Clinical applications of non-invasive prenatal diagnosis from maternal blood: What will bring the future

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Actual situation

- Placental, embryonic and fetal material can be obtained invasively through chorionic villi sampling, amniocentesis or chordocentesis.
- The invasive diagnostic techniques are generally safe and accurate, but harbor a small but measureable chance of miscarriage.

- Cell-free DNA and RNA molecules that circulate in the maternal blood can be obtained noninvasively by maternal venipuncture and therefore, has no associated risk of fetal loss.
- However maternal blood contains a mixture of both embryonic/fetal cell free DNA (predominantly trophoblastic/placental DNA) and maternal DNA, which increases the downstream analytical complexity.

Clinical applications of cffDNA

Complications of pregnancy

RhD diagnostics by RT-PCR

- RhD-testing with specific primer and probe combinations detects RhD+ fetuses in RhD- women with low false positive results (~0,2%).
- NIPD limits RhD prophylaxis only to women who carry a RhD+ fetus.
- Transition to clinical care e.g. in UK and Denmark

Pre-eclampsia

Intrauterine Growth retardation

- subject of research
- potential biomarkers for early detection : e.g. microRNAs are exported by exosomes from syncytiothrophoblast to maternal blood and therefore, may have a functional role in feto-maternal communication or the development of immune tolerance

Clinical applications of cffDNA

Genetic diseases

Autosomal diseases by RT-PCR and RMD

- Few cases described for the qualitative detection of paternally inherited or de novo mutations
- Few cases described for the quantitative detection of maternally inherited mutations by RMD = digital relative mutation dosage testing

Identification of sex by RT-PCR

- Fetal sex determenation for the management of X-linked diseases or of ambigious genitalia detected by sonography.
- Knowledge of fetal sex to determine which women has to take steroids to prevent masculanization of a female fetus that is at risk for congenital adrenal hyperplasia.(CAH)
- Transition to clinical care e.g. in UK

Chromosomal aberrations by NGS

- Autosomal Aneuploidies
- Trisomy 21 testing is offered in China (2011), USA (2011) and Germany (2012)
- Trisomy 13 and 18 testing is also offered in China and USA, but it is more challenging because the diagnostic accuracy is affected by the GC content of an individual chromosome

MPS for non-invasive detection of fetal trisomy 21

Study	cases	Trisomy 21	Sensitivity (False neg.)	Specificity (False pos.)
proof-of-concept				
Fan et al. 2008	18	9	100%	100%
Chiu et al. 2008	28	14	100%	100%
Chiu et al. 2010	15	5	100%	100%
Sehnert et al. 2011	47	13	100%	100%
Sparks et al. 2012	298	89	100%	100%
Stumm et al. 2012	42	8	100%	100%
clinical setting				
Chiu et al. *BMJ* 2011	232*2-plex*	86	100%	97,9% (3)
Ehrich et al. *AJOG* 2011	449	39	100%	99,7% (1)
Palomaki et al. 2011	1696*GC*	212	99,1% (2)	99,9% (1)
Bianchi et al. 2012	532	89	100%	100%

MPS for non-invasive detection of trisomy 18

Study	cases	Trisomy 18	Sensitivity (False neg.)	Specificity (False pos.)
Fan et al. *PNAS* 2008	18	2	100%	100%
Sehnert et al. 2011	47	8	100%	100%
Chen et al. 2011	392	37	91,9%% (3)	98,0% (5)
Sparks et al. 2012	298	7	100%	100%
Palomaki et al. 2012	1971	59	100%	99,7% (5)
Bianchi et al. 2012	532	36	97,2% (1)	100%

Whereas chromosome 21 has a midrange percentage of GC content, chromosome 13 and 18 have a lower percentage, which increases the coefficient of variation in the sequencing reactions of these chromosomes.

MPS for non-invasive detection of fetal trisomy 13

Study	Cases	Trisomy 13	Sensitivity (False neg.)	Specificity (False pos.)
Fan et al. *PNAS* 2008	18	1	100%	100%
Sehnert et al. 2011	47	1	0% (1)	100%
Chen et al. 2010	392	25	100%	98,9% (3)
Palomaki et al. 2012	1971	12	91,7% (1)	99,1% (16)
Bianchi et al. 2012	532	14	78,6% (3)	100%

Specific quantitative correction of the GC content bias in the sequencing data using modified z-score equations has resulted in improved sensitivity and specificity of trisomy 13 and 18.

