Assessment of the fetal *KEL* genotype from cell-free fetal DNA in maternal blood

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BACKGROUND

In pregnant women with diagnosed red blood cell alloantibody anti-K (always "K" negative, genotype *k/k*), the fetuses are at risk of hemolytic disease only if the antigen "K" ("K" positive, genotype *k/K*) is present on their erythrocytes. In reality, however, about 95% of the fetuses are "K" negative (genotype *k/k*) and thus are not at risk of hemolytic disease. The clinical importance of assessment of the fetal *KEL* genotype is to exclude "K" negative fetuses (genotype *k/k*) in "K" negative pregnant women. Noninvasive assessment of the fetal *KEL* genotype is not available in the Czech Republic yet.

The *KEL* gene encodes the Kell antigens and is localized on chromosome 7. The coding sequence consists of 19 exons. The *KEL* gene has two major co-dominant alleles, *K* and *k* (*KEL1* and *KEL2*), which are the result of a single nucleotide polymorphism in the 6th exon. This single nucleotide change causes the amino acid substitution of Methionine (antigen "K") for Threonine (antigen "k"). The complementary antigens "K" and "k" thus differ by a single amino acid change.

OBJECTIVES

Noninvasive assessment of the fetal *KEL* genotype (*k/k* or *k/K*) from cell-free fetal DNA in plasma "K" negative pregnant women (*k/k*).

MATERIALS AND METHODS

In total, 122 women in the 1st trimester of pregnancy (between the 7th and the 14th gestational week) were tested for the *KEL* genotype from leukocytes of the peripheral blood. 95.1% of these women (116/122) were "K" negative (*k/k*), in which case the test of the fetal *KEL* genotype followed from cell-free fetal DNA in the plasma of the peripheral blood. This was further verified by the buccal smear of the newborns.

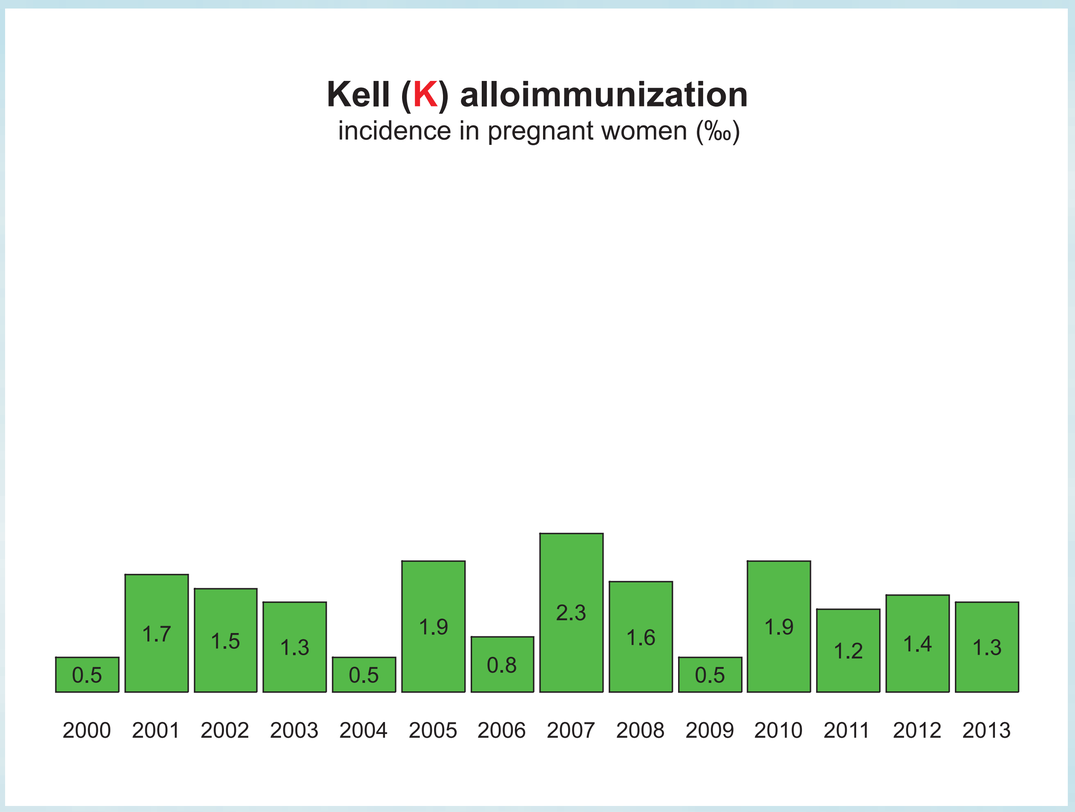
Noninvasive assessment of the fetal *KEL* genotype from cell-free fetal DNA in the plasma of pregnant women was carried out through minisequencing by capillary electrophoresis (so called SNaPshot). The assay is based on extending the sequence-specific DNA primer by one base at the site of the *KEL* polymorphism (*K/k*). On the basis of an incorporated, fluorescently marked base, in cases of *KEL* homozygous pregnant women (*k/k* or *K/K*) the additive of the complementary fetal allele (*K* or *k*) can be identified and the fetal *KEL* genotype can be assessed by detecting the fluorescence of the respective base.

