Single nucleotide polymorphisms in the cyp2C8 and nat2 genes and treatment outcomes in patients suffering from uncomplicated malaria in Garoua, Northern Region of Cameroon

Abstract
The human Cytochrome (cyp2C8) and N-acetyltransferase II (nat2) genes may vary between individuals resulting in differences in anti-plasmodial treatment responses. We set out in this study to determine the association between cyp2C8 and nat2 gene phenotypes, with treatment failures among children with uncomplicated malaria in Cameroon. Treatment outcomes were assessed after, children (n=235) were deparasitized with artemisinin based combination therapies (ACTs) (Artesunate-Amodiaquine or Sulfadoxine-Pyrimethamine-Amodiaquine) and followed up for 28 days. Dried blood spots were used for nested PCR followed by restriction enzyme analysis with KpnI, TaqI, and BamHI for the detection of polymorphisms in cyp2C8 and nat2 genes. Two alleles in the study population were identified with the predominance of the wild-type cyp2C8*1 allele (76%) and the fast metabolisers phenotype (70%). The nat2*6 genotype was highest at 39% and nat2*5/6 highest at 28.12%. Slow metabolizers were found at a frequency of 61.4% and intermediate metabolizers at 28.2%. Prior to PCR corrections 63.5% patients on ACTs had an Adequate Clinical and Parasitological Response (ACPR), while 24% experienced treatment failure (ETF),and 12.5% Late Parasitological Failure (LPF). Individuals with ETF (Odd’s Ratio=4.792 and p-value=0.020) clustered with the fast acetylation phenotype compared to slow and intermediate metaboliser phenotypes. The distance-based clustering analysis using the Jaccard index for calculation of genetic distances showed patients who are intermediate metabolizers and carried wild-type Pfdhfr-S108 and Pfdhps-CS8 formed one cluster. Slow metabolizers carrying the 2 resistance markers 108N and 58R formed another cluster, while fast metabolizers with wild-type genotype at drug resistance codons clustered together. We conclude that the cyp2C8 wild-type allele and the fast metabolizers predominate in the north of Cameroon. Likewise, the nat2*5/6 and its slow metabolizer status. ACPR to antimalarial treatment may be well associated with a nat2 slow metabolizer phenotype.

Keywords: malaria, cyp2C8, nat2, metabolysers, treatment failure, adequate clinical and parasitological response

Introduction
Malaria is an infectious disease transmitted by mosquitoes in humans and other animals caused by the parasitic protozoan, *Plasmodium*. According to the latest statistics of the World Health Organization,2 216 million cases of malaria occurred worldwide in 2016, an increase of about 5 million cases compared to 2015. Most cases in 2016 occurred in the WHO regions of Africa (99.7% *P. falciparum*), South-East Asia (7%) and Eastern Mediterranean (2%). The WHO Cameroon epidemiological bulletin reported an increase of 70,420 new cases (10, 52%) in 2016. Malaria remains a public health problem in Cameroon with enormous morbidity and mortality, partly caused by resistance to conventional antimalarial drugs.2 But the unprecedented malaria epidemic of October 2013 to determine the association between cyp2C8 and nat2 gene phenotypes, with treatment failures among children with uncomplicated malaria in Cameroon. Treatment outcomes were assessed after, children (n=235) were deparasitized with artemisinin based combination therapies (ACTs) (Artesunate-Amodiaquine or Sulfadoxine-Pyrimethamine-Amodiaquine) and followed up for 28 days. Dried blood spots were used for nested PCR followed by restriction enzyme analysis with KpnI, TaqI, and BamHI for the detection of polymorphisms in cyp2C8 and nat2 genes. Two alleles in the study population were identified with the predominance of the wild-type cyp2C8*1 allele (76%) and the fast metabolisers phenotype (70%). The nat2*6 genotype was highest at 39% and nat2*5/6 highest at 28.12%. Slow metabolizers were found at a frequency of 61.4% and intermediate metabolizers at 28.2%. Prior to PCR corrections 63.5% patients on ACTs had an Adequate Clinical and Parasitological Response (ACPR), while 24% experienced treatment failure (ETF),and 12.5% Late Parasitological Failure (LPF). Individuals with ETF (Odd’s Ratio=4.792 and p-value=0.020) clustered with the fast acetylation phenotype compared to slow and intermediate metaboliser phenotypes. The distance-based clustering analysis using the Jaccard index for calculation of genetic distances showed patients who are intermediate metabolizers and carried wild-type Pfdhfr-S108 and Pfdhps-CS8 formed one cluster. Slow metabolizers carrying the 2 resistance markers 108N and 58R formed another cluster, while fast metabolizers with wild-type genotype at drug resistance codons clustered together. We conclude that the cyp2C8 wild-type allele and the fast metabolizers predominate in the north of Cameroon. Likewise, the nat2*5/6 and its slow metabolizer status. ACPR to antimalarial treatment may be well associated with a nat2 slow metabolizer phenotype.

