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# Interaction and association between genetic mutations and clinical risk factors in developing ischemic stroke in Moroccan population

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**ABSTRACT:** In this paper, we focus on the importance of interaction between adverse genetic mutations, and clinical risk factors in the development of ischemic stroke, knowing that this disease is multifactorial common that is affected by a number genetic mutations and environmental factors.

The apolipoprotein E4 allele (APO e4), Factor II G20210A prothrombin (blood clotting protein), and Factor V genotypes were examined in 165 patients with ischemic Stroke group and 165 Control group, at the Laboratory of Human Genetics and Molecular Pathology, University Hassan II- Faculty of Medicine in Casablanca and the department of neurology at the campus teaching hospital of Casablanca and Rabat. Logistics regression models and bivariate correlation analysis with Karl Pearson's method were used to estimate the role of co-occurrences of the clinical risk factors and genetic mutations in ischemic stroke. The presence of the APO e4 is dominant in Stroke group than Control group and its association with hypertension, smoking and cholesterol increases the risk of ischemic stroke. FV mutation in association with hypertension or diabetes mellitus reveals a strong correlation in ischemic stroke. We found no significant relationship between the ACE D / D, FII, MTHFR 677TT genotype and clinical factors.

In some combinations, pairing of adverse genetic factors, which alone confer non-significant risk, with clinical risk factors can greatly increase the risk of having an ischemic stroke.

**KEYWORDS:** Ischemic stroke, Clinical risk factors, Correlation, Logistic regression, Apolipoprotein E (ApoE), FV and Allele frequency

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## 1 INTRODUCTION

Stroke remains a leading cause of death worldwide and the first cause of disability in the western world. Ischemic stroke accounts for almost 80% of the total cases of strokes and is a complex and multifactorial disease caused by the combination of vascular risk factors, environment and genetic factors.

A number of frequent polymorphisms and mutations have been proposed as genetic risk factors. [1], [2] Although some of them can give rise to a specific stroke subtype as minor but significant risk factors, most of them alone have not proved to be significant risk factors for ischemic stroke overall. It has been demonstrated that clustering of unfavorable mutations can lead to strokes or other circulatory disorders even if alone they have not proved to be significant risk factors. [6], [7] The Apolipoprotein e4 allele (APO e4), the Factor V and FII [8], [9] are considered minor or insignificant adverse factors genetic risk for ischemic stroke.

In this current study, we calculated the allele frequencies of Apo E, FII and FV in our population comparing with countries around the world to find the dominant alleles in the cases group of ischemic stroke in our study. Then we examined whether these genetic factors can affect measurable change on clinical risk factors and may increase the relative risk of ischemic stroke.

## 2 MATERIALS AND METHODS

### 2.1 MATÉRIALS

This is a prospective study for 3 years. She focused on 360 subjects whose victims 165 cases of ischemic stroke and admitted in the laboratory of Genetics and Molecular Pathology, Faculty of Medicine, Casablanca LGPM in collaboration with the departments of neurology of the University Hospital of Casablanca and Rabat.

After clinical examination, neurological assessment, cardiovascular and explorations such as biological, physiological and radiological conducted for each patient so different from those balance, patients were classified according to the International Classification TOAST

LGMP (laboratory of Genetics and Molecular Pathology) and neurology CHU Casablanca and Rabat provided us the results of the genetic and clinical analysis of 165 samples, this analysis consisted of 9 genes (MTHFR, FII, ACE, FV, APOE, IPA, ENOS, APOA5, ALOX5AP) and several clinical factors (hypertension, diabetes, smoking, alcoholism and cholesterol ....)

### 2.2 MÉTHODS

The results are presented as percentages. Data are analyzed using the "SPSS" software. Genetic factor data were compared in different categories from test "ANOVA" Logistic regression and Odds ratio (OR) used to analyze associations and interactions between the APOE genetic risk factors, FII, FV ACE and other clinical factors such as hypertension, diabetes, smoking, alcoholism and cholesterol.

We performed a bivariate correlation analysis with the method of Karl Pearson in 1986 which offers a mathematical formula for the concept of correlation and an estimate of this quantity [5], [14];

In the first part we calculated the allele frequency of different genotypes for Stroke group and control group was started by The genetic polymorphism of Apo E that is in the coding portion of the gene and modifies the function of the protein produced. The genetic polymorphism of Apo E has three alleles (E2, E3, E4) encoding three forms of Apo E structurally and functionally different (E2, E3, E4) [10].

