The Role of Erythrocyte Membrane Proteins in Haemolytic Anaemias in South African Populations

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A dissertation submitted to the Faculty of Science, University of the Witwatersrand, in fulfillment of the requirements for the degree of Master of Science.

Johannesburg, 2005

DECLARATION

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

I declare that ethical clearance was obtained from the Medical School Ethics Committee of the University of the Witwatersrand for the purpose of acquiring blood samples from normal and affected individuals. Clearance certificate #25/1/92.

_____

Lara Dominique Vallet

This 31st day of October, 2005

ABSTRACT

The erythrocyte carries gases between the cells and the lungs, and has to distort to negotiate narrow splenic sinuses and capillaries. This distortion necessitates a high surface area to volume ratio that is maintained by the erythrocyte membrane skeleton, a network of proteins including spectrin and protein 4.1. The skeleton anchors the lipid bilayer through attachment to integral membrane proteins, notably the anion exchange protein, band 3. Abnormalities of the erythrocyte membrane proteins cause loss of cell elasticity and ultimately the erythrocytes become prematurely trapped in the spleen where they are phagocytosed, resulting in haemolytic anaemia.

Three mutations causing band 3-deficient hereditary spherocytosis (HS), a haemolytic anaemia characterised by spherical erythrocytes, were located using restriction enzyme analysis and DNA sequencing. Proband A (Black) is heterozygous for band 3 Pinhal (R490H) and has mild clinical symptoms. Proband B and his mother (Caucasian) are heterozygous for band 3 Bicetre (R490C) and have severe anaemia requiring transfusions and splenectomy, respectively. These results confirm codon 490 as a hotspot for mutations and indicate the effect of different amino acid substitutions in the same position on clinical severity. Proband C (Black) is homozygous for a novel mutation (E508K) for which her parents are heterozygous. The proband is severely affected and transfusion-dependent whereas her father has moderate anaemia and her mother is asymptomatic. It is speculated that a secondary factor modulates their clinical symptoms. All of these mutations occur in a CpG dinucleotide, a common source of DNA mutations, and are located within the highly conserved exon 13, which encodes the third to fifth α-helices and the second extracellular loop of the transmembrane region of band 3. The mutations are likely to alter the conformation of band 3, impairing its insertion into the erythrocyte membrane. No causative mutations were located in another 12 band 3-deficient HS kindred using restriction enzymes and single strand conformation polymorphism analysis.

Ten protein 4.1-deficient patients with hereditary elliptocytosis, a haemolytic anaemia characterised by elliptical erythrocytes, were also studied. Immunoblot analyses ruled out abnormally sized protein 4.1 and three known DNA mutations were excluded using restriction enzyme analysis. Further studies are required to elucidate the cause of the haemolytic anaemia in these kindred.

This study advanced our knowledge of the molecular basis of HS in South African kindred and highlighted the susceptibility of CpG dinucleotides to mutations.

CONFERENCE PRESENTATIONS

Poster Presentations

Vallet, L.D.; Lyons, C.; Bracher, N.; Sherman, G.; Coetzer, T.L. A Novel Erythrocyte Band 3 Mutation Modulates Clinical Severity of Hereditary Spherocytosis, **IUBMB/SASBMB Special Meeting on the Biochemical and Molecular Basis of Disease,** Cape Town, RSA, 19-23 November 2001

Vallet, L.D.; Lyons, C.; Bracher, N.; Sherman, G.; Coetzer, T.L. A Novel Erythrocyte Band 3 Mutation Modulates Clinical Severity of Hereditary Spherocytosis, **Wits Medical School Research Day**, August 2003

Vallet, L.D.; Naratam, N.; Coetzer, T.L. Identification Of Two Mutations In The Human Erythrocyte Band 3 Gene In South African Kindred With Hereditary Spherocytosis, **Wits Medical School Research Day,** 04 August 2004

Vallet, L.D.; Naratam, N.; Coetzer, T.L. Identification Of Two Mutations In The Human Erythrocyte Band 3 Gene In South African Kindred With Hereditary Spherocytosis, **SASBMB Molecules-R-Us Conference**, Stellenbosch, RSA, 16-20 January 2005

“Now what I want is, Facts. Teach these boys and girls nothing but Facts. Facts alone are wanted in life. Plant nothing else and root out all else… Stick to the Facts, Sir”

Charles Dickens (1812-1870) in Hard Times (1854)

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ABBREVIATIONS

General Abbreviations

Abbreviation	Meaning
A	Adenine
$A_{260/280}$	Absorbance of UV light at 260nm and 280nm
AE1/2/3	Anion exchange protein 1/2/3
ATP	Adenosine 5'-triphosphate
Bp	Basepair
BSA	Bovine serum albumin
C	Cytosine
cAMP	Adenosine 3', 5'-cyclic monophosphate
cDNA	Complementary deoxyribonucleic acid
Ci	Curie
Cpm	Counts per minute
dATP	Deoxyadenosine triphosphate
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
ddNTP	Dideoxynucleoside triphosphate
$dpm.\mu g^{-1}$	Disintegrations per minute per microgram
DTT	Dithiothreitol
EDTA	Ethylenediamine tetraacetic acid
ERM	Ezrin-Radixin-Moesin
Fl	Femtolitre
G	Guanine
$g.dl^{-1}$	Grams per decilitre
HE	Hereditary Elliptocytosis
HS	Hereditary Spherocytosis
Kb	Kilobases
kD	KiloDalton
M	Molar
mA	MilliAmps
MAGUK	Membrane-associated guanylate kinase
MCV	Mean cell volume
MDE™	Mutation Detection Enhancement
mM	MilliMolar
µCi	MicroCurie
Nm	Nanometre
ORF	Open reading frame
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PTC	Premature termination codon
RNA	Ribonucleic acid
Rpm	Revolutions per minute
SAP	Shrimp alkaline phosphatase

SDS	Sodium dodecylsulphate
SDS-PAGE	Sodium dodecylsulphate polyacrylamide gel electrophoresis
SSCP	Single strand conformational polymorphism
T	Thymine
TAE	Tris-acetate-EDTA
TBE	Tris-borate-EDTA
TE	Tris-EDTA
TEMED	N,N,N',N'-tetramethylethylenediamine
Tris	Hydroxymethyl methylamine
U	Units
UV	Ultra-violet

Amino Acid Abbreviations and Codons

Abbreviation	**Amino Acid**	**Codon***
A	Alanine	GCG/A/T/C
C	Cysteine	TGT/C
D	Aspartic Acid	GAT/C
E	Glutamic Acid	GAG/A
F	Phenylalanine	TTT/C
G	Glycine	GGG/A/T/C
H	Histidine	CAT/C
I	Isoleucine	ATA/T/C
K	Lysine	AAG/A
L	Leucine	TTG/A or CTG/A/T/C
M	**Methionine (Initiation)**	**ATG**
N	Asparagine	AAT/C
P	Proline	CCG/A/T/C
Q	Glutamine	CAG/A
R	Arginine	CGG/A/T/C
S	Serine	AGT/C
T	Threonine	ACG/A/T/C
V	Valine	GTG/A/T/C
W	Tryptophan	TGG
Y	Tyrosine	TAT/C

**In the mRNA thymine is replaced by uracil*

The termination codon (X) is encoded by nucleotides TTG/A or TGA