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The Ministry of Health decided with the support of technical partners to implement WHO’s Seasonal Malaria Chemoprevention (SMC) program;7,8 which recommends that SMC with SPAQ is administered in areas with highly seasonal malaria transmission specifically in the Sahel sub-region of sub-Saharan Africa. *P. falciparum* is still sensitive to both these antimalarial drugs. As part of the implementation of the SMC strategy in Cameroon, a clinical trial was necessary to assess the efficacy of SPAQ versus ASAQ as the comparator drug in the Northern Regions of Cameroon. The age group in this trial was extended from 6 months to 10 years. To this end, two main reactions that take place in the liver are essential; Phase I and Phase II reactions. While Phase I reaction is primarily catalyzed by Cytochrome P450 enzymes (CYP), Phase II reactions are catalyzed by many enzymes including Acetyltransferase (NAT2), Glucuronosyl-transferase, Glutathione-s-transferase and Sulpho-
transf erases. However, the effective metabolism pathway of certain drugs show these drugs are metabolized by a single enzyme (metoprolol by CYP2D6), whereas other drugs involve two or more enzymes (warfarin by CYP1A2, CYP2D6 and CYP3A4).16 The polymorphisms in cyp2C8 and nat2 genes may influence antimalarial treatment outcomes among individuals suffering from uncomplicated malaria. To better understand such differences in the Cameroon setting, we characterized the single nucleotide polymorphisms (SNPs) of cyp2C8, nat2 (nat2*5, nat2*6 and nat2*7) and their relationship with the treatment response of the population of the Northern region.

Materials and methods

Study area

The study was conducted in Garoua, which is situated in the north of Cameroon (06°24′N, 10°46′). Garoua serves as a river port in years when the rainfall is abundant. Situated in the river Benue basin, it receives an average annual rainfall of 380mm. It has about 4 months of rainy season. The mean temperatures for most of the year are about 31°C and the vegetation is guinea-savannah. The population is predominantly of the Fula ethnic group and comprises of cattle grazers. A few have taken to trading in small provision merchandise with neighbouring Nigeria.

Screening and enrolment

Eligible children aged 6 to 120 months with acute uncomplicated falciparum malaria were screened at the outpatient department and informed consent obtained from parents or guardians. Patients meeting the inclusion criteria were randomized to trial allocation and pre-treatment investigations (clinical and laboratory assessments) conducted. Criteria for inclusion were; children of either gender, suffering from acute uncomplicated P. falciparum malaria confirmed by microscopy, or presenting with fever (axillary temperature ≥ 37.5°C) or having a history of fever in the preceding 24 h; ability to ingest tablets orally; willingness and ability to attend the clinic on stipulated follow-up days. A presentation was made of the anticipated risks and benefits, the discomfort to which the subjects were exposed, as well as the right to interrupt the participation at any time on their own free-will. A total of 235 patients were enrolled for the study.

ACT Administration and follow-up

Children (n=235) were randomized to receive Artesunate-Amodiaquine and Sulfadoxine-Pyrimethamine-Amodiaquine in the ratio of 2:2 respectively. The first dose of the anti-malaria’s was administered in the hospital and the rest of the tablets were administered at home, according to the prescription. The medication was administered with water after a meal. Follow up of children was ensured by community health workers (CHW) who visited participants daily in the first three days and reported any complaints from the mother. They also visited these mothers with complaints on other days besides the protocol stipulated days (D7, D14, D28, D35 and D42 after first dose). Complaints included but were not limited to any signs and symptoms suggestive of malaria or toxicity, date of onset, duration of symptoms, severity of malaria or other treatment administered and patient outcomes. Participants were followed up for 42 days post ACT-administration.