The variation of the frequency of alleles of Apolipoprotein E is different from one population to another in the world, in all studies, E3 is the most common allele which varies between 60 and 80% [15], [16] is considered normal, the E2 allele was less frequent. Regarding the E4 allele, frequencies vary from about 5% in the Far Eastern populations to 36.8% in the populations of Polynesia. [11] In most populations studied, three ApoE alleles are present. La E4 allele frequency is low in Japan and raised in Finland [15].

Most studies on the relative frequencies of alleles of the ApoE were made without considering the kind of individual. However, some studies have calculated the allelic frequency of ApoE separating men and women. In these cases the allele frequencies in women were not significantly different from those of men [12], [13].

It was then necessary to calculate the odds ratios and confidence interval in order to find the correlation between the dependent variables (APOE, FII, FV, ACE) and clinical factors hypertension, diabetes, smoking, excessive alcohol and cholesterol. We started by calculating the odds ratio "OR" [3] or relative risk [4], is a statistical measure often used in epidemiology, expressing the degree of dependence between qualitative random variables. It is used in Bayesian inference, and logistic regression, and can measure the effect of a factor.

***Table 1. Contingency table "cross between disease and exposure"***

| | Cases group | Controls group |
|---|---|---|
| Exposed | a | b |
| Not exposed | c | d |
| Total | a+c | b+d |

In contrast, the odds of developing the disease given exposure is a/b and of developing the disease given non-exposure is c/d The odds ratio, OR, is the ratio of the two, [cf. table 1].

Which can be rewritten as : OR = (a/b)/(c/d) or: OR = (a × d)/(b × c)

Given the algebraic rule of cross products, the second formula will produce the same result as the other two formulae for odds ratio and is the more commonly reported formula.

Significance Tests for the Odds Ratio

The first thing to understand when considering a significance test for the OR is that the true neutral value (indicating equal odds for both conditions) is one (1), not 0 (zero). Several significance tests can be used for the Odds Ratio. The most common are the Fisher's Exact Probability test, the Pearson Chi-Square and the Likelihood Ratio Chi-Square.

It should be remembered that the concept of "no difference" in most statistics refers to a difference of zero, and is generally measured with the variable means. The OR is different. The "no difference" value for this statistic is 1 and therefore, when a confidence interval includes the value of 1, the researcher or clinician will know that the odds of the measured outcome are the same for both (or all) treatment groups, even without a significance test.

## 3 RESULTS AND DISCUSSION

The study of the distribution of allele frequencies of ApoE in the Ischemic Stroke in the Moroccan population is 8% for the E2 allele, 82% for the E3 allele and 10% for the E4 allele. Comparison with other populations in the world Table 2 show that in all populations studied E3 allele is the most common (62.6% -87.5%) followed by the E4 allele (4% - 29.7%) and 'E2 allele less frequent (0% -14%). [cf. table 2] and fig.1

Allele frequencies of ApoE of the Moroccan population is E3 = 82%, E4 = 10% and E2 = 8%.

Our results show that the frequency of alleles of the ApoE is similar to what is reported for populations of Southern Europe: Italy, Spain, France, Turkey, and also that of Algeria and Asian populations. Against them by different populations of Europe, North and Sudan and Nigeria or E4 allele is more than twice that of our population (22.7%, 29.1% and 29.7% vs 10%), respectively.

*Table 2. Allelic frequencies of Apo E in different populations*

| Populations | Allelic frequencies | | |
|---|---|---|---|
| | E2 | E3 | E4 |
| Germany [17] | 0.077 | 0.773 | 0.15 |
| Chine [25] | 0.076 | 0.875 | 0.049 |
| Spain [18] | 0.057 | 0.811 | 0.071 |
| France [19] | 0.061 | 0.802 | 0.117 |
| Italy [20] | 0.073 | 0.827 | 0.100 |
| Morocco | 0.080 | 0.820 | 0.100 |
| Finland [17] | 0.041 | 0.733 | 0.227 |
| Nigeria [17] | 0.027 | 0.667 | 0.297 |
| Sudan [17] | 0.083 | 0.626 | 0.291 |
| Turkey [23] | 0.083 | 0.833 | 0.084 |
| Kuwait [21] | 0.057 | 0.854 | 0.090 |
| Saudi Arabia [22] | 0.036 | 0.846 | 0.120 |
| India [24] | 0.140 | 0.810 | 0.040 |
| Amazonian [26] | 0.000 | 0.831 | 0.169 |

