Full Length Research Paper

Application of proteomics for prenatal diagnosis of Down syndrome: Systematic review and a meta-analysis

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We systematically reviewed the available literature and meta-analyzed the data which was specialized in Down syndrome (DS) diagnosis with proteomic techniques. Pubmed, EBSCOhost and ScienceDirect searches for relevant articles published from inception until July 2010 were obtained and ten articles were selected. Many candidate biomarkers were found, which could be used to identify Down syndrome. There were 14 markers noted more than two times and 29 best biomarkers were recommended by the authors particularly for clinical application. Application of proteomics contributed to the finding of novel biomarker for prenatal diagnosis of Down syndrome, providing opportunities for the development of non-invasive prenatal diagnosis.

Key words: Down syndrome, proteomic, aneuploidy, prenatal diagnosis, meta-analysis.

INTRODUCTION

Down syndrome (DS) or trisomy 21 is one of the most prevalent chromosomal disorders, accounting for significant morbidity and mortality. It is caused by the presence of three copies of chromosome 21 and has an incidence of 1 in 700 live births (Roizen and Patterson, 2003). Over the past 25 years, prenatal diagnosis of fetal aneuploidies such as DS relies on the karyotype analysis of fetal cells from mothers, such as amniocentesis or chorionic villous sampling. These invasive prenatal diagnoses are used to achieve over 99% accuracy (Geifman-Holtzman and Ober, 2008). However the invasive procedures will result in severe anxiety of patients (Hewison et al., 2007) and fetal loss or injury (Tabor et al., 2009). Some researches attempted to explore any techniques to make diagnose rapidly. For example, real-time quantitative PCR (RT-PCR) and fluorescence in situ hybridization (FISH), could diagnosed DS in 24 to 48 h (Tabor et al., 2009; Brown et al., 2006), but there were still some defects, which could not be neglected (Karen et al., 2004; Allen et al., 2009).

Current studies focus on new non-invasive prenatal diagnostic techniques which is highly accurate and risk-free. In the past five years, proteomics-based identification of biomarkers for fetal abnormalities in maternal plasma, amniotic fluid and reproductive fluids has made significant progress (Choolani et al., 2006). Despite the fact that it was non-clinically applicable yet, it was described to prenatally diagnose fetal aneuploidies, which mainly lie in DS.

After genomics, proteomics is considered the next step in the study of biological systems. Proteomic is the large-scale study of proteins, particularly their structures and functions (Blackstock and Weir, 1999); include diagnostic pattern proteomics and identification-centred proteomics. Since the establishment of the Human Proteome Organization (HUPO) in 2001, proteomic developed rapidly and penetrated into the various disciplines, especially in cancer research (Thadikkaran et al., 2005). Currently, it is also one hot spot that proteomics as a major platform technology has been applied in perinatal medicine research. Some new biomarkers were found to be associated with fetal genetic diseases or pregnancy complications, such as premature rupture of membrane (Hung and Yu, 2010), preterm birth

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(Buhimschi et al., 2008), preeclampsia (Park et al., 2008) and intra-amniotic infection (Gravett et al., 2004). All these new biomarkers showed great potential in contributing to diagnosis of disease, revealing mechanism and finding new therapeutic targets. In 2004, proteomic were applied in fetal aneuploidies for the first time (Oh et al., 2005). OH et al. (2004) used two-dimensional gel electrophoresis (2-DE) followed by matrix-assisted laser desorption/ionization-time (MALDI) to identify metabolic enzymes of amnion cells after it had been cultivated. The true sense of proteomics applied in the DS diagnosis was carried out (Wang et al., 2005). They first investigated an amniotic fluid (AF) fingerprint in 20 samples obtained from pregnant women known to carry an aneuploid fetus, and got some candidate markers. Their study has brought great hope of identifying novel biomarkers for diagnosis. Nagalla et al. (2007) study was the first attempt of proteomic technology in DS non-invasive prenatal diagnosis. They also performed a comprehensive proteomic analysis to identify potential serum biomarkers to detect DS. From 2004 to 2010, there were several studies reported which focused on the field of diagnosing DS with proteomic (Tsangaris et al., 2006; Mange et al., 2008; Cho et al., 2010; Kolla et al., 2010; Kolialexi et al., 2008; Lopez et al., 2011; Wang et al., 2009). All of the reports showed the hopes of the development of effective non-invasive approaches.

