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Overdominance Effects between Malaria and Visceral Leishmaniasis in the 5q31 Region

[1]Mutaz Amin, [1]Abdelbadea Elhassan, [2]Kirk Rocket and [1]Muntasir Ibrahim

[1]*Department of Molecular Biology, Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan*
[2]*Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford, UK*

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Corresponding Author:
Muntasir Ibrahim
Department of Molecular Biology, Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan
Email: mibrahim@iend.org

Abstract: A group of SNPs in 5q31, genotyped from Hausa tribe of Koka village in eastern Sudan; an area endemic with malaria and visceral leishmaniasis, were analyzed by The Leishmaniasis research group and found to have significant excess of heterozygosis level and departure from HWE and assumed to be due to natural selection resulting from notorious deadly outbreaks since various ethnic groups from western Sudan settled the area although sequence information in the 5q31 region did not detect functional SNPs that could be associated with such drastic phenotypes. Malaria was thought to inflict inferior selective pressure due to the mild clinical phenotype observed. Taking advantage of follow up phenotype data sets available from the Malaria Gen study we re-analyzed these genotypes using different bioinformatics software to determine their effect on the regulation, function and expression of interleukins, miRNA binding and splicing mechanism. The SNPs were found to potentially affect the binding of many transcription factors that regulate the expression of IL-4 and IL-13 and stability of IL-5 mRNA. Association of haplotypes susceptibility revealed that the haplotype of low cytokine TH2 profile is associated with higher risk of malaria infection (P-value = 0.02). Through modulating TH2 cytokine response; the excess heterozygosity in 5q31 is explained with the phenomenon of overdominance between malaria and visceral leishmaniasis, acted by natural selection and driving the locus towards optimum response.

Keywords: Heterozygosity, Natural Selection, Single Nucleotide Polymorphisms, Overdominance

Introduction

The 5q31 in chromosome 5 is of much interest because it encompasses genes of type 2 cytokine responses known to associate with a number of infectious and noninfectious diseases and the fact that a quantitative trait locus associated with inflammatory diseases has been mapped in this region. The region contains several genes that modulate atopic responses, including IL-3, IL-4, IL-13, IL-5, CD14 and granulocyte macrophage-colony-stimulating factor, which are involved in the control of immunity to *P. falciparum* blood stages (Troye-Blomberg *et al*., 1990) and susceptibility to visceral leishmaniasis (Mohamed *at al*., 2003). Elhassan *et al*. (2013) suggested that the region is associated with signals of natural selection in areas endemic with malaria and visceral leishmaniasis. They selected two sets of Single Nucleotide Polymorphisms (SNPS) within the genes of IL-4, IL-5 IL-9 and IL-13, in 57 unrelated individuals. The first subset (4 SNPs) showed significant increase in heterozygosis level and departure from HWE, the second subset (14 SNPs) used to generate haplotype blocks (Elhassan *et al*., 2013).

The studied areas are Koka and Um-Salala villages located in the eastern bank of Rahad River in eastern Sudan; inhabited by Hausa and Massalit tribes respectively. The outbreaks of VL in the eighties and nineties in southern Sudan resulted in probably some of the most severe mortality indexes in modern history with hundreds of thousands reported to have succumbed to the disease (Elhassan *et al*., 2013).

Elhassan *et al*. (2013) suggested that the 5q31 area to be under intense selective pressure as indicated by marked heterozygosity independent of Linkage Disequilibrium (LD); difference in heterozygosity, allele and haplotype frequencies between generations and departure from Hardy-Weinberg Expectations (HWE) (Elhassan *et al*., 2013).

The objective of this study is to try to find out the underlying mechanism of this observed heterozygosity by analyzing the effect of these SNPs on the structure, function, stability and regulation of interleukins using software bioinformatics techniques. We will measure the susceptibility of different genotypes and haplotypes to malaria focusing on Hausa tribe of Koka village because they have clearer genotypes data. Understanding such phenomena will be important for learning the complex interaction between infectious diseases and immunity and the factors that drive human evolution.

