al.**Variant of TYR and autoimmunity susceptibility loci in generalized vitiligo. *N Engl J Med*. 2010;362(18):1686-1697.
20. **BouzidD,AmouriA,FouratiH,MarquesI,Abida O,TahriN,etal.**Polymorphisms in the IL2RA and IL2RB genes in inflammatory bowel disease risk. *Genet Test Mol Biomarkers*. 2013;17(11):833-839.
21. **Lindner E, Weger M, Ardjomand N, Renner W, El-Shabrawi Y.** Associations of Independent IL2RA Gene Variants with Intermediate Uveitis. *PLoS One*. 2015;10(7):e0130737.
22. **FerjaniZ, Bouzid D, Fourati H, Fakhfakh R, Kammoun T, Hachicha M, et al.** Association between the IL2RA polymorphism and type 1 diabetes risk: Family based association study.*Meta Gene*. 2016;10:118–122.
23. **Buhelt S, Søndergaard HB, Oturai A, Ullum H, von Essen MR, Sellebjerg F**. Relationship between Multiple Sclerosis-Associated *IL2RA* Risk Allele Variants and Circulating T Cell Phenotypes in Healthy Genotype-Selected Controls. *Cells.* 2019;8(6):634.
24. **Tishe ZH, Shawkat S, Popy MN, Ahmed A, Mumu SB, Apu MNH, et al.** Interleukin gene polymorphisms and alopecia areata: A systematic review and meta-analysis. *Medicine (Baltimore)*. 2024;103(8):e37300.
25. **Okada Y, Wu D, Trynka G, Raj T,TeraoC,IkariK,et al.** Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature*. 2014;506(7488):376-381.

26. **Hwangbo Y, Park YJ.** Genome-Wide Association Studies of Autoimmune Thyroid Diseases, Thyroid Function, and Thyroid Cancer. *Endocrinol Metab (Seoul)*. 2018;33(2):175-184.
27. **Dimberg J, Shamoun L, Landerholm K, Andersson RE, Kolodziej B, Wågsäter D**. Genetic Variants of the *IL2* Gene Related to Risk and Survival in Patients With Colorectal Cancer. *Anticancer Res*. 2019;39(9):4933-4940.
28. **Goyal M, Khanna D, Rana PS,Khaibullin T, Martynova E, Rizvanov AA,** et al. Computational Intelligence Technique for Prediction of Multiple Sclerosis Based on Serum Cytokines. *Front Neurol*. 2019;10:781.
29. **Inshaw JRJ, Cutler AJ, Crouch DJM, Wicker LS, Todd JA.** Genetic Variants Predisposing Most Strongly to Type 1 Diabetes Diagnosed Under Age 7 Years Lie Near Candidate Genes That Function in the Immune System and in Pancreatic β-Cells. *Diabetes Care*. 2020;43(1):169-177.
30. **Matiello M, Weinshenker BG, Atkinson EJ, Schaefer-Klein J, Kantarci OH**. Association of IL2RA polymorphisms with susceptibility to multiple sclerosis is not explained by missense mutations in IL2RA. *Mult Scler*. 2016;17(5):634–636.
31. **Wang XX, Chen T.** Meta-analysis of the association of IL2RA polymorphisms rs2104286 and rs12722489 with multiple sclerosis risk. *Immunol Invest*. 2018;47(5):431-442.
32. **Rubio JP, Stankovich J, Field J, Tubridy N, Marriott M, Chapman C, et al.** Replication of KIAA0350, IL2RA, RPL5 and CD58 as multiple sclerosis susceptibility genes in Australians. *Genes Immun*. 2008;9(7):624-630.
33. **Cavanillas ML, Alcina A, Núñez C, Bartolomé M, de las Heras V, Fernández- Arquero M, et al.** Polymorphisms in the IL2, IL2RA and IL2RB genes in multiple sclerosis risk. *Eur J Hum Genet*. 2010;18(7):794-799.
34. **Asouri M, Alinejad Rokni H, Sahraian MA, Fattahi S, Motamed N, Doosti R, et al.**Association of *HLA-DRA* and *IL2RA* Polymorphisms with the Severity and Relapses Rate of Multiple Sclerosis in an Iranian Population. *Rep Biochem Mol Biol*. 2020;9(2):129-139.
35. **Stefanović M, Životić I, Stojković L, Dinčić E, Stanković A, Živković M.** The association of genetic variants IL2RA rs2104286, IFI30 rs11554159 and IKZF3 rs12946510 with multiple sclerosis onset and severity in patients from Serbia. *J Neuroimmunol*. 2020;347:577346.
36. **Qiu W, Pham K, James I, Nolan D, Castley A, Christiansen, FT, et al.** The influence of non-HLA gene polymorphisms and interactions on disease risk in a Western Australian multiple sclerosis cohort. *J Neuroimmunol*. 2013;261(1-2):92-97.
37. **Ainiding G, Kawano Y, Sato S, Isobe N, Matsushita T, Yoshimura S, et al.** Interleukin 2 receptor α chain gene polymorphisms and risks of multiple sclerosis and neuromyelitis optica in southern Japanese. *J Neurol Sci*. 2014;337(1-2):147-150.
38. **Ahmadi H, Yassaee VR, Mirfakhraie R, Hashemi-Gorji F**. Association between single nucleotide polymorphisms rs12722489 and multiple sclerosis in Iranian patients with multiple sclerosis. *Curr J Neurol*. 2020;19(1):26-31.
39. **Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T,ComiG,et al.** Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol*. 2018;17(2):162-173.
40. **McGinley MP, Goldschmidt CH, Rae-Grant AD.** Diagnosis and Treatment of Multiple Sclerosis.*JAMA*. 2021;325(8):765-779.
41. **Meyer-Moock S, Feng YS, Maeurer M, Dippel FW, Kohlmann T.** Systematic literature review and validity evaluation of the Expanded Disability Status Scale (EDSS) and the Multiple Sclerosis

