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Comprehensive investigation of DNA methylation and gene expression in trisomy 21 placenta

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ABSTRACT

Introduction: Trisomy 21 (T21) is the most common aneuploidy affecting humans and is caused by an extra copy of all or part of chromosome 21 (chr21). DNA methylation is an epigenetic event that plays an important role in human diseases via regulation of gene expression. However, the integrative association between DNA methylation and gene expression in T21 fetal placenta has yet to be determined.
Methods: We profiled expression of 207 genes on chr21 and their DNA methylation patterns in placenta samples from normal and DS fetuses using microarray analysis and predicted the functions of differentially expressed genes using bioinformatics tools.
Results: We found 47 genes with significantly increased expression in the T21 placenta compared to the normal placenta. Hypomethylation of the 47 genes was observed in the T21 placenta. Most of hypomethylated DNA positions were intragenic regions, i.e. regions inside a gene. Moreover, gene expression and hypomethylated DNA position showed significantly positive associations. By analyzing the properties of the gene-disease network, we found that increased genes in the T21 placenta were significantly associated with T21 and T21 complications such as mental retardation, neurobehavioral manifestations, and congenital abnormalities.
Discussion: To our knowledge, this is the first study to comprehensively survey the association between gene expression and DNA methylation in chr21 of the T21 fetal placenta. Our findings provide a broad overview of the relationships between gene expression and DNA methylation in the placentas of fetuses with T21 and could contribute to future research efforts concerning genes involvement in disease pathogenesis.

1. Introduction

Trisomy 21 (T21), also known as Down syndrome, is the most common chromosome abnormality in humans. It is a genetic disorder caused by the presence of all or part of a third copy of chromosome 21 (chr21). T21 is typically associated with physical growth delays, characteristic facial features, intellectual disability, mental retardation, cognitive impairment, congenital heart defects, childhood leukemia, immune defects, hypotonia, dementia, and early-onset Alzheimer's disease [1]. These complicated and varied phenotypes are generally thought to result from the abnormal dose of trisomic genes on chr21 [2]. Therefore, studies of T21 have focused on changes in expression of chr21 genes in tissues from subjects with T21 such as fibroblasts [3], whole blood [4], T cells [5], brain [6,7], and heart [3,7].

Epigenetics refers to change in gene expression that do not involve changes to the underlying DNA sequence; a change in phenotype without a change in genotype. Epigenetic change is a regular and natural occurrence, but can also be influenced by several factors including age, environment/lifestyle, and disease state. Therefore, new and ongoing research is continuously

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uncovering the role of epigenetics in a variety of human disorders. DNA methylation is one of the most commonly occurring epigenetic events. The DNA methylation on gene activity has been explained by two mechanisms. First is the "critical site" model by the methylation of specific cytosines in transcription-factor binding sites. It means that the methylation of specific cytosines reduces binding affinity of transcription-factor and thus inhibits the transcription of mRNA [8]. Second is the "methylation density" model. It suggests that the proportion of methylated cytosines across a region, rather than at any specific position, controls chromatin conformation and thus the transcriptional potential of the gene [8]. In general, DNA methylation of gene promoters is associated with transcriptional silencing, whereas methylation in gene bodies, i.e. intragenic DNA methylation, is associated with increased expression of gene. However, intragenic DNA methylation can be negatively or positively associated with gene expression according to the methylation dense of CpG islands (CGIs) of gene bodies [9]. Therefore, the complex regulating mechanism between the intragenic CGIs methylation and gene expression needs further investigation.

In humans, DNA methylation plays critical roles in transcriptional regulation, chromosomal stability, genomic imprinting, and X-inactivation [10]. Moreover, it has been reported to be involved in the occurrence and development of various diseases, such as cancer [11], cardiovascular disease [12], developmental delay/intellectual disability [13], fetal growth restriction [14], and T21 [15]. In recent, Jin et al. reported that a global hypermethylation in all genomic regions and all autosomes were observed in T21 placenta villi samples at the second trimester and suggested that global epigenetic changes may occur early in development and contribute to T21 phenotypes [16]. However, the comprehensive association between DNA methylation and gene expression of chr21 in the placenta of fetuses with T21 at the first trimester has yet to be determined, and the functions of these changes are also unclear. Therefore, an integrative investigation on DNA methylation and gene expression is important for understanding the molecular mechanisms involved in disease pathogenesis.