In this systematic review and meta-analysis we performed an updated meta-analysis which was specialized in DS diagnosis with proteomic techniques, including ten studies. We systematically reviewed the available literature and meta-analysed the data.

MATERIALS AND METHODS

This systematic review and meta-analysis was conducted according to a protocol designed by Wang and Yu in August 2010.

Data searches

Electronic searches were performed by two investigators. We performed Pubmed, EBSCOhost and ScienceDirect searches for relevant articles published from inception until July 2010, using the following words: "proteomic or proteomics", "aneuploidy or aneuploid" and "Down syndrome or trisomy 21". We screened all titles and abstracts to determine their suitability and then applied inclusion/exclusion criteria to the complete articles and resolved discrepancies by consensus.

Study selection

Inclusion criteria were (a) original research on diagnosis of fetal aneuploidies or DS with proteomic, (b) use of an analytic design (case-control), (c) English-language articles, (d) non-mechanisms studies and (e) studies about prenatal diagnosis. Review, letters, case reports, brief reports, abstracts and comments were excluded. Articles were independently searched and reviewed by the two investigators. At first, 89 articles were gotten from MEDLINE search. After rapid review, 57 articles were excluded by title or abstracts.

According to the aforementioned criteria, 22 articles were excluded again, because of article type or research filed. Then, ten articles were included in the meta-analysis in the end. A flowchart of the selection process is provided as shown in Figure 1.

Data abstraction, synthesis and analysis

We independently extracted key data from all included studies. The following data were collected from each included studies: first authors, year of publications, study design, study population (fetal aneuploidies or DS), sources of controls, proteomic approach, gestation, candidate biomarkers and recognition capability. All of the candidate biomarkers noted in articles were selected and merged by hand. If the research discussed pooled estimates of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. All of the analyses were performed by Revman4.2.2 software. Since all the data were countable data, the results were expressed as 95% CI, using two-sided P values.

RESULTS

Study characteristics

Based on the search strategy, ten articles selected at the end, described 155 cases of Down syndrome and 240 unaffected fetuses. All of the studies were case-control studies and could be divided into three sample categories: amniotic fluid (n=6, including one study based on amniotic cell), maternal plasma (n=2) and maternal serum (n=1). 70.0% (7/10) studies were carried out in the 2nd trimester, 20.0% (2/10) were in the 1st trimester, while one study was designed from 1st trimester to 2nd trimester. Subjects of two studies were aneuploidies included Down syndrome, trisomy 18 and 13. All of the cases were centrifuged by cytogenetic analysis of the collection of amniocytes. The details of the individual characteristics of the included studies are available in Table 1.

Candidate biomarkers

Many candidate biomarkers were noted in ten studies, which could be used to identify Down syndrome. After selected and merged manual, there were 14 markers that noted more than two times by all the authors (Table 2). Furthermore, the authors recommended particularly 29 best biomarkers for clinical application. Table 3 lists the biomarkers which the authors recommended. Among the 29 candidate biomarkers, 79.3% (23/29) markers were increased in DS group compared with unaffected fetuses, while 20.7% (6/29) proteins decreased. At the same time, alpha-1-microglobulin and serum amyloid P-component were recommended by two different studies.

