Plasmodium falciparum in Brazil: red blood cell variants & parasite invasion

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Malaria in the Brazilian Amazon region

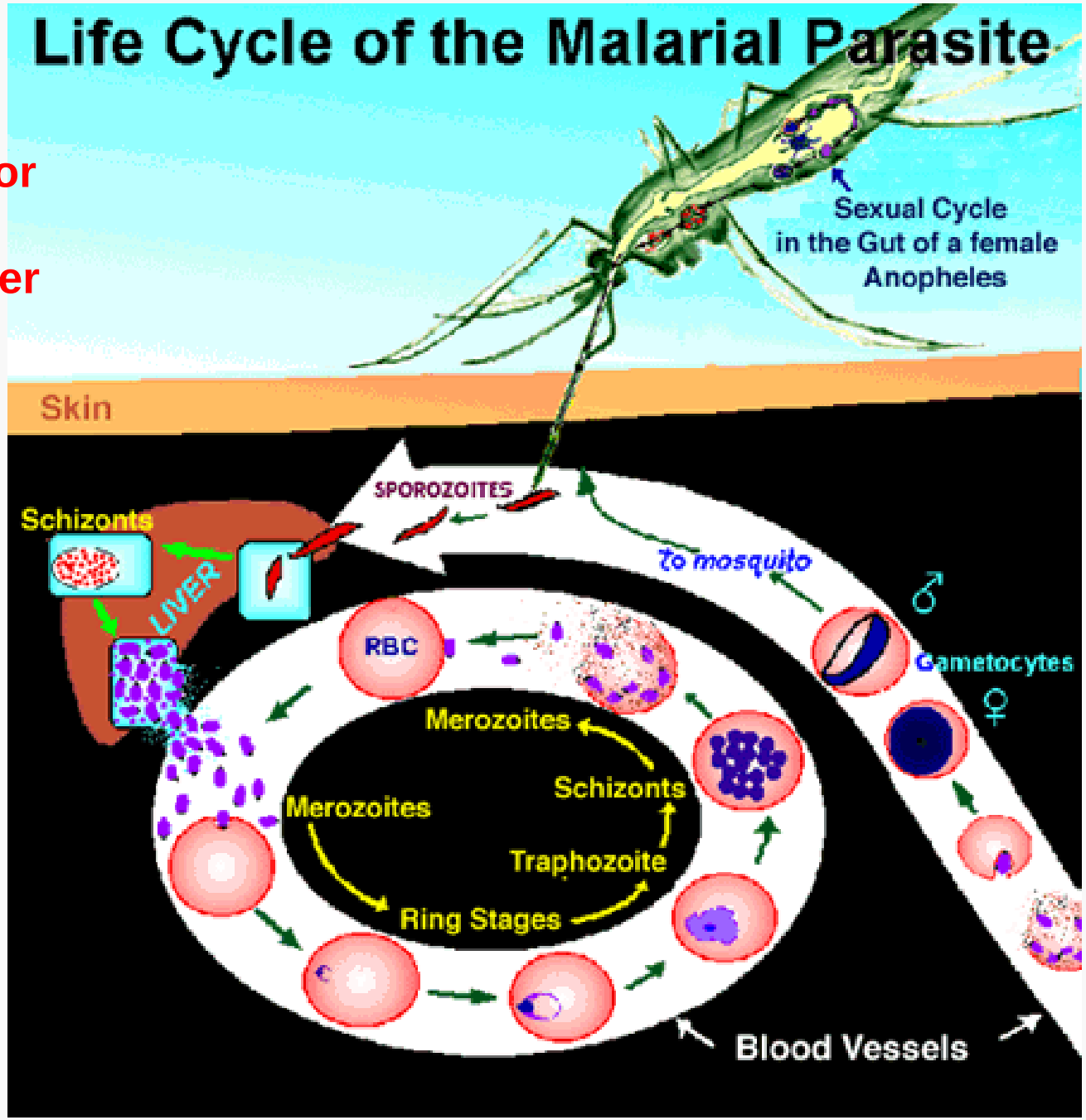
- **Malaria is the most important vector-borne human disease**
  - ***P. falciparum***
  - ***P. vivax***
  - ***P. malariae***
  - ***P. ovale***

- **These parasites are responsible for approximately 300-500 million new infections worldwide annually**

