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Haptoglobin Polymorphism and Disturbance of Lipid Parameters in Victims of Strokes

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Abstract: The objective of our study was to determine the frequency of the main genotypes of haptoglobin and their association with lipid parameters in subjects with stroke. This is a prospective, analytical and case-control study. 46 stroke patients and 46 matched controls were included by sex and age ± 2 years. Genotyping of Hp was performed using standard PCR with Proflex System PCR (Biosystems, Spain) and lipid parameters were assayed using enzymatic techniques with the Cobas c311 system (Roche, Germany). The mean age of our patients was 63 ± 14 years and the sex ratio was 0.70. Ischemic stroke was predominant with a frequency of 60.87% against 30.43% for hemorrhagic stroke. Allelic distribution in patients showed a high frequency of the Hp2 allele (51.09%) compared to control subjects (26.61%) (p <0,0001). The Hp2-2 genotype was more found in stroke subjects (36.96%) than in controls (17.39%) with a p = 0.002. A combination of the Hp2-2 genotype and the increase in mean LDL-c (p = 0.02), Triglycerides (p <0.005) and a decrease in mean HDL-c (p = 0.007) were found. Our study shows that the Hp2-2 genotype would be a predisposing factor to ischemic stroke. Its association with lipid parameters is found and could be considered as an additional risk factor.

Keywords: Stroke, Polymorphism of the Haptoglobin Gene, Lipid Balance

1. Introduction

Stroke is a real public health problem. The World Health Organization (WHO) defines a stroke as "the rapid development of localized or global clinical signs of cerebral dysfunction with symptoms lasting more than 24 hours and that can lead to death without any apparent cause other than vascular" [1]. There are two main types of stroke: ischemic stroke and hemorrhagic stroke.

They are one of the leading causes of morbidity and mortality after heart disease and cancer worldwide. They are the leading cause of acquired adult disability and the second leading cause of disability in the world. According to WHO, there are about 32 million cases, including 12.5 million deaths [2]. They represent 30% of hospitalizations and are responsible for nearly 2/3 of mortality in Senegal with an incidence of 1 to 2% of the general population [3].

The diagnosis is made by medical imaging mainly by CT and MRI. Biology is nowadays particularly absent from the stages of diagnosis and follow-up of the strokes. Thus, the search for biomarkers of prevention and/or follow-up is always more and more relevant.

The objective of our study was to determine the frequency of Hp genotypes and their association with lipid parameters

in stroke patients.

2. Methodology

2.1. Study Population

This is a prospective, analytical and case-control study. Included in our study were 46 stroke patients and 46 controls matched with cases by sex and age ± two years. Free and informed consent of all those involved in this study was obtained in advance in accordance with local ethical considerations. For all of our study population, genotyping of the Hp gene and assay of lipid parameters were performed.

2.2. Sampling

Blood samples were taken from fasting subjects, resting by venipuncture, and blood was collected in a dry tube for determination of lipid status parameters and in a tube with EDTA for genotyping the Hp gene.

2.3. Methods

2.3.1. Determination of Lipid Parameters

The determination of total cholesterol, HDL-c and triglycerides concentrations were carried out from Cobas C-311 (Roche, Germany) with enzymatic techniques. LDL-c was determined by the Friedewald formula.

2.3.2. Genotyping of the Haptoglobin Gene

The extraction of the DNA was carried out using the QIAmp® genomic DNA and RNA kits (QIAGEN, Paris) using the semi-automatic microcentrifuge technique. Genotyping of the Hp gene was performed using conventional PCR without enzymatic digestion with the Proflex System PCR (Biosystems, Spain). The primers A (5'GAGGGGAGCTTGCCTTTCCATTG3') and B (5'GAGATTTTTGAGCCCTGGCTGGT3') were used for the amplification of a 1757pb sequence specific for the Hp1 allele and a 3481pb sequence specific for the Hp2 allele. Primer C (5'CCTGCCTCGTATTAACTGCACCAT3 ') and D (5'CCGAGTGCTCCACATAGCCATGT3') were used to amplify a 349pb sequence specific for the Hp2 allele [4]. The PCR was carried out by adding to 2 µl of DNA, MgCl 2 (1.5 mM), 2.5 µl of 10 mM dNTPs, 12.5 µl of Taq polymerase (Applied Biosystems, USA), each of the primers at a volume of 1 µl and sterile water in a final volume of 25µl. The PCR program used consisted of an initial denaturation at 95°C for 5 min, followed by 35 amplification cycles each comprising DNA denaturation at 95°C for 1 min, primer annealing, and 69° elongation. C for 2 minutes; final elongation was then carried out at 72°C for 10 minutes. The PCR product was visualized on 0.7% agarose gel in the presence of ethidium bromide (BET) and a molecular weight marker.