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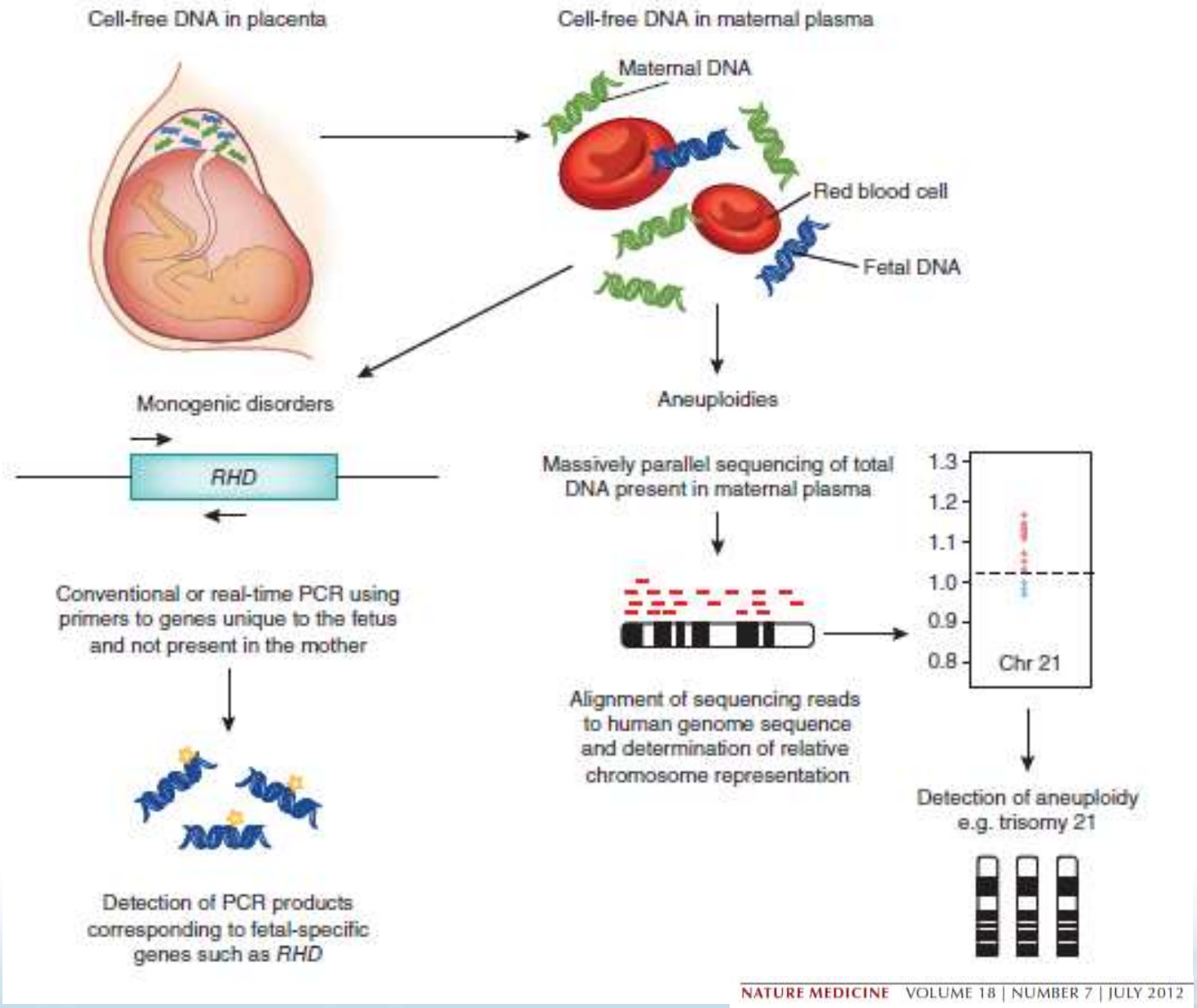
- Transition to clinical care e.g. in UK

Chromosomal aberrations by random MPS

- Autosomal Aneuploidies
- Trisomy 21
- Trisomy 13 and 18
- Other chromosomal aberrations, e.g. mosaic trisomy 9, deletions, duplications and gonosomal aneuploidies where also detected in single studies



Future developments

2010 First successful construction of a genetic map of a fetus by sequencing (65-fold coverage) of cell free fetal DNA from maternal plasma (Lo et al. *Sci Transl Med*)