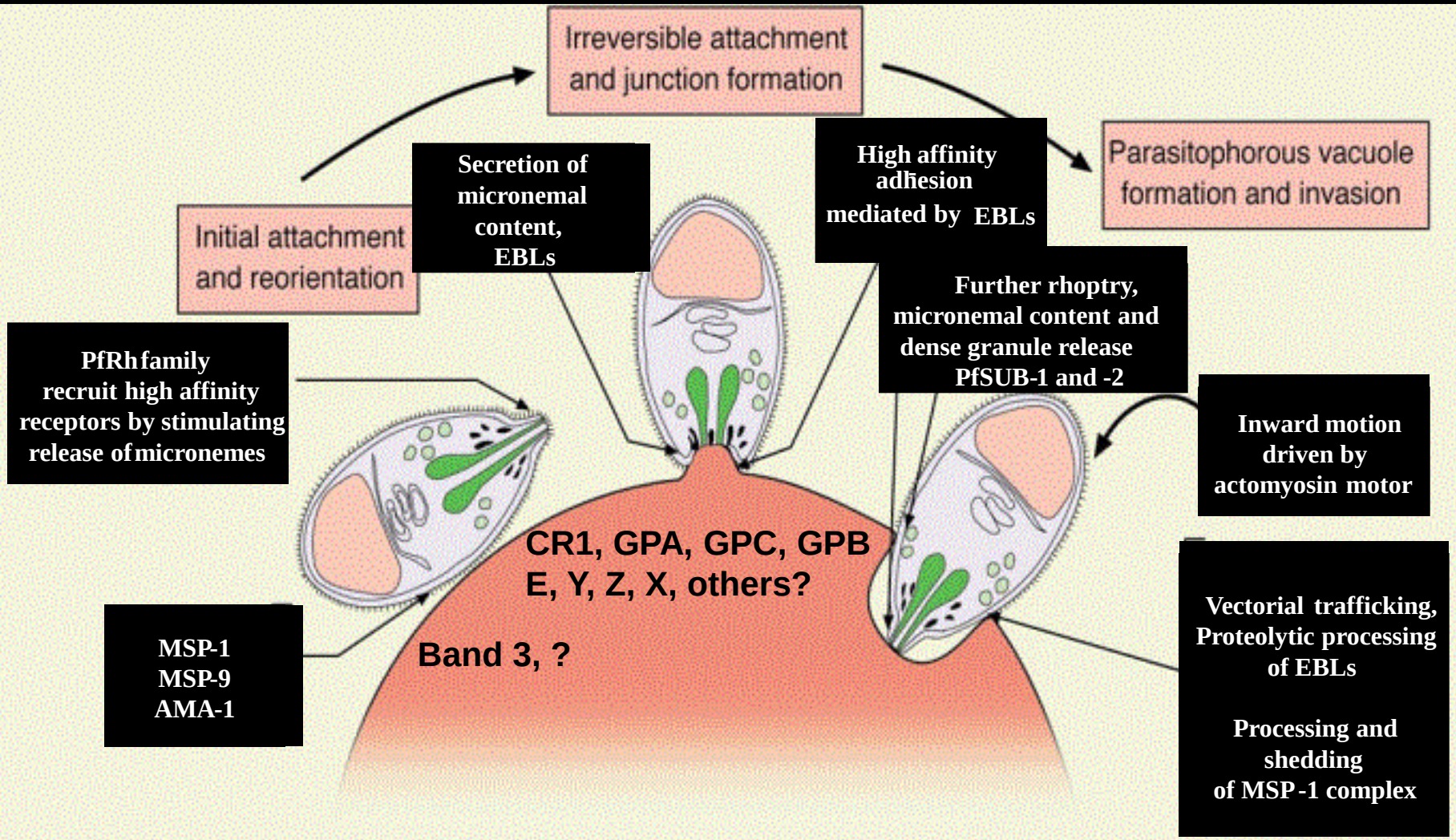
- ***P. falciparum* infection alone accounts for ~1 million deaths annually**

- **In South America a large proportion of all malaria cases occur in the Brazilian Amazon**
  - **Incidence increased significantly between 1970's-1990's**
  - **Approximately 500,000 cases are now reported annually; ~20% of which are of *P. falciparum***
  - **Integrated mix of environmental and sociodemographic risk factors contribute to persistence of malaria transmission in the Amazon region and around the malaria belt**

The specificity of malaria parasites for RBCs appears to depend on a number of ligand-receptor interactions



Parasite invasion into the red cell is a multi-step process involving several specific interactions between receptors on RBCs and parasite ligands



http://www.youtube.com/watch?v=4MYmNaufItU&feature=player_embedded

P. falciparum EBA and PfRh ligands and their RBC receptor interaction profiles

Parasite Ligand	RBC receptor	Binding profile/invasion
EBA-175	GPA	NsTsCr
EBA-140	GPC	NsTsCr
EBA-181	Unknown (Receptor E)	NsTrCs
EBL-1	GPB	NsTrCs
PfRh1	Unknown (Receptor Y)	NsTrCr
PfRh2a	Unknown	NrTrCs
PfRh2b	Unknown (Receptor Z)	NrTs/rCs
PfRh4	CR1	NrTsCs
PfRh5	Unknown	NrTs/rCr
Unknown	Unknown (Receptor X)	NrTsCr/s
Unknown	Unknown (receptor A)	NrTrCs
Unknown	Unknown (Receptor N)	NrTrCr

Red blood cells variants & parasite invasion

- **The use of particular RBC receptor and parasite ligand is not static in *Plasmodium falciparum* partly to provide greater flexibility and advantage in coping with:**
  - **polymorphisms in host red cell surface molecules**
  - **in evading immune responses**
- **Field isolates show greater variability than the laboratory strains**

Advantages of studying malaria in Brazil

The Brazilian population exhibits extensive polymorphism in blood group antigens, some of which are unique to this population and are the result of five centuries of interethnic mixed marriages between Native-American, Black and Caucasian populations.

