SOUTHEAST ASIAN OVALOCYTOSIS IN THE CAPE COLOURED POPULATION

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DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master of Technology at the University of Johannesburg. It has not been submitted before for any degree or examination in any other University or Technikon.

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ABSTRACT

Southeast Asian Ovalocytosis (SAO) is an autosomal dominantly inherited, classically asymptomatic condition, that is widespread in Southeast Asian populations of Malasia, Indonesia, Papua New Guinea and the Philippines. Some regions have close to 30% prevalence and this is thought to be due to SAO providing partial protection against malaria.

SAO is characterized by rigid, spoonshaped, ovalocytic red blood cells. The underlying defect is a deletion of 27bp in the band 3 gene, resulting in the absence of 9 amino acids (400-408) at the boundary of the cytoplasmic and membrane domains of band 3, causing abnormal structure and function. SAO is tightly linked in all cases to the band 3 Memphis 1 polymorphism, which is a lysine 56 (AAG) – glutamic acid (GAG) substitution. This polymorphism can be inherited independently and the prevalence ranges from about 6-30% according to various populations studied.

The presence of SAO in a Cape Coloured family is a recent finding. The purpose of this study was to further investigate the prevalence of SAO and the band 3 Memphis 1 polymorphism in the Cape Coloured population. 20 unrelated individuals with SAO morphology were identified. DNA analysis revealed the 27bp deletion of exon 11 of the band 3 gene in all 20 subjects, which is diagnostic of SAO. This indicates a high occurrence of the SAO mutation in the Cape Coloured population, which is speculated to be due to a founder effect. Some of the clinical features differed from classically described SAO as some individuals showed evidence of haemolysis.

Protein analysis showed all 20 individuals to have a reduced band 3 mobility, indicating the band 3 Memphis 1 polymorphism. Detecting the band 3 Memphis 1 polymorphism on a protein level is time consuming and labour intensive, therefore

a PCR assay, which utilizes DNA, was developed for the rapid screening of this polymorphism. The PCR assay was based on a nucleotide mismatch which created a *Taq* 1 restriction site when combined with the band 3 Memphis 1 allele, but not with the wild type allele. Digestion of the PCR product with *Taq* 1 allowed differentiation between the two alleles. It was established that the band 3 Memphis 1 polymorphism has a high prevalence in the Cape Coloured population as it was detected in 108/326 (33%) of the individuals studied. Analysis indicated the Memphis allele is in Hardy-Weinberg equilibrium.

"Great things are not done by impulse,
but by a series of small things brought together"

Vincent Van Gogh

In loving memory of my father

Richard Cleveland Lyons

1932-1997

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LIST OF ABBREVIATIONS

A	Ampere
$A_{260/280/595}$	absorbance at 260nm, 280nm and 595nm respectively
ACD	acid citrate dextrose
AE1	anion exchange protein 1
APS	ammonium persulphate
Arg	arginine
Asn	asparagine
Asp	aspartic acid
ATP	adenosine triphosphate
b	band
bis	**N,N**-methylene bisacrylamide
bp	base pair
BSA	bovine serum albumin
°C	degrees Celcius
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
EDTA	ethylenediamine tetraacetic acid disodium salt
g	relative centrifugal force
g	gram
Glu	glutamic acid
Hb	haemoglobin
Hct	haematocrit
k	kilo
kb	kilo bases
kDa	kilodalton
l	litre
Lys	lysine
M	molar
µ	micro

m	milli
MCV	mean cell volume
nm	nanometer
OD	optical density
P	primer
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PMSF	phenylmethylsulphonylfluoride
RBC	red blood cell
rpm	revolutions per minute
SAO	southeast asian ovalocytosis
SDS	sodium dodecyl sulphate
TEA	Tris EDTA acetate buffer
TBS	Tris buffered saline
TE	Tris EDTA buffer
TEMED	**N,N,N,N**-tetramethylethylenediamine
T_m	melting temperature
Tris	2-amino-2-(hydroxymethyl)-1,3-propandiol
uv	ultra-violet
V	Volt
v	volume
w	weight
χ^2	chi-squared