Nonivasive prenatal KEL genotyping using TaqMan Real time PCR and by capillary electrophoresis minisequencing

J. Böhmova, R. Vodicka, M. Lubusky, R. Vrtel, I. Holuskova, M. Studnickova, R. Kratochvilova, E. Krejcirikova, M. Janikova

University Hospital and Palacky University Olomouc, Olomouc, Czech Republic

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Introduction: There are two reasons for establishing a methodology for non-invasive determination of KEL genotypes in early pregnancy. To identify fetuses which are at risk of hemolytic disease of fetus and newborn by alloimmunized pregnant women and to prevent alloimmunization during pregnancy.

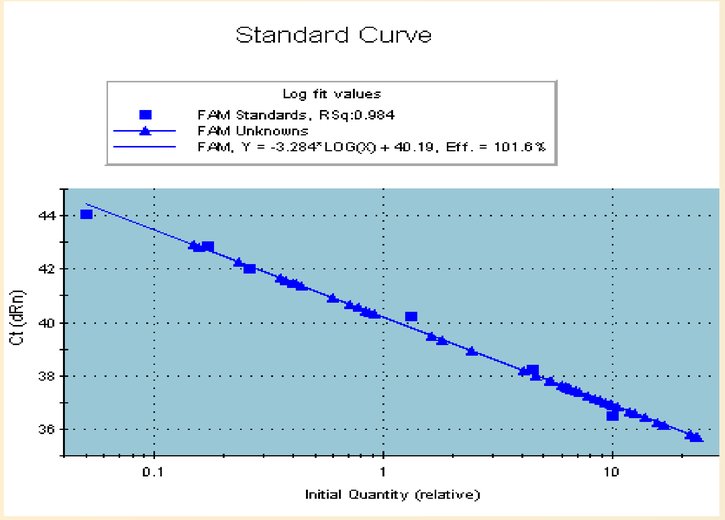
Aim: KEL noninvasively determination of the fetal genotype from fragmented fetal DNA in maternal plasma KEL negative pregnant women using TaqMan Real Time PCR and minisequencing (SNaPshot).

Material and methods:

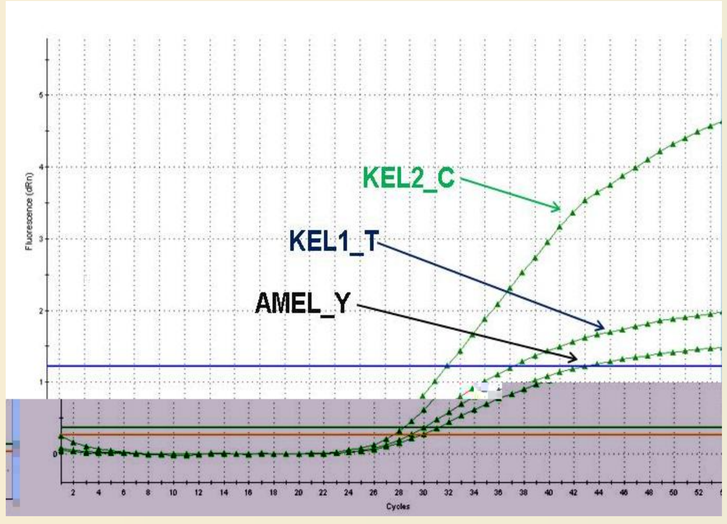
1) TaqMan Real Time PCR: Allelic discrimination by multiplex TaqMan Real Time PCR involves amplification and detection of the region of the KEL 1/2 single nucleotide polymorphism and region of the AMELY gene as an internal amplification control. The methodology was tested on DNA samples isolated from peripheral blood leukocytes of k/k, K/K and k/K men.
2) SNaPshot: Determination of the sensitivity threshold KEL calibration was performed using a dilution series that were prepared using plasma and cellular DNA KEL negative homozygote (KEL2/KEL2;k/k) and KEL heterozygote (KEL1/KEL1;K/k). It was tested a total of 141 random samples of cell free fetal DNA isolated from maternal plasma in the first trimester (from 10th to 15th gestation weeks).

