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## Novel neurodevelopmental information revealed in amniotic fluid supernatant transcripts from fetuses with trisomies 18 and 21

**Lisa Hui,**

Mother Infant Research Institute and the Division of Genetics, Department of Pediatrics, The Floating Hospital for Children at Tufts Medical Center, 800 Washington St, Boston, MA 02111, USA

The Department of Obstetrics, Gynecology and Neonatology, University of Sydney, Sydney, NSW, Australia

**Donna K. Slonim,**

Department of Computer Science, Tufts University, Medford, MA, USA

Department of Pathology, Tufts University School of Medicine, Boston, MA, USA

**Heather C. Wick,**

Department of Computer Science, Tufts University, Medford, MA, USA

**Kirby L. Johnson,**

Mother Infant Research Institute and the Division of Genetics, Department of Pediatrics, The Floating Hospital for Children at Tufts Medical Center, 800 Washington St, Boston, MA 02111, USA

**Keiko Koide,** and

Mother Infant Research Institute and the Division of Genetics, Department of Pediatrics, The Floating Hospital for Children at Tufts Medical Center, 800 Washington St, Boston, MA 02111, USA

**Diana W. Bianchi**

Mother Infant Research Institute and the Division of Genetics, Department of Pediatrics, The Floating Hospital for Children at Tufts Medical Center, 800 Washington St, Boston, MA 02111, USA

Lisa Hui: lisahui77@gmail.com

### Abstract

Trisomies 18 and 21 are the two most common live born autosomal aneuploidies in humans. While the anatomic abnormalities in affected fetuses are well documented, the dysregulated biological pathways associated with the development of the aneuploid phenotype are less clear. Amniotic fluid (AF) cell-free RNA is a valuable source of biological information obtainable from live fetuses. In this study, we mined gene expression data previously produced by our group from mid-trimester AF supernatant samples. We identified the euploid, trisomy 18 and trisomy 21 AF transcriptomes, and analyzed them with a particular focus on the nervous system. We used

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Correspondence to: Lisa Hui, lisahui77@gmail.com.

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multiple bioinformatics resources, including DAVID, Ingenuity Pathway Analysis, and the BioGPS Gene Expression Atlas. Our analyses confirmed that AF supernatant from aneuploid fetuses is enriched for nervous system gene expression and neurological disease pathways. Tissue analysis showed that fetal brain cortex and Cajal–Retzius cells were significantly enriched for genes contained in the AF transcriptomes. We also examined AF transcripts known to be dysregulated in aneuploid fetuses compared with euploid controls and identified several brain-specific transcripts among them. Many of these genes play critical roles in nervous system development. *NEUROD2*, which was downregulated in trisomy 18, induces neurogenic differentiation. *SOX11*, downregulated in trisomy 21, is a transcription factor that is essential for pan-neuronal protein expression and axonal growth of sensory neurons. Our results show that whole transcriptome analysis of cell-free RNA in AF from live pregnancies permits discovery of biomarkers of abnormal human neurodevelopment and advances our understanding of the pathophysiology of aneuploidy.

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## Introduction

Trisomies 18 and 21 are the two most common live born autosomal aneuploidies in humans. While the anatomic abnormalities in affected fetuses have been well documented with prenatal sonography (Bianchi et al. 2010), the dysregulated biological pathways associated with the aneuploid phenotypes are less clear. To study human fetal development, gene expression studies have largely relied on post-partum or post-mortem tissue samples. Samples from live fetuses have almost exclusively been performed using cultured amniocytes or chorionic villi (Altug-Teber et al. 2007; Chung et al. 2005; Chou et al. 2008). These sample sources are all inherently limited in their ability to reflect in vivo physiology. Mid-trimester amniotic fluid (AF) is valuable because it is a pure fetal biofluid that reflects ongoing in utero physiology. The residual cell-free RNA that is present after amniocytes have been removed for clinical diagnosis is an excellent source of biological information (Hui and Bianchi 2011). Prior studies from our group have shown that cell-free RNA from AF can be successfully isolated and used for global gene expression studies of abnormal pregnancies (Larrabee et al. 2005; Slonim et al. 2009; Koide et al. 2011).

In recent work, we analyzed the AF transcriptome in euploid fetuses by mining gene expression data from 12 mid-trimester AF supernatants (Hui et al. 2012). Our results suggested that the cell-free RNA in AF derives from multiple tissues. More specifically, functional analysis indicated that nervous system development and function is enriched in the euploid AF transcriptome. Despite our lack of knowledge about their precise route of entry and their in vivo half-life, brain-derived transcripts may be a novel source of biomarkers of nervous system development that are uniquely accessible from living fetuses. These findings prompted us to establish whether AF supernatant could be a useful tool for studying fetal neurodevelopment in aneuploid pregnancies.

In this study, we mined gene expression data previously produced by our group from aneuploid AF supernatant samples (Koide et al. 2011; Slonim et al. 2009), focusing on the transcripts associated with nervous system development. We newly created the trisomy 18 and 21 transcriptomes, which consist of the universally detected transcripts in AF from mid-trimester trisomy 18 and trisomy 21 fetuses, respectively. Our aims were to determine if nervous system gene expression is enriched in AF supernatant from aneuploid fetuses, and to identify differentially regulated nervous system-specific transcripts in AF that may represent potential biomarkers of abnormal neurodevelopment.