Sample collection and DNA extraction

Finger-prick blood was collected and spotted on filter paper at inclusion and during follow up on days D0 for genomic (or molecular analyses!). Blood spots on the filter paper were excised with a sterile pair of surgical scissors. DNA was extracted from dried blood spots heating at 100°C in Chelex-100 in buffered Tris-EDTA as previously described.17 The DNA was stored in a Tris-EDTA buffer at -20°C until allelic discrimination analysis was done by PCR-RFLP.

Genotyping single nucleotide polymorphisms in cyp2C8 and nat2 gene

Amplification of the cyp2C8 gene was done according to the adapted approach of Dai et al.12 using primers (cyp2C8 F: 5′-AAAGATACATATATCTTATAGCATG-3′ & cyp2C8 R: 5′-ATCCTTAGAAATATTACAGGA GG-3′). For a total of 25μl, the reaction mixture (NEBiolabs) was composed of PCR water, buffer (10Xthermol buffer), 10mM dNTPs (200μM of each deoxyribonucleotide), 0.8μM of each primer, 1.25μl of Taq polymerase DNA and 5μl of DNA extract. The T3 thermal cycler (Biometra, UK) was used for the PCR amplification of target genes. Cycling conditions were as follows: 94°C for 5 minutes (pre-denaturation), 45 cycles of 94°C for 20 seconds (denaturation), 55°C for 20 seconds (annealing), 72°C for 20 seconds (extension). Final extension of amplicons was carried out at 72°C, after which PCR products were stored at 4°C for immediate use or -20/80°C for long-term use. The restriction enzyme Bcl I was used in digesting the cyp2C8 gene according to a previously described protocol.13 Digestion was carried out in a volume of 20μl containing PCR water (NEBiolabs), buffer (10X buffer, manufacturer), 4U/μl of Bcl I restriction enzyme and 8μl of cyp2C8 amplicon. The reaction mixture was incubated at 50°C for 12H.

The most common alleles of nat2 gene in African populations were genotyped/screened for the presence of the following mutations: C481T (rs1799929, amino acid change L161L), G590A (rs1799930, amino acid change R197Q), A803G (rs1208, amino acid change K268R) and G857A (rs1799931, amino acid change G286E). The primers used to amplify the gene were: nat2 (+) 5′-GCCTCAGGTGCCTTGCATTT-3′ and nat2 (-) 5′-CGTGAGGGTAGAGAGGATAT-3′. The amplification was carried out using a T3 thermal cycler (Biometra, UK). Each PCR cycle was performed in a total volume of 25μl containing: nuclease free water, 10Xthermol polymerase buffer, 10 mMdNTPs (200μM of each deoxyribonucleotide), 20 pmol primer and 5 μl Taq polymerase and 3 ng of gDNA. After initial denaturation at 95°C for 5 min, 30 cycles of amplification were carried out with denaturation at 95°C for 50 s, annealing at 55°C for 50 s and extension at 72°C for 50 s, followed by a final extension at 72°C for 5 min. To confirm the presence of nat2 alleles, PCR products were electrophoresed on a 2% agarose gel and polymorphisms determined by restriction endonuclease digestion of amplified gene fragments as described.14 The amplicons were digested under conditions stipulated for the restriction enzymes KpnI and BamHI (NEBiolabs, USA) (37°C for 16 h while Tag I digested at 65°C for 16 h). The digestion reaction was followed by inactivation at 80°C for 20 min. Digested and undigested fragments of each sample were electrophoresed on a 2% agarose gel stained with Ethidium Bromide and the pattern of migration analysed by UV trans-illumination. Different alleles and combinations of alleles of the cyp2C8 and nat2 genes were determined for each sample according to the migration pattern and information indicated.

