ARTICLE ORIGINAL

Severity of coronary artery disease is significantly lowered among carriers of The allele 2 of the common apoE polymorphism

Sévérité amoindrie de l'atteinte coronaire chez les porteurs de l'allèle 2 du polymorphisme 112/158 de l'apoE

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Abstract

Aims

Controversial results about the association between the common apolipoproteinE (apoE) polymorphism and the severity of coronary artery disease have been reported mainly because of the heterogeneous scoring systems used to evaluate the coronary damage. Our study aimed to evaluate the association between the common apoE polymorphism and the severity of coronary damage using the Gensini score.

Methods

Our study involved 172 patients (63 women and 109 men) who benefited from a coronary angiography and a Gensini score. Serum lipid parameters were measured from samples collected before the coronary angiography and apoE genotyping was performed by PCR-RFLP. Patients were divided into tertiles according to their Gensini score. A backward stepwise logistic regression was performed and parameters were adjusted against lipid profile, gender, hypertension, diabetes and age.

Results

Compared to the apoE3, the apoE2 seems to protect against having a Gensini score higher than1 with an odds ratio of 0.191 (IC[0.055-0.668],p=0.010). Compared to apoE4, it seems to protect against having a Gensini score higher than 33 with an odds ratio of 0.09 (IC[0.01-0.79], p=0.012). After adjustment for confounding variables, the common apoE polymorphism remains significantly associated to the severity of the coronary damage.

Conclusion

we came to the conclusion that apoE2 might be an independent protective factor against severe coronary artery disease.

Keywords: *Apolipoprotein E polymorphism - coronary artery disease- coronary artery angiography - Gensini score.*

Résumé

Objectifs

Des résultats controverses ont été rapportés à propos de l'association entre le polymorphisme commun de l'apoE et la sévérité de l'atteinte coronaire probablement à cause de l'hétérogénéité des méthodes d'évaluation des lésions coronaires. Nous nous sommes proposés dans le présent travail d'étudier cette association en utilisant le score de Gensini comme outil d'évaluation de l'atteinte coronaire.

Méthodes

Notre étude compte 172 patients (63 femmes et 109 hommes) ayant tous bénéficié d'une coronarographie et d'un score de Gensini. Les paramètres lipidiques sériques ont été déterminés à partir des prélèvements recueillis avant coronarographie et le génotypage de l'apoE réalisé par PCR-RFLP. Les patients ont été subdivisés en tertiles selon leur score de Gensini. Une régression logistique descendante a été réalisée et les paramètres ont été ajustés par rapport au profil lipidique, au genre, à l'âge, l'hypertension artérielle (l'HTA) et le diabète.

Résultats

comparé à l'apoE3, l'apoE2 semble protéger contre avoir un score de Gensini supérieur à 1 [OR : 0.191 (IC[0.055-0.668], p=0.010].Comparé à l' apoE4, il semble protéger contre avoir un score de Gensini supérieur à 33 [OR :0.09 (IC[0.01-0.79], p=0.012] . Après ajustement par rapport aux facteurs confondants, le polymorphisme commun de l'apoE demeure significativement associé à la sévérité de l'atteinte coronaire.

Conclusion

L'apoE2 semble être un facteur de protection contre une atteinte sévère des coronaires.

Mots clés : *Polymorphisme de l'apolipoprotéine E- Coronaire- coronarographie- score de Gensini*

INTRODUCTION

The apolipoprotein E (apoE) gene is located in chromosome 19q13.2 and is closely linked to the apoC-I/C-II gene complex. It is responsible for the production of an ApoE protein
precursor of 317 aminoacids. ApoE sequences generated from mRNA are truncated and begin at residue 18 [1]. The resulting 299 amino acid polypeptide is synthesized primarily in the liver, but other organs and tissues also synthesize apoE, including the brain, the spleen, the kidneys, the gonads, the adrenal glands and macrophage [2]. The common apoE polymorphism 112/158 leads to three major isoforms E2, E3 and E4 which differ in their amino acid sequence at positions 112 and 158 corresponding to the sites 130 and 176 respectively, in the full un-truncated protein [3].

