Multi-ethnic Lewis phenotype prediction using PCR-SSP genotyping on *FUT2* and *FUT3*

Stefan Meyer[1], Kathrin Neuenschwander[1], Lilian Castilho[2], Beat M. Frey[1], Christoph Gassner[1]

[1] Blood Transfusion Service Zurich, SRC, Department of Molecular Diagnostics (MOC), Schlieren, Switzerland.
[2] Hemocentro Unicamp, Campinas, SP, Brazil.

Background

Lewis antigens are ABH related carbohydrates and their expression is regulated by interaction of the two fucosyltransferases FUT2 (Secretor enzyme, Se) and FUT3 (Lewis enzyme, Le). In principle, active FUT3 transfers a fucose subterminal to type 1 precursor substrates resulting in Le(a+b-) phenotypes (PT). Terminal addition of another fucose by active FUT2 transforms Le^a to Le^b, resulting in Le(a-b+). The third phenotype, Le(a-b-), is the result of an inactive FUT3, completely independent of FUT2 activity (Fig.1). Enzymatic inactivity of FUT2 and FUT3 is caused by a variety of inactivating single nucleotide polymorphisms (SNPs), whose distribution differs in various ethnic groups.

Aims

Since adsorbed onto red blood cells only, serological phenotyping of Lewis antigens is difficult under certain physiological conditions. Therefore, rapid and correct *FUT2/FUT3* genotyping in different ethnic groups would be of great interest.

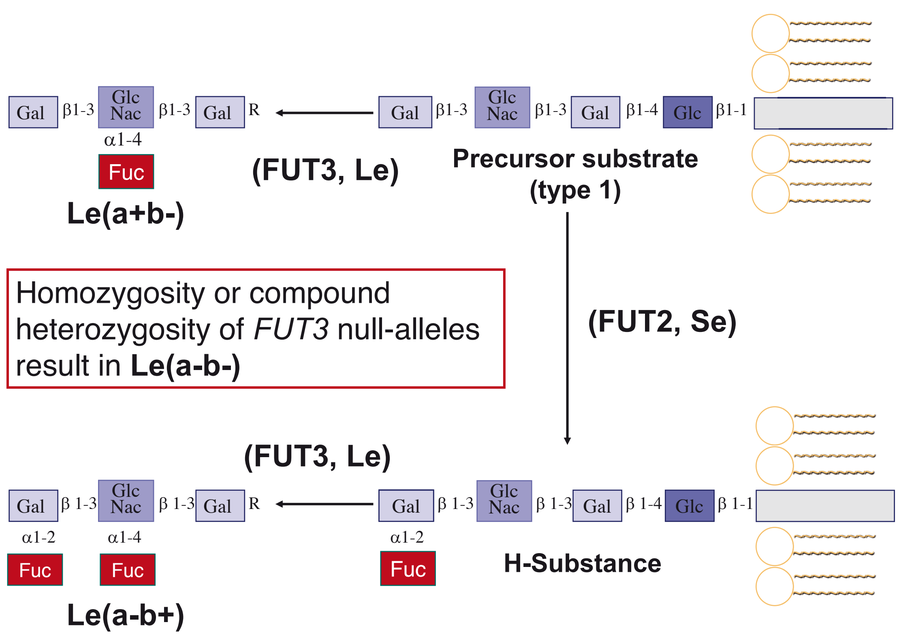


Fig. 1: Lewis antigens - genes and biochemistry.

Methods

The Lewis phenotype was defined using standard serological procedures. For genotyping, an in-house PCR-SSP kit was developed to detect inactivating SNPs 428G>A (5) and 385A>T (6,7) of *FUT2* and 59T>G, 202T>C, 484G>A and 1067T>A of *FUT3*, respectively (Fig.2). To avoid misinterpretation of two *FUT3* null mutant signals from "cis-alleles" as compound heterozygous Le(a-b-) individuals, all *FUT3* specific PCR-SSPs were designed in bi-specific manner, in order to allow for the distinction of "cis-" from "trans-" alleles (Fig.3). Individuals investigated were 150 blood donors of the Zurich area, 16 individuals of presumptive African ancestry, e.g. estimated by the presence of *FY*02N.01* homozygosity, and 56 samples of Brazilian blood donors. All samples had existing serological prevalues of Lewis phenotypes. Additionally, PCR-SSP for the asian specific SNP 385A>T (*FUT2*) was tested on four heterozygous and two homozygous samples of Japanese blood donors, respectively.

25th Regional Congress of the ISBT, June 27 – July 1, 2015, London

Results

Considering above mentioned inactivating mutations and well-known expression-negative haplotypes of *FUT3*, e.g. le59,202, le202,484, le59,1067, for Caucasians, Africans, Asians and Amazonian populations (1-4), 12 specific PCR-SSPs (four for *FUT2* and eight for statistically relevant *FUT3* haplotypes) were developed (Fig.3) and delivered almost 100% (99.55%) concordance with serological prevalues for all samples. Only one sample showed a discrepancy between genotyping (Le(a-b-)) and phenotyping (Le(a-b+)). Subsequent sequencing delivered two different alleles (le59,445; le202,314) both predicited to be null-alleles assuming Le(a-b-) PT, respectively. However, serological retyping on a second sample could not be repeated until now.

