Epigenetic approaches for non-invasive prenatal diagnosis using cell-free fetal DNA present in maternal plasma

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Introduction

Interest in developing non invasive prenatal diagnosis techniques has grown within the past decade, especially since the discovery of cell-free fetal DNA (cffDNA) presence in maternal plasma in 1997.

The potential use of cffDNA presents two main limitations: the development of techniques to allow the distinction between maternal and fetal DNA, and the low presence of cffDNA in maternal plasma, that represents only 10-20% of the fraction. The use of epigenetic markers, which are sequences that contain covalent modifications of DNA that do not change the genome sequence and are stably transmitted during cell division, raised as a suitable choice.

Genetic markers

Absolute discrimination

Y-chromosome-specific loci

Only for male pregnancies

High false negative results

Paternal-inherited loci

Previous knowledge of the parents' polymorphic status needed

Epigenetic markers

Based on different methylation patterns between mother and fetus

Gender independent

Parent of origin specific methylation patterns

Imprinting process: Epigenetic marks depending on the progenitors' sex

Previous knowledge of the parents' polymorphic status required

Placenta specific methylation patterns

Maternal DNA in plasma derived from hematopoietic cells and cffDNA's placental origin provide the different methylation patterns.

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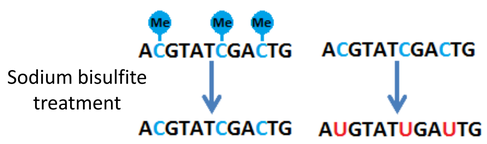
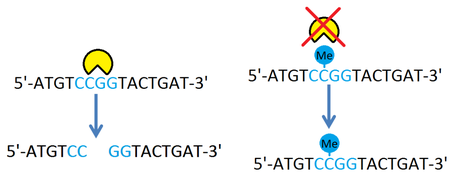
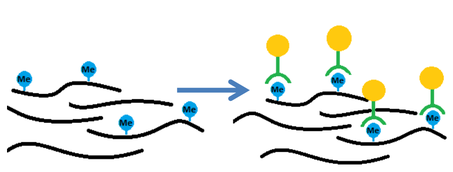
Maspin gene: First universal fetal marker

NO previous information of polymorphic status required

Methylation assays

Sodium bisulfite conversion	Methylation-sensitive restriction enzyme	Methylation DNA immunoprecipitation
Conversion of unmethylated cytosines to uracil.	Enzymes sensitive to methylation	Monoclonal antibodies with magnetic beads attach to methylated cytosines.
		
Epigenetic modifications become genetic modifications	Remove the unmethylated maternal DNA	Immunoprecipitation of methylated sequences
Advantages		
Not sensitive to sample impurities Methylation analysis at base pair level	Easy to perform and low cost Less damage, more molecules available	Low cost assay Not sensitive to sample impurities Can be applied with low starting DNA amounts
Disadvantages		
DNA degradation (>90%) Full conversion rarely achieved	Sensitive to sample impurities Requires high amount of starting DNA Applicable to a limited number of DNA sequences	Depends on antibody efficiency and ideal combination of affinity reagents

Adapted from Patsalis et al. (1)

Methodology

Conduct a literature research using NCBI.

Objectives

Highlight the usefulness and importance of epigenetic markers for non-invasive prenatal testing.

