“OMICS” Assessment of the Placenta in Real Time

Human Placenta Project

May 26-27, 2014

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"The Assignment"

- Develop <u>noninvasive</u> markers of placental development and function
- Markers should permit <u>longitudinal</u> assessment
- Use "omic" tools to improve molecular definitions of placental biology and disease

Outline of Remarks

- "Non-invasive"
  - *In silico,* blood, saliva, urine, cervix
- Intact cells
  - Syncytiotrophoblast, cytotrophoblast
- The genome and epigenome
- The transcriptome
- Importance of annotation
- No time for metabolome, proteome!

In 6 minutes!

Cells: Trophoblasts as a Biomarker of Eclampsia

Georg Schmorl
1861-1932

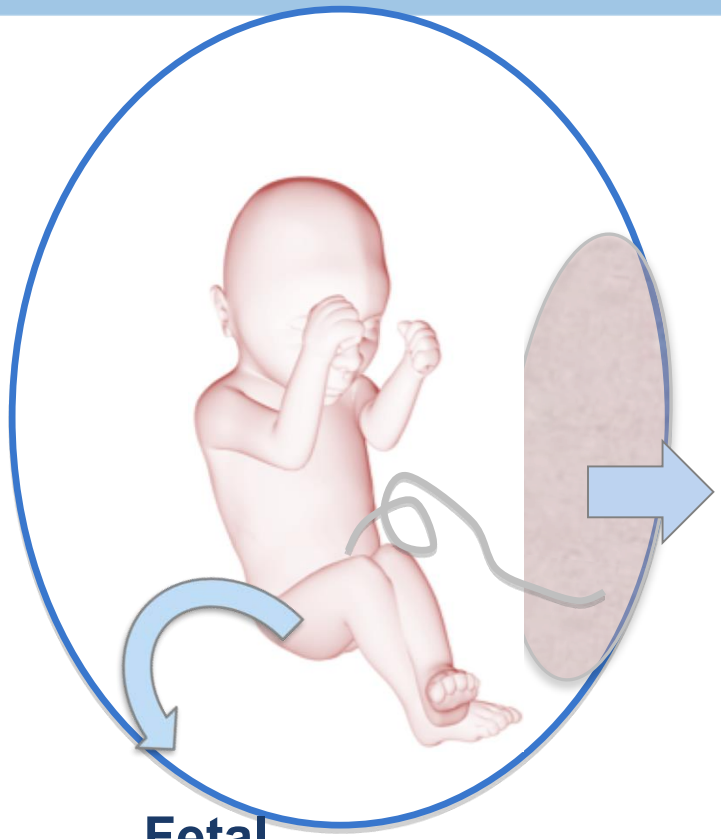
- Multi-nucleated syncytial giant cells in lung capillaries of pregnant women who died of eclampsia
- Women with pre-eclampsia have 5-fold ↑ in number of circulating fetal cells
- Experimental model: injected placental supernatant from pregnant rabbits and caused seizures
- Re-emergence of interest in isolation of intact cells from maternal blood

(Lapaire O et al. Placenta 2007; 28: 1-5)

Cell Free Nucleic Acids as a Biomarker

Cell-free fetal (cff) DNA and RNA are present both in AF and maternal blood

- **Compared with circulating cff nucleic acids, those in AF are:**
  - **in direct physical contact with the fetus**
  - **100-200 fold more concentrated**
  - **uncontaminated by maternal nucleic acids**



