Individual and Combined Effects of *ApoE* and *MTHFR* 677C/T Polymorphisms on Cognitive Performance in Spanish Adolescents: The AVENA Study

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Objective To examine the individual and combined associations of *ApoE* and *MTHFR* 677C/T polymorphisms with cognitive performance in adolescents.

Study design The study comprised 412 Spanish adolescents (13 to 18.5 years of age). Cognitive performance (verbal, numeric and reasoning abilities, and an overall score) was measured by the Spanish-version of the SRA-Test of Educational-Ability.

Results We observed no differences in the cognitive performance study variables in adolescents carrying or not carrying the *ApoE* ϵ4 variant. Adolescents without the *MTHFR* 677TT genotype had significantly better cognitive performance than their TT peers. The analysis of the combined effect of these polymorphisms revealed that those individuals carrying both the *ApoE* ϵ4 variant and the *MTHFR* 677TT genotype had significantly worse cognitive performance than their peers with other genotype combinations. These findings were independent of sex, age pubertal status, socioeconomic status, physical activity, and skipping breakfast.

Conclusions The results of the present study suggest that the *ApoE* ϵ4 alone is not associated with cognitive performance in adolescents. Individuals with the *MTHFR* 677TT genotype had slightly impaired cognitive performance, whereas we observed a combined effect of both the *ApoE* ϵ4 variant and the *MTHFR* 677TT genotype on cognitive performance. More research is needed in larger population samples to corroborate our findings. *(J Pediatr 2010;156:978-84)*.

Apolipoprotein E (ApoE) and its receptors (also known as LDL-receptors) play a pivotal role in neural development, synaptic plasticity, and neuroprotection.[1] The *ApoE* gene is polymorphic, and its 3 common *ApoE* alleles (ϵ2, ϵ3, and ϵ4) have been associated with risks for several diseases.[2,3] The presence of the ϵ4 variant is a strong risk factor for Alzheimer disease.[4,5] Adults who are ϵ4 homozygotes have up to 15 times higher risk of developing Alzheimer disease compared with non-carriers,[6] and their median age of Alzheimer disease onset is 68 years, compared with 84 years in non-ϵ4 carriers.[7] The presence of *ApoE* ϵ4 acts in synergy with several lifestyle factors (eg, fat and alcohol intake, smoking, and physical inactivity) during adult life.[8]

The *ApoE* ϵ4 variant is also associated with normal age-related cognitive decline.[9,10] Whether the *ApoE* ϵ4 influences cognition during youth is, however, a subject of debate. Two studies did not find an association between *ApoE* polymorphisms and cognitive ability in children.[9,10] In contrast, other studies involving healthy young individuals reported a positive association between *ApoE* ϵ4 and intelligence quotient (IQ),[11] predisposition to reach higher levels of education,[12] better performance in some neuropsychological measures after brain injury,[13] improved memory and neural efficiency,[13] or verbal fluency.[14]

Elevated total plasma homocysteine is another strong, independent predictor for the development of vascular dementia and Alzheimer disease later in life.[15] High homocysteine levels are also associated with decreased cognitive function

AVENA	[*Alimentación y Valoración del Estado Nutricional de los Adolescentes Españoles* (Food and Assessment of the Nutritional Status of Spanish Adolescents)] Study
ANCOVA	One-way analysis of covariance
ApoE	Apolipoprotein E
IQ	Intelligence quotient
MTHFR	5,10-Methylenetetrahydrofolate reductase
PCR	Polymerase chain reaction
TEA	Test of Educational Ability

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in the normal aging population.[16-18] Among other mechanisms, elevated homocysteine may negatively affect brain vasculature, resulting in low delivery of nutrients, and could interfere with neurotransmitter formation and DNA repair mechanisms.[19] Plasma homocysteine levels are in turn influenced by the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) 677C/T polymorphism,[20] with the T allele being associated with higher homocysteine levels even in young people.[20,21] Several studies showed no association between the *MTHFR* 677C/T polymorphism and cognitive performance in aging populations,[22-25] and only one report is available in younger cohorts, showing no differences in the distribution of *MTHFR* 677C/T genotypes in children with high IQ compared with peers with average IQ.[26] On the other hand, the possible combined effect of deleterious *ApoE* and *MTHFR* 677C/T polymorphisms on cognitive function in adolescents remains to be elucidated.[27]

The aim of the present study was to examine the association of *ApoE* and *MTHFR* 677C/T polymorphisms with cognitive performance (including verbal, numeric, and reasoning abilities) in Spanish (Caucasian) adolescents. We also examined the combined effects of both polymorphisms on cognitive performance.

