ARTÍCULO ORIGINAL: Determination of ApoE gene in patients with mild cognitive impairment

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RESUMEN

***Introducción*:** La enfermedad de Alzheimer (EA) es la causa más común de demencia. Es una enfermedad multifactorial en la que las condiciones genéticas y ambientales interactúan para presentar una manifestación clínica. El genotipo e4 de la apolipoproteína E (ApoE4) constituye un factor de riesgo para el desarrollo de la EA. Estimaciones indican que el alelo ApoE4 se presenta en el 15-16% de la población en general, con una mayor presencia en poblaciones caucásicas y casi en el 50% de los pacientes con EA. La presencia del genotipo de ApoE4 aumenta el riesgo de desarrollar EA de 3 a 8 veces más y disminuye la edad de aparición de la enfermedad entre 7 a 15 años antes. En forma homocigota el riesgo aumenta 33 veces. En pacientes de aparición tardía se encuentra en el 65% de los casos y el porcentaje se eleva al 80% cuando hay presencia de un familiar con EA. ApoE sigue siendo el biomarcador por excelencia para la predicción y el diagnóstico de EA. ***Objetivo*:** Estandarizar la técnica y determinar la frecuencia de ApoE en los 4 alelos de importancia clínica en los pacientes con deterioro cognitivo leve. ***Métodos*:** Se seleccionó una muestra de 14 pacientes previamente evaluados en la Clínica de Memoria y Envejecimiento del HSJD, diagnosticados con deterioro cognitivo leve amnésico. Se recogieron

muestras de sangre y se realizó el protocolo de extracción de ADN de Miller *et al*. Posteriormente se realizó una PCR múltiple análisis simultáneo de los alelos de ApoE. ***Resultados*:** Se están analizando un total de 14 pacientes seleccionados según los criterios definidos por el equipo interdisciplinario, con el fin de determinar la presencia de los alelos de APOE en esta población. ***Conclusiones*:** La frecuencia de la presencia del gen ApoE permite describir las características de la población de Costa Rica como un factor de riesgo para el desarrollo de AD.

PALABRAS CLAVE

Apoliproteína E. ApoE4. Enfermedad Alzheimer. Deterioro cognitivo leve. Diagnóstico temprano. Biomarcador

ABSTRACT

***Background*:** 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. The e4 genotype for the apolipoprotein E (ApoE4) is a risk factor for developing AD. ApoE4 presents 15-16% of the general population, with greater presence in Caucasian populations and nearly in 50% of subjects with AD. The presence of ApoE4 genotype increases the risk of developing AD 3 to 8 times higher and decreases age onset between 7 to 15 years. In homozygous form the risk increases 33 times. In late-onset AD is found in 65% of the cases and the percentage rises to 80% in presence of a family member with EA. ApoE remains the biomarker for predicting and diagnosing AD. ***Objective*:** To standardize the technique and determine the frequency of the ApoE's 4 alleles of clinical significance in patients with mild cognitive impairment. ***Methods*:** Patients who were previously evaluated in the Memory Clinic-Hospital San Juan de Dios and diagnosed with mild cognitive impairment were selected. We collected blood samples and performed DNA extraction protocol by Miller et al. Multiplex PCR was performed in 14 patients for the simultaneous analysis of gene ApoE genotype of the samples. ***Results*:** We are testing a total of 14 patients diagnosed with mild cognitive impairment who were previously diagnosed by the interdisciplinary team to determine the presence of the ApoE gene. ***Conclusions*:** The frequency of the presence of the ApoE gene allows to describe the characteristics of the Costa Rican population as a risk factor for developing AD

KEY WORDS

Apoliprotein E. ApoE4. Alzheimer's disease, Mild Cognitive Impairment. Early diagnosis.

BACKGROUND

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]. The e4 genotype for the apolipoprotein E (ApoE4) is a risk factor for developing AD. ApoE4 presents 15-16% of the general population[2], with greater presence in Caucasian populations[3] and nearly in 50% of subjects with AD. The presence of ApoE4 genotype increases the risk of developing AD 3 to 8 times higher and decreases age onset between 7 to 15 years[4]. In homozygous form the risk increases 33 times[2,5]. In late-onset AD is found in 65% of the cases[6,7] and the percentage rises to 80% in presence of a family member with EA[8]. ApoE remains the biomarker for predicting and diagnosing AD[10].