2.4. Statistical Evaluation

Our data was collected and used with Microsoft Excel 2013 software. The Student test was used to compare the mean values and the Chi-square test for frequencies. A value of $p < 0.05$ was considered a statistically significant difference.

3. Results

The mean age of our subjects was 63 ± 14 years with extremes of 32 and 87 years. The analysis of the results of our study population showed a female predominance with a rate of 57% against 43% for men. The sex ratio was 0.70. Ischemic stroke was the most found form in our study population with a rate of 60.86% followed by hemorrhagic stroke at 30.43%. The typing of stroke was not done in 8.7% of our subjects (see Figure 1).

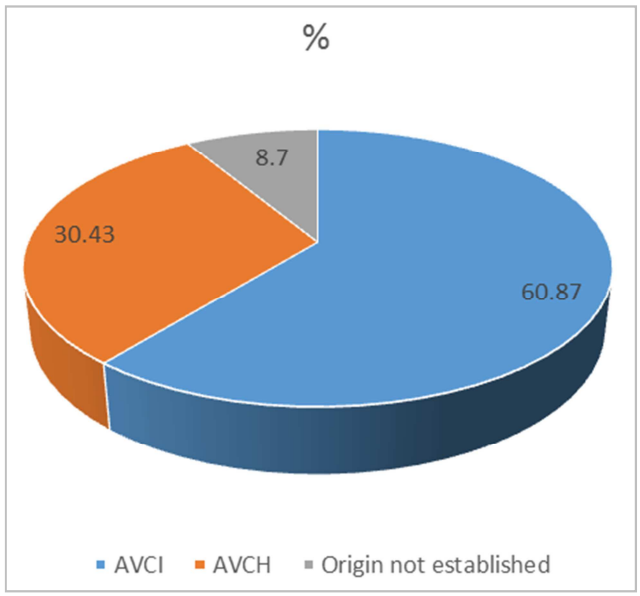

Figure 1. *Distribution of the population according to the type of stroke.*

The distribution of allelic and genomic frequencies of Hp varies between stroke and control. Our analysis showed a high incidence of the Hp2 allele (51.09%) in subjects with stroke compared to control subjects in which the Hp2 allele was a minority (26.61%) with a p-value of 0.001. A predominance of the Hp2-2 genotype was found with a frequency of 37% followed by the genotype Hp1-1 and Hp2-1 with respective rates of 34.7% and 28.3%. On the other hand, in the controls, the Hp1-1 genotype was predominant with a rate of 67% and Hp2-2 was represented at a rate of 17%. Comparative analyses showed that the frequencies of the Hp2 allele and the Hp2-2 genotype increased significantly in patients compared to the control group with $p < 0.05$ values (see Figure 2).

