HHS Public Access

Author manuscript

Hum Genet. Author manuscript; available in PMC 2015 September 01.

Published in final edited form as:

Hum Genet. 2014 September ; 133(9): 1075–1082. doi:10.1007/s00439-014-1448-y.

Amniotic fluid RNA gene expression profiling provides insights into the phenotype of Turner syndrome

Lauren J. Massingham[1], **Kirby L. Johnson**[2], **Thomas M. Scholl**[3], **Donna K. Slonim**[2,4], **Heather C. Wick**[4], and **Diana W. Bianchi**[1]

Lauren J. Massingham: lmassingham@lifespan.org; Kirby L. Johnson: Kirby.Johnson@tufts.edu; Thomas M. Scholl: tmscholl@beckman.com; Donna K. Slonim: slonim@cs.tufts.edu; Heather C. Wick: hwick@cs.tufts.edu; Diana W. Bianchi: dbianchi@tuftsmedicalcenter.org

[1]Mother Infant Research Institute and Department of Pediatrics, Floating Hospital for Children at Tufts Medical Center, Boston, Massachusetts

[2]Tufts University School of Medicine, Boston, MA

[3]Integrated Genetics, Esoterix Genetic Laboratories, LLC, a subsidiary of Laboratory Corporation of America® Holdings, Westborough, MA

[4]Dept. of Computer Science, Tufts University, Medford MA

Abstract

Background—Turner syndrome is a sex chromosome aneuploidy with characteristic malformations. Amniotic fluid, a complex biological material, could contribute to the understanding of Turner syndrome pathogenesis. In this pilot study, global gene expression analysis of cell-free RNA in amniotic fluid supernatant was utilized to identify specific genes/organ systems that may play a role in Turner syndrome pathophysiology.

Methods—Cell-free RNA from amniotic fluid of five mid-trimester Turner syndrome fetuses and five euploid female fetuses matched for gestational age was extracted, amplified, and hybridized onto Affymetrix® U133 Plus 2.0 arrays. Significantly differentially regulated genes were identified using paired t-tests. Biological interpretation was performed using Ingenuity Pathway Analysis and BioGPS gene expression atlas.

Results—There were 470 statistically significantly differentially expressed genes identified. They were widely distributed across the genome. *XIST* was significantly down-regulated ($p<0.0001$); *SHOX* was not differentially expressed. One of the most highly represented organ systems was the hematologic/immune system, distinguishing the Turner syndrome transcriptome from other aneuploidies we previously studied. Manual curation of the differentially expressed

Correspondence to: Lauren J. Massingham, lmassingham@lifespan.org.

Competing interests

The authors declare no conflict of interest.

Authors' contributions

LJM participated in the design of the study, carried out the molecular genetic studies, analyzed the data, and drafted the manuscript. KLJ participated in the design of the study, the analysis of the data, and helped draft the manuscript. TMS participated in the design of the study, provided the samples, and helped revise the manuscript. DKS performed computational analysis and helped revise the manuscript. HCW performed computational analysis and helped revise the manuscript. DWB obtained funding, participated in the design of the study, the analysis of the data, and helped draft the manuscript. All authors read and approved the final manuscript.

gene list identified genes of possible pathologic significance, including *NFATC3*, *IGFBP5*, and *LDLR*.

Conclusions—Transcriptomic differences in the amniotic fluid of Turner syndrome fetuses are due to genome-wide dysregulation. The hematologic/immune system differences may play a role in early-onset autoimmune dysfunction. Other genes identified with possible pathologic significance are associated with cardiac and skeletal systems, which are known to be affected in females with Turner syndrome. The discovery-driven approach described here may be useful in elucidating novel mechanisms of disease in Turner syndrome.

Keywords

Turner syndrome; amniotic fluid; RNA transcriptome; hyperlipidemia; short stature; autoimmune dysfunction

Background

Turner syndrome is a common sex chromosome aneuploidy with a high prevalence (1 in 2500 livebirths) (Hall et al. 1982; Nielsen and Wohlert 1991). The majority of cases (98–99%), however, result in miscarriage (Bianchi et al. 2010). It is not unusual for women with Turner syndrome to be unaware that they have the condition until adolescence and the absence of menarche. Turner syndrome is due to monosomy X in 64% of prenatal cases and 47% of postnatal cases (Gravholt 2004). The remaining cases are due to a variety of X chromosome abnormalities, including deletions and duplications, ring chromosome, or mosaic aneuploidies. There are no pathognomonic clinical features, but there are clinical characteristics and malformations that are typically found, including short stature, webbed neck, coarctation of the aorta, lymphedema and infertility. Associated conditions include obesity, scoliosis, glucose intolerance, atherosclerosis, hyperlipidemia, and juvenile rheumatoid arthritis (Tyler and Edman 2004). Fetal abnormalities that are detectable by prenatal sonography include increased nuchal translucency, hydrops, and cystic hygroma (Papp et al. 2006).