Placental and fetal nucleic acids in maternal blood

Fetal nucleic acids in AF

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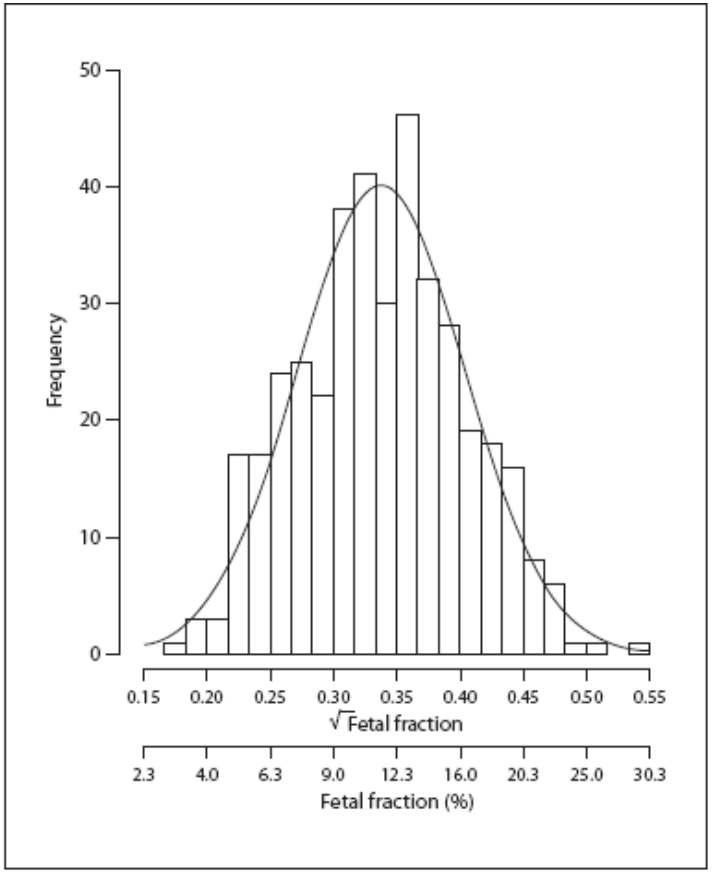
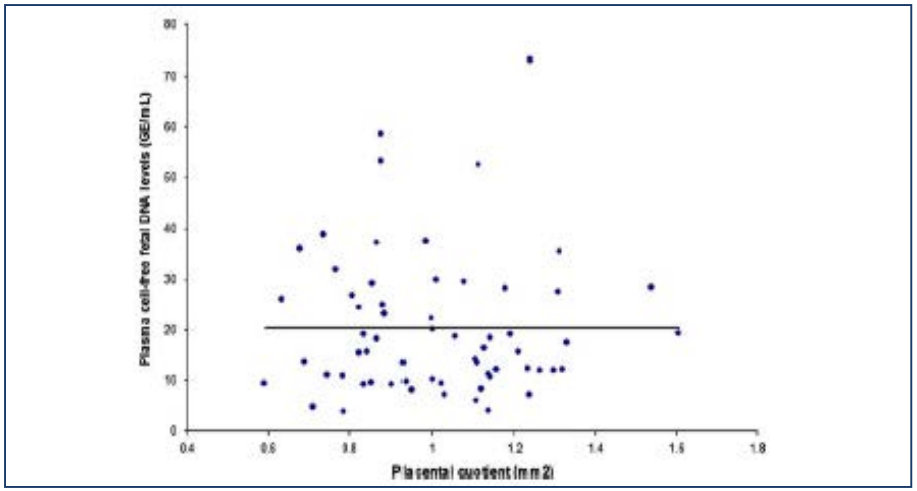
Fetal Fraction is a Placental Biomarker



- Fetal fraction has a normal distribution that peaks between 10 and 20% at 10-21 weeks of gestation
- It is primarily affected by four factors
  - Maternal BMI
  - Gestational age
  - Type of aneuploidy
  - Singleton vs. multiple

