Non-Invasive Testing Beyond Karyotype: What's In It for the Fetal Brain?

Diana W. Bianchi, M.D.

August 25, 2017

NIH *Eunice Kennedy Shriver* National Institute of Child Health and Human Development

The speaker has no financial relationship or off-label usage to disclose.

Fetal Personalized Medicine

nature medicine

2012; 18:1041-1051

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.

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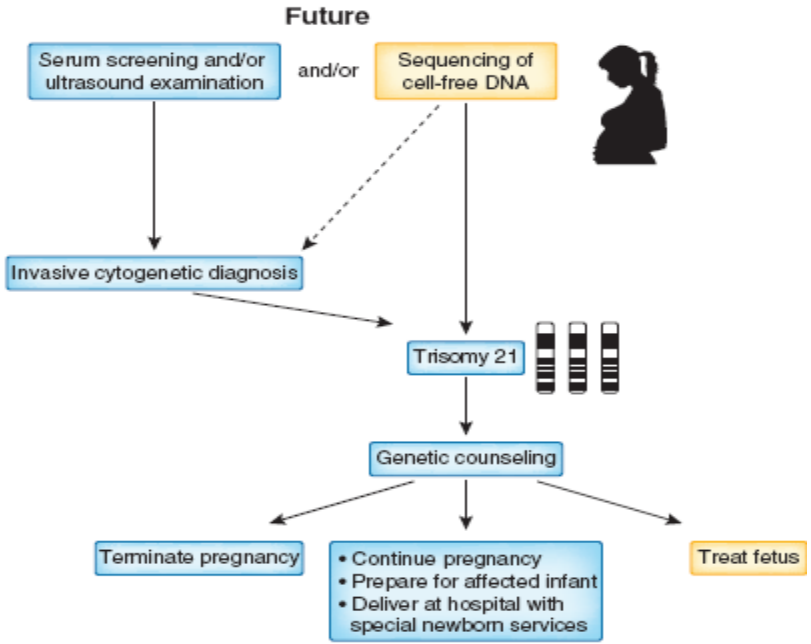
The Vision: Prenatal Testing Creates Opportunities for Fetal Treatment



We hypothesize that prenatal treatment, given to the mother, will result in improved fetal brain growth, connectivity, and ultimately, improved neurocognition

From: Bianchi DW, Nature Medicine 2012; 18: 1041-1051

Outline of My Talk

- **Part 1: “Genomics” = Rapid growth and clinical impact of prenatal cell-free DNA sequencing**
- **Part 2: “Transcriptomics”= Key insights from study of fetal gene expression in amniotic supernatant**
- **Part 3: Using the fetal transcriptomic information to develop novel treatments**

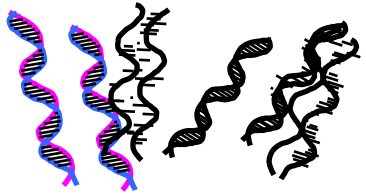
Disruptive Technologies

airbnb

Advances in Technology and an Innovative Concept

MPS used as a counting system rather than as a sequencer *per se*

Sequence, Align, Count



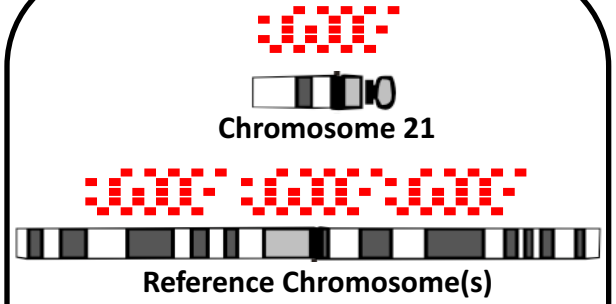
Cell-free DNA in Maternal Plasma

~150-200 bp fragments from both pregnant woman and her fetus

CCCTTAGCGCTTTAACGTACGTAAAA
CCCTTAGCGCTTTAACGTACGTAAAA
ACGGGGTCAAAGGTTCCCACACGTCC
GACTTAAAATCGGAATCGATGCCCAA
GACTTAAAATCGGAATCGATGCCCAA
ACGGGGTCAAAGGTTCCCACACGTCC
CCCTTAGCGCTTTAACGTACGTAAAA
CCCTTAGCGCTTTAACGTACGTAAAA
ACGGGGTCAAAGGTTCCCACACGTCC