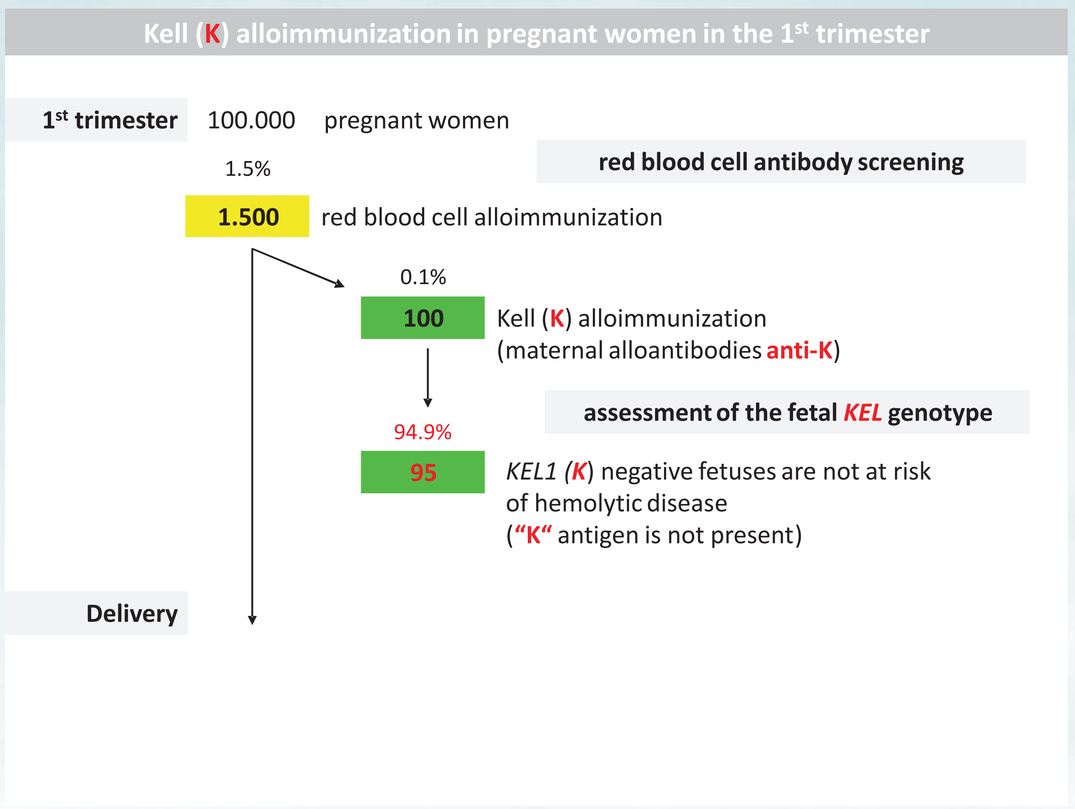
RESULTS

In 97.4% of the "K" negative women (113/116), we were able to assess the fetal *KEL* genotype, which was then verified in newborns. A total of 96.5% fetuses (109/113) were "K" negative (*k/k*), the remaining 3.5% fetuses were "K" positive (*k/K*). Sensitivity and specifity of the method were 100%.

CONCLUSION

Minisequencing by capillary electrophoresis proved to be a reliable method for assessment of the *K* allele from fetal cell-free DNA in the peripheral blood of a "K" negative pregnant woman (*k/k*).





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