Malaria severity: Possible influence of the E670G PCSK9 polymorphism: A preliminary case-control study in Malian children

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Abstract

Aim
Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) is a hepatic secretory protein which promotes the degradation of low-density lipoprotein receptors leading to reduced hepatic uptake of plasma cholesterol. Non-synonymous single-nucleotide polymorphisms in its gene have been linked to hypo- or hyper-cholesterolemia, depending on whether they decrease or increase PCSK9 activity, respectively. Since the proliferation and the infectivity of Plasmodium spp. partially depend on cholesterol from the host, we hypothesize that these PCSK9 genetic polymorphisms could influence the course of malaria infection in individuals who carry them. Here we examined the frequency distribution of one dominant (C679X) and two recessive (A443T, I474V) hypocholesterolemic polymorphisms as well as that of one recessive hypercholesterolemic polymorphism (E670G) among healthy and malaria-infected Malian children.

Methods
Dried blood spots were collected in Bandiagara, Mali, from 752 age, residence and ethnicity-matched children: 253 healthy controls, 246 uncomplicated malaria patients and 253 severe malaria patients. Their genomic DNA was extracted and genotyped for the above PCSK9 polymorphisms using Taqman assays. Associations of genotype distributions and allele frequencies with malaria were evaluated.

Results
The minor allele frequency of the A443T, I474V, E670G, and C679X polymorphisms in the study population sample was 0.12, 0.20, 0.26, and 0.02, respectively. For each
polymorphism, the genotype distribution among the three health conditions was statistically insignificant, but for the hypercholesterolemic E670G polymorphism, a trend towards association of the minor allele with malaria severity was observed ($P = 0.035$). The association proved to be stronger when allele frequencies between healthy controls and severe malaria cases were compared (Odd Ratio: 1.34; 95% Confidence Intervals: 1.04–1.83; $P = 0.031$).

**Conclusions**

Carriers of the minor allele of the E670G PCSK9 polymorphism might be more susceptible to severe malaria. Further investigation of the cholesterol regulating function of PCSK9 in the pathophysiology of malaria is needed.

**Introduction**

Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) is a secretory glycoprotein discovered in 2003 and initially termed Neural Apoptosis-Regulated Convertase 1 (NARC-1) [1]. It belongs to the family of proprotein convertases, the serine endoproteinases involved in the proteolytic activation of a variety of secretory precursor proteins [2, 3]. Its gene is located on human chromosome 1. It was the segregation of missense mutations at its locus with autosomal dominant hypercholesterolemia (ADH) that led to the identification of its function in cholesterol metabolism [4].

PCSK9 is primarily expressed in the liver. It is biosynthesized as a 692-amino acid pre-proPCSK9 within hepatocytes and cleaves itself after the prodomain, forming an enzymatically inactive heteroduplex made of the propeptide and the mature PCSK9. After release into the bloodstream, it acts as an escort protein for low-density lipoprotein receptor (LDLR) to which it binds at the surface of hepatocytes; the binding pair is internalized and directed towards lysosome-like compartments where it is degraded [5]. LDLR mediates hepatic uptake of plasma LDL and thus contributes to the clearance of plasma cholesterol [6]. PCSK9 genetic mutations that strongly enhance its LDLR-degrading activity of the protein have been implicated in autosomal dominant hypercholesterolemia [4, 7]. Furthermore, epidemiological studies have revealed strong associations between several nonsynonymous PCSK9 single-nucleotide polymorphisms (SNPs) with hyper- or hypo-cholesterolemia in humans [8–14]. Gain-of-function (GOF) SNPs accentuate PCSK9 activity, leading to hypercholesterolemia whereas loss-of-function (LOF) SNPs attenuate the activity, leading to hypocholesterolemia. Hypercholesterolemia is a risk factor for atherosclerosis and other cardiovascular diseases [15]. PCSK9 inhibitors have proven to be effective drugs against this condition [16, 17].

Two of the most effective LOF PCSK9 SNPs are c.426C $>$ G and c.2037C $>$ A, which cause the nonsense p.Y142X and p.C679X mutations, respectively. The two mutations are found at a rate of 1 in 40 African Americans and are 100-fold less frequent in European Americans [8]. In a large retrospective study, heterozygosity for these mutations was associated with lifelong hypocholesterolemia and significant protection against coronary heart disease (CHD) [18]. In West African ethnic groups the minor allele frequency (MAF) of the C679X mutation averages 3.3% overall but ranges from 0 to 7% [19].