## Materials and methods

### The euploid, trisomy 21 and trisomy 18 transcriptomes

We newly analyzed gene expression datasets from mid-trimester euploid and aneuploid AF samples produced in prior microarray studies from our group. These data are publicly available at <http://www.ncbi.nlm.nih.gov/geo/> (GSE25634, GSE16176, and GSE33168). Institutional review board approval was obtained for the initial collection and analysis of all samples. The methods of sample collection, RNA extraction, fragmentation, labeling, and hybridization have been previously described (Slonim et al. 2009). In brief, RNA was extracted from 10 ml of AF supernatant from women undergoing fetal testing for clinical indications. The trisomy 21 transcriptome was derived from five male and two female fetuses with trisomy 21 (16–21 weeks), the trisomy 18 transcriptome from five female fetuses with trisomy 18 (17–20 weeks) and the euploid transcriptome from six male and six female euploid fetuses (16–21 weeks). There was no pooling of samples. The final amplified cDNA products were hybridized to Affymetrix U133 Plus 2.0 microarrays (Affymetrix, Santa Clara, CA). Each AF sample was anonymized before being received in the research laboratory. Only gestational age and karyotype were available. No data were available for the presence of structural anomalies at the time of amniocentesis.

Data were normalized using the threestep function from the affyPLM package in Bioconductor (version 2.8.1) (Gentleman et al. 2004), with ideal-mismatch background/signal adjustment, quantile normalization, and the Tukey biweight summary method (Bolstad 2004). This summary method includes a logarithmic transformation that improves the normality of the data. To obtain detection calls consistent with those produced by Affymetrix<sup>®</sup> 5.0 software, we used the mas5calls function from the Bioconductor affy package.

We identified the euploid, trisomy 18 and 21 transcriptomes by selecting those transcripts present in 100 % of euploid, trisomy 18 and trisomy 21 AF supernatant samples, respectively. The enrichment of specific tissue expression was explored using multiple tools. See Table 1 for summary of study methods.

### Comparison of the trisomy 21 transcriptome with a Down syndrome gene expression meta analysis

To determine if our transcriptomic approach produced biologically relevant data, we compared the trisomy 21 AF transcriptome with a meta analysis of 45 trisomy 21 gene expression studies that included many different tissue samples (Vilardell et al. 2011). This meta analysis by Vilardell et al. identified 324 genes with significant genomewide dosage effects in Down syndrome. The authors noted that their meta analysis included many brain experiments and was therefore able to detect a high fraction of genes related to neuron development, synapsis and neurodegeneration. We identified the individual genes and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in common between the trisomy 21 AF transcriptome and the meta analysis results. The KEGG pathways were identified using the functional annotation cluster tool in DAVID and a Benjamini–Hochberg corrected *P* value with a cutoff value <0.05.

### Tissue expression analyses

We used the tissue expression function in the Database for Annotation, Visualization and Integrated Discovery (DAVID) to identify tissues that most highly expressed the genes contained in each AF transcriptome (<http://david.abcc.ncifcrf.gov/>) (Dennis et al. 2003). This tool is designed to facilitate biomarker identification and gene expression pattern discovery. DAVID integrates tissue expression databases from a publicly available Gene

Expression Atlas (Su et al. 2004) (<http://biogps.org/index.html>), the Cancer Genome Anatomy Project (Boon et al. 2002) (<http://cgap.nci.nih.gov/>), Unigene ([ftp://ftp.ncbi.nih.gov/repository/UniGene/Homo\\_sapiens/](ftp://ftp.ncbi.nih.gov/repository/UniGene/Homo_sapiens/)) and UniProt (Consortium 2011) (<http://www.uniprot.org>) to allow the identification of the most enriched gene expression patterns across hundreds of normal and diseased tissues for any given gene list. For each resource, the expression values for a given gene in all tissues are ranked from greatest to least. All tissues with an expression value in the top quartile are associated with that gene and an enrichment *P* value called the EASE score (a modified Fisher exact *P* value) is calculated for that tissue (Hosack et al. 2003). DAVID also calculates a more conservative *P* value using the Benjamini–Hochberg correction to control the false discovery rate of family-wise enriched terms. We defined results as significant if the tissue term had a Benjamini–Hochberg corrected *P* value <0.05. We uploaded the euploid, trisomy 21 and trisomy 18 transcriptome gene lists to DAVID. We reported the top ranked tissues for each database and karyotype, as well as the individual neurological tissues that met statistical significance.

### Identification of tissue-specific genes in the aneuploid transcriptomes

The BioGPS Gene Expression Atlas at <http://biogps.org> identified genes with tissue-specific expression patterns in the trisomy 21 and 18 transcriptomes. This atlas of the human protein-encoding transcriptome used Affymetrix Human Genome-U133A and custom human arrays to map gene expression profiles in 78 normal human tissues (Su et al. 2004). We chose this resource because of its Affymetrix microarray platform, coverage of normal adult and fetal tissues, high reproducibility and good correlation between transcript levels and protein abundance (Kislinger et al. 2006). The BioGPS Gene Expression Atlas allowed us to assess the gene expression patterns of individual Affymetrix probe sets in the trisomy 18 and 21 transcriptomes. We categorized probe sets as highly organ specific if they mapped to a single organ with an expression value >30 multiples of the median (MoM) and had no unrelated tissue expression greater than one-third of the maximum expression level.

