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A Common Variant in the *MC1R* Gene (p.V92M) is associated with Alzheimer's Disease Risk

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Abstract. Despite the recent identification of some novel risk genes for Alzheimer's disease (AD), the genetic etiology of late-onset Alzheimer's disease (LOAD) remains largely unknown. The inclusion of these novel risk genes to the risk attributable to the *APOE* gene accounts for roughly half of the total genetic variance in LOAD. The evidence indicates that undiscovered genetic factors may contribute to AD susceptibility. In the present study, we sequenced the *MC1R* gene in 525 Spanish LOAD patients and in 160 controls. We observed that a common *MC1R* variant p.V92M (rs2228479), not related to pigmentation traits, was present in 72 (14%) patients and 15 (9%) controls and confers increased risk of developing LOAD (OR: 1.99, 95% CI: 1.08–3.64, $p = 0.026$), especially in those patients whose genetic risk could not be explained by *APOE* genotype. This association remains and even increased in the subset of 69 patients with typical AD cerebrospinal fluid profile (OR: 3.40 95% CI: 1.40–8.27, $p = 0.007$). We did not find an association between p.V92M and age of onset of AD. Further studies are necessary to elucidate the role of MC1R in brain cells through the different MC1R pathways.

Keywords: Cerebrospinal fluid biomarkers, common variant, late-onset Alzheimer's disease, melanocortin 1 receptor (*MC1R*) gene, p.V92M, risk

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INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized clinically by memory and cognitive dysfunction and represents the most common form of dementia in the elderly [1]. The prevalence of the disease increases after the age of 65 years and the disease onset is usually after the age of 70 years [2]. However, familial AD (FAD) patients carry autosomal dominant mutations in high-risk Alzheimer susceptibility genes (*APP*, *PSEN1*, and *PSEN2*) and present an early age of onset (<65 years). These genes do not play an important role in either Sporadic AD cases or late onset cases (>65 years) indicating that other genomic variants may be involved in the common forms of the disease [3]. To date, the ε4 allele polymorphism in the Apolipoprotein E (*APOE*) gene has been well established as a risk factor for developing late-onset Alzheimer's disease (LOAD) [3]. The disease-attributable risk in LOAD patients related to the ε4 allele in APOE is less than 50% [4]. Previous genome-wide association studies (GWAS) have identified low-risk variants associated with LOAD [5–10], which account for a small proportion of risk. The inclusion of these novel risk genes to the risk attributable to the *APOE* gene accounts for roughly half of the total genetic variance [11], indicating that additional undiscovered genetic factors may contribute to AD susceptibility.

The main pathological hallmarks of AD are extracellular amyloid plaques, intracellular neurofibrillary tangles, and loss of neurons and synapses, resulting in brain atrophy [12]. Moreover, an elevated level of oxidative damage products has been observed in areas of degeneration in AD brains, suggesting that oxidative stress and consequent protein oxidation may be potential mechanisms of neuronal death in AD [13]. The accumulation of intracellular damage determined by reactive oxygen species might produce the progressive loss of control over biological homeostasis and the functional impairment typical of damaged brain cells in AD [14].

The Melanocortin 1 receptor (*MC1R*) gene encodes for a G protein-coupled seven transmembrane receptor for melanocortin peptides (α-MSH, ACTH) and mediates its effects mainly by activating a cAMP-dependent signaling pathway [15]. MC1R expression is observed in several types of neuronal cells suggesting that it may be a key regulator in brain cell functions and survival [16]. The MC1R activation has anti-inflammatory and immunomodulatory effects in brain cells [16] and promotes pigmentation synthesis in melanocytes [17]. It has been established that several *MC1R* polymorphisms constitute a risk factor to develop skin cancer (melanoma and non-melanoma skin cancer) [18, 19], in part, by promoting an increased oxidative stress in skin cells [20]. Notably, co-occurrence of Parkinson's disease (PD) and cutaneous melanoma (CM) has been reported in epidemiological studies [21] and previous evidence indicates that *MC1R* is involved in the bidirectional link between both diseases [22, 23]. Thus, we hypothesized that certain *MC1R* variants may increase the oxidative damage and/or deregulate inflammatory processes in brain cells, which consequently, increase the susceptibility of developing other neurodegenerative disorders beyond PD. In the present study, we analyzed the role of the *MC1R* gene as a putative genetic risk factor in LOAD patients, and we observed that a common *MC1R* variant, not related to pigmentation traits, confers risk of developing LOAD in a Spanish population.