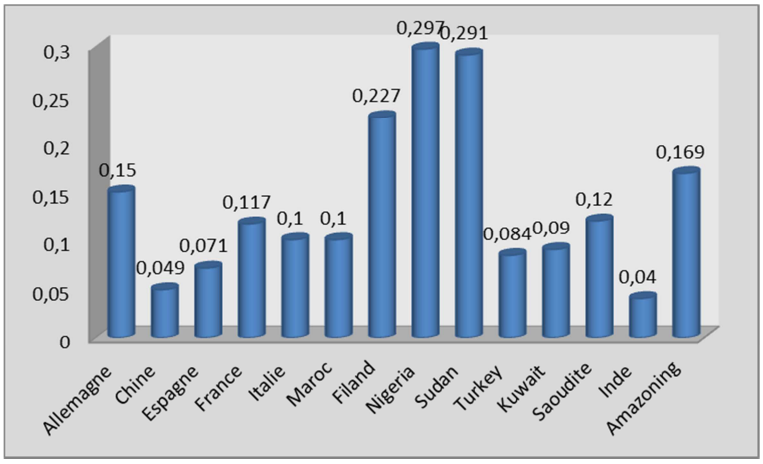


***Fig. 1. E4 allele frequency in different populations.***

In European populations the frequency of the E4 allele is in descending order from north to south, Germany have the highest frequency (15%) than northern populations such as France 11.7%, the Italy 10%, Spain 7.1%.

The distribution of genotype frequencies in ischemic stroke has the same descending order as controls group, but with different frequencies. E4/E4 genotype is more common (12.7%) in this group, it increased by 11.5% compared to controls (1.2%) as the E2/E4 genotype is increased by 22.4% compared to controls [cf. table 3].and fig.2

*Table 3. Genotypic frequencies of Apo E in ischemic stroke*

| Genotypes | Cases | | Controls | |
|---|---|---|---|---|
| | N | (%) | N | (%) |
| E2/E2 | *2* | 1,2 | *2* | 1,2 |
| E2/E3 | *12* | 7,3 | *18* | 10,8 |
| E2/E4 | *42* | 25,4 | *5* | 3 |
| E3/E3 | *75* | 45,4 | *118* | 71,5 |
| E3/E4 | *13* | 7,9 | *20* | 12,3 |
| E4/E4 | *21* | 12,7 | *2* | 1,2 |
| TOTAL | *165* | 100 | *165* | 100 |

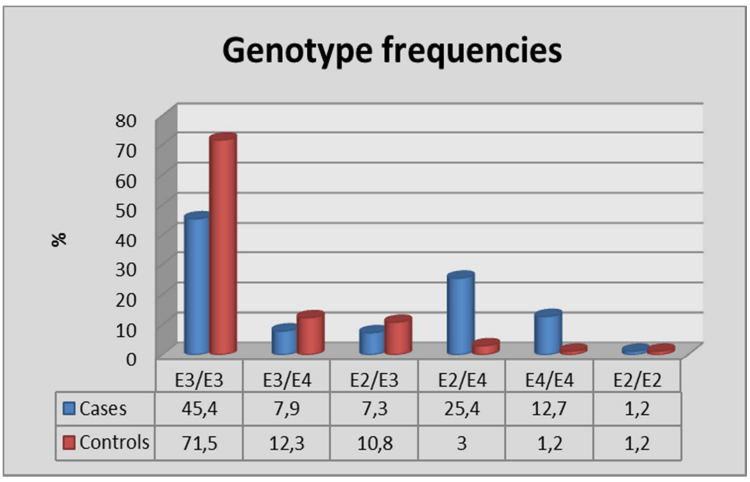


*Fig. 2. Genotypic frequency of ApoE in ischemic stroke in the Moroccan population*

For allelic frequencies of Apo E in the Moroccan population in the ischemic stroke, Table 4 [cf. table 4] and fig.3 show a difference in the increase in allelic frequency E4, compared with controls (29% vs 10%) 3 times higher.

*Table 4. Allelic frequencies of Apo E in Case-Control of ischemic stroke.*

| | E2 | E3 | E4 |
|---|---|---|---|
| **Cases** | 18% | 54% | 29% |
| **Controls** | 8% | 82% | 10% |

