Journal of Integrative Neuroscience

J. Integr. Neurosci. **2022**; 21(4): 99
https://doi.org/10.31083/j.jin2104099

Original Research

Genetic Predictors of Change in Episodic Verbal Memory by Cognitive Intervention: *ACT*, *PICALM*, *BDNF*, *NRG1*, *APOE* Genes and Their Interactions in Situations of Cognitive Demand

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Academic Editor: Robert Friedman
Submitted: 1 December 2021 Revised: 28 January 2022 Accepted: 15 February 2022 Published: 30 May 2022

Abstract

Background: Cognitive interventions (CIs) in the elderly are activities that seek to improve cognitive performance and delay its deterioration. Our objectives were to study potential genetic predictors of how a CI program may influence immediate and delayed episodic verbal memory (EVM). **Methods**: 162 participants were elderly individuals without dementia who were randomized into parallel control and experimental groups. Participants underwent genetic testing to analyze the *PICALM*, *ACT*, *NRG1*, *BDNF* and *APOE* genes. We performed a broad neuropsychological assessment before and 6 months after the CI. The CI involved multifactorial training (30 sessions). The control group undertook the centre's standard activities. The main outcome measures were the genotype studied as a predictor of post-intervention changes in EVM. **Results**: We found the CI was associated with improvements in several cognitive functions, including immediate and delayed EVM. While no individual gene was associated with any such change, the interaction between *PICALM/ACT* ($p = 0.008$; $\text{Eta}^2 = 0.23$) and *PICALM/NRG1* ($p = 0.029$; $\text{Eta}^2 = 0.19$) was associated with improved immediate EVM, and the *NRG1/BDNF* interaction was associated with improved delayed EVM ($p = 0.009$; $\text{Eta}^2 = 0.21$). The *APOEε4* genotype was not associated with any change in EVM. **Conclusions**: Our study shows that the participants' genotype can have an impact on the results of CIs. Cognitive stress may stimulate the interaction of various genes and as such, different types of CI should be established for distinct groups of people taking into account the individual's characteristics, like genotype, to improve the results of this type of health prevention and promotion activity.

Keywords: elderly people; cognitive intervention; genetic predictors; *ACT*; *PICALM*; *BDNF*; *NRG1*; *APOE*; Unidad de Memoria Ayuntamiento de Madrid method (UMAM method)

1. Introduction

Age-related cognitive changes have been paid special attention since it has been recognized that Alzheimer's disease (AD) commences many years before the first symptoms of minor memory loss become evident [1]. Specific activities have been developed that aim to improve memory and cognitive performance, increasing brain reserves while dampening cognitive decline and the evolution of dementia. These activities are generally referred to as Cognitive Interventions (CIs) and they have been the subject of intense development over the past two decades. These interventions usually have positive results and the effect sizes are commonly medium to medium-high when they fulfill certain conditions [2].

One of the important questions regarding CIs is whether elements exist that can predict their performance. What are the characteristics of people who benefit from training [3]? Few studies have been conducted on the modulation by genetic factors in the field of CI. However, of those that have, most focused on *APOE*, *BDNF* and genes involved with dopamine metabolism, catabolism and uptake.

It has been hypothesized that genes associated with cognitive improvements enhance neural plasticity in certain brain regions, facilitating stronger positive change. Polito *et al.* [4] implemented a cognitive stimulation program observing improvement in memory and finding that improvement only occurred in normal subjects who were non-carriers of *APOE* ε4, the most recognizable genetic risk factor associated with AD [4,5]. Accordingly, they suggested that the presence of the ε4 allele makes stimulation less effective in areas where visual attention is involved, which is key to visual memory. There have been other studies into *APOE* with similar results [6], although such outcomes have not been achieved in all studies [7].

BDNF (Brain Derived Neurotrophic Factor) is another gene that has been studied in relation to CIs. This is a growth factor that influences glutamatergic and GABAergic neurons, and its mechanisms of action and differential

expression have been studied in regions thought to be quite important for memory, such as the hippocampus and prefrontal cortex [8]. In a study of a group of healthy senior citizens to determine whether improvement in attention and cognitive flexibility through training was conditioned by *BDNF* [9], it was found that homozygous Val/Val carriers of the most intensely studied single nucleotide polymorphism (SNP) of this gene, rs6265, achieved better results than Met/carriers. Other positive relationships with *BDNF* have been reported [10], and when the effects of *APOE* and *BDNF* on cognitive and physical training were studied, only *APOE* predicted improvements in verbal fluency perhaps because this was the most demanding task tested [11]. In this case an *APOE* and *BDNF* interaction was not considered and in fact, very few studies have contemplated gene associations and interactions in this field, reflecting the need for more efforts along those lines [12].

To date, we have been unable to find any studies on the possible use of *PICALM*, *NRG1* and *ACT* to predict the benefits of training. However, their effects on neurotransmitters and beta-amyloid (Aβ) in the brain, and their wide distribution in memory-related areas like the hippocampus and prefrontal cortex, may justify their study. The *PICALM* gene encodes a protein that intervenes in the endocytosis of several substances, such as lipids and proteins (growth factors, neurotransmitters, etc.). It has been associated with AD [13], essentially due to the possibility that it modulates the production, transport and clearance of Aβ. Some *PICALM* alleles are associated with changes in the thickness of the entorhinal cortex, and in functional connectivity with the hippocampus and cognitive performance [14,15]. *ACT* is a gene from the serine protease inhibitor family and it is produced in the brain near amyloid plaques by activated astrocytes, and expressed in regions like the hippocampus. It is associated with some of the neuropathological changes found with AD, forming a toxic complex with Aβ [16]. The *NRG1* gene codes for a cell-signaling protein that belongs to the neuroregulin family. Some of its activities are memory-related, such as the modulation of long-term potentiation (LTP) and depression (LTD), and others are related to the N-Methyl-D-aspartate (NMDA) and AMPA receptors (NMDARs and AMPARs). Through NMDA, NRG1 also modulates the excitatory neurotransmitter glutamate and the inhibitory transmitter GABA in neurons [17]. Here we studied the rs6994992 variant as it has been associated with spatial working memory and attention in the general population [18]. Indeed, NRG1 interacts with *BDNF* as it activates a receptor of this protein involved in neural plasticity [19].

