Haplotype of *RNASE 3* polymorphisms is associated with severe malaria in an Indian population

Running title: *RNASE 3* gene and susceptibility to severe malaria

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Abstract

Background: Severe malaria (SM) caused by *Plasmodium falciparum* (*Pf*) infection has been associated with life-threatening anemia, metabolic acidosis, cerebral malaria and multiorgan dysfunction. It may lead to death if not treated promptly. *RNASE 3* has been linked to *Pf* growth inhibition and its polymorphisms found associated with SM and cerebral malaria in African populations. This study aimed to assess the association of *RNASE 3* polymorphisms with SM in an Indian population.

Methods and Results: *RNASE 3* gene and flanking regions were amplified followed by direct DNA sequencing in 151 Indian patients who visited Wenlock District Government Hospital, Mangalore, Karnataka, India. Allele, genotype and haplotype frequencies were compared between patients with SM (n=47) and uncomplicated malaria (UM; n=104). Homozygous mutant genotype was only found for rs2233860 (+499G>C) polymorphism (<1% frequency). No significant genetic associations were found for *RNASE 3* polymorphism genotypes and alleles in Indian SM patients using the Fisher's exact test. C-G-G haplotype of rs2233859 (-38C>A), rs2073342 (+371C>G) and rs2233860 (+499G>C) polymorphisms was correlated significantly with SM patients (OR=3.03; p=0.008) after Bonferroni correction.

Conclusions: A haplotype of *RNASE 3* gene was found associated with an increased risk of SM and confirming that *RNASE 3* gene plays a role in susceptibility to SM.

Keywords: Severe malaria; *RNASE 3*; polymorphisms; ECP; *Plasmodium falciparum*

Introduction

Malaria remains to be a major public health problem in low and middle-income countries, especially in the sub-Saharan region. The World Health Organization (WHO) estimated that 228 million cases of malaria and 405,000 related deaths occurred globally in 2018 (WHO, 2019). Nineteen sub-Saharan African countries and India were responsible for carrying approximately 85% of the worldwide burden (WHO, 2019). *Plasmodium falciparum* (*Pf*) malaria is a complex disease with a wide spectrum of clinical manifestations ranging from uncomplicated (UM) to severe malaria (SM). SM is defined by life-threatening anemia, metabolic acidosis, cerebral malaria (CM), and multiorgan system involvement (Wassmer et al., 2015). Sequestration of *Pf*-parasitized erythrocytes within the microvasculature of vital organs in the human host is considered a key pathogenic event leading to SM (Miller et al., 2002; Dondorp AM, 2008). *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) is encoded by the multicopy *var* gene family and mediates the sequestration of parasitized erythrocytes to host receptors (Rowe et al., 2009; Turner et al., 2013).

Both parasite and host factors have been identified as significant contributors to SM (Miller et al., 2002). This includes parasite genes, and *var* groups A, B, as well as domain cassette (DC) 8, DC11 and DC13 have been shown to be associated with SM (Lavstsen et al., 2012; Magallón-Tejada et al., 2016). Similarly, among host genes, polymorphisms in intercellular adhesion molecule 1 (ICAM-1), cluster of differentiation 36 (CD36), tumor necrosis factor-alpha (TNF-α), Interferon-gamma (IFN-γ), interleukin-1β, complement receptor-1 (CR-1), ATP binding cassette subfamily B member 1 (ABCB1) and adenosine A2a receptor (ADORA2A) have linked to the development of SM (Sinha et al., 2008; Olaniyan et al., 2016; Nasr et al., 2014; Gyan et al.,

2002; Ouma et al., 2008; Panda et al., 2012; Gupta et al., 2017; Gupta et al., 2015). Ribonuclease 3 (*RNASE 3*), which encodes eosinophil cationic protein (ECP), an important protein produced by eosinophils during inflammation and infection (Venge et al., 1999; Boix et al., 2012), was found to increase susceptibility to SM (Adu et al., 2011; Diop et al., 2018; Kurtzhals et al., 1998; Waters et al., 1987). Indeed, SM patients had higher ECP levels and hypereosinophilia compared to UM patients (Kurtzhals et al., 1998). In addition, another study demonstrated that ECP can suppress the growth of *Pf* in *in vitro* (Waters et al., 1987). Above findings led to several genetic studies of the *RNASE 3* gene in African populations (Adu et al., 2011; Diop et al., 2018), which all showed an association between *RNASE 3* polymorphisms (+371C>G and +499G>C) and SM (Adu et al., 2011; Diop et al., 2018), further confirming its role in severity of the disease. The minor allele frequencies of +371C>G and +499G>C polymorphisms were more than 0.20 in African populations (Adu et al., 2011; Diop et al., 2018). These *RNASE 3* polymorphisms have also been associated with other disease susceptibility such as allergic asthma (Jönsson et al., 2010) and parasitic-helminth infection (Eriksson et al., 2007).

