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SHORT COMMUNICATION

POLYMORPHISM IN A *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN GENE IN SRI LANKA DEMONSTRATED BY SOUTHERN HYBRIDISATION

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Abstract: The gene for a 45kDa merozoite surface protein of *Plasmodium falciparum* (GYMSSA) was amplified by the polymerase chain reaction in fifteen blood samples collected from patients in Dambulla, Galewela, Kurunegala and Polgahawela hospitals. The amplified DNA was hybridised in Southern blots to repetitive region-based oligonucleotide probes specific for the two major allelic forms of GYMSSA identified in culture-adapted laboratory lines of *P. falciparum*. The FC27 allelic form of GYMSSA was detected in thirteen blood samples and the 3D7 allelic form in the remaining two samples.

Key words: Merozoite surface protein, *Plasmodium falciparum*, polymerase chain reaction, polymorphism, Southern hybridisation.

INTRODUCTION

The 185-200 kDa precursor to the major merozoite surface antigen (termed PMMSA, MSA-1, MSP-1) and the 45kDa glycosylated and myristilated smaller surface antigen (termed GYMSSA, MSA-2 or MSP-2) are candidates for malaria vaccine development since antibodies against the molecules inhibit parasite growth and reinvasion in *in vitro* cultures.[1] Two major allelic forms of GYMSSA, differing chiefly in a centrally located repetitive sequence, have been identified in *in vitro* cultured lines and in patient isolates. These are represented by sequences in the K1 and 3D7 cultured *P. falciparum* isolates.[2,3]

Determining the nature and extent of allelic variation in vaccine candidate molecules is important for vaccine development and may also provide strain-specific markers for epidemiological studies. We report here on the use of repeat-region specific oligonucleotides to examine allele distribution in Southern blots of polymerase chain reaction (PCR) amplified GYMSSA DNA, in fifteen isolates obtained from patients in Dambulla, Galewela, Kurunegala and Polgahawela hospitals located in the central and north-western provinces.

METHODS AND MATERIALS

Isolation of DNA from infected blood: Fifteen 1 ml samples of microscopically confirmed *P. falciparum*-infected blood (parasitaemia 0.06-0.90%) were collected in acid citrate dextrose from the Dambulla (3 samples), Galewela (2 samples),

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Kurunegala (8 samples) and Polgahawela (2 samples) hospitals. After removal of plasma, the cells were treated with 7.5ml of 6M guanidine hydrochloride in 0.1M sodium acetate (pH 5.5) and stored at - 80°C until use. Five hundred µl of lysed blood cells were centrifuged at 100,000xg for 20min at 4°C. The supernatant was transferred to microfuge tubes containing 20µl glassmilk (Prep-a-gene, Biorad, USA) and the DNA purified and collected according to the manufacturer's instructions.

Primers for PCR amplification of GYMSSA DNA: A pair of primers from the N and C terminal conserved regions of GYMSSA in the K1 laboratory isolate of *P. falciparum* were used for PCR amplification.[4] The primers for PCR were synthesised with the M13 forward and reverse sequencing primer sequences located at their 5' ends for possible additional use of the primers in sequencing (M13F = TGTAAAACGACGGCCAG; M13R = CAGGAAACAGCTATGACC). The N-terminal primer, termed VM 591/1, corresponded to nucleotides 100-120 in K1 and had the sequence M13F - GCTTATAATATGAGTATAAGG.[4] The C-terminal primer, termed VM 591/2, corresponded to nucleotides 638-657 in K1 and had the sequence M13R - CATATGTCCATGTTGTCCTG.[4] The two primers were kindly provided by Vicky Marshall of the Walter and Eliza Hall Institute for Medical Research, Melbourne.

PCR amplification: This was carried out essentially as described previously.[4] The PCR mix contained 50mM KCl, 10mM Tris - HCl (pH 8.3), 2.5 mM $MgCl_2$, 0.25mM dNTPs, 1% gelatine, 5 pmol of the primers VM 591/1 and VM 591/2, 2.5 U Taq polymerase and 10µl of template DNA purified from malaria-infected blood. The PCR was performed in a DNA thermal cycler model 480 (Perkin Elmer Cetus, USA) for 40 cycles ; 94°C 45s (denaturation), 55°C 60s (annealing) and 70°C 80s (primer extension). 10 µl of the amplified PCR-1 mix was then subject to a second PCR amplification (PCR-2) using fresh PCR mix and otherwise identical conditions. The PCR-2 product was checked by separating 5µl aliquots on a 1.2% agarose gel by electrophoresis and staining with ethidium bromide. Hind III digested lambda phage DNA was run as a molecular weight marker.