Until recently, CHD was relatively uncommon in sub-Saharan Africa, primarily because of lower prevalence of lifestyle risk factors [20]. Although it is possible that these cardioprotective PCSK9 mutations, may have also contributed to the past low occurrence of CHD, it is probable that these mutations may have been maintained at high frequency in these populations because
they conferred some protection against major causes of mortality before reproductive age. We hypothesize that these causes might have been infectious diseases, malaria in particular [21]. This hypothesis was based on the mounting evidence that host cholesterol significantly contributes to invasion and proliferation of infectious agents [22, 23]. In the case of malaria, studies have shown that hypocholesterolemia conferred protection against malaria by reducing the infectivity of merozoites [24, 25]. Furthermore, the Plasmodium parasite scavenges endogenously produced or LDLR-captured cholesterol for its proficient replication in hepatocytes [26]. Merozoites also need cholesterol for development within the erythrocytes, suggesting that any alteration in cholesterol mobilization and metabolism may influence parasite development, and explaining the fact that reduction of plasma lipoprotein-cholesterol has been observed in individuals with acute malaria [27]. From a therapeutic angle, it has been reported that Atorvastatin, a member of the statin-family of anti-cholesterol drugs, can potentiate the efficacy of standard anti-malarial drug such mefloquine and artemisinin in murine model of cerebral malaria [28, 29].

We wanted to assess whether by affecting cholesterol metabolism, PCSK9 could influence the pathophysiology of malaria. As a first step, we have determined the frequency of four common PCSK9 SNPs with a documented cholesterolemia phenotype in sub-Saharan African children of Mali enrolled in a case-control study evaluating risk and protective factors for severe malaria.

Materials and methods

Ethics

The study protocol was reviewed and approved by the Review Board of the University of Mali Faculty of Medicine, Pharmacy and Dentistry as well as the Research Ethics Committee of the Clinical Research Institute of Montreal. The original case control study protocol and the use of archived samples for malaria studies was approved by the University of Maryland Baltimore Institutional Review Board. Written informed consent was obtained from parents or guardians of all study participants.

Subjects

The subjects were Malian children (n = 752), aged 3 months to 14 years, enrolled in a case-control study evaluating risk and protective factors for severe malaria [30]. Index cases of severe malaria (SM, n = 253) from Bandiagara and surrounding areas were enrolled from July 2000 to December 2001. In all three groups, the mean age was about 40 months, 85% of children were aged less than 5½ years, and nearly 80% of them belonged to the Dogon ethnic group (Supporting information: S1 Table). Cases were classified as severe malaria based on the World Health Organization criteria which include one or more of the following symptoms: impaired consciousness, prostration, multiple convulsions, acidosis, hypoglycemia, renal impairment, severe anemia, jaundice, pulmonary edema, shock, or hyperparasitemia [31]. Each index case was age-, residence-, and ethnicity-matched to a case of uncomplicated malaria (UM) and a healthy control (HC). UM was defined as P. falciparum parasitemia and an axillary temperature ≥ 37.5 °C detected by active surveillance or parasitemia and symptoms leading to treatment-seeking behavior in the absence of other clear cause of fever on passive surveillance. Children were enrolled as HC if they were asymptomatic for acute illness, had no evidence or history of chronic illness, and if the result of their thick blood smears was negative for malaria. All the subjects gave blood samples that were blotted onto Whatman filter papers FTA Classic cards and dried.
Genetic analysis

Genomic DNA was isolated from punctures of the FTA cards (6 mm in diameter) incubated in 0.4 mL of the phosphate buffered saline (1X PBS), pH 7.4, for 20 min at room temperature with gentle agitation. Following centrifugation, the supernatant was discarded and the paper was treated with 40 μL of a solution containing 10 mM NaOH, 200 mM NaCl, and 0.05% sodium dodecyl sulfate for 6 min at 95˚C. After centrifugation for 3 min, the supernatant was collected. Aliquots were diluted 12.5 X in water and used for PCR. TaqMan assays were used for genotyping the SNPs. The assay is based on the presence of fluorescence due to degradation of allele-specific fluorochrome-conjugated probes after annealing to SNP-containing PCR amplicon [32]. It was performed on a Stratagene Mx 3005P thermocycler instrument (Cedar Creek, TX). Table 1 describes the SNPs as well as the sequence context from which allele-specific fluorogenic probes were derived. Primers and fluorogenic probes were purchased from Applied Biosystems (Etobicoke, ON). The probes for common and minor alleles carried at their 5’-end a VIC and a FAM fluorochromes, respectively. They all carried a non-fluorescent quencher (NFQ) at their 3-end. A typical PCR reaction mixture contained 2 μL of DNA sample, 1x FastStart TaqMan ProbeMaster Rox master mix (Roche, Laval, QC), primers at 0.9 μM each and fluorogenic probes at 0.2 μM each. The reaction was run for 45–50 cycles involving a 15-20-sec denaturation at 95˚C, a 20-sec annealing at the appropriate temperature, and 20-sec elongation at 72˚C.

