Received: 2 May 2023 | Revised: 16 July 2023 | Accepted: 13 October 2023

DOI: 10.1002/mgg3.2302

Molecular Genetics & Genomic Medicine Open Access WILEY

ORIGINAL ARTICLE

# Haptoglobin gene polymorphism and iron profile in sickle cell disease patients with inflammation in Yaounde, Cameroon

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## Abstract

**Background:** Major sickle cell syndromes are the most common hemoglobinopathy in the world. The sickle cell patients are subjected to several factors causing inflammation, and the genetic identification of each individual allows to focus the possibility of allelic variations influence of a specific gene and then the polymorphism. This study aims at determining the distribution of *HP* gene (OMIM#140100) and their involvement on hematological parameters and the iron profile in the sickle cell patients presenting an inflammation condition during major sickle cell syndromes in Cameroun.

**Methods:** A case–control analytical study has been conducted over a period of 6 months. Cases consisting of sickle cell patients in a situation of inflammation and control of non-inflamed sickle cell patients. The patients presenting major sickle cell syndromes, interned and/or followed at the Hematology Department of the Regional Hospital of Bafoussam and the Central Hospital of Yaoundé have been recruited. *HP* genotyping was carried out at the Laboratory for Public Health Research Biotechnologies (LAPHER-Biotech) in Yaoundé using allele-specific PCR. Also, inflammatory, hematological parameters and martial assessment were explored by standard methods. Statistical analysis of the data was performed using the statistical tool R version 4.1.1. The comparison of proportions of alleles was made with the chi-square test, and the Wilcoxon test was used to compare the median between different groups using the statistical tool R version 4.1.1.

**Results:** We analyzed the samples of 149 patients. The *HP* polymorphism describes a significant frequency of the "1F" allele (69.8%) followed by the "2" allele (46.31%). In addition, 80 patients (53.69%), 48 (32.21%), and 21 (14.09%) presented the genotype *HP* 1-1, *HP* 2-1, and *HP* 2-2, respectively. And eighty-one percent (81%) patients with genotype *HP* 2-2 showed a significant higher relative

frequency of thrombocytosis compared with the genotype *HP* 1-1 and *HP* 2-1, respectively (51.2% and 68.8%, $p = 0.087$). The proportion of inflammation in the *HP* 2-2 group was higher (57.1%) compared with the other groups (respectively 42.5% and 35.4% in the *HP* 1-1 and *HP* 2-1 groups). Furthermore, the median CRP was significantly higher in the *HP* 2-2 group compared with the other groups ($p = 0.039$). Moreover, the entire population of the *HP* 2-2 group showed an elevation of ferritin and IL6 unlike the *HP* 1-1 and *HP* 2-1 groups.

**Conclusion:** This study demonstrates a higher frequency of genotype *HP* 1-1 followed by the *HP* 2-2 genotype in patients with major sickle cell syndromes. However, a larger proportion of patients with genotype *HP* 2-2 are associated with hematological profile disorders, inflammation, and dysregulation of iron metabolism. Then, the haptoglobin polymorphism contributes to the severity of major sickle cell syndromes.

**KEYWORDS**

haptoglobin polymorphism, hematological profile, inflammation, major sickle cell syndrome, serum iron

## 1 | INTRODUCTION

Sickle cell disease is an inherited and autosomal recessive disease characterized by the presence of abnormal hemoglobin (Hb S) in the blood. Also called major sickle cell syndrome, it is associated with multiple acute and chronic complications such as vaso-occlusive crises, acute anemia, splenic sequestration, and erythroblastopenia (Meher et al., 2020). It is among the most common monogenic diseases worldwide (Nkashama et al., 2015) and an estimated 312,000 people carrying SS hemoglobin are born worldwide each year, with the majority of these 236,000 births in sub-Saharan Africa (Cabannes & Bonhomme, 1972). Sickle cell disease and major sickle cell syndrome (MSCS**)** are real public health problems because of their consequences. Several sources of inflammation are described in patients with major sickle cell syndromes, among other, the alteration of red blood cells containing hemoglobin S in the absence of oxygen, the high frequency of hemolysis release of their contents into the bloodstream which has a proactive action, inflammatory, recurrent infections, vaso-occlusive crises mainly favored by hypo-oxygenation of tissues (Gudjoncik, 2018). Several authors report a significant elevation of pro-inflammatory cytokines in sickle cell patients including Bandeira et al. (2014) and Zahrane et al. (2020).

In a patient with major sickle cell syndrome, during the inflammatory reaction, there is an overproduction of IL 6, the precursor of hepcidin, which is the central molecule of iron metabolism (Gudjoncik, 2018). Previous works showed that hepcidin in serum has a strong positive correlation with serum ferritin levels in patients with anemia associated with inflammatory conditions (Al-Saqladi et al., 2012). The elevation of inflammatory markers in sickle cell patients as described by the authors is associated with dysregulation of the iron profile thus contributing to maintain the inflammatory vicious circle by the oxidizing properties of the iron produced. *HP* gene (OMIM#140100) is a molecule with anti-inflammatory and antioxidant properties which could reduce the consequences of iron from inflammation. Haptoglobin is a serum glycoprotein of hepatocytic origin, migrating electrophoretically in the area of α 2-globulins. It is well known that *HP* possess three phenotypes/proteins, which differ in number, type of chain, and their molecular weight: *HP* 1-1, *HP* 1-2, and *HP* 2-2 (Santos et al., 2011). These proteins are probably not equivalent; indeed, previous studies show that patients with the *HP* 2-2 form would have more vascular complications (Da Guarda et al., 2020; Eric Kerchberger et al., 2019; Soejima & Koda, 2020). Moreover, this genotype would have an oxidative action while the genotype *HP* 1-1 has an antioxidant action (Costacou et al., 2008). According to Langlois et al. (2000), the phenotype 2-2 is associated in adults with a significant elevation of serum iron, an increase in the transferrin saturation coefficient, an increase in ferritin followed however by a decrease in the concentration of soluble transferrin and/or transferrin receptors; Several other authors show the influence and impact of haptoglobin genotypes on the iron profile (Abah et al., 2018; Langlois & Delanghe, 1996; Moreno et al., 2008). Understanding the vascular and inflammatory components of the disease can help in providing

information on the possible genomic sites that influence the phenotype. Studies on the *HP* polymorphisms, an important marker of vascular disease and pro-inflammatory cytokines may therefore provide useful information. This preliminary study aims to determine the distribution of haptoglobin genotypes and their involvement on hematological parameters, and iron profile in Cameroonian patients in inflammation during Major Sickle Cell Syndromes.