A few authors have proposed that the effects of some genes may be more readily detected in situations of demand, change or cognitive effort, such as that occurring in programs of cognitive training or stimulation [20]. However, since the effect sizes of each individual SNP are often low, it may be that the best way to detect any effect caused by them is through their interactions with other genes or SNPs. As a result, we contemplated this possibility in our study. This article is part of a randomized control trial in which multifactorial cognitive training was offered to healthy individuals. We found that, the training program followed, improved several cognitive areas including immediate and delayed episodic verbal memory (EVM). Thus, we set out to examine whether the genes *PICALM* (SNP rs3851179), *ACT* (SNP rs4934), *BDNF* (rs6265; Val66Met), *NRG1* (rs6994992), and *APOE* were associated with the improvements observed in immediate and delayed EVM after training in the experimental group. We hypothesized that while we might not detect single gene effects, we may detect gene interaction effects. Although the various genes and their DNA variants may be conditioned by age, sex, cognitive reserve and other variables, our sole purpose was to study the effect of these genes regardless of these other variables.

This trial was retrospectively registered on January 29, 2020 (ClinicalTrials.gov -NCT04245579).

2. Material and Methods

2.1 Participants

This study was performed on 226 older people, >60 years of age (mean age 71.3, Standard Deviation (SD) 4.7), who were healthy individuals living in the community. The participants were recruited consecutively at the Centre of Prevention of Cognitive Impairment (CPCI), the Psychology Department, and the Clinic Hospital (HCSC). The inclusion criteria for this study were: more than 60 years old, without dementia and with good overall cognitive functioning: Mini Mental State Examination >23 (MMSE) [21], and Functional Activities Questionnaire <6 (FAQ) [22]. We excluded participants with a history of severe psychiatric or neurological disorders (schizophrenia, any type of psychosis, epilepsy, Parkinson's disease, alcoholism, etc.) and with chronic use of sedatives or other psychoactive drugs (anxiolytics, anticonvulsants and neuroleptics). Senior citizens with any other significant impairments (sight or hearing impairments, significant movement difficulties, etc.) were also excluded if the medical staff believed they could hinder any assessments, or if they might interfere with the CI group sessions or the prescribed exercises in which they would need to perform.

2.2 Procedure

The study was approved by the Clinical Investigation Ethics Committee at the HCSC (internal code No. 15/382-E_BS) and it was registered retrospectively on January 29th, 2020 (*ClinicalTrials.gov*-NCT04245579). Participants were informed of the study's characteristics and signed consent forms. Every participant completed a medical record form and was given a structured interview, experienced professionals (a psychiatrist, neurologist and neuropsychologists) evaluating them in three, one-hour sessions. Participants were also examined by Magnetic Resonance Imaging (MRI) and Magnetoencephalography, al-

though these data were not used here. The participants were assigned randomly to either of the two groups, Experimental and Control group, by an independent neuropsychologist blind to the individual process. The participants in the experimental group completed a cognitive intervention program based on the UMAM method, whereas no specific cognitive training was offered to the members of the control group. All the study subjects were assessed for a second time 6 months after the beginning of the study. Both groups followed all the routine activities that those who attend the center are offered (planned consultations, conferences-dialogue, general health recommendations...).

2.3 Evaluation

Each participant was assessed with: the Mini Mental State Examination (MMSE) [21]; the 7Minutes Test (7MT) [23]; Tests of Verbal Memory (Wechsler Logical Memory-EVM and DVM- and the Word List of the Wechsler Memory Scale-III -WMS-III) [24]; the Trail Making Test forms A and B (TMT) [25]; the Rule Shift Cards 1 and 2 from the Behavioral Assessment of the Executive Syndrome (BADS) [26]; the Stroop Test [27]; Rey's Simple Figure Test (form B) [28]; the Rivermead Behavioral Memory Test (RBMT) [29]; the Boston Naming Test (BNT) [30]; the Semantic Verbal Fluency Test and the Phonological Fluency Test [31,32]; and the Functional Activities Questionnaire (FAQ) [22]. Cognitive reserves were assessed using a specific questionnaire (CRQ) [33].

2.4 Genetic Data

2.4.1 Genes Analyzed

Genes and SNPs analyzed were *PICALM* (Chromosome 11; rs3851179, upstream SNP of the gen *PICALM*); *ACT*, also known as *SERPINA3*, (Chromosome 14; rs4934, G (ALA) changes to A (THR) in position 6 of protein SERPINA3 (Mutation missense); *BDNF* (Chromosome 11; rs6265, G (VAL) changes to A (MET) in position 66 of protein BDNF (mutation missense); *NRG1* (Chromosome 8; rs6994992 in the 5-prime promoter region of the *NRG1* gene regulates expression of the *NRG1* type IV isoform); *APOE* (Chromosome 19; rs429358, T (CYS) changes to C (ARG) in position 130 of Apolipoprotein E (mutation missense), and rs7412, C (ARG) changes to T (CYS) in position 176 of Apolipoprotein E (mutation missense).

