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# Research Article
# Haptoglobin Polymorphism and Hyperuricemia among Stroke Patients

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## Abstract

**Background and Objectives:** Stroke is a major public health problem in our regions. In classical risk factors, differences in genetic susceptibility have been reported. So, our study aims to study the polymorphism of the haptoglobin gene as a factor predisposing to the onset of stroke and secondly to evaluate hyperuricemia as an associated risk factor. This is a longitudinal study and control cases. **Materials and Methods:** The number of subjects included was 46 stroke patients and 46 controls matched by sex and age±2 years. The parameters studied were genotyping of the Hp gene and uricemia in all subjects included. **Results:** The mean age of our patients was 63.37±14 years with a sex ratio of 0.70. Ischemic stroke was more represented in our population with a rate of 60.87%. The Hp2-2 genotype was significantly more common in subjects with stroke (36.96%) compared to control subjects (17.39%) and a statistically significant difference was found with $p = 0.002$. The mean serum uterine was 64.81 g $L^{-1}$ in stroke and the values were increased more in patients than in controls ($p<0.0001$). The frequency of hyperuricemia was higher in stroke subjects with genotypes Hp2-2 with a rate of 46.66% against 33.33% for subjects of genotype Hp1-1. **Conclusion:** Our results show that Hp polymorphism and hyperuricemia play a role in the pathogenesis of stroke. However, the impact of these two parameters must be estimated in conjunction with the other genetic, biochemical and associated clinical risk factors.

Key words: Stroke, genes, conjunctions, biochemical, clinical risk factors, patients, significant

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

World Health Organization (WHO) defines stroke as "rapidly developing clinical signs of focal or global disturbance of cerebral function, lasting more than 24 h or leading to death, with no apparent cause other than that of vascular origin[1]. The severity of sequelae is dependent on the affected brain region, the functions it controls and the duration of the stroke. There are two types of stroke: the ischemic stroke (DALYs) and the hemorrhagic stroke (ICH). Worldwide, stroke is the second leading cause of death after infectious diseases such as lung or diarrheic infections, tuberculosis, AIDS or malaria. They are the leading cause of sudden disability among adults and the second most common cause of disability globally[2]. In Senegal, their prevalence is estimated at 45.78% of neurological disorders requiring hospitalization and are responsible for nearly 2/3 of mortality at the Clinic of Neurology of Fann University Teaching Hospital[3-5]. With regard to all these figures, it is more necessary than ever to set up an effective and sustainable prevention policy imperatively based on epidemiological findings. In addition to the traditional risk factors such as diabetes, obesity, high blood pressure, some other factors are being evaluated. Thus, hyperuricemia is increasingly considered as a good marker of cardiovascular risk because of the metabolic abnormalities it can cause.

Besides, there are many arguments explaining the potential involvement of genetic factors directly or indirectly associated with this vascular disease. The haptoglobin gene is thus in top category of the studied molecular markers. On human subjects, Hp genetic polymorphism is mainly associated with three (03) isoforms which are structurally and functionally different. The functional differences between the different Hp genotypes have significant implications in a large number of diseases such as auto-immune diseases, infections but especially in cardiovascular disorders[6-8].

Against this background, our research objective aims for the study of Hp gene polymorphism as a predisposition or protection factor against stroke occurrence and furthermore, the evaluation of hyperuricemia as an associated risk factor.

## MATERIALS AND METHODS

**Study area:** Our study was carried out at the Fann National University Hospital Center in Dakar from 01 January, 2018 to 30 June, 2019.