Diagnostic performance

Nagalla et al. (2007) reported that proteomic discriminated between DS and the controls in both trimesters,

Potentially relevant reports identified and screened for retrieval (n=89)

Reports excluded on title, abstract review (n=57)

Reports retrieved for more detailed evaluation (n=32)

Reports excluded after detailed review (n=22):
Mechanism research (n=11)
Animal mode (n=4)
Reviews article (n=3)
Abstracts (n=2)
Brief report (n=1)
Experiment method (n=1)

Included studies in meta analysis (n = 10)

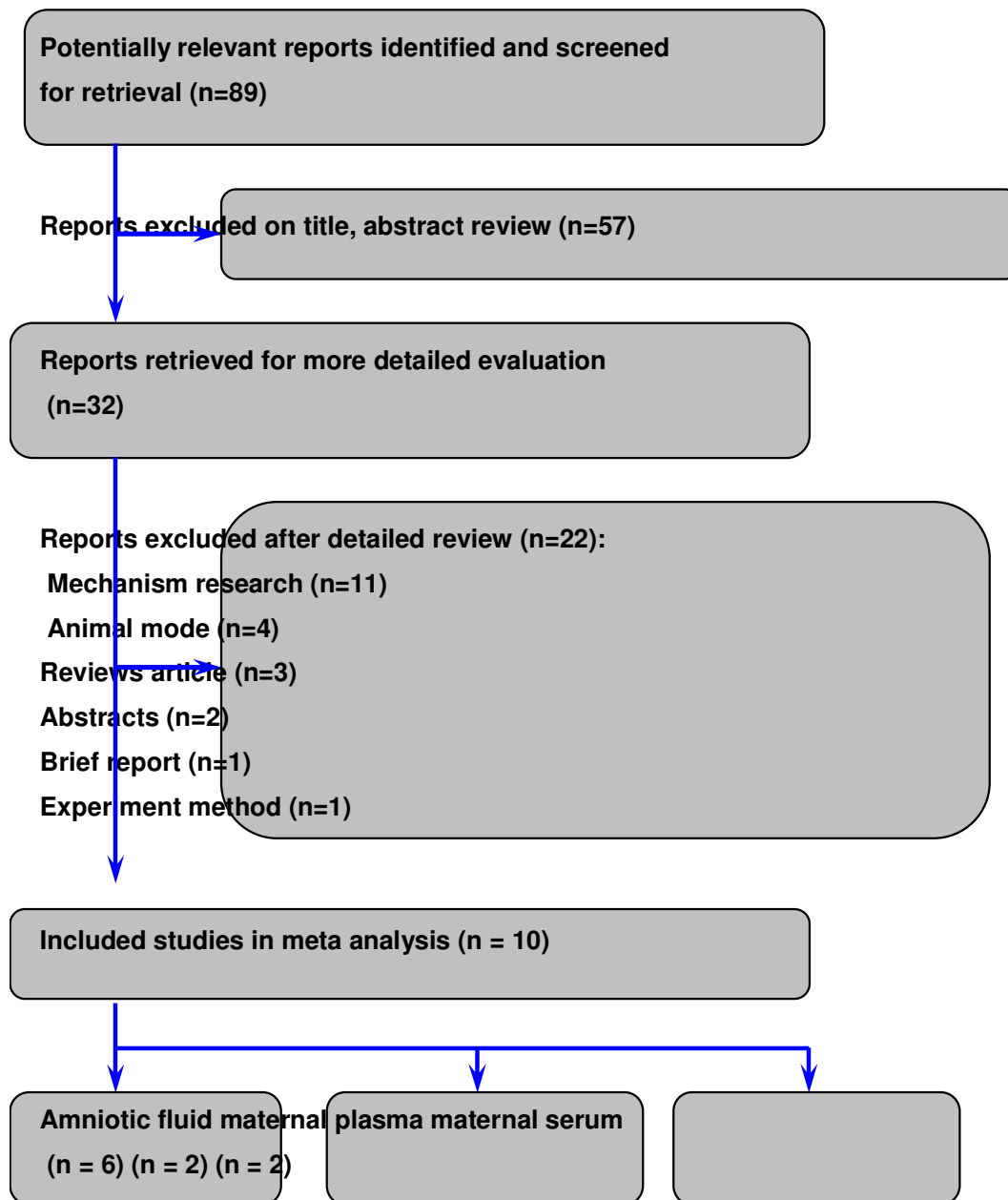**Amniotic fluid maternal plasma maternal serum (n = 6) (n = 2) (n = 2)**

Figure 1. Flowchart of articles selection.

with an average recognition capability approaching 96%. Only two studies (Wang et al., 2005; Mange et al., 2008) provided the data of sensitivity, specificity, PPV and NPV.