2.4.2 Genotyping

Genomic DNA was extracted from 10 mL EDTA-anticoagulated whole blood samples of healthy elders. The detection of *APOE* genotype was performed using a method revised from Zivelin *et al*. [34], although utilizing the forward primer from the technique defined by Hixson and Vernier [35] that produces a 227 bp DNA fragment. Double digestion of this fragment with AflIII and HaeII yields on 4% agarose electrophoresis three specific fragments: 177 bp for ε2 allele, 145bp for ε3 and 195 bp for *APOE* ε4. All polymorphisms were genotyped with TaqMan assays, using an Applied Biosystems 7900 HT Fast Real Time PCR machine (Applied Biosystems, Foster City, CA). A genotyping call rate over 90% per plate, sample controls for each genotype, and negative sample controls were included in each assay. Three well-differentiated genotyping clusters for each SNP were required to validate the results. Intra- and inter-plate duplicates of several DNA samples were also included.

2.5 Training: UMAM Method

The CI program applied to the experimental group is a cognitive program initially designed and implemented in 1994 by the Memory Training Unit of the Madrid City Council. This program is known as the UMAM method and it has been described in a Manual of Evaluation and Memory Training published with explanations of each session and exercise, including booklets, printed sheets, other edited books, slides, etc. [36]. Briefly, the cognitive training program consists of 30 sessions, each 90 minutes long, and the sessions are carried out in groups of 12–18 people. This cognitive program is currently being implemented in 16 municipal health centers run by the Madrid City Council and it has been applied to more than 30,000 individuals.

2.6 Data Analysis

All the statistical analyses were performed using the IBM SPSS Statistics package (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY). To analyze the treatment effect in both immediate and delayed EVM, a General Linear Model with repeated measures was used (post-training minus pre-training). The within-subject factor between the pre- and post-training evaluation, the two-time points examined, was designated as "Time," and the between-subject factor, that is the experimental or control group, was designated as "Group". The difference in response between the experimental and control group was reported as the "Time $\times$ Group" interaction. The effect size was estimated by the standardized mean differences using Cohen's d statistic, specifying a small (d = 0.20), moderate (d = 0.50), or strong effect (d = 0.80) [37].

To study the predictors in the experimental group, we first used an ANOVA test with a partial Eta squared (Eta^2) to reflect the size effects. The estimation was as proposed by Cohen [37]: 0.02 'small', 0.13 'medium', and 0.26 'large'. The possible predictors were the *PICALM*, *NRG1*, *BDNF*, *ACT* and *APOE* genes. *APOE* was coded as 1 for no ε4 allele or 0 for at least one ε4 allele. The dependent variables were the results of the immediate and delayed EVM change (post-training minus pre-training) in the experimental group. The factors were the alleles for each gene. We first investigated the effect of each SNP on the basal score for the immediate and delayed EVM variables, then the interaction of the genes two-by-two. Subsequently, we studied the association of each of the five genes with the change

Table 1. Means, standard deviations, F for the interaction effects (group × time) with *p* values, and effect size, Cohen's "d".

Variable (range)	Experimental group n = 88		Control group n = 74		Time		Group		Interaction (group × time)		
	Pre-training M (SD)	Post-training M (SD)	Pre-training M (SD)	Post-training M (SD)	*F*	*p*	*F*	*p*	*F*	*p*	Cohen's *d*
WLM Immediate (0–75)	34.38 (12.24)	38.59 (11.97)	35.92 (12.89)	36.55 (12.76)	16.28	0.000	0.02	0.903	8.90	0.003	0.26
WLM Delayed (0–50)	19.14 (9.73)	23.30 (9.60)	21.8 (10.38)	22.75 (9.75)	27.29	0.000	0.49	0.487	11.60	0.001	0.34

WLM, Wechsler Logical Memory [Immediate Episodic Verbal Memory (EVM) and Delayed Episodic Verbal Memory (EVM)].

in the immediate and delayed EVM variables, and then the two-by-two interactions.

For those variables with a significant interaction, we analyzed the effect of the alleles and every single gene interaction on the dependent variable using the "Decision Tree" procedure. The gene with the highest F value found by ANOVA was used as the first variable to split the sample in the analysis. It has been pointed out that this procedure allows us to identify different risk profiles and that such partitioning approaches do not imply interaction "per se" but potential interactions [38], which was the meaning we wanted to give to the results of our decision tree.

We used raw scores of all the data in the statistical analysis and the statistical significance was set at $p < 0.05$. Some variables include missing data that correspond to participants who did not perform any of the evaluations and when specific data was missing for any subject, this participant was removed from the corresponding analysis.

3. Results

In this study 226 people aged over 60 years were assessed for eligibility of which 211 were randomized to the two study groups. 38 participants were excluded because of invalid blood samples and 11 because of poor motivation to continue in the study. The final study sample was comprised of 162 individuals: mean age = 71.04 ± 4.75, range = 60–81 years of age, 29.6% males; Caucasian-Europeans; education - university 34.8%, secondary 27.3% (more than 9 years of schooling), elementary 26.7% (7–9 years) and basic 11.2% (less than 7 years); mean MMSE = 28.32 ± 1.6; mean 7MT = 65.31 ± 12.73; mean FAQ = 0.41 ± 1.58. The experimental group that was used for the genetic study was n = 88, mean age = 71.02 (SD 4.63), range = 62–81 years old, 31.8% males. The control sample was n = 74, mean age = 71.07 (SD 4.93), range = 62–80 years old, 27% males. There were no significant differences between the experimental and control groups for any sociodemographic or neuropsychological variable, nor in the questionnaires completed before training. By contrast, significant pre-post differences were detected for several scores after training, reflecting a significant improvement in EVM in the experimental group compared to the control group, in which no significant pre-post differences were found. In the experimental group immediate EVM improved in 67.5% of the participants after training, while it did not change in 10% and it worsened in 22.5%. In terms of delayed EVM, improvement was evident in 76.2% of the participants after training, whereas 5% showed no change and it deteriorated in 18.8% (Table 1).

The genetic data was obtained from all the participants (for the experimental group: Table 2) and the genotype frequency in the cohort did not deviate from the Hardy–Weinberg equilibrium: *NRG1* ($p = 1$); *BDNF* ($p = 0.47$); *PICALM* ($p = 0.49$); *ACT* ($p = 0.07$); *APOE* rs429358 ($p = 1$); *APOE* rs7412 ($p = 1$).