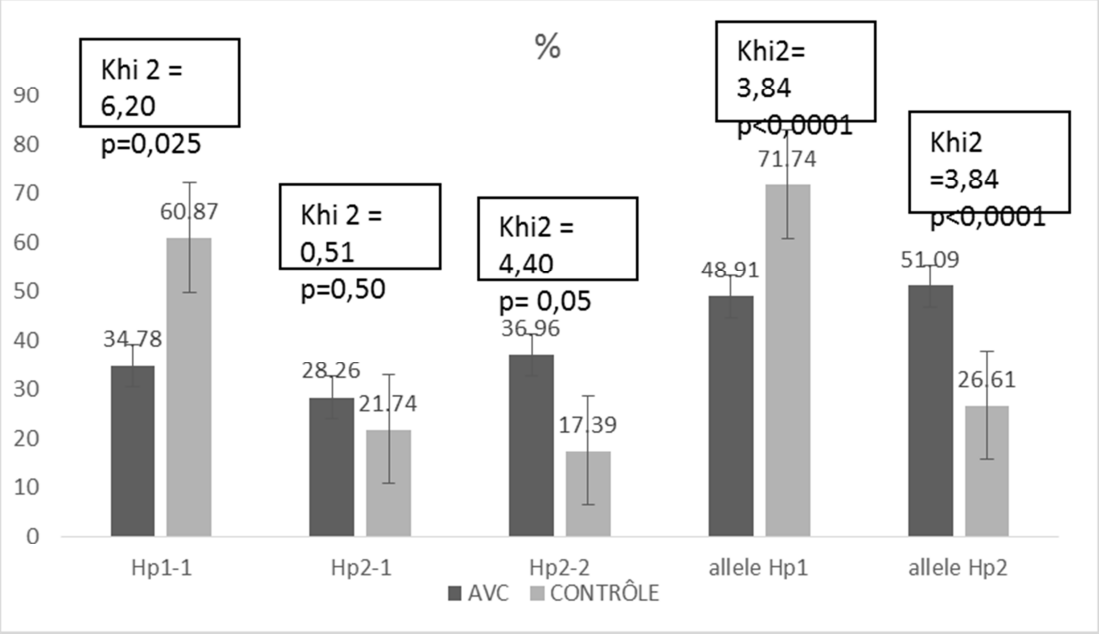

Figure 2. *Allelic and genotypic frequencies of Hp in stroke and control subjects.*

The statistical analysis of the data showed that the average total cholesterol level in patients with stroke was 1.81g / l against 1.60g / l for controls (p = 0.057).

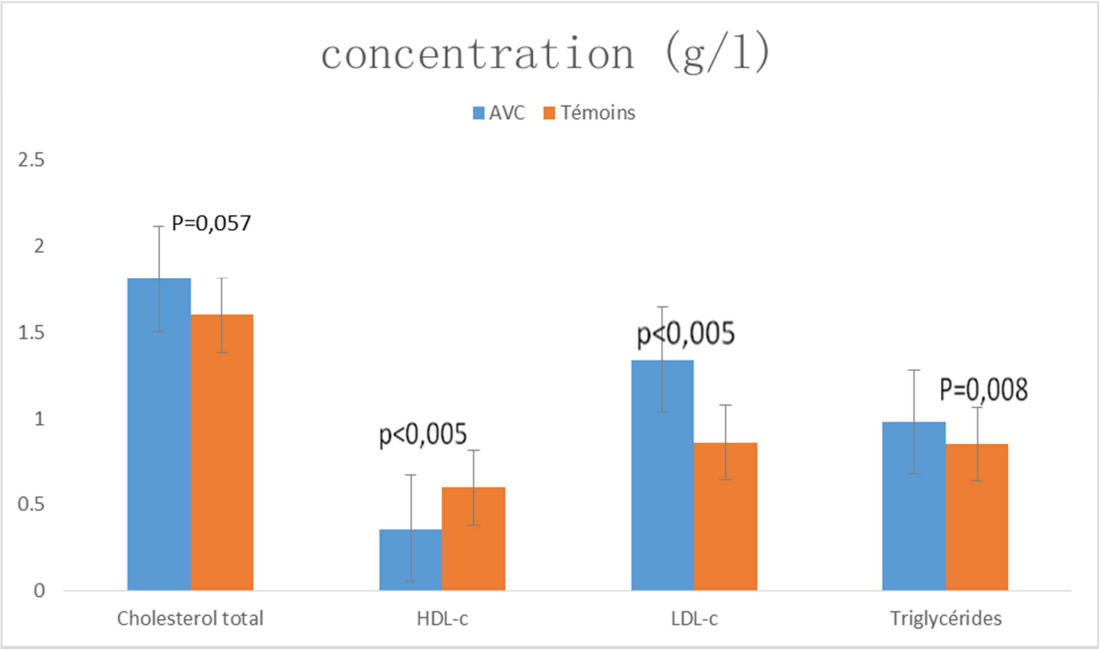

Figure 3. *Valeurs moyennes des parametres du bilan lipidique ches les AVC et les témoins.*

In addition, no significant difference was found between stroke subjects and controls by dividing the subjects according to the three Hp genotypes. The mean HDL-c level was 0.36g / l in stroke and 0.60g / l in control subjects with p <0.001. The comparison between these two groups showed significant differences for all three genotypes (see Figure 3). The subjects with stroke had an average LDL-c level of 1.34g / l for a mean level in the controls of 0.80g / l with a p <0.001 showing a significant difference. The comparison between stroke and control subjects by genotype showed significant differences for Hp1-1 (p <0.001) and Hp2-2 (p = 0.02) (see Table 1). The mean value of triglycerides in subjects with stroke (0.98 g / l) was increased more than in control subjects (0.85 g / l) with a p = 0.008 showing a significant difference. Mean TG levels for the different genotypes were 0.7g / l, 1.1g / l and 0.9g / l for Hp1-1, Hp2-1 and Hp 2-2, respectively. Comparison with control subjects showed a significant difference only for the Hp2-2 genotype (p <0.001) (See Table 1).

