Identification of Trophoblast Markers for application in (Tel Hashomer)
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Non-Invasive Prenatal Diagnosis (NIPD)

Dr. Esther Gurtta and Dr. Liat Abo-Gutstein, Sheba Medical Center.

Categories	Noninvasive Prenatal Diagnosis (NIPD), Fetal Cells in Maternal Blood
Development Stage	Clinical stage , Tested in human blood samples
Patent Status	Approved patent

Background and Innovation of the Technology

Prenatal diagnosis needs to be differentiated from routine antenatal screening. The National Institute for Health and Care Excellence (NICE) and the UK National Screening Committee (UK NSC) have laid down standards for antenatal care, including the screening tests that should be offered to all pregnant women. These **screening tests do not give a definitive prenatal diagnosis** but give a risk/probability of a problem with the fetus - for example, Down's syndrome. **Further diagnostic tests are required to confirm and diagnose the fetal abnormality**.

To date, prenatal diagnosis is offered to all pregnant women if they have positive antenatal screening results. However, some women may be offered definitive prenatal diagnosis from the outset without any preceding screening tests; for example:

- If there is a family history of an inherited condition.
- If they have had a previous pregnancy with fetal abnormality.
- If they have been exposed to illness such as toxoplasmosis or rubella during the pregnancy.
- If they have been exposed to teratogens, such as certain drugs or radiation, during the pregnancy.
- If the woman has type 1 diabetes mellitus, epilepsy or myotonic dystrophy.

The primary aim of a prenatal diagnosis is to provide an **accurate diagnosis** that will allow the widest possible range of informed choice to those at increased risk of having children with genetic disorders or with congenital abnormalities.

Invasive procedures, such as amniocentesis or chorionic villus sampling (CVS),are routinely applied to diagnose fetal chromosomal abnormalities and genetic diseases, for example, Down syndrome, fragile X syndrome, cystic fibrosis, Tay-Sachs disease and many others. Such procedures involve a risk of fetal loss, and are applied only in high-risk cases.

Therefore, non-invasive prenatal diagnosis (NIPD), based on the isolation of rare fetal cells from maternal blood, has been the source of anticipation over the last few decades. However, two major obstacles have hampered the development of a robust protocol that can be applied in the clinical laboratory. First, the **fetal cells**, although proven unequivocally to be present in maternal blood, are **very rare** and can be found at a frequency of 1 fetal cell to106-107 nucleated maternal blood cells.

And second, until our recent progress, **specific fetal cell markers have yet to be identified**, thus accurate identification has not been possible among the immense background of maternal blood cells.

The development of a reproducible, reliable, non-invasive method based on retrieval of rare fetal cells from the maternal circulation for down-stream genetic diagnosis would render safer and more feasible testing. Several cell types have been targeted to this end such as nucleated red blood cells, CD34+ and Trophoblasts.

Trophoblasts present an attractive target cell type for non-invasive prenatal diagnosis as they can be **isolated from maternal blood** early in the first trimester. Trophoblasts are distinguishable from maternal blood cells due to their unique structure and are **absent in normal adult blood**. Trophoblast cells have been isolated from maternal blood by several different methods related to surface antigen expression, for example HLA-G and cell size. However, these approaches have yet to be implemented in clinical practice of prenatal diagnosis due to major obstacles: cell rarity, difficulty in determining definitive fetal source and the challenge of efficient retrieval.

Our invention consists of a new method for noninvasive prenatal diagnosis based on enrichment of fetal Trophoblasts from maternal blood. The method is based on cell separation with specific trophoblast markers (annexin IV, cytokeratin 7, cytokeratin 8, keratin19). Following our novel enrichment protocol and retrieval, the cells can be used for genetic and cytogenetic diagnosis.

The Need

Technological advances in prenatal genetic diagnosis continue to evolve, increasing diagnostic accuracy and broadening indications. However, **unanimity does not exist**. Genetic prenatal diagnosis is an extremely dynamic and expanding field, requested most Obstetrics and gynecology (OB/GYN), depending of course on the medical details of the couple. The application in prenatal diagnosis of new advanced methods, for example, cytogenetic microarray (CMA) is increasing exponentially.

NIPT avoids the risk of miscarriage associated with invasive testing procedures (e.g. amniocentesis or chorionic villus sampling). and the sensitivity and specificity of NIPT approaches 90% for detecting single gene for Down syndrome in maternal serum DNA, providing the sequencing is successful. However, technical, financial and ethical issues remain as NIPT becomes a primary screen for those women who wish to know about fetal all chromosomal abnormality.

Currently, two broad categories of massively parallel sequencing (MPS)-based approaches are being used to provide plasma-DNA-based NIPT clinical services for patients:

Shotgun sequencing where DNA molecules contained in a maternal plasma sample are sequenced at random. The proportional representation of DNA molecules sequenced from the chromosome of interest (for example, chromosome 21) is compared with those sequenced from elsewhere in the genome. The disadvantage of this approach is that genomic regions that are not directly relevant to NIPT are also analyzed as the sequencing is random.

