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Article

The relation of Interleukin18 (IL18) gene polymorphisms and Mild Cognitive Impairment and their impact on physical outcomes in elderly

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Abstract

Background: Mild Cognitive Impairment (MCI) is an important medical problem in elderly which may progress to dementia. Early recognition and intervention are important to prevent dementia harmful consequences. Its prevalence in Egyptian governorates ranges from1.7% to 39.3%. Frailty is an important geriatric giant that increase probability of disabilities and diseases. Both cognitive impairment and frailty are thought to be related to each other. Co-presence of MCI and physical

frailty yield cognitive frailty which considered cumulative risk for disabilities. Inflammation plays a vital role in cognitive impairment and thought to mediate frailty in elderly population. *Objective*: In our study we investigated the relationship between Interleukin-18 gene polymorphisms at 607 C/A and 137 C/G and MCI and physical frailty. *Methods*: 90 elderly patients were assessed for cognitive status by Arabic version of Montreal Cognitive Assessment tool (MoCA) and presence of physical frailty. Participants were categorized into Group I: patients with both MCI and physical frailty or prefrailty (Cognitive frailty), Group II: patients with MCI without physical frailty or prefrailty, and Group III: Participants with normal cognition. Genotyping of interleukin-18 gene polymorphism by sequence-specific PCR were done for all patients. *Results*: CC genotype at position 607 C/A is associated with lower MoCA score and higher physical frailty score than other genotypes (p= <0.001 and 0.013) respectively, while GG genotype at position 137 C/G is associated with lower MoCA score (P <0.001) and this remained significant after adjustment of confounding factors (OR: 44.990, 95% CI:(1.498 – 1351.44), p=0.028, with no substantial relation to physical frailty (P=0.545) respectively. *Conclusion*: Interleukin- 18 gene polymorphisms play an important role in mild cognitive impairment. However, their role in physical frailty is still questionable.

Keywords: Mild Cognitive Impairment (MCI); physical frailty; cognitive frailty; Interleukin-18 gene polymorphism, elderly.

Introduction

Ageing is a worldwide concern, and it's associated with major health burden. There was 2.5% increase in number of Egyptian elderly population from 2006 to 2017 census.(1)

Mild cognitive impairment (MCI) is an intermediate stage between normal cognitive decline and dementia but not always progress to dementia and may become stationary or even reverts to normal.(2)

Prevalence of MCI in some governorates in Egypt ranges from 1.7% to 39.3%.(3, 4)

MCI can be identified clinically by personal or family history of memory deficits, abnormal cognitive testing, and preservation of independence in daily activities, occupational, and social life that exclude dementia.(5)

It can be the early stage of any type of dementia either AD, multi-infarct dementia, or other neurodegenerative disorders.(6)

Previous research had found an interrelationship between cognitive impairment and frailty.(7)

Frailty is a dysfunctional ageing process that is an intermediate stage between normal aging and disability or death; hence, early recognition and treatment can enhance the patient's quality of life and minimize the patient's severe health impacts.

Four models of frailty are known, physical, cognitive, social, and psychosocial frailty which comprises the recently suggested depressive frail phenotype. (8)

Physical frailty can be assessed by Fried's criteria suggested by Cardiovascular Health Study (CHS), the individual who has ≥3 of those 5 items (exhaustion, unintentional losing weight, weak

hand grip, low energy consumption and sluggish gait speed) defined as frail, prefrail has 1-2 items, or non-frail has none of those items.(9)

Neuro-inflammation is being highly considered as a possible mediator of cognitive decline. Ageing itself participates in microglial stimulation, increased output of pro-inflammatory mediators, and abnormal signaling of neurons, which contribute to accelerated cognitive impairment.(10)

Interleukin-18 is one of important pro-inflammatory mediators that found to be increased in AD. (11)

There are five distinct single nucleotide polymorphism (SNP) locations in the promoter region: 656 G/T, 607 C/A, 137 G/C, +113 T/G, and +127 C/T. Prior research have shown that only SNPs at locations 137 (rs187238) and 607 (rs1946518) influence IL-18 gene function,(12) and these are thought to impact the probability of developing AD in different races.(13)

Additionally, IL-18 gene polymorphism was found to be associated with many physical disorders such as rheumatoid arthritis, atopic disorders, and idiopathic joint inflammation and (14-16)

So, it is crucial to study the relationship between IL-18 gene polymorphisms and MCI and physical frailty in elderly Egyptians which hadn't been studied before.

Objective

Our study was conducted to investigate the effect of the IL-18 gene polymorphisms on cognition and frailty.

