Identification of Two Mutations in the Human Erythrocyte Band 3 Gene in South African Kindred with Hereditary Spherocytosis

Lara D. Vallet, Neelam Naratam and Theresa L. Coetzer

Department of Molecular Medicine and Haematology, NHLS, School of Pathology, University of the Witwatersrand, Johannesburg, South Africa

Hereditary spherocytosis is a haemolytic anaemia characterized by defective erythrocyte membrane proteins. One family (mother and son) of Caucasian origin with severe anaemia and one proband of Black origin with moderate anaemia were examined. Protein studies indicated that all patients had a band 3 deficiency. DNA from the patients was amplified and screened for known hotspots using restriction enzyme digest analysis. Each of the three patients was heterozygous for a change in codon 490 in exon 13 according to an *AciI* digest. DNA sequencing identified these changes as Band 3 Pinhal (CGC to CAC; arginine to histidine) in the Black proband and Band 3 Bicetre I (CGC to TGC; arginine to cysteine) in the Caucasian kindred. The mutant mRNA is stable, indicating that the band 3 deficiency arises due to protein degradation in the cytoplasm or defective protein insertion into the erythrocyte membrane. Erythrocyte band 3 is a multi-pass transmembrane protein and highly-conserved amino acid 490 lies at the extracellular border of the second transmembrane loop of band 3. It is hypothesised to be an important signal for the band 3 to re-enter the lipid bilayer. An amino acid substitution could affect this ability and hence the spatial arrangement of band 3. The variable clinical severity is associated with the level of protein deficiency which is speculated to relate directly to the charge and structure of the amino acid replacing arginine 490.