In this study, we performed an integrative analysis of DNA methylation and gene expression of chr21 in placentas of euploid and T21 fetuses using microarray technology and identified genes that were aberrantly expressed in placentas from fetuses with T21. In addition, we analyzed the association between gene expression and DNA methylation patterns and explored the biological function and molecular pathways of the identified genes using various bioinformatics tools.

2. Materials and methods

2.1. Study subjects

This study was conducted according to the principles expressed in the Declaration of Helsinki. Appropriate institutional review board approval for this study was obtained from the Ethics Committee at Cheil General Hospital (#CGH-IRB-2011-85). All patients provided written informed consent for the collection of samples and subsequent analysis. Pregnant women with normal and T21 fetuses who attended the Department of Obstetrics and Gynecology, Cheil General Hospital, Korea were recruited between March 2011 and December 2013. All placenta samples were obtained by chorionic villus samples (CVS) in the first trimester and stored in liquid nitrogen until analysis.

2.2. Cytogenetic analysis for fetal karyotype

Chromosomal analyses of fetal CVS were carried out using standard protocols as in our previous study [17]. Cells were cultured in the AmnioMAX-C100 culture medium (Invitrogen, Carlsbad, CA, USA). Metaphase chromosomes were stained using the GTG banding method, and 20 metaphases per sample were analyzed. All T21 samples used in this study were complete T21 and all normal samples were complete euploid.

2.3. Gene expression profiling using microarray

2.3.1. Array hybridizations

Total RNA was extracted from the placentas using the TRI Reagent (Molecular Research Center, Inc., Cincinnati, OH, USA) according to the manufacturer's instructions and purified using the RNeasy MinElute Cleanup Kit (QIAGEN, Hilden, Germany) as recommended by the manufacturers. Quantity and quality measurements were carried out using a NanoVue™ Plus Spectrophotometer (GE, London, UK) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). An RNA integrity number $\geq$7.0 was considered acceptable for the microarray analysis. Gene expression profiles were determined using Affymetrix GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix Inc., Santa Clara, CA, USA). The array was used to investigate expression of the 207 genes on chr21. For each gene, eleven pairs of oligonucleotide probes were synthesized in situ on the arrays. GeneChips were washed and stained using the Affymetrix Fluidics Station 450 (Affymetrix Inc.) and scanned using the Affymetrix GeneChip Scanner 3000 7G (Affymetrix Inc.).

2.3.2. Gene expression analysis

Expression data were extracted from the scanned images using Expression Console 1.3.1 software (Affymetrix Inc.) and analyzed with robust multichip analysis using Affymetrix default analysis settings and global scaling as the normalization method. The trimmed mean target intensity of each array was arbitrarily set to 100. The normalized, and log transformed intensity values were then analyzed using GeneSpring GX 12.6.1 (Agilent Technologies). The expression levels of genes were considered statistically significant between the T21 and normal groups for P values less than 0.05 using student t-test; the P values were corrected using the Benjamini and Hochberg false discovery rate (FDR) method to control false positive results from multiple testing [18]. Significant candidate genes (FDR < 0.05 with 1.5-fold or 0.5-fold expression change) were selected for association analysis with DNA methylated regions.

2.4. Array-based DNA-methylation analysis of chr21

2.4.1. Methylated DNA tiling array hybridizations

Each DNA sample was extracted from euploid placenta samples and T21 placenta samples and subjected to the methylated-CpG binding domain (MBD) and oligonucleotide tiling array. Briefly, the subsequent product was amplified and labeled using a Genomic DNA Enzymatic Labeling Kit (Agilent Technologies, Wilmington, DE, USA) and hybridized to a customized chip specific for chr21. The array platforms were composed of 0.4 million 50 to 60-mer oligonucleotides covering total chr21 at a median probe density of 1 per 60 bp. Microarray protocols, including labeling, hybridization and post-hybridization washing procedures, were performed as in our previous study [19].

2.4.2. Methylated region analysis

The two signal values were normalized using background subtraction. Signal ratio (MBD/input), signal log ratio [$\log_2$(MBD/input)], P[X], and P were obtained using Agilent Genomic Workbench software (Agilent Technologies). The $\log_2$ value is the