Studies in several populations have indicated that genetic variation at the apoE structural locus influences the risk of coronary artery disease (CAD) [4-7]. and even the risk of premature coronary artery disease (PCDA) [8].This may be mediated by lipid parameters such as total cholesterol whose increased levels are reported to be associated with CAD and which metabolism is influenced by the common apoE polymorphism [9,10].

Because coronary angiography is an important tool for the quantifying of CAD damage in both clinical practice and scientific investigation, researchers have attempted to define angiographic CAD damage using quantitative scoring systems [11].

Even though the existing scoring systems are still heterogeneous, some of them, such us the Gensini score which reflects the degree of reduction of the arterial lumen and the location of coronary disease - validated in multiple settings - such us the Gensini score which reflects the degree of reduction of the arterial lumen and the location of coronary disease, are easily reproducible and provide prognostic value [12, 13].

Our study aimed to evaluate the association between the common apoE polymorphism and the severity of coronary damage using the Gensini score.

MATERIAL AND METHODS

Subjects

Our study involved 172 Tunisian patients (63 women and 109 men) aged between 29 and 79 years (mean 59.00 ± 9.719). All patients benefited from a coronary angiography because they were highly suspected of having a CAD. Sample collection

A 12-hour overnight fasting was respected and venous blood was taken before the coronary
angiography. For every patient, blood was collected into two tubes with EDTA anticoagulant
for apoE genotyping and without anticoagulant for the determination of lipid parameters. Tubes with EDTA were stored at -80°C until the moment of extraction of DNA. Tubes without anticoagulant were centrifuged and sera were separated to be analyzed the same day as sample collection.

DNA analysis: Genomic DNA was extracted from leucocytes by a salting out method using the guanidium chloride. apo E genotyping was performed as described by Hixson and Vernier [14].

Amplification was performed using the following oligonucleotides primers:

Forward: 5' TAAGCTTGGCACGGCTGTCCAAGGA 3'
Reverse: 5' ACAGAATTCGCCCCGGCCTGGTACAC 3'

The amplification product was digested by CfoI restriction enzyme.Digested products were then resolved by polyacrylamid gel electrophoresis revealed by ethidium bromide staining.

Twenty eight patients were not genotyped because the DNA was missing or damaged.

Lipid analysis: Lipid parameters were measured (total cholesterol, triglycerides, HDL cholesterol, apolipoprotein AI and apolipoprotein B). LDL-cholesterol, (total cholesterol/HDL-Cholesterol) and (apolipoprotein B/apoplipoprotein AI) ratios were then calculated.

Coronary angiography: Each angiography was evaluated by two cardiologists from the cardiology department and coronary stenosis was measured by a software that was integrated in the cardiovascular Imaging INNOVA 2000.

Gensini score: For the estimation of the severity of coronary artery disease, we used the Gensini score. This scoring system assigns to each of the arterial luminal reduction a number of points (severity score):

- A score of 1 to a narrowing of 1-25%.
- A score of 2 to a narrowing of 26-50%.
- A score of 4 for a narrowing of 51-75%.
- A score of 8 for a narrowing of 76-90%.
- A score of 16 for a narrowing of 91-99%.
- A score of 32 for a total occlusion.

The score is subsequently multiplied by a coefficient that takes into account the importance of the position of the coronary artery disease at the branches of the coronary arteries.

The Score calculation is done by applying the following formula:

Gensini score = ∑(Severity score X coefficient).

This score, thus reflects at the same time the degree of reduction of the arterial lumen and the geographical location of coronary disease [15].

Statistical analysis: The statistical analysis was performed using SPSS 16 (SPSS, Chicago, USA). Kolmogorov-Smirnov test was used to check the distribution of parameters. Discrete variables were analyzed by the chi-square test and odds ratios were also determined. We conducted a backward stepwise logistic regression

and we introduced confounding factors reported in the literature as coronary heart disease risk factors (lipid profile, diabetes, hypertension, sex and age) to identify an independent association of apoE genotype with severity of coronary disease.

