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Original Article 1

Key genetic variants with multiple sclerosis risk in Egyptian patients

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Background
Multiple sclerosis (MS) is an autoimmune demyelinating disease that affects the central nervous system (CNS) and is becoming more prevalent globally. The interaction between genetic predisposition and environmental factors plays a significant role in the etiopathogenesis of MS.
Objectives
The study aims to investigate the association between MS likelihood in the Egyptian population and the *EVI5* rs11808092, *GSTT1* null, and HindIII C>G *PAI-1* polymorphisms. This cohort included 50 multiple sclerosis patients and 100 healthy controls matching age and gender.
Results
The 50 MS patients (35 females and 15 males) had a mean age of 36.0±6.6 years and 100 healthy controls. The two groups were matched regarding age and sex; the p-value was above 0.05. The types of multiple sclerosis were relapsing-remitting multiple sclerosis (56.0%), secondary progressive multiple sclerosis (38.0%), primary progressive multiple sclerosis (6.0%), and Expanded Disability Status Scale (EDSS). The HindIII C>G PAI-1 GC and rs11808092 AA and A allele were significantly more frequent in the multiple sclerosis group. No statistically significant association was detected between the type of multiple sclerosis and the studied polymorphisms.
Conclusion
Multiple sclerosis results from environmental, genetic, and epigenetic factors. This study found that the HindIII C>G PAI-1 GC genotype, the rs11808092 AA genotype, and the A allele are strongly associated with an increased risk of multiple sclerosis. However, no correlation was observed between the *GSTT1* null polymorphism and susceptibility to MS risk.

Keywords: Autoimmune Disease, Multiple Sclerosis, *GSTT1* gene, HindIII C>G PAI-1 GC, rs11808092 AA, and A allele.

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Introduction

Multiple sclerosis (MS) is one of the most prevalent causes of neurodegenerative autoimmune impairment in young adults (between 18 and 40 years). MS impacts 2.3 million individuals globally [1]. New pathophysiological discoveries highlight how crucial the interplay between the environment and genetics. Multiple sclerosis (MS) is a chronic autoimmune disease characterized by inflammation, demyelination, neuronal loss, and gliosis [2]. Advancements in diagnostic criteria, standardized MRI protocols, and global treatment recommendations have enabled accurate diagnoses and earlier initiation of effective immunomodulatory treatment, significantly enhancing the quality of life for multiple sclerosis patients [3].

The MS incidence in women is double that of men [4]. Tiredness, tremors, numbness, motor difficulties, nystagmus, loss of integration, speech and sight defects, cognitive dysfunction, and acute paralysis constitute the key manifestations [5]. Fortunately, the position of the MS lesions in the central nervous system specifies the signs and symptoms [5]. There are three phenotypes of multiple sclerosis depending on the patterns of cognitive or physical impairment progression: primary, secondary progressive, and relapsing-remitting (the most prevalent) [6]. The *EVI5* rs11808092 polymorphism is situated in the 3′-end intron of the *EVI5* gene, which mimics an enhancer domain and has been proven to serve as an effective enhancer element on the adjoining gene (*GFI1*)'s promoter corresponding to MS risk. Based on reports, *EVI5* rs11808092 and other polymorphisms

in *EVI5* contribute to MS risk and its clinical manifestations [7].

Increased levels of oxidative stress are the hallmark of chronic inflammatory diseases like multiple sclerosis. The glutathione antioxidant mechanism, consisting of superoxide dismutase (SOD), catalase, glutathione reductase, glutathione peroxidase, and glutathione s-transferase (GST), exists in tissues to stop ROS-induced deterioration [8]. The initial and acute stages of multiple sclerosis are greatly impacted by oxidative stress. Additionally, oxidative stress is a contributor to nerve damage during the latter phase of multiple sclerosis [9]. Genetic predisposition is a major variable controlling the detoxification of toxins produced through oxidative stress [10]. Disturbances in glutathione (GSH) homeostasis destroy nerve cells at different rates and, finally, dementia. GSH metabolism also takes part in anti-oxidative protection and sustaining the longevity of cells [11]. The *GSTT1* null genotype is markedly more prevalent in MS patients than in controls [12].