The endemic area is characterized by a hypo-endemic pattern of malaria infection, due mainly to its low demographic index, which provides a special advantage: despite having a highly divergent population of malaria parasites, patients are infected by single clones.

Invasion profiles of MG field isolates into enzyme treated RBCs

Invasion	Parasite	Invasion Rate (%) in treated RBCs		
		Neuraminidase [N]	Trypsin [T]	Chymotrypsin [C]
Type 1:				
NsTsCr	3D7	42 ± 9.5	34 ± 33	74 ± 26
	JSL	49 ± 18	49 ± 22	103 ± 20
	ALR	12 ± 0	5 ± 0	61 ± 0
	PSS1	25 ± 0	41 ± 8	76 ± 36
	BHZ	43 ± 12	24 ± 3	100 ± 36
	04Q	30 ± 23	38 ± 2	81 ± 6
	FFS	35 ± 2	37 ± 2.4	121 ± 36
	GVM	37.5 ± 12.5	42 ± 4.5	134 ± 18
Type 2:				
NsTrCr	Dd2	8.6 ± 2.8	70 ± 4	56 ± 4
	GOS	3.6 ± 2.8	100 ± 30	69 ± 10
	LANA	0 ± 0	50 ± 3.5	125 ± 17
	NS	1 ± 1	125 ± 27	102 ± 16
	IPN	10.4 ± 3.9	63 ± 15	78 ± 16
	35Q	25 ± 0	150 ± 50	165 ± 35
Type 3:				
NrTsCr	7G8	65 ± 8	9 ± 4.6	52 ±11
	21Q	77.5 ± 2.5	45 ± 5	98 ± 17
Type 4				
NsTsCs	RPN	36 ± 11	21 ± 6.5	27 ±1.5

Lobo C et al. 2004

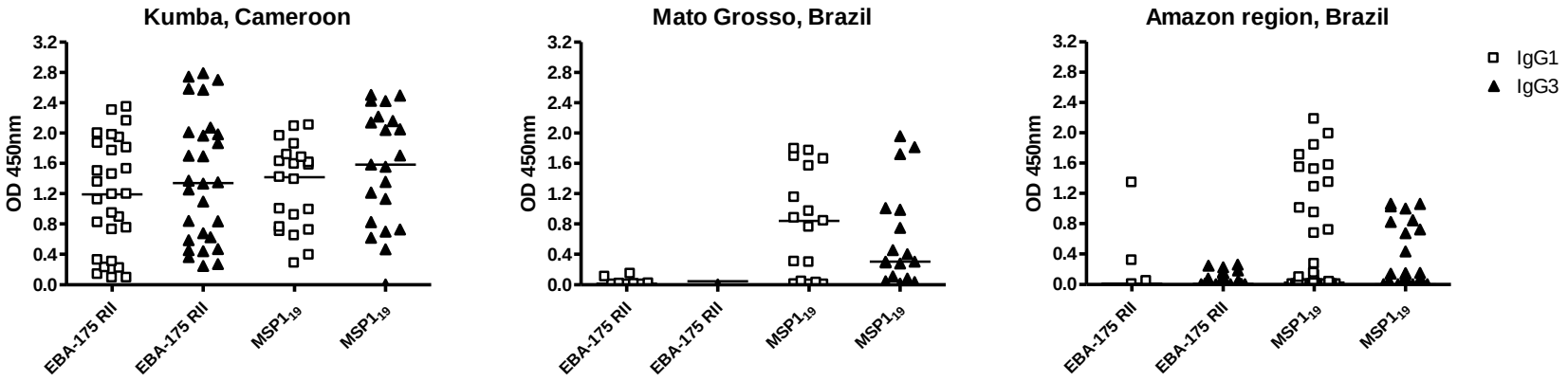
Invasion of NsTsCr parasites into En(a-) mutant RBCs (GPA deficient)

Parasite	WT	FrWT	En(a-)
3D7	2.3	1.5	0.8
ALR	1.4	1.2	0.05
JSL	1.8	1.6	0.9
GVM	1.4	1.0	0.9
FFS	0.8	0.8	0.15
04Q	1.3	1.5	0.9
BHZ	1	0.9	0.7
PSS1	1.3	1.2	1.2

Only 2/7 field isolates are GPA- dependent

Lobo C et al. 2004

Antibody responses to EBA-175 and MSP-1_{19} in MG, Brazilian Amazon vs. Cameroon (N = 17, 39 vs. 28)



Ford L et al 2007

The antibody responses to EBA-175, the ligand of GPA, are much less prevalent in the Brazilian infected population

We hypothesized that the GPA/EBA-175 pathway of invasion in not used predominately by Brazilian isolates