Here, we conducted a case-control study to assess the association of *RNASE 3* polymorphisms with SM in India, as the same polymorphism can have heterogeneous effect in two populations due to genetic differences (Lin et al., 2007). *RNASE 3* polymorphism alleles, genotypes and haplotypes frequencies were compared between falciparum malaria patients with SM and UM.

Materials and methods

Ethical Statement

All subjects were recruited from the Department of Medicine, Wenlock District Government Hospital, Mangalore, Karnataka, India. Prior written informed consent was obtained from each adult patient, or informed assent from a parent or legal guardian if the individual was ≤ 18 years. The research and ethics committee of the Kasturba Medical College (KMC) under Manipal Academy of Higher Education, Mangalore, Karnataka, India, approved the study (IEC KMC MLR 03-16/49). The Institutional review board of ICMR-National Institute of Malaria Research, New Delhi, India also reviewed and approved the study (ECR/NIMR/EC/2012/39). Patient data obtained in this study was kept confidential and unique laboratory code was used for laboratory and dataset analyses.

Study design and population

For this case-control study, we used a convenience sampling method, whereby all available malaria subjects in a specific area are included. We enrolled patients with SM and UM caused by *P. falciparum* who were admitted or visited to the Department of Medicine, Wenlock District Government Hospital, the largest health facility in Mangalore and the main malaria referral hospital in the region, Karnataka, India, from July 2015 to December 2018. UM patients were used as controls to understand the role of *RNASE 3* polymorphisms in severe malaria. SM was defined based on the modified WHO criteria (WHO, 2015). A total of 151 patients were recruited (age range: 1-75 years), including 19 (12.6%) children (≤ 18 years, median: 12;

interquartile range: 8) and 132 (87.4%) adults (>18, median: 32; interquartile range: 22) participants; 19 patients were female (12.6%). All the participants were from the same Tuluva ethnic group, as determined on the basis of shared history, food habit, language and habitat region. Patients with axillary temperature >37.5°C and confirmed mono-infection of *Pf* by expert microscopy and rapid diagnostic tests (RDTs) were included in the study. *P. falciparum* positive patients with other *Plasmodium species*, HIV, HBsAg, HCV, pneumonia, bacterial meningitis, sepsis and tuberculosis co-infections were excluded from the study.

Parasite quantification and treatment

Giemsa-stained thick and thin blood smears were air-dried, and tested for the presence of *Pf* parasites under a light microscope fitted with a 100X oil immersion lens and a 10X eyepiece (Zeiss Primo Star, Germany), and parasitemia was quantified as previously described (Punnath et al., 2019). In addition, the National Vector Borne Disease Control Programme (NVBDCP) approved RDT kits were used as per the manufacturer's instructions to confirm *Plasmodium* infections. These kits were FalciVax™ Rapid Test for Malaria Pv/Pf (Ref. No.: 50301002), Onsite Malaria Pf/Pv Ag Rapid Test (Ref. No.: R0112C) and SD Bioline Malaria Ag P.f/P.v (Ref. No.: 05FK80) targeting both *P. vivax*- specific pLDH and *P. falciparum*-specific HRP-2 antigens. All the cases were successfully treated with the artemisinin-based combination therapy (ACT) as prescribed by the National Vector Borne Control Programme.

Laboratory procedures

Patients positive for mono-*Pf* infections were subjected to venipuncture; 4ml of whole blood was collected in EDTA Vacutainers (BD Vacutainer®) for hematological tests, and a further 4ml blood was taken for biochemical liver and kidney function tests using Clot Activator Vacutainers (BD Vacutainer®). DxH 800 Hematology (Beckman Coulter) and Cobas® 6000 (Roche) analyzers were used for hematological and biochemical tests, respectively.