MATERIAL AND METHODS

We performed a case-control study of 525 unrelated LOAD patients (Mean age ± SD, 76.35 ± 5.61 years (the age of onset was after 65 years in all patients studied; male 29.7%/female 70.3%) and 160 controls (Mean age ± SD, 73.81 ± 5.87 years; male 36.3%/female 63.7%). All patients studied were recruited from two hospital-based series from the same geographical area: the Alzheimer's Disease and Other Cognitive Disorders Unit at Clinic Hospital of Barcelona and from the Memory Unit at Sant Pau Hospital of Barcelona. All AD patients were diagnosed using the NINCSDS-ADRDA criteria [24]. Furthermore, 69/525 had a CSF biomarker profile typical of AD ($A\beta_{42}$/p-tau ratio <6.43) [25].

The control group included healthy individuals without signs of neurodegenerative or psychiatric disorders obtained from three independent control series from Spain: Hospital Clinic of Barcelona (N = 13), Sant Pau Hospital of Barcelona (N = 85), and the Spanish National Bank of DNA (N = 62) (Table 1).

All individuals included in the study gave their written informed consent according to the Declaration of Helsinki. The Ethical Committee of Clinical Investigation at the Hospital Clinic of Barcelona approved the study.

APOE genotype analysis

DNA was isolated from blood samples using the Wizard® Genomic DNA Purification Kit (Promega,

Table 1
Demographic data and APOE genotype of the samples analyzed

			LOAD (N = 525)	Controls (N = 160)
AOO		Mean ± SD*	76.35 ± 5.61	73.81 ± 5.87
Gender	Male	N (%)	156 (29.7)	58 (36.3)
	Female	N (%)	369 (70.3)	102 (63.7)
[a]APOE	APOE –/–	N (%)	283 (53.9)	128 (80)
	APOE –/+	N (%)	218 (41.5)	32 (20)
	APOE +/+	N (%)	24 (4.6)	0 (0)
Hospital based-series	Hospital Clinic of Barcelona	N (%)	110 (21)	13 (8)
	Sant Pau Hospital of Barcelona	N (%)	415 (79)	85 (53)
	National Bank of DNA[b]	N (%)	0 (0)	62 (39)

[a] –/– individuals who carry no ε4 allele. –/+individuals who carry one ε4 allele. +/+individuals who carry two ε4 alleles [b]from The University of Salamanca. *SD standard deviation.

Fitchburg, Wisconsin, USA). *APOE* genotype was determined through the analysis of rs429358 and rs7412 using TaqMan (Applied Biosystems) genotyping technologies.

CSF biomarkers determination

69 subjects underwent a spinal tap during the morning. The samples were centrifuged and stored in polypropylene tubes at –80°C within 2 h. Levels of $A\beta_{42}$, t-tau, and p-tau were measured by experienced laboratory personnel using commercial sandwich ELISA kits (Innogenetics, Ghent, Belgium) [26]. We are participants of the QC program, and $A\beta_{42}$, t-tau, and p-tau levels obtained in our lab for the Alzheimer's Association QC samples were within mean ± 2 SD.