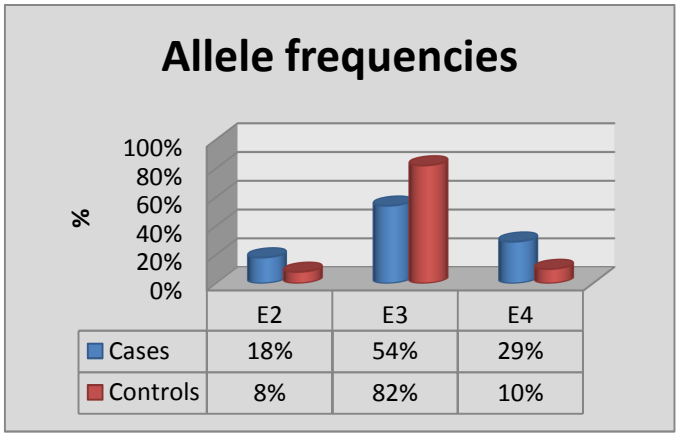


*Fig. 3. Allele frequencies of Moroccan population in ischemic stroke*

Our results show that E4 allele frequency is dominant in cases group than controls group and its association with hypertension, smoking and cholesterol increases the risk of ischemic stroke. [cf. table 5].In this case OR > 1 and 1 ∉ IC95% → statistically significant association between hypertension and APO E4 allele with same for smoking and cholesterol

***Table 5. The relative frequencies of hypertension, diabetes, smoking, drinking and cholesterol in cases of Ischemic stroke with and without the APO e4 allele***

| | | Ischemic stroke cases with APO e4 allele n= 76 | Ischemic stroke cases without APO e4 allele n= 89 |
|---|---|---|---|
| **Hypertension*** | Oui | 45 | 38 |
| | Non | 31 | 51 |
| | | *OR = 1.95 IC 95% =[1.05 ; 3.64]* ***p ≤ 0.01*** | |
| **Diabetes** | Oui | 12 | 22 |
| | Non | 64 | 67 |
| | | *OR = 0.55 IC 95% =[0.25 ; 1.21] p < 0.20* | |
| **Smoking*** | Oui | 32 | 24 |
| | Non | 44 | 65 |
| | | *OR = 1.97 IC 95% =[1.04 ; 3.80]* ***p ≤ 0.02*** | |
| **Drinking** | Oui | 3 | 8 |
| | Non | 74 | 80 |
| | | *OR = 0.41 IC 95% =[0.1 ; 1.59] p < 0.10* | |
| **Cholesterol*** | Oui | 13 | 5 |
| | Non | 63 | 84 |
| | | *OR = 3.47 IC 95% =[1.18 ; 10.23]* ***p ≤ 0.01*** | |

FV mutation in association with hypertension or diabetes mellitus reveals a strong correlation in ischemic stroke. [cf. table 6]

***Table 6. The relative frequencies of hypertension, diabetes, smoking, drinking and cholesterol in cases of Ischemic stroke with and without the FV mutation***

| | | Ischemic stroke cases with FV mutation n= 112 | Ischemic stroke cases without FV mutation n= 53 |
|---|---|---|---|
| **Hypertension*** | Oui | 63 | 20 |
| | Non | 49 | 33 |
| | | *OR = 2.16 IC 95% =[1.10 ; 4.14]* ***p ≤ 0.01*** | |
| **Diabetes*** | Oui | 29 | 6 |
| | Non | 83 | 47 |
| | | *OR = 2.74 IC 95% =[1.06 ; 7.07]* ***p ≤ 0.01*** | |
| ***Smoking*** | Oui | 36 | 20 |
| | Non | 76 | 33 |
| | | *OR = 0.78 IC 95% =[0.39 ; 1.55] p < 0.50* | |
| ***Drinking*** | Oui | 11 | 3 |
| | Non | 101 | 50 |
| | | *OR = 1.82 IC 95% =[0.48 ; 6.8] p < 0.50* | |
| **Cholesterol** | Oui | 12 | 5 |
| | Non | 100 | 46 |
| | | *OR = 1.1 IC 95% =[0.37 ; 3.32] p < 0.50* | |

We found no significant relationship between the ACE D / D, FII, MTHFR 677TT genotype and clinical factors.

Comparing our results with those of other populations in the world as a study was made in Hungary [27] show that V Leiden mutation in association with hypertension or diabetes mellitus increases the risk of ischemic stroke. They found synergistic effects between the ACE D / D and MTHFR 677TT genotypes and drinking or smoking, what is not the case in our study. The presence of the APO e4 reveals a strong correlation of hypertension and smoking and the incidence of ischemic stroke, what is similar to that we found in our population.

## 4 CONCLUSION

In addition to the classical clinical risk factors there are unfavorable genetic mutations that will increase the risk of ischemic stroke, and this can lead to more effective prevention.

Our results may be useful in everyday medical practice, and the detection of some adverse change, in combination with certain clinical risk factors could be an alarm and draw attention to the need for strict preventive measures.

A better understanding of the interaction between genotypes and clinical risk factors may facilitate the detection of the appropriate mutation.

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