Amniotic fluid (AF) is a routinely collected clinical material that could contribute to the understanding of the pathogenesis of Turner syndrome in the fetus. Typically discarded, the residual AF supernatant is an abundant source of cell-free mRNA (Larrabee et al. 2005). Previous studies investigating the AF transcriptome of fetuses with trisomies 21 and 18 demonstrated widespread expression differences between affected and normal fetuses (Slonim et al. 2009; Koide et al. 2011). Prior examination of the common genes expressed in healthy euploid fetuses suggested that multiple organ systems were overrepresented in the AF transcriptome, including the musculoskeletal, hematologic, and nervous systems (Hui et al. 2012a).

In this pilot study, we utilized global gene expression analysis to interrogate the cell-free mRNA transcriptome from AF supernatants of fetuses with Turner syndrome in order to understand pathophysiologic changes that are already present during the second trimester of gestation.

Methods

Subjects

The Institutional Review Board at Tufts Medical Center approved this research. The fully anonymized specimens were obtained from Integrated Genetics/LabCorp (Westborough, MA), a CLIA-approved diagnostic laboratory. The anonymized residual AF supernatant samples (gestational ages between 15 and 17 6/7 weeks) were collected from women undergoing genetic testing for routine clinical indications. Only the karyotypes and gestational ages associated with the samples were known; the laboratory team was blinded to results of any ultrasound findings. AF supernatant was stored at −80°C at Integrated Genetics for two to three months, shipped on dry ice, and again stored at −80°C at Tufts prior to processing. Once the samples arrived at Tufts, RNA was extracted within 1 week. The original study set consisted of seven 45, X samples and seven 46, XX samples matched for gestational age. Due to poor hybridization quality, two 45, X samples and their controls were excluded from analysis, resulting in an analytic cohort of five 45, X samples and five 46, XX samples. Such sample sizes have been observed to offer moderate levels of stability and reproducibility in other microarray experiments (Pavlidis et al., 2003).

Sample Preparation

RNA was extracted from 5ml AF supernatant with the QIAamp Circulating Nucleic Acid Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol under the section "Purification of circulating RNA from 5ml serum or plasma" and as described in Dietz et al. (2011). Samples were stored at −80° C until further analysis.

cDNA was synthesized from extracted RNA and amplified using the WT-Ovation Pico RNA Amplification System (NuGEN, San Carlos, CA) according to the manufacturer's instructions. Amplifed cDNA was purified by adding 800μl PB buffer (Qiagen, Valencia, CA), vortexed, and centrifuged in QIAquick spin columns for one minute at 14,000g. 700μl of 80% ethanol was added to the spin column, centrifuged for one minute at 14,000g and repeated once. Columns were transferred to clean collection tubes, and 30μl nuclease-free water was added and incubated for five minutes at room temperature. Purified cDNA was eluted by centrifuging for one minute at 14,000g. Samples were then stored at −80°C until further processing.

cDNA was fragmented and labeled using the Encore Biotin Module (NuGEN) according to manufacturer's instructions. Fragmented and labeled cDNA was hybridized on GeneChip® Human Genome U133 Plus 2.0 arrays (Affymetrix®, Santa Clara, CA) according to the GeneChip® Expression Analysis Technical Manual, with the following changes: the Encore Biotin Module (NuGEN) hybridization cocktail assembly was used for a standard array, and the array was placed in the hybridization oven for 18–20 hours. Arrays were washed and stained with streptavidin-phycoerythrin and scanned with a GeneChip® Scanner.

Data Analysis

The data were analyzed using GeneChip® Microarray Suite 5.0 (Affymetrix®) and normalized as previously described (Hui et al. 2012b). This step includes a log-

transformation, improving the normality of the data. Individual differentially-expressed probe sets were identified via paired *t*-tests implemented using the Bioconductor package in R, pairing each Turner syndrome sample with a control matched for gestational age. Array quality was also assessed in Bioconductor using the simpleaffy package, which reported that an average of 28.7% of the probe sets on these arrays were called "present." Probe sets were considered differentially-expressed if their Benjamini-Hochberg adjusted p-values were below 0.015. We also compared the Turner syndrome cohort differentially-regulated probe set lists with previous data from our laboratory obtained from the AF from fetuses with trisomy 21 and trisomy 18. Probe sets were manually converted to genes using Affymetrix® NetAffx™ (http://www.affymetrix.com).

