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Frequency of APOE, ACE, MTHFR an CCR5 Polymorphisms in Patients with Mild Cognitive Impairment in Costa Rican Population

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Abstract

Background: This is a descriptive cross-sectional epidemiological study describing the prevalence of polymorphisms within the Apolipoprotein E (ApoE), Methylenetetrahydrofolate reductase (MTHFR), Angiotensin converting enzyme (ACE), and Chemokine receptor 5 (CCR5) genes in patients with mild-cognitive impairment (MCI).

Methods: The study analyzed 84 blood samples from patients diagnosed with MCI at the Memory and Aging Clinic at the Hospital San Juan de Dios in Costa Rica. The authors performed genetic analysis to determine and compare the genotypic and allelic frequencies in the MCI patients versus those reported for the Costa Rican population.

Results: Genotypic and allelic frequencies obtained were compared to reports in Costa Rican population, and a gender-based analysis. There was significant difference in the APOE and MTHFR polymorphism (p=0.007446 and p=0.003329, respectively).

Discussion: The study found a statistical difference in prevalence of the ApoE (ε2, ε3, ε4 alleles) and MTHFR (C677T) polymorphisms in the MCI patients. The study lacks a cohort of age-matched control subjects that do not have MCI. However, this study is very relevant to our understanding the role played by these genes in the etiopathogenesis of MCI.

Keywords: Mild cognitive impairment; Alzheimer's disease; ApoE; MTHFR; ACE; CCR5; Polymorphisms

Introduction

Mild cognitive impairment and Alzheimer´s disease

Alzheimer's disease (AD) is the most common cause of dementia. It is a multifactorial disease in which genetic and environmental conditions interact to present a clinical manifestation [1]. It is characterized by progressive cognitive impairment and memory damage. Patients meeting criteria for Mild Cognitive Impairment (MCI) are at an increased risk for developing to diagnosable AD [2] and are considered to be a transitional phase between healthy cognitive aging and dementia up to 60% of the cases within a ten year period [3].

MCI is defined as a subtle but measurable memory disorder, and represent an important step forward in diagnosing AD in its earliest stage. Diagnoses based mainly on cognitive performance present limitations, associated to the variability in methods and tests employed in the evaluation and how they are interpreted. Recent advances in understanding of imaging and biochemical changes in early stages of the disease have improved the possibility to diagnose AD in earlier stages [4].

Genetic polymorphisms such as Apolipoprotein E (ApoE), Methylenetetrahydrofolate reductase (MTHFR), Angiotensin I-converting enzyme (ACE) and Chemokine receptor 5 (CCR5) have been associated with age-related disorders, due to their implications in various complex disorders such as cerebrovascular disease (CVD), coronary artery disease (CAD) and AD [5].

Apolipoprotein E (rs429358 and rs7412)

The ε4 genotype for the ApoE is a risk factor for developing AD. ApoE-ε4 presents 15-16% of the general population [6], with greater presence in Caucasian populations [7] and nearly in 50% of subjects with AD. The presence of ApoE-ε4 genotype increases the risk of developing AD 3 to 8 times higher and decreases age onset between 7 to 15 years [8]. In homozygous form, the risk increases 33 times [9]. In late-onset AD is found in 65% of the cases [10,11] and the percentage rises to 80% in presence of a family member with EA [12]. ApoE remains the biomarker for predicting and diagnosing AD [13].

Methylenetetrahydrofolate reductase (rs1801133)

Several studies support that the polymorphism C677T in the MTHFR promotes brain atrophy associated with cognitive impairment [14]. MTHFR is an enzyme responsible for intracellular folate homeostasis and metabolism. The most common functional variant in the MTHFR gene is the polymorphism C677T (rs1801133). It has been shown that de homozygous (T/T) and heterozygous (T/C) variants of the C677T polymorphism are responsible for 30% and 65%, respectively, of the activity of the MTHFR enzyme, in comparison to subjects with the homozygous wild type variant. As a result, variants T/T and C/T have been associated with lower levels of serum folate and higher homocysteine levels. These levels can cause neurotoxic effects

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such as damage at DNA level (synthesis transmethylation), imbalance in neurotransmitters, and alteration in the basal ganglia, among others. This relates to the onset of neurodegenerative disorders such as AD [14]. In Costa Rica, previous research estimated that the prevalence of the C677T polymorphism have a similar trend reported for Latin American populations with a frequency of 677 genotype C/T of 45.9%, genotype T/T of 26.8% and for wild type (677 C/C) of 27.3% [15].