Total DNA is sequenced

25-36 base pair reads

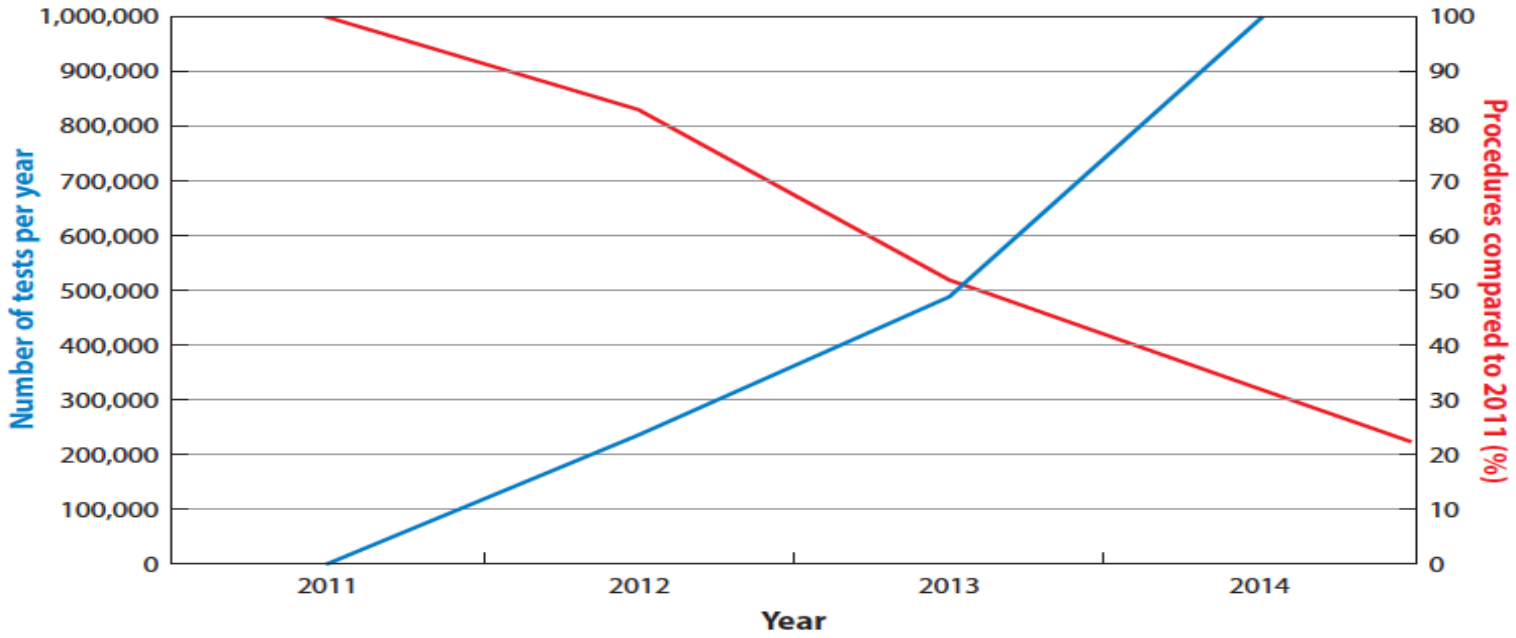


Alignment of reads

Measure counts relative to a reference

Prenatal Genomics Has Transformed Care

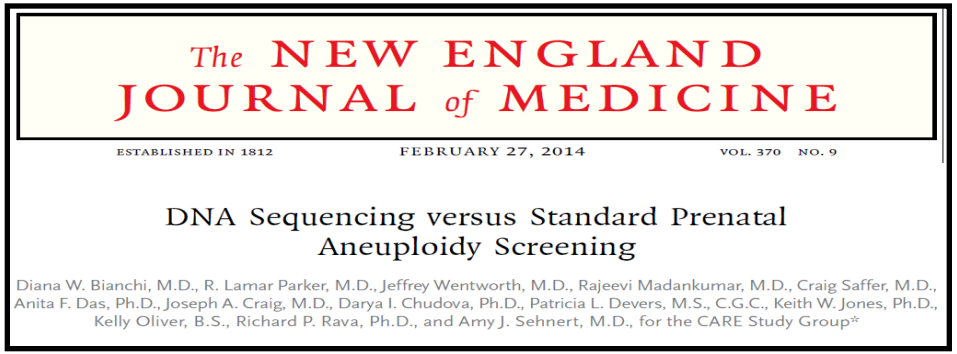
Hui and Bianchi, *Ann Rev Med* 2017; 68: 459-472



Positive and Negative Predictive Values

- **PPV= # of true positives**
  - **# of true positives + # of false positives**
- **NPV= # of true negatives**
  - **# of true negatives + # of false negatives**
- **Importance of Positive Predictive Values**
  - **Depends on prevalence of disease in the population**
  - **Rarer diseases will have ⇧ rates of false positives**

NIPT as a Primary Screen in Low-Risk Women



The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 FEBRUARY 27, 2014 VOL. 370 NO. 9

DNA Sequencing versus Standard Prenatal Aneuploidy Screening

Diana W. Bianchi, M.D., R. Lamar Parker, M.D., Jeffrey Wentworth, M.D., Rajeevi Madankumar, M.D., Craig Saffer, M.D., Anita F. Das, Ph.D., Joseph A. Craig, M.D., Darya I. Chudova, Ph.D., Patricia L. Devers, M.S., C.G.C., Keith W. Jones, Ph.D., Kelly Oliver, B.S., Richard P. Rava, Ph.D., and Amy J. Sehnert, M.D., for the CARE Study Group*

For Trisomy 21

PPV of standard screens:

4.2%

PPV: 45.5% (62.5%)

NPV: >99%

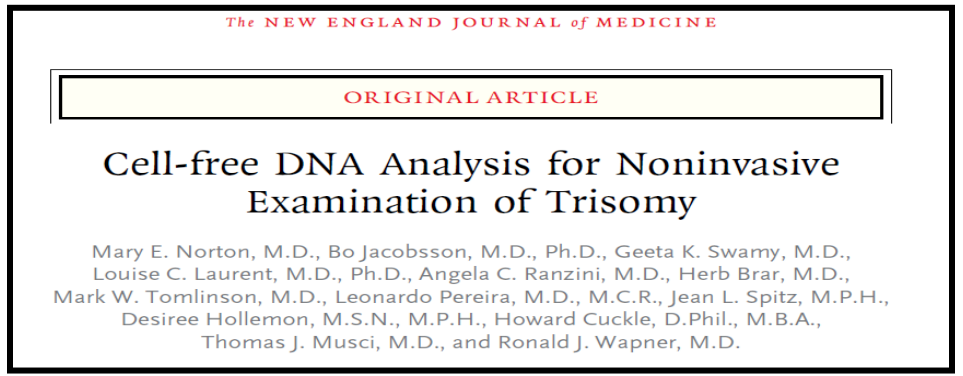
For Trisomy 21

PPV of standard screens:

3.4%

PPV:80.9%

NPV:>99%



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Cell-free DNA Analysis for Noninvasive Examination of Trisomy

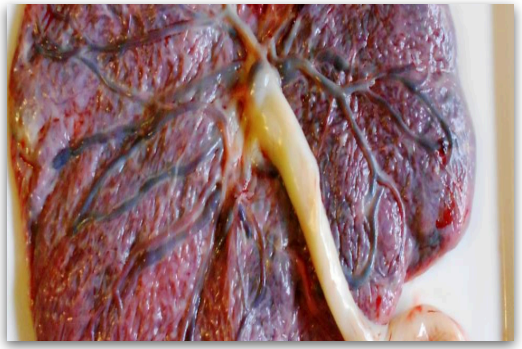
Mary E. Norton, M.D., Bo Jacobsson, M.D., Ph.D., Geeta K. Swamy, M.D., Louise C. Laurent, M.D., Ph.D., Angela C. Ranzini, M.D., Herb Brar, M.D., Mark W. Tomlinson, M.D., Leonardo Pereira, M.D., M.C.R., Jean L. Spitz, M.P.H., Desiree Hollemon, M.S.N., M.P.H., Howard Cuckle, D.Phil., M.B.A., Thomas J. Musci, M.D., and Ronald J. Wapner, M.D.

Current Recommendations for Obstetric Practice

- NIPT using sequencing of maternal plasma DNA is routinely offered as an alternative to invasive procedure for all women who are high risk
  - Positive screens require diagnostic confirmation
- Low risk pregnant women can have test, but generally self-pay
- Pre and post test genetic counseling are provided for all
- Sex chromosome aneuploidy testing offered as an option
  - No extra charge, fetal sex provided
- Microdeletion testing offered as an option
  - Extra charge, not recommended by most societies
- Evolving area: rare autosomal trisomies

“False Positive” cfDNA Screen Results

Up to 2% of high-risk cases are “discordant” with the fetal karyotype
Most have an underlying biologic mechanism



Confined Placental Mosaicism

Twin Demise

COMMENT

BIOGRAPHY The monumental life of August Weismann, hereditary-science pioneer p.31 | FILM Palaeontology adviser to *Jurassic World* talks GM dinosaurs p.32 | GENOMICS Ten thousand bird genomes to be sequenced over next five years p.34 | GEOLOGY A call to regulate fracking in China to reduc earthquake risk p.34

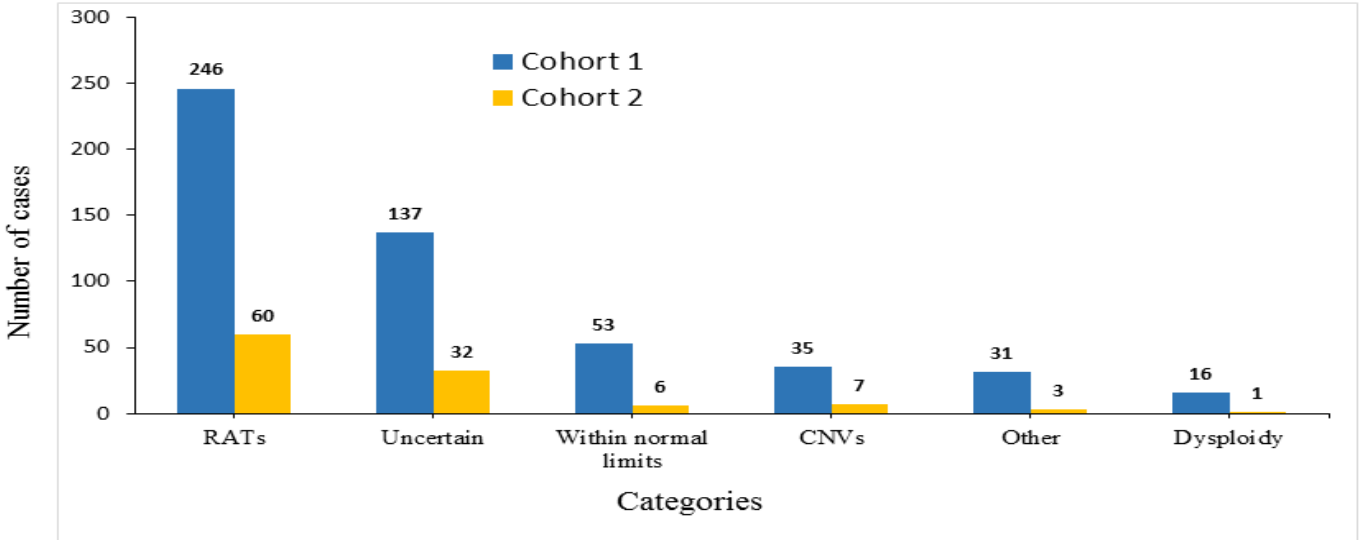
Prepare for unexpected prenatal test results