Each differentially expressed gene in the dataset was mapped to a specific chromosome location by utilizing the Online Mendelian Inheritance in Man (OMIM) database (http://www.omim.org). The number of known genes on each chromosome was obtained from the Human Genome (Build 36.3) in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov). The expected number of altered gene expression differences was calculated based on the number of known genes on each chromosome. A chi-square statistical analysis utilizing the observed and the expected number of altered gene expression differences was performed to determine if the distribution of differentially expressed genes was random.

Probe sets with Benjamini-Hochberg corrected p-values of <0.015 and the median fold-change expression difference for each of those genes were uploaded to the Ingenuity Pathway Analysis® (IPA) system (Content version 12710793, Ingenuity Systems, Redwood City, CA, USA, http://ingenuity.com). IPA is a software tool that uses a manually curated collection of biological interactions and functional annotations to identify significantly over-represented signaling pathways and biological processes in a given gene set. Networks of differentially regulated molecules were generated using the IPA software. A right-tailed Fisher's exact test is used by IPA to calculate a p-value representing the probability that each reported network would be implicated by chance alone.

The BioGPS Gene Expression Atlas (http://biogps.org) is a web resource for gene and protein annotation, which includes access to tissue-specific gene expression profiles for 79 human tissues (Su et al. 2004; Wu et al. 2009). Transcripts that mapped to a single organ system in the BioGPS data set with an expression value of >30 multiples of the median (MoM), whose second-most-abundant tissue's expression was no more than a third as high, and whose median expression across all tissues was at least a value of 30 MoMs, were identified as highly organ specific. The "Turner syndrome transcriptome" was defined as transcripts that were called present by Affymetrix® software in all five Turner syndrome samples. The data obtained from the BioGPS analysis were utilized to identify the tissue specific transcripts in the Turner syndrome transcriptome and to compare these results with similar analyses of the euploid (Hui et al. 2012a), trisomy 21, and trisomy 18 AF transcriptomes (Hui et al. 2012b).

Genes of interest were identified by narrowing down the entire differentially regulated IPA gene list by requiring a p-value of <0.015 and a >five fold-change expression difference.

Dysregulated genes were further categorized based on organ specificity in order to narrow down the gene list to a reasonable number for subsequent manual curation. All genes that met these criteria and were included in the top five most common organ systems were compared with the known gene functions as described in the OMIM database and the typical clinical phenotypic features that have been reported in women with Turner syndrome.

Results

Differentially expressed genes

The GeneChip® Human Genome U133 Plus 2.0 arrays analyze expression levels of more than 38,500 well-characterized transcripts and UniGene clusters. (www.affymetrix.com). Comparison of Turner syndrome and euploid transcriptomes revealed 807 statistically-significantly differentially expressed probe sets, corresponding to 470 annotated genes. There were 272 up-regulated (Online Resource 1) and 198 down-regulated genes (Online Resource 2). Chi-square analysis revealed that there was no chromosome with a significantly higher concentration of differentially-expressed genes than expected (Online Resource 3). Sixteen (3.4%) of the 470 differentially regulated genes were on the X chromosome (Table 1); most of these genes function in general cellular activities such as transcription mediation and signal transduction. The immune system was highly represented in each of the methods utilized to analyze the data. Four of the dysregulated X-chromosome genes (*NKRF*, *ELF4*, *RBM3*, and *FRMPD4)* play a role in immune system mediation. The X-inactive specific transcript gene (*XIST)* was down-regulated and the Benjamini-Hochberg corrected p-value was highly significant ($p<0.0001$). The short stature homeobox (*SHOX*) gene was not differentially regulated ($p=0.99$) in our AF samples but the raw values were highly variable (Table 2).

In the comparison of differentially-regulated probe sets between Turner syndrome and trisomies 18 and 21 no genes were common to all three aneuploidies. Three genes were differentially expressed in both Turner syndrome and trisomy 21 (*ZNF2, SLC48A1, NOVA2*). One gene was differentially expressed in both Turner syndrome and trisomy 18 (*SLC35G1*).