Materials and Methods

A data set comprising information on age, gender; genotypes of SNPs in IL-4, IL-5, IL-9 and IL-13, of 57 unrelated subjects from Hausa tribe published previously (Elhassan *et al*., (2013) were reanalyzed with updated information regarding malaria affection status (positive or negative by ICT) and serum IgE level. The SNPs studied, heterozygosity level and HWE is shown in Table 1. The SNP rs1799962 in IL-9 is excluded from subsequent analysis because its heterozygosity level was found statistically non-significant (Elhassan *et al*., 2013). The list of bioinformatics tools used in subsequent analyses is shown in Table 2.

Results

Malaria infection was more common in children (≤15 years) than adults (>15 years), but the result was statistically non-significant (p-value =0.09). Association between malaria/gender and malaria/serum Ig-E level was non-significant either (p-value = 0.24 and 0.07 respectively).

The SNP rs1800474 in IL-5 (heterozygotes) was significantly protective against malaria (p-value = 0.008), compared to IL-4 (rs734244) and IL-13 (rs1881457), p-value = 0.11 and 0.24 respectively, Fig. 1a, 1b and 1c. The results' significance persisted after permutation. The haplotype GCG of IL-4, IL-13 and IL-5 respectively was associated with significantly lower risk of malaria infection, Table 3.

The SNPs rs734244, rs1881457 and rs2070874 (the latter is found to be in perfect LD to rs734244 in YRI population) were found to affect many transcription factor binding sites, the transcription factors and binding scores varied between different software tools, Table 4. The effect of SNPs on transcription factor binding was verified using Alibaba 2.1 and TFSEARCH tools.rs1800474 was found to affect the thermodynamic stability of IL-5 mRNA by changing the free energy of the molecule from-189.3 kcal/mol to-191.9 kcal/mol.

Table 1. Shows the studied SNPs, located genes, position and variation, observed and expected heterozygosity and p-value for DHWE

SNP	Gene	Position	Var	Obs Hz	Exp Hz	*p-value*
rs734244	Il-4	Intronic	A/G	0.85	0.49	2.8E-9
rs1800474	Il-5	Exonic	A/G	0.71	0.45	4.9E-5
rs1799962	Il-9	Intronic	A/G	0.48	0.38	0.09
rs1881457	IL-13	Promoter	A/C	0.56	0.40	0.014

Table 2. Shows the list of bioinformatics tools used in this article, their functions and websites

Software	Function	website
SNP function prediction	Transcription factor binding	http://genome.ucsc.edu/).
Is-rSNP	Transcription factor binding	http://bioinformatics.research.nicta.com.au/software/is-rsnp/
RegulomeDB	Transcription factor binding	http://regulomedb.org/
r-SNPBase	Transcription factor binding	http://rsnp.psych.ac.cn/
AliBaba2.1	Transcription factor binding	http://labmom.com/link/alibaba_2_1_tf_binding_prediction
TFSEARCH	Transcription factor binding	http://diyhpl.us/~bryan/irc/protocol-online/protocol-cache/TFSEARCH.html
mirSNP	mi-RNA binding prediction	http://bioinfo.bjmu.edu.cn/mirsnp/search/
Spliceport	Spicing prediction	http://spliceport.cbcb.umd.edu/
RNAfold	mRNA 2^{ry} structure	http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi
PLINK	SNP-SNP interaction	http://pngu.mgh.harvard.edu/~purcell/plink/
SNAP	SNPs LD blocks	https://www.broadinstitute.org/mpg/snap/ldsearch.php

Table 3. Shows association of different haplotypes of IL-4, IL-5 and IL-13 with malaria, (F-A=Frequency in affected, F-U= Frequency in unaffected)

Haplotype	F-A	F-U	*p-value*
GCG	0.00	0.24	0.02
GTG	0.11	0.04	0.25
ATG	0.07	0.02	0.27
GCT	0.05	0.11	0.50
ACT	0.06	0.03	0.50
GTT	0.13	0.05	0.20
ATT	0.55	0.49	0.60

Table 4. Transcription factors predicted by the different softwares

Tool SNP	SNP function prediction	IsrSNP	(score)*	RegulomeDB/ rSNPBASE	Regulatory region	Ensemble Alibaba2.1	TFSEARCH	Potential score	Regulatory Conservation
rs734244 (IL-4)	-	-	POLR2A, GATA2, GATA1 4	+	ENSR00001290157	SP-1	SP-1	0.19	0.004
rs1800474 (IL-5)	-	LM7	-	-	-	-	-	0.22	0.934
rs1881457 (IL-13)	+	-	GATA1 (3a)	-	ENSR00001290150	SP-1	SP-1	0.00	0.000
rs2070874 (IL-4)	+	LM101	MAX (2b)	+	-	Ftz	Ftz	0.09	0.725