Methods

Study design, setting and participants

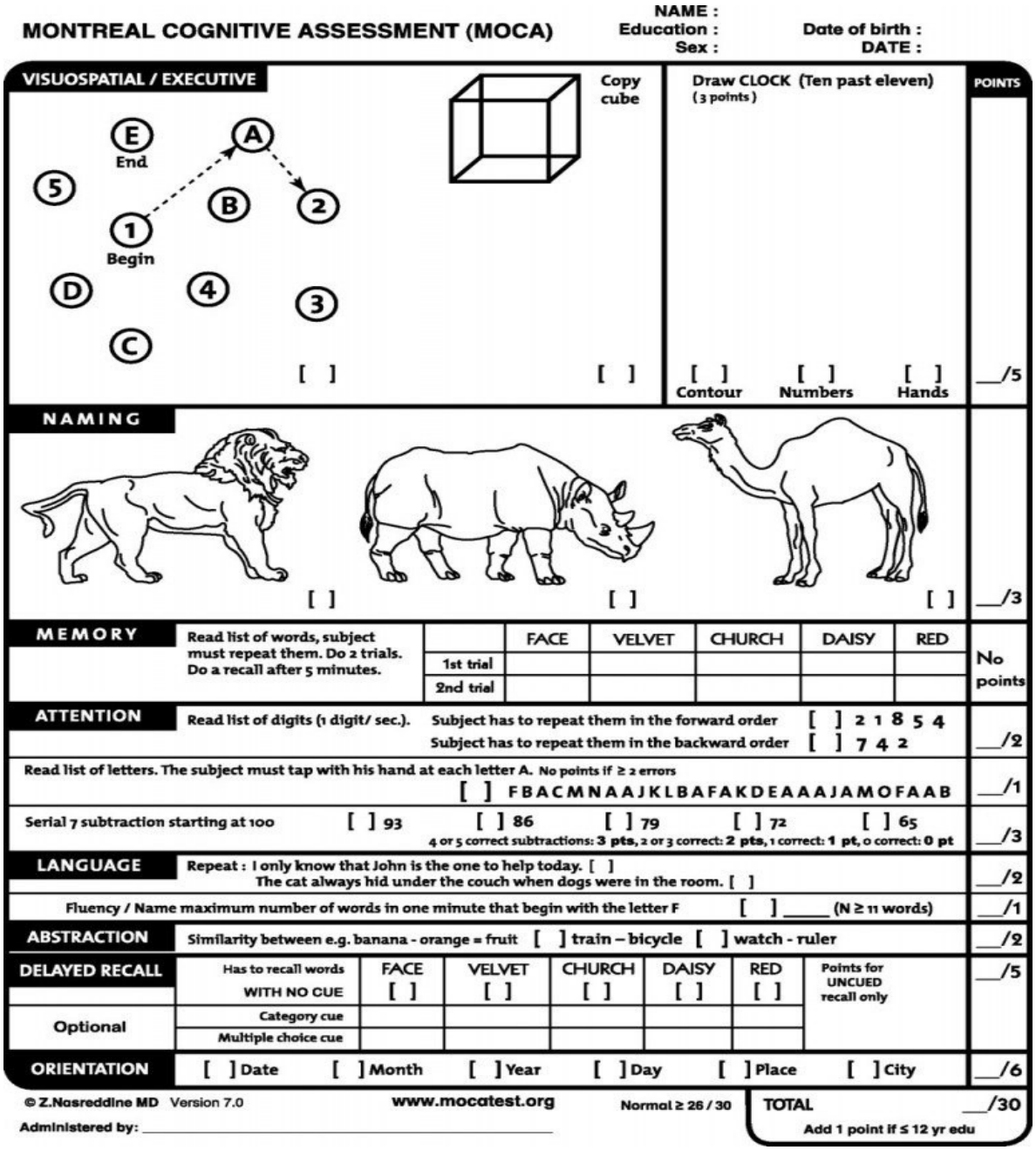
Our cross-sectional study was conducted on 90 elderly patients (over 65 yrs.) attended geriatric outpatient clinic at Alexandria Main University Hospital (AMUH), Egypt to detect an assumed average proportional difference in cognitive function using MoCA compared to Null hypothesis taking in consideration 5% level of significance and 90% power using Z-test (PASS program version 20). The Faculty of Medicine of Alexandria University ethics committee had approved our study and it followed the declaration of Helsinki.

We excluded patients with neuropsychiatric disorders (previous history of cerebrovascular stroke, Parkinson's disease, and schizophrenia), major organ failure, and severe anaemia (Haemoglobin level< 8 gm/dl).

Written informed consent was taken from all participants, complete history and full clinical examination were done.

Arabic version of Montreal Cognitive Assessment (MoCA) tool (17) which was validated and tested for its reliability(5) (**Appendix I**) was used for cognitive assessment which included 8 cognitive domains, visuo-spatial ability, attention, executive function, immediate memory, delayed memory, language, abstraction, calculation, and orientation, for a maximum total score of 30, with one point added if the formal education was fewer than 12 years. Those scored below 17 was excluded from the study.

