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

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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 Lea to Leb, 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.

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 and 385A>T 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 a 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 prevalences of Lewis phenotypes. Additionally, PCR-SSP for the asian specific SNP 385A>T (FUT2) was tested on four homozygous and two homozygous samples of Japanese blood donors, respectively.

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 prevalences 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 predicted to be null-alleles assuming Le(a-b-) PT, respectively. However, serological retyping on a second sample could not be repeated until now.

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
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(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.