*RegulomeDB score:
Score: Supporting evidence
2b: TF binding + any motif + Dnase Footprint + Dnase peak
3a: TF binding + any motif + Dnase peak
4: TF binding + Dnase peak

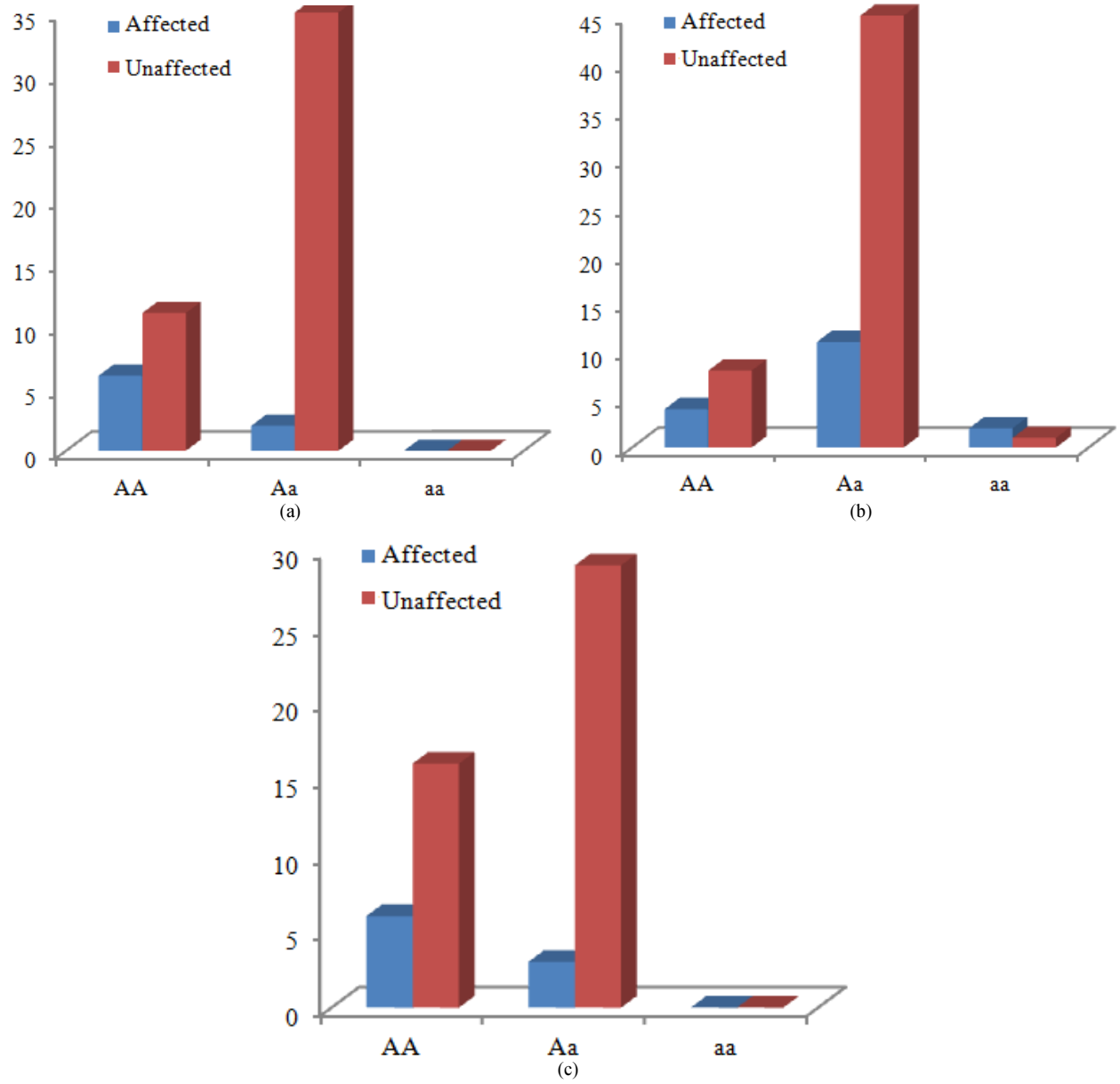

Fig. 1. (a) Frequency of malaria for different IL-5 rs1800474 genotypes, odd ratio: 0.11, p-value = 0.008, (b) Frequency of malaria for different IL-4 rs734244 genotypes, p-value = 0.11, (c) Frequency of malaria for different IL-13 rs1881457 genotypes, odd ratio: 0.33, p-value = 0.24