Angiotensin I-converting enzyme (rs1799752)

The ACE (dipeptidyl carboxypeptidase, EC 3.4.15.1) is a membrane-bound ectoenzyme. ACE is a very important component of the renin-angiotensin system (RAS) promoting the formation of angiotensin II from angiotensin I and inactivating the vasodilator bradykinin [16]. In addition, a local RAS in the brain plays an important role in the central nervous system. In addition, the angiotensin present in astrocytes is required for the functional maintenance of the blood brain barrier [17], which is affected in AD [18]. The activity of the ACE in the brain varies significantly between individuals with AD or in patients with MCI and healthy controls subjects [19] and has been demonstrated that ACE inhibit amyloid-beta peptide aggregation and plaque formation in vitro [20]. The I/D polymorphism in intron 16 of the ACE gene on chromosome 17q23 [21] is associated with lower ACE level, mainly those subjects who carry the I allele have a lower ACE level than subjects bearing the D allele [22]. Arregui et al. [23] observed an increase in ACE activity in patients with AD.

Chemokine receptor 5 (rs333)

Chemokines are produced due to the activation of a wide spectrum of inflammatory cells including the astrocytes [24]. It is been found that some chemokines, their receptors and ligands are found in brain with AD. The monocyte chemoattractant protein 1 (MCP-1) and ligand of CC chemokine receptor 2 (CCR2) are found in senile plaque, and the reactive microglia promotes de activation of the astrocytes, suggesting neuroinflamation [25]. It is been found that CCR5 deficiency activated astrocytes and Aβ accumulation *via* upregulation of CCR2 [26]. These findings suggest that chemokines and their receptors and ligands may contribute to the development and/or the progression of AD through modification of astrocyte activation.

Memory and aging clinic

In Costa Rica, the first prevalence study conducted in a community (Santo Domingo, Heredia) with a sample of 400 subjects, showed a 4.2% prevalence of probable dementia (in any of its subtypes). Among the subjects evaluated 41 were diagnosed with AD (n=14) and MCI (n=27) [27].

At the Memory and Aging Clinic of the Hospital San Juan de Dios (CMEC) interdisciplinary diagnosis by consensus assessments have been conducted for the past 7 years, using a protocol established by the our team of neurologists, geriatricians and clinical psychologists. In 2012 the CMEC reports a first analysis of the prevalence of MCI an AD in the population served by the clinic (n=128), during 2010-2011. The most frequent diagnosis was MCI (44.5%), while dementia was found in 30.5% of cases, where the AD (43.6%) and vascular dementia (25.6%) predominated.

These results showed that the CMEC is attracting patients at early stages, so our efforts should focus on this population. This highlights the importance of providing follow-up to patients and reinforces the need to implement the detection of biomarkers and the presence of other genetic mutations considered as risk factors for dementia (ApoE), as a part of the diagnostic process. Thus, during 2012 there was a national campaign for early diagnosis, promoted by the Alzheimer and Other Dementias Association (ASCADA), which gave the first donation of the ApoE detection kits. In this study, we aimed to establish the prevalence of ApoE, MTHFR C677T, ACE I/D and CCR5 Δ32 in DNA samples of patients with MCI in Costa Rican population, evaluated and diagnosed by the CMEC and compare them with previous reports in control subjects in this country.

Methods and Materials

Sample collection

This is a descriptive and transversal study. Patients were previously evaluated in the Memory Clinic-Hospital San Juan de Dios using a protocol established by our team of neurologists, geriatricians and clinical psychologists, which includes a battery of tests for assessing cognitive and functional performance (screening, medical history, neuropsychological assessment, neurological examination, review of the patient's records, molecular biology studies and neuroimaging). Subjects gave written informed consent and the University of Costa Rica's institutional Bioethics review board approved the study. Patients diagnosed with MCI were randomly selected.

We collected blood samples with EDTA and DNA isolation was obtained following the standard NaCl precipitation method [28] and analyzed 84 anonymous samples for the presence of ApoE, ECA, CCR5 and MTHFR in patients diagnosed with MCI during 2012-2014.

Genetic analysis

ApoE polymorphism was amplified by RFLP-PCR method [29]. Amplification of ApoE products that were suitable for HhaI digestion proved successful for most DNA samples, with the exception of samples that were extensively degraded prior to amplification. The products were detected in 8% polyacrylamide gel by electrophoresis. Fragments of 72 bp and 48 bp are produced in ApoE-ε4, fragments of 91 bp and 83 bp are produced in ApoE-ε2 in 91 bp and 48 bp are generated in ApoE-ε3.

MTHFR polymorphism was performed by RFLP-PCR method was used [30]. After amplification using HinfI restriction enzyme to identify the mutation. The mutant allele generated two fragments: 175 bp and 23bp, while the wild-type is not cleaved and is identified by a 198 bp fragment.