Table 2. Descriptive data of alleles of studied genes (experimental group).

Gene	N	%	Gene	N	%
NRG1			*BDNF*		
CC	23	26.4	AA	4	4.6
CT	43	49.4	AG	24	27.6
TT	21	24.1	GG	59	67.8
ACT			*PICALM*		
GG	27	31.4	AA	10	11.4
AG	50	58.1	AG	45	51.1
AA	9	10.5	GG	33	37.5
APOE (haplotype)					
e2e3	7	8.1			
e3e3	60	69.8			
e3e4	18	20.9			
e4e4	1	1.2			

We analyzed the effect of each SNP on the basal immediate and delayed EVM scores of experimental group, and no significant associations were observed, although the effect sizes remained small. While none of the two-by-two gene interactions produced any significant association either, the largest effect sizes (Eta^2 between 0.12 and 0.13) were observed with the *PICALM* + *NRG1* and *PICALM* + *BDNF* interactions (Table 3).

We studied each of the five genes to analyze their association with the post-training changes in immediate and delayed EVM (Table 4). No significant association was observed for any single gene and the effect sizes were very small. The largest effect sizes for immediate EVM were evident with the *NRG1* + *PICALM* interaction, as was the case

Table 3. Associations of genes with basal evaluation data.

Association of Genes with basal data (Evaluation Pre)						
	WLM Immediate			WLM Delayed		
Single Genes	F	Sig. (*p*)	Eta^2	F	Sig. (*p*)	Eta^2
ACT	0.297	0.744	0.007	0.939	0.395	0.022
BDNF	2.221	0.115	0.050	2.259	0.122	0.049
NRG1	0.332	0.718	0.008	0.130	0.878	0.003
PICALM	0.718	0.491	0.017	0.950	0.391	0.022
APOE	0.086	0.770	0.001	0.031	0.860	0.000
Interactions						
NRG1 + BDNF	1,017	0.426	0.084	1.230	0.297	0.099
NRG1 + ACT	0.629	0.751	0.062	0.982	0.457	0.094
NRG1 + PICALM	1.490	0.175	0.133	1.365	0.225	0.123
NRG1 + APOE	0.166	0.974	0.010	0.255	0.936	0.016
BDNF + ACT	0.772	0.613	0.066	0.848	0.551	0.072
BDNF + PICALM	1.537	0.167	0.120	1.652	0.133	0.128
BDNF + APOE	1.154	0.337	0.055	1.178	0.327	0.056
PICALM + APOE	1.291	0.276	0.075	0.867	0.507	0.051
ACT + APOE	0.318	0.901	0.020	0.502	0.774	0.031
ACT + PICALM	0.460	0.860	0.040	0.652	0.712	0.055

WLM, Wechsler Logical Memory.

Table 4. Association of single genes and interactions with change in WLM immediate and delayed.

Association of Genes with Change by training (Differences Pre-Post)						
	Change in WLM Immediate Pre-Post			Change in WLM Delayed Pre-Post		
Gene	F	Sig. (*p*)	Eta^2	F	Sig. (*p*)	Eta^2
NRG1	2.605	0.080	0.064	2.451	0.093	0.061
BDNF	0.037	0.964	0.001	0.089	0.915	0.002
ACT	1.457	0.239	0.037	2.462	0.092	0.062
PICALM	2.188	0.119	0.054	0.541	0.585	0.014
APOE	0.000	0.994	0.000	0.081	0.776	0.001
Interactions						
PICALM + ACT	**3.002**	**0.008**	**0.231**	1.461	0.195	0.127
PICALM + APOE	1.412	0.230	0.088	0.206	0.959	0.014
NRG1 + BDNF	0.953	0.463	0.075	**3.114**	**0.009**	**0.208**
NRG1 + ACT	1.656	0.125	0.163	1.326	0.246	0.135
NRG1 + PICALM	**2.398**	**0.029**	**0.191**	1.297	0.264	0.113
NRG1 + APOE	1.206	0.315	0.077	0.918	0.474	0.060
BDNF + ACT	0.650	0.713	0.062	1.137	0.351	0.103
BDNF + PICALM	0.959	0.468	0.086	0.609	0.747	0.057
BDNF + APOE	0.243	0.421	0.013	0.138	0.968	0.008
ACT + APOE	0.968	0.443	0.064	1.186	0.325	0.077

WLM, Wechsler Logical Memory. Bold data correspond to significant associations.

for the *NRG1 + ACT* interaction, which was also associated with changes in delayed EVM. Upon studying *PICALM* in more detail, and focusing on the AA allele as opposed to the AG/GG allele, a significant association with EVM was found (F = 4.432; $p = 0.038$; Eta^2 = 0.05). Table 5 shows the ANOVA post-hoc analysis and the interactions of the different alleles. With respect to *APOE* the mean change in immediate EVM was not significantly different in the carriers of the *APOE* ε4 allele (4.27 $\pm$ 5.51) from that in non-carriers (4.26 $\pm$ 7.89; $p = 0.99$), as was also the case in delayed memory, with a mean change for carriers of the *APOE* ε4 allele of 3.88 $\pm$ 4.36 and of 4.34 $\pm$ 6.33 ($p = 0.77$) for non-carriers. As such, there appeared to be no association between *APOE* and the change in immediate or delayed EVM.