Wang et al. (2005) reported that the proteomics analysis could be used to detect aneuploid AF at 3.3% disease prevalence rate with 100% sensitivity, 72 to 96% specificity, 11 to 50% PPV and 100% NPV. Mange et al. (2008) used two class predictor models to classify the test and found that the overall classification accuracies were maintained in the validation phase with 87.5% (83.33% sensitivity, 83.33% specificity, 83.33% PPV, and 83.33%NPV) and 91.67% (83.33%sensitivity, 100% specificity, 100% PPV, and 87.71% NPV) for SVM (support vector machine) classification and logistic regression indexes, respectively.

DISCUSSION

The science of proteomic has been applied to the search for biomarkers and generation of protein profiles that can rapidly aid the prediction, early diagnosis and treatment of human diseases (Papadopoulos et al., 2004; Petricoin et al., 2002; Chen et al., 2004). It can also be divided into

Table1. Description of the studies including in the meta-analysis.

Author	Year	Country	Design	Sample	DS Cases	Unaffected fetus	Trimester	Technology used
Cho et al	2010	Canada	Case-control random	AF	10	10	2nd	LTQ-Orbitrap MS
Wang et al*	2009	China Taiwan	Case-control	AF	19	34	2nd	MALDI-TOF-MS
Mang et al*	2008	France	Case-control	AF	17	25	2nd	SELDI-TOF/MS
Tsangaris et al	2006	Greece	Case-control	AF	6	12	2nd	MALDI-TOF-MS nano-ESI-MS/MS
Wang et al *	2005	China Taiwan	Case-control	AF	6	60	2nd	MALDI-TOF-MS
Oh et al	2004	Austria	Case-control	AF Cell	3	4	2nd	MALDI -MS
Kolla et al	2010	Switzerland	Case-control	Maternal plasma	6	6	1st	iTRAQ LC-MALDI-MS/MS
Kolialexi et al	2008	Greece	Case-control	Maternal plasma	8	12	2nd	MALDI-TOF-MS
Lopez et al	2011#	UK	Case-control random	Maternal serum	24	21	1st	LC-MS/MS
Nagalla et al	2007	USA	Case-control	Maternal serum	56	56	1st 2nd	LC-MS/MS MALDI-TOF-MS

* Subjects of the study were aneuploidies and included Down syndrome, trisomy and trisomy 13. It was publish on-line in 2010

two major groups, such as: techniques used for profiling and techniques used for differential protein detection. The most common approach for the analysis of reproduction-related biological fluids relies upon a coordinated used of 2-DE, image analysis, mass spectrometry (MS) protein identification and bioinformatics/database construction (Park et al., 2006; Tsangaris et al., 2006). In general, four different types of MS-based proteomic technologies are used in proteomics, namely, two-dimensional gel electrophoresis coupled to mass spectrometry (2DE-MS), surface-enhanced laser desorption/ionization coupled to mass spectrometry (SELDI-MS), liquid chromatography coupled to mass spectrometry (LC-MS) and capillary electrophoresis coupled to mass spectrometry (CE-MS) (Wu et al., 2010). Among the nine studies included in the meta-analysis, the first three were reported to use MS-based techniques, respectively, while the others used CE-MS.