Molecular procedures

EDTA blood collected for each patient was sent to Chromous Biotech, Pvt. LTD, Bangalore, India (http://www.chromous.com/index.php?q=chromous-biotech/about-us), for DNA isolation, *RNASE 3* gene amplification and DNA Sanger sequencing. In brief, 100 - 250µl of blood was used for DNA isolation using the Chromous Biotech DNA extraction kit (Cat. No.: RKN25/26). Finally, DNA was eluted in 35µl of elution buffer available in the kit. *RNASE* 3 gene was amplified using two primer sets (**Set-1**: Fw: 5'-TCCAGCAAGAGTGGTGGATGAGAT-3' and Rv: 5'-CTGTTGTCACATGCAACTACATAG-3'; **Set-2**: Fw: 5'-TTGCCATCCAGCACATCAGTCTGA and Rv: 5'-CTGGTTCCACCTCTATTACGATTGC-3') covering 2047bp region (chr14: 20890932 – 20892978, including upstream and downstream sequences) of the *RNASE* 3 gene and targeting more than 150 reported polymorphisms (as per the Ensembl release 101; Human GRCh38.p13). In brief, *RNASE* 3 gene fragments were amplified separately in 50µl reactions including 200ng of each forward and reverse primers of set-1 and set-2, 5µl of 10x PCR buffer, 2µl of dNTPs (10mM), 2µl of template DNA and 3 units of Taq® DNA polymerase, reaction volume was raised by PCR-grade water. The reaction volume was prepared with PCR-grade water. The PCR reaction was performed with the initial denaturation at 94°C for 5min, followed by 35 cycles of 94°C for 30 sec, 52°C for 30 sec, 72°C

for 2min, followed by final extension at 72°C for 7min. DNA sequencing (Sanger et al., 1977) was performed using ABI Prism (Applied Biosystems, USA) 3500 genetic analyzer automated DNA sequencer using ABI Prism BigDye Terminator v3.1 cycle sequencing kit. The direct DNA sequencing was performed for the two amplicons described above, of 1177bp and 1180bp long regions of *RNASE 3* containing all the reported gene polymorphisms. The variations in the sequences were identified by sequence alignment using NCBI blast with reference sequence NC_000014.9.

Statistical analysis

To compare continuous data and categorical data between groups, respectively, we performed Mann Whitney U test and Fisher's exact test. Odds ratio (OR), 95% confidence interval (95% CI) and p value for the mutant allele of each *RNASE 3* polymorphisms were calculated using online version of MedCalc software (https://www.medcalc.org/calc/odds_ratio.php). The SHEsis (http://analysis.bio-x.cn/SHEsisMain.htm), an online software tool, was used for haplotype and linkage disequilibrium (LD) analyses. The software uses a partition–ligation–combination–subdivision expectation maximization algorithm for haplotype inference with multiallelic markers for haplotype analysis. All haplotypes with a frequency below 0.03 were discarded (Shi and He, 2005; Li et al., 2009). We defined statistical significance as $p<0.05$. Bonferroni correction was applied for multiple comparisons.

Results

Patient

All study participants (n=151) were positive for *P. falciparum* mono-infection, and no other species including *P. vivax*, *P. malariae, P. ovale* or *P. knowlesi* were identified. Among participants, median (± IQR) parasitemia was 39446 ± 52106 parasites/µL and median (± IQR) age was 27 ± 21years. Thrombocytopenia (<150,000 platelets/µL) was found in 60.9% of patients (92/151), and 10.6% (16/151) had severe thrombocytopenia (<50,000 platelets/µL). 31.1% (47/151) and 68.9% (104/151) of all patients were diagnosed with SM and UM, respectively. Comparisons between the demographic, hematological and biochemical laboratory findings of the participants with SM and UM was performed and are shown in **Table 1**. Statistically significant differences were only found for age, parasitemia, red blood cell counts, urea, bilirubin, AST, ALT, albumin levels and ratios of albumin and globulin (**Table 1**). Among patients with SM, 10.6% (5/47) and 23.4% (11/47) patients had multiple organ dysfunction and splenomegaly, respectively. Information on symptoms of severity of the patients as per the WHO criteria (WHO, 2015) is presented in **Table 2**.