Organ-specific differentially expressed genes

We identified 57 genes in the “Turner syndrome transcriptome” that are expressed in a specific tissue or organ system (Online Resource 4). The most highly represented systems were the hematologic/immune (24 genes) and neurologic (19 genes). The respiratory/tongue/skin systems had a relatively low percentage (7%) of tissue specific genes, but this is due to increased representation of the other organ systems in Turner syndrome. In the raw data, the Turner syndrome AF samples had similar numbers of tissue specific respiratory/tongue/skin genes as euploid (4 and 6 respectively). In addition, a comparison between Turner syndrome and euploid, trisomy 21, and trisomy 18 transcriptomes revealed an increased percentage of differentially-expressed genes in the hematologic/immune system (42.1%) compared with the other three transcriptomes (17.4–29.5%) (Table 3). The neurologic system was also more highly represented in the Turner syndrome (33.3%) and euploid (30.4%) datasets compared with the trisomy 21 (19.7%) and trisomy 18 (16.7%) datasets.

Pathways enriched for differentially expressed genes

Using IPA, the most enriched biological functions in the AF of Turner syndrome fetuses were identified. Results were divided by IPA into 77 different categories that had considerable overlap (Online Resource 5). We therefore condensed the categories into general organ systems and functionality. The top 13 general organ systems and functionality with p-values <0.01 are shown in Table 4. Cellular functions, such as cell growth, cell morphology, cell cycle, and cell death, had the most significant p-value ranges as well as the highest number of genes. The highly statistically significant hematologic/immune category included functions such as antigen presentation, humoral immune response, and immune cell trafficking. In the gastrointestinal/metabolic category, lipid metabolism was one of the most significant functions (p-value range of 9.56×10^{-4} to 3.10×10^{-2}).

Identification of genes of interest

Genes of interest from the IPA analysis, organized by p-values, most frequently represented organ systems, and fold change are listed in Table 5. The functions and pathways identified by IPA analysis in Table 5 are very similar to those identified by BioGPS in the Turner syndrome column of Table 3. Using OMIM descriptions of gene function, manual curation identified three additional genes (*NFATC3*, *IGFBP5*, and *LDLR)* of potential interest with regard to Turner syndrome pathogenesis.

Discussion

In this pilot study we present an analysis of the cell-free RNA transcripts in the AF of fetuses with Turner syndrome. Comparison of Turner syndrome and euploid gene expression profiles identified several novel genes that have not been previously known to be associated with this condition. This discovery-driven analysis allowed for an unbiased, genome-wide examination of fetal development, which may be helpful in elucidating mechanisms of disease in Turner syndrome. Comparison between the differentially expressed genes in the AF from fetuses with Turner syndrome and trisomies 21 and 18 revealed only a few common genes, suggesting a unique specific molecular phenotype for Turner syndrome.

The *XIST* gene is involved in the X-inactivation process and is expressed universally in all cells, thus it is the only true positive control. Decreased *XIST* expression in Turner syndrome samples was expected and was demonstrated, further corroborating the AF RNA gene expression technique. The *SHOX* gene, which is also on the X chromosome, was not significantly differentially expressed in our study set. It was measured by a single probe set on the microarray and was called “present” in both some of the Turner syndrome and the euploid samples. There are multiple potential explanations for the lack of statistical significance. Decreased expression of *SHOX* has historically been thought to be one cause of the short stature present in women with Turner syndrome (Hintz 2002). During fetal life, shorter fetal long bones have been described as early as the second trimester in Turner syndrome (FitzSimmons et al. 1994). It is possible that genes other than *SHOX* are involved in fetal bone growth. Also, differential expression of *SHOX* might not be reflected in AF RNA because osteogenic tissue may not contribute to nucleic acids found in AF, causing

low expression levels. Another explanation is that the *SHOX* gene coverage on the U133 Plus 2.0 array is insufficient as there is only one probe set for the *SHOX* gene and there may have been non-specific hybridization in the Turner syndrome samples.

Genes that were differentially expressed in AF of fetuses with Turner syndrome were distributed uniformly across the genome. In the past, a gene dosage effect was hypothesized to explain aneuploid phenotypes. However, as seen in our previous studies of AF of fetuses with trisomies 18 and 21, the changes in gene expression in this study occurred across all the chromosomes and were not confined to the aneuploid chromosome (Slonim et al. 2009; Koide et al. 2011). These combined results suggest genome-wide, rather than chromosome-specific dysregulation.