A serine protease enzyme named tissue plasminogen activator (tPA) induces the decomposition of inactive plasminogen into active plasmin [13]. Plasmin is an essential enzyme in the fragmentation of fibrin. The tPA includes the control of direct fibrinolysis and fibrin accumulation, where the impairment in its breakdown has been demonstrated to affect the demyelination process [14]. The plasminogen activator inhibitor 1 (PAI-1) exists on chromosome 7 q21.3-22. The transforming growth factor TGF-β controls PAI-1 transcription [15]. Multiple PAI-1 polymorphisms have been identified to modulate gene expression and protein function; of these, the HindIII polymorphism C>G is related to increased PAI-1 concentrations with the GG genotype. A considerable correlation has been observed between MS incidence and the CG genotype of the HindIII C>G PAI-1 variant [16].

The current research investigates the association between MS likelihood in the Egyptian population and the *EVI5* rs11808092, *GSTT1* null, *HindIII* C>G PAI-1 polymorphisms.

Subjects and methods

This cohort included 50 multiple sclerosis patients and 100 healthy controls matching age and gender. Patients were recruited from the Neurology department and the outpatient clinic. The study was approved by the Medical Research Ethics Committee at the National Research Centre (Approval no. 02410724). Before their inclusion in the study, Informed consent was obtained from all participants.

This study includes clinically definite MS patients according to the revised McDonald criteria from 2017 [17]. Their ages ranged from 20 to 45 years, and all phenotypes of MS were included (RRMS, SPMS, and PPMS). Only MS patients with other associated autoimmune illnesses were excluded from the study.

The included patients presented with different clinical attacks, including optic neuritis, motor weakness, unsteadiness of gait, sensory manifestations, and sphincter dysfunction. All patients were diagnosed with clinically definite MS by using an MRI of the brain and spine with contrast (MS protocol) and cerebrospinal fluid (CSF) analysis for oligoclonal bands (OCB) and the IgG index to achieve the criteria for dissemination in space and time for MS.

All patients were subjected to thorough neurological examination and history taking, including the patient's age, positive consanguinity, presence of similar conditions in the family, age of onset, disease duration, number of relapses/year, associated other chronic diseases (hypertension, diabetes, thyroid disorders) and assessment of clinical disability using the Expanded Disability Status Scale (EDSS) [18].

Genomic DNA was extracted from peripheral blood lymphocytes of 50 MS patients, and 100 normal healthy controls were tested using the Puregene DNA extraction kit (Gentra Systems Inc., Minneapolis, MN) after written informed consent following the Medical Research Ethics Committee's specifications. The following polymorphisms underwent analysis in the current study: *GSTT1* null, *EVI5* rs11808092, *and HindIII* C>G PAI-1.

The study utilized a duplex polymerase chain reaction (PCR) assay for detecting the homozygous deletion of *GSTT1*, using the primers designed for the *GSTT1* gene and the housekeeping gene *β-globin* as an internal control (Table 1). The sizes of the bands were 480 and 268 bp for *GSTT1* and *β-globin*, respectively. The band at 268 bp, reflecting the *β-globin* gene, is formed in the case of homozygous deletion of the *GSTT1* gene, while the two bands at 268 bp and 480 bp demonstrate the occurrence of the *GSTT1* gene.

The Hind III PAI-1 C>G locus has been investigated using polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) with appropriately designed primers, producing 600 bp amplification fragments (Table 1). The *HindIII* restriction enzyme was used to digest the 600 bp PCR products for one hour at 37°C (New England Biolabs, Beverly, Mass). The presence of a 600 bp fragment after digestion indicates the existence of the G allele, while the C allele creates a *HindIII* restriction site that produces two fragments of 371 bp and 229 bp.

Table 1. Represents the primer sequence of *GSTT1*, *β-globin* and Hind III PAI-1 C>G polymorphisms.