### Functional analyses of the trisomy 18 and 21 transcriptomes

The web-based software tool Ingenuity Pathway Analysis Version 9.0 (IPA) (Content Version 11904312) was used for the biological interpretation of the trisomy 18 and trisomy 21 AF transcriptome gene lists (Ingenuity Systems, Redwood City, CA, USA; <http://www.ingenuity.com>). Functional analysis of the euploid transcriptome has been previously reported by our group and was not repeated here (Hui et al. 2012). IPA uses a manually curated repository of biological interactions and functional annotations to identify the most statistically significant signaling pathways and biological processes represented in a given gene set. The right-tailed Fisher's exact test is used by IPA to calculate a *P* value representing the probability that a biological function not really relevant to the AF transcriptome is reported as relevant. Controlling for multiple pathway testing is performed with the Benjamini–Hochberg correction. Diseases and disorder pathways that contained at least one functional annotation with a Benjamini–Hochberg corrected *P* value <0.05 were considered statistically significant.

### Identification of differentially regulated tissue-specific genes in aneuploid fetuses

Genes that were significantly differentially expressed in trisomies 18 or 21 AF supernatants compared with euploid controls matched for gestational age and fetal sex were obtained from prior published work from our group (Slonim et al. 2009; Koide et al. 2011). We used the BioGPS Gene Expression Atlas to identify the differentially regulated genes that were specifically expressed by the nervous system according to the method described above for the aneuploid transcriptomes.

## Results

In the trisomy 21 dataset, 1,644 probe sets received a present call in all seven samples, corresponding to 1,184 individual genes in the transcriptome (Supplemental Table 1). In the trisomy 18 dataset, 1,076 probe sets were present in all five samples, corresponding to 746 genes (Supplemental Table 2). The euploid dataset contained 806 probe sets that were present in all 12 samples, corresponding to 536 genes. Nervous system gene expression was consistently represented in our analyses of these datasets.

### Comparison of the trisomy 21 AF transcriptome to the Down syndrome gene expression meta analysis

Results from the statistical meta analysis of 45 heterogeneous trisomy 21 data sets were compared to the trisomy 21 AF transcriptome (Vilardell et al. 2011). Fifty of 324 (15.4 %) genes identified in the meta analysis were present in trisomy 21 AF transcriptome, including genes physically located on chromosome 21 (*APP*, *SOD1*, *DYRK1A*, and *RCAN1*) (Supplemental Table 3). In contrast, only 17 (5.2 %) of the meta analysis genes were universally detectable in the euploid AF samples.

Analysis of KEGG pathways in the trisomy 21 AF transcriptome using the functional annotation tool in DAVID showed one statistically significant functional cluster (Enrichment score >1.3). This single cluster contained three of the four neuropathological KEGG pathways present in the meta analysis gene list (Table 2).

### Tissue expression analyses

The DAVID tissue expression analysis showed that transcripts from multiple organs are enriched in AF, including the fetal nervous system, embryonic stem cells, pooled cell lines, umbilical cord, and epithelia (Table 3). Fetal brain cortex and Cajal–Retzius cells consistently ranked in the top four tissues in the UniProt database (Table 4). A Unigene expressed sequence tag library derived primarily from fetal cochlea was also highly ranked. Many other nervous system tissues met statistical significance across all DAVID databases, including adult caudate nucleus, whole brain, whole fetal brain, spinal cord, retina and many neurological malignancies (Supplemental Table 4).

### Tissue-specific genes in the aneuploid transcriptomes

The search for tissue-specific genes using the Gene Expression Atlas at BioGPS identified genes in the trisomy 21 and 18 AF transcriptomes that were specifically expressed by the nervous system. A total of 61 organ-specific genes were identified in the trisomy 21 AF transcriptome gene list. The most highly represented tissues were hematopoietic/immune cells (19 genes), epithelial cells from skin, oral cavity and respiratory tract (14 genes) and nervous system (12 genes). These results are consistent with the tissues identified in the DAVID tissue expression analysis. The trisomy 18 transcriptome contained six nervous system-specific genes among the total of 36 tissue-specific genes. The complete lists of tissue-specific genes are available in Supplemental Tables 6 and 7.

### Functional analyses of trisomy 21 and trisomy 18 AF transcriptomes

IPA identified neurological disease as the first and second most highly enriched disease pathway in the trisomy 21 and 18 datasets, respectively (Tables 5, 6). There was a considerable overlap of specific functional annotations within the neurological disease category between the two aneuploid groups (Supplemental Table 8). The infectious diseases annotations that were highly ranked in both groups related primarily to immune mechanisms against infection or cell surface proteins involved in the mechanisms of viral infection. We specifically excluded the biological category of cancer because cell proliferation functions

that are normal in the developing human cause the IPA disease category “cancer” to be highly significant in fetal datasets (unpublished observations).