Results:

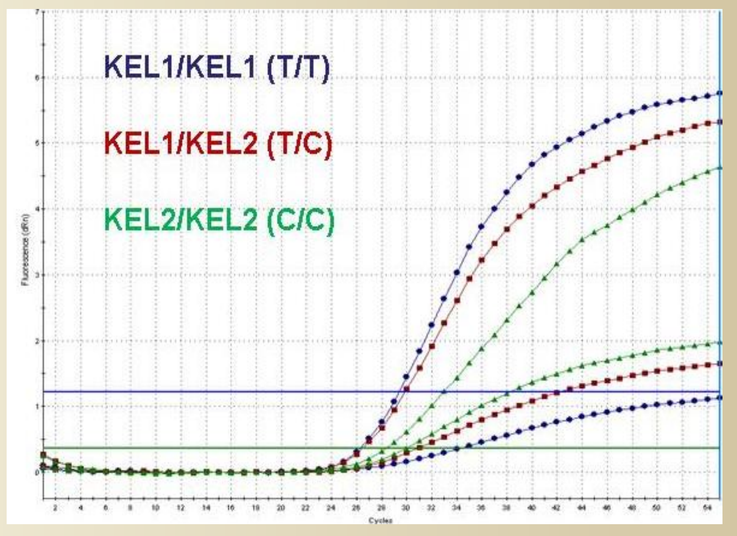
1) TaqMan based Real Time PCR was able to detect 0,22% of artificial KEL1 admixture (Graph 1). Disadvantage of this approach is fluorescent background particularly in KEL negative samples (k/k; KEL2/KEL2) (Picture 1, 2). For this reason, the contribution of KEL1 fetal allele may be confused with fluorescent background non-specifically bounded probe. TaqMan Real Time PCR is not able to clearly distinguish fetal KEL genotype.
2) Minisequencing (SNaPshot) based quantification was able to detect 1,7% of artificial KEL1 admixtures (Graph 2; Pictures 3). In addition, SNaPshot assay is suitable for heterozygozity from homozygozity recognition (Pictures 4).
   It was detected 5 KEL1 positive fetuses (KEL heterozygous fetuses) of the 141 randomly selected plasma samples. In 3 plasma samples was not possible to establish the genotype of the fetus. In 9 samples was not possible to determine the KEL genotype of the fetus because of the heterozygous genotype of the mother. To confirm the results of the analysis were available 129 buccal swabs from newborns after delivery. By the analysis of 118 buccal swabs we confirmed 4 positive plasma samples. The fifth positive fetus and the rest of the negative fetuses will be confirmed after delivery (Table 1).
   Following dilution series it was possible to detect less than 0.78 % admixture K allele corresponding DNA concentration of 0.04 ng / ml (Graph 3). This method can also provide for fetal allele KEL2 for rarely occurring maternal genotype KEL1 / KEL1 (Picture 5).
   Capillary electrophoresis minisequencing is suitable for the detection of K fetal allele from cffDNA in maternal plasma.



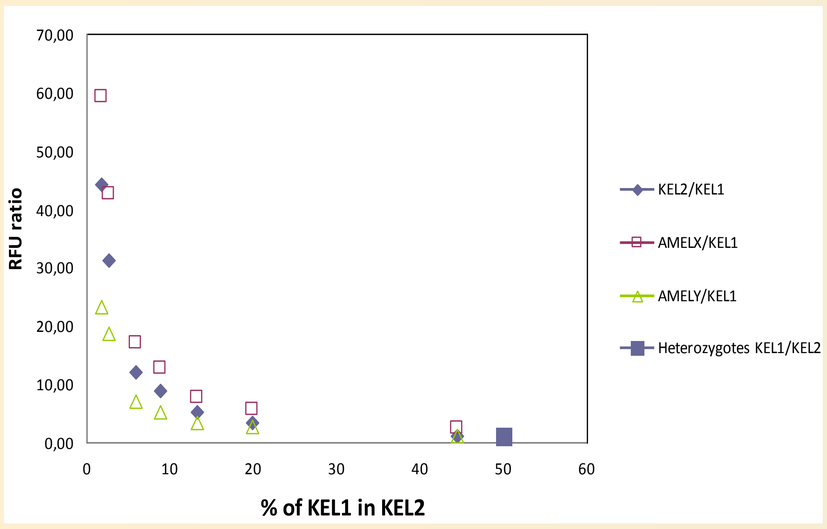
Graph 1: KELL comparative quantification using calibration curve prepared from KEL1 and KEL2 samples



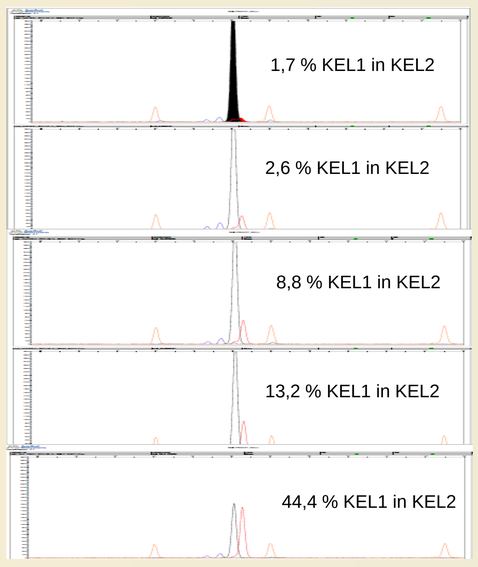
Picture 1: KEL2/KEL2 male genotype and region of the AMELY gene as an internal amplification control using TaqMan Real Time PCR