Table 1. *Mean values of lipid status parameters according to Hp genotypes.*

	Cholestérol total			HDL-cholestérol			LDL-cholestérol			Triglycérides		
	AVC	**témoins**	**p**	**AVC**	**témoins**	**p**	**AVC**	**témoins**	**p**	**AVC**	**témoins**	**p**
Allèle Hp1	2,04	1,65	<0,001	0,38	0,59	<0,001	1,43	0,88	<0,001	0,94	0,89	0,288
Allèle Hp2	1,79	1,70	0,222	0,38	0,61	<0,001	1,19	0,95	0,03	1,01	0,76	<0,001
Hp1-1	1,9±0,64	1,6±0,30	0,06	0,3± 0,98	0,6± 0,24	0,04	1,8± 0,62	0,8 ± 0,35	<0,001	0,7± 0,20	0,9 ± 0,28	0,22
Hp-2-1	1,8±0,39	1,86±0,30	0,38	0,3± 0,15	0,56±0,15	<0,001	1,2±0,59	1,1±0,35	0,30	1,1±0,19	0,8±0,24	0,02
Hp2-2	1,7±0,42	1,5±0,29	0,06	0,5± 0,16	0,7 ± 0,27	0,007	1,2 ±0,63	0,7 ± 0,35	0,02	0,9 ± 0,20	0,7 ± 0,27	<0,001

A value of p <0.05 was considered a statistically significant difference.

4. Discussion

Our study focused on a population of stroke patients in the search for biomarkers predisposing to this pathology. The average age of our subjects was 63 ± 14 years with a predominance of subjects aged 60 to 80 years. The distribution of subjects by sex showed a female predominance (59% vs. 41%) with a sex ratio of 0.70. In Senegal, the Diouf et al study reported a similar sex ratio (0.68) [5], however, other studies have shown a male predominance [6-7]. Nevertheless, according to the literature, there is variability in the prevalence of stroke in Africa according to sex with a predominance of studies in favor of the male gender [8].

The study of the type of stroke showed that the ischemic form was more frequent with a rate of 60.87% against 30.43% for the hemorrhagic form. This predominance of the ischemic form has been found in similar studies including those reported by Balogou et al [9], Diarra et al [7] and Bugnicourt et al [10].

The study of polymorphism of the Hp gene in subjects with stroke showed a higher frequency for the genotype Hp2-2 with a rate of 36.96%, followed by the genotype Hp1-1 with 34.78 %. As observed in our study, the predominance of the Hp2-2 genotype was found and associated in the literature with cardiovascular diseases but also in the context of other pathologies such as cancers [11]. According to MacKellar and Vigerust, people with genotype Hp2-2 have a much higher risk of being exposed to neurological, infectious, renal, but also to cardiovascular complications such as myocardial infarction or stroke [12]. Indeed, Hp2-2 is incriminated in the onset of diabetes with a risk of increased inflammation, oxidative stress, and instability of the atheromatous plaque [13]. Analysis of Hp genotypes in the controls established the following order of frequency Hp1-1> Hp2-1> Hp2-2 with respectively 60.87%, 21.74%, and 17.39 %. Similar results were found by Harris et al who found in a study in Gambia a predominance of the Hp1-1 genotype with a rate of 30.57% followed by the Hp2-1 genotype with 21.66% and 7.01% for the genotype Hp2-2 [14]. Studies have shown that in Africa there is a predominance of the Hp1-1 form; while in Europe the predominant form is the genotype Hp 2-1 [15].

Study of lipid status results showed significant differences between mean values of LDL-C (p <0.005), HDL-C (p <0.005) and TG (p = 0.008) for subjects with stroke compared to control subjects. However, for total cholesterolemia, a non-significant difference was found (p = 0.057).