Genetic association analyses

We successfully amplified 151 samples for *RNASE 3* gene followed by direct DNA sequencing, covering more than 150 polymorphisms. All the reported polymorphisms were present in the wild type form except for three polymorphisms [rs2233860 (+499G>C), rs2233859 (-38C>A) and rs2073342 (+371C>G)], and no novel polymorphisms were identified in our samples.

Among +499G>C, -38C>A and +371C>G polymorphisms (**Fig. S1**), homozygous mutant genotype was only found for +499G>C polymorphism, only 0.7% (1/151) patients possessed a mutant genotype (CC). However, -38C>A and +371C>G polymorphisms were either present in the form of homozygous wild-type or heterozygous genotypes in the studied participants. Thus, -38C>A, +371C>G and +499G>C were further considered for odds ratio, haplotype and linkage disequilibrium analyses. The minor allele and genotype frequencies of the entire studied population are shown in **Table 3**.

Odds ratio was calculated for different genetic models, neither alleles nor genotypes of -38C>A, +371C>G and +499G>C polymorphisms were found associated with SM and UM patients (**Table 4)**.

The haplotype analysis was performed for three (-38C>A, +371C>G and +499G>C) polymorphisms of *RNASE 3* gene by using SHEsis. Among the 8 possible haplotypes comprising the three polymorphic loci, C-G-G haplotype was associated with SM patients (OR=3.03; p=0.008) after Bonferroni correction. No, haplotypes were found associated with UM. The results of the haplotype analysis are shown in **Table 5**.

LD was estimated for the three polymorphisms using the D′ values. The analysis showed strong linkage disequilibrium (D′ = 1 or > 0.75) between rs2233859 (-38C>A), rs2073342 (+371C>G) and rs2233860 (+499G>C) polymorphisms in UM patients. However, no complete linkage between the three polymorphisms was observed in the SM group due to absence of LD between the polymorphisms rs2233859 and rs2073342 (D′ = 0.57). The results of the linkage analysis are shown in **Fig. S2**.

Discussion

In this first study aiming to decipher the effect of *RNASE 3* polymorphisms on SM susceptibility in India, we used the direct DNA sequencing of clinical samples collected using a convenience sampling method from *Pf*-positive patients residing in Mangalore, Karnataka, on the Western coast of India. We show evidence for the absence of homozygous mutant genotypes for *RNASE 3* reported polymorphisms, except for 3' UTR +499G>C polymorphism, which was only found in one patient. No significant genetic association was found for genotypes and alleles of *RNASE 3* polymorphisms with SM in the studied Indian population. This contrasts markedly with two reports on African populations, which showed an association between *RNASE 3* polymorphisms and SM susceptibility (Adu et al., 2011; Diop et al., 2018).

In Ghana, +371C>G polymorphism was found associated with CM, a neurological type of SM, and +499G>C polymorphism was associated with SM in a Senegalese population (Adu et al., 2011; Diop et al., 2018). No such associations were found in the present study. These differing results may be due to several reasons. First, the same polymorphism can have heterogeneous effect in two geographically and genetically distinct populations, despite the endemicity of *P. falciparum* at both sites (Lin et al., 2007). Second, the sample size used in the present study was small compared to previous studies (Adu et al., 2011; Diop et al., 2018). Third, the present study did not include participants with CM and no past history of malaria, which could have provided higher granularity in our association study. Indeed, the SM patients enrolled in the Senegalese study consisted primarily of CM and severe anemia cases (Diop et al., 2018); it is therefore possible that the reported association was driven by a high number of CM patients, which would be in line with the findings from Ghana (Adu et al., 2011). It has to be noted that Mangalore city

alone contributes about 72% of total malaria in Karnataka. In the last five years, the local district health department instead of the local municipal authority governs the malaria control operation. Following this, active and passive fever surveys through digital surveillance devices are in function throughout the city (Baliga et al., 2019). Thus, prompt diagnosis and treatment are provided. This may be one of the reasons for not finding CM cases in the present study. However, further studies including large groups of different SM subtypes are needed to test this hypothesis in Indian populations.