Ford L et al. 2007

Invasion profiles of Belém field isolates

		% invasion vs. untreated RBCs			
Invasion pathway	Isolate	Neuraminidase	Trypsin	Chymotrypsin	pathway/potential receptor
NrTrCs	262	57.4%	76.4%	17.5%	New pathway or GPB/EBL-1; Rec Z/PfRh2b
NsTrCs	BEL00509	39.3%	63.1%	8.3%	New pathway or GPB, EBA-181/Rec E
NrTrCr	279	78.4%	60.4%	63.0%	New pathway
NsTsCr	1076	46.9%	33.1%	77.2%	GPA/GPC 3D7-like pathway
NsTrCr	1107	38.0%	60.8%	55.7%	Unknown Dd2-like pathway
NrTsCs	516	86.7%	19.5%	44.2%	New pathway or Rec X/CR1/GPB
	526	66.7%	0.0%	34.0%	
	1038	52.5%	22.7%	24.3%	

Neuraminidase (N); Trypsin (T); α-Chymotrypsin (C)
Resistance (r), > 50% invasion vs. untreated WT RBCs
Sensitive (s), < 50% invasion vs. untreated WT RBCs
Yellow highlights, sensitivity to treatment = <50% invasion vs. untreated RBCs

Invasion is mediated by a combined EBA/PfRh pathways

Ricardo Machado et al., unpublished

% responders to EBA and PfRh ligands in infected individuals living in the Brazilian Amazon vs. Cameroon

	Kumba, Cameroon		Amazon, Brazil	
	IgG1	IgG3	IgG1	IgG3
Antigen/ligand	Responders (%)	Responders (%)	Responders (%)	Responders (%)
EBA-181	38.1	65	10.3	69.2
EBA-140	96.4	100	25.6	38.5
PfRh1	39.4	96.1	31.2	25.0
PfRh2a	29.4	16.1	15.0	0
PfRh2b	10.0	49.4	15.0	47.5
PfRh5	36.7	66.1	20.0	25.0

Ricardo Machado et al. unpublished

Summary

- **Differences in the immunoreactivity could be linked to:**
  - **heterogeneity of the RBCs between the two endemic populations in Brazil and Africa**
    - **dictate parasite's choice of invasion pathways**
    - **expression of specific EBLs and/or PfRh ligands**
    - **induction of ligand specific antibody responses**
- **The preferred invasion pathways used by Brazilian isolates:**
  - **a low usage of the EBA-175/GPA invasion pathway**
  - **use of GPA-independent pathways possibly GPC, GPB, EBA-181, PfRh2b and/or other novel pathways**

How do we link invasion profiles seen in different parasite isolates with the use of distinct ligands for invasion?

- **Screen for polymorphisms in the potential binding domains of the different invasion ligands**
- **If evidence of positive selection is obtained, one can study if it is linked to host immune response or host receptor heterogeneity**
- **Since these proteins are vaccine candidates, information on sequence polymorphism in the binding domains of these ligands will be highly relevant to vaccine development studies**

Polymorphisms of EBA-181 in Brazilian field isolates

EBA-181	27/52: KVIQN (NsTsCs; GPA independent)
	9/52: RVIQN (NsTrCs)
	8/52: RVNKN (NrTrCr; GPB independent)
	7/52: RVNQN (NrTsCs)
	1/52: RVNKK (NsTsCs; GPA independent)

	EBA-181 RII	# samples
BEL	RVNQN	2
	KVIQN	6
MCP	RVIQN	2
	KVIQN	8
	RVNKN	2
	RVNQN	2
PVL	RVIQN	3
	KVIQN	5
	RVNKN	1
RBR	KVIQN	6
	RVNKN	1
MG	RVIQN	4
	KVIQN	2
	RVNKN	4
	RVNQN	3
	RVNKK	1
		52

Ricardo Machado et al., unpublished

Polymorphism of PfRh1 in field isolates from 5 Brazilian regions

PfRh1 Polymorphism	amino acid # as in HB3										
Isolates (# isolates having the variant)	2830	2831	2832		2849	2850		(code)	2851	2852	2853
HB3	V	I	(H	N)	x9	(Q	N)	X1	Q	K	D
Dd2, 3D7, MG (6), PVL (2), RBR (3)	V		(H	N)	x4	(Q	N)	x1 (10D)	Q	K	D
7G8, MG (4), BEL (5)	V		(H	N)	x6	(Q	N)	x2 (4D)	Q	K	D
MG (1), MCP (2), PVL (6), RBR (4)	V		(H	N)	x4	(Q	N)	x3 (6D)	Q	K	D
MG (1), MCP (2), RBR (1)	V		(H	N)	x5	(Q	N)	x2 (6D*)	Q	K	D
MG (2), MCP (5), BEL (8), PVL (8)	V		(H	N)	x3	(Q	N)	x2 (10D*)	Q	K	D

- 2 variants are 3D7/Dd2- (11) or 7G8-like (9)
- none are HB3-like
- majority of the isolates have 3 novel variants (13, 4, 23)
- in MCP only new variants are present

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Polymorphism of PfRh2a in field isolates from 5 Brazilian regions