We then analyzed the gene interactions that might predict a change with training in immediate EVM (pre-post). The association between the genes *PICALM + ACT* was significant, both in terms of the effect of each gene individually as well as their interaction, with an overall effect size of 0.23 (see Table 4). Upon introducing *APOE* into the *PICALM + ACT* model and studying the interactions between the three genes, the equation continued to be significant (F = 2.062; $p = 0.029$; Eta^2 = 0.299), although with *APOE* in the equation there was a significant effect of *ACT* (F = 3.263; $p = 0.045$; Eta^2 = 0.09) but no longer of *PICALM* ($p = 0.171$; Eta^2 = 0.054). Neither APOE ($p = 0.839$; Eta^2 = 0.001), nor did the interactions have a statistically significant influence (*APOE + ACT*, $p = 0.248$; Eta^2 = 0.043; *APOE + PICALM*, $p = 0.429$; Eta^2 = 0.027). The *NRG1 + PICALM* association also produced a statistically significant change. We then studied the gene interactions that might predict a change with training in delayed EVM (pre-post). None of these interactions were significant except for that of *BDNF + NRG1*, although upon introducing *APOE* this model ceased to be significant (F = 1.648; $p = 0.101$; Eta^2 = 0.236; although the statistical power for *APOE* and their interactions <0.20). We added the variables of age and sex separately to the significant interaction equations for *PICALM + ACT* and *NRG1 + PICALM* (immediate EVM) and *BDNF + NRG1* (delayed EVM) with no significant effects evident for either of these variables.

To determine whether any of the alleles might be associated with changes in immediate and delayed EVM, we followed the "Decision Tree" procedure using the groups of variables for which a significant interaction was identified. Analyzing *PICALM* and *NRG1* (Fig. 1), we can observe that when the mean improvement in "immediate EVM" was assessed for the whole group (Fig. 1), the performance of individuals with the AA alleles (node 1) did not improve but rather worsened (note that the negative sign mean indicates a decline in performance, –0.875: Fig. 1). The interaction of the *PICALM* AG and GG carriers with the participants carrying the *NRG1* CT alleles performed three times better than those with the CC and TT alleles, who also performed worse than the mean of the entire sample (node 4) (Fig. 1).

Interacting with *PICALM* (Fig. 2), the carriers of the AA and GA, *ACT* variants (node 3) improved nearly three

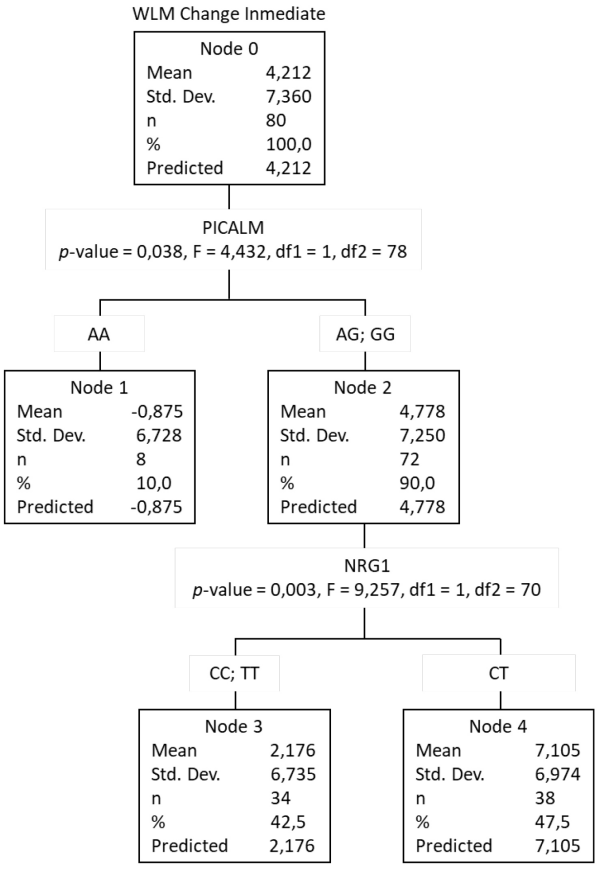

Fig. 1. Decision Tree Change in WLM immediate as dependent variable split by alleles of the two genes with interaction significant effects. Over each node the correspondent PICALM and NRG1 alleles is shown, inside the node is the mean of change in the dependent variable, with its SD, the total "n" in the node, and the percentage (%) of participants in this node with respect to the sample of each specific gene; *p*-value; df, degrees of freedom.

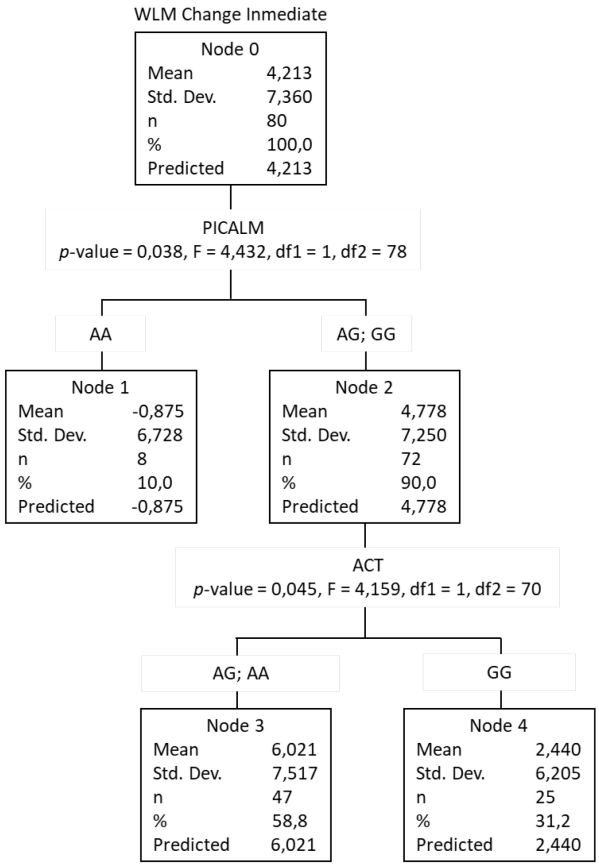

Fig. 2. Decision Tree Change in WLM immediate as dependent variable split by alleles of the two genes with interaction significant effects. Over each node the correspondent PICALM and ACT alleles is shown, inside the node is the mean of change in the dependent variable, with its SD, the total "n" in the node, and the percentage (%) of participants in this node with respect to the sample of each specific gene; *p*-value; df, degrees of freedom.

times more than carriers of the GG alleles (node 4).