We show that in SM patients from India, there is a lack of homozygous mutant genotypes of +371C>G and +499G>C polymorphisms. This absence may indicate that mutant alleles may have a deleterious effect on the Indian population. This could be explained by the presence of heterozygous genotypes in our cohort, which may be to balance the deleterious effect of these mutant alleles, especially for +371C>G missense-polymorphism that has been associated with SM in Ghanaian population (Adu et al., 2011) and with parasitic-helminth infection in Ugandan population (Eriksson et al., 2007). The +371C>G polymorphism, resulting in an Arg/Thr substitution, drastically reduces the protein cytotoxicity. The change in cytotoxic activity could be due to the substitution, which creates a potentially new glycosylation site in ECP (Trulson et al., 2007; Eriksson et al., 2007; Salazar et al., 2014). According to the dbSNP database, a 0.73 minor allele (G) frequency of +371C>G polymorphism has been found in Asia, suggesting that it may increase in Indian population in the future.

Haplotype analysis can provide pivotal evidence on human evolution, and the identification of genetic variants causing specific human traits through linkage disequilibrium (Liu et al., 2008). C-G-G haplotype of -38C>A, +371C>G and +499G>C polymorphisms were found associated

with SM in this study. However, no complete linkage between the three polymorphisms was observed in the SM group. Therefore, in the absence of homozygous genotype for the mutant alleles of -38C>A and +371C>G polymorphisms, any conclusion on the association of C-G-G haplotype with SM would be speculative. Further genetic analyses involving a larger sample size in India are warranted to further explore the possible association we describe here.

SM predominantly affected adults in this study (median age 36 years, interquartile range 26 years), indicating a probable age shift in anti-malarial immunity (Fowkes et al., 2016). This may have resulted from the recent decrease in transmission due to India's largest national malaria control program (Ghosh and Rahi, 2019). Higher bilirubin, AST and ALT levels were found in SM patients, confirming the association between hepatic injury and severe *Pf* infection described in previous studies (Anand et al., 1992; Chawla et al., 1989; Kochar et al., 2003a; 2003b). In addition, low albumin levels, albumin:globulin ratio, and high urea levels were noted in SM patients compared to UM, confirming the presence of liver and kidney injuries (**Table 2**). Parasitemia was also found significantly increased in SM patients. However, peripheral parasitemia may not be a true indicator of disease severity due to the sequestration of parasitized erythrocytes in the microvasculature of vital organs (Dondorp et al., 2005).

This study has a few limitations. In the era of next-generation sequencing and genome wide association studies, we performed a case-control study to assess the role of *RNASE 3* polymorphisms in SM susceptibility in Indian population using Sanger DNA sequencing. This option was chosen as case-control studies using Sanger sequencing assays are less time consuming, cost-effective, reliable, and can be useful for recognizing associating factors of disease outbreaks, as well as current cases, and allow the assessment of multiple risk factors at

once (Tenny and Hoffman, 2020). Another limitation was the lack of CM patients included in the study, which may explain the discrepancies between our findings and the ones reported in Ghana and Senegal. Lastly, ECP levels were not assessed in plasma samples collected from study participants due to limited funding.

In conclusion, we tested the hypothesis if *RNASE 3* polymorphisms have a role in determining the SM susceptibility in an India population, and demonstrated that the homozygous mutant genotype of *RNASE 3* polymorphisms (+371C>G and +499G>C), which were shown to be associated with CM and SM in the population of Ghana and Senegal, respectively, were absent in our cohort of SM patients. However, C-G-G haplotype (-38C>A, +371C>G and +499G>C polymorphisms) frequency was significantly higher in SM patients compared to UM, suggesting its association with an increased risk of SM, and further confirming a role of *RNASE 3* gene in SM.

Acknowledgment

We thank all study participants as well as physicians, technician, nurses from Wenlock District Government Hospital, Mangalore, Karnataka, India, and everyone who supported this study directly or indirectly. We thank the Director, National Institute of Malaria Research, New Delhi, for the opportunity to complete the research work. We also thank Dr. Rajeshwari Devi (District Surgeon and Medical Superintendent, Wenlock District Government Hospital, Mangalore) and Dr. K. Chakrapani (Associate Dean and Head of Medicine department) Kasturba Medical College (Manipal University), Mangalore, Karnataka, India, for their support on this study. We

also acknowledge Indian Council of Medical Research, New Delhi, for funding. SCW is supported by a grant from the UK Medical Research Council, award number MR/S009450/1.