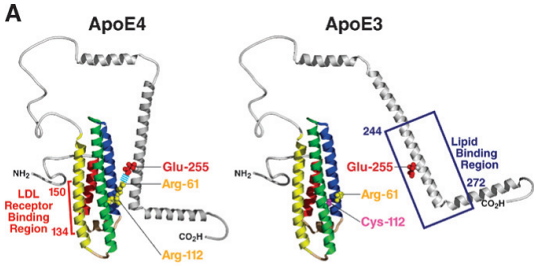


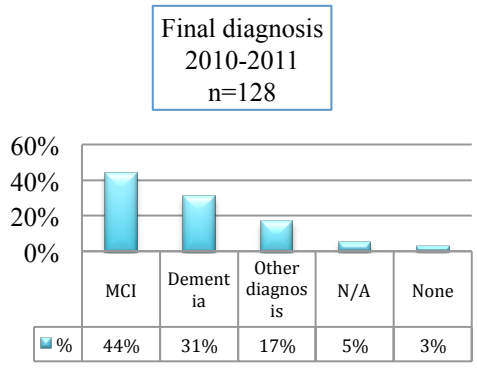
Figure 1. Structural protein conformation of the E4 and E3 haplotypes of ApoE
Source: http://biomodel.uah.es/model2/lip/apo-e4.htm

In Costa Rica, the first prevalence study conducted in a community (Santo Domingo de Heredia) with a sample of 400 subjects, showed a 4.2% prevalence of probable dementia (in any of its subtypes)[12]. Among the subjects evaluated 41 were diagnosed with AD (n = 14) and mild cognitive impairment (MCI, n = 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, which includes a battery of tests for assessing cognitive and functional performance (screening, medical history, neuropsychological assessment, neurological examination, review of the patients record, molecular biology studies and neuroimaging).

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 were found in 30.5% of cases, where the AD (43,6%) and vascular dementia (25,6%) predominated.

Graphic 1. Final diagnosis



Source: Memory and Aging Clinic, HJSD.

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 also 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 Costarrican Alzheimer and Other Dementias Association (ASCADA), which gave the first donation of the ApoE detection kits.

The aim of this study is to standardize the technique for detection of the ApoE haplotypes in DNA samples, and determine the frequency of the ApoE 4 alleles of clinical significance in patients with Amnesic Mild Cognitive Impairment (MCI-A), evaluated and diagnosed by the CMEC.

METHODS AND MATERIALS

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 record, 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-A were randomly selected.

We collected blood samples and performed DNA extraction protocol by Miller *et al*, 1988[11]. Multiplex PCR was performed in 14 anonymous samples for the simultaneous analysis of gene ApoE genotype of the samples. PCR product ApoE was then analyzed by electrophoresis at 105 V for 35 min through a 2% agarose gel and stained with ethidium bromide.

RESULTS

We are testing a total of 14 anonymous samples of individuals diagnosed with MCI-A who were previously diagnosed by the interdisciplinary team to determine the presence of the ApoE gene. They were divided by according to amnesic MCI's subtypes (simple or multodomain).

Table 1. Distribution of Mild Cognitive Impairment (MCI) in patients previously evaluated in the Memory Clinic-Hospital San Juan de Dios (n = 14).

MCI*	Frequency
MCI-Am	71,43
MCI-A	28,57

(*)MCI-Am: Amnestic multidomain MCI, MCI-A: Amnestic MCI.

All of the samples were processed, but only fourth were able to determine their ApoE genotype. This due to a complex process of standardization that took longer than initially expected. The following are the preliminary results of the samples analyzed.

Table 2. Distribution of ApoE genotypes in patients previously evaluated in the Memory Clinic-Hospital San Juan de Dios (n = 14).

Genotype/Haplotypes	Frequency
In process[1]	71,43
E3/E4	14,29
E3/E3	14,29
112cys[2]	92,86
Other	7,14

1) Samples still not totally processed.
2) Protein conformation associated with E2 and E3 haplotypes.

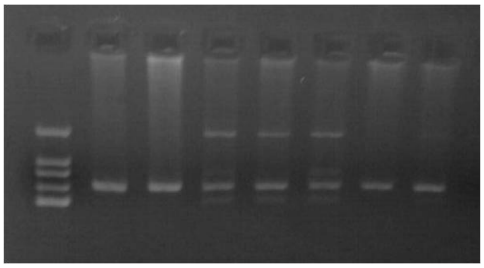


Figure 2. Multiplex PCR amplification of ApoE gene

DISCUSSION AND CONCLUSIONS

This is the first study of standardization of the test for ApoE genotype in Costa Rica. Despite the constraints faced, we now have solved the problems with the standardization of the technique and have the capacity to continue the analysis of the patients.

We overcome the most complicated stage for the application, which constituted the adaptation of the technique to our environment. This is a breakthrough because it is the stage that more time and resources involve.

Already achieved preliminary results of 4 samples of a new technique in the country, representing a pioneering project in our field, allowing it to provide the first results which will be statistically significant once more samples are processed.

ACKNOWLEDGMENTS

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