Polymorphism	Primer sequence	Amplification size (bp)	Annealing temperature	Primer concentration
GST T1	F:5'-TTCCTTACTGGTCCTCACATCTC-3' R:5'-TCACCGGATCATGGCCAGCA-3'	480	61	1 μM 1 μM
β-globin	F:5'-CAACTTCATCCACGTTCACC-3' R:5'-GAAGAGCCAAGGACAGGTAC-3'	268	61	1 μM 1 μM
***Hind III* PAI-1 C>G**	F: 5′-CTGGCCCGCTGTTCTTTAG-3′ R:5′-GGTTTCACGCCATTCTCCTG-3′	600	58	1 μM 1 μM

To study the *EVI5* rs11808092, *EVI5:* c.1884G>T, we have amplified the G and T alleles employing two duplex PCRs with an annealing temperature of 58°C. The non-allele distinct primers (forward outer and reverse outer) were performed to check the success of PCR giving a fragment of 589 bp.

To check the presence of the G allele, we use the non-allele distinct primers (forward outer and reverse outer) and the inner forward primer. Inner forward primer gives 266 bp with the reverse outer primer. Therefore, the existence of both fragments, 266 bp, and 589 bp, confirms the presence of the G allele, while the existence of 589 bp reveals the absence of the G allele.

- Forward outer primer (5' - AATACTAGTAAATGCCAATCCAGGAAA - 3') with 1 μM concentration
- Reverse outer primer (5' - TCAGCCTTGAATGACTTGATTTTAA - 3') with 2 μM concentration.
- Forward inner primer (G allele): (5' - CAGAAAGATACTGCACTTTCCCC- 3') with 1 μM concentration

The non-allele-specific primers (forward outer and reverse outer) and the inner reverse primer were used to amplify the T allele, the inner reverse primer and the forward outer primer, producing 373 bp. Consequently, the presence of the T allele was verified by the production of the longest fragments of 373 bp and 589 bp, while the absence of the T allele was identified by producing a fragment of 589 bp.

- Forward outer primer (5' - AATACTAGTAAATGCCAATCCAGGAAA - 3') with 2 μM concentration
- Reverse outer primer (5' - TCAGCCTTGAATGACTTGATTTTAA - 3') with 1 μM concentration.
- Reverse inner primer (T allele): (5'-GCAGAACAAGAGGTGATTAGCCTATAT-3') with 1 μM concentration.

The standard PCR protocol used an initial denaturation of 95°C for 5 minutes, then 35 cycles, including 95°C denaturation for 30 seconds, then an annealing step for 30 seconds using an optimized annealing temperature, and an extension step with 72°C for 30 seconds, and finally a final extension at 72°C for 7 minutes. Gel electrophoresis with 2.5% agarose gel stained with ethidium bromide was performed to visualize the PCR products.

Statistical analysis

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS Statistics (Statistical Package for Social Sciences) software version 28.0, IBM Corp., Chicago, USA, 2021. Quantitative data were described as mean±SD (standard deviation), minimum and maximum of the range, then compared using independent t-test and ANOVA tests. Qualitative data was defined as numbers and percentages and compared using the Chi-square and Fisher's Exact tests. The Bonferroni test was used for post hoc comparisons. The level of significance was taken at p-value ≤ 0.050.

Results

The current research investigated 100 normal controls (61 females and 39 males) with a mean age of 34.5±4.9 years and 50 MS cases (35 females and 15 males) with a mean age of 36.0±6.6 years. The two groups were matched regarding age and sex; the p-value was above 0.05 (Table 2).

The MS patients were categorized into 28 patients (56%) having RRMS, 19 patients (38%) with SPMS, and only three patients (6%) with PPMS. Two MS patients are diabetic, whilst four MS instances had elevated blood pressure. The mean age of onset and illness duration were 30.3±6.0 and 5.7±2.4 years, correspondingly, with a range of 20–40 years and 1–13 years. The EDSS score ranged from 1 to 6.5, with a mean of 3.5±1.9, and the number of relapses per year ranged from 0 to 3 (Table 3).

Table 2 Represents the relation of age and sex between multiple sclerosis and control groups.