Methods

Participants were recruited from the AVENA [*Alimentación y Valoración del Estado Nutricional de los Adolescentes Españoles* (Food and Assessment of the Nutritional Status of Spanish Adolescents)] Study. This is a cross-sectional study that was primarily designed to assess the nutritional status of a sample of urban Spanish adolescents ages 13 to 18.5 years. Data collection took place from 2000 to 2002 in 5 Spanish cities (Madrid, Murcia, Granada, Santander, and Zaragoza). The complete methodology of the study is detailed elsewhere.[28-30] The number of adolescents included in the AVENA Study was 2859. Blood samples were randomly obtained from 581 participants. The subgroup from which blood samples were obtained was similar to the remaining subjects with regard to the variable selected to calculate the number of participants to be included in the study, for example, body mass index[29] and age and sex proportions (all $P > .2$). The present study comprised 412 adolescents (204 boys and 210 girls) for whom we obtained complete data on cognitive performance and *ApoE* and *MTHFR* 677C/T genotypes.

A comprehensive verbal description of the nature and purpose of the study was given to the parents, school supervisors, and adolescents. Written consent to participate was requested from both parents and adolescents. Adolescents with personal history of cardiovascular disease, cognitive dysfunction, on medication at the time of the study, or those who were pregnant, were excluded. The study protocol was performed in accordance with the ethical standards laid down in the 1961 Declaration of Helsinki (as revised in 2000) and approved by the Review Committee for Research Involving Human Subjects of the Hospital Universitario Marqués de Valdecilla (Santander, Spain).

We assessed cognitive performance with the Spanish version of the SRA Test of Educational Ability (TEA).[31] The TEA measures the subject's ability to learn, by evaluating 3 areas: (1) verbal, command of language; (2) numeric, speed and precision in performing operations with numbers and quantitative concepts; and (3) reasoning, the ability to find logical ordination criteria in sets of figures, numbers, or letters. Direct scores were obtained for each of these parameters. We also computed an overall cognitive performance score by summing up the individual scores of the 3 items.

Genomic DNA for polymorphism analysis was extracted from EDTA-collected peripheral blood using the Quiagen procedure.[32] The *ApoE* (rs7412 and rs429358) genotypes were determined by polymerase chain reaction (PCR) and allele-specific restriction digestion of the amplified products with the restriction enzyme *HhaI*.[33] Genotyping of the 677C/T variant in the *MTHFR* gene (rs1801133) was performed with PCR and allele-specific restriction digestion of the amplified products with the restriction enzyme *Hinf I* (GE Healthcare, Madrid, Spain).[20] More detailed information about the *ApoE*[30] and *MTHFR* genotyping procedures is available.[34] The genotypes were in Hardy-Weinberg equilibrium ($P > .1$).

Potential Confounding Factors

Before any testing was performed, the parents completed a questionnaire that addressed the adolescents' previous and current health status and socioeconomic status, as defined by the educational level and occupation of the father. According to this information, and following the recommendation of the Spanish Society for Epidemiology,[35] the adolescents were classified into 5 categories: (I) low, (II) medium-low, (III) medium, (IV) medium-high, and (V) high socioeconomic status. We also obtained information regarding maternal education level (primary, secondary or university).

We obtained information about family structure through the aforementioned questionnaire. Family structure was defined as living with both mother and father or any other arrangement (only mother, only father, grandparents, others).

We assessed leisure physical activity by means of a questionnaire in which the adolescents answered the following question: "Do you practice any type of physical activity outside school time?" The possible answers were 0 (no) or 1 (yes).

We assessed whether the adolescents skipped breakfast by means of a questionnaire in which they answered the following question: "Do you have breakfast?" The possible answers were either 0 (no) or 1 (yes).

We assessed pubertal development according to Tanner and Whitehouse.[36]

Anthropometric measurements were obtained.[37] Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Skinfold thickness was measured at the biceps, triceps, subscapular, suprailiac, thigh, and calf on the left side of the body to the nearest 0.2 mm using a Holtain skinfold caliper. All measurements were taken twice and in rotation, and the mean value was calculated. The sum of 6 skinfold, as well as BMI, was used as marker of body fat.