## 2 | MATERIALS AND METHODS

### 2.1 | Ethical compliance

This study was approved by the Centre's Regional Research Ethics Committee (AUTHORIZATION No. E210/CRERSHC2021). Authorization to collect the data was obtained from the Regional Hospital of Bafoussam (No. 005/L/MINSANTE/SG/DRSPO/HRB/D), from the Central Hospital of Yaoundé (No. 276/21/AR/MINSANTE/SG/DHCY/CM/SM). Authorization for analysis of the samples was obtained from the University Hospital of Yaoundé (No. 74/AR/CHUY/DG/DGA/CAPRC) and the Laboratory for Public Health Research Biotechnologies (LAPHER-Biotech). Before initiating the study, participants were given a newsletter about the objectives of the study, its benefits, and its risks. For eligible participants, we obtained their free and informed consent through their signatures. The confidentiality of the research results was respected by the use of a unique code for each patient.

### 2.2 | Sampling and study site

A case–control analytical study has been conducted for 6 months at the Bafoussam Regional Hospital (BRH) and the Yaoundé Central Hospital (YCH) where participants have been recruited at BRH and YCH. Cases consisting of sickle cell patients in a situation of inflammation and control of non-inflamed sickle cell patients. All the participants presented major sickle cell syndromes in stationary phase interned and/or followed in the hematological department of the above-mentioned hospitals. A total of 149 sickle cell patients above 0.5 years with major sickle cell syndrome were included in the study according to the Lorentz formula.

Information on the study was given to the potential participants and their legal guardians (if need be) in their first official languages. Patients read and signed the informed consent form. For each participant, demographic, and clinical data were obtained and noted on a pre-structured data collection sheet. Homozygous sickle cell (SS), male and female, above 0.5 years old, accepted to participate in the study were included. All sickle cell patients with other pathologies were not included in the study such as patient's smoker, alcoholic, taking of oral contraceptives for female, having had a blood transfusion for less than 4 months, hypertension, hyperthyroidism, obesity, undernutrition, hepatocellular insufficiency, pregnant women, under estrogenic treatment.

### 2.3 | Biological analysis

Four milliliter of blood was collected in tubes containing Ethylene Diamine Tetra Acetic (EDTA) and tubes without anticoagulant from study participants. Each tube of blood sample collected was sent to the Hematology and Medial Biochemistry laboratories of the University Hospital of Yaoundé for analysis. Then, simultaneously during blood sampling, 40 µL of whole blood was impregnated on Whatman filter paper with a micropipette. The filter paper was then fed into an envelope containing silicate and stored at room temperature (22°C) and the samples were sent to the Laboratory for Public Health Research Biotechnologies (LAPHER-Biotech) for Haptoglobin genotyping.

As well as the hematological analyses were concerned, a complete blood count was performed on the HumaCount 30TS hematology automaton. Beside this later, a blood smears were done in order to assess the quality of blood cells and the vital staining to classify the type of anemia. The slides colored with May Grunwald Giemsa and brilliant cresyl blue were read under a binocular microscope from the manufacturer "Irmerco." The results obtained were discussed according to WHO usual values. Subsequently, quantitative electrophoresis was performed on alkaline agarose of hemoglobin in each patient to determine the proportion of different hemoglobin fractions according to the "HELLABIO" protocol.

Regarding the biochemical and immunological analyses, the concentration of the C-Reactive Protein was determined according to the method of the "Genrui" Kit, using the semi-automatic protein analyzer "PA54" calibrated by magnetic card whose operating principle is nephelometry. This parameter was used as an inflammatory index because C-reactive protein (CRP) is the major human acute-phase reactant and is the most sensitive acute-phase protein reflecting inflammatory activity; it has been implicated previously in the prediction of morbidity and outcome in chronic disease states (Al-Saqladi et al., 2012). Furthermore, the concentration of interleukin 6 was determined in patients according to the protocol of the reagent "Elabscience$^{R}$" and on the system

Elisa *ELx50TM Automated Strip Washer* brand *BIOTEK*. The determination of the ferric profile was done by serum iron assessment according to the "Biolabo" kit using spectrophotometric method. Also, the determination of transferrin, ferritin were carried out respectively according to the Human kit by immunoturbidimetric method and the "*CALBIOTECH*" kit Elisa sandwich method. The total iron binding capacity (TIBC) according to the Biolabo kit was deduced from the determination of transferrinemia using the formula: TIBC (mg/L) = transferrin (g/L) × 1395 (Beaune et al., 2009); and the transferrin or siderophilin saturation coefficient (TSC) according to the Biolabo kit was determined using the formula TSC (%) = (serum iron/TIBC) × 100 (Beaune et al., 2009).

### 2.4 | Genotyping of the haptoglobin: DNA extraction, amplification, and electrophoresis (Yano et al., 1998)

The GenBank reference of *HP* is DY802087 and NCBI Ref Seq: NM_001040470.2. DNA extraction was done using the Chelex method from dried blood stains on filter papers (Choi et al., 2014). This method is based on breaking the cell membrane and nuclear membrane to release DNA under the action of saponin as a lysis agent and phosphate buffer saline (PBS) which works by removing saponin from the tubes and further removing contaminants and cellular residue such as proteins and other molecules by washing at "Chelex 100" (Walsh et al., 2013). Subsequently, a polymerase chain reaction was performed using allele-specific primer pairs and primers, as described by Yano et al. (1998). This method allowed to obtain the six main genotypes of Hp. For the three alleles, *HP* 1S, *HP* 1F, and *HP* 2, three reactions were performed; reaction 2, reaction S, and reaction F, with a few sets of primers each (Tables S1 and S2). After amplification, the amplicons were subjected to electrophoresis at 50 V for 1 h on agarose gel 2% Tris Borate EDTA and Hp genotypes were observed on gels under Ultraviolet light according to different band sizes.