On studying the change in the delayed EVM (Fig. 3), we observed that the CT carriers of the *NRG1* gene (node 2) performed better. Regarding the *BDNF* gene and its interaction with the *NRG1* gene, carriers of the AG alleles of *BDNF* showed the greatest improvement after training in node 6, whereas in node 4 the carriers of this *BDNF* allele (AG) showed a decline in performance. Carriers of *BDNF* AA were not studied here as they comprised only 4.6% of the sample and there were no carriers of *NRG1* CT who were also *BDNF* AA.

4. Discussion

We studied here a group of elderly individuals without dementia who were randomly assigned to either a control or experimental group. The experimental group participated in a memory training and cognitive stimulation program based on the UMAM method, which achieved improvements in immediate and delayed EVM, as measured by the WLM (immediate and delayed scoring). We set out to analyze whether the *ACT*, *PICALM, BDNF*, *NRG1* and *APOE* genes could serve as predictors of this change or the improvement following training. The results showed that none of these genes were associated with the participants' basal scoring in immediate or delayed EVM, neither individually or via two-by-two interactions. Furthermore, there was no association between the individual genes and the change induced by training. However, when the interaction between genes was studied, the interaction between *PICALM* and *ACT* was significantly associated with the change in immediate EVM (beneficial alleles: *PICALM* AG/GG and *ACT* AA/AG), as was the interaction between *PICALM* and *NRG1* (beneficial alleles: *NRG1* CT, *PICALM* AG/GG). The interaction between *NRG1* and *BDNF* proved to be significantly associated with the change in delayed EVM. The *APOE* gene did not appear to be significantly associated with any improvement or decline in EVM performance, either alone or in any interaction, although the data avail-

Table 5. Alleles with significant interactions (ANOVA, Post-hoc Analysis).

PICALM + ACT (WLM Change Immediate)					
PICALM	*(I) ACT*	*(J) ACT*	Mean Difference (I-J)	Std. Error	Sig. (*p*)
GG	AA	AG	14.471*	5.067	0.017
		GG	17.364*	5.210	0.004
	AG	AA	–14.471*	5.067	0.017
		GG	2.893	2.623	0.821
	GG	AA	–17.364*	5.210	0.004
		AG	–2.893	2.623	0.821
NRG1 + PICALM (WLM Change Immediate)					
NRG1	*(I) PICALM*	*(J) PICALM*	Mean Difference (I-J)	Std. Error	Sig. (*p*)
CT	AA	AG	–11.202*	3.794	0.013
		GG	–11.375*	3.888	0.014
	AG	AA	11.202*	3.794	0.013
		GG	–0.173	2.308	1.000
	GG	AA	11.375*	3.888	0.014
		AG	0.173	2.308	1.000
NRG1 + BDNF (WLM Change Delayed)					
NRG1	*(I) BDNF*	*(J)BDNF*	Mean Difference (I-J)	Std. Error	Sig. (*p*)
CT	AA	AG	–11.202*	3.794	0.013
		GG	–11.375*	3.888	0.014
	AG	AA	11.202*	3.794	0.013
		GG	–0.173	2.308	1.000
	GG	AA	11.375*	3.888	0.014
		AG	0.173	2.308	1.000

* The mean difference is significant at the 0.05 level.

Adjustment for multiple comparisons: Bonferroni.

able may not have had sufficient statistical power to fully analyze all such interactions. It is interesting to note that the *NRG1* gene is involved with two interactions (with *PICALM* and *BDNF*) associated with an improvement in immediate and delayed EVM, and that the same CT allele is involved in the interactions with both genes, indicating it could be considered a "beneficial" allele in terms of such tasks. Interestingly, we have found that some heterozygotes perform better than homozygotes, which is less usual [39,40].

In terms of the basal scores of the participants for immediate or delayed EVM, associations between cognitive ("basal") performance and some genes have been found previously in adults [41], and the inheritability of general memory is usually considered to be around 50% [42]. However, elsewhere studies of post-training predictors have found similar results to those seen here, as no association was found between the basal scores and the genes studied. When training working memory in children, two SNPs were seen to predict the results but none were associated with basal performance [12]. No such association was found for a single gene in an earlier study on EVM, although combinations of genes did seem to have some effect [39]. While this failure to detect an association may be due to the lack of any association, it may also just reflect the low effect size [43]. Nevertheless, the best results are generally found in gene association studies.

4.1 APOE and its Interactions

We did not find an *APOE* allelic variant to be a predictor of improvement in EVM despite the supposed evidence that non-carriers of the ε4 allele have enhanced neuroplasticity. However, the data from other studies is not consistent, as ε4 carriers have worse results in visuospatial memory when assessed post-intervention but not in other cognitive areas [5]. A post-intervention improvement in verbal memory was observed when assessed after 6 but not 12 months, and not immediately either [6]. A CI with cognitively stimulating lifestyle activities improved verbal fluency task, word recall and fact recall in non-carriers of the ε4 allele [44]. Elsewhere no differences between carriers and non-carriers were seen in any cognitive function post-intervention [7]. As a result, several hypotheses can be formulated. Based on results similar to ours, it was proposed that the non-concordance in different characteristics of the training activities may explain these differences and influence how *APOE* intervenes, such as the longer time spent on some cognitive processes than others, the number of sessions and other training variables [45]. It is also possible that *APOE* affects the individuals' baseline EVM perfor-