Statistical analysis

A preliminary quality control of all data was conducted (Supporting information: S1 Text). Conformity of each SNP to Hardy-Weinberg equilibrium (HWE) was independently evaluated using an online software (www.oege.org). Chi-square tests or Fisher’ exact tests were used to compare genotype distributions or allele frequencies between cases and controls. The strength of allelic association with malaria was expressed as odds ratios (OR) and 95% confidence intervals (95% CI). A P value of < 0.05 was set for significance in all analyses. The data were analyzed by GraphPad Prism 5 software.

Results

A total of 752 DNA samples were genotyped for the rs28362263 (G>A), rs562556 (A>G), rs505151 (A>G), and rs28362286 (C>A) SNPs leading to A443T, I474V, E670G, and C679X PCSK9 polymorphisms, respectively. Their genotype distribution in this population sample did not deviate from HWE and their MAFs were 0.12, 0.20, 0.26, and 0.022, respectively (see Table 1).

Since we presumed that plasma cholesterol level could influence the vulnerability to malaria, we examined the frequency of these cholesterolemia-modifying PCSK9 SNPs among healthy and malaria-stricken children in our cohort. The results per genotype and per health condition severity are presented in Table 2.

The percent of heterozygotes for the dominant 679X variant were 5.9%, 3.2, and 3.2% among HC, UM, and SM groups, respectively. Although the difference was not significant, the trend would be expected if low cholesterol offered relative protection against malaria. A similar trend was observed for homozygotes for the recessive 474V variant (HC/UM/SM: 5.5/4.1/3.2%), but not for homozygotes for the recessive 443T variant (HC/UM/SM: 3.58/0.81/3.16%). Interestingly, an opposite trend was noted for the recessive GOF 670G variant (HC/UM/SM: 3.6/7.9/8.7, Chi-square for trend P = 0.035), suggesting that hypercholesterolemia may render individuals relatively more susceptible to severe malaria.
When we grouped all malaria cases and compared the genotype distribution and allele frequencies for each SNP, no association was observed (Supporting information: S2 Table). However, when we compared healthy controls to severe malaria cases or uncomplicated malaria, we noted a significant association of the GOF 670G variant with susceptibility to severe malaria (OR: 1.38; 95% CI: 1.04–1.83, \(P = 0.031\)) (Table 3), and a significant association of the LOF 443T variant with protection from uncomplicated malaria (OR: 0.63; 95% CI: 0.43–0.94, \(P = 0.024\)) (Table 4).

Interestingly, when the allele distribution of the E670G polymorphism was considered by gender, the association of the G variant with severe malaria was significant in males (OR: 2.70; 95% CI: 1.74–4.19; \(P < 0.0001\)), not in females (OR: 1.37; 96% CI: 0.89–2.11; \(P = 0.186\)). Indeed, 15 out the 20 homozygous carriers of the 670G variation (75%) were males. No such gender dichotomy was observed for the A443T polymorphism in relation with uncomplicated malaria (Supporting information: S3 Table). No association was detected when UM cases were compared to SM cases for any of the SNPs (see Supporting information: S2 Table). The E670G genotypes did not influence the manifestation of malaria symptoms (Supporting information: S4 Table), nor the levels of blood glucose, hemoglobin, white blood cells or parasites (Supporting information: S1 Fig).

### Table 1. PCSK9 SNPs under study.

| SNP ID | c.#N1>N2 (p.AA1>AA2)\(^a\) | Context Sequence: 5’-3’\(^b\) | Exon | Phenotype\(^c\) | MAF\(^d\) | HWE\(^e\) |
|--------|----------------------------|---------------------------------|------|---------------|----------|-------|
| rs28362263 | c.1327G>A (p.A443T) | cccgacctgtggtgcc[g/a]ccctgccccccagca | 8    | LOF           | 0.12     | 0.23  |
| rs562556   | c.1420A>G (p.I474V) | cggatgccacagcc[a/g]tcgccctctgcccc | 9    | LOF           | 0.20     | 0.96  |
| rs505151   | c.2009A>G (p.E670G) | gcagcaccacgcaag[a/g]gcgcgtgacagctgt | 12   | GOF           | 0.26     | 0.71  |
| rs28362286 | c.2037C>A (p.C679X) | cgttgcacatgtgctg[c/a]cgagccgacacctg | 12   | LOF           | 0.02     | 0.90  |

\(^a\) Codon.# common nucleotide>variant nucleotide (protein.common amino acid>variant amino acid).