### Identification of differentially regulated tissue-specific genes in aneuploid fetuses

There was significant differential regulation of several nervous system-specific genes in trisomy 21 and 18 fetuses, including transcription factors with critical roles in neuronal differentiation. These genes represent potential biomarkers of abnormal neurodevelopment (Table 7). Of the 318 genes that were differentially regulated in trisomy 21 fetuses compared with matched euploid controls, 22 were tissue specific (Supplemental Table 9). Four genes of these genes were specifically expressed by the nervous system: *SOX11* and *DAAM2* were downregulated, and *MEF2C* and *CELSR2* were upregulated in trisomy 21 fetuses, respectively.

Of the 264 genes that were differentially regulated in trisomy 18 fetuses compared with matched euploid controls, there were 13 organ-specific genes (Supplemental Table 10). Three neurological tissue-specific genes were upregulated (*PLEKHA4*, *PRPH2*, and *GPM6Z*) and three were downregulated (*NEUROD2*, *SOBP* and *PTPRD*). There was no overlap between the trisomy 21 and trisomy 18 differentially regulated nervous system-specific genes.

### Discussion

We applied multiple bioinformatic tools to explore the gene expression information contained in AF cell-free RNA from euploid, trisomy 21 and trisomy 18 fetuses. In this study, we focused on the neurodevelopmental data in the aneuploid groups because of the importance of developmental disability to the phenotypes of trisomy 21 and 18. We showed that the transcripts consistently present in AF are enriched for nervous system tissue expression and identified nervous-specific genes that are differentially regulated in aneuploid fetuses compared with euploid controls.

The significant result for the KEGG pathway for Alzheimer’s disease in both the trisomy 21 AF transcriptome and the Down syndrome meta analysis is consistent with the known neuropathology of Down syndrome. This suggests that AF supernatant reflects real tissue biology and is therefore valid for studying human neurodevelopment. Of particular interest was the finding from the DAVID tissue expression analysis that Cajal–Retzius cells and fetal cortex had highly enriched expression of genes in the AF transcriptomes. Cajal–Retzius cells are reelin-producing neurons found in the marginal zone of the fetal brain that play an essential role in neuronal migration and cortical development (Fig. 1 Cajal–Retzius cell in the developing human fetal cerebral cortex at 13 weeks’ gestation). The fact that nervous system tissues were enriched in AF across multiple independent databases gives us greater confidence that these findings reflect actual fetal biology and are not the result of noise inherent in high throughput gene expression data.

The significant neurological disease annotations obtained in the IPA functional analysis of the aneuploid transcriptomes provided additional confirmation that nervous system pathophysiology can be reflected in AF. However, it is important to note that the functional annotation of diseases within IPA is largely based on adult studies. We do not propose that all the features of specific disorders such as Huntington’s disease or Parkinson’s disease occur in aneuploid fetuses. Rather, we believe that the significant results for these categories are a result of the fact that many of the genes involved in these adult diseases are essential for normal neurodevelopment and are dysregulated in aneuploid fetuses.

We identified brain-specific genes that were dysregulated in aneuploid fetuses compared with euploid controls. Many of these genes play critical roles in nervous system development, as summarized in Table 7. *NEUROD2*, which was downregulated in trisomy 18, is most highly expressed in the marginal zone in week 19 of human gestation (Franklin et al. 2001) and induces neurogenic differentiation (Ravanpay et al. 2010; Mattar et al. 2008). *SOBP*, also downregulated in Trisomy 18, is involved in development of the cochlea, and defects in this gene have been linked to developmental disability (Birk et al. 2010; Chen et al. 2008). Both hearing and intellectual impairment are consistent features of the trisomy 18 phenotype. *SOX11*, downregulated in trisomy 21, is a transcription factor expressed in the embryonic central and peripheral nervous systems and is of critical importance for the establishment of pan-neuronal protein expression (Bergsland et al. 2006) and axonal growth of embryonic sensory neurons (Lin et al. 2011). These findings, uniquely derived from live human fetuses, provide novel information about the altered neurodevelopmental gene expression in trisomies 18 and 21.

There is still a general paucity of information on the biology of cell-free RNA in AF (Hui and Bianchi 2011). The mechanisms by which fetal transcripts enter the AF are still unknown. It is possible that differential entry or degradation of cell-free transcripts could influence the results of our analysis. Furthermore, cell-free RNA is inherently more degraded and generally produces lower hybridization rates than cellular RNA. Fresh tissue would be an ideal source of gene expression information, but given the practical issues associated with human studies, AF provides an important window on fetal development.