Our data also revealed that the hematologic/immune system is significantly dysregulated during fetal development. Since the fetus is a “foreign object” from the perspective of the maternal immune system, maternal-fetal immune tolerance is required to prevent immune rejection (Gobert et al 2012). However, the overexpression of immune system transcripts in the Turner syndrome transcriptome compared with the euploid, trisomies 21 and 18 transcriptomes, may be an early indication of autoimmune dysregulation that could be related to the later onset of autoimmune disease seen in women with Turner syndrome (Jørgensen et al. 2010; Bakalov et al. 2012). Our results suggest that prevention of autoimmune disease might be a potential target for *in utero* therapy of affected fetuses. We have previously utilized the Connectivity Map (http://www.broadinstitute.org/cmap/) to identify drugs approved by the Food and Drug Administration that reverse the abnormal transcriptomic effects observed in the AF of Down syndrome fetuses (Slonim et al. 2009). Such an approach could also be used in Turner syndrome.

Another phenotypic characteristic of Turner syndrome is aortic coarctation and hypertension. Our manual curation of upregulated genes identified *NFATC3*, which has been associated with perivascular tissue remodeling (Horsley and Pavlath 2012). *NFATC3* is activated due to intermittent hypoxia, which causes vascular remodeling by increasing arterial wall thickness (de Frutos et al. 2010; Bierer et al. 2011). The pathophysiology of coarctation of the aorta has been theorized to be due to vascular endothelial dysfunction (Kenny et al. 2011). Individuals with coarctation of the aorta are also at risk for hypertension, regardless of whether or not the coarctation is repaired (Clarkson et al. 1983). These data suggest that *NFATC3* dysregulation could play a role in the increased risk of coarctation and hypertension found in women with Turner syndrome.

The low-density lipoprotein receptor (*LDLR*) was also found to be up-regulated in AF from affected fetuses. A prior study revealed that women with Turner syndrome had significantly higher LDL levels than controls (Van et al. 2006). The cause of hyperlipidemia in individuals with Turner syndrome is not currently known, but the increased expression of *LDLR* may provide a clue to the dysregulation. Hyperlipidemia and atherosclerosis are well known to be associated with Turner syndrome (Ross et al. 1995; Elsheikh et al. 2002; Bondy 2007; Giordano et al. 2011; Gravholt et al. 1998). The risk of hypertension is increased threefold in individuals with Turner syndrome (Gravholt et al. 1998). Further work needs to be done to investigate the link between *LDLR* expression and Turner syndrome.

The common association between short stature and women with Turner syndrome and the over-expression of *IGFBP5* in the Turner syndrome transcriptome imply that an impaired osteoblast/osteoclast relationship could play a role in the pathophysiology of this condition. Over-expression of *IGFBP5* increases osteoclast formation, associated with increased absorption (Kanatani et al. 2000), impairs osteoblast function (Devlin et al. 2002), and increases cellular senescence (Kim et al. 2007; Kojima et al. 2012). Transgenic mice that overexpress *IGFBP5* have inhibition of growth, increased neonatal mortality, and reduced female fertility (Salih et al. 2004). These data suggest that *IGFBP5* is an interesting gene to further explore in relation to the Turner syndrome phenotype.

Conclusions

Unlike other aneuploidies, such as trisomies 13 or 18, life expectancy and intelligence are generally normal in individuals with Turner syndrome. This makes it an important aneuploidy to study, because awareness and treatment of gene expression abnormalities could potentially reduce some of the morbidities associated with this condition. Here we utilized a discovery-driven approach that identified several differentially expressed genes and pathways that could contribute to the pathophysiology of Turner syndrome, even as early as the second trimester. Further investigation of these genes and pathways may lead to novel approaches to antenatal and postnatal treatment for fetuses and individuals diagnosed with Turner syndrome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding for this study was provided by the Eunice Kennedy Shriver National Institute of Child Health and Human Development [R01 HD42053-10 to DWB and R01 HD058880-04 to DKS].

References

Bakalov VK, Gutin L, Cheng CM, Zhou J, Sheth P, Shah K, Arepalli S, Vanderhoof V, Nelson LM, Bondy CA. Autoimmune disorders in women with Turner syndrome and women with karyotypically normal primary ovarian insufficiency. J Autoimmun. 2012; 38:315–321. [PubMed: 22342295]

Bianchi, D.; Crombleholme, T.; D'Alton, M.; Malone, F. 45, X (Turner syndrome). In: Fried, A.; Davis, K., editors. Fetology: Diagnosis and management of the fetal patient. 2. McGraw-Hill Companies; 2010.