Since the establishment of the Human Proteome Organization (HUPO) in 2001, proteomic developed rapidly and penetrated into the various disciplines, for example gynecologic oncology. There were many studies that focused on ovarian cancer (Kim et al., 2009; Wang et al., 2008) and cervical cancer (Lee et al., 2005; Zhu et al., 2009). All the studies showed that proteomic techniques contribute to the finding of the potential diagnostic and therapeutic targets and improve individual patient outcome. Currently, it is also one of the hot spots that proteomics as a major platform of technology has been applied in perinatal medicine research. Liberatori et al. (1997) identified human proteins in AF supernatant by immunoblot analysis and reported a 2-DE protein map of human AF in the second trimester of gestation. As the most important maternal-fetal medium, AF plays a key role in some pregnancy-related diseases. Therefore, the proteomic based on AF shows a huge space. With it, many new biomarkers for maternal-fetal diseases were found and applied in clinic gradually (Hung and Yu, 2010; Buhimschi et al., 2008; Park et al., 2008; Gravett et al., 2004). The prenatal diagnosis of fetal aneuploidies (Down syndrome) is one of the most important researches filed.

Oh et al. (2004) attempted to find a screening method for a large series of metabolic enzymes with proteomic. They used 2-DE followed by MALDI to compared

Table2. Candidate biomarkers noted in the studies (≥ 2 times).

Protein name	Protein symbol	Swiss-Prot ID	Biological processes	Molecular functions	Fold change	Coverage (%)
Complement component C8 beta chain	CO8B	P07358	Complement mediated immunity	Complement component	2.3 (Cho et al., 2010) 2.7 (Nagalla et al., 2007)	36.5 (Kolla et al., 2010)
Serum amyloid A	SAA	P02735	Acute phase	G-protein-coupled receptor binding	11.2 (Nagalla et al., 2007) 0.71* (Lopez et al., 2011)	--
Serum amyloid P-component	APCS	P02743	Amino acid biosynthesis	Synthase	--	54.3 (Kolla et al., 2010) 42.0 (Kolialexi et al., 2008)
Alpha-1-antitrypsin	SERPINA1	P01009	Nerve-nerve synaptic transmission	Glutamate receptor	--	32.8 (Kolla et al., 2010) 46.0 (Kolialexi et al., 2008)
Alpha-2-macroglobulin	A2M	P01023	Developmental processes	Serine/threonine kinase	0.62* (Lopez et al., 2011)	39.8 (Kolla et al., 2010)
AMBP protein	AMBP	P02760	Host-virus interaction	Protease inhibitor	5.71[#] (Tsangaris et al., 2006)	35.0 (Kolialexi et al., 2008)
Apolipoprotein C-I	APOC1	P02654	Lipid transport	fatty acid binding	2.5 (Nagalla et al., 2007) 0.66* (Lopez et al., 2011)	--
Carbonic anhydrase 1	CAH1	P00915	one-carbon metabolic process	carbonate dehydratase activity	3.9 (Cho et al., 2010) -4.3 (Nagalla et al., 2007)	--
Choriogonadotropin subunit beta	CGB	P01233	mRNA transcription	Other signaling molecule	2.0 (Cho et al., 2010)	26.1 (Kolla et al., 2010)
Fibronectin	FINC	P02751	Extracellular matrix protein	Cell adhesion molecule	1.8 (Nagalla et al., 2007)	49.4 (Kolla et al., 2010)
Histidine-rich glycoprotein precursor	HRG	P04196	Blood coagulation	cysteine-type endopeptidase inhibitor activity	0.65* (Lopez et al., 2011)	23.0 (Kolialexi et al., 2008)
Platelet basic protein	SCYB7	P02775	Pyrimidine metabolism	Phosphorylase	3.4 (Nagalla et al., 2007)	41.4 (Kolla et al., 2010)
Afamin	AFAM	P43652	Transport	transfer/carrier protein	--	47.7(Kolla et al., 2010) 43.0 (Kolialexi et al., 2008)
Transthyretin	TTHY	P02766	Transport	Thyroid hormone	2.2 (Nagalla et al., 2007)	68.0 (Kolialexi et al., 2008)

* Area-under ROC curve; [#] Expression level.

Table 3. The list of biomarkers recommended by the authors.