PfRh2a Polymorphism	amino acid # as in 7G8														
Isolates (# isolates having the variant)	2548	2699	to	2713	2734	2735		2736	2737	2738	2739	2740	2741	(code)	2803	to	2852
7G8, MG(1), MCP(9), BEL(1), PVL(6), RBR(5)	D	no Del			K	K		E	A	L	K	K	Q	(pepA)	50 aa Del		
HB3, MG(8), MCP(5), BEL(10), PVL(9), RBR(4)	D	15 aa Del			K	K		E	A	L	K	K	Q	(pepA)	50 aa Del		
Dd2, MG(5)	D	15 aa Del			Q	K	E	E	E	L	K	R	Q	(pepB)	50 aa Del		
3D7	A	15 aa Del			K	K	E	E	E	L	R	K	K	(pepC)	no Del		

- 3 variants are Dd2- (5, MG), HB3- (36) or 7G8-like (22)
- none are 3D7-like
- The peptide variants (2734-2741) are part of a 21 aa peptide (LEREKQEQL**QKEEELKRQ**EQY) that was shown to bind to RBCs and thus potentially is involved in the ligand receptor interaction

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Polymorphism of PfRh2b in field isolates from 5 Brazilian regions

PfRH2b Polymorphism	amino acid # as in 3D7														
Isolates (# isolates having the variant)	2547	2635	2715 TO 2766			2769	2770	2771	2772	2773	2774	2775	2776	2777	(code)
3D7	A	K	no del			Q	K	E	E	E	L	R	K	K	(pepC*)
7G8,MG(3),MCP(2)	A	E	no del			Q	K	E	E	E	L	R	K	K	(pepC*)
HB3,MCP(4),BEL(1),PVL(8),RBR(6)	D	E	no del			Q	K	E	E	E	L	R	K	K	(pepC*)
Dd2,MG(4),MCP(3),BEL(3),PVL(1)	D	E	52 aa del			Q	K	E	E	E	L	K	R	Q	(pepB)
MG(7),BEL(2),PVL(3),RBR(2)	D	E	52 aa del			Q	K	E	E	E	L	R	K	K	(pepC*)
PVL(1)	D	E	52 aa del			Q	K	E	E	E	L	R	K	Q	(pepD)
BEL(7)	D	E	52 aa del			K	K		E	A	L	K	K	Q	(pepA)

- 3 variants are 7G8- (5), Dd2- (11) or HB3-like (19)
- none are 3D7-like
- 3 novel variants (14, 1, 7)
- The new variants have a distinct 52 aa deletion as Dd2
- The peptide variants (2734-2741) are part of a 21 aa peptide (LEREKQEQL**QKEEELKRQ**EQY) that was shown to bind to RBCs and thus potentially is involved in the ligand receptor interaction

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Polymorphism of PfRh4 in field isolates from 5 Brazilian regions

PfRh4 Polymorphism	amino acid # as in 3D7																					
Isolates (# isolates having the variant)	1482	1501					1510	1528	1537	1686	1687	1688	1689	1690	1691	1692	1693	1694	1695	1696	1697	(Code	1702	1703	1704	1705
3D7,MG(1)	K									D	E	V	E	D	E	V	E	D	E	V	E	3	N	E	N	E
Dd2,7G8,MG(6),MCP(7),BELL(12),PVL(3),RBR(9)	R									D	E	V	E	D	E	V	E					2	N	E	N	E
HB3	R									D	E	V	E	D	E	V	E					2			N	E
MG(1)	R	10 aa insertion								D	E	V	E	D	E	V	E					2	N	E	N	E
MG(4),MCP(3),PVL(2)	R	10 aa insertion								D	E	V	E									1	N	E	N	E
MG(2)	R									D	E	V	E	D	Q	V	E					1+1	N	E	N	E
PVL(6),RBR(2)	R							10 aa insertion		D	E	V	E	D	E	V	E					2+1	N	E	N	E

- 2 variants are 3D7- (1) or Dd2/7G8-like (37)
- none are HB3-like
- 4 novel variants (1, 9, 2, 8)
- 3 of the new variants have each a distinct 10 aa insertion

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Summary

- Not all PfRh variants reported for lab strains were found in the Brazilian field isolates
- New variants of PfRh1, PfRh2b and PfRh4 were found -- in Macapá, for example, only new PfRh1 variants are expressed
- Each isolate has a unique combination of EBA-140, EBA-181, EBL-1 and PfRh polymorphic variants
- Future analysis of parasite isolates with naturally modified ligands might reveal differential associations between invasion pathways and the corresponding defined native ligand variants
- Majority of the isolates express EBL-1, the ligand for GPB
  - in other Amazonian regions (Peru and Colombia) EBL-1 is mostly not expressed
  - is GPB an important receptor for Brazilian parasites?