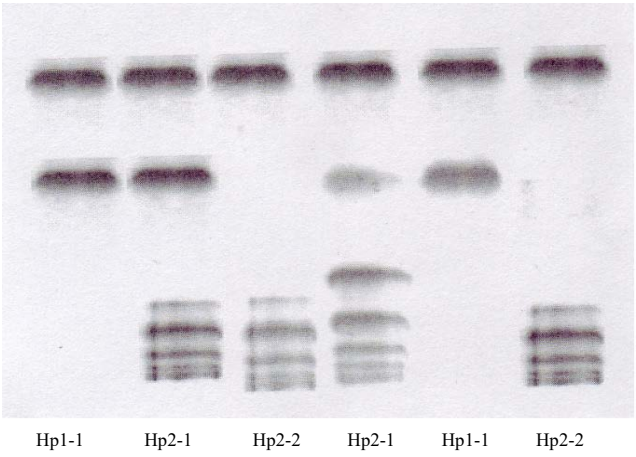


Fig. 1: Revelation of PCR products by agarose gel electrophoresis

**Research procedure:** This was an analytical case-control study[9]. It included 46 stroke patients matched to 46 control subjects according to sex and age ±2 years old. Samples were collected in a dry tube for the determination of uric acid and in a tube with EDTA for haptoglobin gene genotyping. The uric acid determination was performed using an enzymatic method with Cobas c 311 system (Roche, Germany). In this study we considered the reference range of uric acid from 40-60 mg $L^{-1}$ for men and 30-50 mg $L^{-1}$ for women.

DNA extraction was performed from whole blood using the QIAmp® genomic DNA and RNA kits (Paris, QIAGEN) according to the semi-automatic microcentrifuge method. Hp gene genotyping was performed using classic PCR with Proflex system. The A (5'GAGGGGGAGCTTGCCCCTTTCCATTG3') and B (5'GAGATTTTTGAGCCCTGCTGCTGGTGT3') primers were used to amplify a 1757 pb sequence specific for the Hp1 allele and a 3481 pb sequence specific for the Hp2 allele. The C (5'CCTGCCTCGTATTAACTGCACCAT3') and D (5'CCGAGTGCTCTCCACACATAGCCATGTGT3') primers were used to amplify a 349 pb sequence specific for the Hp2 allele. The PCR product was visualized on agarose gel in the presence of ethidium bromide (BET) and a molecular weight marker (Fig. 1).

**Statistical analysis:** Our data were collected using Microsoft Excel 2013 software. The collected data were processed using XLSTAT 2018 software. The Student T-test was used to compare the mean values and the Chi-square test for frequencies. A value of $p<0.05$ was considered as a statistically significant difference.

## RESULTS

The average age of our patients was 63.37±years old with extremes of 32 and 87 years old. The study population was characterized by a female predominance with a sex ratio of 0.70. Our findings showed that the DALY was the most highly represented type with 60.87% as the ICH represented 30.43% of the subjects and patients whose type of stroke the study failed to determine represented 8.7%.

Our data analysis showed an increased frequency of the Hp2-2 genotype with a rate of 36.96%, followed by the Hp1-1 and Hp2-1 genotypes with 34.78 and 28.26%, respectively. In contrast, among the controls, the Hp1-1 genotype was more common with a rate of 67% as the less common Hp2-2 was of 17%. When we compared these frequencies between stroke subjects and controls, we found significant differences except for the Hp2-1 genotype (Table 1).

The mean uric acid level was 64.81 mg $L^{-1}$ in stroke patients against 36.58 mg $L^{-1}$ in controls (Fig. 2). Comparison of the mean uric acid values between cases and controls showed a statistically significant difference ($p<0.005$). When the distribution was made according to stroke type, a significant difference was found between DALYs and controls with a $p<0.005$.

However, the comparison of mean uric acid values in ICHs and controls showed no significant difference ($p = 0.41$) (Fig. 3). In addition, mean uric acid with statistically significant differences were obtained in patients compared to controls according to Hp genotypes; Hp1-1 ($p = 0.0003$), Hp2-1 ($p = 0.0037$) and Hp2-2 ($p = 0.0038$). In our study population, hyperuricemia was frequently found in stroke with a rate of 32.60%. This frequency was more marked in subjects with Hp2-2 genotypes (46.66%) than among subjects with Hp1-1 (33.33%) or Hp2-1 (20%) genotypes (Table 2).