### 2.5 | Statistical analysis

The data collected were saved in Microsoft Excel 2016 software. Statistical analysis was performed using the statistical tool R version 4.1.1. The qualitative variables studied were body mass index, clinical history of participants, sex, the different alleles, and phenotypes of haptoglobin; and quantitative variables were age, hemoglobin level, red blood cell count, leukocytes, platelets, the proportion of different hemoglobin, CRP concentration, Interleukin 6, serum iron, transferrin, total iron binding capacity, transferrin saturation coefficient, and ferritin. The body mass index has been categorized into lean, thin, normal, overweight, obesity and morbid obesity according to the intervals and cutoff defined by the WHO. Also, each of the hematological, biochemical, and immunological parameters studied has been dichotomized and groups into "normal, high or low" according to the reference values and cutoff defined by the WHO, the manufacturer of each reagents used and the scientific literature. Qualitative variables were presented as frequency while quantitative variables were presented as medians and their interquartile range (IQR) P25–P75. The comparison of proportions was made with the chi-square test when the expected numbers were greater than 5 and the Fisher test when not. The Wilcoxon test was used to compare the median between different groups. All these tests were done at a risk threshold of $\alpha = 5\%$.

## 3 | RESULTS

### 3.1 | Haptoglobin polymorphism in sickle cell disease

Table 1 describes the haptoglobin polymorphism in the study population. It reveals that the most frequent allele was "$HP^{1F}$" (69.8%) followed by "$HP^{2}$" (46.31%). In addition, 53.69% of the population had genotype HP 1-1 and 14.09% of the population had HP 2-2.

### 3.2 | Sociodemographic characteristics and clinical history of the participants

Table 2 presents the sociodemographic characteristics and clinical of the patient registered during the study period depending on haptoglobin polymorphism. It shows that 149 major sickle cell syndromes (MSCS) recorded. The median and interquartile interval age of the population was 13 [4.00–13.0]. The sex ratio was 1.01 in favor of men (50.3%; 75/149) and 19.7% of women (74/149). Ninety-eight percent (98%) of the population are single. In all the population, 38.5% were on iron intake and 10.5% on hydroxyurea. 38.5% on iron intake and 10.5% on hydroxyurea. The study population was from the West and Central Cameroon region for various ethnicities.

It also appears that the average BMI was 17.2 ± 3.07 and was not influenced by the genotypes; 65.77% of the population was thin. Patients had a history of infectious

**TABLE 1** Distribution of major sickle cell syndrome patients from RHB and CHY according to haptoglobin polymorphism.

| Variables (alleles and genotypes) | *HP* 1-1 *N* = 80 *n* (%) | *HP* 2-1 *N* = 48 *n* (%) | *HP* 2-2 *N* = 21 *n* (%) | Total *N* = 149 *n* (%) | *p*-value |
|---|---|---|---|---|---|
| *HP*_2 | | | | | |
| No | 80 (100) | 0 (0.00) | 0 (0.00) | 80 (53.69) | <0.001* |
| Yes | 0 (0.00) | 48 (100) | 21 (100) | 69 (46.31) | |
| *HP*_1S | | | | | |
| No | 40 (50.0) | 35 (72.9) | 21 (100) | 96 (64.43) | <0.001* |
| Yes | 40 (50.0) | 13 (27.1) | 0 (0.00) | 53 (35.57) | |
| *HP*_1F | | | | | |
| No | 11 (13.8) | 13 (27.1) | 21 (100) | 45 (30.2) | <0.001* |
| Yes | 69 (86.2) | 35 (72.9) | 0 (0.00) | 104 (69.8) | |
| *HP*_2_2 | | | | | |
| No | 80 (100) | 48 (100) | 0 (0.00) | 128 (85.9) | <0.001* |
| Yes | 0 (0.00) | 0 (0.00) | 21 (100) | 21 (14.09) | |
| *HP*_1S_1S | | | | | |
| No | 69 (86.2) | 48 (100) | 21 (100) | 138 (92.62) | 0.005* |
| Yes | 11 (13.8) | 0 (0.00) | 0 (0.00) | 11 (7.4) | |
| *HP*_2_1S | | | | | |
| No | 80 (100) | 35 (72.9) | 21 (100) | 136 (91.27) | <0.001* |
| Yes | 0 (0.00) | 13 (27.1) | 0 (0.00) | 13 (8.7) | |
| *HP*_1S_1F | | | | | |
| No | 51 (63.7) | 48 (100) | 21 (100) | 120 (81.08) | <0.001* |
| Yes | 29 (36.2) | 0 (0.00) | 0 (0.00) | 29 (19.46) | |
| *HP*_1F_1F | | | | | |
| No | 40 (50.0) | 48 (100) | 21 (100) | 109 (73.15) | <0.001* |
| Yes | 40 (50.0) | 0 (0.00) | 0 (0.00) | 40 (26.85) | |
| *HP*_2_1F | | | | | |
| No | 80 (100) | 13 (27.1) | 21 (100) | 114 (76.51) | <0.001* |
| Yes | 0 (0.00) | 35 (72.9) | 0 (0.00) | 35 (23.49) | |
| Total | 80 (53.69) | 48 (32.21) | 21 (14.09) | 149 (100) | |

*Note*: The GenBank reference of *HP* is DY802087 and NCBI RefSeq: NM_001040470.2.

Abbreviations: *HP*, Haptoglobin gene; 1F, 1S, 2 = allele; Fisher test.

*Significant difference at $p < 0.05$.

seizures (32.2%), anemic attacks (52.3%), vaso-occlusive seizures (54.4), and cardiovascular disease (2.06%), although no significant difference was observed according to the genotypes.