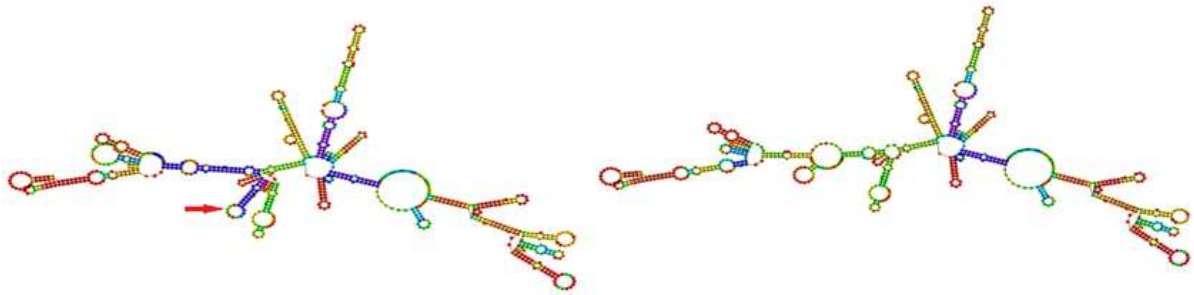

Fig. 2. To the left: The secondary structure of IL-5 mRNA, to the right: The structure of the same molecule with the variation rs1800474. Arrow indicates the site of the stem loop

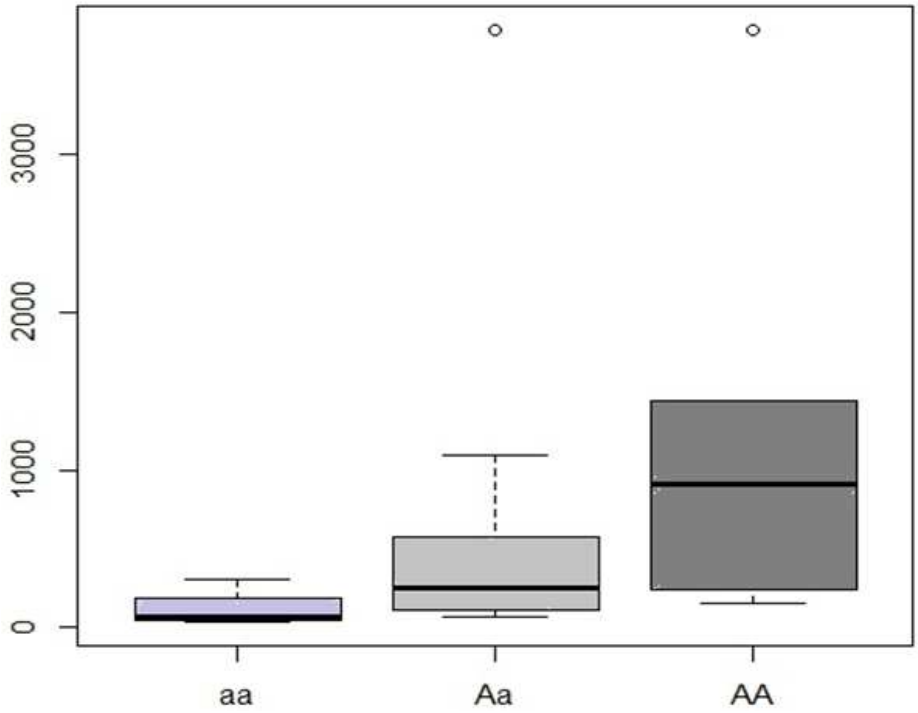

Fig. 3. The correlation between different IL-4 rs734244 genotypes and mean serum IgE level (IU/mL), (p-value=0.32)

In addition, rs1800474 was found to change the secondary structure of IL-5 mRNA by changing a stem loop structure in the center of the molecule, Fig. 2. There was stepwise increase in mean serum IgE level from homozygous minor (aa) to heterozygous (Aa) and homozygous major (AA), p-value = 0.32 Fig. 3. The latter genotype is associated with (A) allele which binds many transcription factors. In another unpublished data of ours we found that IL-4 mRNA level is twice that of the control (the samples were taken randomly from Hausa population).