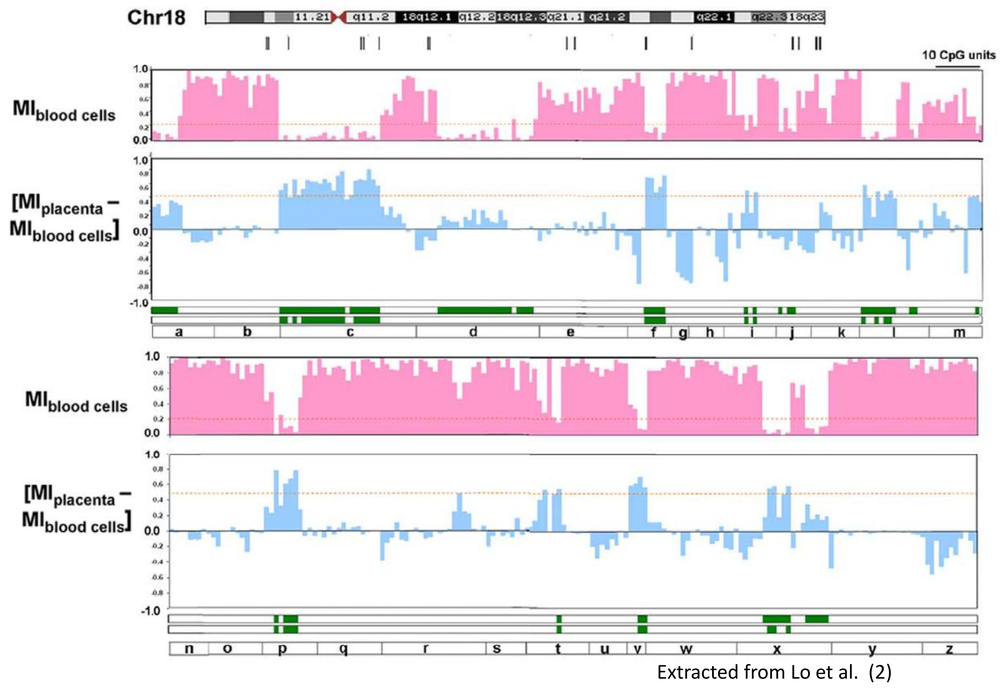
Describe the approaches and techniques used to perform the assays, its advantages, limitations and clinical applications.

New epigenetic markers

Detection of differently methylated regions.

Increase the number of regions known to expand the disease application range.

Performed with methylation array analysis.



Extracted from Lo et al. (2)

Search for suitable epigenetic markers

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Special consideration needs to be taken:

- Methylation patterns are susceptible to external agents.
- Methylation status can change depending on pregnancy state.
- Individual methylation variation.

Applications

Sex determination

Rheshus D status

Epigenetic markers as positive control

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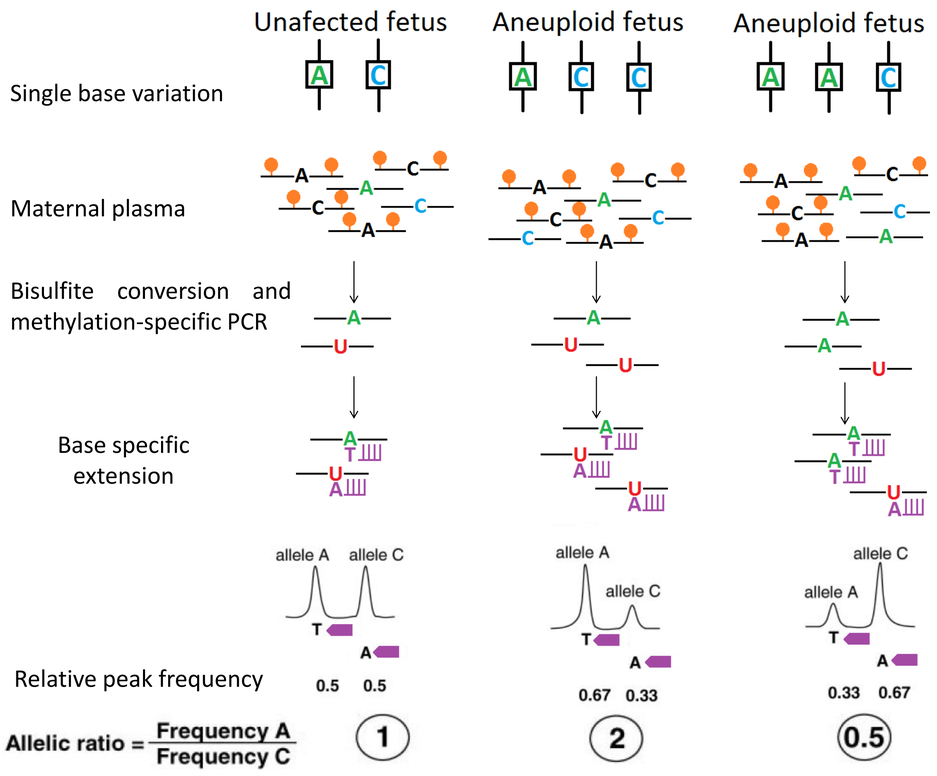
Address the false negative results