Women are learning about their own health problems through fetal screening. Revise consent forms and raise awareness, urges **Diana W. Bianchi**.

Maternal

Rare Autosomal Trisomies (RATs) Explain Most QC Failures

Cohort 1: 328/72,932 (0.45%) **Cohort 2: 71/16,885 (0.42%%)**



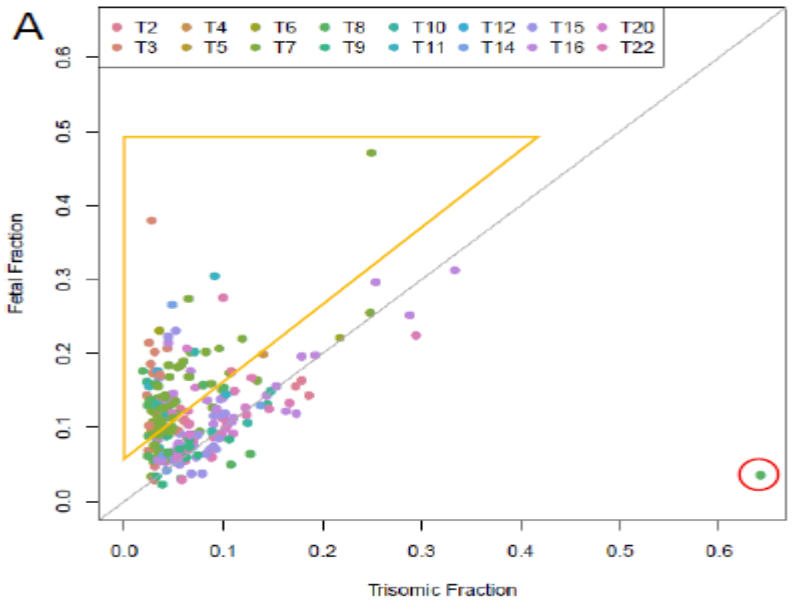
(Pertile and Halks-Miller *et al.*, Science Transl Med 2017; Aug 30)

Clinical Outcome Data: 60 Cases of RATs

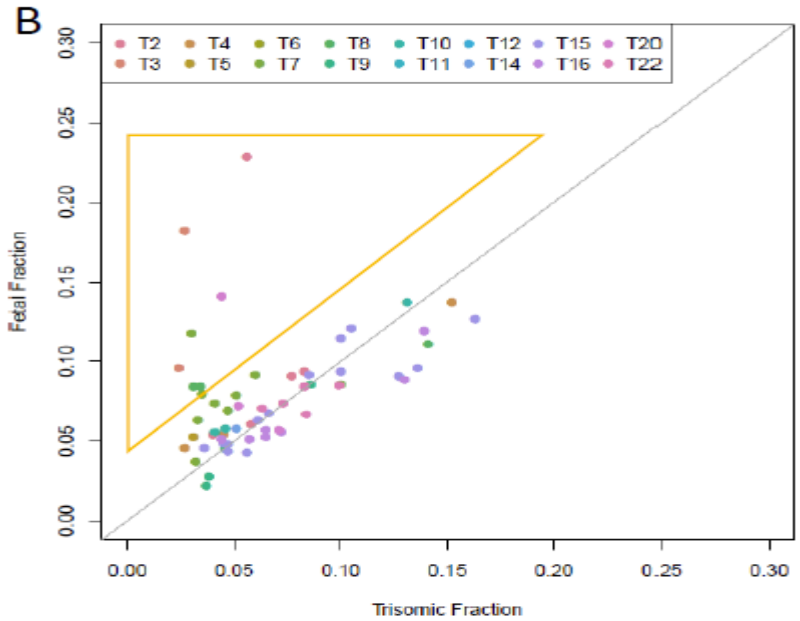
(Pertile and Halks-Miller *et al.*, Science Transl Med 2017; Aug 30)

	Missed or early misc.	TFM	UPD	IUFD	IUGR	MCA	NLB	NA	Mat	Total
Trisomy 2		3	(2*)	1	(1*, 1†)		1			5
Trisomy 3							1	1		2
Trisomy 4			2					1		3
Trisomy 5								1		1
Trisomy 7		1			(1*)		5	3		9
Trisomy 8							1	1	1	3
Trisomy 9	2					1	1			4
Trisomy 10	1						2			3
Trisomy 14	1									1
Trisomy 15	13		1							14
Trisomy 16	2	1	2	(2#)	(1#)		2			7
Trisomy 20	1				1			1		3
Trisomy 22	3	1^					1	1		5
Total	23	5 (+1^)	5 (+2*)	1 (+2#)	1 (+4**†#)	1	14	9	1	**60**

Fetal Fraction: Trisomic Fraction Ratios Predict Clinical Outcomes



Higher fetal: trisomic ratio suggests CPM



Equal fetal and trisomic ratio suggests poor clinical outcome

Clinical Utility of Reporting RATs

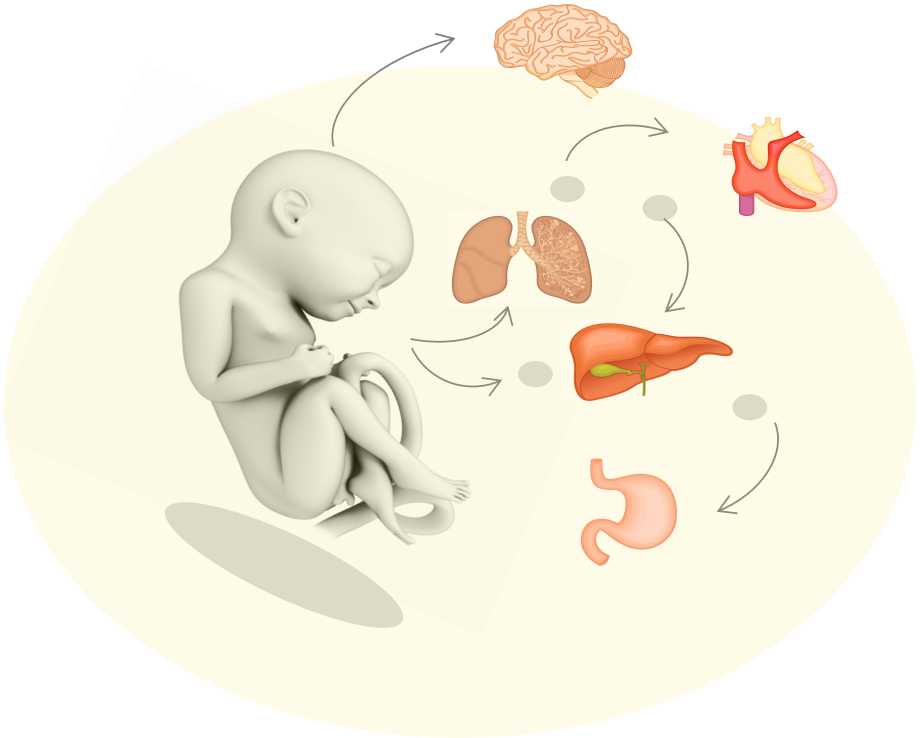
- **Identify cases at high risk of miscarriage**
  - If RAT detected, have U/S for fetal viability
  - Counsel regarding high rate of miscarriage (esp. T15)
  - If fetus is viable, consider amnio to r/o true fetal mosaicism
- **Identify cases at risk for uniparental disomy**
  - Some conditions, like Prader-Willi syndrome, affect brain
- **Can potentially explain other adverse events associated with feto-placental pathology**
  - Fetal growth restriction
  - ?Effects on developing fetal brain