Classification of acetylator genotypes

The genotypic frequency of the cyp2C8 genes were distinguished as fast metabolizers (cyp2C8*1) or slow metabolizers (cyp2C8*2). The
Single nucleotide polymorphisms in the cyp2C8 and nat2 genes and treatment outcomes in patients suffering from uncomplicated malaria in Garoua, Northern Region of Cameroon


nat2 acetylator genotypes were established according to previously published data. Homozygotes (nat2*4/nat2*4) or heterozygotes (nat2*4/nat2*5, nat2*4/nat2*6 and nat2*4/nat2*7 combinations) for the dominant nat2*4 wildtype allele were classified as fast acetylator genotypes, while homozygotes of the mutant alleles (nat2*5, nat2*6 and nat2*7) were classified as slow acetylator genotypes.

Patients harbouring the cyp2C8*1 genotype were phenotypically distinguished/categorized as fast metabolizers, while patients typed with cyp2C8*2 as slow metabolizers. nat2 acetylator genotypes were established according to previously published data. Patients were classified as fast acetylators, if they were homozygous (nat2*4/nat2*4) or heterozygous (a combination of nat2*4/nat2*5, nat2*4/nat2*6, and nat2*4/nat2*7) to the dominant nat2*4 wild-type allele. On the converse, they were classified as slow acetylators if they were homozygous with the mutant alleles (nat2*5, nat2*6 and nat2*7).

**Distance-based clustering analysis**

Genetic distances that have served for the establishment of the phylogenetic tree were calculated with the formula below:

$$D_{XY} = N_x(N_x+N_y-N_{xy}) / N_x$$

- $D_{XY}$ - the genetic distance between X and Y,
- $N_x$ - the number of electrophoresis bands of X,
- $N_y$ - the number of electrophoresis bands of Y,
- $X$ & $Y$ represent the different haplotypes basis on nat2 genes, Pfdhfr and Pfdhps. These distances were used to draw a distance tree using the software MEGA 5.05.

**Data analysis**

Data was entered using Microsoft Access 2007, CS-Pro and analysed using Statistical Package for the Social Sciences (SPSS). The distance migrated by each gene was measured against that of molecular weight marker and recorded using a Microsoft 2016 Excel spreadsheet. The molecular size of each gene was determined graphically from the curve of log$_{10}$ of molecular weight marker against the distance of migration.

**Efficacy of anti-malaria drugs**: PCR-adjusted parasitological cure rates were analysed using Fisher’s exact test, odds ratios for likelihood of cure with 95% confidence intervals, Kaplan-Meier survival analysis.

**Safety of anti-malaria drugs**: Prevalence of Adverse Events were analysed by the Fisher’s exact test. Changes of biological safety tests & vital signs: Wilcoxon’s Signed Rank test or paired t-test. Statistical significance was set at $\alpha=0.05$ for all tests.

Allelic frequencies for the cyp2C8 and nat2 gene were obtained using the Hardy-Weinberg equilibrium formula. Phenotypic frequencies were obtained by dividing the total number of a particular phenotype by the total number of patients. The relationship between the cyp2C8, nat2 gene and treatment outcomes were analyzed by the $\chi^2$ test. The odds ratio (OR) was evaluated using a confidence interval of 95%, for the susceptibility of early treatment failure for fast acetylators and late parasitological failure for slow acetylators. A p-value less than 0.05 were considered statistically significant.

**Ethical clearance**

Ethical approval was obtained for the study from the National Ethics Committee for Health Research on human subjects in Cameroon.

**Results**

A total of 235 children were enrolled from North regions. The number of children randomised to the ASAQ group was 117 while those assigned to the SPAQ group was 118. There were 14 withdrawals or lost to follow up in the ASAQ group as opposed to 13 in the SPAQ group. Finally, 85 children completed the study in the ASAQ group and 80 in the SPAQ group. Follow-up was in accordance with the protocol.