Allele - specific oligonucleotide probes: Two oligonucleotide probes described previously were used[3]: The K1 repeat-specific probe R92/3 (ATCACAAACTACTACTC) and the 3D7 repeat-specific probe R92/4 (GGTGGTAGTGCTGGTGGTAG). The oligonucleotides were synthesised at the La Trobe University, Department of Biochemistry, Australia. 10 pmoles of the probes were 5' end labelled with 50µCi of [α-^{32}P] ATP (specific activity 3000 Ci/mmole, Amersham, UK) using T4 polynucleotide kinase according to standard procedures.[5]

Southern hybridisation: Eight µl of PCR-2 amplified product separated on 1.2% agarose gels were denatured with 0.5N NaOH and then transferred to nitrocellulose membranes by standard procedures.[5] The membranes were treated with pre-hybridisation solution containing 6 x SSC (standard saline citrate pH7.0), 5 x Denhardts solution (0.5g ficoll, 0.5g polyvinyl pyrollidone and 0.5g bovine serum albumin), 0.5% sodium lauryl sulphate and 50 µg/ml salmon sperm DNA,

for 1h at 37°C. Hybridisation was carried out for 16 h at 37°C by adding 10 pmoles of the [^{32}P] labelled probe to the pre-hybridisation solution. After hybridisation, the membrane was washed twice at 37°C in 6xSSC, 0.1% sodium lauryl sulphate and autoradiographed for 16 h.

RESULTS

The PCR amplified GYMSSA DNA gave a single major band of approximately 500bp on agarose gels stained with ethidium bromide. In Southern blots of the amplified DNA, R92/3 reacted with thirteen of the fifteen samples (Fig. 1). The two R92/3 non-reactive samples termed D3 (Dambulla) and P2 (Polgahawela), reacted with probe R92/4 (Fig. 2).

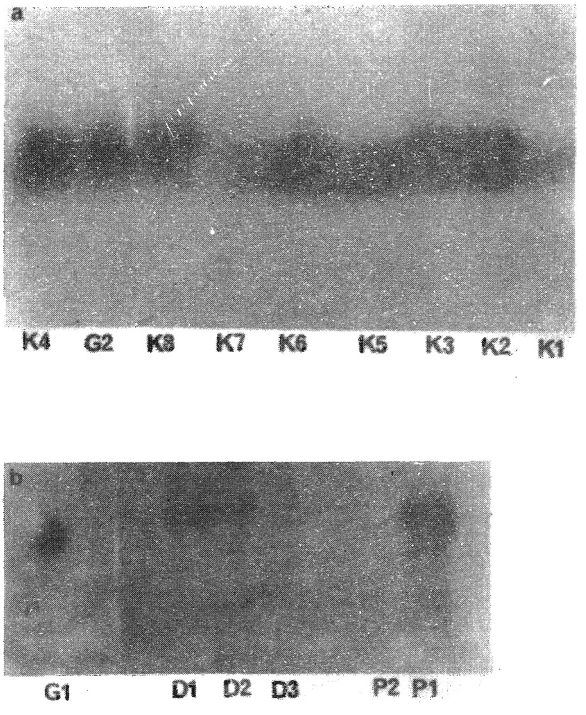

Figure 1a & b: Southern hybridisation of R92/3 with GYMSSA DNA amplified from patients. D1-D3 (Dambulla), G1-G2 (Galewela), K1-K8 (Kurunegala) and P1-P2 (Polgahawela).

DISCUSSION

The results show that GYMSSA in *P. falciparum* isolated from patients in Sri Lanka is represented by at least the two major allelic families (K1 and 3D7) that were previously detected in cultured lines. Since this work was initiated, similar GYMSSA alleles were reported in *P. falciparum* isolates from patients in

Colombia,[6] Irian Jaya[7] and Papua New Guinea.[8] The complete sequence of the GYMSSA gene in two isolates, D2 (Dambulla) and P2 (Polgahawela), have been determined confirming the presence of the two types of allelic families in Sri Lanka.[4] We did not investigate, in our samples, the occurrence of sub-families within the two major allelic types and the limited recombination between alleles that have been reported.[9] The presence of possible mixed infections in some of the blood samples was also not excluded. A larger number of blood samples need to be examined in order to determine the relative prevalences of the K1 and 3D7 allotypes of GYMSSA in Sri Lanka. However our results suggest that a malaria vaccine for use in Sri Lanka should preferably be based on N and C terminal sequences of GYMSSA that are conserved between alleles and that probes specific for the repetitive region of GYMSSA are useful in differentiating *P. falciparum* strains in Sri Lanka.

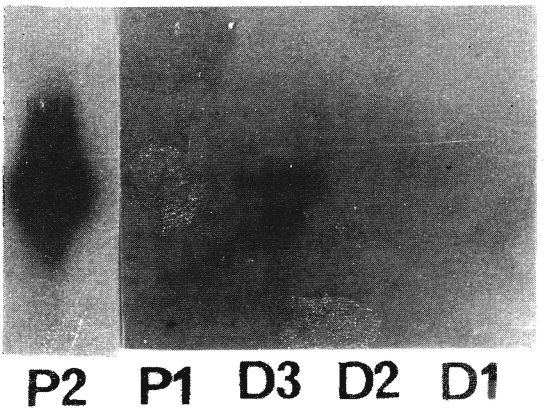

Figure 2: Southern hybridisation of R92/4 with GYMSSA DNA amplified from patients. P1-P2 (Polgahawela), D1-D3 (Dambulla).

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