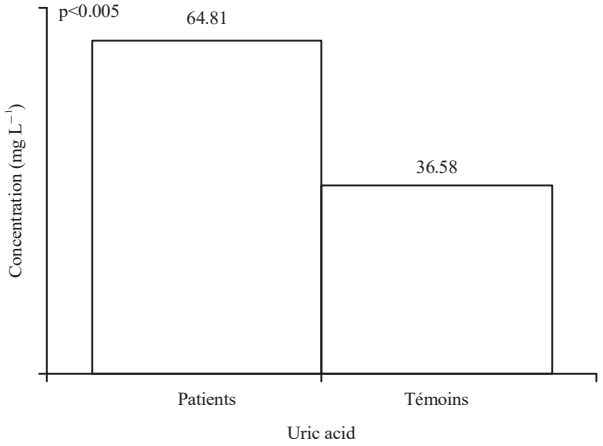


Fig. 2: Comparison of means of uricemia between stroke patients and controls

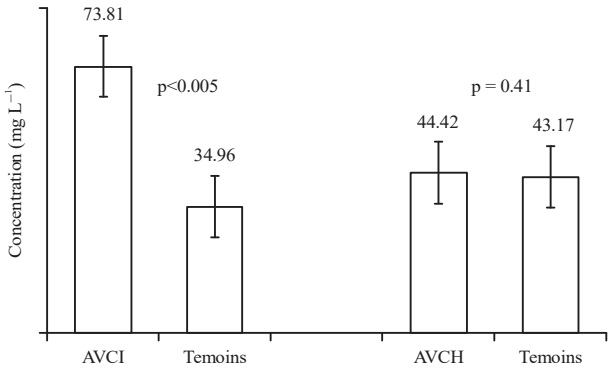


Fig. 3: Mean plasma uric acid (mg $L^{-1}$) values in cases and controls by stroke type

Table 1: Allelic and genotypic frequencies of Hp in stroke and control subjects

| Genotypes | Stroke subjects | Control subjects | p-value |
|---|---|---|---|
| Allele Hp1 | 48.91 | 71.74 | <0.0001 |
| Allele Hp2 | 51.09 | 26.61 | <0.0001 |
| Genotype Hp1-1 | 34.78 | 60.87 | 0.025 |
| Genotype Hp2-1 | 28.26 | 21.74 | 0.5 |
| Genotype Hp2-2 | 36.96 | 17.39 | 0.002 |

Table 2: Frequency of hyperuricemia according to Hp genotypes

| Genotypes | Normal uricemia (%) | Hyperuricemia (%) |
|---|---|---|
| Hp1-1 | 35.48 | 33.33 |
| Hp2-1 | 32.25 | 20.00 |
| Hp2-2 | 32.25 | 46.66 |

## DISCUSSION

The evaluation of the epidemiologic features of our study population found similar results with the data cited in the literature. It thus showed a female predominance and an average age of 63.37 years old, which is in line with findings from many other studies[10-12].

The study of both types of stroke showed a higher frequency of DALYs with a rate of 60.87% of the population. This finding is corroborated by the study by Mbonda *et al.*[5], which found a frequency of 65.8%[7]. Other studies had also found a similar result.

In our cohort, the study of Hp polymorphism revealed a predominance of the Hp2-2 genotype with a frequency of 36.96%. While in the control subjects, the Hp1-1 genotype was found predominant with a frequency of 67% of the population. Consistent findings have been reported by other authors namely Home and Harris[13,14]. Moreover, according to MacKellar and Vigerust[14], Hp2-2 genotype subjects present a much higher risk of exposure to neurological, infectious and renal diseases, but also to cardiovascular complications such

as myocardial infarction or stroke[15,16]. And also, Hp2-2 is implicated in the occurrence of diabetes with a risk of increased inflammation, oxidative stress and atheroma plaque instability[8].