These data confirm the results of many studies conducted in the context of the link between dyslipidemias and stroke [16-18]. Indeed, authors have noted that the efflux capacity of cholesterol was inversely related to the incidence of cardiovascular diseases [19]. However, this relationship between stroke and HDL-C is more complex and not universally established. The increase in LDL-C noted in our study is also found in the literature with several references [20-21]. Indeed, LDL-C is an important vector of cholesterol. When there is a decrease in specialized LDL-C receptors, its concentration increases and moreover its accumulation at the level of the artery wall, resulting in the formation of an atheromatous plaque whose rupture has many pathological consequences. It is in this sense that many studies have shown a strong and gradual correlation between LDL-C levels and the risk of atherosclerosis and cardiovascular diseases [22].

In our series, the comparison of mean TG rates between subjects with stroke and control subjects showed a significant difference (p <0.05) which may define a relationship between triglyceride levels and the risk of stroke, more specifically ischemic strokes. However, epidemiological studies of this relationship between ischemic stroke and TG had found controversial results [23]. According to a meta-analysis (64 studies), there would be a correlation between increased triglyceride levels and the risk of stroke for any 10 mg/dl increase in basal triglyceride. In addition, the study of the influence of the major genotypes of haptoglobin on the variations of the lipid parameters showed statistically significant differences. An association between the Hp2-2 genotype and the increase of the average level of LDL-C and Triglycerides and a decrease in the HDL-C level was found. These findings were also found in other studies including that of Sow A D et al., which showed the same link between these two parameters in the context of cardiovascular risk but in epileptic subjects [24]. In addition, the scarcity of studies associating these two parameters in the literature should be noted.

5. Conclusion

The polymorphism of Hp is always interesting to study because carriers of genotype Hp2-2 are more likely to

develop the disease. However, the impact of this polymorphism must be estimated in conjunction with the other genetic, metabolic and clinical risk factors associated with the pathogenesis of stroke. In this study, the Hp2-2 genotype comes out as a predisposing factor to stroke. Its association with the lipid parameters is found but must be confirmed in other studies with a larger cohort and in other cardiovascular pathologies.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Faye E. H. O. «Aspects Tomodensitométriques des Accidents Vasculaires Cérébraux à l'hôpital régional de Kaolack» Thése d'exercice: Medecine, Dakar-Senegal: UCAD; 2016. 16

[2] Adoukonou T. A., Vallat J. M., Joubert J., Macian F., Kabore R., Magy L., Houinato D., et Preux P-M. «Prise en charge des accidents vasculaires cérébraux en Afrique subsaharienne». Revue neurologique 2010; 166 (11): 882‑93.

[3] Gaye A. «Accident Vasculaire Cérébral de la femme jeune à propos de 109 cas». Thése d'exercice: Medecine, Dakar-Senegal, UCAD; 2014.253.

[4] Koch W., Latz W., Eichinger M., Roguin A., Levy A. P., Schömig A., Kastrati A. «Genotyping of the Common Haptoglobin Hp 1/2 Polymorphism Based on PCR». Clinical Chemistry 2002; 48 (9): 1377‑82.

[5] Diouf Basse A. M., Ndao A. K., Ndiaye M., Touré K., Thiam A., Ndiaye M. M., Diop A. G., Ndiaye I. P.« Pronostic fonctionnel des accidents vasculaires cérébraux dans les pays en voie de développement: Sénégal». Annales de Réadaptation et de Médecine Physique 2006; 49 (3): 100‑104.

[6] Mboup M. C., Sarr S. A., Dia K., Fall P. D. «Aspects étiologiques des accidents vasculaires cérébraux ischémiques au Sénégal». Pan African Medical Journal 2015; 22 (201).

[7] Diarra E. A. A., Assouan A. E. K., Yao R. B., Kouame L. K., Kajo C., Tanoh C. «Épidémiologie des AVC en Côte d'Ivoire et perspectives». Revue Neurologique, Journées de Neurologie de langue française 2016;172: 164.

[8] N'goran Y. N. K., Traore F., Tano M., Kramoh K, E., Kakou J. B. A., Konin C., Kakou M. G. «Aspects épidémiologiques des accidents vasculaires cérébraux (AVC) aux urgences de l'institut de cardiologie d'Abidjan (ICA)». The Pan African Medical Journal 2015;21.

[9] Balogou A. A. K., Belo M. «Epidémiologie récente des accidents vasculaires cérébraux au Togo». Revue Neurologique, Journées de Neurologie de langue française 2015;171: 235‑36.