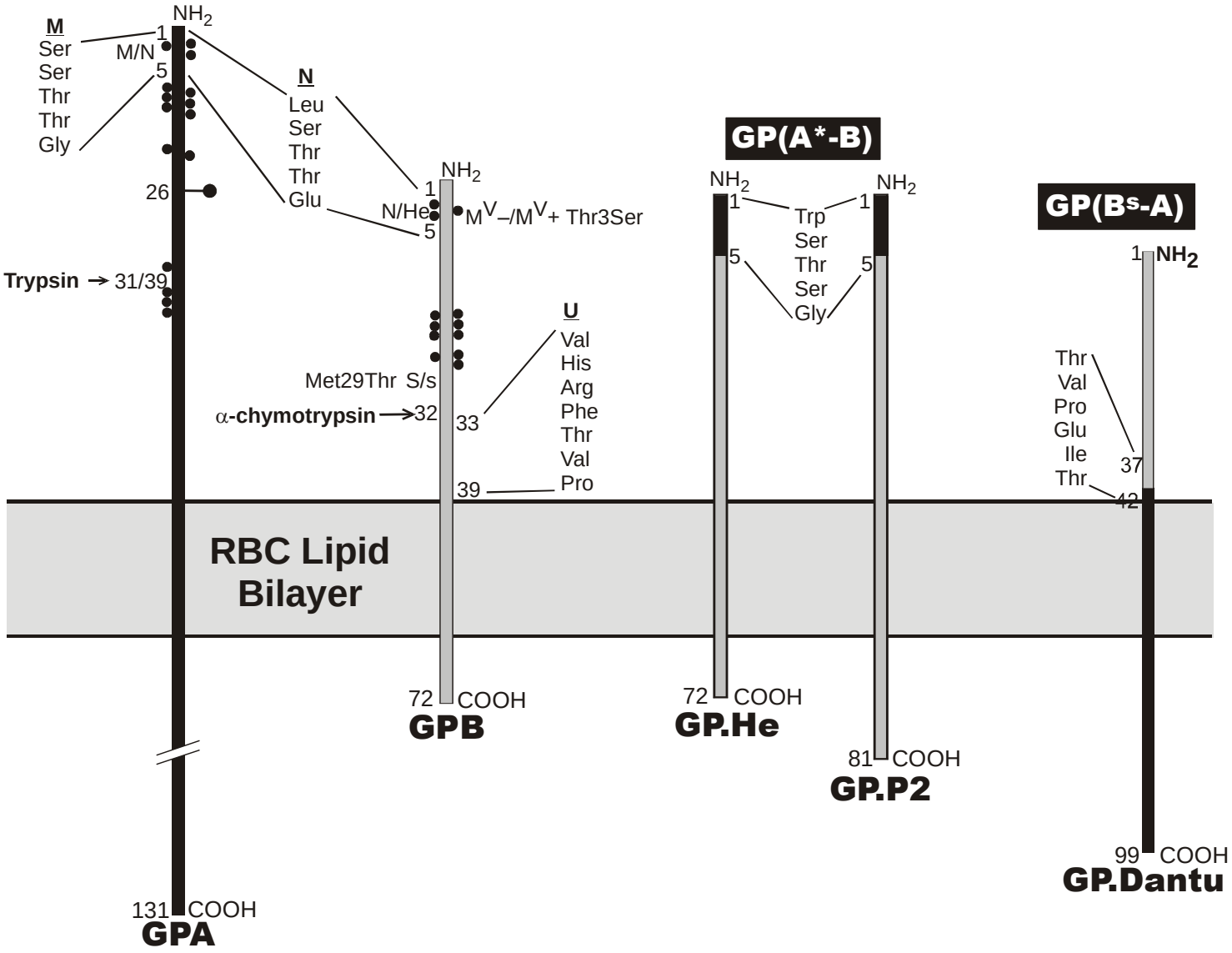
Erythrocyte receptor polymorphisms and malaria

- **RBC mutations that provide protective effects against illness and death caused by malaria:**
  - **thalassaemia**
  - **sickle cell disease**
  - **hereditary ovalocytosis**
  - **glucose-6-phosphate dehydrogenase (G6PD) deficiency**

- **Duffy null phenotype provides a protective effect against *P. vivax* infection and is highly prevalent in black people**

- **RBC phenotype containing a deletion in Band 3 (SLC4A1D27) or in GPC (Ge phenotype, GYPCDex3) is highly prevalent in Papua New Guinea**

The GPA/GPB glycophorins



Polymorphisms in the erythrocyte GPA and GPB in highly endemic areas for malaria

- **Higher prevalence of the GPB S–s–U– phenotype in Africa (2-8%)**
  - **20% among the pygmies**
  - **up to 37% of West Africans**
- **The GP.Dantu variant (a GPB-GPA hybrid) is more prevalent in Africa (4%) in comparison to European people (0%)**
- **The Henshaw phenotype (GP.He; GPA/GPB hybrid) is only found in Blacks:**
  - **African Americans -- 3%**
  - **In Natal, Brazil, 7% of African donors have the Dantu variant**
- **A novel *GYPB-A-B* recombinant allele (Morobe allele) was found in a highly endemic area in Papua New Guinea**
- **In South and SE Asia significantly higher prevalence of MUT, MINY, HIL, Hop, St^a, Mur and Mi^a antigens carried by GPA/GPB hybrids are present (0.68-15% vs. 0% in Caucasian), some of which also affect the expression of the GPB S antigen on RBC surface**
- **The existence of these GPB variants in people of African origin has led to the speculation that these variants may have been selected as a result of the relative resistance that they confer against *P. falciparum* malaria**