Variables		Multiple sclerosis group (Total=50)	Control group (Total=100)	p-value
Age (years)	**Mean±SD**	36.0±6.6	34.5±4.9	^0.127
	Range	23.0–48.0	26.0–47.0	
Sex (n, %)	**Male**	15 (30.0%)	39 (39.0%)	#0.279
	Female	35 (70.0%)	61 (61.0%)	

^Independent t-test. #Chi square test.

The frequencies of the genotypes in the study, involving *EVI5* rs11808092, *GSTT1* null, and *HindIII* C>G *PAI-1* polymorphisms, were presented in Table 4. Comparing the MS patients and the control groups, the *HindIII* C>G PAI-1 GC and rs11808092 AA and A alleles were significantly more frequent in the MS group (P = 0.05). At the same time, there was no significant variation in the *GSTT1* null polymorphism frequencies (P = 0.417) between the MS and control groups. There was a deficiency of statistical significance between the MS types and clinical features, including age of onset, duration of illness, number of relapses annually, EDSS score, and the studied polymorphisms (Tables 5 and 6). Table 2 showed no statistically significant differences between the studied groups regarding age and sex.

Table 3 shows that the types of multiple sclerosis were relapsing-remitting multiple sclerosis (56.0%), secondary progressive multiple (38.0%), primary progressive multiple sclerosis (6.0%), and Expanded Disability Status Scale (EDSS).

Table 4 showed that *HindIII* C>G PAI-1 GC and rs11808092 AA and A allele were significantly more frequent in the multiple sclerosis group.

Table 5 showed no statistically significant association between types of multiple sclerosis and the studied polymorphisms.

Table 6 showed no statistically significant differences according to the studied polymorphisms compared to clinical characteristics.

Table 3 Represents clinical characteristics of multiple sclerosis group.

Variables		Number	Percentage
Type of multiple sclerosis	**RRMS**	28	56.0%
	SPMS	19	38.0%
	PPMS	3	6.0%
Hypertension		4	8.0%
Diabetes mellitus		2	4.0%
		Mean±SD	**Range**
Age of onset (years)		30.3±6.0	20.0–40.0
Duration of illness (years)		5.7±2.4	1.0–13.0
Relapses per year		1.8±0.8	0.0–3.0
EDSS score		3.5±1.9	1.0–6.5

Table 4. Represents molecular findings in multiple sclerosis patients versus control group.

Variables		Multiple sclerosis group (Total=50)	Control group (Total=100)	p-value	Odds ratio (95% CI)
GSTT1	**Positive**	36 (72.0%)	78 (78.0%)	#0.417	Reference
	Negative	14 (28.0%)	22 (22.0%)		0.73 (0.33– 1.58)
HindIII C>G PAI-1	**GG**	8 (16.0%)	34 (34.0%)	**#0.021***	Reference
	GC	42 (84.0%)	66 (66.0%)		2.71 (1.14– 6.40)
rs11808092	**CC**	23 (46.0%) a	58 (58.0%) a	**#0.028***	Reference
	CA	21 (42.0%) a	40 (40.0%) a		1.32 (0.65– 2.71)
	AA	6 (12.0%) a	2 (2.0%) b		7.75 (1.42– 40.25)
		Total=100	**Total=200**		
***HindIII* C>G PAI-1**	**G**	58 (58.0%)	134 (67.0%)	#0.126	Reference
	C	42 (42.0%)	66 (33.0%)		1.47 (0.90– 2.41)
rs11808092	**C**	67 (67.0%)	156 (78.0%)	**#0.040***	Reference
	A	33 (33.0%)	44 (22.0%)		1.75 (1.02– 2.98)

#Chi square test. *Significant. Homogenous groups had the same symbol "a and b" based on the post hoc Bonferroni test. CI: Confidence Interval.

Table 5. Represents molecular findings in patients with different types of multiple sclerosis.