- status of a familiar ß-thalassemia mutation detectable

2011 Detection of a familiar inherited microdeletion (Peters et al. *JAMA*)

- 4.2 Mbp deletion in chromosome 12p detectable

2012 Complete whole-genome sequence reconstruction of two fetal genomes by genome sequencing of two parents, genome wide maternal haplotyping (32-fold coverage) and deep sequencing of ceel free DNA from maternal plasma (72-fold coverage) (Kitzman et al. *Sci Transl Med*)

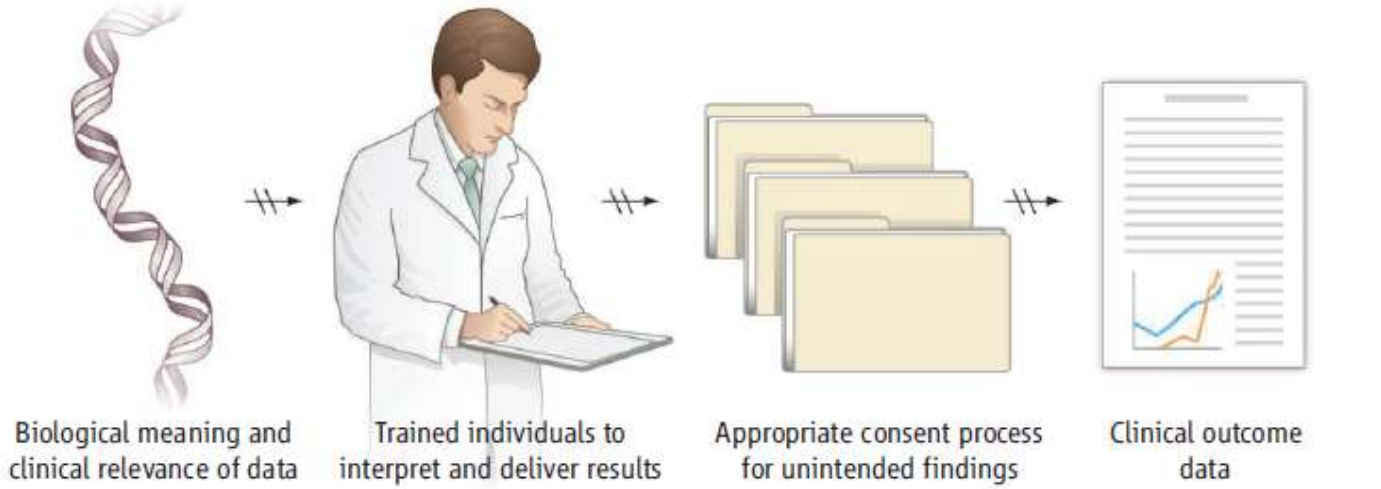
- genome wide detection of fetal de novo mutations becomes possible

2012 Analysis of the complete fetal genome by massively parallel sequencing of cell free maternal plasma DNA (Fan et al. Nature)

- 2,85 Mbp deletion in chromosome 22q11.2 detectable
- non-invasive exome screening of all clinical relevant and deleterious alleles becomes possible

Future developments

- It is theoretically possible to noninvasively screen maternal blood for both fetal DNA copy number variation and single gene disorders by random MPS strategies.



Gaps in the delivery of routine WGS of healthy individuals. 1 JUNE 2012 VOL 336 SCIENCE www.sciencemag.org

- But in the near future, targeted MPS approaches may be applied for the NIPD of groups of common single-gene disorders, microdeletion/-duplication syndromes and aneuploidies.

From prenatal genomic diagnosis to fetal personalized medicine: progress and challenges

Diana W Bianchi

Thus far, the focus of personalized medicine has been the prevention and treatment of conditions that affect adults. Although advances in genetic technology have been applied more frequently to prenatal diagnosis than to fetal treatment, genetic and genomic information is beginning to influence pregnancy management. Recent developments in sequencing the fetal genome combined with progress in understanding fetal physiology using gene expression arrays indicate that we could have the technical capabilities to apply an individualized medicine approach to the fetus. Here I review recent advances in prenatal genetic diagnostics, the challenges associated with these new technologies and how the information derived from them can be used to advance fetal care. Historically, the goal of prenatal diagnosis has been to provide an informed choice to prospective parents. We are now at a point where that goal can and should be expanded to incorporate genetic, genomic and transcriptomic data to develop new approaches to fetal treatment.

NATURE MEDICINE VOLUME 18 | NUMBER 7 | JULY 2012

Thank you

for your attention!