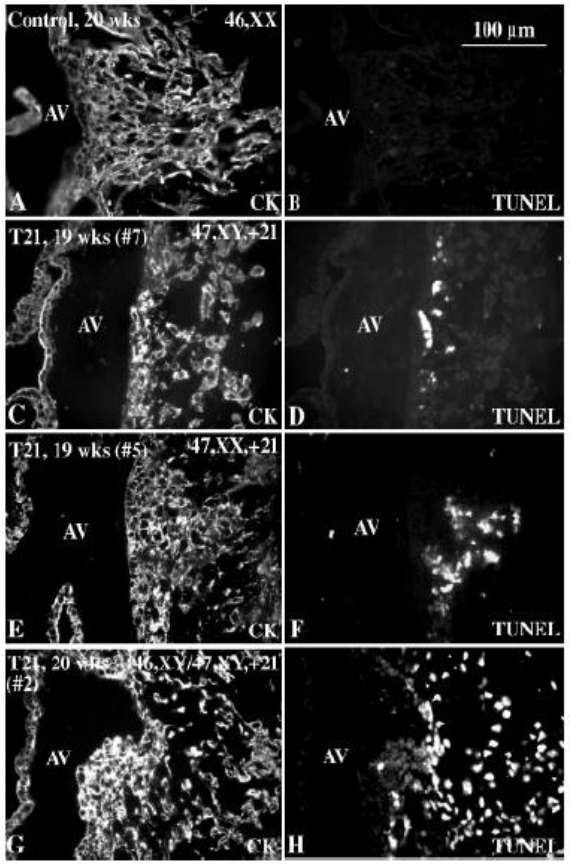
Ashoor et al. Fetal Diagn Ther 2012

Cell-Free Fetal (Placental) DNA in Maternal Blood



T. Wataganara, Am J Obstet Gynecol 2005

- Does not reflect placental volume
- Does reflect oxidative stress and apoptosis



A. Wright, Am J Med Genet 2004

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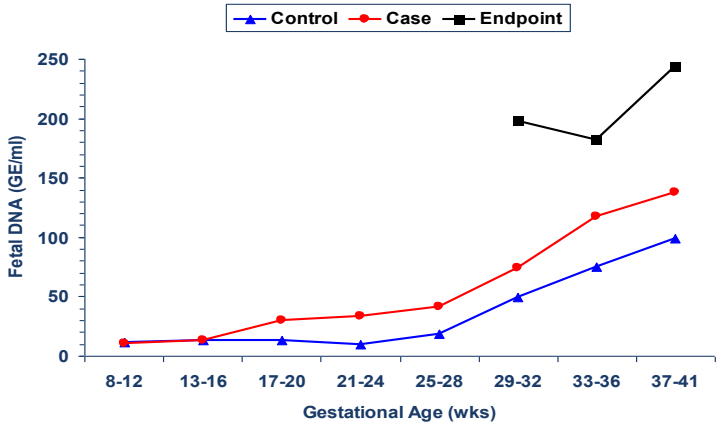
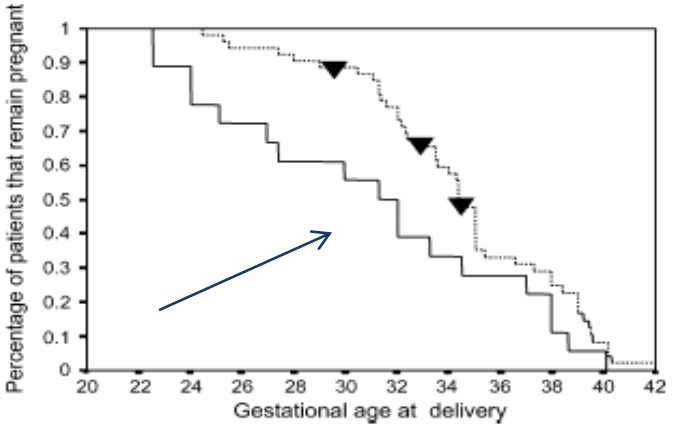
Cell-free DNA as a placental biomarker





- Increased DNA levels in maternal plasma in pre-eclampsia, unstoppable preterm labor, IUGR
- Increased tag counts can suggest the presence of confined placental mosaicism for aneuploidy
- Differential methylation occurs in placenta (epigenetics)