Some “surprises” we’ve learned from NIPT

1. Women with sex chromosome aneuploidies are more fertile than we previously thought
2. Adult women with autosomal aneuploidy mosaicism can be asymptomatic
3. Confined placental mosaicism can occur with microdeletions
4. Pregnant women can have a significant tumor burden and be clinically asymptomatic

Part 2: Key Insights From Gene Expression Studies in Amniotic Fluid

Cell-Free Fetal RNA in Amniotic Fluid is Derived From Many Sources



Genes Related to Fetal Neurodevelopment in AF

Hui et al. Hum Genet 2012

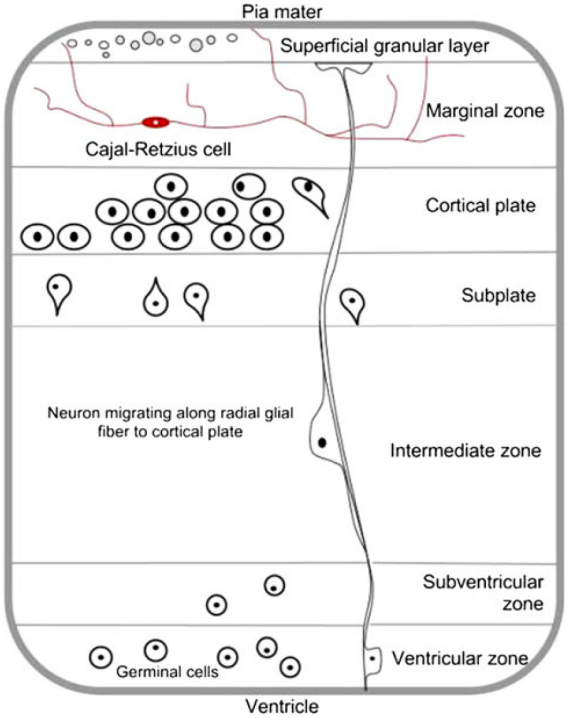


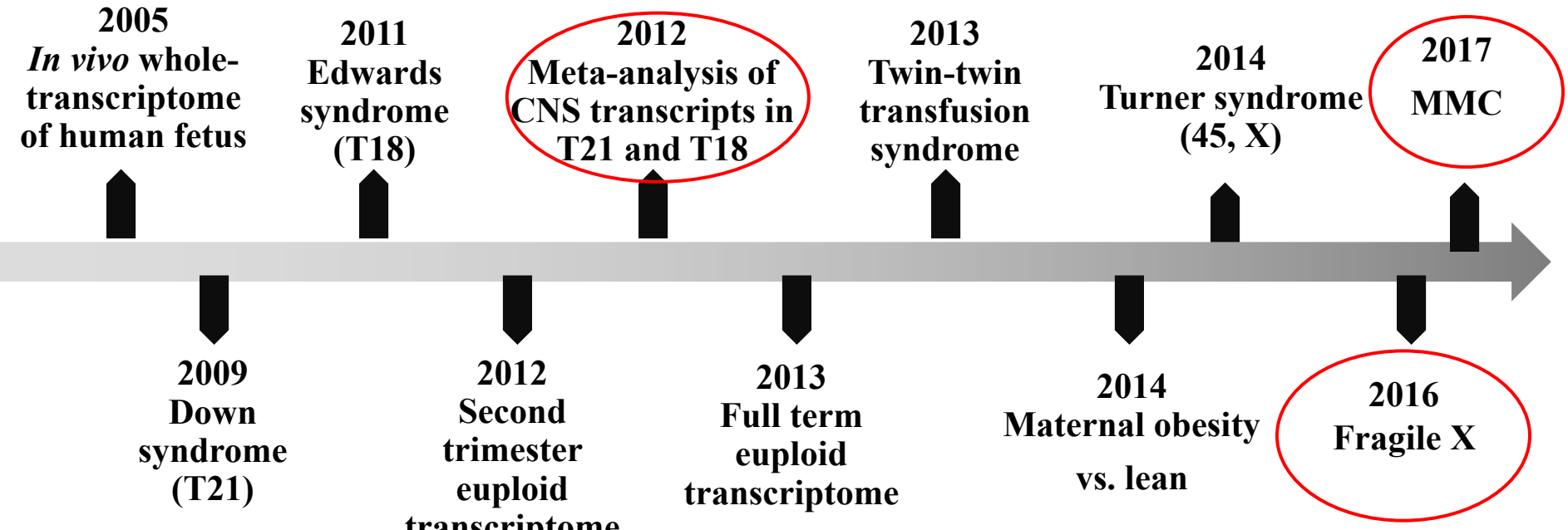
Fig. 1 Cajal–Retzius cell in the developing human fetal cerebral cortex at 13 weeks of gestation

Table 4 Top five terms in UniProt tissue expression chart by karyotype

Karyotype	UniProt KB term	No. of genes	*P* value*
Euploid	Fetal brain cortex	41	6.34E−18
	Cajal–Retzius cell[a]	35	2.83E−14
	Epithelium	154	1.23E−13
	B-cell lymphoma	24	5.86E−10
	Skin	102	1.71E−07
Trisomy 21	Epithelium	342	1.21E−35
	Placenta	377	6.06E−27
	Fetal brain cortex	63	4.24E−22
	Cajal–Retzius cell[a]	59	3.20E−21
	Skin	216	1.26E−16
Trisomy 18	Epithelium	224	1.11E−23
	Fetal brain cortex	50	1.37E−20
	Cajal–Retzius cell[a]	44	2.39E−17
	Skin	140	2.05E−10
	Placenta	220	2.24E−10

* Benjamini–Hochberg corrected modified Fisher exact *P* value

Amniotic Fluid Gene Expression Studies: Bianchi Lab 2005-2017



Data are publicly shared via Gene Expression Omnibus

Transcriptomic Analyses in 2nd Trimester Fetuses

L. Zwemer and D. Bianchi, Cold Spring Harbor Perspect Med 2015;

T21

Oxidative stress
Ion transport
G protein signaling
Cell proliferation
Neuro-inflammation

T18

NOTCH signaling
Adrenal development
T cell-mediated immunity

45, X

Cholesterol homeostasis
Autoimmune disease
Perivascular tissue remodeling

Part 3: Using Transcriptomics to Develop Novel Therapies to Improve Neurocognition

Is Prenatal Treatment Ethical? If so, under what conditions?