## 3.3 | Profile of blood count in sickle cell patients according to haptoglobin phenotypes

Tables 3 and 4 describe the blood count profile in the study population according to *HP* polymorphism. Its appears that a high frequency of anemia in the population but is not significantly associated with a particular genotype (median hemoglobin of 7.8 g/dL [7.1–8.4] and red blood cells of 2.7 T/L). In addition, this table (Table 3) shows a higher proportion of leukocytosis in the group of patients with genotype *HP* 2-2 (95.2%) with 85% and 89.6% in the *HP* 1-1 and *HP* 2-1 groups, respectively. Moreover, thrombocytosis was also more associated with the *HP* 2-2 genotype group (81%) unlike the other *HP* 1-1 and *HP* 2-1 groups (51.2% and 68.8%, respectively; $p = 0.087$). From Table 4, the median platelet in the population was 460 g/mL [323–575] with 532 g/mL in the *HP* 2-2 genotype group and 404 g/mL and 466 g/mL respectively in the *HP* 1-1 and Hp2-1 groups.

**TABLE 2** Distribution of major sickle cell syndrome patients from RHB and CHY by sociodemographic characteristics and clinical history to polymorphism of haptoglobin.

| Variables | *HP* 1-1 *N* = 80 *n* (%) | *HP* 2-1 *N* = 48 *n* (%) | *HP* 2-2 *N* = 21 *n* (%) | Total *N* = 149 *n* (%) | *p*-value |
|---|---|---|---|---|---|
| Sex | | | | | |
| F | 39 (48.8) | 20 (41.7) | 15 (71.4) | 74 (49.7) | 0.073 |
| M | 41 (51.2) | 28 (58.3) | 6 (28.6) | 75 (50.3) | |
| Age, Med [q1–q3] | 8.00 [4.00–14.0] | 10.0 [6.00–13.0] | 7.00 [4.00–11.0] | 9.0 [4.0–13.0] | 0.542 |
| BMI, Mean ± SD | 16.7 ± 3.28 | 17.7 ± 2.82 | 17.7 ± 2.63 | 17.2 ± 3.07 | 0.179 |
| BMI | | | | | |
| Thin | 51 (63.7) | 31 (64.6) | 15 (71.4) | 98 (65.77) | 0.120 |
| Normal | 29 (26.2) | 17 (25.55) | 6 (28.6) | 51 (34.23) | |
| History of infectious crises | | | | | |
| No | 52 (65.0) | 38 (79.2) | 11 (52.4) | 101 (67.8) | 0.067 |
| Yes | 28 (35.0) | 10 (20.8) | 10 (47.6) | 48 (32.2) | |
| History of anemic crises | | | | | |
| No | 35 (43.8) | 27 (56.2) | 9 (42.9) | 71 (47.7) | 0.349 |
| Yes | 45 (56.2) | 21 (43.8) | 12 (57.1) | 78 (52.3) | |
| History of VOS | | | | | |
| No | 39 (48.8) | 26 (54.2) | 3 (14.3) | 68 (45.6) | 0.007 |
| Yes | 41 (51.2) | 22 (45.8) | 18 (85.7) | 81 (54.4) | |
| Bacterial and parasitic infection | | | | | |
| Yes | 5 (6.25) | 1 (2.08) | 4 (19.0) | 7 (6.71) | 0.022* |
| No | 75 (93.8) | 47 (97.9) | 17 (81.0) | 139 (93.3) | |
| Cardiovascular disease and stroke | | | | | |
| No | 78 (97.5) | 47 (97.9) | 21 (100) | 146 (98.0) | 1.000 |
| Yes | 2 (2.50) | 1 (2.08) | 0 (0.00) | 3 (2.01) | |
| Mean vos/month, Med (IQR) | 1.00 [0.00–2.00] | 0.00 [0.00–1.00] | 1.00 [1.00–2.00] | 1.0 [0.0–2.0] | 0.003* |

*Note*: The GenBank reference of *HP* is DY802087 and NCBI RefSeq: NM_001040470.2.

Abbreviations: BMI, body mass index; CHY, Central Hospital of Yaounde; RHB, Regional Hospital of Bafoussam; VOS, vaso-occlusive seizures; Fisher test, Wilcoxon test.

*Significant difference at $p < 0.05$.

### 3.4 | Association between biochemical, immunological parameters and the genotypes in the population

Table 5 describes the association and relationship between haptoglobin genotypes and population plasma marker concentration. It appears from this table that the proportion of inflammation in the HP 2-2 group is higher (57.1%) than in the other groups ($p = 0.23$) with respectively 42.5% and 35.4% in the HP 1-1 and HP 2-1 groups. Furthermore, the median CRP was significantly higher in the HP 2-2 group compared with the other groups ($p = 0.039$). Moreover, the entire population (100%) of the HP 2-2 group showed an elevation of ferritin and IL6 unlike the HP 1-1 and HP 2-1 groups.

## 4 | DISCUSSION

The present study aims to determine the distribution of haptoglobin genotypes and their involvement in hematological parameters, and the ferric profile in the Cameroonian patient in inflammation during major sickle cell syndromes. SCA is a monogenic disease with exceptional phenotypic variability that is regulated by several known and unknown genetic factors. Understanding the vascular and inflammatory components of the disease can help in providing information on the possible genomic sites that influence the phenotype. Studies on the *HP* polymorphisms, an important marker of vascular disease and pro-inflammatory cytokines, may therefore provide useful information. This