## Conclusions

Multiple independent bioinformatics analyses confirm that AF supernatant of aneuploid fetuses is enriched for nervous system gene expression and neurological disease pathways. We identified nervous system-specific transcription factors with key roles in brain development that are differentially regulated in aneuploid fetuses compared with euploid controls. Whole transcriptome analysis of cell-free RNA in AF from live pregnancies permits discovery of biomarkers of abnormal human neurodevelopment and advances understanding of the pathophysiology of human aneuploidy.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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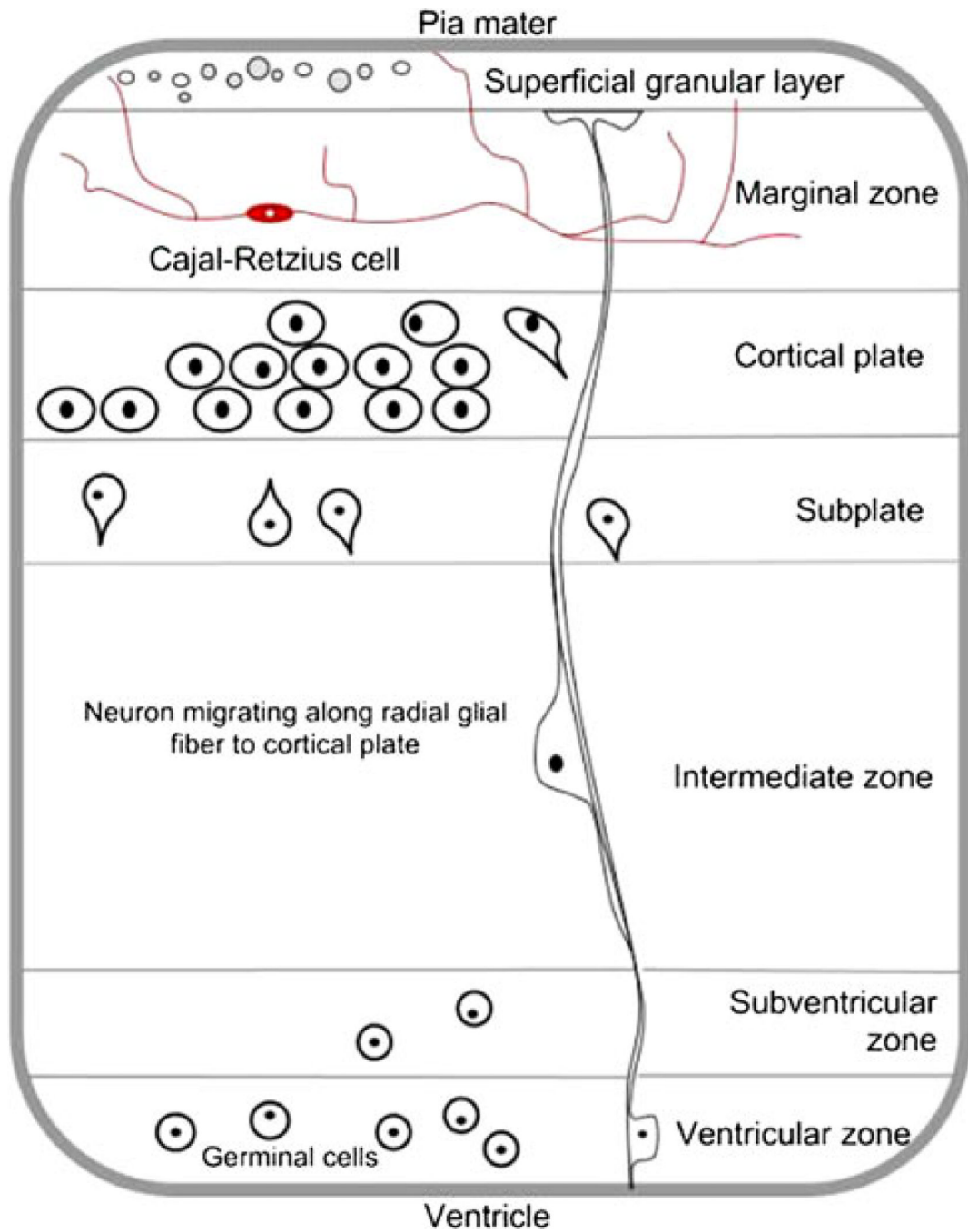
## References

- Altug-Teber O, Bonin M, Walter M, Mau-Holzmann UA, Dufke A, Stappert H, Tekesin I, Heilbronner H, Nieselt K, Riess O. Specific transcriptional changes in human fetuses with autosomal trisomies. *Cytogenet Genome Res.* 2007; 119(3–4):171–184. [PubMed: 18253026]
- Bergsland M, Werme M, Malewicz M, Perlmann T, Muhr J. The establishment of neuronal properties is controlled by *Sox4* and *Sox11*. *Gene Dev.* 2006; 20(24):3475–3486. [PubMed: 17182872]
- Bianchi, DW.; Crombleholme, TM.; D'Alton, ME.; Malone, FD. *Fetology*. 2nd edn.. USA: McGraw-Hill Companies, Inc; 2010. p. 910-925.