A p value of less than 0.05 was considered statistically significant for all the tests.

To study the associations of the common apoE polymorphism with the severity of CAD, the population was divided into three tertiles according to their Gensini score.

RESULTS

Among the apoE genotypes noted by our study, E3E3 was the most frequent (72.22%) followed by E3 E4 (17.36%), E3E2 (8.33%),E4E4 (1.38 %) and than by E2E2 (0.69%) (Table 1).

We noted that our population was in hardy-weinberg equilibrium [$\chi^2 = 2.89 < \chi^2$ threshold value (α=5% ; ddl=3) = ^{7,81}].

As ε3 allele was hypothesized to be neutral, we considered apoE genotypes as follows:

apoE2= (apoE2E3 and apoE2E2); apoE3 = E3E3 and apoE4 = (apoE3E4 and apoE4E4).

Association between the common apoE polymorphism and the severity of CAD

We divided the population into three tertiles: first tertile (T1) with a Gensini score lower or

equal to 1, second tertile (T2) with a Gensini score higher than 1 and lower or equal to 33 and third tertile with a Gensini score higher than 33.

The association of the common apoE polymorphism to score is shown in table 2

We found a significant association between the Gensini

Table 1
Population characteristics.

Age (years)	N : 172 59.00 ±9.719
Sex Men Women	N : 172 109 (63.4%) 63 (36.6%)
Diabetes Yes No	N : 172 62 (36%) 110 (64%)
Hypertension Yes No	N : 172 89 (51.7%) 83 (48.3%)
Lipidloweringtreatment* Yes No	N : 155 46 (29.7%) 109 (70.3%)
Gensini score** **(min-max)**	N : 171 9.5 (0 – 516)
Stenosis degree (%)**	N : 171 55 ± 26.62
apoE genotypes*** E3 E3 E3 E4 E3 E2 E4 E4 E2 E2	N: 144 72.22% 17.36% 8.33% 1.38 % 0.69%

*: No mention about followed treatment in 17 patients
**: Coronary angiography result was missing in 1 patient folder
***: Twenty eight patients were not genotyped because the DNA was missing or damaged.

Table 2
Association between the common apoE polymorphism and Gensini score

apoE genotypes	T1 N=50	T2 N=47	T3 N=46	p
apoE2 (%)	9 (18)	3 (6.38)	1 (2.17)	
apoE3 (%)	31 (62)	40 (85.1)	32 (69.56)	0.008
apoE4 (%)	10 (20)	4(8.51)	13 (28.26)	

T1 (tertile 1): Gensini score lower or equal to 1, T2 (tertile 2): Gensini score higher than 1 and lower or equal to 33, T3 (tertile 3): Gensini score over 33

score and the common apoE polymorphism (p=0.008, Pearson's R = 0.187).
Results of pairwise comparisons of apoE allele carriers and Gensini score tertiles are resumed in table 3.
Compared to the apoE3, the apoE2 seems to protect against having a Gensini score higher than1 with an odds ratio of 0.191 (IC[0.055-0.668], p=0.010). Compared to the apoE4, it seems to protect against having a Gensini score higher than 33 with an odds ratio of 0.09 (IC[0.01-0.79], p=0.012).
Results of lipid parameters are not shown and used only as confounding factors in a backward stepwise logistic regression to study the association of apoE genotype with severity of coronary disease.
After adjustment for the confounding variable (lipid profile, age, sex, diabetes, hypertension), the common apoE polymorphism remains significantly associated to the severity of the coronary damage.
Indeed, by adopting the apoE4 as a reference indicator, the polymorphism of apoE [(E2 vs E4) and (E3 vs E4)] influence independently the severity of coronary artery disease (T3 vs (T1 +T2)).Compared to apoE4, apoE2 seems to significantly reduce the risk of having severe coronary artery disease. The part of the common apoE polymorphism to explain the coronary artery disease severity is about 11% (R^2= 0.11) (Table 4).