**TABLE 3** Distribution major sickle cell syndrome patients by blood count profile versus haptoglobin polymorphism.

| Variables | *HP* 1-1 *N* = 80 *n* (%) | *HP* 2-1 *N* = 48 *n* (%) | *HP* 2-2 *N* = 21 *n* (%) | *p*-value |
|---|---|---|---|---|
| RBC | | | | |
| Low | 75 (93.8) | 43 (89.6) | 17 (81.0) | 0.048* |
| High | 0 (0.00) | 0 (0.00) | 2 (9.52) | |
| Normal | 5 (6.25) | 5 (10.4) | 2 (9.52) | |
| Reticulocyte | | | | |
| High | 76 (95.0) | 47 (97.9) | 19 (90.5) | 0.353 |
| Normal | 4 (5.00) | 1 (2.08) | 2 (9.52) | |
| HB | | | | |
| Low | 76 (95.0) | 47 (97.9) | 19 (90.5) | 0.353 |
| Normal | 4 (5.00) | 1 (2.08) | 2 (9.52) | |
| Anemia | | | | |
| Slight | 26 (32.5) | 20 (41.7) | 6 (28.6) | 0.675 |
| Moderate | 43 (53.8) | 25 (52.1) | 11 (52.4) | |
| Severe | 7 (8.75) | 2 (4.17) | 2 (9.52) | |
| Hématocrit | | | | |
| Low | 76 (95.0) | 47 (97.9) | 19 (90.5) | 0.056 |
| High | 0 (0.00) | 0 (0.00) | 2 (9.52) | |
| Normal | 4 (5.00) | 1 (2.08) | 0 (0.00) | |
| MCV | | | | |
| Microcytosis | 31 (38.8) | 23 (47.9) | 3 (14.3) | 0.030* |
| Normocytosis | 49 (61.3) | 25 (52.1) | 18 (85.7) | |
| MCH | | | | |
| Low | 25 (31.2) | 18 (37.5) | 7 (33.3) | 0.769 |
| Normal | 55 (68.8) | 30 (62.5) | 14 (66.7) | |
| MCHC | | | | |
| Hypochromia | 3 (3.75) | 1 (2.08) | 4 (19.0) | 0.025* |
| Normal | 77 (96.2) | 47 (97.9) | 17 (81.0) | |
| WBC | | | | |
| Leukocytosis | 68 (85.0) | 43 (89.6) | 20 (95.2) | 0.472 |
| Normal | 12 (15.0) | 5 (10.4) | 1 (4.76) | |
| Granulocytes | | | | |
| Low | 0 (0.00) | 1 (2.08) | 0 (0.00) | 0.245 |
| High | 31 (38.8) | 17 (35.4) | 12 (57.1) | |
| Normal | 49 (61.3) | 30 (62.5) | 9 (42.9) | |
| Lymphocyte | | | | |
| Lymphocytosis | 65 (81.2) | 36 (75.0) | 18 (85.7) | 0.605 |
| Normal | 15 (18.8) | 12 (25.0) | 3 (14.3) | |
| Monocyte | | | | |
| Monocytosis | 78 (97.5) | 45 (93.8) | 21 (100) | 0.429 |
| Normal | 2 (2.50) | 3 (6.25) | 0 (0.00) | |
| Neutrophil | | | | |
| Neutropenia | 1 (1.25) | 1 (2.08) | 0 (0.00) | 0.624 |
| Neutrophilia | 33 (41.2) | 19 (39.6) | 12 (57.1) | |
| Normal | 46 (57.5) | 28 (58.3) | 9 (42.9) | |

(Continues)

TABLE 3 (Continued)

| Variables | *HP* 1-1 *N* = 80 *n* (%) | *HP* 2-1 *N* = 48 *n* (%) | *HP* 2-2 *N* = 21 *n* (%) | *p*-value |
|---|---|---|---|---|
| Eosinophil | | | | |
| Eosinophilia | 38 (47.5) | 23 (47.9) | 12 (57.1) | 0.722 |
| Normal | 42 (52.5) | 25 (52.1) | 9 (42.9) | |
| Basophil | | | | |
| Basophilia | 4 (5.00) | 2 (4.17) | 5 (23.8) | 0.725 |
| Normal | 76 (95.0) | 46 (95.8) | 16 (76.2) | |
| Thrombocyte | | | | |
| Thrombocytopenia | 4 (5.00) | 1 (2.08) | 0 (0.00) | 0.087 |
| Thrombocytosis | 41 (51.2) | 33 (68.8) | 17 (81.0) | |
| Normal | 35 (43.8) | 14 (29.2) | 4 (19.0) | |

*Note*: The GenBank reference of *HP* is DY802087 and NCBI RefSeq: NM_001040470.2.

Abbreviations: HB, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin content; MCHC, mean corpuscular hemoglobin concentration; RBC, red blood cell; WBC, white blood cells; Fisher test, Wilcoxon test.

*Significant difference at $p < 0.05$.

TABLE 4 Distribution of hematological markers of patients according to haptoglobin polymorphism.

| Variables | *HP* 1-1 *N* = 80 *n* (%) | *HP* 2-1 *N* = 48 *n* (%) | *HP* 2-2 *N* = 21 *n* (%) | *p*-value |
|---|---|---|---|---|
| RBC (T/L) | 2.72 [2.40–3.03] | 2.75 [2.59–3.29] | 2.62 [2.27–3.09] | 0.229 |
| Reticulocytes (g/L) | 352.5 [283–371.2] | 350 [273.7–377] | 305 [230–370] | 0.658 |
| HB (g/dL) | 7.80 [7.00–8.30] | 7.95 [7.40–8.45] | 7.50 [7.00–9.30] | 0.805 |
| HTE (%) | 21.9 [19.5–23.2] | 22.0 [20.1–24.7] | 22.2 [20.7–25.6] | 0.709 |
| MCV (fL) | 80.0 [75.8–87.0] | 80.5 [73.0–83.4] | 85.0 [83.0–89.0] | 0.013* |
| MCHC (pg/c) | 29.2 [26.3–31.4] | 28.0 [25.0–31.2] | 31.1 [24.1–32.2] | 0.256 |
| MCH (g/L) | 36.0 [34.8–36.6] | 35.4 [34.3–37.2] | 35.6 [33.6–37.8] | 0.930 |
| WBC (g/L) | 15.8 [13.5–21.3] | 14.8 [12.3–17.9] | 20.4 [15.8–23.9] | 0.024* |
| Granulocytes (g/L) | 6.5 [4.7–8.5] | 6.6 [5.1–8.6] | 9.5 [6.1–10.7] | 0.063 |
| Lymphocytes (g/L) | 6.7 [5.2–9.6] | 5.6 [4.1–6.9] | 7.8 [6.1–8.9] | 0.014* |
| Monocytes (g/L) | 2.2 [1.6–3.1] | 1.8 [1.3–3] | 2.1 [1.9–2.3] | 0.164 |
| Neutrophils (g/L) | 6.1 [4406–8] | 6.2 [4.7–8.1] | 8.9 [5.7–10] | 0.063 |
| Eosinophils (/mL) | 381 [275–499] | 388 [295–505] | 555 [357–627] | 0.063 |
| Basophil (/mL) | 105 [75.8–138] | 107 [81.4–139] | 153 [98.4–173] | 0.063 |
| Thrombocytes (g/mL) | 404 [285–571] | 466 [388–589] | 532 [408–598] | 0.030* |

*Note*: The GenBank reference of *HP* is DY802087 and NCBI RefSeq: NM_001040470.2.