Variables		RRMS (Total=28)	SPMS (Total=19)	PPMS (Total=3)	p-value
GSTT1	**Positive**	20 (71.4%)	14 (73.7%)	2 (66.7%)	§0.999
	Negative	8 (28.6%)	5 (26.3%)	1 (33.3%)	
***HindIII* C>G PAI-1**	**GG**	5 (17.9%)	3 (15.8%)	0 (0.0%)	§0.999
	GC	23 (82.1%)	16 (84.2%)	3 (100.0%)	
rs11808092	**CC**	10 (35.7%)	11 (57.9%)	2 (66.7%)	§0.203
	CA	15 (53.6%)	6 (31.6%)	0 (0.0%)	
	AA	3 (10.7%)	2 (10.5%)	1 (33.3%)	
Alleles		**Total=56**	**Total=38**	**Total=6**	
***HindIII* C>G PAI-1**	**G**	33 (58.9%)	22 (57.9%)	3 (50.0%)	§0.949
	C	23 (41.1%)	16 (42.1%)	3 (50.0%)	
rs11808092	**C**	35 (62.5%)	28 (73.7%)	4 (66.7%)	§0.479
	A	21 (37.5%)	10 (26.3%)	2 (33.3%)	

§Fisher's Exact test.

Table 6. Represents clinical characteristics according to the studied polymorphisms among the multiple sclerosis patients.

Variables		n	Age of onset (years)	Duration of illness (years)	Relapses per year	EDSS score
GSTT1	**Positive**	**36**	29.5±6.2	6.0±2.5	1.9±0.8	3.4±1.8
	Negative	**14**	32.4±4.8	5.1±1.9	1.6±0.8	3.7±2.2
	^p-value		0.117	0.219	0.349	0.554
***HindIII* C>G PAI-1**	**GG**	**8**	29.6±6.0	5.3±2.4	1.9±0.8	3.0±1.9
	GC	**42**	30.4±6.0	5.8±2.4	1.8±0.8	3.5±1.9
	^p-value		0.731	0.531	0.840	0.469
rs11808092	**CC**	**23**	32.0±5.8	5.8±2.6	1.9±0.9	3.9±1.9
	CA	**21**	28.0±5.3	5.3±2.2	1.8±0.7	2.8±1.8
	AA	**6**	31.8±7.2	7.2±2.1	1.7±1.0	4.3±1.8
	⌂p-value		0.065	0.235	0.868	0.083

^Independent t-test. ⌂ANOVA test.

The image of gel electrophoresis of the rs11808092: *EVI5*: c.1884G>T using a 2.5% agarose gel stained by ethidium bromide is illustrated in Figure 1. While those represents the homozygous deletion of *GSTT1* and *Hind III* PAI-1 C>G are shown in Figure 2.