The basic characteristics of the population as seen in Table 1, shows the different characteristics of the study participants on day 0. All participants were positive for malaria after a thick blood smear (mean parasitaemia = 10799 parasites/µl) on the first day of recruitment. The female-to-male ratio was 55:54 in the SPAQ group and 51:58 in the ASAQ group. Unfortunately, there were 8 unrecorded sex status in the SPAQ group and 9 in the ASAQ group. There was no significant difference in sex and age between both groups. The mean hemoglobin level at baseline was 10.24±2.3g/dl in the SPAQ group and 10.14±2.4g/dl in the ASAQ group. There was however no significant difference between the two groups (p=0.75). The mean temperature in the ASAQ group was 38.6±0.9°C and 37.9±0.8°C in the SPAQ group. This difference was also not significant between the two groups (p=0.47).

Table 1 Baseline Characteristics of the enrolled study participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment Group</th>
<th>Artesunate-Amodiaquine (AS AQ)</th>
<th>Sulfadoxine-Pyrimethamine-Amodiaquine (SPA Q)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female: Male ratio*</td>
<td>55:54:00</td>
<td>51:58:00</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>North: Far North</td>
<td>54:63</td>
<td>55:63</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Mean age (months)±SD</td>
<td>48.0±28.7</td>
<td>46.3±31.4</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Mean Hb(g/dl)±SD</td>
<td>10.2±2.3</td>
<td>10.1±2.4</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Mean temperature (°C)±SD</td>
<td>38.6±0.9</td>
<td>37.9±0.8</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Weight(kg)±SD</td>
<td>15.3±7.5</td>
<td>14.9±6.1</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>Geometric mean Parasite count(µl)±SD</td>
<td>398±3</td>
<td>380±3</td>
<td>0.38</td>
<td></td>
</tr>
</tbody>
</table>

F:M, female: male ratio; * indicates that sex status missing for 8 in SPAQ group and 9 in ASAQ group; Hb, hemoglobin; SD, standard deviation.
Allelic, genotype and phenotype frequencies of the cyp2C8 gene

We determined that there were 2 alleles in our study population with the predominance of the wild-type cyp2C8*1 allele, at a frequency of 76% (Figure 1). In this study population of cyp2C8, we could distinguish between fast, intermediate and slow metabolisers with a predominance of fast metabolisers at a frequency of 70% (Figure 2).

Allelic, genotype and phenotype frequencies of the nat2 gene

The nat2*4, nat2*5, nat2*6 and nat2*7 alleles were found, with nat2*6 being predominant at 39% and allele nat2*7 less found at 4% (Figure 3), of which nat2*5/6 was dominant at 28.12% (Figure 4). Slow metabolizers (nat2*5/5, nat2*5/6, nat2*5/7, nat2*6/6, nat2*6/7) were found at a frequency of 61.4% and intermediate metabolizers (nat2*4/5, nat2*4/6, nat2*4/7) at 28.2% (Figure 5).

Treatment outcomes

The treatment outcomes were classified before PCR corrections, according to the WHO Guidelines as Adequate Clinical and Parasitological Response (ACPR), Early Treatment Failure (ETF), and Late Parasitological Failure (LPF). ACPR to SPAQ was much more dominant (63.5%), with respect to ETF (24%) and LPF (12.5%). The ASAQ drug demonstrated an ACPR of 98% (Figure 6).

ACPR was much more dominant (63.5%), with respect to ETF (24%) and LPF (12.5%) (Table 2). Individuals with the fast acetylation were much more susceptible to experience early treatment failures (Odd’s Ratio=4.792 and p-value=0.020) with respect to individuals with the slow and intermediate phenotype (Table 3) (Table 4).
Single nucleotide polymorphisms in the cyp2C8 and nat2 genes and treatment outcomes in patients suffering from uncomplicated malaria in Garoua, Northern Region of Cameroon

Table 2 Relationship between cyp2C8 phenotypes and treatment outcomes

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>No. examined (%)</th>
<th>Treatment outcomes (%)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow</td>
<td>3 (3.3)</td>
<td>ETF: 0 (0.0) LPF: 3 (100.0) ACPR: 0 (0.0)</td>
<td>p=0.000</td>
</tr>
<tr>
<td>Intermediate</td>
<td>24 (26.7)</td>
<td>ETF: 23 (95.8) LPF: 0 (0.0) ACPR: 1 (4.2)</td>
<td>p=0.000</td>
</tr>
<tr>
<td>Fast</td>
<td>63 (70.0)</td>
<td>ETF: 0 (0.0) LPF: 0 (0.0) ACPR: 63 (100.0)</td>
<td>p=0.000</td>
</tr>
</tbody>
</table>