Targeted sequencing where genomic regions containing the chromosomes at risk of the aneuploidy, as well as a selected group of reference regions, are selectively targeted for sequencing. To date, there are two different implementations of this approach, one involving targets that do not vary from individual to individual and another involving allelic ratio analysis for targets that are polymorphic within a population. The disadvantage of the targeted sequencing approach is that the targeting steps (for example, DNA hybridization probes or PCR primers) need to be tailor-made for every woman.

Currently, only fetal trisomies 21, 18, 13 , the common sex chromosome aneuploidies and paternity test from maternal blood are using massively parallel genomic sequencing of DNA.

One should remember that the proportion of maternal plasma DNA that is fetal is affected by several maternal characteristics, including maternal weight, *Multiple pregnancies* and additional maladies.

We have developed a method to enrich fetal cells from maternal blood. Our methods can be **applied in single cells or in very low numbers of cells**, to perform variety of prenatal diagnosis' including hole genome sequencing.

Development Stage

Development of methods for isolation, identification and diagnosis of rare fetal cells in maternal peripheral blood has been our ongoing clinical research focus for over a decade.

1- We have developed a method enabling placental Trophoblast cells derived from maternal blood to proliferate under in-vitro culture conditions, thereby increasing fetal cell yields and allowing fetal cell metaphase karyotyping.

2- The detection for fetal cells presence was demonstrated in about 90% over 50 maternal blood samples tested with the novel trophoblast markers we identified (IP).

3- We developed a novel protocol for cell enrichment with intracellular markers.

4- We have validated the enriched Fetal cells for the fetal cells, using various FISH genetic probes and tests.

Advantages:

Noninvasive retrieval of fetal cells from maternal blood offers 100% safety for the fetus and the mother. This approach eliminates the need for invasive procedures and allows all genetic analysis:

1. Single cell PCR DNA analysis of fetal Cells
2. FISH analysis
3. Karyotyping

The Market

Πρεναταλ τεστσ ασσεσσ τηε ρισκ ορ τηε πρεσενχε οφ χηρομοσομαλ αβνορμαλιτιεσ ιν α φετυσ. Χοπψ νυμβερ ϖαριατιον ορ χηρομοσομαλ ιμβαλανχεσ (αμπλιφιχατιονσ ορ δελετιονσ) χαν ηαϖε δεϖαστατινγ εφφεχτσ ον α πρεγνανχψ εϖεν χαυσινγ λοσσ οφ τηε φετυσ.

As Women Delay Motherhood, Demand for a Broader Range of Products Increases Significantly. In 2012, the worldwide annual birth reached 140 M Births with 8% Born with Birth Defects. Today we can analyze over 1,800 Genetic Disorders that can be tested.

Currently, genetic testing on the fetus involves sampling material by amniocentesis or chorionic villus sampling (CVS). Due to the slight risks of miscarriage and potential complications associated with both procedures, maternal blood tests were developed to screen the larger population for the at-risk subset that should have an invasive procedure performed for conclusive diagnostic testing.

U.S. prenatal testing was a $0.8 billion market in 2012. Prenatal screens provided 75% of revenues, and prenatal diagnostic testing generated the remaining 25%. Considering that 60% to 70% of the 5 million births in the U.S. receive prenatal care, the screening segment would be large relative to the diagnostic segment. However, the clinical utility of microarrays and sequencing in prenatal applications will lay the foundation for tremendous growth in the prenatal diagnostic segment.

The market for noninvasive prenatal testing is gaining momentum, analysts say, and a new report from market researchers shows that the demand for such tests is set to triple over the next 5 years, potentially becoming a $3.6 billion industry by 2019. The market is evolving rapidly due the advantages it offers over the conventional prenatal screening and diagnostic methods such as maternal serum screening, nuchal translucency (NT) scan, amniocentesis and chorionic villus sampling (CVS). Overcoming technology difficulties , looking directly at Fetal cells and its genome , will divers the market.

High incidence rate of babies born with certain type of chromosomal abnormalities, growing trend of child bearing at advanced maternal age and enhanced popularity of NIPT among gynecologists and high risk pregnant women are some of the major factors anticipated to drive growth of the NIPT market during the forecast period. Some of the most commonly detected chromosomal aneuploidies include Down syndrome, Edwards syndrome, Patau syndrome and monosomy X.

Geographically, North America was the largest regional market in 2012 with a market share of 64.5% of the total revenue generated globally, followed by Europe. Almost every company operating in this market is adopting the same business model of marketing their tests via alliances with hospitals, diagnostic laboratories and physician offices. The increasing penetration by test developers in European, Asia-Pacific and Middle-East countries indicates promising growth in the near future in these markets. Sequenom, Inc., Verinata Health, Inc. (now a part of Illumina, Inc.), Ariosa Diagnostics, Natera, Inc., BGI Health, LifeCodexx and Berry Genomics are the companies engaged in providing non-invasive prenatal tests worldwide.

The exciting technological developments in the prenatal diagnostic segment make this one of the

most anticipated clinical diagnostic segments, which will contribute to the long-term health of prenatal testing markets.

IP Status: Approved in US, Israel and Europe

Contact

Sylvie Luria PhD.

Technology Transfer and Business & Development Manager

Tel Hashomer Medical Research, Infrastructure and Services Ltd.

Tel: +972-3-5305998 Fax: +972-3-5305944 Cell: 052-6667277

sylvie.luria@sheba.health.gov.il http://research.sheba.co.il/e/