\(^b\) The polymorphic nucleotides are written in bold and bracketed.

\(^c\) LDLR-degrading activity: loss-of-function (LOF) or gain-of-function (GOF).

\(^d\) Minor allele frequency in the whole population sample.

\(^e\) Hardy-Weinberg equilibrium.

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### Table 2. Genotype distribution among healthy and malaria children.

| SNP | Healthy Controls (HC, N = 253) | Uncomplicated Malaria (UM, N = 246) | Severe Malaria (SM, N = 253) | Statistics\(^b\) |
|-----|--------------------------------|------------------------------------|-----------------------------|------------------|
|     | G:\(^a\) | 0 | 1 | 2 | 0 | 1 | 2 | 0 | 1 | 2 | \(P_{\text{exact}}\) | \(P_{\text{trend}}\) |
| A443T | N | 191 | 53 | 9 | 202 | 42 | 2 | 197 | 47 | 8 | 0.112 | 0.888 |
| I474V | N | 162 | 77 | 14 | 151 | 85 | 10 | 168 | 76 | 8 | 0.437 | 0.226 |
| E670G | N | 145 | 99 | 9 | 140 | 92 | 14 | 126 | 106 | 20 | 0.110 | 0.035 |
| C679X | N | 237 | 15 | 0 | 237 | 8 | 1 | 244 | 8 | 0 | 0.269 | 0.126 |

\(^a\) G, genotypes by number of variant allele: 0 homozygotes for the common allele; 1, heterozygotes; 2, homozygotes for variant allele; N, number of subjects; % of subjects per genotype.

\(^b\) \(P_{\text{exact}}\) or trend was determined by Chi\(^2\), Strong trends among health conditions is shaded.

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The E670G PCSK9 polymorphism increases the risk for severe malaria

Discussion

The E670G PCSK9 polymorphism is located in the cysteine/histidine-rich domain C-terminal domain (CHRD) of PCSK9. This domain is required for the LDLR-degrading activity of the protein [33]. The substitution of the charged Glu by a neutral Glycine at position 670 increases this activity presumably by altering CHRD conformation. The E670G polymorphism has been associated with hypercholesterolemia and increased risk of coronary artery disease [34]. In adults, for unknown reasons, the association with high plasma cholesterol appears to be male-gender specific [35]. In our children study, homozygous carriers of the minor allele, were mostly males and there were more of them among severe malaria patients than among healthy controls. These observations call for further studies that include lipid profiling to verify whether E670G-linked chronic hypercholesterolemia is indeed associated with a greater risk of severe malaria. In patients under septic shock, the GOF 670G variation was associated with greater blood levels of pro-inflammatory cytokines and greater mortality [36]. In mice, increased PCSK9 activity was linked to inflammation and septic shock lethality; its deficiency reversed these adverse outcomes [36, 37]. Therefore it is possible that the E670G polymorphism contributes to the inflammatory responses that commonly accompany and sometimes aggravate malaria [38, 39].

It is intriguing that the risk allele of the E670G polymorphism should be found at such a high frequency (MAF: 0.26) if it is associated with child morbidity and mortality due to endemic malaria. As in the case of balanced polymorphisms [40], it could provide some yet unknown biological benefits to carriers. On the other hand, using long-range haplotype test on the PCSK9 locus, Ding and Kullo [41] have observed that the ancestral (major) allele of the

Table 3. Association analysis of PCSK9 SNPs and severe malaria.

| SNP     | (PCSK9) | Healthy Controls (N = 253) | Severe Malaria Cases (N = 253) | Statisticsb |
|---------|---------|----------------------------|-------------------------------|-------------|
|         |         | 0  | 1  | 2  | 0  | 1  | 2  | Pg | Pa | OR | 96% CI |
| rs28362263 | (A443T) | 191 | 53 | 9  | 197 | 47 | 8  | 0.775 | 0.516 | 0.88 | (0.61–1.26) |
| rs562556  | (I474V) | 162 | 77 | 14 | 168 | 76 | 8  | 0.417 | 0.340 | 0.85 | (0.62–1.17) |
| rs505151  | (E670G) | 145 | 99 | 9  | 126 | 106| 20 | 0.057 | 0.031 | 1.38 | (1.04–1.83) |
| rs28362286 | (C679X) | 237 | 15 | 0  | 244 | 8  | 0  | 0.200 | 0.205 | 0.53 | (0.22–1.25) |

a G, genotypes by number of variant allele: 0, homozygotes for common allele; 1, heterozygotes; 2, homozygotes for variant allele.
b Pg, statistical differences of genotypes distribution (Chi² test); Pa, statistical differences of allelic frequencies (Fisher’s exact test); OR, odds ratio; CI, confidence interval. Significant differences between cases and controls are shaded.