ETF, early treatment failure; LPF, late parasitological failure; ACPR, adequate clinical and parasitological response

Table 3 Relationship between nat2 phenotypes and treatment outcomes

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>No. examined (%)</th>
<th>Treatment outcomes (%)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow</td>
<td>66 (68.75)</td>
<td>ETF: 0 (0.0) LPF: 3 (100.0) ACPR: 0 (0.0)</td>
<td>p=0.02</td>
</tr>
<tr>
<td>Intermediate</td>
<td>21 (21.88)</td>
<td>ETF: 23 (95.8) LPF: 0 (0.0) ACPR: 1 (4.2)</td>
<td>p=0.02</td>
</tr>
<tr>
<td>Fast</td>
<td>9 (9.37)</td>
<td>ETF: 0 (0.0) LPF: 0 (0.0) ACPR: 63 (100.0)</td>
<td>p=0.02</td>
</tr>
</tbody>
</table>

ETF, early treatment failure; LPF, late parasitological failure; ACPR, adequate clinical and parasitological response

Table 4 Relationship between nat2 Phenotypes and LPF

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>LATE PARASITOLOGICAL FAILURE (LPF) Present</th>
<th>Absent</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow</td>
<td>2</td>
<td>19</td>
<td>0.684</td>
<td>0.138-3.395</td>
<td>0.641</td>
</tr>
<tr>
<td>Fast</td>
<td>6</td>
<td>3</td>
<td>4.333*</td>
<td>0.921-20.379</td>
<td>0.047*</td>
</tr>
<tr>
<td>Intermediate</td>
<td>7</td>
<td>59</td>
<td>0.593</td>
<td>0.172-2.048</td>
<td>0.405</td>
</tr>
</tbody>
</table>

OR, Odd’s ratio; CI, confidence interval; (*), statistically significant

Clustering analysis

The distance tree presents three clusters:

a. Cluster A - of patients who are intermediate metabolizers with wild type Pf dhfr-S108 and Pf dhps-C58, (without resistance markers),

b. Cluster B - of slow metabolizers with the 2 resistance markers 108N & 58R

c. Cluster C - of patients who are rapid metabolizers, without the resistance markers S108 & C58

As depicted in Figure 7, slow metabolizers clustered with resistance mutation (cluster B)

Figure 7 Distance tree of patient’s phenotypic status according to nat2, Pf dhfr and Pf dhps genes.

A, intermediate metabolizers without resistance mutations; B, slow metabolizers with resistance mutations; C, fast metabolizers without resistance mutations; Yagoua (Y) & Garoua (G) sites.

Discussion

Malaria remains a cause of mortality and morbidity worldwide. The consensus view of recent studies and reviews is that, malaria accounts for about 429,000 deaths annually, Sub-Saharan Africa bearing the heaviest burden. In Cameroon, malaria is also a public health problem, responsible for about 49% of hospitalisations. Artemisinin-based combination treatments are now generally accepted as the best treatments for uncomplicated falciparum malaria since the early 2000 and implemented in Cameroon in 2006. They have a rapid and reliable efficacy, although several cases of poor treatment outcomes as well as adverse effects have been noted in some patients. One of the challenges in the effective treatment outcomes is mutant polymorphisms in genes encoding enzymes responsible for metabolism of ACTs, notably the cyp2C8 and nat2 gene, wherein individuals could either be slow, fast or intermediate metabolizers.