Normal cognition score was ≥26 and mild cognitive impairment (MCI) score was 18-25.



MONTREAL COGNITIVE ASSESSMENT (MOCA)

NAME :
Education :
Sex :
Date of birth :
DATE :

VISUOSPATIAL / EXECUTIVE

Copy cube [ ]

Draw CLOCK (Ten past eleven) (3 points) [ ] Contour [ ] Numbers [ ] Hands

POINTS ___/5

NAMING

___/3

MEMORY — Read list of words, subject must repeat them. Do 2 trials. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION — Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order [ ] 2 1 8 5 4
Subject has to repeat them in the backward order [ ] 7 4 2 ___/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors
[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B ___/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65
4 or 5 correct subtractions: 3 pts, 2 or 3 correct: 2 pts, 1 correct: 1 pt, 0 correct: 0 pt ___/3

LANGUAGE — Repeat : I only know that John is the one to help today. [ ]
The cat always hid under the couch when dogs were in the room. [ ] ___/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] _____ (N ≥ 11 words) ___/1

ABSTRACTION — Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler ___/2

DELAYED RECALL

	Has to recall words WITH NO CUE	FACE [ ]	VELVET [ ]	CHURCH [ ]	DAISY [ ]	RED [ ]	Points for UNCUED recall only
Optional	Category cue						
	Multiple choice cue						

___/5

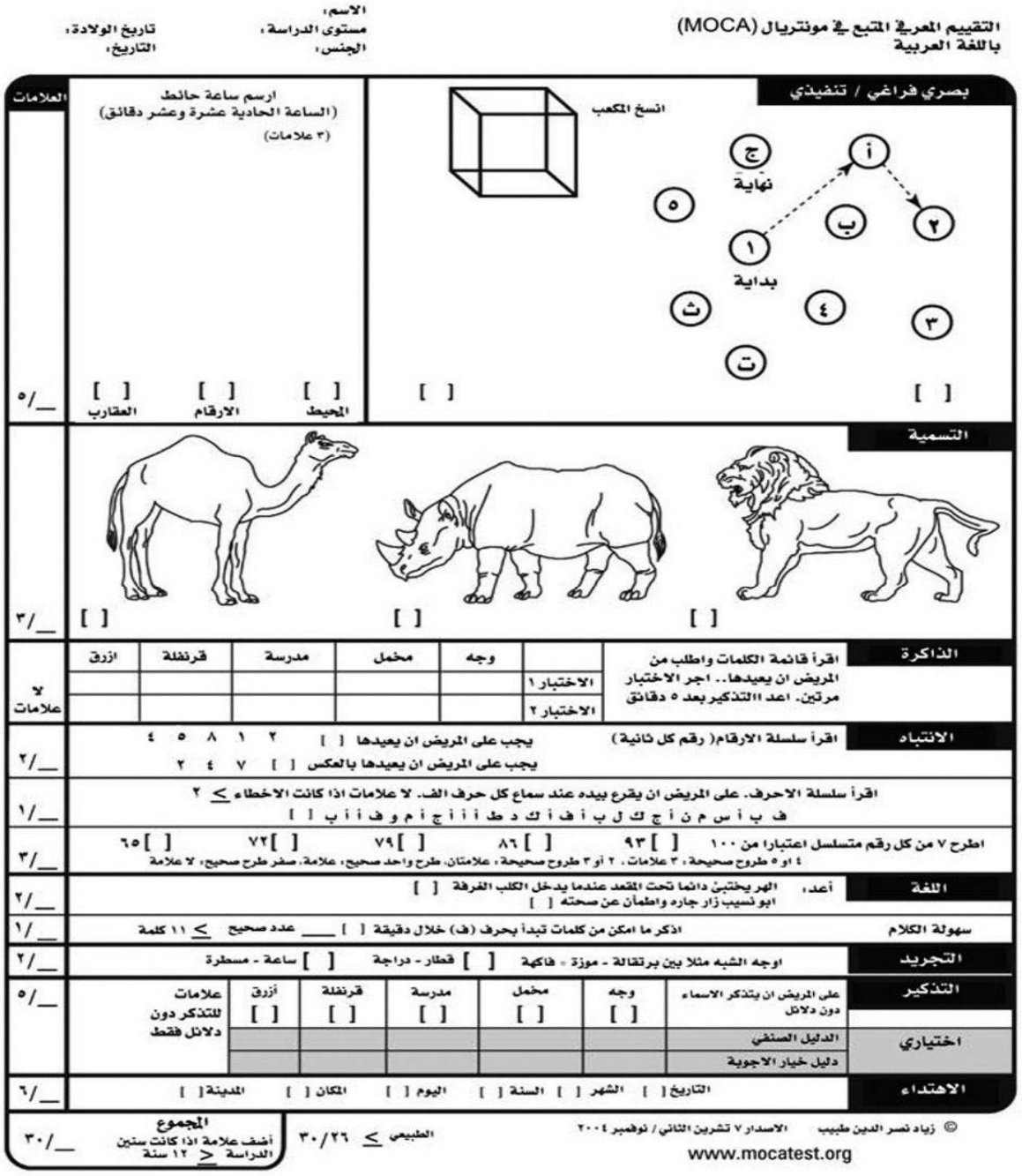
ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City ___/6