Bierer R, Nitta CH, Friedman J, Codianni S, de Frutos S, Dominguez-Bautista JA, Howard TA, Resta TC, Gonzalez Bosc LV. NFATc3 is required for chronic hypoxia-induced pulmonary hypertension in adult and neonatal mice. Am J Physiol Lung Cell Mol Physiol. 2011; 301:L872–L880. [PubMed: 21908592]

Bondy CA. Turner syndrome study group: care of girls and women with Turner syndrome: a guideline of the Turner syndrome study group. J Clin Endocrinol Metab. 2007; 92:10–25. [PubMed: 17047017]

Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, Stoeckert C, Aach J, Ansorge W, Ball CA, Causton HC, Gaasterland T, Glenisson P, Holstege FC, Kim IF, Markowitz V, Matese JC, Parkinson H, Robinson A, Sarkans U, Schulze-Kremer S, Stewart J, Taylor R, Vilo J, Vingron M.

Minimum information about a microarray experiment (MIAME)- toward standards for microarray data. Nat Genet. 2001; 29:365–371. [PubMed: 11726920]

Clarkson PM, Nicholson MR, Barratt-Boyes BG, Neutze JM, Whitlock RM. Results after repair of coarctation of the aorta beyond infancy: a 10 to 28 year follow-up with particular reference to late systemic hypertension. Am J Cardiol. 1983; 51:1481–1488. [PubMed: 6846181]

de Frutos S, Caldwell E, Nitta CH, Kanagy NL, Wang J, Wang W, Walker MK, Gonzalez Bosc LV. NFATc3 contributes to intermittent hypoxia-induced arterial remodeling in mice. Am J Physiol Heart Circ Physiol. 2010; 299:H356–H363. [PubMed: 20495147]

Devlin RD, Du Z, Buccilli V, Jorgetti V, Canalis E. Transgenic mice overexpressing insulin-like growth factor binding protein-5 display transiently decreased osteoblastic function and osteopenia. Endocrinology. 2002; 143:3955–3962. [PubMed: 12239107]

Dietz JA, Johnson KL, Massingham LJ, Schaper J, Horlitz M, Cowan J, Bianchi DW. Comparison of extraction techniques for amniotic fluid supernatant demonstrates improved yield of cell-free fetal RNA. Prenat Diagn. 2011; 31:598–599. [PubMed: 21480301]

Elsheikh M, Dunger DB, Conway GS, Wass JA. Turner's syndrome in adulthood. Endocr Rev. 2002; 23:120–140. [PubMed: 11844747]

FitzSimmons J, Fantel A, Shepard TH. Growth parameters in mid-trimester fetal Turner syndrome. Early Hum Dev. 1994; 38:121–129. [PubMed: 7851305]

Giordano R, Forno D, Lanfranco F, Manieri C, Ghizzoni L, Ghigo E. Metabolic and cardiovascular outcomes in a group of adult patients with Turner's syndrome under hormonal replacement therapy. Eur J Endocrinol. 2011; 164:819–826. [PubMed: 21378088]

Gobert M, Lafaille JJ. Maternal-fetal immune tolerance, block by block. Cell. 2012; 150:7–9. [PubMed: 22770210]

Gravholt CH, Juul S, Naeraa RW, Hansen J. Morbidity in Turner syndrome. J Clin Epidemiol. 1998; 51:147–158. [PubMed: 9474075]

Gravholt CH. Epidemiological, endocrine, and metabolic features in Turner syndrome. Eur J Endocrinol. 2004; 151:657–687. [PubMed: 15588233]

Hall JG, Sybert VP, Williamson RA, Risher NL, Reed SD. Turner's syndrome. West J Med. 1982; 137:32–44. [PubMed: 6753342]

Hintz RL. SHOX mutations. Rev Endocr Metab Disord. 2002; 3:363–367. [PubMed: 12424438]

Horsley V, Pavlath GK. NFAT: ubiquitous regulator of cell differentiation and adaptation. J Cell Biol. 2002; 156:771–774. [PubMed: 11877454]

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. 2012a; 119:111–118. [PubMed: 22183218]

Hui L, Slonin DK, Wick HC, Johnson KL, Koide K, Bianchi DW. Novel neurodevelopmental information revealed in amniotic fluid supernatant transcripts from fetuses with trisomies 18 and 21. Hum Genet. 2012b; 131:1751–1759. [PubMed: 22752091]

Jørgensen KT, Rostgaard K, Bache I, Biggar RJ, Nielsen NM, Tommerup N, Frisch M. Autoimmune diseases in women with Turner's syndrome. Arthritis Rheum. 2010; 62:658–666. [PubMed: 20187158]