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Table 4. Association analysis of PCSK9 SNPs and uncomplicated malaria.

| SNP     | (PCSK9) | Healthy Controls (N = 253) | Uncomplicated Malaria Cases (N = 246) | Statisticsb |
|---------|---------|----------------------------|--------------------------------------|-------------|
|         |         | 0  | 1  | 2  | 0  | 1  | 2  | Pg  | Pa  | OR  | (%CI) |
| rs28362263 | (A443T) | 191 | 53 | 9  | 202 | 42 | 2  | 0.051 | 0.024 | 0.63 | (0.43–0.94) |
| rs562556  | (I474V) | 162 | 77 | 14 | 185 | 85 | 10 | 0.591 | 0.877 | 1.04 | (0.76–1.41) |
| rs505151  | (E670G) | 145 | 99 | 9  | 140 | 92 | 14 | 0.913 | 0.605 | 1.09 | (0.81–1.46) |
| rs28362286 | (C679X) | 237 | 15 | 0  | 237 | 8  | 1  | 0.297 | 0.302 | 0.61 | (0.26–1.40) |

a G, genotypes by number of variant allele: 0, homozygotes for common allele; 1, heterozygotes; 2, homozygotes for variant allele.
b Pg, statistical differences of genotypes distribution (Chi² test); Pa, statistical differences of allelic frequencies (Fisher’s exact test); OR, odds ratio; CI, confidence interval. Significant differences between cases and controls are shaded.

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E670G SNP may have been under positive selection in African-Americans, not in European-Americans, suggesting that it may have been advantageous for the survival or the reproduction of their African ancestors.

It was expected that minor allele of the rs28362286 (C>A) nonsense SNP would be less frequent among severe malaria patients than among healthy controls. It was not to a statistically significant extent. This SNP was initially identified among African-Americans at MAFs of 0.018 [8, 18]; it was observed in Zimbabwe and West Africa at MAFs of 0.04 and 0.033, respectively [19, 42]. The transversion introduces a premature termination codon leading to the production of a truncated protein that is not secreted and thus is incapable of promoting LDLR degradation [43, 44]. It is associated with a remarkable 28% reduction of mean plasma cholesterol in heterozygotes [8, 9, 18, 42]. The lower MAF of this SNP would require a larger population sample to establish any protective effect against severe malaria.

The rs28362263 (G>A) and rs562556 (A>G) LOF SNPs are found in many populations at varying frequencies [9, 11, 12, 45, 46]. Their minor alleles encode 443T and 474V LOF PCSK9 variants, respectively. These alleles are recessive since they are associated with significant reduction of plasma LDL-C level only in homozygotes [9, 45, 46]. The 443T variant has been shown to be more susceptible to proteolytic inactivation by the furin convertase [44], explaining its LOF phenotype. Why it appears to protect against uncomplicated malaria and not against the severe form remains to be investigated.

Conclusions

Data presented in this preliminary report support the notion that PCSK9 activity could affect susceptibility to malaria. This finding should be corroborated by prospectively studying a larger population sample. It should be pointed out that sub-Saharan Africans commonly carry more than one non-synonymous LOF and GOF PCSK9 SNPs in various combinations [12]. In the present cohort, 17.6% of subjects carried two of the four SNPs examined. In such cases, the resultant phenotype could be difficult to predict unless they are correlated with cholesterolemia. A substantial deviation from normal cholesterolemia in the absence of malaria may be a valid predictor of susceptibility or resistance to it. Furthermore, induction of normcholesterolemia with anti-PCSK9 drugs in hypercholesterolemic individuals could be useful for anti-malaria prophylaxis and therapy.

Supporting information

S1 Text. Statistical analysis. (DOCX)

S1 Table. Cohort age, gender et ethnicity. (DOCX)

S2 Table. SNP association analysis HC vs all malaria and UM vs SM. (DOCX)

S3 Table. Association analysis of E670G and A443T SNPs with malaria per gender. (DOCX)

S4 Table. Malaria symptoms per E670G genotypes. (DOCX)

S1 Fig. Blood parameters per E670G genotypes. Blood parameters per genotype of the rs505151 (A>G) PCSK9 SNP (E670G) in uncomplicated and severe malaria. No significant
difference was observed among genotype within either malaria conditions.