© Z.Nasreddine MD Version 7.0 www.mocatest.org Normal ≥ 26 / 30

TOTAL ___/30
Add 1 point if ≤ 12 yr edu

Administered by: _____

Arabic version



التقييم المعرفي المتبع في مونتريال (MOCA)
باللغة العربية

الاسم:
مستوى الدراسة:
الجنس:

تاريخ الولادة:
التاريخ:

العلامات

بصري فراغي / تنفيذي

انسخ المكعب

[ ]

نهاية
بداية

[ ]

٥/__

ارسم ساعة حائط
(الساعة الحادية عشرة وعشر دقائق)
(٣ علامات)

[ ] المحيط [ ] الارقام [ ] العقارب

التسمية

[ ] [ ] [ ]

٣/__

الذاكرة

اقرأ قائمة الكلمات واطلب من المريض ان يعيدها.. اجر الاختبار مرتين. اعد االتذكير بعد ٥ دقائق

	وجه	مخمل	مدرسة	قرنفلة	ازرق
الاختبار ١					
الاختبار ٢					

لا علامات

الانتباه

اقرأ سلسلة الارقام(رقم كل ثانية)

يجب على المريض ان يعيدها [ ] ٢ ١ ٨ ٥ ٤

يجب على المريض ان يعيدها بالعكس [ ] ٧ ٤ ٢

٢/__

اقرأ سلسلة الاحرف. على المريض ان يقرع بيده عند سماع كل حرف الف. لا علامات اذا كانت الاخطاء ≤ ٢

ف ب أ س م ن أ ج ك ل ب أ ف أ ك د ط ا ا ا ج أ م و ف ا أ ب [ ]

١/__

اطرح ٧ من كل رقم متسلسل اعتبارا من ١٠٠ [ ] ٩٣ [ ] ٨٦ [ ] ٧٩ [ ] ٧٢ [ ] ٦٥

٤ او ٥ طروح صحيحة، ٣ علامات، ٢ أو ٣ طروح صحيحة، علامتان، طرح واحد صحيح، علامة، صفر طرح صحيح، لا علامة

٣/__

اللغة

أعد: الهر يختبئ دائما تحت المقعد عندما يدخل الكلب الغرفة [ ]

ابو نسيب زار جاره واطمأن عن صحته [ ]

٢/__

سهولة الكلام

اذكر ما امكن من كلمات تبدأ بحرف (ف) خلال دقيقة [ ] ____ عدد صحيح ≤ ١١ كلمة

١/__

التجريد

اوجه الشبه مثلا بين برتقالة - موزة = فاكهة [ ] قطار - دراجة [ ] ساعة - مسطرة

٢/__

التذكير

على المريض ان يتذكر الاسماء دون دلائل	وجه	مخمل	مدرسة	قرنفلة	أزرق	علامات للتذكر دون دلائل فقط
	[ ]	[ ]	[ ]	[ ]	[ ]	
اختياري: الدليل الصنفي						
دليل خيار الاجوبة						

٥/__

الاهتداء

التاريخ [ ] الشهر [ ] السنة [ ] اليوم [ ] المكان [ ] المدينة [ ]

٦/__

المجموع
أضف علامة اذا كانت سنين الدراسة ≤ ١٢ سنة

٣٠/__

الطبيعي ≤ ٢٦/٣٠

Physical Frailty assessed by Fried's criteria:

- **a.** Shrinkage (losing weight) was described as a history of a new onset of unintended weight reduction ≥ 3 kg or a BMI < 21 kg/m2(18)
- **b.** Gait speed: Participants were directed to walk 4 meters linear path by their usual pace and time was calculated by a stopwatch. Gait speed was considered slow if walking time ≥6.153 sec i.e. gait speed ≤0.65 m/s (male height ≤173 cm, female height ≤159 cm) and walking time ≥5.263 sec i.e. gait speed ≤0.76 m/s (male height >173 cm, female height >159 cm).(19)
- **c.** Physical activity was evaluated by the participants answer to the question of "Number of times per week do you practice with medium intensity (working till one becomes sweating)?", their responses by zero&<1/week described as poor physical activity for Fried's criteria.(20)
- **d.** A hand grip dynamometer was used to assess muscular endurance. While the participant was sitting and the elbow 110° flexed, he/she was asked to press the handle as firm as possible with the dominant hand for 3-5 seconds. After a 30-second rest period, the test was redone and if there was a difference > 10%, a third testing was performed, and best performance was selected.(21) Weak hand grip was considered if: ≤ 29 kg for BMI ≤ 24 kg/m2, ≤30 kg for BMI 24.1-26 kg/m2, ≤ 30 kg for BMI 26.1-28 kg/m2, and ≤ 32 kg for BMI > 28 kg/m2 in men. Women with grip strength ≤ 17 kg for BMI ≤ 23 kg/m2, ≤ 17.3 kg for BMI 23.1-26 kg/m2, ≤ 18 kg for BMI 26.1-29 kg/m2, and ≤ 21 kg for BMI > 29 kg/m2 was considered weak. (19)
- **c.** Fatigue was measured using two questions from Depression Scale of the Center of Epidemiological Studies (CES-D): (22) "I felt like everything I did was an effort" & "I couldn't get going." The answers were evaluated on a scale from 0 to 3, the response of 2 (moderate amount of time) or 3 (almost all of time) in any of 2 questions was considered positive for frailty.(23)

Participant had ≥3 of those 5 items was classified as frail, 1-2 items as pre-frail, none as robust.(9) Participants were categorized into Group I: patients with both MCI and physical frailty or prefrailty (Cognitive frailty), Group II: patients with MCI without physical frailty or prefrailty, and Group III: Participants with normal cognition.

Genotyping

Genomic DNA was extracted from peripheral blood samples using QIAamp DNA Blood Mini Kit (Qiagen, USA) according to the manufacturer's instructions. The quantity and purity of the extracted DNA were assessed using the NanoDrop 2000 (Thermo Scientific, USA).

The two polymorphisms were genotyped by sequence-specific PCR (PCR-SSP). For the position 607 C/A-specific PCR, a common reverse primer 5'-TAACCTCATTCAGGACTTCC-30 and two sequence-specific forward primers 5' -GTTGCAGAAAGTGTAAAAATTATTAC-3' and 5'-GTTGCAGAAAGTGTAAAAATT ATTAA-3' were used.

A control forward primer 5'-CTTTGCTATCATTCCAGGAA-3' was used to amplify a 301-bp fragment covering the polymorphic site as an internal positive amplification control.

All reactions were carried out in using SimpliAmp thermal cycler (Applied Biosystems, USA). Samples were initially denatured at 95°C for 3 min, followed by 40 cycles including denaturation at 95°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 60 s followed by final step of elongation in 1 cycle at 72°C for 5 min.

For the position 137 G/C -specific PCR genotyping, a common reverse primer 5'-AGGAGGGCAAAATGCACTGG-3' and two sequence-specific forward primers 50-CCCCAACTTTTACGGAAGAAAAG -3' and 5'- CCCCAACTTTTACGGAAGAAAAC -3' were used.

A control forward primer 5'-CCAATAGGACTGATTATTCCGCA-3' was used to amplify a 446-bp fragment covering the polymorphic site to serve as an internal positive amplification control.

Samples were initially denatured at 95°C for 3 min, followed by 40 cycles including denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 60 s followed by final step of elongation in 1 cycle at 72°C for 5 min.

All PCR products were visualized by 2% agarose gel electrophoresis stained by ethidium bromide.

Statistical analysis

The data was introduced into the computer and analyzed with the IBM SPSS software version 20.0. (Armonk, NY: IBM Corp). Numbers and percentages were used to describe qualitative data. The Kolmogorov- Smirnov and Shapiro-Wilk test was done to ensure that the distribution was normal. Range, mean (standard deviation), and median (interquartile range, IQR) were used to describe quantitative data. 5% level of significance was used to assess the given results.

For categorical variables, Chi-square test was used to compare between different groups, with Fisher's Exact or Monte Carlo correction for chi-square when more than 20% of the cells have expected count less than 5.

For normally distributed quantitative variables, Student t-test was used to compare between two groups, F-test (ANOVA) was used to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons and Pearson coefficient was used to correlate between two variables.

For abnormally distributed quantitative variables, Kruskal Wallis test was used to compare between more than two groups, and Post Hoc test (Dunn's multiple comparisons test) for pairwise comparisons.

Logistic regression analysis was used to assess the association between risk factors and MCI.

The Hardy-Weinberg equation was used to determine equilibrium of analyzed population sample.