Functional Composite (MSFC) in patients with multiple sclerosis. *BMC Neurol*. 2014;14:58.

42. **Hauser SL, Bar-Or A, Cohen JA, Comi G, Correale J, Coyle PK, et al**. Ofatumumab versus Teriflunomide in Multiple Sclerosis. *N Engl J Med*. 2020;383(6):546-557.
43. **Weerasinghe-Mudiyanselage PDE, Kim JS, Shin T, Moon C.** Understanding the spectrum of non-motor symptoms in multiple sclerosis: insights from animal models. *Neural Regen Res*. 2024;19(1):84-91.
44. **Manuel AM, Dai Y, Jia P, Freeman LA, Zhao Z.** A gene regulatory network approach harmonizes genetic and epigenetic signals and reveals repurposable drug candidates for multiple sclerosis. *Hum Mol Genet*. 2023;32(6):998-1009.
45. **Weber F, Fontaine B, Cournu-Rebeix I, Kroner A, Knop M, Lutz S, et al.** IL2RA and IL7RA genes confer susceptibility for multiple sclerosis in two independent European populations. *Genes Immun*. 2008;9(3):259-263. doi:10.1038/gene.2008.14
46. **Hoffjan S, Akkad DA.** The genetics of multiple sclerosis: an update 2010. *Mol Cell Probes*. 2010;24(5):237-243.
47. **Xia ZL, Qin QM, Zhao QY.** A genetic link between CXCR5 and IL2RA gene polymorphisms and susceptibility to multiple sclerosis. *Neurol Res*. 2018;40(12):1040-1047.
48. **Moradi N, Sharmin S, Malpas C,Ozakbas S, Shaygannejad V, Terzi M,et al.** Utilization of Multiple Sclerosis Therapies in the Middle East Over a Decade: 2009-2018. *CNS Drugs*. 2021;35(10):1097-1106.
49. **Farghaly M, Langdon DW, Shalaby NM, Shehata HS, Abokrysha NT, Hassan A, et al.**Reliability and validity of Arabicversionofthebriefinternationalcognitiveasses smentformultiple sclerosis: Egyptian dialect.*Egypt J Neurol Psychiatry Neurosurg*. 2021;57(1).
50. **Khedr EM, El Malky I, Hussein HB, Mahmoud DM, Gamea A.** Multiple sclerosis diagnostic delay and its associated factors in Upper Egyptian patients. *Sci Rep*. 2023;13(1):2249.
51. **El-Samahy M, Anani M, Attia F, Abdulrehim M, Abdul Y, Gaber A,et al.** Assessment of H. pylori seropositivity in Multiple sclerosis and its relationship with disease severity in Egypt. *Egyptian Journal of Medical Microbiology*, 2024; 33(3): 103-109.
52. **Jahromi AS, Erfanian S, Roustazadeh A.** Association of *OX40L* gene polymorphism with multiple sclerosis in Iranians. *Heliyon*. 2024;10(6):e27304.
53. **Maier LM, Lowe CE, Cooper J, Downes K, Anderson DE, Severson C, et al.** IL2RA genetic heterogeneity in multiple sclerosis and type 1 diabetes susceptibility and soluble interleukin-2 receptor production. *PLoS Genet*. 2009;5(1):e1000322.
54. **Schwartz AM, Demin DE, Vorontsov IE, Kasyanov AS, Putlyaeva LV, Tatosyan KA, etal.** Multiple single nucleotide polymorphisms in the first intron of the IL2RA gene affect transcription factor binding and enhancer activity. *Gene*. 2017;602:50-56.
55. **Buhelt S, Laigaard HM, von Essen MR, Ullum H, Oturai A, Sellebjerg F, et al.** IL2RA Methylation and Gene Expression in Relation to the Multiple Sclerosis-Associated Gene Variant rs2104286 and Soluble IL-2Rα in $CD8^+$ T Cells. *Front Immunol*. 2021;12:676141.
56. **Elghzaly AA, Metwally SS, El-Chennawi FA, Elgayaar MA, MosaadYM,El-TorabyEE,et al.** IRF5, PTPN22, CD28, IL2RA, KIF5A, BLK and TNFAIP3 genes polymorphisms and lupus susceptibility in a cohort from the Egypt Delta; relation to other ethnic groups. *Hum Immunol*. 2015;76(7):525-531.
57. **NCBI.** The frequency of genotypes of rs2104286 SNP [Internet]. 2024 [cited 2024 Nov 1]. Available from: https://www.ncbi.nlm.nih.gov/snp/rs2104286.