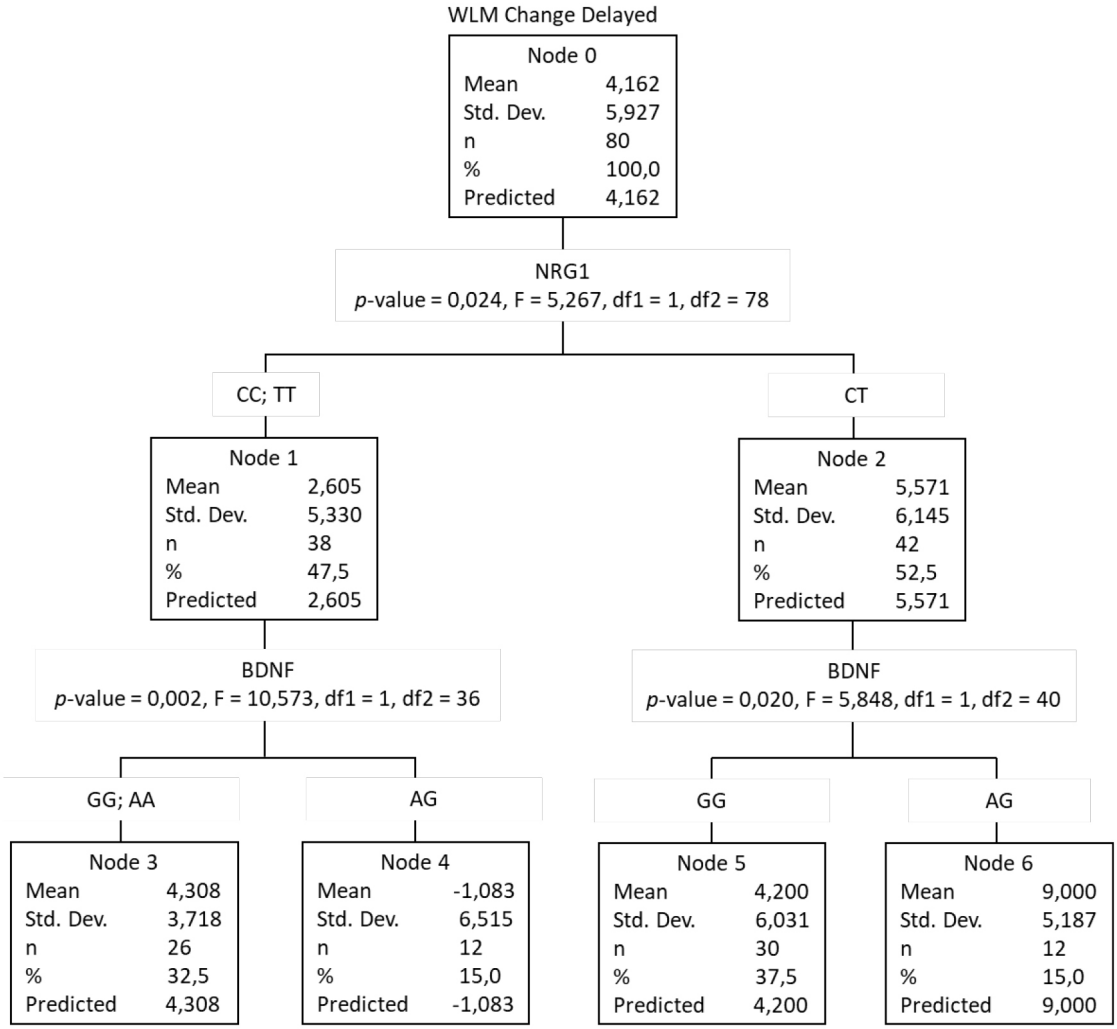

Fig. 3. Decision Tree Change in WLM immediate as dependent variable split by alleles of the two genes with interaction significant effects. Over each node the correspondent NRG1 and BDNF alleles are shown, inside the node is the mean of change in the dependent variable, with its SD, the total "n" in the node, and the percentage (%) of participants in this node with respect to the sample of each specific gene; *p*-value; df, degrees of freedom.

mance but not any gains in stressful or demanding situations. Furthermore, we must take into account that neither the effect sizes nor the changes due to training are sufficiently large, which increases the probability of a type II error.

4.2 Change in Immediate Episodic Verbal Memory

4.2.1 The PICALM/ACT Interactions

In a previous study of the *PICALM*, *CR1*, *BIN1*, *CLU* and *APOE* genes, Barral *et al*. [39] did not find any single SNP associated with EVM in a non-AD population, although some association with poorer performance was detected for some alleles in interactions with other genes: e.g., *PICALM* GG, *CR1* GG and *APOE ε4*. In this interaction, the *PICALM* G allele would have a negative effect and the A allele would have a positive one. Other authors also found that the A allele is protective and that the G allele poses a risk [46]. However, the opposite results have also been found or no effects in relation to the *PICALM* alleles were seen [47]. In terms of the interaction with *NRG1*, we found that the A alleles are not protective but rather, they are associated with risk.

In relation to the *ACT* gene and the rs4934SNP, it is unclear whether there is an association with risk or protection. It has been proposed that the onset of AD is earlier in subjects with the GG alleles and that their life expectancy is lower [48], yet in a different study AA was considered to be a risk allele [49]. The most severe association was also reported to be the *ACT 5* G allele plus the *ACT 7* C allele [50]. Here, the *ACT* allele associated with worst performance was the GG allele (which would be considered the risk allele), conditioned by *PICALM* AG/GG.

How can the *PICALM*/*ACT* interaction be explained? Again, a large number of studies of these genes are related

to AD in which the events underlying this interaction remain unclear. Both genes are expressed in regions of the brain linked to AD, both are associated with actions on Aβ and some of their effects are complementary. Moreover, both these genes have alleles that are considered to be protective. Nonetheless, we have not found any study in which *PICALM* was associated with *ACT* in influencing EVM or the prediction of post-training results. One hypothesis might consider the enhanced formation of toxic protein polymers linked to *ACT* [51], particularly since *PICALM* has been found at the walls of blood vessels and associated with weaker endocytosis, which could reduce the clearing of these toxic substances [46], leading to worse cognitive performance and progressive cognitive decline.