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References

1. Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, et al. The secretory pro-protein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. Proc Natl Acad Sci U S A. 2003; 100: 928–933. https://doi.org/10.1073/pnas.0335507100 PMID: 12552133

2. Seidah NG, Chrétien M. Proprotein and prohormone convertases: a family of subtilases generating diverse bioactive polypeptides. Brain Res. 1999; 848: 45–62. PMID: 10701998

3. Seidah NG. The proprotein convertases, 20 years later. Methods Mol Biol. 2011; 768: 23–57. https://doi.org/10.1007/978-1-61779-204-5_3 PMID: 21805237

4. Abifadel M, Varret M, Rabes JP, Allard D, Ouguerram K, Devillers M, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet. 2003; 34: 154–156. https://doi.org/10.1038/ng1161 PMID: 12730697

5. Mbikay M, Mayne J, Chrétien M. Proprotein convertases subtilisin/kexin type 9, an enzyme turned escort protein: hepatic and extra hepatic functions. J Diabetes. 2013; 5: 391–405. https://doi.org/10.1111/1753-0407.12064 PMID: 23714208

6. Goldstein JL, Brown MS. The LDL receptor. Arterioscler Thromb Vasc Biol. 2009; 29: 431–438. https://doi.org/10.1161/ATVBAHA.108.179964 PMID: 19299327

7. Abifadel M, Rabes JP, Devillers M, Munnich A, Erlich D, Junien C, et al. Mutations and polymorphisms in the proprotein convertase subtilisin kexin 9 (PCSK9) gene in cholesterol metabolism and disease. Hum Mutat. 2009; 30: 520–529. https://doi.org/10.1002/humu.20882 PMID: 19191301

8. Cohen J, Pertsemidis A, Kotowski IK, Graham R, Garcia CK, Hobbs HH. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. Nat Genet. 2005; 37: 161–165. https://doi.org/10.1038/ng1509 PMID: 15654334

9. Kotowski IK, Pertsemidis A, Luke A, Cooper RS, Vega GL, Cohen JC, et al. A spectrum of PCSK9 alleles contributes to plasma levels of low-density lipoprotein cholesterol. Am J Hum Genet. 2006; 78: 410–422. https://doi.org/10.1086/500615 PMID: 16465619

10. Baass A, Dubuc G, Tremblay M, Delvin EE, O’Loughlin J, Levy E, et al. Plasma PCSK9 is associated with age, sex, and multiple metabolic markers in a population-based sample of children and
adolescents. Clin Chem. 2009; 55: 1637–1645. https://doi.org/10.1373/clinchem.2009.126987 PMID: 19628659

11. Miyake Y, Kimura R, Kokubo Y, Okayama A, Tomoike H, Yamamura T, et al. Genetic variants in PCSK9 in the Japanese population: rare genetic variants in PCSK9 might collectively contribute to plasma LDL cholesterol levels in the general population. Atherosclerosis. 2008; 196: 29–36. https://doi.org/10.1016/j.atherosclerosis.2006.12.035 PMID: 17316651

12. Mayne J, Ooi TC, Raymond A, Cousins M, Bernier L, Dewpura T, et al. Differential effects of PCSK9 loss of function variants on serum lipid and PCSK9 levels in Caucasian and African Canadian populations. Lipids Health Dis. 2013; 12: 70. https://doi.org/10.1186/1476-511X-12-70 PMID: 2363650

13. Mayne J, Raymond A, Chaplin A, Cousins M, Kaefner N, Gyamerah-Acheampong C, et al. Plasma PCSK9 levels correlate with cholesterol in men but not in women. Biochem Biophys Res Commun. 2007; 361: 451–456. https://doi.org/10.1016/j.bbrc.2007.07.029 PMID: 17645871

14. Mayne J, Dewpura T, Raymond A, Bernier L, Cousins M, Ooi TC, et al. Novel loss-of-function PCSK9 variant is associated with low plasma LDL cholesterol in a French-Canadian family and with impaired processing and secretion in cell culture. Clin Chem. 2011; 57: 1415–1423. https://doi.org/10.1373/clinchem.2011.165191 PMID: 21813713

15. Miller M, Seidler A, Kwiterovich PO, Pearson TA. Long-term predictors of subsequent cardiovascular events with coronary artery disease and ‘desirable’ levels of plasma total cholesterol. Circulation. 1992; 86: 1165–1170. PMID: 1394924

16. Brown WV, Moriarty PM, McKenney JM. JCL roundtable: PCSK9 inhibitors in clinical practice. J Clin Lipidol. 2016; 10: 5–14. https://doi.org/10.1016/j.jcllip.2015.12.004 PMID: 26892118