In our study population, 44.8% of patients were men and 55.1% were women. This could be explained by the natural predominance of women in any given population. Malaria was prevalent in both sexes with the mean parasitaemia being 10,799 parasites/μL. This study allowed for the characterization of cyp2C8. The relatively low frequency of the cyp2C8*2 allele (24%) observed in this study population is similar to that observed in Senegal (15%) and Madagascar (15%),13 Tanzania (19%),14 Burkina Faso (15.5%)17 and Ghana (17.5%).18 The genotypic frequencies observed in the study population are partially consistent with other available data: the cyp2C8*1/*1 (70%) genotype obtained, corresponding to the rapid metabolisers, corroborates those observed in several countries, particularly in Senegal (55.7%), Uganda (79.7%), Madagascar (73.7%) and Nigeria (9%).19 The frequency of the cyp2C8*1/*2 genotype (26.67%) corresponding to the intermediate metaboliser phenotype is on the same line as that obtained in Senegal (44.3%)20 but different from those observed in Uganda (19.9%) and Madagascar (22.2%).15

In this study, nat2*4, nat2*5, nat2*6 and nat2*7 were found in the north of Cameroon. nat2*6 was predominant (39%) whereas nat2*7 was the least found (4%), compared with findings in Bangolan, wherein nat2*6 was found at 24.2%.19 The nat2*6 allele was among the most frequent alleles in populations of European and African descent, whereas nat2*7 was specific to the Asian population.20,21 With respect to nat2 gene phenotypes, intermediate metabolizers were dominant (61.4%) in the north of Cameroon. This results were similar to those obtained previously in Garoua, Mutengene and Bangolan, where slow and intermediate metabolizers dominated.22 Furthermore, studies in the north, east and west of Nigeria as well as in Kenya assessing the polymorphism of the nat2 gene had similar results in accordance with findings showing that Africans are predominantly slow and intermediate metabolizers.16,23

An inter-individual difference in drug metabolism rate is likely due to human genetic polymorphisms.24 With this in mind, we sought to determine the relationship between metabolism rates and treatment outcomes. According to our study, individuals with a fast metabolizer status seemed to experience early treatment failures (OR=4.792, p=0.02), contrary to those with a slow or intermediate metabolizer status. This relationship indicates that rapid metabolizers are more likely to have poor treatment outcomes. With regard to late parasitological failures, again, individuals with the nat2 fast metabolizer status were liable to have late parasitological failures (OR=4.333, p=0.047), contrary to those with the slow and intermediate metabolizer status. This is in concordance with a 6-months trial conducted in Japan, on tuberculosis patients using Isoniazid, in which patients with the fast metabolizer status were more susceptible to early treatment failures.25 While individuals with a fast metabolizer status were prone to poor treatment outcomes (early treatment failures and late parasitological failures), those with a slow and intermediate metabolizer status were much more susceptible to experiencing adverse effects to the prescribed drugs as demonstrated by.22 This can be explained by the fact that fast acetylators metabolize xenobiotes faster than the required time for therapeutic action to occur, hence, early treatment failures or late parasitological failures. Slow and intermediate acetylators on the other hand metabolize and eliminate xenobiotics slower than normal which may expose the human being to drug toxicity from accumulation of metabolites, and leading to adverse effects.15-17 The clustering pattern of responders is indicative of the fact that slow metabolizers clustered with the resistance mutation genes; this is not the case for rapid and intermediates metabolizers and the reasons for this observation needs to be investigated further.

Conclusion

From the investigation of the influence of single nucleotide polymorphisms in the cyp2C8 and nat2 genes on treatment outcomes in patients suffering from uncomplicated malaria in the north of Cameroon, we found that, there are two allelic frequencies with the wild type of cyp2C8*1 being at 76% and the cyp2C8*2 at 24%. Of these 70% were fast metabolizers. No trend with respect to treatment response was found for the cyp2C8. However, the main genotype in the north was nat2*5/6 for the acetylators, with the slow metabolizer status being the main phenotype found at 61.4% which also clustered with adequate clinical and parasitological response to antimalarial drugs.

Authors’ contributions

WFM, RNM, JPC, AN, IA, OA contributed to the design of the study. WFM coordinated the study. RNM, IA, supervised the enrolment, clinical coordination and follow-up of patients. AMN, JPC, EMA participated in data entry, collection and analysis of data and writing up the manuscript alongside RNM, JPC, DNM, performed the molecular analysis. All authors contributed in the revision of the manuscript and approved the final version of the manuscript prior to submission.

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Conflicts of interest

Authors declare that there is no conflict of interest.
Single nucleotide polymorphisms in the cyp2C8 and nat2 genes and treatment outcomes in patients suffering from uncomplicated malaria in Garoua, Northern Region of Cameroon

References