- Birk E, Har-Zahav A, Manzini CM, Pasmanik-Chor M, Kornreich L, Walsh CA, Noben-Trauth K, Albin A, Simon AJ, Colleaux L, Morad Y, Rainshtein L, Tischfield DJ, Wang P, Magal N, Maya I, Shoshani N, Rechavi G, Gothelf D, Maydan G, Shohat M, Basel-Vanagaite L. *SOBP* is mutated in syndromic and nonsyndromic intellectual disability and is highly expressed in the brain limbic system. *Am J Hum Genet.* 2010; 87(5):694–700. [PubMed: 21035105]
- Bolstad, BM. Low level analysis of high-density oligonucleotide array data: background, normalization and summarization. Berkeley: University of California; 2004.
- Boon K, Osorio EC, Greenhut SF, Schaefer CF, Shoemaker J, Polyak K, Morin PJ, Buetow KH, Strausberg RL, De Souza SJ, Riggins GJ. An anatomy of normal and malignant gene expression. *Proc Natl Acad Sci USA.* 2002; 99(17):11287–11292. [PubMed: 12119410]
- Chen Z, Montcouquiol M, Calderon R, Jenkins NA, Copeland NG, Kelley MW, Noben-Trauth K. *Jxc1/Sobp*, encoding a nuclear zinc finger protein, is critical for cochlear growth, cell fate, and patterning of the organ of corti. *J Neurosci.* 2008; 28(26):6633–6641. [PubMed: 18579736]
- Chou CY, Liu LY, Chen CY, Tsai CH, Hwa HL, Chang LY, Lin YS, Hsieh FJ. Gene expression variation increase in trisomy 21 tissues. *Mamm Genome.* 2008; 19(6):398–405. [PubMed: 18594911]
- Chung IH, Lee SH, Lee KW, Park SH, Cha KY, Kim NS, Yoo HS, Kim YS, Lee S. Gene expression analysis of cultured amniotic fluid cell with Down syndrome by DNA microarray. *J Korean Med Sci.* 2005; 20(1):82–87. [PubMed: 15716609]
- Consortium U. Ongoing and future developments at the Universal Protein Resource. *Nucleic Acids Res.* 2011; 39:D214–D219. ((Database issue)). [PubMed: 21051339]
- Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: database for annotation, visualization, and integrated discovery. *Genome Biol.* 2003; 4(5):P3. [PubMed: 12734009]
- Franklin A, Kao A, Tapscott S, Unis A. NeuroD homologue expression during cortical development in the human brain. *J Child Neurol.* 2001; 16(11):849–853. [PubMed: 11732772]
- Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol.* 2004; 5(10):R80. [PubMed: 15461798]
- Hosack DA, Dennis G Jr, Sherman BT, Lane HC, Lempicki RA. Identifying biological themes within lists of genes with EASE. *Genome Biol.* 2003; 4(10):R70. [PubMed: 14519205]
- Hui L, Bianchi DW. Cell-free fetal nucleic acids in amniotic fluid. *Hum Reprod Update.* 2011; 17(3):362–371. [PubMed: 20923874]
- Hui L, Slonim DK, Wick HC, Johnson KL, Bianchi DW. The amniotic fluid transcriptome: a source of novel information about human fetal development. *Obstet Gynecol.* 2012; 119(1):111–118. [PubMed: 22183218]
- Kida Y, Shiraishi T, Ogura T. Identification of chick and mouse Daam1 and Daam2 genes and their expression patterns in the central nervous system. *Brain Res Dev Brain Res.* 2004; 153(1):143–150.
- Kislinger T, Cox B, Kannan A, Chung C, Hu P, Ignatchenko A, Scott MS, Gramolini AO, Morris Q, Hallett MT, Rossant J, Hughes TR, Frey B, Emili A. Global survey of organ and organelle protein expression in mouse: combined proteomic and transcriptomic profiling. *Cell.* 2006; 125(1):173–186. [PubMed: 16615898]
- Koide K, Slonim DK, Johnson KL, Tantravahi U, Cowan JM, Bianchi DW. Transcriptomic analysis of cell-free fetal RNA suggests a specific molecular phenotype in trisomy 18. *Hum Genet.* 2011; 129(3):295–305. [PubMed: 21152935]
- Larrabee PB, Johnson KL, Lai C, Ordovas J, Cowan JM, Tantravahi U, Bianchi DW. Global gene expression analysis of the living human fetus using cell-free messenger RNA in amniotic fluid. *JAMA.* 2005; 293(7):836–842. [PubMed: 15713773]
- Lin L, Lee VM, Wang Y, Lin JS, Sock E, Wegner M, Lei L. Sox11 regulates survival and axonal growth of embryonic sensory neurons. *Dev Dyn.* 2011; 240(1):52–64. [PubMed: 21117150]