The primers and PCR conditions for the ACE polymorphism were based on those described by Rigat et al. [31], obtaining a 190 bp for DD genotype and a fragment of 490 bp in the presence of the corresponding insertion genotype II; heterozygous individuals have both bands (I/D). To avoid false-positive DD genotype, a second amplification [32] was performed, attempting to obtain a band of 300 bp for the heterozygous genotype (I/D) and the homozygous deletion allele (D/D) a band of 200 bp. The bands obtained were analyzed in 2% agarose gels by electrophoresis.

CCR5 polymorphism was performed by PCR which flank the deletion of 32 bp [33]. The products were detected in 4% agarose gel by electrophoresis. The genotyping was performed according to the size of the amplification products (CCR5 1/1) a band of 184 bp was observed for the homozygous, deletion of 32 bp (Δ32/ Δ32); the expected product was 152 bp and heterozygotes (1/Δ32) 2 bands: 184 and 152 bp.

Statistical Analysis

Chi square test was used to compare the frequencies between

populations. This test takes large (>30), independent samples, and categorical variables and random sampling. Confidence intervals were calculated for the difference of proportions that turned out significant using Tukey multiple comparison method, with a global confidence of 95%.

Results

A description of the demographic and genotypic characteristics in patients with mild cognitive impairment diagnosed at the Memory Clinic of San Juan de Dios Hospital is presented in Table 1. The age average of the population (n=84) is 62.8 years (SD: 13.2), with a mean of education of 7.3 years (SD: 5.4). The gender distribution consisted in 67% women and 34% men.

For the ApoE analysis the ε2/ε2 genotype was absent in our sample. The ε4 allele frequency is 0.14, where the genotype ε3/ε4 predominates (22.62%). The most frequent genotype was ε3/ε3 (67.86%). The ε3 allele of ApoE polymorphism was the most frequent (0.83), while the ε2 allele had a frequency of 0.04.

The C allele of the MTHFR C677T polymorphism was similar to the T allele with a frequency of 0.46 and 0.54, respectively. The most frequent genotype was the C677T (64.29%) and the wild type CC 677 the less frequent (14.29%).

For the ACE polymorphism, the D allele was also similar to I allele with a frequency of 0.46 and 0.54, respectively. The most frequent genotype was I/D (53.57%), and the less frequent was the D/D genotype (19.05%). For both MTHFR and ACE polymorphisms, it is important to notice that they present a similar behavior, having frequencies near 50%.

The distribution of the CCR5 allele frequencies was 0.96 for the 1 allele and 0.04 for the Δ32 allele, which is not usual taking into account the sample size (n=84). The CCR5 1/1 genotype was de most frequent (91.67%) and the Δ32/Δ32 genotype was absent in our sample.

Significant differences were found when comparing the control population versus population with MCI in the ApoE and MTHFR polymorphism, assuming an alpha =0.05 (maximum error allowed type I), with p-values of 0.007446 and 0.003329, respectively.

Confidence intervals (CI) were calculated. For the ε3 polymorphism of ApoE, the difference between the proportions of the two samples is 0.0821429 (CI=-0.1601294 to -0.00797766), which means that the presence of the ε3 allele is 0.16% to 0.079% less than in the control sample. Note that this interval is very close to 0, implying that it is not significant.

For the ε4 polymorphism, the difference is 0.09404762 (CI= 0.02618271 to 0.1659556), implying that in the sample evaluated in comparison to the control sample, the presence of ε4 allele is between 2.6% to 16.6% higher than in the general population.

Regarding MTHFR, the difference in ratio for the allele with the C variant for T is 0.1388071, (CI=0.04852785 to 0.2271886). This indicates that in the sample evaluated, in comparison to the control sample, this variant is 4.8% to 22.7% more prevalent.

In gender-based analysis, Table 2 presents the distribution of the allelic and genotypic frequencies of the ApoE, ACE, MTHFR, CCR5 polymorphisms by gender. Allelic distribution is very homogeneous in most polymorphisms. In the genotypic frequency, is important to highlight that the ApoE ε3/ε4 genotype is more frequent in males

Demographic Variables					
N-Sample		84			
Male (%)		28(33,3)			
Female (%)		56(66,67)			
Age, years, mean (SD)		62.83(13,25)			
Years of education mean (SD)		7.33(5,44)			
Polymorphisms	**Genotypic frequencies n (f)**	**Allelic frequencies n(f)**			
ApoE					
E2/E2	0	Alleles	Cases	Control	P
E2/E3	6 (7,14)			**Marca et al, 2011 [34]**	
E2/E4	0	E2	6 (0,04)	13(0,03)	
E3/E3	57 (67,86)	E3	139 (0,83)	382(0,91)	0.007446*
E3/E4	19 (22,62)	E4	23 (0,14)	25(0,06)	
E4/E4	2 (2,38)				
ACE				**Salazar et al, 2009 [35]**	
I/I	23 (27,38)	I	91 (0,54)	76(0,57)	0,7442
I/D	45 (53,57)	D	77 (0,46)	58(0,43)	
D/D	16 (19,05)				
MTHFR				**Herrmann et al, 2001 [36]**	
CC677	12 (14,29)	C	78 (0,46)	234(0,60)	0.003329*
C677T	54 (64,29)	T	90 (0,54)	154(0,40)	
677TT	18 (21,43)				
CCR5					
01-Jan	77 (91,67)	1	161 (0,96)	283(0,95)	0,8444
1/Δ32	7 (8,33)	Δ32	7 (0,04)	15(0,05)	
Δ32/Δ32	0				