  RASSF1A is hyper-methylated in placenta, hypo-methylated in blood

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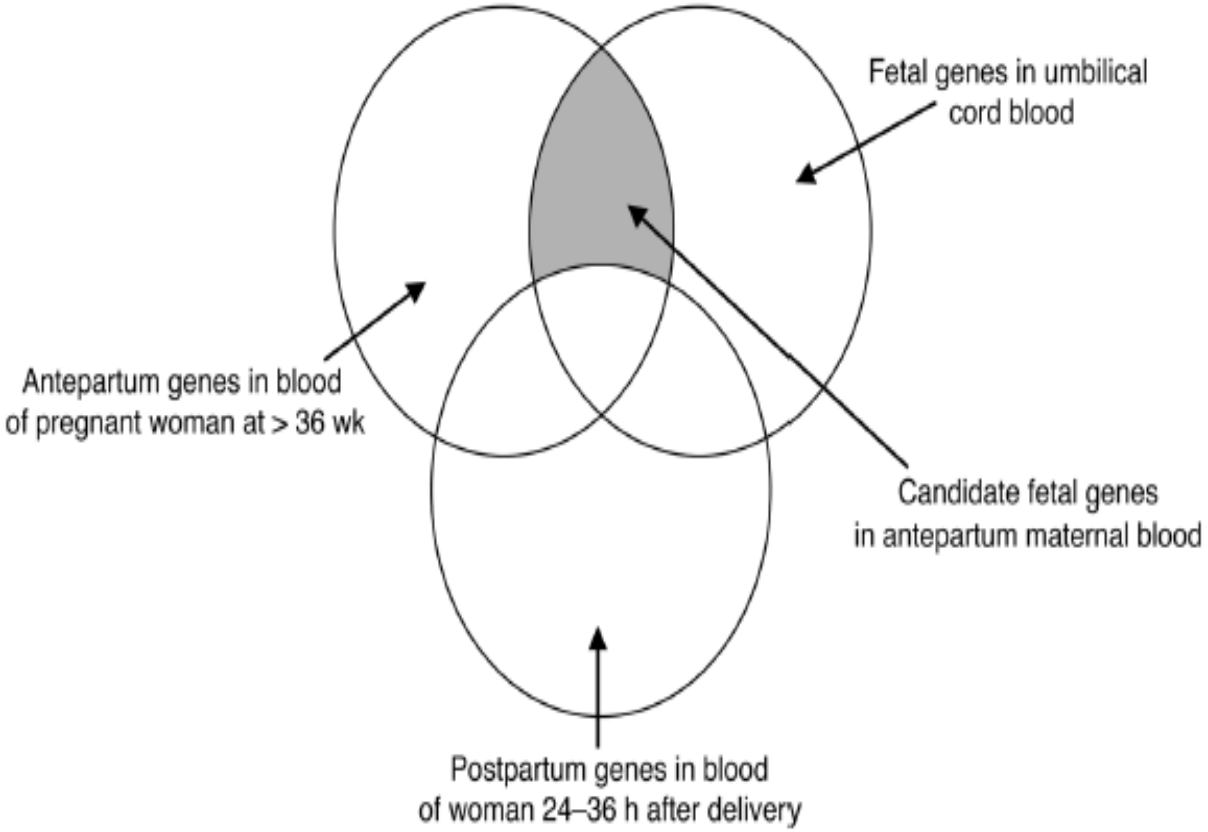
Approach to detection of feto-placental transcripts in maternal blood using cell-free RNA