Significant correlation between GPB S+s+ and S+s- phenotypes and malaria infection in Rio Branco, Porto Velho and Belem

	Belém		Macapá		Porto Velho		Rio Branco	
	Para State		Amapá State		Rondonia State		Acre State	
Group	Donors	Patients	Donors	Patients	Donors	Patients	Donors	Patients
N=	100	100	117	94	100	64	100	51
Blood Group	Frequency (%)							
S^-s^+	50**	38	41	39.3	62**	46.9	62**	39.2
S^+s^+ or S^+s^-	50	62*	59	60.7	38	53.1*	38	60.8*
**↑ in donor	P < 0.05		NS		P < 0.05		P < 0.05	
*↑ patients	P < 0.05		NS		P < 0.05		P < 0.05	

The GPB S-s+ phenotype was hypothesized to potentially contribute to resistance to *P. falciparum* malaria in Rio Branco, Porto Velho and Belem

Cavasini CE et al 2006

Association study between polymorphisms in GPB and susceptibility to *P. falciparum* infection in in Porto Velho, Rondônia

- **To establish whether molecular variation in the *GYPB* gene, particularly the one that generates the *GYPB*S/s* alleles, influences host susceptibility to infection with *P. falciparum*, taking into account the possible confounding factor of ethnicity, age and sex**

- **To address the possibility that the *GYPB**S/s alleles might not be the only functional GPB variants that can modify invasion efficiencies via the GPB pathway and thus prevalence, but rather other *GYPB* variants, some of which might be in linkage disequilibrium (LD) with *GYPB**S/s, or their haplotypes, are in fact responsible for these functional consequences**

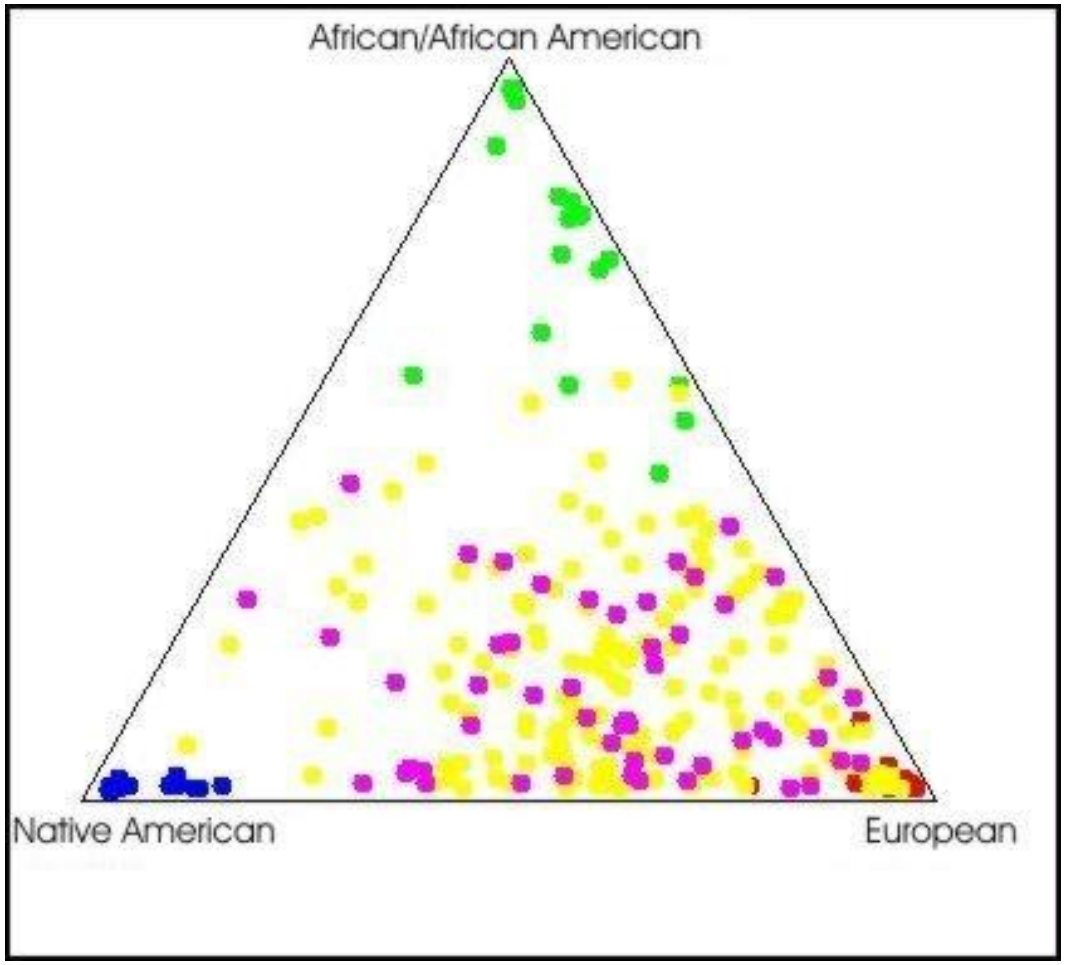
- **Two groups of individuals were recruited in Porto Velho, Rondonia during 2006 – 2007. All consenting individuals have been interviewed and information regarding their gender, age, date of birth, place of birth, ethnic origin of their parents and grandparents, length of residence in their present locality, history of exposure and past treatment for malaria were recorded**

