Non-invasive prenatal testing of HPA*1A in Poland

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Fetal/Neonatal AlloImmune Thrombocytopenia = FNAIT

- pathogenesis: maternal alloantibodies against fetal platelet antigen(s)
- frequency: 1/1000-1/2000 delivered babies,
- FNAIT: 85% anti-HPA-1a antibodies,
- HPA-1a negative (HPA-1b/b) women: 2%
- in Poland FNAIT highly underdiagnosed and inappropriately treated
PREVFNAIT
Prevention of Fetal/Neonatal Alloimmune Thrombocytopenia in Polish newborns

- screening of pregnant women in order to identify HPA-1a negative mothers
- further management of „at risk” pregnancies (monitoring of anti-HPA-1a antibodies, antenatal intervention in reference hospitals)
- non-invasive prenatal testing (NIPT) of fetal HPA*1A (~30% HPA-1a/b heterozygous fathers – 50% chance of HPA-1a negative fetus)
Non-invasive prenatal testing of *HPA*1A

**MATERIAL**

1) Sample collection:

**Mother (plasma):**
- 5ml of EDTA blood from 125 HPA-1a negative women in 28th week of gestation
- transportation/storage at 4°C
- 2ml plasma separation by centrifugation not later than 48 hours after blood collection

**Father:** 1.5 ml of EDTA blood if available

**Neonate:** 60 cord blood samples

**Control DNA** from HPA-1b/b and HPA-1a/b donors
METHODS

2) DNA isolation:
   • 2 x 1 ml maternal plasma with Nuclisens easyMAG extractor (Biomerieux), with 2 x 25μl elution
   • whole blood - Nucleospin Blood Kit

3) pre-PCR digestion of HPA*1B allele in plasma DNA (Scheffer et al., 2011)

HPA*1A: \(\ldots\text{GCCTCTGGGCT}\ldots\)

HPA*1B: \(\ldots\text{GCCTCCC}GGG\text{GCT}\ldots\)

\(<\text{Msp1 enzyme}\)
METHODS

4) Amplification:

- real-time PCR with TaqMan technology (LightCycler 480):
  - **HPA*1A** using 10ul digested DNA in triplicate
  - **CCR5** using 2ul undigested DNA
  - SRY or ins/del polymorphisms (after pre-typing of parental DNA) using 10ul undigested DNA

- 25ul final reaction volume
- PCR profile: 95°C 10 min, 95°C 15sec, 60°C 1min - 45cycles

In each setting we test:
- **HPA-1a/b** DNA 0.5ng/ul
- **HPA-1b/b** DNA 5ng/ul
- Water control
Results of fetal *HPA*1A genotyping in 60 HPA-1a negative pregnant women

In 60 cases, where neonatal *HPA*1A genotype was available, NIPT gave correct fetal *HPA*1A results. Fetus was *HPA*1A positive in 47 cases, *HPA*1A negative in 13:

- *SRY* (6 cases),
- other paternal polymorphisms (7 cases)

In one case the presence of *HPA*1A variant in maternal genome made NIPT impossible.
Figure: Ct value of fetal *HPA*\(^{*}1A\) genotyping from plasma DNA of women who delivered HPA-1a positive or HPA-1a negative neonates.

- **HPA-1a pos neonate**
  - n=47
  - Ct CCR5: 29.7-34.0

- **HPA-1a neg neonate**
  - n=13
  - Ct CCR5: 29.7-32.5
Summary:

- Real-time PCR combined with digestion of maternal *HPA*1*B* allele is a highly reliable method for predicting fetal *HPA*1*A* status. This method is of clinical importance in the diagnosis of FNAIT.

- The fetal and maternal *HPA*1*A* genotypes were compatible in 25% of pregnancies
  - In 47 (75%) of cases the fetus was incompatible with mother
  - In 8 mothers anti-HPA-1a was present and they were treated (IVIG) and the neonates were born with no thrombocytopenia (6 cases) or with mild thrombocytopenia (3 cases)
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