PREVFNAIT
konflikt płytkowy

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

Agnieszka Orzińska[1], Katarzyna Guz[1], Małgorzata Uhrynowska[1], Marzena Dębska[2], Anne Husebekk[3], Ewa Brojer[1]

[1] Institute of Hematology and Transfusion Medicine, Warsaw,
[2] Medical Centre of Postgraduate Education, Warsaw, Poland
[3] University of Tromsø, Tromsø, Norway

norway grants

Polish-Norwegian Research Programme

INSTYTUT HEMATOLOGII I TRANSFUZJOLOGII

IHiT

INSTITUTE OF HEMATOLOGY AND TRANSFUSION MEDICINE

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
konflikt płytkowy

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^{o}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: …GCCTC**T**GGGCT…

HPA*1B: …GCCTC**C**GGGCT…

✂ ***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.

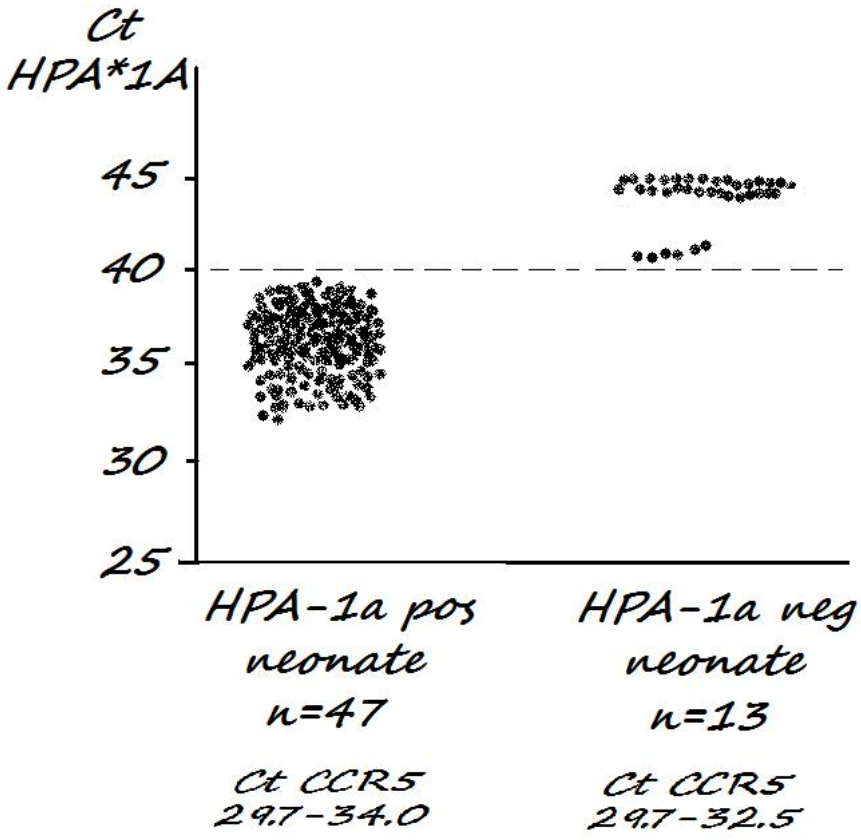
Fetus was *HPA*1A* positive in 47 cases,

*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



Summary:

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

• The fetal and maternal *HPA*1A* 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)

PREVFNAIT
konflikt płytkowy

THANK YOU FOR YOUR ATTENTION

The research leading to these results has received funding from the Polish-Norwegian Research Programme operated by the National Centre for Research and Development under the Norwegian Financial Mechanism 2009-2014 in the frame of Project Contract No Pol-Nor/203111/69/2013