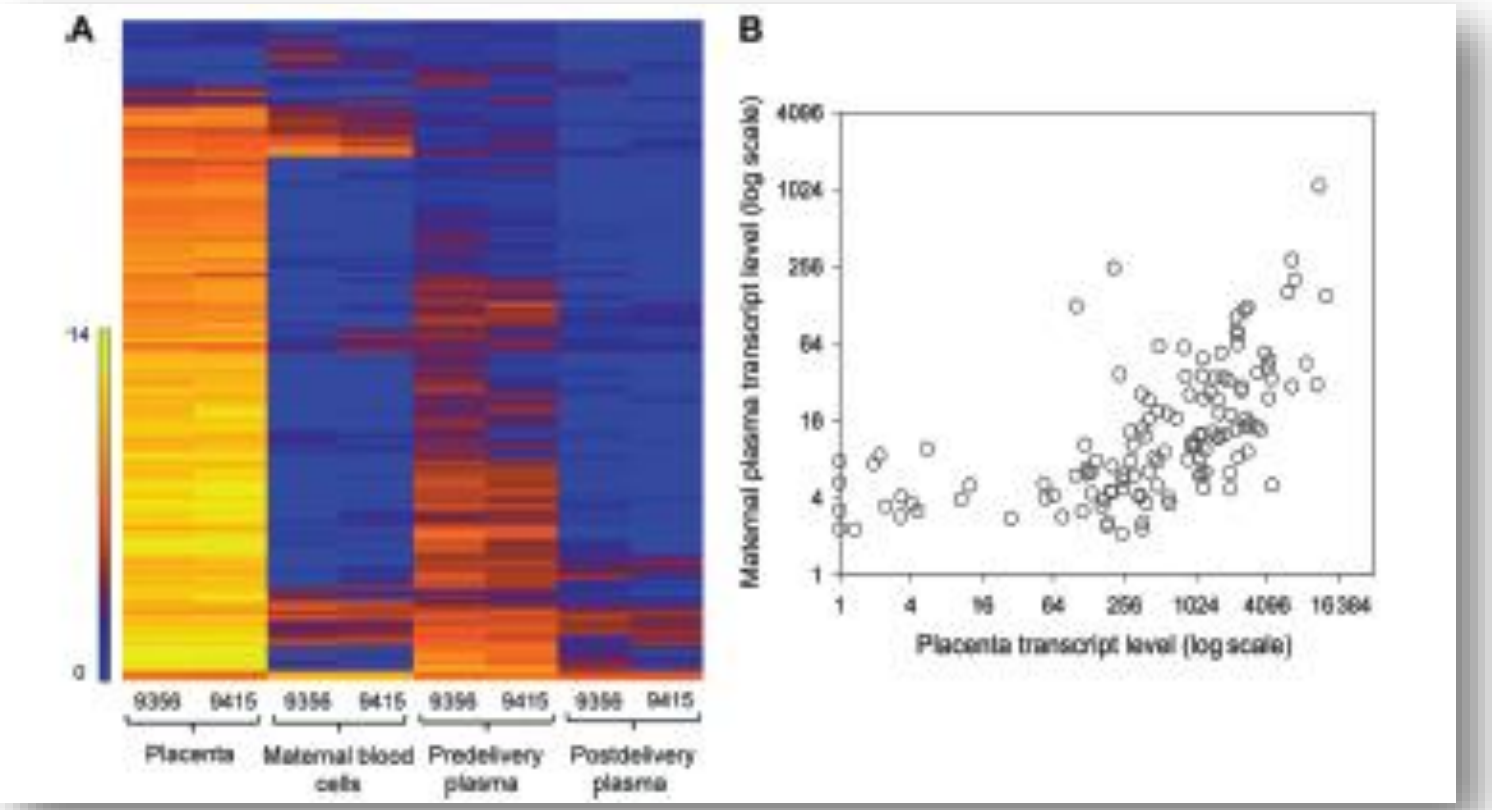
Maron et al. J Clin Invest 2007; 117: 3007-3019



- Studied whole blood and placental gene transcripts at term using microarrays
- Sensory perception genes up-regulated
- Fetal nervous system genes
- Immune-related genes
- Placenta-specific genes more easily detected in plasma

Placental-specific genes are more easily detected in maternal plasma compared to maternal blood

Tsui et al. Clin Chem 2014; April 16



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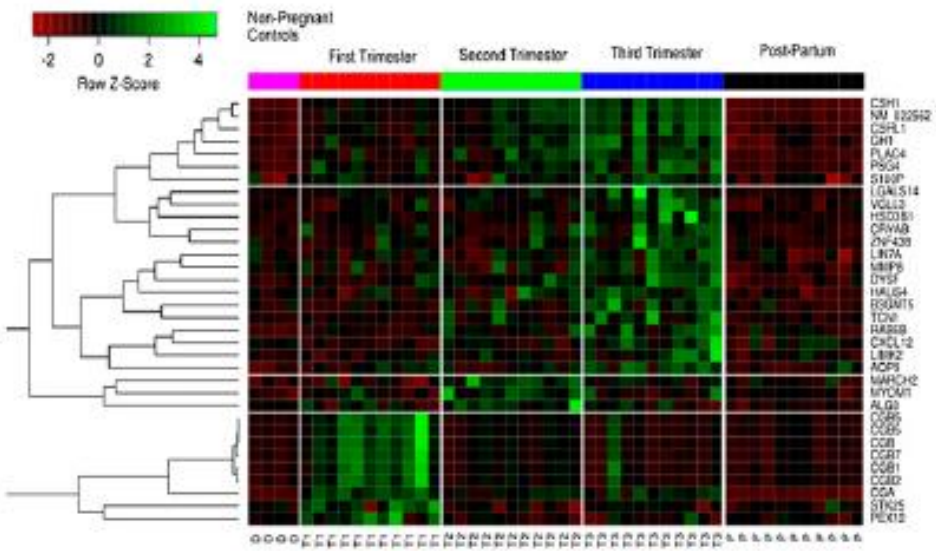
Placental Transcripts Detected in Maternal Plasma