Abbreviations: MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin content; MCHC, mean corpuscular hemoglobin concentration; RBC, red blood cell; WBC, white blood cells; Wilcoxon test.

*Significant difference at $p < 0.05$.

study is also the first in Cameroon to determine the role of haptoglobin polymorphism in relation to inflammation and iron profile in sickle cell disease. The ability to predict the phenotype of SCA during the first months of life or even in the prenatal period will allow a more precise prognosis and individualized treatment.

From the study, the sex ratio was 1.01 in favor of men which suggests equitable genetic transmission of the "S" allele regardless of sex. The following authors Dahmani et al. (2016) and Doupa et al. (2017) also reported equitable transmission of the condition between the two sexes. The study population was relatively young with a

**TABLE 5** Repartition of the immunological and Biochemical markers according to the haptoglobin genotypes.

| Variables | *HP* 1-1 *N* = 80 *n* (%) | *HP* 2-1 *N* = 48 *n* (%) | *HP* 2-2 *N* = 21 *n* (%) | *p*-value |
|---|---|---|---|---|
| Iron (mg/L), a[xxx] | 1.96 [1.46–2.40] | 1.82 [1.50–2.74] | 1.86 [1.58–1.96] | 0.842 |
| Iron | | | | |
| Low | 1 (1.25) | 0 (0.00) | 0 (0.00) | 0.109 |
| High | 71 (88.8) | 39 (81.2) | 21 (100) | |
| Normal | 8 (10.0) | 9 (18.8) | 0 (0.00) | |
| Ferritin (ng/mL), a[xxx] | 523 [279–735] | 442 [351–747] | 730 [642–781] | 0.101 |
| Ferri | | | | |
| High | 66 (82.5) | 40 (83.3) | 21 (100) | 0.094 |
| Normal | 14 (17.5) | 8 (16.7) | 0 (0.00) | |
| Tranferrin (g/L), a[xxx] | 2.67 [1.75–3.27] | 2.31 [1.59–3.05] | 2.08 [1.93–3.93] | 0.396 |
| Tranferrin | | | | |
| Low | 26 (32.5) | 20 (41.7) | 7 (33.3) | 0.626 |
| High | 9 (11.2) | 4 (8.33) | 4 (19.0) | |
| Normal | 45 (56.2) | 24 (50.0) | 10 (47.6) | |
| TIBC, a[xxx] | 66.8 [43.9–81.7] | 57.8 [39.7–76.2] | 51.9 [48.2–98.2] | 0.396 |
| TSC, a[xxx] | 58.0 [35.4–101] | 65.6 [43.2–100] | 63.6 [35.7–69.3] | 0.207 |
| CST | | | | |
| High | 66 (82.5) | 44 (91.7) | 16 (76.2) | 0.167 |
| Normal | 14 (17.5) | 4 (8.33) | 5 (23.8) | |
| Low | 22 (27.5) | 17 (35.4) | 4 (19.0) | |
| CTF | | | | |
| High | 31 (38.8) | 16 (33.3) | 9 (42.9) | 0.720 |
| Normal | 27 (33.8) | 15 (31.2) | 8 (38.1) | |
| CRP (mg/L), a[xxx] | 5.00 [3.39–9.92] | 3.60 [2.00–8.67] | 9.00 [3.99–17.5] | 0.039* |
| CRP | | | | |
| High | 34 (42.5) | 17 (35.4) | 12 (57.1) | 0.243 |
| Normal | 46 (57.5) | 31 (64.6) | 9 (42.9) | |
| IL6 (pg/mL), a[xxx] | 33.7 [14.0–60.6] | 37.8 [14.1–50.7] | 50.6 [23.7–110] | 0.083 |
| IL6 | | | | |
| High | 71 (88.8) | 42 (87.5) | 21 (100) | 0.283 |
| Normal | 9 (11.2) | 6 (12.5) | 0 (0.00) | |
| Inflammation | | | | |
| Oui | 34 (42.5) | 17 (35.4) | 12 (57.1) | 0.243 |
| Non | 46 (57.5) | 31 (64.6) | 9 (42.9) | |

*Note*: The GenBank reference of *HP* is DY802087 and NCBI RefSeq: NM_001040470.2.

Abbreviations: a[xxx], Med (IQR); TIBC, total iron binding capacity of siderophilin (μmol/L); TSC, transferrin saturation coefficient (%); Fisher test, Wilcoxon test.

*Significant difference at $p < 0.05$.

median age of 9 years (Al-Saqladi et al., 2012; Bandeira et al., 2014; Costacou et al., 2008; Da Guarda et al., 2020; Eric Kerchberger et al., 2019; Gudjoncik, 2018; Langlois & Delanghe, 1996; Santos et al., 2011; Soejima & Koda, 2020; Zahran et al., 2020).

The *HP* polymorphism during this study describes a significant frequency of the "1F" allele (69.8%) followed by the "2" allele (46.31%) (Table 1). In addition, 53.69% of the population had genotype *HP* 1-1, 32.21% had genotype *HP* 2-1, and 14.09% of the population had

*HP* 2-2. This distribution of genotypes is close to that obtained by Olatunya et al. (2020) in Nigeria which reported respective frequencies of 42.6%, 39.6%, and 17.8% for *HP* 1-1, *HP* 2-1, and *HP* 2-2. It is also similar to that of Khalid and Khalil (2016) in Sudan, which reported respective frequencies of 67.5% for *HP* 1-1, 26.5% for *HP* 2-1, and 6.3% for *HP* 2-2 and that of Ostrowski et al. (1987) which reported 72% for *HP* 1-1, 11% for *HP* 2-1, and 12% for *HP* 2-2. In contrast, these results are different from those of Bruna et al. (2015) which reported 16.7% of *HP* 1-1, 56.7% of *HP* 2-1, and 26.6% of *HP* 2-2 (Pierrot-Gallo et al., 2015) and those of Kengne et al. (2021) which reported 31.4% of *HP* 1-1, 14.7% of *HP* 2-1, and 53.9% of *HP* 2-2 (Bernard et al., 2021). A variability in the distribution of haptoglobin genotypes in the population is reported also by Santos et al. (2011) in Brazil. This multiplicity and variability of results demonstrate significant genetic segregation and variability depending on the geographical location on the one hand, and on the other hand, reflects the need for several studies and cohorts on populations of diverse origins and ethnicities to better understand the polymorphism of haptoglobin in sickle cell patients. However, the results obtained during this study compared with those of other authors demonstrate that the distribution of haptoglobin in sickle cell patients shows a predominance of the *HP* 1-1 genotype, followed by *HP* 2-1 and *HP* 2-2.