*p values were calculated X^2-test, p significant <0.05

Table 1: Demographic characteristics, genotypic and allele frequencies of the polymorphisms studied in patients with MCI and controls in Costa Rica population reported in other studies.

Polymorphism		Genotypic frequencies n (f)			Allelic Frequencies n (f)		
		Male	Female		Male	Female	P
ApoE	E2/E2	0	0	E2	2 (0,04)	4 (0,04)	
	E2/E3	2 (7,14)	4 (7,14)	E3	44 (0,79)	95 (0,85)	0,5375
	E2/E4	0	0				
	E3/E3	16 (57,14)	41 (73,21)				
	E3/E4	10 (35,71)	9 (16,07)				
	E4/E4	0	2 (3,57)				
ACE							
	I/I	7 (25,00)	16 (28,57)	I	30 (0,54)	61 (0,54)	
	I/D	16 (57,14)	29 (51,79)	D	26 (0,46)	51 (0,46)	0,9998
	D/D	5 (17,86)	11 (19,64)				
MTHFR							
	CC677	3 (10,71)	9 (16,07)	C	23 (0,41)	55 (0,49)	0,412
	C677T	17 (60,71)	37 (66,07)		33 (0,59)	57 (0,51)	
	677TT	8 (28,57)	10 (17,86)				
CCR5							
	01-Jan	25 (89,29)	52 (92,86)	1	53 (0,95)	108 (0,96)	0,8914
	1/Δ32	3 (10,71)	4 (7,14)		3 (0,05)	4 (0,04)	
	Δ32/Δ32	0	0				

Table 2: Gender frequencies of the ApoE, ACE, MTHFR, CCR5 polymorphism in patients with mild cognitive impairment in Costa Rica population.

(35.71%) than in females (16.07%). No significant differences were obtained.

Discussion

According to the demographic characteristics analyzed, in the relation of MCI, AD and gender, our population behaves as quoted by the literature, where the reported incidence is higher in women [37,38] (MCI: 67% in our sample; 69% [38,39]). We analyzed for the first time the frequency of the polymorphisms for ApoE, MTHFR, ACE and CCR5 genes in Costa Rican population with mild cognitive impairment. This effort represents a first approach to the study of genetic factors considered as risk factors for diseases such as AD and other dementia syndromes. The study of different genes to determine risk factors is of great importance for establishing treatment and management strategies [1,3]. In this article, we contemplated the analysis of genes with vascular effect such as ApoE [7], MTHFR [40,41], ACE [19, 20] and inflammatory effect in CCR5 [26] that are associated with cognitive impairment.

Two of the polymorphisms analyzed, presented a significant statistical difference. The T allele in the MTHFR gene (p=0.003329) is associated with a reduced activity and increased thermolability of the enzyme and therefore an increase in oxidative damage, cellular DNA damage and blood vessel damage related to homocysteine increase [40,41]. Also, is associated with cerebral atrophy even independent at the level of homocysteine [42] and it is possible to be considered an independent risk factor for MCI [43]. The ε4 allele of the ApoE gene (p= 0.007446) is related to a decrease in the transportation of lipoproteins and directly associated with neurodegenerative processes, such as AD [7,9,13]. The presence of the ε4 allele in our study with a value of 0.14 is consistent with the reports for the American region by Ward et al. [39], in a systematic review and meta-analysis, reporting prevalence from 0.11 to 0.14 in North America, and from 0.09 to 0.13 in South America.

The presence of the ApoE ε4 genotype is associated with its double structural conformation of domains interacting with each other by salt bridges between N terminal and C terminal contributing to neurodegeneration including mitochondrial dysfunction. It has been linked to deposition of cholesterol in areas previously damaged or complexing with beta-amyloid protein which reduces the capacity of synaptic plasticity contributing to cognitive failure [42,43].

The statistical significant differences indicates that the control group reported in other studies in Costa Rica and the MCI population analyzed, behave differently with respect to the general allele frequency for ApoE and MTHFR genes. Further analysis is important to determine which of those alleles differ from a control and a case. It is known that the presence of a matched control is necessary to limit bias in the variables. Currently the CMEC is selecting a control group matched to analyze these polymorphisms and thus carry out a comparative study directly with our population.

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