- Mattar P, Langevin LM, Markham K, Klenin N, Shivji S, Zinyk D, Schuurmans C. Basic helix-loop-helix transcription factors cooperate to specify a cortical projection neuron identity. *Mol Cell Biol.* 2008; 28(5):1456–1469. [PubMed: 18160702]
- Michibata H, Okuno T, Konishi N, Kyono K, Wakimoto K, Aoki K, Kondo Y, Takata K, Kitamura Y, Taniguchi T. Human GPM6A is associated with differentiation and neuronal migration of neurons derived from human embryonic stem cells. *Stem Cells & Dev.* 2009; 18(4):629–639.
- Ravanpay AC, Hansen SJ, Olson JM. Transcriptional inhibition of REST by NeuroD2 during neuronal differentiation. *Mol Cell Neurosci.* 2010; 44(2):178–189. [PubMed: 20346398]
- Slonim DK, Koide K, Johnson KL, Tantravahi U, Cowan JM, Jarrah Z, Bianchi DW. Functional genomic analysis of amniotic fluid cell-free mRNA suggests that oxidative stress is significant in Down syndrome fetuses. *Proc Natl Acad Sci USA.* 2009; 106(23):9425–9429. [PubMed: 19474297]
- Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D, Zhang J, Soden R, Hayakawa M, Kreiman G, Cooke MP, Walker JR, Hogenesch JB. A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc Natl Acad Sci USA.* 2004; 101(16):6062–6067. [PubMed: 15075390]
- Vilardell M, Rasche A, Thormann A, Maschke-Dutz E, Perez-Jurado LA, Lehrach H, Herwig R. Meta-analysis of heterogeneous Down syndrome data reveals consistent genome-wide dosage effects related to neurological processes. *BMC Genomics.* 2011; 12:229. [PubMed: 21569303]
- Woo J-M, Park SJ, Kang HI, Kim BG, Shim SB, Jee SW, Lee SH, Sin JS, Bae CJ, Jang MK, Cho C, Hwang DY, Kim CK. Characterization of changes in global gene expression in the brain of neuron-specific enolase/human Tau23 transgenic mice in response to overexpression of Tau protein. *Int J Mol Med.* 2010; 25(5):667–675. [PubMed: 20372808]



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

**Table 1**

## Summary of methods

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*Identification of the euploid, trisomy 21 and 18 AF transcriptomes*Trisomy 21 AF transcriptome compared with Down syndrome meta analysis<sup>a</sup>DAVID<sup>b</sup> tissue expression analysis of euploid, trisomy 21 and 18 AF transcriptomesBioGPS analysis for tissue-specific genes in the trisomy 21 and 18 AF transcriptomes<sup>c</sup>IPA<sup>d</sup> functional analysis of trisomy 21 and 18 AF transcriptomes<sup>c</sup>*Genes differentially regulated in trisomy 21 and 18 compared with euploid fetuses<sup>e</sup>*Identification of nervous system-specific genes that were up or down regulated in aneuploid compared with euploid fetuses

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<sup>a</sup>Vilardell et al. (2011)<sup>b</sup>Database for annotation, visualization and integrated discovery<sup>c</sup>We have recently reported these results for the euploid AF transcriptome (Hui et al. 2012)<sup>d</sup>Ingenuity Pathway Analysis™<sup>e</sup>As previously published by our group in Koide et al. (2011), Slonim et al. (2009)

**Table 2**

Significant functional cluster of KEGG pathways in the trisomy 21 AF transcriptome

Annotation Cluster 1 Category	Enrichment score: 6.520 <sup>a</sup> Term	Count	<i>P</i> value*
KEGG_PATHWAY	hsa05016: Huntington's disease	41	1.46E-06
KEGG_PATHWAY	hsa05012: Parkinson's disease	32	1.03E-06
KEGG_PATHWAY	hsa00190: Oxidative phosphorylation	29	9.79E-05
KEGG_PATHWAY	hsa05010: Alzheimer's disease	33	3.46E-04

\* Benjamini-Hochberg corrected *P* value

<sup>a</sup> Group Enrichment Score is the geometric mean (in -log scale) of member's *P* values in a corresponding annotation cluster, and is used to rank their biological significance

**Table 3**

DAVID tissue expression results for AF transcriptomes: top ranking tissues by database and karyotype

Database	Euploid	Trisomy 21	Trisomy 18
BioGPS Gene Expression Atlas	Caudate nucleus	Caudate nucleus	Caudate nucleus
UniProt tissue	Fetal brain cortex	Epithelium	Epithelium
CGAP EST <sup>a</sup>	Pooled tissue normal <sup>c</sup>	Pooled tissue normal <sup>c</sup>	Embryonic tissue
CGAP SAGE <sup>b</sup>	Embryonic stem cell	Embryonic stem cell	Embryonic stem cell
Unigene EST	Umbilical cord	Ear <sup>d</sup>	Ear <sup>d</sup>

<sup>a</sup>Cancer Genome Anatomy Project expressed sequence tag library

<sup>b</sup>Cancer Genome Anatomy Project serial analysis of gene expression library

<sup>c</sup>Library 10550 (dbEST ID) pooled from 40 cell lines including brain

<sup>d</sup>Only two Unigene EST ear libraries contained >1,000 sequences. Both were derived from fetal cochlea: Lib.371 Morton Fetal Cochlea (14805) and Lib.18222 Human Fetal Cochlea cDNA Library (3880)

**Table 4**

Top five terms in UniProt tissue expression chart by karyotype

<b>Karyotype</b>	<b>UniProt KB term</b>	<b>No. of genes</b>	<b><i>P</i> value*</b>
Euploid	Fetal brain cortex	41	6.34E-18
	Cajal–Retzius cell <sup>a</sup>	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 <sup>a</sup>	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 <sup>a</sup>	44	2.39E-17
	Skin	140	2.05E-10
	Placenta	220	2.24E-10