MC1R molecular screening

The *MC1R* gene, which consists of one single exon encoding a 317 amino-acid protein (ENST00000 555147), was sequenced using 50–100 ng of total DNA per sample. PCR amplification was carried out as previously described [27] using an initial denaturizing step at 95°C 5 min, followed by 35 PCR cycles (94°C 1 min, 55°C 1 min, 72°C 3 min), and a final extension at 72°C 10 min. PCR products were purified using Multiscreen Filter plates (Millipore). We sequenced the entire coding region of *MC1R* (a 1,107 bp fragment) using the following internal primers (TM-F: 5'AACCTGCACTCACCCATGTA3' and TM-R: 5'TTTAAGGCCAAAGCCCTGGT3') and the BigDye Terminator v3.1 Cycle Sequencing kit, according to manufacturer's instructions (Applied Biosystems, Foster City, CA). Sequences were run on an ABI3100 automatic sequencer (Applied Biosystems) and analyzed using the SeqPilot 4.0.1 software (JSI Medical Systems). The entire coding region was sequenced in 110 LOAD patients and in all controls. In 415 LOAD patients, the *MC1R* gene was exclusively sequenced using TM-R primer. This strategy allows us to detect all *MC1R* variants, except for the presence of p.D294H and p.T314T variants.

Statistical analysis

We focused the analysis on the non-synonymous *MC1R* variants with an observed minor allele frequency (MAF) in at least 1% of cases. Synonymous variants were considered as wild-type *MC1R* alleles. Public databases such as dbSNP (http://www.ncbi.nlm.nih.gov/), MelGene DB (http://www.melgene.org/), and Ensembl genome browser (http://www.ensembl.org/) were used to determine whether the detected non-synonymous variants have been previously described. *In-silico* analysis of each rare non-synonymous variant was carried out using software Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) [28].

The genotypic association analysis was performed using multiple logistic regression models (co-dominant, dominant, recessive, over-dominant, and log-additive) in the whole set of patients (N = 525) and in the subset of patients with CSF biomarkers data (N = 69). The selection of the most suitable model of inheritance was performed based on both Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). All reported odds ratios (ORs), 95% confidence intervals (CI), and *p*-values were adjusted for age, gender, and *APOE* genotype.

The goodness-of-fit of our logistic regression model was evaluated by the Hosmer-Lemeshow test. In the genotypic association analysis with the whole set of samples (525 patients and 160 controls), we obtained the best fit of our model by categorical transformation of the age. We converted the numerical

Table 2
Genetic variants detected in the *MC1R* gene

MC1R variants				Subjects		
				LOAD (N = 525)	Controls (N = 160)	Polyphen
rs	MAF (%) (EA/AA/All) [#]	AA change	cDNAchange	N (%)	N (%)	Score 2
Synonymous						
rs146544450	0.2151/0.0969/0.1761	p.Q233Q	(c.699G>A)Het	4 (0.7)	–	–
rs181269865	0.1064/0.0712/0.0947	p.I264I	(c.792C>T)Het	2 (0.4)	–	–
UN	UN	**p.L309L**	**(c.927C>G)Het**	1 (0.2)	–	–
rs2228478	10.7936/40.8062/20.8104	^p.T314T[a]	(c.942A>G)Het	21 (19)[a]	24 (15)[a]	–
			(c.942A>G)Homo	2 (2)[a]	–	
Non-synonymous						
rs1805005	13.3419/2.2612/9.6064	^p.V60L	(c.178T>G)Het	137 (26)	40 (25)	0.988
			(c.178T>G)Homo	18 (3)	5 (3)	
rs372590533	0.0117/0.0/0.0078	p.R67Q	(c.200G>A)Het	–	3 (2)	0.744
rs34474212	0.1051/0.0228/0.0773	p.S83P	(c.247T>C)Het	1 (0.2)	–	0.999
rs1805006	1.0028/0.1826/0.7254	^p.D84E	(c.252C>A)Het	1 (0.2)	–	1.000
rs2228479	8.5784/1.9389/6.3311	^p.V92M	(c.274G>A)Het	72 (14)	15 (9)	0.015
			(c.274G>A)Homo	2 (0.4)	–	
rs201192930	0.0116/0.0228/0.0154	p.V122M	(c.364G>A)Het	1 (0.2)	–	0.126
rs374235260	0.0116/0.0/0.0077	p.M128T	(c.383T>C)Het	–	1 (0.6)	0.235
rs11547464	0.7442/0.0682/0.5155	^p.R142H	(c.425G>A)Het	6 (1)	1 (0.6)	1.000
rs1805007	7.6163/1.7061/5.6171	^p.R151C	(c.451C>T)Het	26 (5)	9 (6)	1.000
rs201326893	0.0349/0.0/0.0231	p.Y152*	(c.456C>A)Het	1 (0.2)	–	1.000
rs1110400	1.0468/0.2959/0.7928	^p.I155T	(c.464T>C)Het	6 (1)	5 (3)	0.986
UN	UN	**p.V156E**	**(c.467T>A)Het**	1 (0.2)	–	0.981
rs1805008	7.7147/1.3452/5.5624	^p.R160W	(c.478C>T)Het	18 (3)	4 (2)	0.861
rs885479	4.8207/1.5269/3.7068	^p.R163Q	(c.488G>A)Het	24 (5)	3 (2)	0.004
UN	UN	**p.V174I**	**(c.520G>A)Het**	–	1 (0.6)	0.002
rs200000734	0.0594/0.0239/0.0476	p.R213W	(c.637C>T)Het	1 (0.2)	–	0.019
rs1805009	2.0716/0.6859/1.6111	^p.D294H[a]	(c.880G>C)Het	5 (4)[a]	6 (4)[a]	1.000
			(c.880G>C)Homo	1 (0.9)[a]	–	
UN	UN	**p.Y298H**	**(c.892T>C)Het**	–	2 (1.2)	1.000