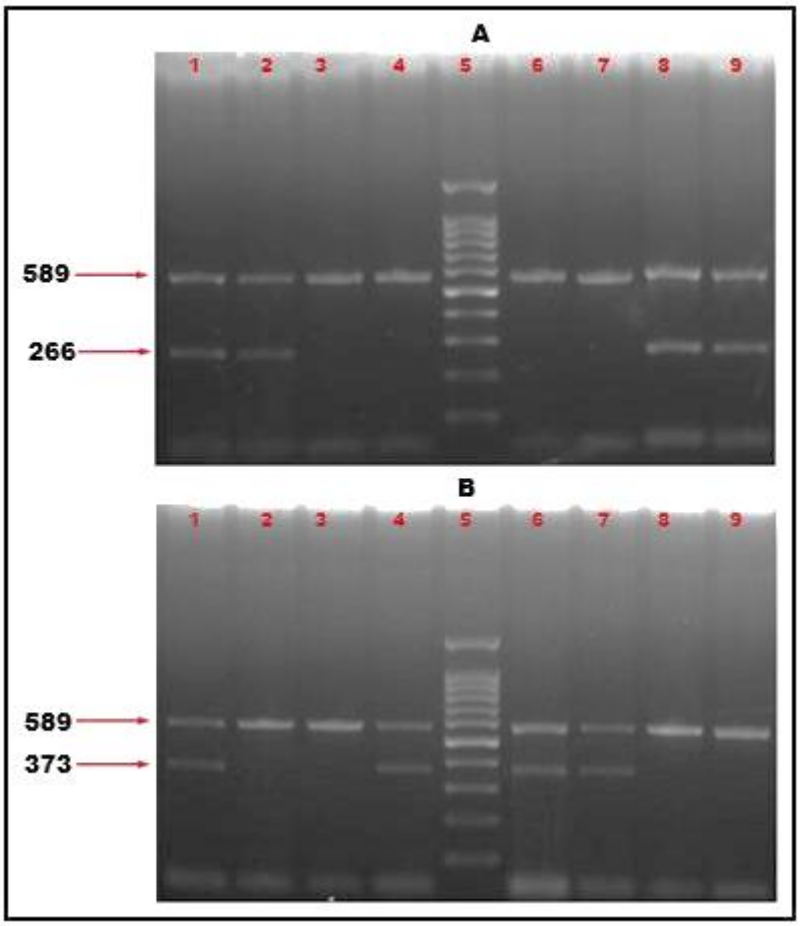

Fig. 1 The image of gel electrophoresis represents the rs11808092: *EVI5*: c.1884G>T using a 2.5% agarose gel stained by ethidium bromide.
A) Lanes 1, 2, 8, and 9 show 589 bp and 266 bp, which indicates the presence of the G allele in the rs11808092 polymorphism, while lanes 3, 4, 6, and 7 have a single band of 589 bp, indicating the absence of the G allele in the rs11808092 polymorphism. Finally, Lane 5 represents a 100 bp marker. **B)** Lanes 1, 4, 6, and 7 show 589 bp and 373 bp, showing the existence of the T allele in the rs11808092 polymorphism, while lanes 2, 3, 8, and 9 show one band of 589 bp, so T allele is absent in the rs11808092 polymorphism, while lane 5 presents a 100 bp marker.