17. Everett BM, Smith RJ, Hiatt WR. Reducing LDL with PCSK9 Inhibitors—The Clinical Benefit of Lipid Drugs. N Engl J Med. 2015; 373: 1588–1591. https://doi.org/10.1056/NEJMp1508120 PMID: 2644323

18. Cohen JC, Boenwinkel E, Mosley TH Jr., Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med. 2006; 354: 1264–1272. https://doi.org/10.1056/NEJMo054013 PMID: 16554528

19. Sirois F, Gbeha E, Sanni A, Chretien M, Labuda D, Mbikay M. Ethnic differences in the frequency of the cardioprotective C679X PCSK9 mutation in a West African population. Genet Test. 2008; 12: 377–380. https://doi.org/10.1089/gte.2008.0013 PMID: 18652535

20. Marjoni E, Trinquart L, Jani D, Joudrier H, Garbarz E, Mocumbi AC, et al. Coronary heart disease and associated risk factors in sub-Saharan Africans. J Hum Hypertens. 2007; 21: 411–414. https://doi.org/10.1038/sj.jhh.1002146 PMID: 17287844

21. Mbikay M, Mayne J, Seidah NG, Chretien M. Of PCSK9, cholesterol homeostasis and parasitic infections: possible survival benefits of loss-of-function PCSK9 genetic polymorphisms. Med Hypotheses. 2007; 69: 1010–1017. https://doi.org/10.1016/j.mehy.2007.03.018 PMID: 17502126

22. Sviridov D, Bukrinsky M. Interaction of pathogens with host cholesterol metabolism. Curr Opin Lipidol. 2014; 25: 333–338. PMID: 25036592

23. Kumar GA, Jafurulla M, Chatterj A. The membrane as the gatekeeper of infection: Cholesterol in host-pathogen interaction. Chem Phys Lipids. 2016; 199: 179–185. https://doi.org/10.1016/j.chemphyslip.2016.02.007 PMID: 26902688

24. Samuel BU, Mohandas N, Harrison T, McManus H, Rosse W, Reid M, et al. The role of cholesterol and glycosylphosphatidylinositol-anchored proteins of erythrocyte rafts in regulating raft protein content and malarial infection. J Biol Chem. 2001; 276: 29319–29329. https://doi.org/10.1074/jbc.M101268200 PMID: 11352913

25. Frankland S, Adisa A, Horrocks P, Taraschi TF, Schneider T, Elliott SR, et al. Delivery of the malaria virulence protein PiEMP1 to the erythrocyte surface requires cholesterol-rich domains. Eukaryot Cell. 2006; 5: 849–860. https://doi.org/10.1128/EC.5.5.849-860.2006 PMID: 16682462

26. Labaied M, Jayabalasingham B, Bano N, Cha SJ, Sandoval J, Guan G, et al. Plasmodium salvages cholesterol internalized by LDL and synthesized de novo in the liver. Cell Microbiol. 2011; 13: 569–586. https://doi.org/10.1111/j.1462-5822.2010.01555.x PMID: 21105984

27. Visser BJ, Wieten RW, Nagel IM, Grobusch MP. Serum lipids and lipoproteins in malaria—a systematic review and meta-analysis. Malar J. 2013; 12: 442. https://doi.org/10.1186/1475-2875-12-442 PMID: 24314058

28. Dormio J, Briolant S, Pascual A, Desgrouas C, Travaille C, Pradines B. Improvement of the efficacy of dihydroartemisinin with atorvastatin in an experimental cerebral malaria murine model. Malar J. 2013; 12: 302. https://doi.org/10.1186/1475-2875-12-302 PMID: 23988087

29. Souraud JB, Briolant S, Dormoi J, Msonjer J, Savini H, Baret E, et al. Atorvastatin treatment is effective when used in combination with mefloquine in an experimental cerebral malaria murine model. Malar J. 2012; 11: 13. https://doi.org/10.1186/1475-2875-11-13 PMID: 22233563
30. Lyke KE, Diallo DA, Dicko A, Kone A, Coulibaly D, Guindo A, et al. Association of intraleukocytic Plasmodium falciparum malaria pigment with disease severity, clinical manifestations, and prognosis in severe malaria. Am J Trop Med Hyg. 2003; 69: 253–259. PMID: 14628940

31. WHO. Guidelines for the treatment of malaria. Third edition. 3 ed2015. 316 p.

32. Morin PA, Saiz R, Monjazeb A. High-throughput single nucleotide polymorphism genotyping by fluorescent 5’ exonuclease assay. Biotechniques. 1999; 27: 538–540, 542, 544. PMID: 10489614

