Towards a vaccine against pregnancy associated malaria

B-cell epitopes and sequence variation in the Plasmodium falciparum VAR2CSA DBL3X domain

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Malaria – life cycle

Anopheles

Source: Miller LH (2002)
Malaria

- Genus Plasmodium
  - Species: *falciparum*, vivax, ovale, malariae

- Annual clinical cases/deaths: 3-500,000,000/1-2,700,000
  - 2% of all deaths (90% of these in sub-Saharan Africa)
  - Only matched by HIV (2.8 megadeaths) and TB (1.7 megadeaths)

Malaria – one mechanism of immunity

Source: Miller LH (2002)
Malaria - PfEMP1 and the var genes

Each parasite has ~60 var genes encoding PfEMP1s

Each PfEMP1 has 3-8 domains

200-400kD

High intra/inter genomic sequence variation

Expression of a var gene is **mutually exclusive** in one iRBC

Source: Adapted from Wahlgren et al (sites.huji.ac.il/malaria/maps/PfEMP1.html)
Pregnancy associated malaria (PAM)

Pregnancy: placenta a new receptor, a new niche

P = does not bind any tissue receptor (low conc = low Ab)

Y = receptor on brain tissue
X = receptor on placental tissue

Spleen
Brain tissue
Placental tissue
Pregnancy associated malaria (PAM)

- Maternal anaemia and death (10,000)
- Premature delivery
- Intrauterine growth retardation
- Stillbirth
- ~200,000 annual perinatal deaths due to PAM
- In some endemic regions, 40% of all newborns have low birth weight caused by PAM
- **IgG against CSA-binding iRBC protects against lbw and maternal anaemia = vaccine possible**

PfEMP-1 VAR2CSA

- Upregulated in iRBC adhering to CSA and in placental isolates
- Ab to VAR2CSA are acquired by pregnant women exposed to malaria
- VAR2CSA IgG protects against lbw
- Disruption of var2csa => marked reduction in CSA adherence

Recombinant DBL3 binds CSA

- Highly conserved between falciparum strains compared to most other var genes (nucleotide sequence diversity is 10-30%)

The VAR2CSA DBL3X domain – Placental cDNA sequencing

- Parasites from Senegalese placental samples were sequenced from cDNA

Homology model from Erythrocyte Binding Antigen 175 (EBA-175)
The VAR2CSA DBL3X domain – Sequence Variation

- For DBL3X: $\pi = 8.5\%$ (low)
- No obvious subgrouping:
  Local Senegalese variation ~ global variation
The VAR2CSA DBL3X domain – Selection analysis

- Diversifying selection may be seen in connection with immune escape (epitopes)
- Non-synonymous/Synonymous mutation rates ($\omega = dN/dS$)
  - neutral evolution ($\omega=1$)
  - purifying (negative) selection ($\omega<1$)
  - diversifying (positive) selection ($\omega>1$)

- Positive selection found in hypervariable blocks, but no clear picture
• Sites under positive selection are mainly surface exposed and on one side of the DBL3X domain

• Conserved side may be functionally constrained
The VAR2CSA DBL3X domain – Recombination

The VAR2CSA DBL3X domain – Recombination

- Variations in $r$ estimated over DBL3X
  ($r =$ per generation cross-over recombination rate)

- Recombinational hotspots found in association with region V1 and V3
  - V1 contains variable number of tandem repeats (VNTR) = microsatellite
  - V3 contains deletions in several of the sequences

- Deletions/insertions may be caused by unequal crossover during recombination at the hotspots
The VAR2CSA DBL3X domain – Parity motif EIEKD / GIEGE

- 2 groups:
  - Primigravidae
  - Multigravidae

- Sequence differences determined by the symmetric Kullback-Leibler distance:

\[
D_{KL} = \sum_{AA} (p - p') \cdot \log\left(\frac{p}{p'}\right)
\]
The VAR2CSA DBL3X domain –
Parity motif EIEKD / GIEGE

Evolutionary pathway on position four in motif:
• lysine (AAA or AAG) ↔ arginine (AGG) ↔ glycine (GGG)

lysine+arginine+glutamate (primi):
• large charged sidechains

glycine (multi):
• no sidechain

Function of motif:
• CSA-binding affinity?
• Epitope?
• 3rd function eg. structural?
The VAR2CSA DBL3X domain – B-cell epitope prediction

- **BepiPred** – a linear B-cell epitope prediction server
- Uses a combination of a hidden Markov model and propensity scale method

Server address: http://www.cbs.dtu.dk/services/bepipred/
- 430 overlapping 30-mer peptides based on 3D7 sequence
- Bound to plate by middle cysteine
- Exposed to Ghanian and Tanzanian PAM patient serum
- Each 30-mer gets a value indicating the amount of binding IgG
The VAR2CSA DBL3X domain –
Peptide array with patient sera

- Eight Ghanian women with PAM history
- V1 and V2 is target. V3 partly deleted in 3D7 = no data
- Conserved α-helix is target for IgG
- Same targets were predicted with BepiPred
The VAR2CSA DBL3X domain – Peptide array with patient sera

- Affinity purification on recombinant DBL3X to identify surface exposure
  - SE1 and SE3 forms structural epitope
  - The α-helix in SE3 is very conserved (C2)
- Depletion by VAR2CSA expressing iRBC to determine binding of native protein
The VAR2CSA DBL3X domain – Surface exposed epitopes

• Most pronounced blocks of positive selection at SE2 and SE3
• Bepipred is optimistic, but finds the surface exposed epitopes
  – SE epitopes (green)
  – Non-SE epit. (blue)
  – Non-3D7 epit. (cyan)
  – Gaps (red)
The VAR2CSA DBL3X domain – Conclusion

• **Causes of sequence variation**
  – Positive selection in blocks (Largest blocks coincide with determined epitopes in V1+V2, but generally not clear correlation)
  – Recombination hotspots => deletions/insertions

• **Structural localization of variation in DBL-domain**
  – Mainly flexible loops (and surface of α-helix)

• **Local African seq. variation = global variation**
  – Most of the sequence variants have probably been observed

• **We have determined surface exposed B-cell epitopes for use in a PAM vaccine**
  – Conserved epitope (SE3)
  – Structural epitope which may include SE1, SE3 and the parity motif
  – SE2 for which we know the possible sequence variants

• **Similar analysis should be performed for remaining VAR2CSA**
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