[#]The minor-allele frequency in percent listed in the order of European American (EA), African American (AA) and all populations (All) (delimited by /). Variants in bold have not been reported before. UN = Unknown. Het: variant in heterozygosis. Homo: variant in homozygosis.^ *MC1R* common variants.Score Polyphen2: predicts possible impact of an amino acid substitution on the structure and function of a human protein (scores close to 0.0, indicate a benign mutation; score close to 1.0, damaging mutation) [a] *MC1R* variants evaluated in 110 LOAD patients and 160 controls.

variable (age) into a categorical variable, dividing the age into different categories. In this analysis, the logistic regression model was adjusted for age (as categorical variable), gender, and APOE genotype. Otherwise, in the genotypic association analysis with the subset of patients with typical AD CSF biomarkers (69 patients and 160 controls), we obtained the best fit of our model, including the age as a numerical variable. In this second analysis, the logistic regression model was adjusted for age (as numerical variable), gender, and APOE genotype.

We evaluated whether *MC1R* and *APOE* genotypes modify age of onset (AOO) among the Spanish LOAD group using the Student's *t*-test. We evaluated whether interaction between *MC1R* variants and *APOE* alleles exists using the two-tail χ^2 test.

p-values less than 0.05 were considered statistically significant. All tests were two sided, and Bonferroni correction for multiple comparisons was applied to all *p*-values <0.05. We performed all the analyses using STATA v.11 software.

RESULTS

MC1R genotyping was carried out in 525 LOAD patients and in 160 control subjects. Non-significant difference in the gender distribution was detected between patients and controls ($p = 0.118$). The study identified 22 *MC1R* variants (4 synonymous and 18 non-synonymous), four rare variants (p.V156E, p.V174E, p.Y298H, and p.L309L) had not been previously identified (Table 2). Five recurrent non-synonymous variants showed a frequency $\geq$1% in LOAD patients: p.V60L (16%), p.V92M (7%), p.R151C (2%), p.R160W (2%), and p.R163Q (2%). All variants were in Hardy-Weinberg equilibrium within both the control population and LOAD