33. Saavedra YG, Day R, Seidah NG. The M2 module of the Cys-His-rich domain (CHRD) of PCSK9 protein is needed for the extracellular low-density lipoprotein receptor (LDLR) degradation pathway. J Biol Chem. 2012; 287: 43492–43501. https://doi.org/10.1074/jbc.M112.394023 PMID: 23105118

34. Cai G, Zhang B, Shi G, Weng W, Ma C, Song Y, et al. The associations between proprotein convertase subtilisin/kexin type 9 E670G polymorphism and the risk of coronary artery disease and serum lipid levels: a meta-analysis. Lipids Health Dis. 2015; 14: 149. https://doi.org/10.1186/s12944-015-0154-7 PMID: 26576960

35. Evans D, Beil FU. The E670G SNP in the PCSK9 gene is associated with polygenic hypercholesterolemia in men but not in women. BMC Med Genet. 2006; 7: 66. https://doi.org/10.1186/1471-2350-7-66 PMID: 16875509

36. Walley KR, Thain KR, Russell JA, Reilly MP, Meyer NJ, Ferguson JF, et al. PCSK9 is a critical regulator of the innate immune response and septic shock outcome. Sci Transl Med. 2014; 6: 258ra143. https://doi.org/10.1126/scitranslmed.3008782 PMID: 25320235

37. Dwivedi DJ, Grin PM, Khan M, Prat A, Zhou J, Fox-Robichaud AE, et al. Differential Expression of PCSK9 Modulates Infection, Inflammation, and Coagulation in a Murine Model of Sepsis. Shock. 2016; 46: 672–680. https://doi.org/10.1097/SHK.0000000000000682 PMID: 27405064

38. Francischetti IM, Seydel KB, Monteiro RQ. Blood coagulation, inflammation, and malaria. Microcirculation. 2008; 15: 81–107. https://doi.org/10.1080/10739680701451516 PMID: 18260002

39. Dieye Y, Mbengue B, Dagamajalu S, Fall MM, Loke MF, Nguer CM, et al. Cytokine response during non-cerebral and cerebral malaria: evidence of a failure to control inflammation as a cause of death in African adults. PeerJ. 2016; 4: e1965. https://doi.org/10.7717/peerj.1965 PMID: 27168977

40. Dean M, Carrington M, O’Brien SJ. Balanced polymorphism selected by genetic versus infectious human disease. Annu Rev Genomics Hum Genet. 2002; 3: 263–292. https://doi.org/10.1146/annurev.genom.3.022502.103149 PMID: 12142357

41. Ding K, Kullo IJ. Molecular population genetics of PCSK9: a signature of recent positive selection. Pharmacogenet Genomics. 2008; 18: 169–179. https://doi.org/10.1097/FPC.0b013e3282f444d9 PMID: 18300938

42. Hooper AJ, Marais AD, Tanyaniyiwa DM, Burnett JR. The C679X mutation in PCSK9 is present and lowers blood cholesterol in a Southern African population. Atherosclerosis. 2007; 193: 445–448. https://doi.org/10.1016/j.atherosclerosis.2006.08.039 PMID: 16989838

43. Zhao Z, Tuakli-Wosornu Y, Lagace TA, Kinch L, Grishin NV, Horton JD, et al. Molecular characterization of loss-of-function mutations in PCSK9 and identification of a compound heterozygote. Am J Hum Genet. 2006; 79: 514–523. https://doi.org/10.1086/507488 PMID: 16909389

44. Benjannet S, Rahinds D, Hamelin J, Nassoury N, Seidah NG. The proprotein convertase (PC) PCSK9 is inactivated by furin and/or PCS5/6A: functional consequences of natural mutations and post-translational modifications. J Biol Chem. 2006; 281: 30561–30572. https://doi.org/10.1074/jbc.M606495200 PMID: 16912035

45. Dwivedi DJ, Grin PM, Khan M, Prat A, Zhou J, Fox-Robichaud AE, et al. Differentia l Expression of PCSK9 Modulate s Infection, Inflammation , and Coagulati on in a Murine Model of Sepsis. Shock. 2016; 46: 672–680. https://doi.or g/10.1097/SHK.0000000000000682 PMID: 27405064

46. Anderson JM, Cerda A, Hirata MH, Rodrigues AC, Dorea EL, Bernik MM, et al. Influence of PCSK9 polymorphisms on plasma lipids and response to atorvastatin treatment in Brazilian subjects. J Clin Lipidol. 2014; 8: 256–264. https://doi.org/10.1016/j.jcllip.2014.02.008 PMID: 24793346