Mir SNP predicted no result for miRNA binding sites and Splice Port predicted no effect for splicing variations. PLINK tool showed that there is no SNP to SNP interaction; every SNP has a unique effect.

Discussion

Visceral Leishmaniasis (VL) and malaria are two major parasitic diseases which overlap geographically and may co-exist in the same patients. Van den Bogaart *et al*. (2014) compared the analysis of cytokine profiles from co-and mono-infected patients and highlighted significant differences in the immune response mounted upon co-infection, confirming the ability of L. Donovani and *P. falciparum* to mutually interact at the immunological level. The study clearly revealed the positive role of TH1 cytokine response and TNF-alpha in both malaria and visceral leishmaniasis, but the role of TH2 cytokine response in co-infected individuals is yet to be determined (Van den Bogaart *et al*., 2014). Many studies revealed the positive role of TH2 cytokine

response in the clearance of malaria but not visceral leishmaniasis (see below). The different cytokine TH2 response in these two diseases may result in variable pressures in 5q31 region in areas where malaria and visceral leishmaniasis are both endemic. In Koka village there was no evidence of overt co-infection of malaria and VL, but occult infections remain to be shown.

In this study we suggested that the observed excess in heterozygosity in 5q31.1 region is due to unbalanced polymorphism (overdominance) between two major endemic diseases; malaria and visceral leishmaniasis. But the model cannot be simple as in the case of sickle cell anemia and malaria; because cytokine responses for different environmental triggers are very complex and many contradicting factors favor up or down regulations of different immunological responses, malaria favors a strong TH2 response; high level of IL-4 and IL-5 in malaria positive patients correlates with mild symptoms and rapid parasite clearance (Prakash *et al*., 2006; Troye-Blomberg *et al*., 1990). We failed to detect significant association between risk of malaria and serum IgE level (p-value = 0.07) probably due to the small sample size, but Interestingly, we found that the haplotype GCG of IL-4, IL-5 and IL-13 respectively which is associated with greater risk of malaria (p-value = 0.02), is the haplotype associated with little/no transcription factor binding and decreased mRNA stability (i.e., low cytokine TH2 profile) as predicted by the different tools. On the other hand in visceral leishmaniasis TH2 response has negative outcome; it correlates with progressive lesions and disseminated leishmaniasis (Ribeiro-de-Jesus *et al*., 1998). In Sudan, showed that IL-4 contributes to the susceptibility of visceral leishmaniasis (Mohamed *et al*., 2013).

These two factors (malaria and VL) acting differently probably result in a TH2 cytokine response that is optimum for these strong forces and manifested in the form of excess heterozygosity for the SNPs that have direct effect on the expression, regulation and function of interleukins. There are probably other factors besides malaria and visceral leishmaniasis; like schistosomiasis, asthma and childhood respiratory infections acting on 5q31 chromosomal region forming a complex interplay driving the locus towards excess heterozygosity. This illustrates how complex gene-environment interaction is and understanding it allows us to comprehend the factors that shaped human evolution both at molecular and phenotypic level.

Conclusion

Immunological interaction between malaria and visceral leishmaniasis is very complex, both diseases require strong cytokine TH1 response but for cytokine TH2 the story is different. SNPs in 5q31 which have direct role in the expression of interleukins are subject to balancing selection (heterozygosity) that drives the locus towards optimum response.

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Author's Contributions

Mutaz Amin: Analysed the data and wrote and submitted the manuscript.

Abdelbadea Elhassan: Provided the raw data,

Kirk Rocket: Edited the manuscript

Muntasir Ibrahim: Revised and supervised the work.

Ethics

The study has been approved by the Ethics Review Committee of the Institute of Endemic Diseases, University of Khartoum. No ethical issues were aroused during or after publication of the manuscript.

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