

SEROLOGY HELPS MOLECULAR DIAGNOSTICS TO SOLVE A RARE CASE OF HOMOZYGOUS *FY*X/FY*X* – CONSEQUENCES FOR TRANSFUSION RECOMMENDATIONS

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Background: The DUFFY (*FY*) blood group system is expressed on the DARC protein (Duffy antigen/receptor for chemokines, CD234), which is a 336 AA multipass glycoprotein type I of the red blood cell (RBC) membrane and contains 6'000 – 13'000 copies of DARC. DARC is encoded by two exons on chromosome 1q22-23 and expresses 7 antigens, namely Fya (G125), Fyb (A125), Fyx (A125, T265, A298), Fy3, Fy4, Fy5, Fy6. *FY*X* is a variant *FY*B* allele with dramatically weakened antigen expression. The Fy(a-b-) phenotype is also prevalent among Africans and as such mainly due to an upstream promoter mutation T-67C (GATA-1, assigned as *FY*Fy(Bnull-67C)*) which prevents expression of DARC on erythropoietic tissue. In our case, however, we observed a patient with Fy(a-b-) phenotype due to the very rare homozygous *FY*X/FY*X* genotype which is distinct from the Fy(a-b-) phenotype due to GATA-1 mutation.

Case presentation: The 58 years old woman was hospitalized for elective hip replacement. Preoperatively, anti-c, anti-Fya, anti-E and anti-Leb were found, corresponding to the phenotype CCD. ee, Fy(a-b-), Le(a-b-). The Fy(a-b-) phenotype suggested various alternative *FY* genotypes: *FY*Fy(Bnull-67C)/FY*Fy(Bnull-67C)*; *FY*Fy(Bnull-67C)/FY*X* or *FY*X/FY*X*, or a very rare homozygous, or compound heterozygous combination of truly unexpressed *FY* alleles. By *FY* genotyping using commercial genotyping kit (Inno-Train, Kronberg i.T., Germany), *FY*B/FY*X*, or *FY*X/FY*X* was identified. To tell the correct genotype, serology was used, either to show weak expression of Fyb antigen on RBCs (indicative of *FY*X/FY*X*), or apparently regular Fyb expression (indicative of *FY*B/FY*X*). In contrast to the findings with standard serological techniques (ID gel sedimentation using Gel Station[®], DiaMed, Cressier, CH, and standard tube hemagglutination assay), which were repeatedly negative, we were able to identify microaggregates of patient's RBC applying indirect agglutination testing with monoclonal anti-Fyb and microscopic reading as well as very faint reaction in the ID gel card by eye reading. These findings confirm weak Fyb expression on the patient's RBCs and confirm the rare *FY*X/FY*X* genotype.

Conclusion:

- According to the manufacturer of the genotyping kit (Inno-Train), unambiguous genotyping for *FY*X* homozygosity would technically be possible, but was waived in favour to stay with the current microtiter-plate format of 8 PCR-SSPs in one row. This decision was also reasoned by the low abundance of *FY*X* homozygous individuals, which is estimated to be about 2.5 to 4 per 10'000 Caucasians.
- Sophisticated search for mikrohemagglutination by microscopic reading may demonstrate weak antigen expression confirming unusual phenotypes suggested by molecular genotyping. This applies similarly to other weakly expressed blood group antigens e.g. weakD, weakLub and others.
- Phenotypic distinction between weak antigen expression and absent antigen expression is of practical importance for transfusion recommendation.
- The differential expression of Fyb antigen in erythropoietic versus non-erythropoietic tissue based on the underlying *FY* genotype (*FY*X* versus *FY*Fy(Bnull-67C)*) remains to be defined. However, in medical patient care, carriers of either of these genotypes may be transfused with Fyb positive RBCs without running the risk of anti-Fyb formation since Fyb will be expressed at least on non-erythropoietic cells.