DOI: 10.1002/pd.4995

PRENATAL DIAGNOSIS

ORIGINAL ARTICLE

Fetal therapy for Down syndrome: an ethical exploration

Guido de Wert[1], Wybo Dondorp[1]* and Diana W. Bianchi[2]

[1]Department of Health, Ethics & Society, School for Oncology and Developmental Biology (GROW), School for Public Health and Primary Care (CAPHRI), Maastricht University, Maastricht, The Netherlands
[2]Mother Infant Research Institute, Tufts Medical Center and Tufts University School of Medicine, Boston, MA, US
*Correspondence to: Wybo Dondorp. E-mail: w.dondorp@maastrichtuniversity.nl

ABSTRACT

Background Parallel to recent advances in prenatal screening for Down syndrome (DS), therapies for different aspects of the condition have become available. As intellectual disability is a key aspect, this is an active area for research. Several groups have hypothesized that prenatal interventions will give better chances at improving cognitive functioning in persons with DS than postnatal treatment. Clinical trials are being developed.

Method We first discuss the ethical pros and cons of trying to improve cognitive functioning in persons with DS to see if there are categorical objections to the general idea, and then move on to explore ethically relevant aspects of the prospect of developing fetal therapy for DS (FTDS).

Results Only on the basis of a one-dimensional emphasis on the social model of disability would (fetal) therapy aimed at cognitive improvement be inherently problematic.

Conclusions Inviting pregnant women to participate in FTDS-research should be based on adequate pre-clinical trials, as well as information aimed at avoiding the so-called 'therapeutic misconception'. Should FTDS be proven to be effective and safe, women carrying a fetus with trisomy 21 who have decided to continue the pregnancy may have a moral obligation to make use of this option. © 2016 John Wiley & Sons, Ltd.

De Wert *et al.* Prenat Diagn 2017; 37: 222-228

Pregnant Women Already Use the Internet to Seek Prenatal Therapies for T21

From: XXXX

Sent: Thursday, April 30, 2015 7:25 PM
To: Bianchi, Diana
Subject: Down syndrome and prenatal therapies

Hello Dr. Bianchi,

My name is XXXX. I am currently 19 weeks pregnant and just learned that our son has trisomy 21. As a physician assistant I have a medical background and have been scouring the internet for any therapies that could help improve his cognitive function, starting in utero. After coming across this article I decided to contact you for more information. I realize that you can't recommend anything not tested and approved for humans, but I was wondering if you know of any modalities that may help (or at least wouldn't hurt) to try. I am sure you are very busy, but any advice, further reading, or other contacts would mean a great deal to me. I would love to feel like I am doing something to help him. Currently I am on a prenatal vitamin as well as prenatal DHA. Thank you so much for your time and I hope to hear from you.
Sincerely, XXXX

PAUL WATSON'S FUNDRAISER:

UT SOUTHWESTERN DOWN SYNDROME CLINICAL STUDY

ABOUT THIS CAMPAIGN | THE TEAM

PAUL
Great news--we started recruiting patients for the trial in the DFW last week of April!
via Crowdrise 2 months ago

BENEFITING: SOUTHWESTERN MEDICAL FOUNDATION
THE STORY:

We are charitably funding a 3 year pilot study at the University of TX Southwestern Medical Center to improve memory and learning in young children with Down syndrome. This groundbreaking study will start treatment during pregnancy and continue to age 2 with the common medication, Prozac. In 7 studies on mouse models of Down syndrome, the use of Prozac was highly effective in improving the memory and learning of the mice. Prozac has been safely used for years in humans, and we hope that this will lead to effective treatments that help our loved ones with DS to better learn in school and work. For a study summary, please email me: pjbwatson@yahoo.com. 501c3 #75-0945939,

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TO THIS FUNDRAISER

$85,303
MONEY RAISED

Watson family	$5,000
Brinks, Inc	$5,000
Watson kids	$665
Brinks, Inc	$665
Watson Golf tourney	$4,241

114% Raised of $75,000 Goal

Support

Unique Challenges for Prenatal Rx

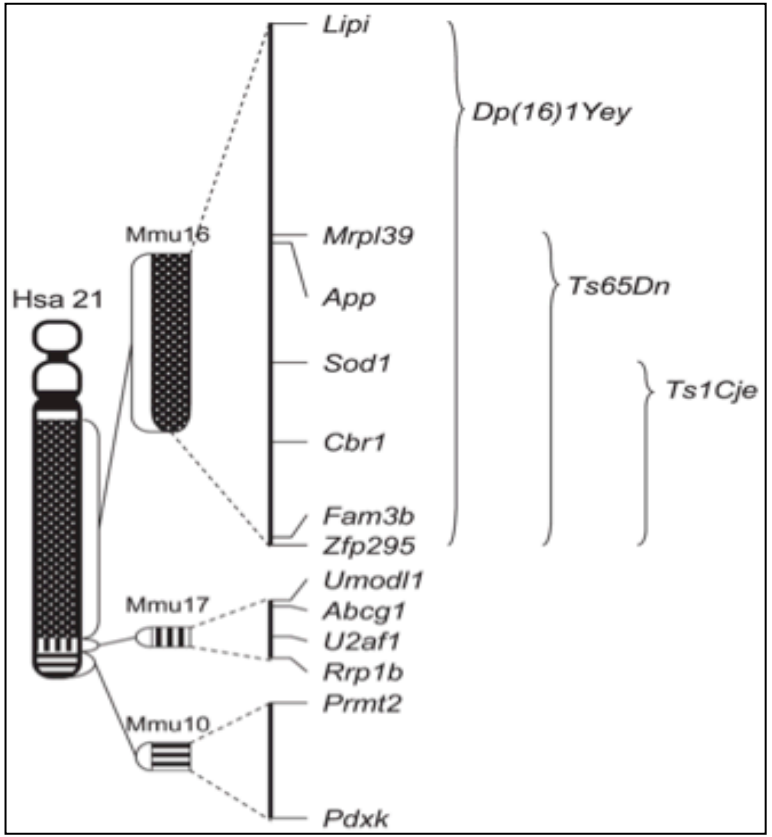
- **The primary concern is safety.**
- **Two individuals are treated simultaneously.**
  - **We cannot**
    - **harm the mother or**
    - **cause adverse effects to the developing fetus**
- **Achieving therapeutic levels of drugs is especially challenging during pregnancy.**
  - **To treat the fetus, drug must cross both the placenta and the blood-brain barrier.**

Our Overall Translational Research Plan

- ✓Compare phenotypes of 3 most commonly used mouse models of “DS”
- ✓Determine best endpoints to use
- ✓Analyze integrated transcriptomes of human and mice
- ✓Perform proof of principle experiments using candidate therapies in mice and amniocytes

Best Mouse Model to Use

Parameter	Ts65Dn	Ts1Cje	Dp(16)1Yey
Year Created	1995	1998	2007
PubMed Entries	372	53	11
Triplicated MMU16	128	77	145
Extra-MMU16 (non HSA21)	50 MMU17	7 MMU12	0
Males	Sterile	Fertile	Fertile
Oxidative Stress	Present	Present	Not tested
Prenatal Brain Phenotype	Present	Present	Absent
Adult Cognitive Phenotype	Present	Mild	Present