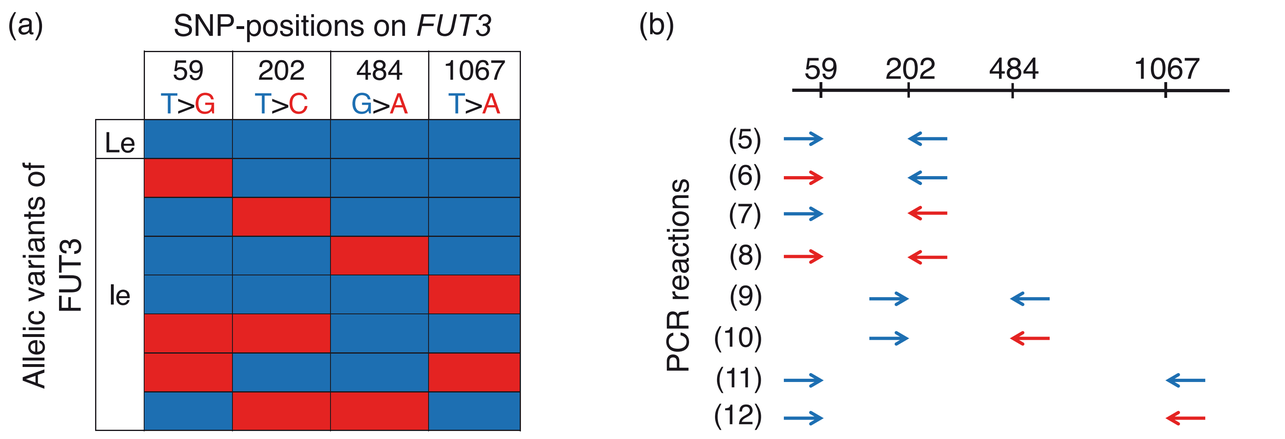
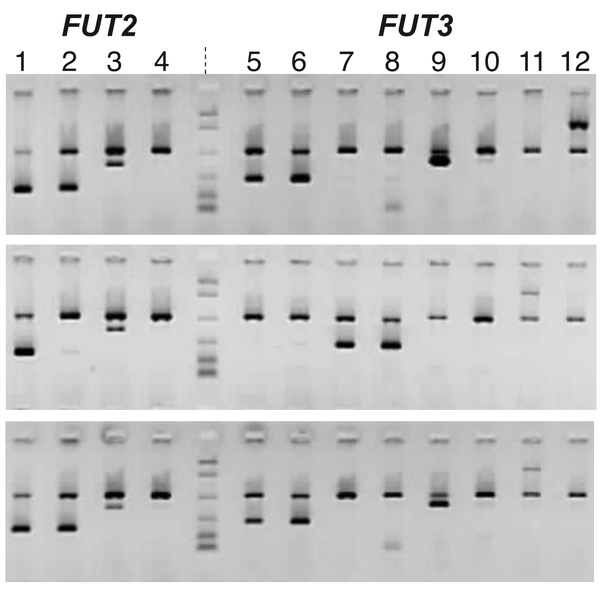


Fig. 2: (a) Allelic variants and SNP-positions of *FUT3* relevant for Lewis PT prediction. (b) PCR- reactions designed for Lewis PT-prediction allowing for the distinction of "cis-" from "trans-" alleles (numbering according to Fig.3). Arrows mark position of forward and reverse primer. Wild-type SNPs/arrows are marked in blue, mutated SNPs/arrows in red.

FUT2				*FUT3*							
1	2	3	4	5	6	7	8	9	10	11	12
Se	se	Se	se	Le	le	le	le	Le	le	Le	le
428	428	385	385	59	59	59	59	202	202	59	59
				202	202	202	202	484	484	1067	1067
Secretor (Se/se)				Lewis (Le/le)							

Fig. 3: 12 PCR-SSPs reactions allowing for multi-ethnic Lewis PT prediction including inactivating SNPs 428G>A and 385A>T of *FUT2* and well-known expression-negative haplotypes of *FUT3.*



Se/se (*FUT2*)		Le/le (*FUT3*)				Lewis PT
385	428	59	202	484	1067	Le(a-b-)
385	428	59	202	484	1067	
385	428	59	202	484	1067	Le(a-b-)
385	428	59	202	484	1067	
385	428	59	202	484	1067	Le(a-b+)
385	428	59	202	484	1067	

Fig. 4: PCR-SSP kit-representative examples.

Conclusion

The Lewis blood group system comprises the three common phenotypes Le(a+b-), Le(a-b+) and Le(a-b-). The kit consists of 12 PCR-SSPs and provides a helpful and highly accurate diagnostic tool for Lewis genotyping with consecutive phenotype prediction. Since the Lewis blood group phenotype is difficult to assess in situations when affected by certain diseases and under atypical physiological conditions, genotyping the Secretor and Lewis genes *FUT2* and *FUT3* is therefore an attractive and accurate alternative.

References and Acknowledgements

We thank Dr. Koda for providing us with DNA of Japanese donors.
(1) Soejima *et al.*, (2009); (2) Matzhold *et al.*, 2009; (3) Pang *et al.*, 1998; (4) Corvelo *et al.*, 2013; (5) Koda *et al.*, 2001; (6) Liu *et al.*, 1999; (7) Koda *et al.*, 1996.