Integrated analysis of DNA methylation and RNA-sequencing data in Down syndrome

JIANTAO ZHANG[1], WENLI ZHOU[2], YING LIU[2] and NAN LI[2]

Departments of [1]Colorectal Anal Surgery and [2]Neonatology, The First Hospital of Jilin University, Changchun, Jilin 130021, P.R. China

Received August 28, 2015; Accepted August 30, 2016

DOI: 10.3892/mmr.2016.5778

Abstract. Down syndrome (DS) is the most common birth defect in children. To investigate the mechanisms of DS, the present study analyzed the bisulfite-sequencing (seq) data GSE42144, which was downloaded from the Gene Expression Omnibus. GSE42144 included DNA methylation data of three DS samples and three control samples, and RNA-seq data of two DS samples and five control samples. The methylated sites in the bisulfite-seq data were detected using Bismark and Bowtie2. The BiSeq tool was applied to determine differentially methylated regions and to identify adjacent genes. Using the Database for Annotation, Visualization and Integrated Discovery, the functions of the abnormal demethylated genes were predicted by functional enrichment analyses. Differentially expressed genes (DEGs) were then screened using a paired *t*-test. Furthermore, the interactions of the proteins encoded by selected genes were determined using the Search Tool for the Retrieval of Interacting Genes, and a protein-protein interaction (PPI) network was constructed using Cytoscape. A total of 74 CpG regions showed significant differential DNA methylation between the DS and normal samples. There were five abnormal demethylated DNA regions in chromosome 21. In the DS samples, a total of 43 adjacent genes were identified with demethylation in their promoter regions and one adjacent gene was identified with upregulated methylation in its promoter regions. In addition, 584 upregulated genes were identified, including 24 genes with transcriptional regulatory function. In particular, upregulated Runt-related transcription factor 1 (*RUNX1*) was located on chromosome 21. Functional enrichment analysis indicated that inhibitor of DNA binding 4 (*ID4*) was involved in neuronal differentiation and transcriptional suppression. In the PPI network, genes may be involved in DS by interacting with others, including nuclear receptor subfamily 4 group A member 2 (NR4A2)-early growth response (EGR)2 and NR4A2-EGR3. Therefore, *RUNX1*, *NR4A2*, *EGR2*, *EGR3* and *ID4* may be key genes associated with the pathogenesis of DS.

Correspondence to: Ms. Wenli Zhou, Department of Neonatology, The First Hospital of Jilin University, 71 Xinmin Street, Changchun, Jilin 130021, P.R. China
E-mail: zhouwenli48@hotmail.com

Key words: down syndrome, differentially methylated region, adjacent gene, differentially expressed genes, protein-protein interaction network

Introduction

Down syndrome (DS) or Down's syndrome, also known as trisomy 21, is an autosomal abnormality induced by an extra copy of chromosome 21 and is the most common birth defect among children worldwide (1,2). Children with DS usually have severe mental retardation (3) and delayed development (4), and are prone to gastrointestinal malformations (5). In children, almost 50% of DS cases are accompanied with congenital heart disease (6), and the risk of developing acute leukemia is 20 times higher than that of the normal population globally (7). In addition, patients with DS generally have shorter life expectancy (8,9).

DS is the most common cause of mental retardation and malformation in newborns. During meiosis, chromosome 21 in the egg does not separate, therefore, an extra copy of chromosome 21 is produced (10). When the sperm and the egg fuse, the embryo has 47 chromosomes, with three copies of chromosome 21 (11). An extra chromosome 21 leads to the overexpression of its genes, causing nerve dysfunction *in vivo*, and affecting the normal growth and development of children (12). At present, prenatal diagnosis is the optimal approach in preventing DS, however, there are no effective drugs for treatment of the disease. Thus, it is important to investigate the molecular mechanisms of DS.

