NOVEL HIGH INCIDENCE ANTIGEN IN THE DIEGO BLOOD GROUP SYSTEM (DISK) AND CLINICAL SIGNIFICANCE OF ANTI-DISK

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Background: Antigens of the Diego blood group system reside on the red cell membrane protein AE1 (band 3). The system currently comprises 21 antigens with two sets of antithetical antigens, Di^a /Di^b and Wr^a/Wr^b, of low and high incidence, respectively. The 17 remaining antigens are all of low incidence and no antithetical high incidence antigens are described. Many of these antigens reside on loop 3 of the band 3 protein which has a chymotrypsin cleavage site. All Diego antigens arise from a single nucleotide polymorphism in the *SLC4A1* (*DI*) gene. We describe here a novel high incidence antigen which is antithetical to the low incidence Wu (DI9) antigen.

Case Study and Methods: Blood samples from a healthy, untransfused, 34 year old primigravida (SK) were investigated for identification of an antibody detected following a miscarriage at 12 weeks gestation. Standard serological methods were used. The antiglobulin test (IAT) was carried out by LISS tube and DiaMed gel methods. DNA was extracted and genomic DNA sequencing was performed for exon 14 of *SLC4A1*, which encodes the third extracellular loop of the band 3 protein.

Results: SK plasma contained a very strong alloantibody reacting with all red cells tested by direct agglutination at 18^{o}C and 37^{o}C and marginally weaker by IAT. The cells of her brother were more weakly reactive than random control cells. Following extensive investigation and exclusion of all known antibodies to high incidence antigens we concluded that a novel antigen/antibody was involved. Testing against a range of proteolytic enzyme treated and chemically modified cells showed the determinant to be resistant to papain, trypsin and AET and sensitive to chymotrypsin. Serological testing for low incidence Diego antigens revealed a positive reaction with one multispecfic

antiserum containing, amongst other specificities, anti-Wu. A homozygous G>C mutation was identified in exon 14 of *SLC4A1* at position 1694 for SK. This results in a glycine to alanine change at position 565 of the band 3 protein (G565A) and corresponds to homozygosity for the allele encoding the Wu antigen. The 1694 G>C mutation was found in the heterozygous state in SK's brother.

Summary/Conclusions: We conclude that SK lacks a novel high incidence Diego antigen which is antithetical to Wu. We propose to call the antigen DISK (DI22). SK's Diego phenotype is DI:9,-22 and that of her brother DI:9,22. Wu and DISK represent the third pair of alleles in the Diego system. SK's antibody (anti-DISK) is extremely potent and has all the characteristics of a naturally occurring antibody, with similarities to anti-P,P1,P^K made in pp individuals . It shows dosage in being more strongly reactive with DI:-9,22 cells than DI:9,22 cells. The serological characteristics suggest a highly clinically significant antibody but it is not known whether it played any part in her early miscarriage. Autologous units of SK are being frozen down should they be needed in the future.