58. **Akkad DA, Hoffjan S, Petrasch-Parwez E, Beygo J, Gold R, Epplen JT.** Variation in the IL7RA and IL2RA genes in German multiple sclerosis patients. *J Autoimmun*. 2009;32(2):110-115.

59. **D'Cunha MA, Pandit L, Malli C.** CD6 gene polymorphism rs17824933 is associated with multiple sclerosis in Indian population. *Ann Indian Acad Neurol*. 2016;19(4):491-494.

60. **Traboulsee AL, Bernales CQ, Ross JP, Lee JD, Sadovnick AD, Vilariño-Güell C.** Genetic variants in IL2RA and IL7R affect multiple sclerosis disease risk and progression. *Neurogenetics*. 2014;15(3):165-169.

61. **Wang LM, Zhang DM, Xu YM, Sun SL.** Interleukin 2 receptor α gene polymorphism and risk of multiple sclerosis: a meta-analysis. *J Int Med Res*. 2011;39(5):1625-1635.

62. **Cerosaletti K, Schneider A, Schwedhelm K, Frank I, Tatum M, Wei S, et al.** Multiple autoimmune-associated variants confer decreased IL-2R signaling in CD4+ CD25(hi) T cells of type 1 diabetic and multiple sclerosis patients. *PLoS One*. 2013;8(12):e83811.

63. **Zhou W, Hu W, Tang L, Ma X, Liao J, Yu Z, et al.** Meta-analysis of the Selected Genetic Variants in Immune-Related Genes and Multiple Sclerosis Risk. *Mol Neurobiol*. 2024;61(10):8175-8187.

64. **Zakaria M, Zamzam DA, Abdel Hafeez MA, Swelam MS, Khater SS, Fahmy MF, et al.** Clinical characteristics of patients with multiple sclerosis enrolled in a new registry in Egypt. *Mult SclerRelatDisord*. 2016;10:30-35.

65. **Gyu Song G, Ho Lee Y.** CTLA-4 +49 A/G and -318 C/T polymorphisms and susceptibility to multiple sclerosis: a meta-analysis. *Immunol Invest*. 2013;42(5):409-422.