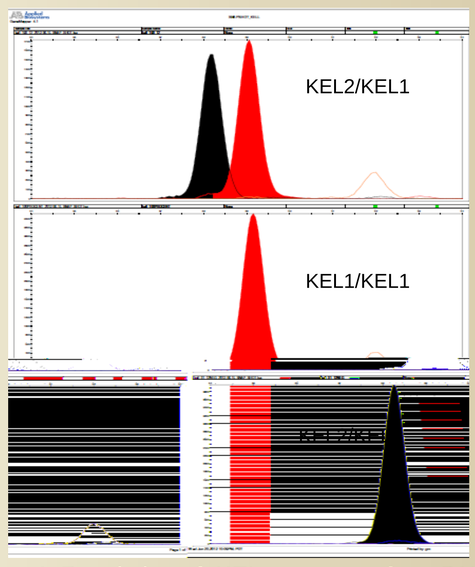
Picture 2: Resolution of KEL genotypes using TaqMan Real Time PCR



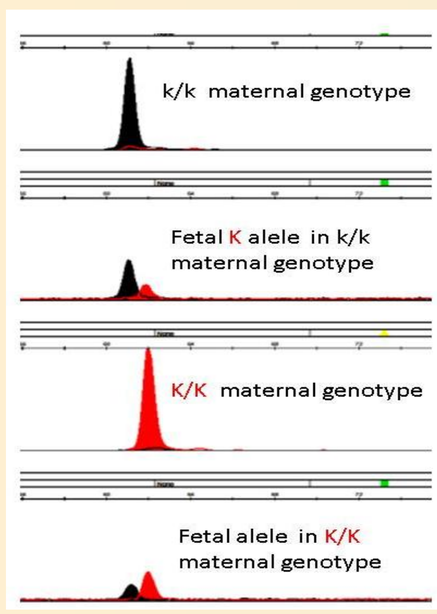
Graph 2: Artificial genotype mixture KEL positive heterozygote (KEL1/KEL2) in KEL negative sample (KEL2/KEL2)



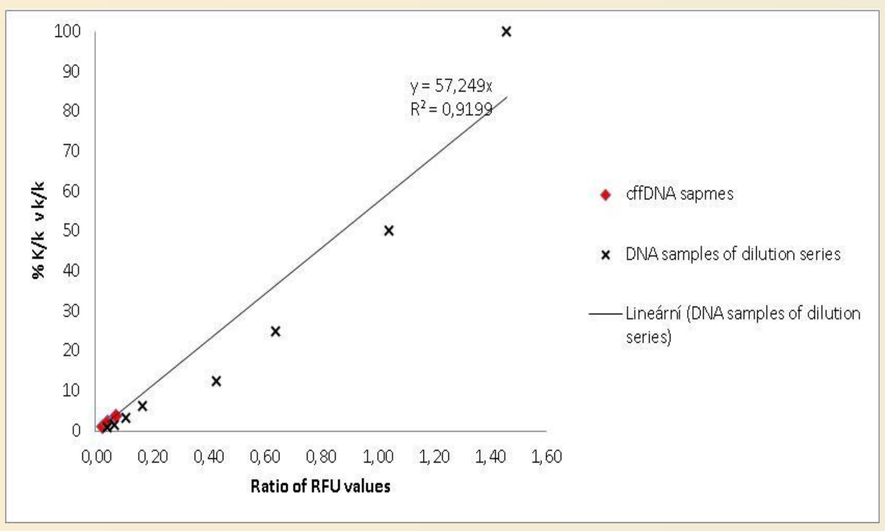
Picture 3: Detection sensitivity using SNaPshot of 1,7% of artificial KEL1 admixtures



Picture 4: Resolution of KEL genotypes from DNA blood samples using SNaPshot



Picture 5 : Detection of fetalKEL alele from DNA plazma samples using SNaPshot



Graph 3: KEL quantification of four K/k fetuses using dilution series from plasma DNA samples of KELL homozygous k/k and heterozygous K/k

	Plasma samples (cffDNA)	Buccal swabs of born children	Waiting to delivery
Total of collected samples	**141**	**129**	**12**
Total of analysed samples	**141**	**118**	
Nonanalyzable samples	**3**	**0**	
KEL heterozygous pregnant women	**9**	**---**	
KEL1/KEL2 fetuses (children)	**5**	**4**	**1?**

Table 1: Analysed plasma and buccal swab samples