Table 3
Results of association analysis of *MC1R* variants and LOAD

AA change	Controls (N = 160)				LOAD patients (N = 525)					
	[a]MAF	[a]Genotype frequency			MAF	Genotype frequency			CONTROL vs LOAD patients	
	2	1–1	1–2	2–2	2	1–1	1–2	2–2	OR (95% CI)[b]	*p-value*[b]
p.V60L	0.16	0.72	0.25	0.03	0.16	0.71	0.26	0.03	0.93 (0.69–1.33)	0.695
p.V92M	0.05	0.9	0.1	0	0.07	0.859	0.137	0.004	**1.99 (1.08–3.64)**	**0.026**
p.R163Q	0.01	0.98	0.02	0	0.02	0.95	0.05	0	2.47 (0.69–8.81)	0.162
p.R151C	0.03	0.94	0.06	0	0.025	0.95	0.05	0	1.01 (0.44–2.30)	0.979
p.R160W	0.01	0.975	0.025	0	0.02	0.966	0.034	0	0.92 (0.29–2.91)	0.882

MAF: Minor allele frequency. [a]Allele described as 1 (wild type allele) or 2 (variant allele). [b]Adjusted for gender, age (converted in a categorical variable) and APOE genotype. The genetic model used was log-additive, the homozygous for 'variant allele' (a/a) has double the risk of the heterozygous (A/a). The statistically significant result is highlighted in bold.

patients (allelic and genotypic frequencies are listed in Table 3). Moreover, frequencies of *MC1R* variants observed in control subjects were compared with those observed in other set of controls (N = 736) free from neurodegenerative disorders used in other study [29]. No statistical significant differences were observed between both groups.

The *APOE* genotype was obtained for all cases and controls. The frequency of heterozygous *APOE* ε4 carriers was 41.5% (218/525) in LOAD patients and 20% (32/160) in controls. The *APOE* ε4/ε4 genotype was only detected in 4.6% (24/525) of LOAD patients. The carriers of at least one APOE ε4 allele had a higher than three-fold increased risk of developing LOAD (OR: 3.47, 95% CI: 2.24–5.39, $p < 0.0001$).

We evaluated the 5 most common *MC1R* variants detected in the study with the risk of developing LOAD (Table 3). We detected that variant p.V92M was enriched in LOAD patients compared to controls, being associated with an increased risk of developing LOAD under the log-additive genetic model (OR: 1.99, 95% CI: 1.08–3.64, $p = 0.026$), after Bonferroni correction this association did not reach statistical significance (Bonferroni corrected $p = 0.13$).

We re-evaluated the association between *MC1R* variant p.V92M and LOAD risk within the subset of 69 patients with typical AD CSF profile (Table 4) and we found it was statistically significant and we evidenced an even higher OR (OR: 3.40 95% CI: 1.40–8.27, $p = 0.007$). We did not detect statistically significant differences in the different CSF biomarkers levels between p.V92M carriers and non-carriers (data not shown).

In order to identify whether the p.V92M association with LOAD risk was modulated by the presence of the APOE ε4 allele, we evaluated the interaction between both alleles within the whole set of LOAD patients (Table 5). The frequency of variant p.V92M in the *MC1R* gene was significantly lower ($p = 0.041$) in carriers of at least one APOE ε4 allele compared to non-carriers (35.1% and 64.9%, respectively). This result suggests an inverse correlation between both alleles.

Table 4
Demographic data and CSF biomarkers levels of LOAD patients subset confirmed by CSF analysis

			LOAD (N = 69)
AOO		Mean ± SD*	71.46 ± 4.74
Gender	Male	N (%)	26 (37.7%)
	Female	N (%)	43 (62.3%)
[a]APOE	APOE –/–	N (%)	30 (43.5%)
	APOE –/+	N (%)	31 (44.9%)
	APOE +/+	N (%)	8 (11.6%)
CSF protein levels	$A\beta_{42}$	Mean ± SD	348.64 ± 143.14
	t-tau	Mean ± SD	711.25 ± 385.51
	p-tau	Mean ± SD	100.46 ± 42.002

[a] –/– patients who carry no ε4 allele. –/+patients who carry one ε4 allele. +/+patients who carry two ε4 alleles. *SD standard deviation.