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

<sup>a</sup> See Supplemental Table 5 for genes expressed by Cajal–Retzius cells that were detected in the euploid, trisomy 21 and trisomy 18 AF transcriptomes

**Table 5**

Trisomy 21 IPA significant diseases and disorders

Category	<i>P</i> value range *	No. of genes
Neurological disease	2.76E-12 to 4.61E-02	201
Dermatological diseases and conditions	7.05E-06 to 3.06E-02	114
Infectious disease	6.52E-05 to 4.20E-02	177
Reproductive system disease	1.97E-04 to 2.51E-04	96
Connective tissue disorders	7.38E-03 to 4.61E-02	6
Respiratory disease	4.61E-02	6
Cardiovascular disease	4.20E-02	4

\* Benjamini-Hochberg corrected right-tailed Fisher exact *P* value ranges for individual functional annotations within each category

**Table 6**

Trisomy 18 IPA significant diseases and disorders

Category	<i>P</i> value range *	No. of genes
Infectious disease	1.61E-05 to 2.82E-02	119
Neurological disease	3.80E-05 to 3.76E-02	107
Dermatological diseases and conditions	1.67E-03 to 3.79E-03	45
Skeletal and muscular disorders	8.49E-03 to 3.45E-02	9
Connective tissue disorders	2.00E-02 to 4.64E-02	5

\* Benjamini-Hochberg corrected right-tailed Fisher exact *P* value ranges for individual functional annotations within each category



Table 7

Differentially regulated nervous system-specific genes detected in trisomy 18 and 21 AF supernatants

Differential regulation <sup>a</sup>	Gene	Gene name	Tissues expressing gene >30 MoM <sup>b</sup>	Gene function in nervous system <sup>c</sup>
Downregulated in trisomy 18	<i>PTPRD</i>	Protein tyrosine phosphatase, receptor type, D	Fetal brain, prefrontal cortex, hypothalamus, amygdala, spinal cord	Implicated in axonal growth and guidance of motor neurons in mammals
	<i>SOBP</i>	Sine oculis binding protein homolog ( <i>Drosophila</i> )	Fetal brain, amygdala, prefrontal cortex, whole brain	The protein encoded by this gene is a nuclear zinc finger protein that is involved in development of the cochlea. Defects in this gene linked to intellectual disability
	<i>NEUROD2</i>	Neurogenic differentiation 2	Cerebellum peduncles, fetal brain, cerebellum	Expression of this gene can induce transcription from neuron-specific promoters. The product of the human gene can induce neurogenic differentiation in non-neuronal cells in <i>Xenopus</i> embryos, and is thought to play a role in the determination and maintenance of neuronal cell fates
Upregulated in trisomy 18	<i>PLEKHA4</i>	Pleckstrin homology domain containing, family A (phosphoinositide binding-specific) member 4	Olfactory bulb	The second messenger phosphatidylinositol 3-phosphate regulates a plethora of cellular processes
	<i>PRPH2</i>	Peripherin 2 (retinal degeneration, slow)	Pineal (day), pineal (night)	The proteins mediate signal transduction events that play a role in the regulation of cell development, activation, growth and motility. This encoded protein is a cell surface glycoprotein found in the outer segment of both rod and cone photoreceptor cells. Defects in this gene are associated with both central and peripheral retinal degenerations
	<i>GPM6A</i>	Glycoprotein M6A	Amygdala, fetal brain, prefrontal cortex, occipital lobe, whole brain	An abundant cell surface protein on neurons in the central nervous system associated with differentiation and neuronal migration (Michibata et al. 2009). Downregulated in the brains of transgenic mice that overexpress tau, the neuronal phosphoprotein associated with Alzheimer's disease (Woo et al. 2010)
Downregulated in trisomy 21	<i>SOX11</i>	SRY (sex determining region Y)-box 11	Fetal brain	A transcription factor involved in the regulation of embryonic development and in the determination of the cell fate; promotes neuronal maturation in the developing neural tube and is essential for establishment of pan-neuronal protein expression (Bergsland et al. 2006)
	<i>DAAM2</i>	Disheveled associated activator of morphogenesis 2	Spinal cord, hypothalamus, prefrontal cortex, retina, thalamus, caudate nucleus	Disheveled-binding protein transducing WNT signals to the PCP pathway. Involved in pivotal steps in neuronal cell differentiation and movement (Kida et al. 2004)
Upregulated in trisomy 21	<i>MEF2C</i>	Myocyte enhancer factor 2C	Fetal brain	Crucial for normal neuronal development, distribution, and electrical activity in the neocortex. May also be involved in neurogenesis and in the development of cortical architecture
	<i>CELSR2</i>	Cadherin, EGF LAG seven-pass G-type receptor 2 (flamingo homolog, <i>Drosophila</i> )	Whole brain, prefrontal cortex, amygdala, medulla oblongata, cingulate cortex, parietal lobe	Receptor involved in cell/cell signaling during nervous system formation

<sup>a</sup>As identified from Slonim et al. 2009, Koide et al. 2011

<sup>b</sup>BioGPS Gene Expression Atlas

<sup>c</sup>Entrez gene or UniProt summaries unless references given