Regarding the evaluation of uricemia, the comparison of mean serum values between controls and stroke patients showed a significant difference with a $p<0.005$. When we performed the evaluation of uricemia according to the type of stroke, our results showed different levels of association between the two factors. In daily patients and controls, the comparison of averages showed a statistically significant difference ($p<0.005$) while in ICH patients and controls we found a value of $p = 0.41$. Our findings thus corroborate those by Richette and Bardin[17] who clearly demonstrated the association between hyperuricemia and cardiovascular events, but also with many other metabolic abnormalities as well[17]. According to the 2006 Rotterdam study report, hyperuricemia was found to be a strong predictor of the risk of heart attack (myocardial infarction) and stroke. In addition, Chalès and Richette[18] reported in an American cross-sectional study (NHANES-III) that cardiovascular risk increased substantially with a uricemia value equal to or higher than 100 mg $L^{-1}$ compared to individuals with uricemia below 60 mg $L^{-1}$. He also stipulated that this risk increases twice as much in hyperuricemic subjects compared to normouricemic subjects[18]. To clarify the predictive value of uric acid level on cardiovascular mortality among stroke survivors, a Scottish team followed a cohort of 354 stroke survivors over an average period of 2.8 years old. When the overall cohort was considered, patients with serum urate concentrations higher than 0.32 mmol $L^{-1}$ had a risk of fatal cardiovascular event more than 3 times higher, even when adjusted for conventional risk factors such as age, gender, blood glucose, pulse pressure and cholesterol[19]. Presently, hyperuricemia is recognized as a major factor in the occurrence of high blood pressure but also in hyperinsulinemia, which are in turn considered to be classic risk factors for cardiovascular disease[20]. Hyperuricemia is reported to induce endothelial dysfunction via two mechanisms. First, uric acid inhibits the production of nitric oxide (NO) induced by VEGF (vascular endothelial growth factor) in endothelial cells[21,22]. Secondarily, uric acid also has an action on the smooth muscle vascular cell involving multiple intracellular messengers. It enters the cell through an anionic organic transporter (OATs) which then activates specific kinases and nuclear transcription factors leading to the synthesis of thromboxane (TXA2) and PDGF (platelet-derived growth factor) allowing cell proliferation. Uric acid also acts on drivers of inflammation, such as MCP-1 (chemoattractant protein-1 monocyte) and stimulates macrophages to produce Il 1-6 and TNF-$\alpha$[21].

Our finding also shows that hyperuricemia was more frequent in subjects with Hp2-2 genotype with a rate of 46.66% of patients. The simultaneous presence of these 2 independent risk factors in the patient thus seems to increase the risk of cardiovascular events occurrence. However, very few studies have been done to highlight the association of these two bio-markers in stroke or even more broadly in cardiovascular disease occurrence.

## CONCLUSION

The results of our study show that the Hp2 allele and the Hp2-2 genotype were significantly associated with stroke. in addition, hyperuricemia is an additional risk factor in stroke subjects of Hp2-2 genotype. However, additional genetic association studies are needed to confirm these findings.

## SIGNIFICANCE STATEMENT

This study discovered that the polymorphism of the haptoglobin gene plays a significant role in the pathogenesis of stroke, which may be beneficial for better patient management. This study will help the researchers to discover the critical areas of genomics and metabolic biochemistry that many researchers have not been able to explore.

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## REFERENCES

1. Faye, E.H.O., 2016. Aspects tomodensitométriques des accidents vasculaires cérébraux à l'hôpital régional de Kaolack. Ph.D. Thése, UCAD., Dakar-Senegal.
2. Sagui, E., 2007. Stroke in sub-Saharan Africa. Méd. Trop.: Rev. Corps Santé Colonial, 67: 596-600.
3. Diop, D.S., 2009. Évaluation des facteurs de risques cardiovasculaires et du risque cardiovasculaire global de l'hypertendu: Étude transversale chez 1229 travailleurs sénégalais. Ph.D. Thése, UCAD., Dakar.
4. Guetta, J., M. Strauss, N.S. Levy, L. Fahoum and A.P. Levy, 2007. Haptoglobin genotype modulates the balance of Th1/Th2 cytokines produced by macrophages exposed to free hemoglobin. Atherosclerosis, 191: 48-53.