Results

For the studied population, mean age (SD) was 69.44(5.04), 51(56.7%) females, 30(33.3%) smokers, 40(44.4%) diabetics, and 38(42.2%) hypertensives, 36 (40.0%) were physically frail patients with mean education years (SD) was 11.9(3.76), mean MoCA score (SD) 22.34 (2.88), and there was substantial difference between the 3 groups as regards all these variables. (**Table1**)

Table (1): Comparison between the three studied groups according to different parameters

	Total (n = 90)	Group I (n = 65)	Group II (n = 13)	Group III (n = 12)	Test of Sig.	p_1	p_2	p_3
Age (years)								
Mean ± SD.	69.44 ± 5.04	70.46 ± 5.52	67.08 ± 1.89	66.50 ± 1.45	F=5.273* p=0.007*	0.059	0.028*	0.952
Sex								
Male	39 (43.3%)	24 (36.9%)	10 (76.9%)	5 (41.7%)	χ^2=7.074* p=0.029*	0.008*	FEp= 0.756	FEp= 0.111
Female	51 (56.7%)	41 (63.1%)	3 (23.1%)	7 (58.3%)				
Level Education (years)								
Mean ± SD.	11.90 ± 3.76	11.37 ± 3.89	11.85 ± 2.85	14.83 ± 2.52	F=4.663* p=0.012*	0.901	0.008*	0.103
Smoking	30 (33.3%)	21 (32.3%)	9 (69.2%)	0 (0%)	χ^2=13.91* MCp0.001*	0.012*	FEp= 0.030*	FEp <0.001*
Diabetes mellitus	40 (44.4%)	34 (52.3%)	5 (38.5%)	1 (8.3%)	χ^2=8.154* p=0.017*	0.362	0.005*	FEp= 0.160
Hypertension	38 (42.2%)	31 (47.7%)	6 (46.2%)	1 (8.3%)	χ^2=6.529* MCp0.038*	0.919	FEp= 0.012*	FEp= 0.073
MOCA								
Mean ± SD.	22.34 ± 2.88	21.49 ± 2.44	22.23 ± 1.69	27.08 ± 0.79	F=32.702* p<0.001*	0.514	<0.001*	<0.001*
Physical frailty score								
Robust	19 (21.1%)	0 (0%)	13 (100%)	6 (50.0%)	χ^2=69.61* MC<0.001*	<0.001*	<0.001*	MCp= 0.005*
Pre frail	35 (38.9%)	30 (46.2%)	0 (0%)	5 (41.7%)				
Frail	36 (40.0%)	35 (53.8%)	0 (0%)	1 (8.3%)				

SD: Standard deviation χ^2: Chi square test FE: Fisher Exact MC: Monte Carlo

F: F for One way ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)

p: p value for comparing between the studied groups

*: Statistically significant at $p \leq 0.05$

Group I: Cognitive frail

Group II: MCI without physical frail

Group III: Normal cognition

Regarding interleukin-18 gene polymorphism, distribution of genotypes and frequency of alleles in 3 different groups shown below in **Table 2.**

At position -137 G/C**,** GG genotype was more prevalent in cognitive frail group than normal cognition one 35 (53.8%) vs 2(16.7%) while CC genotype was more prevalent in normal cognition group than cognitive frail one 6(50.0%) vs 5(7.7%), p_2= 0.001. In addition, G allele was more prevalent in cognitive frail and MCI patients than normal cognition group (95(73.1%) and 19(73.1%) vs 8 (33.3%), p_2=<0.001 and p_3=0.005) respectively.

At position -607 C/A, CC genotype was more prevalent in cognitive frail and MCI patients than normal cognition group 39(60.0%) and 7(53.8%) vs 1 (8.3%) while AA genotype was more prevalent in normal cognition group than cognitive frail and MCI patients 7(58.3%) vs 3 (4.6%) and 2 (15.4%), p_2= <0.001, and p_3=0.042 respectively. Additionally, C allele was more prevalent in cognitive frail and MCI patients than normal cognition group (101 (77.7%) and 18 (69.2%) vs 6 (25.0%), p_2=<0.001 and p_3=0.002) respectively.

Table (2): Comparison between the three studied groups according to IL-18 gene polymorphisms

	Total (n = 90)	**Group I (n = 65)**	**Group II (n = 13)**	**Group III (n = 12)**	χ^2	p_1	p_2	p_3
-137 G/C								
Genotype								
CC	13 (14.4%)	5 (7.7%)	2 (15.4%)	6 (50.0%)	13.531*	MCp=	MCp=	MCp=
GC	32 (35.6%)	25 (38.5%)	3 (23.1%)	4 (33.3%)	MCp0.004*	0.380	0.001*	0.064
GG	45 (50.0%)	35 (53.8%)	8 (61.5%)	2 (16.7%)				
HWχ^2 (p)	3.113 (0.078)	0.033 (0.856)	2.223 (0.136)	0.750 (0.386)				
Allele	**(n = 180)**	**(n = 130)**	**(n = 26)**	**(n = 24)**				
C	58 (32.2%)	35 (26.9%)	7 (26.9%)	16 (66.7%)	15.044*	1.000	<0.001*	0.005*
G	122 (67.8%)	95 (73.1%)	19 (73.1%)	8 (33.3%)	p=0.001*			
-607 C/A								
Genotype								
AA	12 (13.3%)	3 (4.6%)	2 (15.4%)	7 (58.3%)	21.697*	MCp=	MCp	MCp=
CA	31 (34.4%)	23 (35.4%)	4 (30.8%)	4 (33.3%)	MC<0.001*	0.356	<0.001*	0.042*
CC	47 (52.2%)	39 (60.0%)	7 (53.8%)	1 (8.3%)				
HWχ^2 (p)	3.193 (0.074)	0.028 (0.867)	1.003 (0.317)	0.148 (0.700)				
Allele	**(n = 180)**	**(n = 130)**	**(n = 26)**	**(n = 24)**				
A	55 (30.6%)	29 (22.3%)	8 (30.8%)	18 (75.0%)	26.510*	0.354	<0.001*	0.002*
C	125 (69.4%)	101 (77.7%)	18 (69.2%)	6 (25.0%)	p<0.001*			