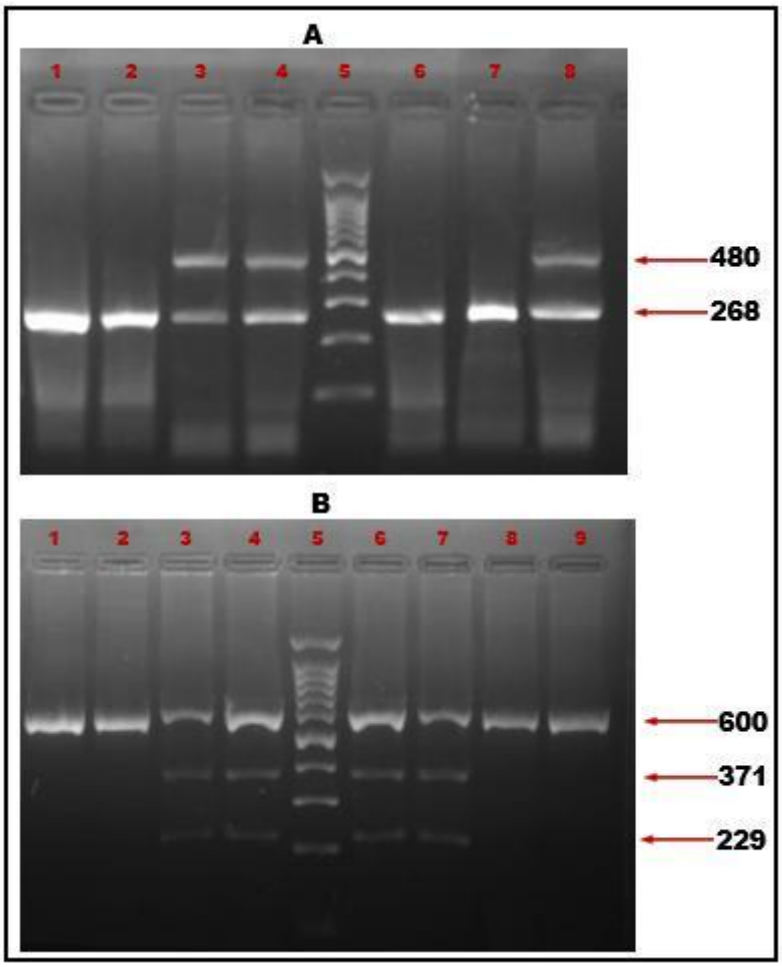

Fig. 2 The image of gel electrophoresis refers to the detection of homozygous deletion of *GSTT1* and *Hind III* PAI-1 C>G using a 2.5% agarose gel stained by ethidium bromide.
A) lanes 1, 2, 6, and 7 show a single band of 268 bp, which indicates the presence of the β-globin gene without the *GSTT1* gene. Lanes 3, 4, and 8 imply two bands of 480 and 268, indicating the presence of the *GSTT1* gene. The 100 bp marker is present in lane 5. **B)** Lanes 1, 2, 8, and 9 have one band of 600 bp, which represents the GG genotype, while lanes 2, 4, 6, and 7 show three bands of 600 bp, 371 bp, and 229 bp, implying the existence of the GC genotype. The 100 bp marker is in lane 5.

Discussion

Inflammatory demyelination is a hallmark of multiple sclerosis (MS), an autoimmune, degenerative disease of the central nervous system that mainly affects women of childbearing age [19]. The current cohort studied 100 normal controls (61 females and 39 males) with a mean age of 34.5±4.9 years and 50 MS cases (35 females and 15 males) with a mean age of 36.0±6.6 years. There were no statistically significant differences between the studied groups regarding age and sex. This is similar to the findings of Mohamed et al., 2023, who described that 71% of their patients were females, and the mean age was 28.1 ±7.4 [20].

Relapsing-Remitting Multiple Sclerosis (RRMS) is the most common form of multiple sclerosis, affecting approximately 85% of patients. The second type is Primary Progressive Multiple Sclerosis (PPMS), which progresses continuously from the onset of symptoms without any relapses, although there may be periods of stability. This type affects around 15% of MS patients. The third type is Secondary Progressive Multiple Sclerosis (SPMS), which is characterized by disease progression that includes acute relapses, resulting in disability that impacts mobility, autonomic functions, and cognitive abilities [21]. The MS cases in our study were categorized into 28 patients (56%) having RRMS, 19 cases (38%) with SPMS, and just 3 cases (6%) with PPMS phenotypes.

Two MS patients were diabetic, whilst four MS patients had elevated blood pressure. Similarly, Marrie et al. (2020) indicated their association with MS [22]. Ramachandran et al. (2014) elucidated that patients older than 30 years at onset accumulate disability at a higher rate [23]. The mean age of onset and illness duration were 30.3±6.0 and 5.7±2.4 years, correspondingly, with a range of 20–40 years and 1–13 years. The EDSS is widely used to measure disease severity and the efficiency of therapeutic interference [24]. In our report, the EDSS score ranged from 1 to 6.5, with a mean of 3.5±1.9, and the number of relapses per year ranged from 0 to 3.

Multiple sclerosis (MS) is an inflammatory demyelinating condition that strikes the central nervous system triggered by T-cell autoimmunity [25]. MS is also a complicated disorder brought about by factors that are genetic and environmental. Based on previous reports, MS cases' siblings and twins were more prone to the sickness than people in general, implying that genetic over environmental causes led to MS being concentrated within families [26]. Prior studies showed the potential impacts of the three polymorphisms we studied in the *EVI5, GSTT1, and PAI-1* genes on the susceptibility to increased risk of MS disease. However, no studies have investigated the association of these polymorphisms with Egyptian MS cases.

The *EVI5* rs11808092 polymorphism is located in the 3′-end intron of the *EVI5* gene. It functions as an enhancer element and has been shown to act as a potent enhancer factor on the promoter of a nearby gene (*GFI1*) associated with MS [27]. *Evi5* may also be related to MS as it could influence innate immunity components, such as toll-like receptor signaling via Rab11, which is known to regulate receptor transport through endosome recycling [28].