Finally, we observed a significantly lower AOO associated with APOE ε4 allele ($p < 0.0001$), in contrast, no significant effect on AOO was observed for *MC1R* variant p.V92M (Table 6).

DISCUSSION

LOAD form accounts for more than 90% of AD cases [30]. To date, the *APOE* gene is the major genetic factor in LOAD susceptibility [31], while other genetic factors related with LOAD susceptibility remain largely unknown. Thus, identification of novel genetic factors may be crucial to detect individuals with an inherited AD risk.

In this study, we report a novel association between the p.V92M variant in the *MC1R* gene and the risk of developing LOAD. After Bonferroni correction, the association detected in the overall set of patients did not reach statistical significance. However, the

Table 5
Analysis of p.V92M distribution regarding APOE genotype within LOAD patients (N = 525)

	^p.V92M				*p-value*
	0		≥1		
APOE genotype	N	%	N	%	
APOE ε4 (–)	235	52.1	48	64.9	0.041
APOE ε4 (+)	216	47.9	26	35.1	

APOE ε4 (–): non carriers of APOE ε4 allele. (+): carriers of at least one APOE ε4 allele. ^ p.V92M (0): non carriers of variant p.V92M. (≥1): carriers of at least one p.V92M allele.

Table 6
Analysis of AOO regarding APOE genotype and p.V92M variant genotype

	Age of onset		*p-value*
[a]APOE genotype	N	Mean ± SD*	
APOE ε4 –	283	77.36 ± 5.33	<0.0001
APOE ε4 +	242	75.17 ± 5.70	
^MC1R genotype			
p.V92M (–)	451	76.38 ± 5.64	0.758
p.V92M (+)	74	76.16 ± 5.43	

*SD, standard deviation.[a]–/– patients who carry no ε4 allele. –/+ patients who carry one ε4 allele. +/+ patients who carry two ε4 alleles. ^ p.V92M (–): non carriers of p.V92M variant. (+): carriers of at least one p.V92M allele.

association in the subset of biochemically confirmed AD patients was even stronger than in the overall set of patients, suggesting that the *MC1R* variant plays a role in the etiology of AD. This gene is highly polymorphic, and p.V92M and other variants are common in the Caucasian population. During the last few years, several GWAS have been conducted to identify common LOAD risk variants [5–10], and none of these studies reported an association between *MC1R* and the disease. This could be caused by methodological issues (coverage level of *MC1R* in SNP-array platforms or conservative statistical correction procedures) or by the molecular or clinical heterogeneity of patients included. Interestingly, we observed an inverse tendency between the *APOE* genotype and p.V92M suggesting that the presence of the *MC1R* variant could contribute to AD susceptibility, especially in those patients whose genetic risk could be not attributable to the *APOE* genotype. Further studies restricted to *MC1R* variants and *APOE* alleles should be conducted using previous GWAS data to elucidate such an inverse correlation.

Clinicopathological studies have shown that the sensitivity and specificity of the classical clinical criteria for AD diagnosis are roughly 85% and 70%, respectively [32]. CSF studies measuring $A\beta_{42}$ and tau protein levels in AD patients with confirmed pathology have demonstrated that abnormal levels of both biomarkers constitute a specific signature of the underlying AD-pathology (senile plaques and neurofibrillary tangles, respectively). Furthermore, multiple studies have shown that the sensitivity and specificity with the inclusion of specific CSF biomarkers profiles are roughly 90% and 85%, respectively [33, 34]. In accordance with this evidence, a recent study has found after inclusion of CSF results, 90% of amnestic and 82% of the non-amnestic AD presentation could be categorized as "high probability of AD etiology", while 3% of AD patients fit into the category "dementia probably not due to AD" [35]. Thus, we included a subset of patients with typical CSF AD biomarkers, demonstrating evidence of AD pathophysiological process and increased probability of AD etiology as a cause of symptomatology of the patient according to NIA-AA criteria [36]. This fact is relevant, because different studies have demonstrated neuropathological changes that sometimes do not correlate with clinical diagnosis [37]. Therefore, the role of the *MC1R* variant p.V92M in the AD risk was re-analyzed in these well-characterized AD patients. Notably, in spite of the sample size reduction, the association remains statistically significant and the LOAD risk in p.V92M carriers increased more than three fold. Thereby, the risk of p.V92M is more evident within more accurately diagnosed AD patients.