Kanatani M, Sugimoto T, Nishiyama K, Chihara K. Stimulatory effect of insulin-like growth factor binding protein-5 on mouse osteoclast formation and osteoclastic bone-resorbing activity. J Bone Miner Res. 2000; 15:902–910. [PubMed: 10804020]

Kenny D, Polson JW, Martin RP, Paton JF, Wolf AR. Hypertension and coarctation of the aorta: an inevitable consequence of developmental pathophysiology. Hypertens Res. 2011; 34:543–547. [PubMed: 21412243]

Kim KS, Seu YB, Baek SH, Kim MJ, Kim KJ, Kim JH, Kim JR. Induction of cellular senescence by insulin-like growth factor binding protein-5 through a p53-dependent mechanism. Mol Biol Cell. 2007; 18:4543–4552. [PubMed: 17804819]

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; 129:295–305. [PubMed: 21152935]

Kojima H, Kunimoto H, Inoue T, Nakajima K. The STAT3-IGFBP5 axis is critical for IL-6/gp130-induced premature senescence in human fibroblasts. Cell Cycle. 2012; 11:730–739. [PubMed: 22374671]

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; 293:836–842. [PubMed: 15713773]

Nielsen J, Wohlert M. Chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. Hum Genet. 1991; 87:81–83. [PubMed: 2037286]

Papp C, Beke A, Mezi G, Szigeti Z, Ban Z, Papp Z. Prenatal diagnosis of Turner syndrome; report on 69 cases. J Ultrasound Med. 2006; 25:711–717. [PubMed: 16731887]

Pavlidis P, Li Q, Noble WS. The effect of replication on gene expression microarray experiments. Bioinformatics. 2003; 19(13):1620–1627. [PubMed: 12967957]

Ross JL, Feuillan P, Long LM, Kowal K, Kushner H, Cutler GB Jr. Lipid abnormalities in Turner syndrome. J Pediatr. 1995; 126:242–245. [PubMed: 7844670]

Salih DA, Tripathi G, Holding C, Szestak TA, Gonzalez MI, Carter EJ, Cobb LJ, Eisemann JE, Pell JM. Insulin-like growth factor-binding protein 5 (Igfbp5) compromises survival, growth, muscle development, and fertility in mice. Proc Natl Acad Sci USA. 2004; 101:4314–4319. [PubMed: 15010534]

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; 106:9425–9429. [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; 101:6062–6067. [PubMed: 15075390]

Tyler C, Edman JC. Down syndrome, Turner syndrome, and Klinefelter syndrome: primary care throughout the life span. Prim Care. 2004; 31:627–648. [PubMed: 15331252]

Van PL, Bakalov VD, Bondy CA. Monosomy for the X-chromosome is associated with an atherogenic lipid profile. J Clin Endocrinol Metab. 2006; 91:2867–2870. [PubMed: 16705071]

Wu C, Orozco C, Boyer J, Leglise M, Goodale J, Batalov S, Hodge CL, Haase J, Janes J, Huss JW 3rd, Su AI. BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources. Genome Biol. 2009; 10:R130. [PubMed: 19919682]

Table 1

Differentially regulated Turner syndrome genes on the X chromosome

	Symbol	Fold Change	BH value	Entrez Gene Name
Up Regulated Genes				
1	*SEPT6*	5.951	0.0043	septin 6
2	*SLC6A8*	4.109	0.0014	solute carrier family 6 (neurotransmitter transporter, creatine), member 8
3	*NKRF**	2.647	0.0043	NFKB repressing factor
4	*ELF4**	2.596	<0.0001	E74-like factor 4 (ets domain transcription factor)
5	*RBM3**	2.132	0.0007	RNA binding motif (RNP1, RRM) protein 3
6	*GPKOW*	1.91	0.0034	G patch domain and KOW motifs
7	*FRMPD4**	1.857	0.0014	FERM and PDZ domain containing 4
8	*MAGEC2/MAGEC3*	1.703	<0.0001	melanoma antigen family C, 2
9	*CASK*	1.685	<0.0001	calcium/calmodulin-dependent serine protein kinase (MAGUK family)
10	*FAM3A*	1.384	0.0002	family with sequence similarity 3, member A
11	*TSR2*	1.139	0.0004	TSR2, 20S rRNA accumulation, homolog (S. cerevisiae)
Down Regulated Genes				
1	*XIST*	−5.897	<0.0001	X (inactive)-specific transcript (non-protein coding)
2	*MID1*	−3.752	<0.0001	midline 1 (Opitz/BBB syndrome)
3	*ZNF157*	−1.916	0.0004	zinc finger protein 157
4	*NXF2/NXF2B*	−1.694	0.0002	nuclear RNA export factor 2
5	*CAPN6*	−1.508	0.0004	calpain 6