Previous studies have suggested that the elevated gene expression of human chromosome 21 (*HSA21*) is responsible for specific aspects of the DS phenotype. Arron *et al* (13) showed that certain characteristics of the DS phenotype can be associated with the increased expressions of two *HSA21* genes, namely those encoding the transcriptional activator, regulator of calcineurin 1 (DSCR1-RCAN1), and the protein kinase, dual-specificity tyrosine phosphorylation-regulated kinase (DYRK)1A. The overexpression of a number of *HSA21* genes, including *DYRK1a*, synaptogenin 1 and single-minded homolog 2, results in learning and memory defects in mouse models, suggesting that trisomy of these

genes may contribute to learning disability in patients with DS (14,15).

The abnormal copy number of chromosome 21 is the primary genetic characteristics of DS. Therefore, the present study applied a variety of bioinformatics tools to determine the genetic fragments in chromosome 21. The methylated sites in bisulfite-sequencing (seq) data were detected, differentially methylated regions between DS and control samples were determined, and the adjacent genes of differential DNA methylation regions were identified. Subsequently, the functions of the abnormal demethylated genes were predicted using Gene Ontology (GO) enrichment analyses. The differentially expressed genes (DEGs) between the DS and control samples were screened. Furthermore, the interactions/associations between the proteins encoded by selected genes were determined, and a protein-protein interaction (PPI) network was constructed. The present study aimed to identify the key genes involved in DS, and may be able to establish the theoretical foundation for the targeted therapy of DS.

Materials and methods

Data sources. The bisulfite-seq data GSE42144 deposited by Jin *et al* (16) was downloaded from the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) of the National Center for Biotechnology Information, which was based on the platform of the Illumina Genome Analyzer IIx (Illumina, Inc., San Diego, CA, USA). GSE42144 included three placental samples (GSM1032059, GSM1032060 and GSM1032061) from patients with DS and three normal control samples (GSM1032070, GSM1032071 and GSM1032072). According to the quality control results of RNA-seq data, two RNA-seq data from patients with DS (GSM1033476 and GSM1033478) and five RNA-seq data from normal control samples (GSM1033470, GSM1033471, GSM1033472, GSM1033473 and GSM1033474) were also used in the present study.

Alignment of bisulfite-seq data and detection of DNA methylation. For all bisulfite-seq data, Bismark (www.bioinformatics.bbsrc.ac.uk/projects/bismark/) (17) and Bowtie2(bowtie-bio.sourceforge.net/bowtie2/index.shtml)(18) software were applied to perform read alignment, analyze methylated DNA signaling and output cytosine methylation sites in the genome. All parameters were set at default values.

Differential DNA methylation and corresponding adjacent gene analysis. The BiSeq tool (19) was used to determine differentially methylated regions between the placenta samples of patients with DS and normal control samples. The false discovery rate of each significant differentially methylated CpG cluster was ≤0.1. The methylated CpG clusters with a length of <100 bp were merged and defined as a differentially methylated DNA region. In addition, the length of each differentially methylated DNA region was required to be ≥50 bp. If the distance between the center of the differentially methylated DNA region and the transcription start site of a specific gene ranged between 3,000 and 500 bp, the differentially methylated DNA region was considered to have the potential to affect the gene, and this gene was defined as an adjacent gene of the differentially methylated DNA region.

Alignment of RNA-seq data and calculation of gene expression. Tophat (4) software was used to perform read alignment, with the University of California Santa Cruz (genome.ucsc.edu) hg19 genome sequences as a reference. For read alignment, up to two base mismatches were permitted in one read. Only the reads which mapped to specific genome locations were retained for further analysis. The other parameters were set to the defaults. On combining with the Refseq gene annotations, the transcripts were assembled and gene expression values were calculated using Cufflinks and Cuffdiff tools (5). The calculated gene expression values were based on the fragments per kilobase of transcript per million fragments mapped method (20).

Analysis of DEGs. The paired *t*-test (21) was used to identify DEGs between the DS and control samples. $P<0.01$ and $|\log_2\text{fold-change}|\geq 2$ were used as the cut-off criteria.