Authors take this opportunity to applaud and thank health care workers all around the world for their hard work in the COVID-19 crisis.

Conflict of interest

The authors declare that they have no conflict of interest.

Availability of data and material – Anonymized data is available on request

Author contributions

BM participated in fieldwork, collected clinical and epidemiological data, laboratory analyses, and together with HG wrote the first draft of this manuscript. HG also participated in data curation, sequencing and statistical analyses as well as in results interpretation. SCW and AKRA participated in data interpretation and critically reviewing this article. SKG participated in the study design, supervision, generate the resources, manuscript review, project administration and coordinated all the stages of the project. All authors read and approved the final manuscript.

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Table 1 Demographic, hematological and biochemical laboratory findings of patients at the time of admission, 2015-2018. For continuous variables, values are expressed in median and interquartile range in bracket.

	SM (n=47)	UM (n=104)	p-value
Female, no. (%)	6 (12.8)	13 (12.5)	1.000
Age (year)	36 (26.0)	26 (20.0)	0.007
Hemoglobin levels (g/dL)	12.3 (3.4)	12.2 (3.5)	0.828
Total RBC counts (million/mm^3)	4.48 (1.1)	4.92 (1.1)	0.049
Platelet counts (per µL)	121000 (107000)	112000 (65750)	0.419
Blood glucose (mg/dL)	74 (37)	79 (35.8)	0.633
Blood urea (mg/dL)	37.5 (17.6)	28.8 (16.1)	0.002
Serum creatinine (mg/dL)	0.90 (0.7)	0.88 (0.4)	0.266
Serum bilirubin (mg/dL)	2.7 (2.8)	1.6 (1.2)	< 0.001
AST levels (IU/L)	68.0 (61.5)	56.7 (60.9)	0.021
ALT levels (IU/L)	63.5 (40.5)	41.5 (51.2)	0.008
Alkaline phosphatase (IU/L)	310.4 (195.9)	257.5 (198.7)	0.092
Total protein levels (g/dL)	7.0 (1.3)	7.1 (0.8)	0.881
Albumin levels (g/dL)	3.7 (0.5)	3.9 (0.7)	0.038
Globulin levels (g/dL)	3.2 (1.2)	3.1 (0.9)	0.186
A:G Ratio	1.1 (0.4)	1.2 (0.5)	0.002
Parasitemia (per µL)	76357 (104280)	28735 (40816)	< 0.001

Severe malaria (SM); Uncomplicated malaria (UM); Albumin:globulin (A:G) ratio

Table 2 Severe malaria symptoms according to WHO criteria in patients enrolled in the study.

Severe malaria symptoms	Patients, n(%)
Metabolic acidosis	30 (63.8)
Jaundice	9 (19.1)
Hypoglycemia	1 (2.1)
Severe anemia	1 (2.1)
Acute kidney injury	1 (2.1)
Pulmonary edema	18 (38.3)
Multiple convulsions	3 (6.4)
Hyperparasitemia	1 (2.1)

Table 3 Genotype frequencies of studied polymorphisms in the Indian population.

rsIDs	Location	Amino acid	Wild	Heterozygous	Mutant	MAF[#]	Asia MAF (dbSNP)
			Genotype frequency (%)[#]				
rs2233859 (-38C>A)	Intron *chr14:20891649		42.4	57.6	0	A (0.288)	0.312
rs2073342 (+371C>G)	Exon2 *chr14:20892057	T/R	20.5	79.5	0	G (0.397)	0.73
rs2233860 (+499G>C)	3'UTR *chr14:20892185		83.4	15.9	0.7	C (0.086)	0.1

*[#]present study data; MAF: minor allele frequency; *GRCh38.p12*

Table 4 Odds ratio analysis of *RNASE 3* polymorphisms in severe and uncomplicated malaria patients.