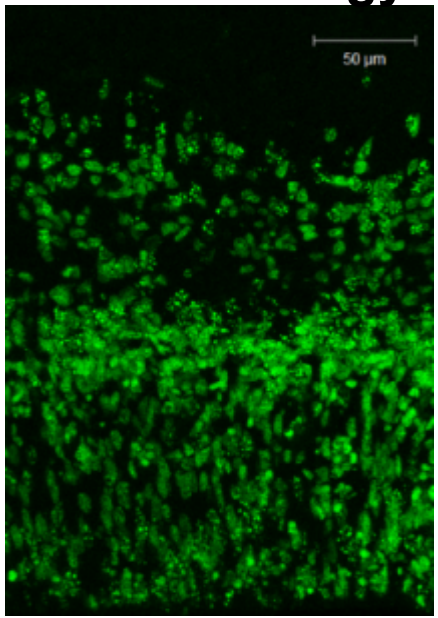


Cross-Model Comparison: Bianchi and Haydar labs

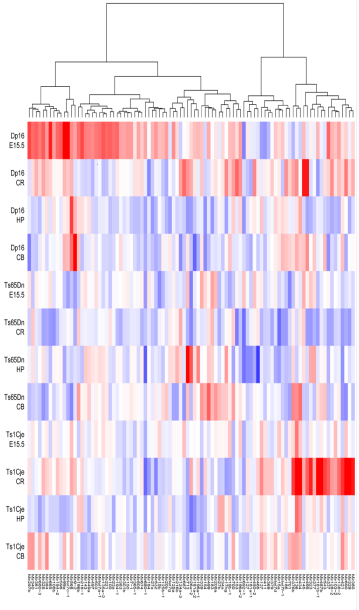
Age and Sex Matched Ts65Dn, Ts1Cje and Dp16 vs WT
Lifespan approach: E15.5, Neonatal and Adult Mice

Anatomy/Growth

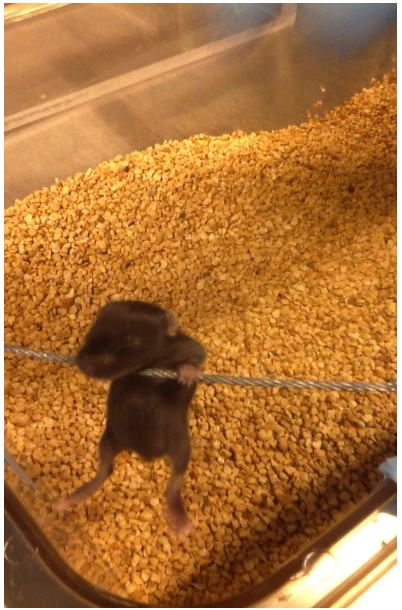
CNS Histology



Transcriptome



Behavior



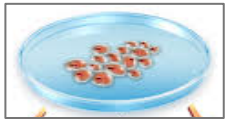
Aziz and Guedj et al., submitted 2017

Analysis of RNA from 9 Tissue Sources to Identify Candidate Therapies

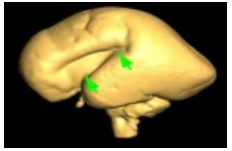
Step I: Gene Expression Microarrays

Humans with DS

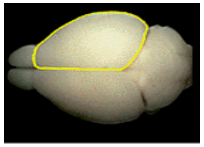
AF & Amniocytes
(Slonim et al, 2009)



iPSCs & Neurons
(Weick et al, 2013)

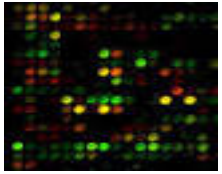


Fetal Cerebrum & Cerebellum
(Mao et al, 2005)

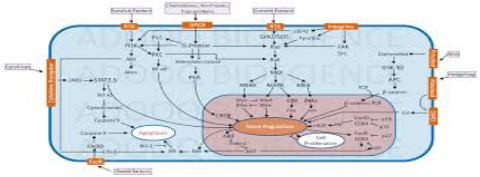


Ts1Cje, Ts65Dn, Dp16
Mouse models of DS

Step II: Differentially Expressed Genes

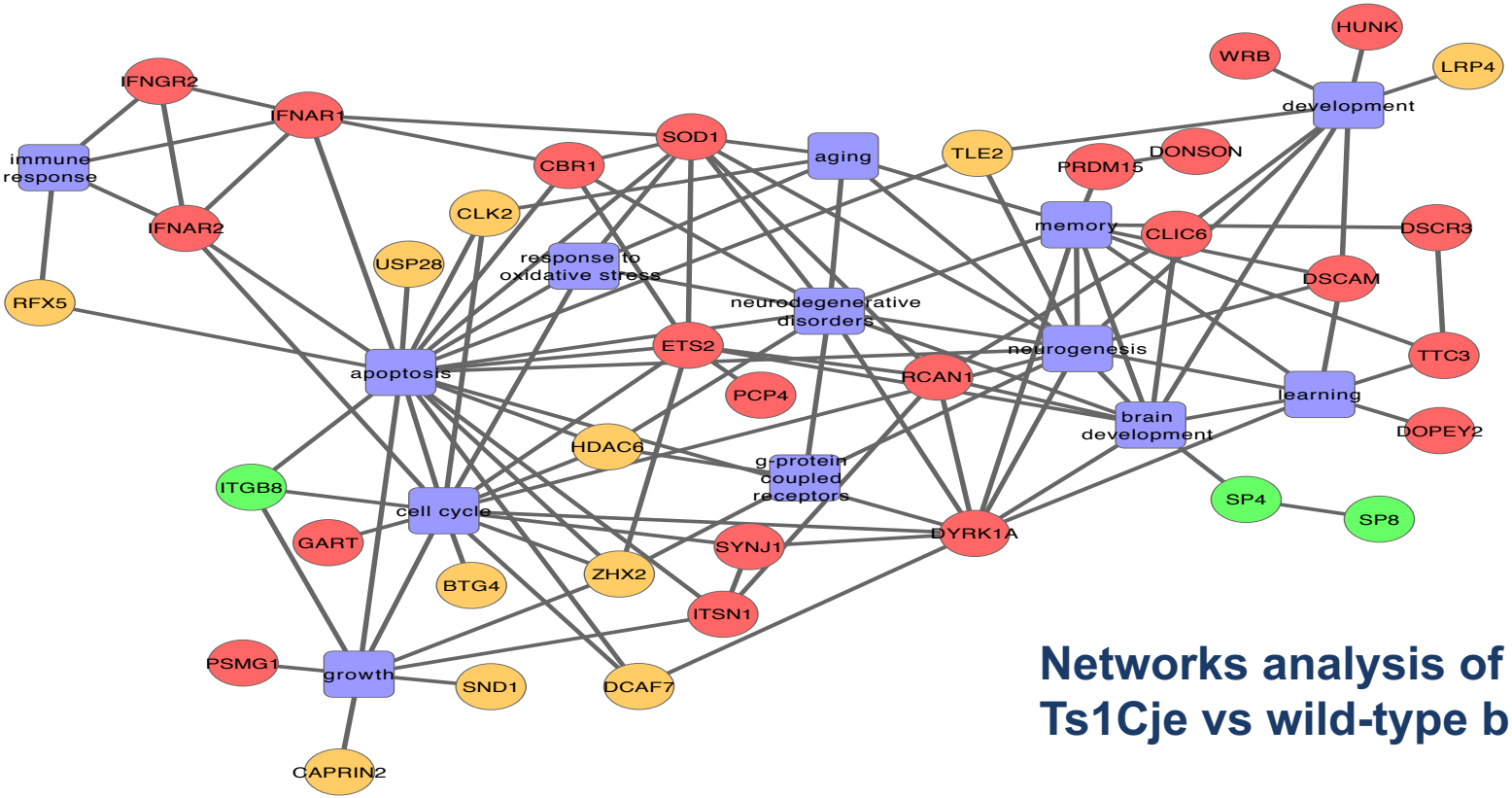


Step III: Dysregulated Signaling Pathways



Step IV: Screen for FDA-Approved Drugs and Test Effects

Goal is to Treat Pathways and not Single Genes



Networks analysis of Ts1Cje vs wild-type brain

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Received: 04 April 2016
Accepted: 27 July 2016
Published: 02 September 2016