The clinical (vaso-occlusive crises, history of cardiovascular and cerebrovascular disease, hemolytic and anemic crises, infectious crises) and anthropometric characteristics observed in sickle cell patients during this study describe those commonly encountered in sickle cell disease. The average BMI was $17.2 \pm 3.07$ (Table 2); 65.77 of the population were meager; this reflects the significant state of malnutrition in the sickle cell population. This observation was also made by Sombodi et al. (2015). However, these clinical features and anthropometric parameters were not significantly associated with a particular haptoglobin genotype ($p > 0.05$); we can also note significant differences for bacterial infections and vaso-occlusive crises suggesting that the presence of the "$HP^2$" allele is a parameter favoring these clinical manifestations. This observation has also been reported by several authors (Bernard et al., 2021; Ostrowski et al., 1987; Park et al., 2004; Soejima & Koda, 2020).

The blood count describes a high frequency of anemia with median hemoglobin of 7.8 g/dL [7.1–8.4] and red blood cells of 2.7 T/L (Table 4). This observation is similar to that made by Omena et al. (2018) who reported a median of 8 g/dL. Anemias were mainly moderate, microcytic, normochromic, and regenerative anemias. They are the result of a high frequency of hemolysis which is the direct result of the polymerization of HbS which damages the sickle cell erythrocyte membrane (Houwing et al., 2019). Indeed, at birth, people with sickle cell disease do not suffer from anemia, but with the synthesis of adult hemoglobin, they develop chronic hemolytic anemia present throughout life. This can be interspersed with acute episodes of hemoglobin reduction "anemic attacks." The high frequency of anemia is explained by the rigidity of red blood cells in sickle cell patients reducing the half-life of peripheral red blood cells to 10–20 days instead of 120 days (Houwing et al., 2019). In addition, this study reports a lower median hemoglobin and red blood cells in the *HP* 2-2 group and also a higher proportion of severe anemia compared with the other groups, suggesting that the latter would have a higher frequency of hemolysis than those of the other groups. However, despite the weak influence of haptoglobin genotypes on hemoglobin, sickle cell patients with genotypes *HP* 1-1 have a high hemoglobin level compared with other phenotypes suggesting a protective effect. Anemias were mainly regenerative; several other studies also report cases of reticulocytosis and regenerative anemia in sickle cell patients (Sombodi et al., 2015; Yahouédéhou et al., 2019);

Moreover, the median white blood cell was differing significantly from the *HP* 2-2 group (20.4 g/L) compared with the *HP* 1-1 (15.8 g/L [13.5–21.3]) and *HP* 2-1 (14.8 g/L [12.3–17.9]) groups ($p = 0.024$) (Table 4). Several studies also report hyperleukocytosis in sickle cell patients (Makulo et al., 2019; Um et al., 2019). Moreover, although it is an inflammatory disease, the more pronounced hyperleukocytosis in the *HP* 2-2 genotype group suggests that in the latter, the increase in the number and activation of leukocytes and important mediators of inflammation are more important. Moreover, leukocytes can adhere to each other, sickle and non-sickle erythrocytes, platelets, and the vascular endothelium. In addition, in acute hemolysis, strong bone marrow regeneration is responsible for erythroblastosis causing false hyperleukocytosis, since erythroblasts because of their nucleus are counted as leukocytes by automata. And this uncorrected leukocytosis would therefore be more important in the group of sickle cell patients with genotype *HP* 2-2 (Makani et al., 2013).

The median platelet in the population was 460 g/mL [323–575] with 532 g/mL in the *HP* 2-2 genotype group and 404 g/mL and 466 g/mL, respectively in the *HP* 1-1 and *HP* 2-1 groups; thus reflecting a greater thrombocytosis in the *HP* 2-2 genotype group ($p = 0.03$). Thrombocytosis in sickle cell patients is a factor promoting vaso-occlusive crises because activated platelets secrete thrombospondin (TSP) involved in GR-endothelium bypass and participate in the hypercoagulability state of sickle cell disease. It would also be the result of the occurrence of functional or organic asplenia or would be the consequence of hyposplenism

(Doupa et al., 2017; Houwing et al., 2019). These previous descriptions made and reports suggest that in sickle cell disease, the "$HP^2$" allele of haptoglobin would have a negative influence on the hematological profile. Regarding hematological and biochemical disorders, the impaired ability of the *HP* 2-2 protein to prevent Hb-driven oxidation as compared with the Hp1-1 protein might constitute the mechanism by which oxidative stress and vascular risk in *HP* 2-2 individuals are increased (Goldenstein et al., 2018). Individuals with the *HP* 2-2 genotype have reduced clearance of the macrophage-*HP*-Hb complex, which affects iron deposition, oxidative stress, and active macrophage accumulation. These changes would be consistent with an increased risk of atherosclerotic cardiovascular diseases (Hamdy et al., 2014). Therefore, the mechanism by with SCA patients with *HP* 1-1 is more prone to oxidative stress than those with *HP* 2-1 genotype should be investigated. Since a large body of evidence suggests that the *HP* 2 allele is a major susceptibility gene for the development of vascular complications and of oxidative stress, a special focus should be given to the particular population of SS patients with *HP* 2-2 genotype.