Function annotation of the adjacent genes. The Database for Annotation, Visualization, and Integrated Discovery (22) was used to perform GO enrichment analysis for the adjacent genes of the differentially methylated DNA regions. The GO terms were classified into biological process, molecular function and cellular component categories. $P<0.05$ was used as the cut-off criterion.

PPI network construction. The interaction associations of the proteins encoded by selected genes were determined using the Search Tool for the Retrieval of Interacting Genes (STRING) database (23). All parameters were set to defaults. A PPI network was then constructed using Cytoscape (24).

Results

Identification of differential DNA methylation regions in DS. Based on the Bisulfite-seq data, a total of 74 CpG regions had significant differential DNA methylation between the DS and normal samples, including 68 demethylated regions, accounting for 92%, and six regions with higher levels of methylation, compared with those of the normal samples.

For a single chromosome, the majority of the abnormal DNA methylation regions were detected in chromosomes 7 and 17, showing a total of seven aberrantly methylated DNA regions. In chromosome 21, five abnormal demethylated DNA regions were found (Fig. 1).

Identification of adjacent genes of the differentially methylated DNA. Compared with the control samples, a total of 43 adjacent (protein-coding) genes were identified in the DS samples with demethylated promoter regions and one adjacent gene, chromosome 19 open reading frame 80, which is known to be located in chromosome 19, was identified with upregulated methylation in its promoter region (Table I).

In the autosomal chromosomes, there were six DS-associated genes with demethylated promoter regions in chromosome 17. The number of abnormal genes in other chromosomes ranged between one and three. No genes were found to be affected by abnormal DNA demethylation in the sex chromosomes.

The distributions of the genes on chromosomes are listed in Table II. Among these, only Runt-related transcription

Table I. Number of adjacent genes with differentially methylated DNA regions in Down syndrome samples.

Methylation	Protein-coding genes (n)	Noncoding genes (n)
Hypomethylated	43	0
Hypermethylated	1	0

Table II. Distribution of adjacent genes with demethylated regions in DS sample chromosomes.

Chromosome	n	Gene
1	2	LOC730144, EIF1
2	3	ATG4B, LRRFIP1, STRADB
3	1	EXOG
4	1	FRYL
5	3	BOD1, B4GALT7, BRD9
6	2	MAP3K4, ID4
7	4	PAXIP1, POLM, FAM131B, AGAP3
8	2	ATAD2, REEP4
9	2	BARX1, KIAA1539
10	2	OTUD1, GPRIN2
11	1	SLC1A2
12	1	ALDH2
13	3	HMGB1, HMGB1L10, FARP1
14	1	RTN1
15	2	TYRO3, PCSK6
16	1	FA2H
17	6	UTP18, LOC730144, C17ORF56, EIF1, TIMP2, MRM1
18	1	ABHD3
20	2	CBFA2T2, C20ORF20
21	1	RUNX1
22	3	HMGB1, OSBP2, HMGB1L10

factor 1 (*RUNX1)* was found to be located on chromosome 21, and the demethylation of the promoter region of this gene was significant (Table II).

Functional enrichment of abnormal demethylated genes. The present study subsequently analyzed the functions of the 43 abnormally demethylated genes. Combined with GO functional annotation, the five genes, high mobility group box (*HMGB)1*, *HMGB1L10*, inhibitor of DNA binding 4 (*ID4*), leucine-rich repeat flightless-interacting protein 1 and core-binding factor, Runt domain, α subunit 2; translocated to, 2 (*CBFA2T2*) were found to be involved in the biological process of negative regulation of transcription, whereas the three genes, BARX homeobox 1, DNA polymerase mu and *RUNX1*, were associated with immune system development. In addition, the present study found that solute carrier