An Integrated Human/Murine Transcriptome and Pathway Approach To Identify Prenatal Treatments For Down Syndrome

Faycal Guedj[1], Jeroen LA Pennings[2], Lauren J. Massingham[1], Heather C. Wick[3], Ashley E. Siegel[1], Umadevi Tantravahi[4] & Diana W. Bianchi[1]

Anatomical and functional brain abnormalities begin during fetal life in Down syndrome (DS). We hypothesize that novel prenatal treatments can be identified by targeting signaling pathways that are consistently perturbed in cell types/tissues obtained from human fetuses with DS and mouse embryos. We analyzed transcriptome data from fetuses with trisomy 21, age and sex-matched euploid controls, and embryonic day 15.5 forebrains from Ts1Cje, Ts65Dn, and Dp16 mice. The new datasets were compared to other publicly available datasets from humans with DS. We used the human Connectivity Map (CMap) database and created a murine adaptation to identify FDA-approved drugs that can rescue affected pathways. *USP16* and *TTC3* were dysregulated in all affected human cells and two mouse models. DS-associated pathway abnormalities were either the result of gene dosage specific effects or the consequence of a global cell stress response with activation of compensatory mechanisms. CMap analyses identified 56 molecules with high predictive scores to rescue abnormal gene expression in both species. Our novel integrated human/murine systems biology approach identified commonly dysregulated genes and pathways. This can help to prioritize therapeutic molecules on which to further test safety and efficacy. Additional studies in human cells are ongoing prior to pre-clinical prenatal treatment in mice.

Guedj *et al.* Sci Reports 6:32353/doi:10.1038/srep32353

Connectivity Map Scores of Previously Tested Therapies

Drug Name (Category)	DS AF	DS Amniocytes	DS iPSCs	DS Neurons	DS Cerebellum	DS Cerebrum	Ts1Cje E15.5 Brain	Ts65Dn E15.5 Brain	Dp16 E15.5 brain	Average Score
Apigenin (Flavone)	0.50	−0.862	−0.671	−0.536	0.576	−0.782	−0.659	−0.748	−0.668	**−0.417**
MK-801 (NMDAR agonist)	−0.265	−0.454	0.298	−0.311	0.270	−0.446	−0.567	−0.580	−0.63	**−0.298**
Vitamins A (Antioxidant)	0.150	−0.267	−0.251	−0.190	−0.174	−0.327	−0.412	−0.284	−0.488	**−0.249**
Vitamins C (Antioxidant)	−0.267	0.575	0.526	−0.384	−0.339	−0.579	−0.585	−0.444	−0.441	**−0.215**
Minocycline (Anti-inflammatory)	−0.327	0.324	0.368	−0.282	−0.327	−0.279	−0.505	−0.397	−0.362	**−0.199**
Piracetam (Derivative of GABA)	0.378	−0.289	−0.392	−0.431	−0.224	−0.29	−0.367	−0.575	−0.276	**−0.178**
Memantine (NMDAR antagonist)	0.354	0.288	−0.350	−0.523	−0.476	0.508	0.281	0.287	−0.219	**0.017**
Vitamins E (Antioxidant)	0.349	0.363	0.396	−0.220	−0.442	−0.241	0.298	0.297	0.292	**0.121**
Metformin (Type 2 Diabetes)	0.351	0.385	0.355	0.215	−0.222	−0.217	0.290	0.286	0.187	**0.181**
Riluzole (Autism spectrum disorder)	0.258	−0.251	0.175	0.382	0.258	0.354	0.319	0.549	0.266	**0.256**
Folinic Acid	0.476	0.743	0.747	0.752	−0.572	0.266	0.545	0.352	−0.205	**0.345**
Streptomycin (Antibiotic)	0.509	0.492	−0.253	0.647	0.283	0.570	0.366	0.423	0.621	**0.406**
Fluoxetine (Serotonin reuptake inhibitor)	0.436	0.370	0.424	0.747	0.209	0.444	0.482	0.433	0.426	**0.441**

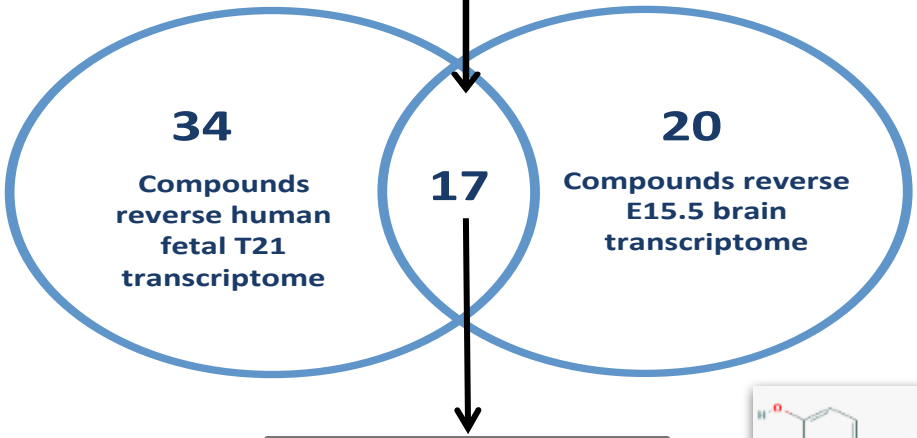
Guedj *et al.* Sci Reports 6:32353/doi:10.1038/srep32353