The frequency of inflammation in the population was 42.3%, which is relatively high. In addition, the proportion of inflammation in the *HP* 2-2 group is higher (57.1%) than in the other groups with respectively 42.5% and 35.4% in the *HP* 1-1 and *HP* 2-1 groups (Table 5). The inflammation would be explained first of all by the large proportion of hemoglobin S in the population promoting polymerization and destruction of the sickle cell membrane. Added to this, there is a high frequency of infections, vaso-occlusive crises, and oxidative stress in sickle cell patients (Rees & Gibson, 2012). These results are close to those of Beaune et al. (2009) who report 1 in 2 sickle cell patient is in inflammation. In addition, the higher proportion of inflammation in patients in the *HP* 2-2 group suggests that they may be subject to more factors in favor of inflammation (Houwing et al., 2019) and conversely the presence of the $HP^1$ allele. Moreover, the median CRP was significantly higher in the *HP* 2-2 group compared with the other groups ($p = 0.039$). Also, the entire population (100%) of the *HP* 2-2 group had an elevation of interleukin 6 and ferritin unlike the other groups. These protein markers of inflammation are therefore higher in the *HP* 2-2 group and conversely suggest that patients with genotypes *HP* 1-1 would be protected against inflammation and therefore would be subject to less hemolysis and factors in favor of hemolysis. This observation was also made by Pierrot-Gallot et al. (2015) who also report an elevation of interleukin 6 in sickle cell patients with *HP* 2-2 genotypes compared with those of other genotypes. Indeed, intravascular hemolysis produces a state of endothelial dysfunction, vascular proliferation, inflammation, and oxidative stress. Recent studies have demonstrated the important roles of free plasma Hb in reducing NO. Intravascular hemolysis and the reduction in NO cause increased expression of endothelin-1 and activation of endothelial adhesion molecules and platelets. Thus, not only do they cause vaso- constriction but also NO is involved in endothelial activation and proliferation that eventually contributes to the pathogenesis of SCA (Belcher et al., 2006; Houwing et al., 2019; Schaer et al., 2013). It is known that sickled red cells bind to the vascular endothelium cells by integrin alpha-4 beta-1. This membrane protein then acts as a receptor for either fibronectin or VCAM-1. In the endothelium, VCAM-1 is stimulated by inflammatory cytokines, such as IL-6 and IL-8 which are released by the activated leukocytes. Heme and hemin, which are released into the circulation during the active phase of sickle cell disease, also contribute to inflammatory states in individuals with SCA by increasing the expression of endothelial adhesion molecules and adherence of leukocytes and reticulocytes to endothelial cells (Pierrot-Gallo et al., 2015). Considering that the transcription of cytokines is regulated by a great number of genetic mechanisms, this study intended to verify whether the *HP* polymorphisms influence cytokine secretion. *HP* has emerged as one of the most important SCD phenotypic modulators, as it is a protein with high capability to bind to free Hb in the plasma, forming the *HP*–Hb complex and thereby preventing heme-catalyzed oxidative damage (Buehler et al., 2020; Graves & Vigerust, 2016; Santos et al., 2011). Although the three *HP* genotypes have the same ability to bind to free Hb, the speed of heme release can be related to differences in their molecular size. *HP* 2-2, which is a more complex polypeptide, removes iron more slowly to the extravascular space thereby allowing free Hb to be in circulation for a longer period, causing more oxidative stress. On the contrary, *HP* 1-1 protects the endothelium against oxidative damage, as it is the most biologically active (Buehler et al., 2020). In 2011, Santos et al. reported that the *HP 2-2* genotype in Brazilian SCA patients was less frequent than the *HP 1-1* genotype and hypothesized that *HP* 2-2 might be associated with worse prognosis because of lower antioxidant capacity and higher inflammatory response (Santos et al., 2011). It therefore becomes imperative at this level to determine or even systematically the genotype of haptoglobin in sickle cell patients in order to improve control and prevent inflammatory events that may occur.

Furthermore, few or no studies describe the association of haptoglobin genotypes with iron in sickle cell patients. In this study, in addition to ferritin having a difference

according to *HP* genotypes, other markers of the ferric profile including transferrin saturation coefficient, transferrin, siderophilin binding total capacity, and serum iron did not show a significant association with a particular haptoglobin genotype ($p > 0.05$).

One of the limitations of this study is the small size of the population. Cohort studies of a larger size will show greater power and may better present the associations of haptoglobin phenotypes with the parameters studied.

## 5 | CONCLUSION

This study describes the distribution of *HP* genotypes and their involvement on hematological parameters and the iron profile in the sickle cell patients presenting an inflammation condition during major sickle cell syndromes in Cameroun. The higher genotype frequency is *HP* 1-1 followed by *HP* 2-2. However, a larger proportion of the *HP* 2-2 genotype is associated with hematological profile disorders, inflammation, and achievement of ferric parameters; In addition, the results did not show a significant association with clinical parameters such as infectious seizures, anemic attacks, vaso-occlusive seizures, and cardiovascular disease. These results suggest that haptoglobin polymorphism contributes to the severity of major sickle cell syndromes.

### AUTHOR CONTRIBUTIONS

Romaric De Manfouo Tuono conducted item retention, data interpretation, statistical analysis, and a major contribution to manuscript writing. Jean-Paul Chedjou, Calvino Fomboh Tah, and Wilfried Fon Mbatcham supervised the realization of Molecular Biology. Bernard Claude Chetcha, Josué Louokdom Simo, Prosper Cabral Biapa Nya, Constant Anatole Pieme, and Claude Tagny Tayou designed the study and contributed to the data interpretation, and the manuscript writing. All authors read and approved the final manuscript.

### ACKNOWLEDGMENTS

We address our sincere thanks to all the laboratory staff of the Faculty of Medicine, Laboratory for Public Health Research Biotechnologies (LAPHER-Biotech), and Biomedical Sciences of the University of Yaounde I and *Université des Montagnes* for their help in carrying out this work.

### FUNDING INFORMATION

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

### CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

### DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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## SUPPORTING INFORMATION

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**How to cite this article:** Tuono, R. D. M., Simo, J. L., Nya, P. C. B., Chedjou, J. P., Fotsing, C. B. K., Chetcha, B. C., Tah, C. F., Tayou, C. T., Mbatcham, W. F., & Pieme, C. A. (2024). Haptoglobin gene polymorphism and iron profile in sickle cell disease patients with inflammation in Yaounde, Cameroon. *Molecular Genetics & Genomic Medicine*, *12*, e2302. https://doi.org/10.1002/mgg3.2302