In the current study, the rs11808092 AA genotype and A allele were significantly more frequent in the multiple sclerosis group compared to the control group: allelic A vs. C: OR = 1.75, 95% CI = 1.02−2.98, p-value < 0.040; the genotype AA vs. AC+CC: OR = 7.75, 95% CI = 1.42−40.25, p-value < 0.028. These findings align with Liu et al.'s 2016 meta-analysis [29], indicating a statistically significant correlation between the *EVI5* rs11808092 polymorphism and the susceptibility to multiple sclerosis. Allele A vs. C: OR = 1.17, 95% CI = 1.10–1.24, p-value < 0.01; homozygous AA vs. CC: OR = 1.28, 95% CI = 1.11–1.48, p-value < 0.01. Several studies have reported a significant association between the A allele and AA genotype and increased risk of MS in the European race, New Zealand, Australia, the UK, the USA, and Spain [29], as well as in the Asian race including Kuwait [7].

Being an inflammatory disease, the pathological and inflammatory processes of multiple sclerosis are strongly affected by oxidative stress. In response to oxidative stress, cells activate antioxidant functions. One of the brain's antioxidants, glutathione, is a coenzyme for the enzyme glutathione s-transferase (GST), which cleanses nerve cells from reactive oxygen species. One of these enzymes, GSTT1, loses its function because of structural homozygous deletion of the *GSTT1* gene. The *GSTT1* null polymorphism frequencies were not substantially different compared to the MS and control groups (P = 0.417). This finding is consistent with a Brazilian study on ALS that demonstrated no link between the *GSTT1* null polymorphism (p = 0.90) and ALS likelihood [30]. In contrast to our findings, an Iranian study by Parchami et al. (2017) revealed a strong association between *GSTT1* null polymorphisms and MS risk (p = 0.0001) [12]. Therefore, it is crucial to investigate the variations in different populations due to ethnic variances and the effect of the genotypes of enzymes involved in glutathione metabolism.

The plasminogen activator inhibitor 1 (PAI-1) lowers the production of plasmin by suppressing tissue plasminogen activator (tPA). Plasmin is a crucial enzyme in the disintegration of fibrin; subsequently, fibrin aggregation impacts the demyelination process, which has a critical role in MS pathogenesis [14]. Various *PAI-1* polymorphisms have modified the protein function and gene expression. Moreover, the *HindIII* polymorphism C>G was shown to raise *PAI-1* concentrations with the GG genotype [16]. In the current study, the CG genotype of the *HindIII* C>G *PAI-1* polymorphism has been proven to be significantly associated with an increased risk of MS (p=0.021). This finding is consistent with the results of Valdés-Alvarado et al. (2019) (p=0.3) in MS patients from western Mexico [16].

Conclusion

The results of this study indicate a significant association between the *HindIII* C>G PAI-1 GC genotype, as well as the rs11808092 AA genotype and A alleles, and a heightened risk of multiple sclerosis (MS) in the Egyptian patient cohort. Conversely, no correlation was observed between the *GSTT1* null polymorphism and an increased likelihood of MS. This study offers further insight into the disease's pathogenesis and possible therapeutic targets.

Authors' contributions

This work was carried out in collaboration between all authors. T.H.A.A. designed, wrote the protocol and coordinate the study. H.T.E., H.E., and K.H. performed the clinical study for the patients and their families and recruited the patients samples. T.H.A.A. and E.E.A.M. performed the molecular analysis, results interpretation, literature searches, and wrote the first draft of the manuscript. H.M.H. provided statistical data analysis. All authors read, revised, and approved the submitted manuscript. T.H.A.A. (guarantor) has the responsibility for the integrity of the work as a whole from inception to published article.

The manuscript has been read and approved by all the authors, that the requirements for authorship as stated earlier in this document have been met, and

that each author believes that the manuscript represents honest work.

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Conflict of interest

The Authors declare that there is no conflict of interest.

Data Availability

All study data are upon request and can be accessed by contacting the corresponding author.

Declarations

The study has been approved by the NRC Medical Research Ethics Committee (Approval no. 02410724). All study participants signed a written informed consent.

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