rsIDs	Genotypes/alleles	SM (n=47)	UM (n=104)	Models	OR (95%CI), p-value
rs2233859 (-38C>A)	CC	23	41		
	CA	24	63		
	AA	0	0	Additive	NA
	CA+AA	24	63	Dominant	0.7 (0.3-1.3), p=0.274
	CC+CA	47	104	Recessive	NA
	CC+AA	23	41	Co-dominant	1.5 (0.7-2.9), p=0.274
	C	70	145		
	A	24	63	Allele	0.8 (0.4-1.4), p=0.399
rs2073342 (+371C>G)	CC	6	25		
	CG	41	79		
	GG	0	0	Additive	NA
	CG+GG	41	79	Dominant	2.2 (0.8-5.7), p=0.118
	CC+CG	47	104	Recessive	NA
	CC+GG	6	25	Co-dominant	0.5 (0.2-1.2), p=0.118
	C	53	129		
	G	41	79	Allele	1.3 (0.8-2.1), p=0.354
rs2233860 (+499G>C)	GG	40	86		
	GC	7	17		
	CC	0	1	Additive	NA
	GC+CC	7	18	Dominant	0.8 (0.3-2.2), p=0.712
	GG+GC	47	103	Recessive	NA
	GG+CC	40	87	Co-dominant	1.1 (0.4-2.9), p=0.821
	G	87	189		
	C	7	19	Allele	0.8 (0.3-2.0), p=0.629

NA: not applicable due to the presence of 0 value; OR: odds ratio; 95%CI: 95% confidence interval; Severe malaria (SM); Uncomplicated malaria (UM)

Table 5 Predicted haplotypes of *RNASE 3* polymorphisms [rs2233859 (-38C>A), rs2073342 (+371C>G) and rs2233860 (+499G>C)] association with severe and uncomplicated malaria.

Haplotype	SM (frequency)	UM (frequency)	OR [95%CI]	Chi^2	p value	[a]p value
A-C-C	0.0 (0.0)	0.18 (0.001)	-	-	-	-
A-C-G	6.24 (0.066)	5.92 (0.028)	2.402 [0.759-7.603]	2.35	0.125	NS
A-G-C	0.0 (0.0)	0.04 (0.0)	-	-	-	-
A-G-G	17.76 (0.189)	56.9 (0.274)	0.610 [0.335-1.111]	2.64	0.104	NS
C-C-C	7.0 (0.074)	16.84 (0.081)	0.903 [0.361-2.261]	0.05	0.827	NS
C-C-G	39.76 (0.423)	106.1 (0.510)	0.690 [0.422-1.128]	2.2	0.138	NS
C-G-C	0.0 (0.0)	1.98 (0.01)	-	-	-	-
C-G-G	23.24 (0.247)	20.12 (0.097)	3.031 [1.572-5.845]	11.66	<0.001	**0.008**

OR: odds ratio; 95%CI: 95% confidence interval; Severe malaria (SM); Uncomplicated malaria (UM)

[a]p value = p value after Bonferroni correction < 0.05 (in bold) considered to be significant. NS = non-significant

Supplementary material

Fig. S1: Schematic representation of the *RNASE 3* gene indicating location of rs2233859, rs2073342 and rs2233860 polymorphisms found in Indian population. Exons are indicated as boxes.

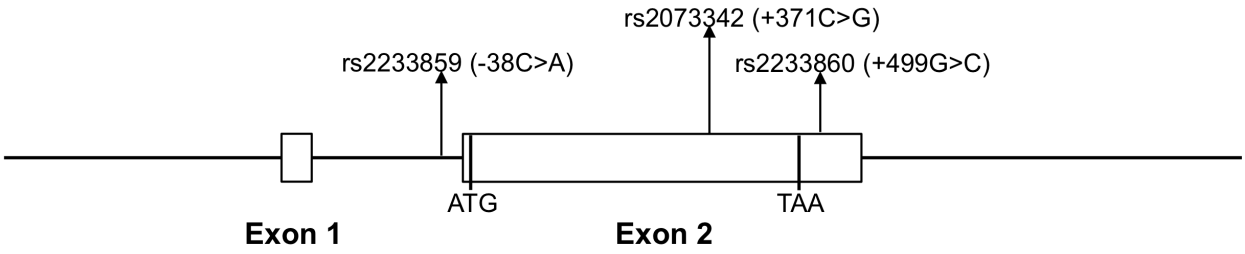

Fig. S2: Linkage disequilibrium (LD) mapping of rs2233859 (-38C>A), rs2073342 (+371C>G) and rs2233860 (+499G>C) polymorphisms of *RNASE 3* gene in patients with severe malaria (Panel A) and uncomplicated malaria (Panel B), the darkest shade indicating LD of 100 percent.