5. Strandhave, C., M. Vensson, H. Krarup and J.H. Christensen, 2013. Haptoglobin genotype and risk markers of cardiovascular disease in patients with chronic kidney disease. Int. J. Nephrol., Vol. 2013. 10.1155/2013/650847.
6. Koch, W., W. Latz, M. Eichinger, A. Roguin, A.P. Levy, A. Schömig and A. Kastrati, 2002. Genotyping of the common haptoglobin Hp 1/2 polymorphism based on PCR. Clin. Chem., 48: 1377-1382.
7. Chimi, P.C.M., K. Toure, A. Sow, L.B. Seck, N.S. Diagne, M. Ndiaye and M. Ndiaye, 2013. Aspects épidémiologiques et pronostiques des Accidents Vasculaires Cérébraux (AVC) de la personne âgée à la clinique neurologique du CHU de Fann, Dakar-Sénégal. Rev. Neurol., 169: A89-A89.
8. Kolié, P., 2016. Étude des facteurs de risque cardiovasculaire chez les patients atteints d'accidents vasculaires cérébraux au CHNUF. Ph.D. Thèse, UCAD., Dakar.
9. Ndao, M., 2016. Evaluation du profil lipidique et de l'index d'athérogénicité plasmatique (IAP=log TG/HDL-C) chez des patients victims d'AVC au CHNU de Fann. Ph.D. Thèse, Universite Cheikh Anta Diop de Dakar, Dakar.
10. Benoit, M.O., 2001. CRP et prévention cardiovasculaire chez le sujet âgé. Ann. Biol. Clin., 4: 469-473.
11. Diouf, F.S., A.M. Basse, A.K. Ndao, M. Ndiaye and K. Touré *et al*., 2006. Pronostic fonctionnel des accidents vasculaires cérébraux dans les pays en voie de développement: Sénégal Functional prognosis of stroke in countries in the process of development: Senegal. Ann. Réadaptation Méd. Phys., 49: 100-104.
12. Harris, H., E.B. Robson and M. Siniscalco, 1959. Genetics of the Plasma Protein Variants. In: Ciba Foundation Symposium Biochemistry of Human Genetics, Wolstenholme, G.E.W. and C.M. O'Connor (Eds.)., John Wiley & Sons, New York, pp: 151-173.
13. Holme, I., A.H. Aastveit, N. Hammar, I. Jungner and G. Walldius, 2009. Haptoglobin and risk of myocardial infarction, stroke and congestive heart failure in 342,125 men and women in the Apolipoprotein MOrtality RISk study (AMORIS). Ann. Med., 41: 522-532.
14. MacKellar, M. and D.J. Vigerust, 2016. Role of haptoglobin in health and disease: A focus on diabetes. Clin. Diabetes, 34: 148-157.
15. Strandhave, C., M. Vensson, H. Krarup and J.H. Christensen, 2013. Haptoglobin genotype and risk markers of cardiovascular disease in patients with chronic kidney disease. Int. J. Nephrol., Vol. 2013. 10.1155/2013/650847.
16. Gueye, P.M., 2007. Phénotypes majeurs de l'haptoglobine humaine et stress oxydant induit par l'hémoglobine extra érythrocytaire sur le globule rouge. Ph.D. Thèse, Université Louis Pasteur de Strasbourg, Strasbourg.
17. Richette, P. and T. Bardin, 2010. Gout. Lancet, 375 : 318-328.
18. Chalès, G. and P. Richette, 2016. Obesity, hyperuricemia and gout. Rev. Rhumatisme Monographies, 83: 44-49.
19. Wong, K.Y.K., R.S. Macwalter, H.W. Fraser, I. Crombie, S.A. Ogston and A.D. Struthers, 2002. Urate predicts subsequent cardiac death in stroke survivors. Eur. Heart J., 23: 788-793.
20. Bardin, T. and A. Cohen-Solal, 2011. Goutte et pathologies cardiovasculaires. Rev. Rhumatisme, 78: S129-S133.
21. Feig, D.I., D.H. Kang and R.J. Johnson, 2008. Uric acid and cardiovascular risk. N. Engl. J. Med., 359: 1811-1821.
22. Ejaz, A.A., W. Mu, D.H. Kang, C. Roncal and Y.Y. Sautin *et al*., 2007. Could uric acid have a role in acute renal failure? Clin. J. Am. Soc. Nephrol., 2: 16-21.