χ^2: **Chi square test** MC: **Monte Carlo**

HWχ^2**:** Chi square for goodness of fit for Hardy-Weinberg equilibrium (If P < 0.05 - not consistent with HWE.)

p: p value for comparing between the studied groups

*: Statistically significant at p ≤ 0.05

Group I: Cognitive frail

Group II: MCI without physical frail

Group III: Normal cognition

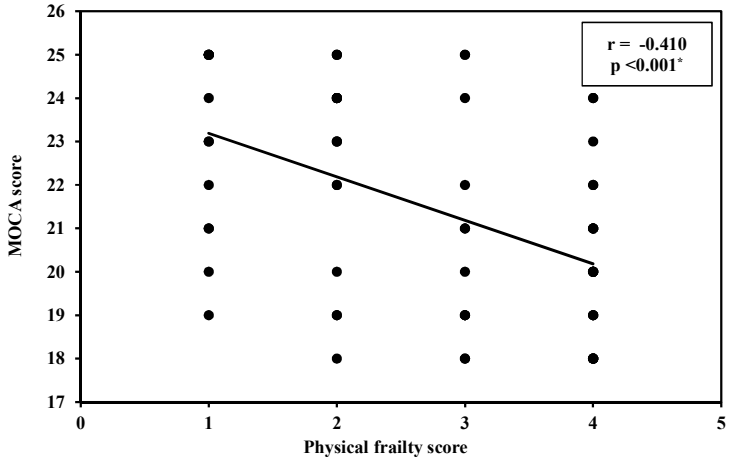


Figure (1): Correlation between Physical frailty score with MOCA score in all MCI group (n=78)

In all MCI patients, physical frailty score was negatively associated with MoCA **score. (Figure1**)

Mean MoCA score (SD) differed significantly between the 6 genotypes at position 137 C/G and 607 C/A *(P* <.001), while median physical frailty score (range) of patients differed significantly between the 3 genotypes at position 607 C/A only. (**Table 3**)

Table (3): Relation between IL-18 genotypes at position-137 G/C, 607 C/A with MOCA, and physical frailty score in all MCI patients (n = 78)

	-137 G/C						
	GG	**GC**	**CC**	**Test of sig. (p)**	**GG vs. GC**	**GG vs. CC**	**GC vs. CC**
MOCA	**(n = 43)**	**(n = 28)**	**(n = 7)**				
Mean ± SD.	20.60 ± 2.33	22.79 ± 1.47	23.14 ± 2.41	F=11.49* p<0.001*	<0.001*	0.010*	0.912
Physical frailty score	**(n = 43)**	**(n = 28)**	**(n = 7)**				
Mean ± SD.	2.40 ± 1.55	2.07 ± 1.33	2.0 ± 1.53	H=1.213	>0.05	>0.05	>0.05
Median (Min. – Max.)	3 (0 – 4)	2 (0 – 4)	2 (0 – 4)	p=0.545			
	-607 C/A						
	CC	**CA**	**AA**	**Test of sig. (p)**	**CC vs. CA**	**CC vs. AA**	**CA vs. AA**
MOCA	**(n = 46)**	**(n = 27)**	**(n = 5)**				
Mean ± SD.	20.52 ± 2.18	23.0 ± 1.52	24.20 ± 1.30	F=18.756* p<0.001*	<0.001*	<0.001*	0.414
Physical frailty score							
Mean ± SD.	2.61 ± 1.48	1.85 ± 1.29	1.0 ± 1.0	H=8.738*	0.027*	0.019*	0.246
Median (Min. – Max.)	3 (0 – 4)	2 (0 – 4)	1 (0 – 2)	p=0.013*			

SD: **Standard deviation** **F**: **F for One way ANOVA test**, pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Tukey)**