From Koh et al. Proc Natl Acad Sci USA 2014;doi/10.1073/pnas.1405528111

- *PLAC1*
- *PLAC4*
- *hCG*
- *PSG4*
- *GH1*
- *CSHL1*
- *PAPP-A*



-MicroRNAs: very stable in plasma
-Many placenta-specific miRNAs
-Placental-miRNA expression associated with infant behavior

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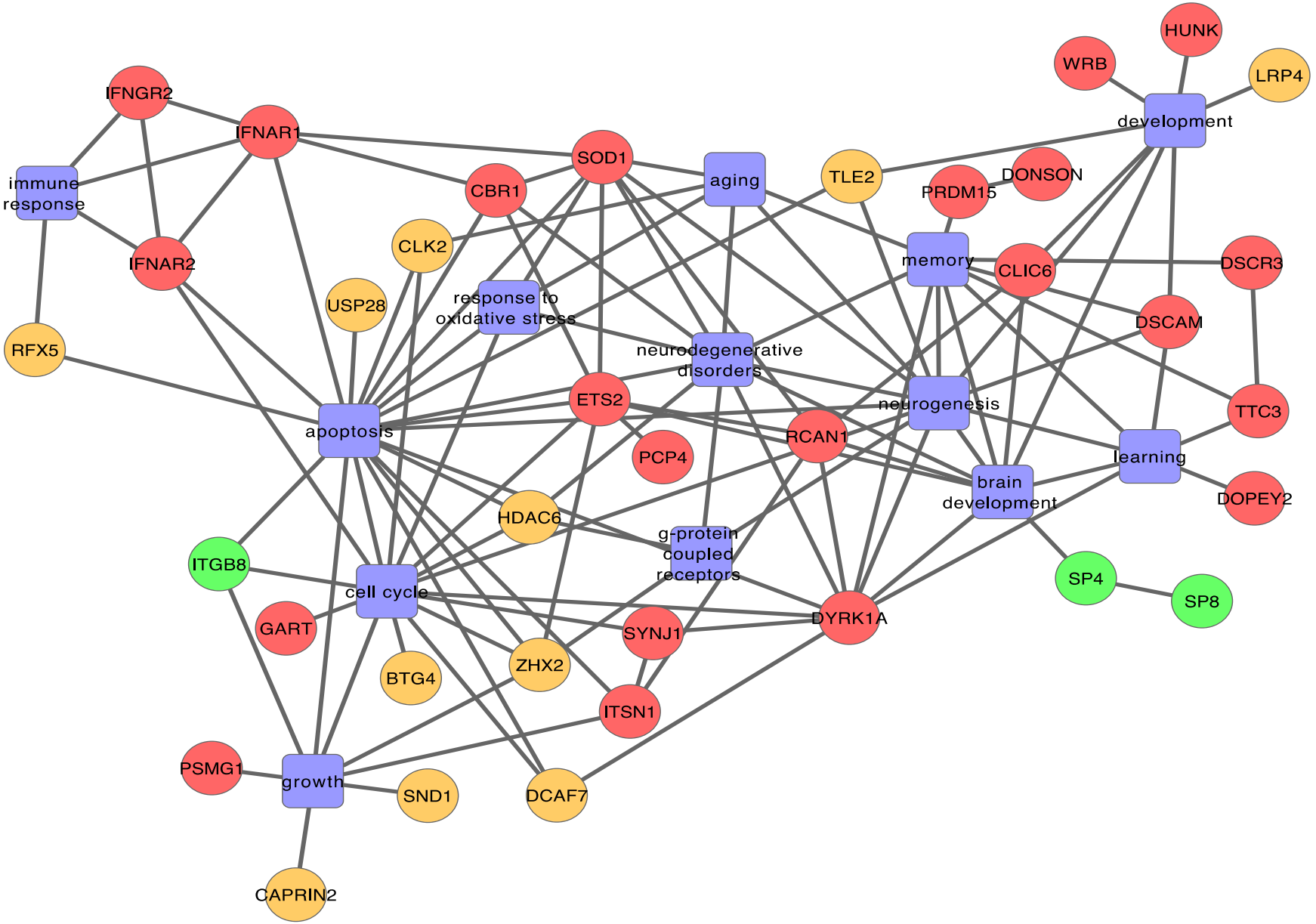
What if You Want to do Network and Pathway Analysis?