Goal: Identify FDA-Approved Drugs Based on Integrated Dysregulated Signatures

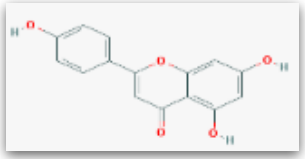
Humans with DS

Amniotic Fluid
Amniocytes
iPSCs
Fetal Brain

Common compounds: highest priority to evaluate



Apigenin

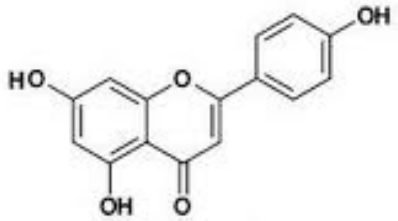


Mouse Models

(Ts1Cje, Ts65Dn, Dp16)
Forebrain at E15.5

1st Test Drug: Apigenin

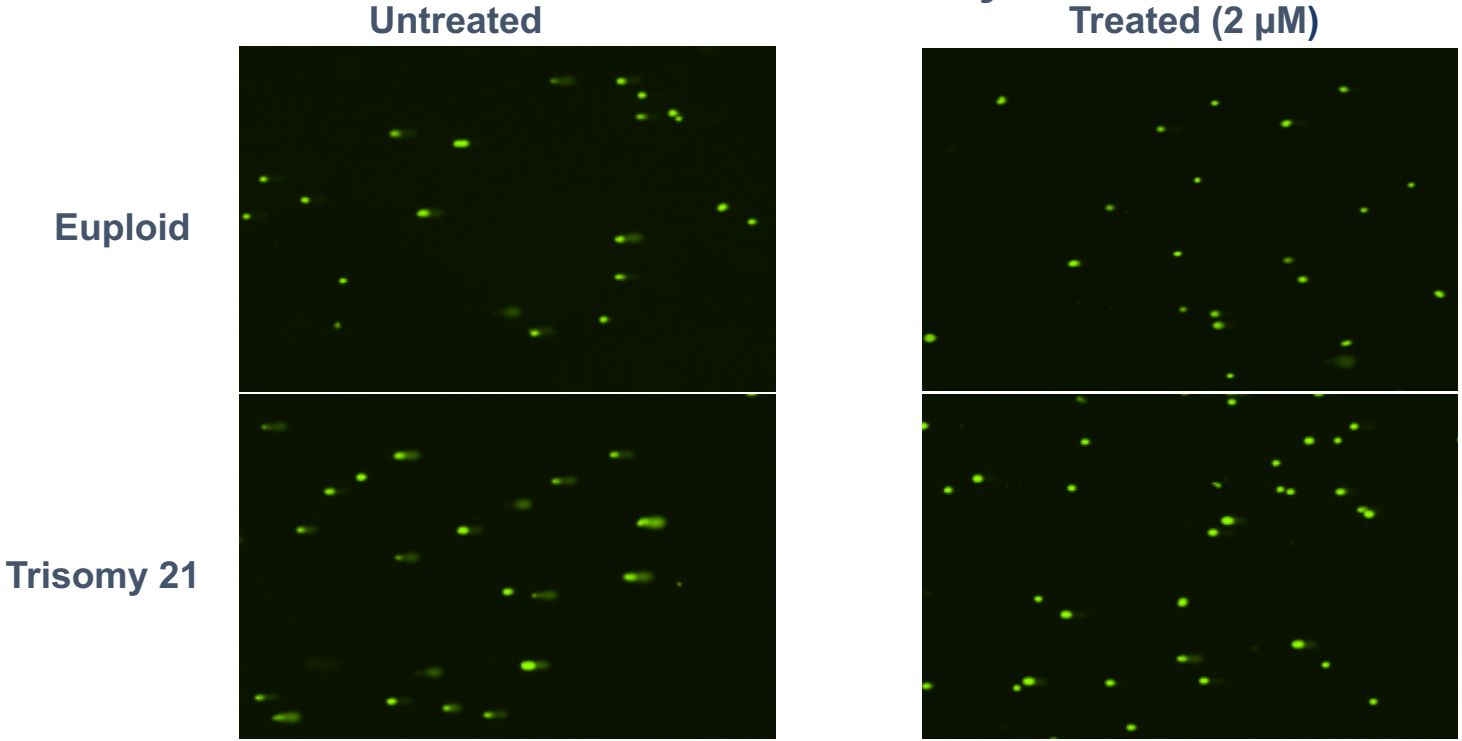
- A naturally occurring flavonoid and potent anti-oxidant
- Inhibits oxidative stress and apoptosis
- Reduces inflammation
- Crosses the blood-brain barrier
- Promotes neurogenesis in adults, no teratogenic effects



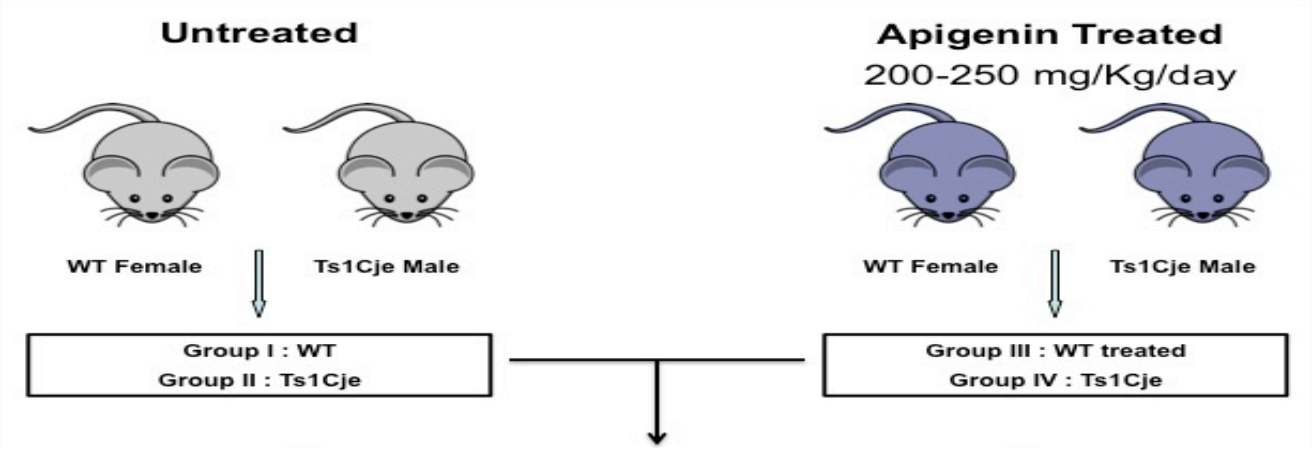
Apigenin

Results of Apigenin Treatment on Human Cells

The Comet Assay



Prenatal Treatment of Ts1Cje Mice with Apigenin



Prenatal Effects of Apigenin on Ts1Cje Mice

(Proof of Principle Demonstrated)

- **Embryonic Brain Gene Expression**
  - Normalizes gene expression in many, but not all genes
  - No change in expression in some genes, worsens a few
- **Neonatal Effects**
  - Shortens time it takes to achieve developmental milestones
  - Improves coordination (surface righting) and strength (cliff aversion test)
- **Adult Effects**
  - Improvement in open field test
  - Significant improvement in memory (fear conditioning test)
  - Effect is greater in males compared with females
  - No effects on motor coordination (rotarod test)

Summary: Part 1 (Genomics)

- Rapid incorporation of NIPT has transformed prenatal care in only 6 years (~7 million tests performed)
  - Well integrated into care for women at high-risk for aneuploidy
  - Performs equally well for women at low-risk, but PPVs are lower due to decreased prevalence of fetal aneuploidy
- High NPVs have significantly reduced need for diagnostic procedures
- False positive cases have uncovered maternal and placental abnormalities
  - NIPT demonstrates power to detect occult cancers, maternal DNA variants
  - RATs may be a marker for IUGR and pregnancy loss

Summary: Parts 2 and 3 (Transcriptomics)

- **Gene expression analysis of amniotic fluid supernatant shows many transcripts related to the developing brain**
- **A systems biology approach identified oxidative stress as the major fetal functional abnormality in trisomy 21 human cells, cell-free RNA, and murine brain**
  - **FDA-approved anti-oxidant molecules suggested to reverse abnormal gene expression.**
- ***In vitro* and *in vivo* experiments with apigenin show:**
  - **No significant toxic effects on human cell proliferation;**
  - **Statistically significant reduction of oxidative stress in DS amniocytes;**
  - **Improvement in gene expression and some behaviors in the Ts1Cje mouse model of Down syndrome.**

Future Work

- ***In Vitro***:
  - Test other high priority drugs identified by the Connectivity Map to reverse the abnormal prenatal transcriptome in trisomy 21
- ***In Vivo***:
  - Compare the effects of prenatal treatment alone versus prenatal and postnatal treatment
- **Prepare for Human Prenatal Clinical Trials**:
  - Identify noninvasive markers to potentially follow effects of treatment
  - Demonstrate that drugs cross the placenta and do no harm

Thank You!

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