[10] Bugnicourt J. M., Tchouata-Ngandjouc L., Beugre K., Chillon J-M., Godefroy O. «A comparison of stroke patients' characteristics between a sub-Saharan Africa population (Abidjan) and a French stroke unit (Amiens): An observational study». Revue Neurologique 2013;169 (3): 258‑65.

[11] Holme I., Aastveit A. H., Hammar N., Jungner I., Walldius G. «Haptoglobin and Risk of Myocardial Infarction, Stroke, and Congestive Heart Failure in 342,125 Men and Women in the Apolipoprotein MOrtality RISk Study (AMORIS)». Annals of Medicine 2009; 41 (7): 522‑32.

[12] Standhave C., Svenson M., Kranup H., Christensen J. H. «Haptoglobine Genotype and Risk markers of CVD in patients with chronic kidney diseases». Hindavi Publishing Corporation, 2013; n° 650847: 7pages.

[13] Gueye PM, Bertrand F, Duportail G, Lessinger JM. Extracellular haemoglobin, oxidative stress and quality of red blood cells relative to perioperative blood salvage. Clin Chem Lab Med. 2010 May;48(5):677-83.,

[14] Harris H., Robson E. B., Siniscalco M. «Genetics of the Plasma Protein Variants». In Ciba Foundation Symposium - Biochemistry of Human Genetics, édité par G. E. W. Wolstenholme O. B. E. M. R. C. P M. A., M. B. et Cecilia M. O'Connor B.Sc, 1959: 151‑77.

[15] Moullec J., Fine J.-M., Linhard J. «Les groupes d'haptoglobine, moyen d'étude des populations humaines.» Bulletins et Mémoires de la Société d'anthropologie de Paris 1961; 2 (1): 109‑24.

[16] Gordon D. J., Rifkinds B. M. « High-Density Lipoprotein--the Clinical Implications of Recent Studies ». The New England Journal of Medicine 1989; 321 (19): 1311‑16.

[17] Soyama Y., Miura K., Morikawa Y., Nishijo M., Nakanishi Y., Naruse Y., Kagamimori S., Nakagawa H. «High-Density Lipoprotein Cholesterol and Risk of Stroke in Japanese Men and Women». Stroke 2003; 34 (4): 863‑68.

[18] McGrowder D., Riley C., Morrison E. Y. St A., Gordon L. «The Role of High-Density Lipoproteins in Reducing the Risk of Vascular Diseases, Neurogenerative Disorders, and Cancer». Cholesterol 2010: 1-9.

[19] Rohatgi A., Khera A., Berry J. D., Givens E. G., Ayers C. R., Wedin K. E., Ian J. Neeland I. J., Yuhunna I. S., Rader D. R., De emos J. A., Shaul P. W. «HDL Cholesterol Efflux Capacity and Incident Cardiovascular Events». New England Journal of Medicine 2014; 371 (25): 2383‑93.

[20] Cisse O., Lemine D. S. M., Ba F., Ba E. H. M., Diop M. S., Diagne N. S., Sow A. D., Basse A. M., Touré K., Ndiaye M., Diop G. A., Ndiaye M. M. «Le profil lipidique et glucidique des accidents vasculaires cérébraux ischémiques à Dakar». The Pan African Medical Journal 2016;25.

[21] Gotto Jr A. M., Brinton E. A. «Assessing low levels of high-density lipoprotein cholesterol as a risk factor in coronary heart disease: A working group report and update». Journal of the American College of Cardiology 2004; 43 (5): 717‑24.

[22] Hovingh G. K., Hutten B. A., Holleboom A. G., Petersen W., Rol P., Stalenhoef A., Zwinderman A. H., De Groot E., Kastelein J. J. P., Kuivenhoven J. A.«Compromised LCAT Function Is Associated With Increased Atherosclerosis». Circulation 2005; 112 (6): 879‑84.

[23] Lindenstrøm E., Boysen G., Nyboe J. «Influence of Total Cholesterol, High Density Lipoprotein Cholesterol, and Triglycerides on Risk of Cerebrovascular Disease: The Copenhagen City Heart Study». BMJ (Clinical Research Ed.) 1994; 309 (6946): 11‑15.

[24] Sow A. D., Basse A. M., Sarr M. M., Cisse O., Moustapha N., Diop A. G., & Mouhamadou M. N. Variations métaboliques, place de l'haptoglobine chez des épileptiques, versus témoins, suivis au CHUN de Fann; Dakar, Senegal. Revue Neurologique 2017; 173 (2): 46.