In this study, decreasing age of onset was restricted to the APOE ε4 allele. Thus, the p.V92M variant should be considered like those prior variants which increase the risk of developing AD but do not modulate AOO [38].

The *MC1R* gene encodes a membrane receptor, which is expressed, in neurons of the periaqueductal gray matter, astrocytes, and Schwann cells activated by melanocortin peptides [16, 39]. This receptor may have an important role in the anti-inflammatory brain response [16] and in female specific mediation mechanisms of analgesia [40]. MC1R is also expressed in melanocytes, a cell type with a common embryonic origin with brain cells [41], which determine hair and skin color [42], and certain variants increase the risk for skin cancer (melanoma and non-melanoma skin cancer) [18, 19]. Functional studies of *MC1R* variants conducted in melanocytes, reveal that certain variants reduce cell surface protein expression and diminished capacity to stimulate cAMP, resulting in the red hair color phenotype [17]. Interestingly, two *MC1R* variants related to red hair color phenotype modulate the risk to develop PD [22, 29]. These findings partially explain the previous epidemiological evidence

describing a bidirectional link between PD and CM [21]. In contrast to PD, an increased incidence of CM among AD patients and overrepresentation of individuals with natural red hair within AD patients compared to control population has not been reported. This can be explained as the p.V92M variant does not confer a risk to develop CM [18] and by the fact that variant p.V92M promotes a decrease in the affinity of the receptor for its ligand α-MSH, but showed normal cell surface expression and normal capacity to stimulate cAMP, consequently it does not impact on the phenotype [17, 39]. Notably, a functional deficiency of α-MSH in the brain cells of LOAD patients had been previously reported, suggesting that α-MSH may be critical in the development of LOAD [43].

To date, functional evaluation of *MC1R* variants in other cell types such as the nervous system cells is limited. However, there is evidence that certain variants may also impact physiological conditions beyond skin and hair pigmentation, such as risk of depression disorders [44], pain response [40], and anesthetic requirement [45]. Interestingly, a case-control study indicates that variant p.V92M is associated with the response of desipramine treatment in depression disorder [44].

Our study, although exploratory, has some limitations. The major one being sample size, which is not large enough to provide reliable evidence for a genetic AD risk factor, especially if we focus on the controls size (N = 160) which is small. However, our work provides positive results from our hypothesis and highlights a putative role of the *MC1R* gene in the genetic susceptibility to developing neurodegenerative diseases, which is in the same line as previously published works [22, 23].

Another minor limitation is the exclusion of the p.D294H *MC1R* variant from the genetic association analysis. As we explained in the Material and Methods section, the entire coding region was sequenced in 110 LOAD patients, but the other 415 LOAD patients were exclusively sequenced using TM-R primer. This strategy allowed us to detect all *MC1R* variants except one common non-synonymous variant (p.D294H). However, we compared the frequency of the p.D294H variant observed in 110 LOAD patients (minor allele frequency was 3%) with the frequency observed in 160 controls (minor allele frequency was 2%), and we did not detect any statistically significant difference.

In conclusion, the present study suggests that *MC1R* variant p.V92M may increase the risk to develop LOAD. Although, the molecular mechanisms underlying the increased risk of LOAD associated with p.V92M variant are not known, this variant may have biological relevance through non-pigmentation pathways involved in inflammatory or immunomodulatory processes.

Larger genetic studies are necessary to confirm the association of p.V92M with AD. Additionally, further functional studies should be carried out to elucidate the role of the MC1R receptor in brain cells.

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