4.2.2 PICALM/NRG1 Interactions

We did not find any studies into interactions between *PICALM* and *NRG1* in relation to cognitive performance in any area. If we try and consider the nature of their interaction, important features of these two genes can be taken into account, such as their strong expression in the hippocampus and frontal cortex [52,53]. Some of the actions of *PICALM* occur in the pre- and post-synaptic regions, regulating aspects of exocytosis/endocytosis. Indeed, *PICALM* has been implicated in the mechanisms by which neurotransmitter vesicles associate with the presynapse [54], a key aspect of the Long-Term Potentiation (LTP) linked to memory regulation by *NRG1* [17].

4.3 Changes in Delayed Episodic Verbal Memory

NRG1/BDNF Interaction

The *NRG1* CT alleles could be a "beneficial" variant in delayed EVM when this gene is associated with *BDNF*, whereas the improvement associated to the CC and TT alleles represents about half that of the heterozygote, representing risk alleles. We did not find any other study of this gene interaction effect, although many studies (some related to schizophrenia) have associated *NRG1* with structural differences in the brain, identifying the TT alleles as risk alleles associated with a loss of gray and white matter in several regions of the human brain [55].

Altered *BDNF* activity in the hippocampus can lead to a decline in memory tasks [56] and in most cases, the adenine allele (encoding Met) is associated with worse cognitive performance than the guanine allele (encoding Val). This may be associated with the reduction in hippocampal volume, as occurs in subjects with a high level of Aβ but not in those with low Aβ. Regarding the predictive capacity of BDNF protein in blood, both low [11] and high levels [10] have been associated with improved cognitive performance post-CI.

As far as the effects following training are concerned, *BDNF* was not seen to be a predictor in a previous study [12], although a predictor effect was observed in our results when interacting with *NRG1*. In an attempt to understand this finding, we can consider the relationships between these two genes. Some of their activities are similar or complementary, especially their effects on neural plasticity and LTP [17]. The NRG1 receptor ErbB4 activates a BDNF protein receptor, TrkB, indicating that an increase in NRG1 will also increase the levels of BDNF. Both *NRG1* and *BDNF* induce myelination in the central nervous system (CNS) by increasing the number of NMDARs, and *BDNF* plays a compensatory role in this myelination when the activity of *NRG1* is dampened or absent [57]. The hippocampus and frontal region, common predominant sites of their activity, are important areas for memory and particularly, delayed memory. BDNF has been found to enhance cognitive performance post-physical or cognitive exercise [58] and NRG1 levels may increase with neuronal activity [59]. Despite these possible functional and physical interactions, we have not found a truly plausible explanation for the specific interaction observed in the improvement of delayed memory. Might this interaction influence LTP? If the *NRG1* CC/TT alleles were to reduce the levels of BDNF in AG carriers, it may have an adverse effect on the post-training influence on delayed EVM memory. Conversely, the effects may be enhanced in the two heterozygous types as the *NRG1* CT could produce an increase of BDNF in subjects who are carriers of AG alleles.

4.4 Limitations

There are several limitations to our study that should be highlighted. We are aware that some factors that are associated with an improvement or a decline were not analyzed in this work (cognitive reserve, brain volume, education level, motivation, etc.). While this may be viewed as a limitation, we wanted to focus our work about CI specifically on the influence of genotype, irrespective of other factors, and even their possible influence on the expression of these genes. Given the number of participants, it is difficult to conduct studies on the interactions of more than two genes and moreover, this was a possible cause of the inconsistent results from *BDNF* AA carriers. Furthermore, our explanations for the physiological effect of the genes must be understood as possibilities or hypotheses that require further study. As such future efforts should be specifically designed to confirm or reject the relationships identified here.

5. Conclusions

Recent genetic studies, including genotyping in specific diseases [60], have highlighted the role of oxidative stress as an inducer of neurodegeneration, causing toxicity and promoting cell death [61,62]. Learning and memory are possible only because of the neural plasticity; genetic and environmental factors are involved both in pathological processes and in slowing or preventing cognitive decline; molecular biomarkers can contribute to the diagnosis of neurodegeneration and guide pharmacological interventions [63]. Here we aimed to open new avenues in the

studies of the influence of specific genes on the benefits of non-pharmacological therapies.

Our study reinforces the concept that gene interactions may be better studied in situations of stress or high demand as their expression could be boosted in such situations. However, this study has practical applications as while a large proportion of the sample improved with the CI in terms of the variables analyzed, immediate and delayed EVM, some participants did not change or even achieved lower scores when assessed six months after training. Therefore, we must adapt the cognitive training and stimulation to each participant's characteristics, including their genotype, which has not been intensely studied. If the results obtained are taken into consideration, it should be possible to achieve better results with these Health Prevention and Promotion activities.

Author contributions

PMC, MMP and DPC, were responsible for the design of the study, carried out the statistical data analysis and wrote the paper; CMR and MLDL supervised the data collection, revised the manuscript for important clinical and scientific content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Clinical Investigation Ethics Committee of the Clinic Hospital San Carlos (HCSC) (internal code nº 15/382-E_BS). Participants were informed of the study characteristics and signed consent forms. **Trial registration**: Trial retrospectively registered on January 29, 2020; ClinicalTrials.gov-NCT04245579.

Acknowledgment

Not applicable.

Funding

This work was supported by the Ministry of Science and Innovation of Spain: PSI 2015-68793-C3-3-R and PSI 2015-68793-C1-3-R.

Conflict of interest

The authors declare no conflict of interest.

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