*Indicates genes that are involved in immune system mediation

Table 2

Raw XIST and SHOX gene expression values

XIST[^]	
46, XX control samples	**Turner syndrome samples**
5.231	0.662
2.961	0.688
4.639	0.792
7.256	2.965
2.218	0.787

SHOX[#]	
46, XX control samples	**Turner syndrome samples**
74.056[*]	74.577[*]
5.545	0.664
14.663[*]	34.365[*]
7.655[*]	2.092
0.714	20.100[*]

[^] adjusted p-value 0.0000000267

[*] expression values called present

[#] adjusted p-value of 0.99

Table 3

Tissue-specificity comparison (by BioGPS) between euploid and aneuploid AF transcriptomes*

	Euploid		Turner Syndrome		Trisomy 21		Trisomy 18	
	Genes	**Percentage**	**Genes**	**Percentage**	**Genes**	**Percentage**	**Genes**	**Percentage**
Hematologic/Immune	4	17.4%	24	42.1%	18	29.5%	8	22.2%
Respiratory/Tongue/Skin	6	26.1%	4	7.0%	15	24.6%	12	33.3%
Neurologic	7	30.4%	19	33.3%	12	19.7%	6	16.7%
Gastrointestinal/Liver	2	8.7%	5	8.8%	7	11.5%	5	13.9%
Placenta	2	8.7%	0	0.0%	5	8.2%	2	5.6%
Reproductive/Endocrine	2	8.7%	3	5.3%	4	6.6%	3	8.3%
Cardiac	0	0.0%	1	1.8%	0	0.0%	0	0.0%
Fat	0	0.0%	1	1.8%	0	0.0%	0	0.0%

* number of Euploid samples =12, number of Turner samples =5, number of Trisomy 21 samples =7, number of Trisomy 18 samples = 5

Table 4

IPA Biological Functions

System	p-value range	Genes
Cellular function	0.00016 – 0.031	3264
Hematologic/Immune	0.00064 – 0.031	661
Gastrointestinal/Metabolism	0.00088 – 0.031	239
Dermatologic	0.00096 – 0.031	25
Cardiovascular	0.00131 – 0.031	91
Oncology	0.00162 – 0.031	99
Skeletal/Connective Tissue	0.00162 – 0.031	193
Neurologic	0.00281 – 0.014	188
Ophthalmology	0.00281 – 0.031	8
Renal/Urologic	0.00301 – 0.031	51
Reproductive	0.00810 – 0.031	61

Table 5

IPA genes of interest

Up Regulated		Down Regulated	
Gene	**Fold change**	**Gene**	**Fold change**
Cellular function			
MBD1	19.237	*LUZP1*	−7.835
DNAJB11	16.875	*WHSC1*	−6.289
HMG20B	12.089	*XIST*	−5.897
FEN1	8.71	*ATP8B3*	−5.503
USP10	8.404	*DDX10*	−5.441
ZNF2	8.165	*GOSR2*	−5.614
COPA	7.171	*KIAA0391*	−5.101
PCDHGC3	5.967		
ABCC1	5.952		
SEPT6	5.951		
ARNTL2	5.872		
N6AMT1	5.824		
LIG3	5.612		
CELF1	5.596		
BCL2L11	5.558		
MED9	5.535		
SLC25A1	5.517		
ZNF652	5.344		
BCLAF1	5.266		
ZBTB17	5.135		
Hematologic/Immune system			
NFATC3	17.232	*IFNAR1*	−8.258
CXADR	7.898	*PSG3*	−6.326
STAMBP	8.472	*PILRA*	−5.462
SDC2	5.252		
EHMT2	5.134		
GI/Metabolism			
GMDS	52.447	*PEX13*	−15.376
LDLR	8.152	*NDUFV1*	−7.83
PEX3	7.06	*GSK3B*	−6.158
PFKFB4	6.58		
Neurologic			
BAIAP2	7.453		
LMO3	5.854		
SYNJ1	5.217		
ALX4	5.127		

Up Regulated		Down Regulated	
Gene	**Fold change**	**Gene**	**Fold change**
Skeletal/Connective Tissue			
IGFBP5	16.783	*NFIX*	−6.026
SERPINH1	11.302		
XYLT2	7.035		