H: H for **Kruskal Wallis test,** pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**

p: p value for comparing between the different genes

*: Statistically significant at $p \leq 0.05$

Univariate analysis revealed that the education, diabetes, hypertension, physical frailty, IL-18 gene polymorphisms at both positions -137 C/A and 607 C/G were significantly associated with Mild Cognitive Impairment (MCI). However, multivariate analysis revealed that IL-18 gene polymorphisms at position -137 C/A was the only associated factor with MCI (OR= 44.990, 95% CI:1.498 – 1351.44, p=0.028). (**Table 4**)

Table (4): Univariate and multivariate Logistic regression analysis for the parameters affecting MCI patients (n = 78 vs. 12)

	Univariate		**#Multivariate**	
	p	**OR (95%C.I) LL–UL**	**p**	**OR (95%C.I) LL–UL**
Age (years)	0.065	1.308 (0.983 – 1.740)		
Male	0.900	1.082 (0.316 – 3.708)		
Level Education (years)	0.007*	0.715 (0.560 – 0.913)	0.335	0.840 (0.588–1.198)
Diabetes mellitus	0.025*	11.0 (1.354 – 89.351)	0.688	4.960 (0.002–12354.2)
Hypertension	0.032*	9.927 (1.222 – 80.644)	0.703	4.689 (0.002–13078.9)
Genotyping at -137 C/A				
CC®		1.000		
GC	0.020*	6.000 (1.323 – 27.219)	0.098	17.238 (0.590 – 503.72)
GG	0.001*	18.429 (3.081 – 110.223)	0.028*	44.990 (1.498 – 1351.44)
Genotyping at -607 C/G				
AA®		1.000		
CA	0.005*	9.450 (1.995 – 44.771)	0.456	2.393 (0.241 – 23.733)
CC	<0.001*	64.40 (6.524 – 635.660)	0.051	17.105 (0.993 – 294.559)
Physical frailty score				
Robust®		1.000		
Pre frail	0.140	2.769 (0.715 – 10.720)	0.382	2.422 (0.334 – 17.587)
Frail	0.014*	16.154 (1.771 – 147.349)	0.204	6.160 (0.372 – 102.096)

OR: Odd`s ratio

C.I: Confidence interval LL: Lower limit UL: Upper Limit

#: All variables with p<0.05 was included in the multivariate

*: Statistically significant at p ≤ 0.05

Discussion

In our study, cognitive frail patients included more patients with GG genotype at position 137 and more with CC genotype at position 607 than normal cognition group. Also, G and C alleles were repeated more frequently at position 137 and 607 respectively and patients with GG genotype at position -137 C/G was associated with lower MoCA score than both GC and CC genotype and those with CC genotype at position -607 C/A was associated with lower MoCA score in all MCI patients.

An Italian cohort reported similar finding, AD patients included significantly more patients with CC genotype at position 607 than healthy participants. Also, participants with CC genotype at position 607 was associated with high probability of experiencing AD on follow-up. On the contrary, there was no significant difference between AD group and healthy participants as regarding IL-18 gene polymorphism at position-137 and there was no significant association between it and AD development with longititudnal follw-up.(24) In the contrary, Segat L et. al reported that there was no relationship between IL-18 SNP at both positions and development of AD in the same race after follow-up.(25)

Findings from case control chinese study go with our findings, AD patients included more participants with CC and GG genotype than controls at position 607 C/A and 137 respectively.

Also, G allele at position 137 and C allele at position 607 were associated with more susceptibility for late onset Alzheimer's Disease (LOAD) and this association may be related to positivity of ApoE ε4 alleles.(26) However, another Chinese study reported that these alleles were not linked to higher association with LOAD.(27)

Two meta-analytic reviews had found that those harboring the C allele at position 137 and A alleles at position 607 had a considerably lower likelihood for AD development than G and C alleles, respectively.(13, 28)

Our study revealed that CC genotype at position 607 of IL-18 gene was associated with significantly higher physical frailty score than CA and AA genotypes in all MCI patients (P=.013). However, there was no relationship between physical frailty score and IL-18 -137 C/G polymorphism.

Till now, there is no studies examine the association between these two SNPs and physical frailty. Meanwhile, Mekli K and colleagues reported a significant inverse relationship between A allele of interleukin-18 gene (rs360722) and frailty index.(29)

There was no significant relationship between IL-18 polymorphism at position 137 and hand grip strength in both males and females in a previous study conducted by Dato S and colleagues.(30)

Our study is considered the first one to assess the relationship of MCI and IL-18 SNPs -137 G/C and 607 C/A and its relationship to physical frailty in Egyptian elderly population, which is considered the major strength point.

Collection of participants from single centre, limited number of participants are considered limitations of our study.

Conclusions

Our study concluded that IL-18 gene polymorphism may be an important risk factor for MCI. Prospective and large-scale studies are needed to assess a causal relationship.

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Conflict of interest

The authors declare no conflict of interest.

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