DISCUSSION

Controversial results about the association between the common apoE polymorphism and the severity of CAD have been reported [16, 17]. We think that these results may differ depending on the study population, environmental risk factors and mainly on the scoring system used to evaluate the severity of the coronary damage. Indeed heterogenous methods exist based on the number of significantly stenosed coronarien arteries, extension of stenosis or both.
In this study we have analyzed the association between

Table 3
Pairwise comparisons of the common apoE polymorphism and Gensini score tertiles

	T1 vs (T2+T3)			T3 vs (T1+T2)		
	OR	**IC**	**p**	**OR**	**IC**	**p**
apoE2 vs apoE3	0.191	0.055-0.668	0.010	0.185	0.023 – 1.48	0.078
apoE2 vs apoE4	0.261	0.064-1.074	0.091	0.09	0.01-0.79	0.012
apoE3 vs apoE4	0.732	0.301-1.77	0.49	0.485	0.205-1.15	0.097

T1 (tertile 1): Gensini score lower or equal to 1, T2 (tertile 2): Gensini score higher than 1 and lower or equal to 33, T3 (tertile 3): Gensini score over 33, OR: odds ratio, CI: confidence interval, P: P-value.

Table 4
Association between common apoE polymorphism and the Gensini score after adjustment for lipid profile, sex, age, and hypertension

	T3 vs (T1 +T2)			
	OR	**IC**	**p**	**R^2**
ApoE2 vs ApoE4	0.081	0.008 – 0.786	0.03	0.11
ApoE3 vs ApoE4	0.425	0.144 – 1.253	0.121	

T1 (tertile 1): Gensini score lower or equal to 1, T2 (tertile 2): Gensini score higher than 1 and lower or equal to 33, T3 (tertile 3): Gensini score over 33, OR: odds ratio, CI: confidence interval, P: P-value, R2: R-square.

the common apoE polymorphism and the severity of coronary artery disease evaluated by Gensini score which considers the stenosis site in addition to the amount of the arteries diameter reduction [15].After division of our population into tertiles according to their Gensini score and compared to the apoE3, the apoE2 seems to protect against having a Gensini score higher than1. Whereas, compared to the apoE4, the apoE2 seems to protect against having a Gensini score higher than 33.

We noted that even after adjustment for the confounding variables (lipid profile, age, sex, diabetes, hypertension), the common apoE polymorphism remains significantly associated with the severity of the coronary damage.

Another study conducted on a Tunisian population reported that apoE genotypes were significantly associated with the severity of CAD and the frequency of allele ε4 linearly increased with the number of damaged coronary arteries [16].

Li and al have shown such an association among Chinese men. Severity of CAD was evaluated by three methods considering: the number of affected vessels severity, the length of the longest damaged artery and the Gensini score. They reported that ε4 allele wan independent risk factor for the progression of the CAD [18]. They showed that the ε2 allele was associated with a decreased severity of stenosis and shorter vessel disease and they postulate that its protective role may be due to the fact that the E2 isoform has within its structure two sulfhydryl groups that allow it to have a more powerful antioxidant effect compared to the other two isoforms [18].

In 2007, one meta-analysis showed that compared to individuals with the ε3ε3 genotype, individuals who carry ε2 have a 20% lower risk of CAD and those carrying ε4 have a slightly higher risk. This observation suggests that the ε2 allele might be considered as a protective factor against CAD [7]. This conclusion opposes the results found by another meta-analysis showing that the E2 allele has a neutral effect [5]. However, with all the meta-analyses of the association between APOE and CAD, the studies that were pooled had included a variety of covariates in their analyses and the specific effect of adjustment for lipid profile on an individual basis was less clear. Karahan et al, investigated the relation between ApoE polymorphism and severity of CAD in patients with acute MI by using the Gensini Score [19]. They don't found statistically significant association between ApoE genotypes and severity of coronary artery disease.

CONCLUSION

Differences in allele frequency, geographic and ethnic background, study design may have contributed to the conflicting results about the association between the common apoE polymorphism and the severity of CAD. We came to the conclusion that compared to apoE3 and apoE4; apoE2 might be an independent protective factor against severe CAD in a Tunisian population.

COMPETING INTERESTS

No competing interests exist

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