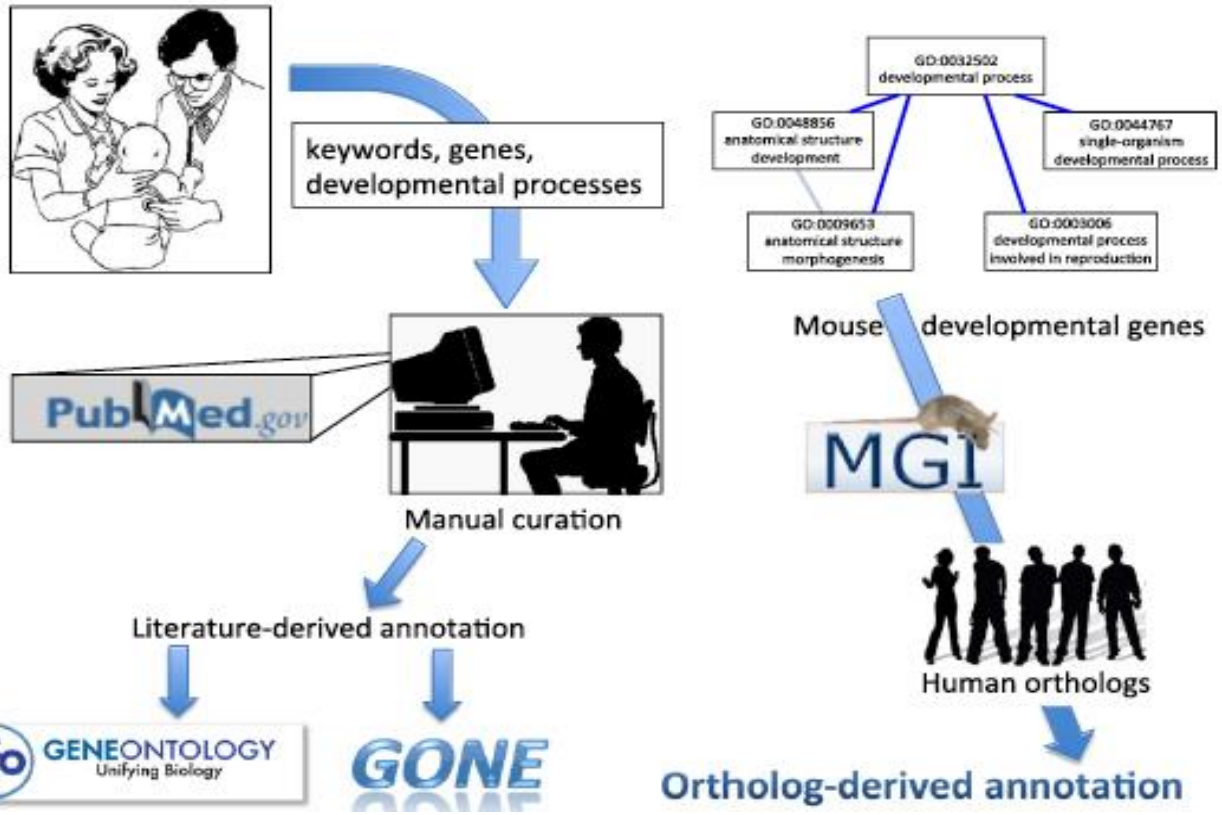


BioGPS Gene Expression Atlas

- How do you know if a transcript is unique to the placenta?
- Publicly-available atlas of human protein-encoding transcriptome (Su *PNAS* 2004)
- Relative tissue expression patterns of individual gene probes across 78 normal human tissues including fetal brain, liver, thyroid, lung (15-33 wks) and placenta (n=4)
- Definition of organ-specific gene expression
  - expression value > 30 MoM in a single tissue type
  - no unrelated tissue expression > 1/3 of max. expression value

Developmentally-appropriate annotation is critical!

http://dflat.cs.tufts.edu



From: HC Wick et al. Bioinformatics 2014; 15:45

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