  - **83 Pf infected individuals (cases)**
    - **Pf Giemsa +**
    - **Pf PCR+**

  - **199 uninfected individuals who were born and/or lived in the same endemic region for over ten years, exposed to infection but not had malaria in the past or over the 2-3 year study period (control)**
    - **OptiMal®-**
    - **Pf PCR-**
    - **Anti-MSP-1 negative**

Tarazona-Santos E et al 2011

The infected and non-infected are ethnically similar



genotyping 62 Ancestry Information Markers (AIMs): 14 SNPs and 42 INDELS; cases (pink) and controls (yellow)

Significant association between *GYPB*S/GYPB*S* and *GYPB*S/GYPB*s* prevalence and *P. falciparum* infection

	Mean Age (SD*)	Females/ Males	African ancestry (SD)	European ancestry (SD)	Native American ancestry (SD)	SS/Ss (%)	ss (%)	Hardy-Weinberg equilibrium
Controls (n=199)	28.29 (9.16)	97/102	0.18 (0.14)	0.54 (0.19)	0.28 (0.17)	99 (49.75)	100 (50.25)	P = 0.65
Cases (n=83)	31.76 (11.99)	27/56	0.18 (0.14)	0.54 (0.18)	0.28 (0.18)	58 (69.88)	25 (30.12)	P < 0.01
Total (n=282)	29.30 (10.16)	124/158	0.18 (0.14)	0.54 (0.19)	0.28 (0.17)	Association test assuming dominance of S P = 0.02**		

The frequencies of the *GYPB*S/GYPB*S*, *GYPB*S/GYPB*s* and *GYPB*s/GYPB*s* were determined by:

- **Allele-specific PCR; *GYPB* Exon 5 combination AS/PCR-RFLP assay**
- **Human Erythrocyte Antigen BeadChip DNA analysis**
- **Sequencing of *GYPB* exon 4**

GYPB haplotypes frequencies in the PVL Brazilian population

Linkage disequilibrium among common SNPs in *GYPB*

	rs4835511	rs12499907	rs12499906	rs41338748	rs7662277	rs7683365[a]	rs7661933	rs1132783[b]	Cases	Controls	Total
Ancestral allele	C	T	T	T	T	C	T	G			
GYPB-s1	T	.	.	.	.	.	.	.	5	13	18
GYPB-s2	.	.	.	.	.	.	.	.	37[c]	109	146
GYPB-s3	.	.	.	.	.	.	.	C	4	0	4
GYPB-s4	.	.	.	A	.	.	.	C	6	19	25
GYPB-s5	.	.	.	A	.	.	.	.	0	1	1
GYPB-S6	.	G	.	.	A	T	A	.	1	1	2
GYPB-S7	.	.	G	.	A	T	A	.	0	1	1
GYPB-S8	.	G	G	.	A	T	A	.	26	49	75
GYPB-S9	.	G	G	A	A	T	A	.	3	7	10
Number of chromosomes									**82**	**200**	**282**

Non-synonymous substitutions are underlined

[a] SNP accounting for S (Met) and s (Thr) phenotypes

[b] Ser(G)/Thr(C) – in the transmemberane region of GPB

[c] The modal haplotype in each group is underlined

The Ss SNP (rs7683365) is in LD with only few common silent SNPs in exon 4 and its adjacent introns, but not with all

No LD between the S/s alleles (rs7683365) and the non-synonymous common SNP rs1132783 (Ser/Thr) in exon 5

Summary

- **Our study has verified for the first time that molecular variation in the *GYPB* gene, particularly in the *GYPB**S/s alleles, can influence host susceptibility to infection with *P. falciparum* (Porto Velho, Rondônia)**
  - **Parasites that invade RBCs through GPB might be less successful in S-s+ individuals – needs to be confirmed experimentally**
  - **This Met29Thr polymorphism might influence the structure of the GPB ; GPB s+ (Thr) vs. GPB S+ (Met) gains potentially a new site for O-glycosylation, which can likely alter protein conformation and thus influence the efficiency of invasion by the parasites and ultimately, susceptibility to infection**
  - **This Met29Thr polymorphism may also affect dimerization of GPB with other GPB and/or with GPA molecules**
- **The GPB invasion pathway might be more dominant in this region**
  - **To identify isolates that preferentially utilize GPB for invasion**
  - **Use specific GPB peptides and antibodies to block invasion**
  - **Differential binding of the EBL-1 ligand to variant GPB RBCs**

Acknowledgements

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