Aneuploidies

Epigenetic allelic ratio



Adapted from Lo et al. (3)

Depending on SNP heterogeneity

Epigenetic-genetic chromoseme dosage

Ratio value calculation:

$$\text{Ratio} = \frac{[\text{Epigenetic fetal marker}]}{[\text{Fetal-specific genetic marker}]}$$

Genetic marker unaffected by individual methylation variation

$$\text{Ratio} = \frac{[\text{Epigenetic fetal marker on the affected chromosome}]}{[\text{Epigenetic fetal marker on unaffected chromosome}]}$$

Not depending on SNP heterogeneity

Fetal-specific DNA methylation ratio

Multiple markers analyzed

Similar to epigenetic-genetic chromosome dosage

Discrimination value is achieved considering the discriminative coefficient for each marker:

$$D = -6{,}331 + 0{,}959\,X_{EP4} + 1{,}188\,X_{EP5} + 0{,}424\,X_{EP6} + 0{,}621\,X_{EP7} + 0{,}028\,X_{EP8} + 0{,}387\,X_{EP10} - 0{,}683\,X_{EP11} + 0{,}897\,X_{EP12}$$

where X_{EPn} = ratio value$^{\text{Sample; EP}n}$, n = 1–12

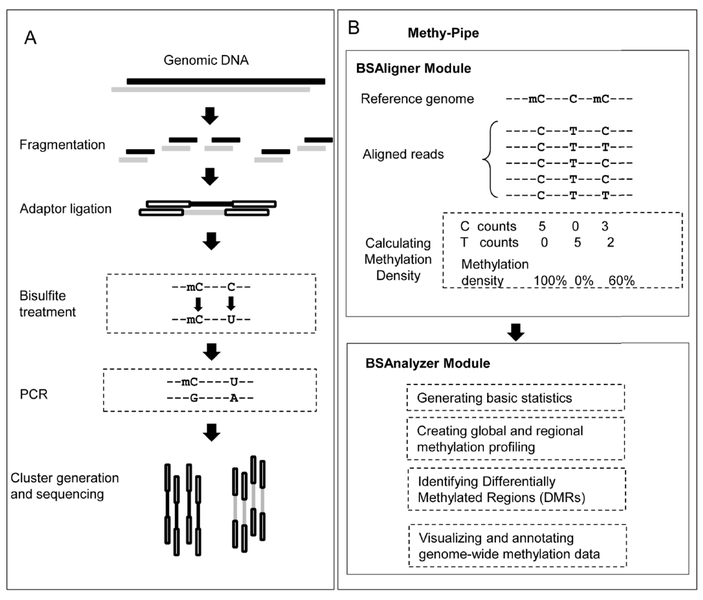
Extracted from Patsalis et al. (4)

Discriminative coefficients have to be precisely selected

Next generation sequencing

Combination of methylation status of the sequences and genome-wide sequencing

Bioinformatics modules to process and analyze the data



Extracted from Sun et al. (5)

Depending on bisulfit conversion efficacy and expensive

Discussion

- Epigenetic approaches have successfully defeated the restrictions that absolute discriminative genetic markers presented, allowing the application of non invasive diagnosis to all pregnancies by using universal fetal markers.
- Clinical implementation of epigenetic approaches has to overcome a few limitations, since all the described techniques are useful but none of them are optimal. Even though, this techniques present more potential to be implemented on global scale than currently available sequencing procedures, because they are easier and less expensive to perform, and the necessary equipment is present in more laboratories.
- Further validation of potential epigenetic markers and improvement of ratio values should be performed.
- The following years genome wide arrays will provide more differentially methylated regions that could be used as epigenetic biomarkers for other diseases, increasing its interest to be applied to clinics.

References

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