Article	Sample	Protein name	Up-regulated	Down-regulated
Nagalla et al., 2007	Maternal serum	serum glycoproteins	↑&	
Tsangaris et al., 2006	AF	Splicing factor arginine/serine-rich 4	↑	
		Alpha-1-microglobulin	↑	
		Collagen alpha 1 (I) chain	↑	
		Collagen alpha 1 (III) chain	↑	
		Collagen alpha 1 (V) chain	↑	
		Basement membranespecific heparin sulfate proteoglycan core protein	↑	
		protein IBP-1		↓$
Cho et al., 2010	AF	Amyloid precursor protein(APP)	↑*	
		Tenascin-C(TNC-C)	↑#	
Wang et al., 2009	AF	Antitrypsin	↑	
		Prealbumin	↑	
		Transferrin	↑	
		Apolipoprotein A1		↓
Kolla et al., 2008	Maternal plasma	Ig lambda chain C region	↑	
		Serum amyloid P-component	↑	
		Amyloid beta A4	↑	
		gamma-actin		↓
		titin		↓
Kolialexi et al., 2008	Maternal plasma	Transthyretin	↑	
		Ceruloplasmin	↑	
		Afamin	↑	
		Alpha-1-microglobulin	↑	
		Apolipoprotein E	↑	
		Serum amyloid P-component	↑	
		Histidine-rich glycoprotein	↑	
		Alpha-1-antitrypsin	↑	
		Clusterin		↓
Lopez et al., 2011	Maternal serum	Serum amyloid A4		↓

*An increase of 63% of APP levels in DS group; #the mean concentration of TNC-C was significantly higher in the DS group ($p<0.004$); $Protein IBP-1 (P08833) was decreased by 40%; &The fold change (DS/control) of serum glycoproteins was 2.7.

metabolic proteins in amnion cells from controls with those from Down syndrome, and found that the protein levels of several enzymes were significantly deranged in DS group. But the true sense of proteomics applied in the DS diagnosis was carried out (Wang et al., 2005). They first investigated an AF fingerprint in 20 samples obtained from pregnant women known to carry an aneuploid fetus, and took some candidate markers. In 2009, they did the network analyses of differentially expressed proteins in DS amniotic fluid more deeply (Wang et al., 2009). According to their results, apolipoprotein A1 was decreased in DS, but antitrypsin, prealbumin and transferrin were increased. These proteins were associated with dysfunctional lipid and cholesterol metabolism, processes of metal ion transport, adenosine triphosphate metabolism and energy-coupled protein transport. Some new biomarkers were also reported in AF which could be used as potential markers for prenatal diagnosis (Tsangaris et al., 2006; Mange et al., 2008; Cho et al., 2010).

Nagalla et al. (2007) continued the proteomic analysis of maternal serum. They found that 28 and 26 proteins

were differentially present in first- and second-trimester maternal serum of DS. Of these, 19 were specific for the first trimester and 16 for the second trimester and ten were differentially present in both trimesters, and the average recognition capability approached was 96%. Lopez et al. (2011) reported 12 proteins in maternal serum as candidates were decreased in trisomy 21 vs normal samples. On the other hand, Kolla et al. (2010) and Kolialexi et al. (2008) made similar proteomics analysis of maternal plasma in Down syndrome pregnancies. These studies showed that all differentially expressed proteins are candidate biomarkers for DS, providing opportunities for the development of non-invasive prenatal diagnosis.

Although proteomic has brought with it the hope of identifying novel biomarkers for the prenatal diagnosis of Down syndrome, there are many factors that make this research very challenging, such as beginning with standardization of sample collection, consistent sample preparation and continuing through the entire analytical process. The use of maternal blood samples for diffe-rential proteomic analysis raises the question of whether plasma or serum should be used. Recently, some researches considered that plasma maybe is the better one, because fragments of proteins will be detectable in serum (Avent et al., 2008).

In conclusions, based on the present meta-analysis of studies, we concluded that application of proteomics can contribute to the finding of novel biomarker for prenatal diagnosis of Down syndrome. Further characterization and quantification of these markers in a larger cohort of subjects may provide the basis for new tests for improved DS screening and non-invasive prenatal diagnosis.

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