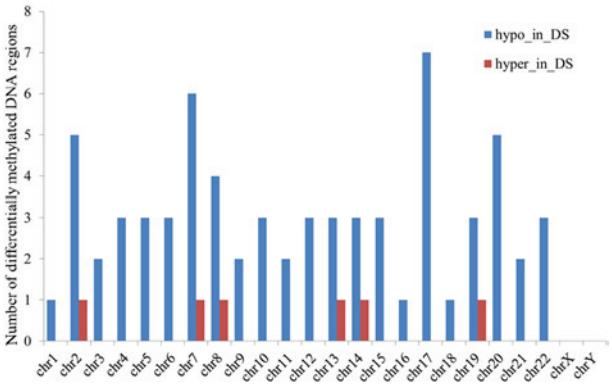

Figure 1. Distribution of differentially methylated DNA regions in each chromosome. Blue indicates hypomethylated DNA regions and red indicates hypermethylated DNA regions. DS, Down syndrome; chr, chomosome.

family 1 member 2, *ID4* and tissue inhibitor of matrix metalloproteinase 2 were predominantly involved in forebrain development and regulation of neurogenesis. The *HMGB1*, *HMGB1L10*, *ID4*, *RUNX1* and *CBFA2T2* genes possessed the capabilities of transcription factor binding, according to the molecular function terms (Table III).

Functional enrichment analysis showed that *ID4* was not only involved in neuronal differentiation, but also functioned in transcriptional suppression. The demethylation of its promoter region led to the increased expression level of *ID4* (Table III).

Analysis of DEGs. Combined with the RNA-seq data, the present study analyzed transcriptome differences between the DS samples and normal samples, and identified a total of 584 DEGs, including *RUNX1*, which were upregulated (Table IV).

Based on the detection of tissue-specific gene database, 208 of the 584 DEGs (36%) were found to have specific expression in brain tissue. By contrast, 52 DEGs in the DS samples were expressed specifically in neutrophils, the pituitary, peripheral nervous system, stomach and T-cells, which was substantially lower than the number of brain tissue-specific genes (Fig. 2).

Finally, with the addition of transcription factor data, the present study identified 24 DEGs with transcriptional regulatory function, of which the eight transcription factors, zinc finger protein 43, early growth response (*EGR)3*, nuclear receptor subfamily 4, group A, member 2 (*NR4A2*), nuclear receptor subfamily 3, group C, member 2 (*NR3C2*), LIM homeobox 2, gastrulation brain homeobox 2, pentraxin-related gene, rapidly induced by interleukin-1β and nuclear factor I/A, were brain-tissue specific (Table V).

Association between differential methylation and dysregulation. In order to examine the potential link between abnormal methylation and dysregulation in DS samples, the present study integrated their data combined with protein-protein interactions the STRING database, and found only one PPI network (Fig. 3). The network contained five genes, including NR4A2, EGR2, EGR3, RUNX1 and hepatocyte nuclear factor 4, γ (HNF4G). There were several interactions, including RUNX1-NR4A2, NR4A2-EGR2 and NR4A2-EGR3, in the PPI network.

Table III. Functional annotation of the adjacent genes with demethylated promoter regions in Down syndrome samples.

Category	GO term	Genes (n)	Gene
BP	GO:0016481~negative regulation of transcription	5	HMGB1, HMGB1L10, ID4, LRRFIP1, CBFA2T2
BP	GO:0002520~immune system development	3	BARX1, POLM, RUNX1
BP	GO:0032147~activation of protein kinase activity	2	MAP3K4, STRADB
BP	GO:0030855~epithelial cell differentiation	2	BARX1, CBFA2T2
BP	GO:0030900~forebrain development	2	SLC1A2, ID4
BP	GO:0050767~regulation of neurogenesis	2	ID4, TIMP2
CC	GO:0005694~chromosome	4	HMGB1, BOD1, BARX1, HMGB1L10
CC	GO:0019898~extrinsic to membrane	3	OSBP2, PCSK6, FARP1
MF	GO:0008134~transcription factor binding	5	HMGB1, HMGB1L10, ID4, RUNX1, CBFA2T2
MF	GO:0016564~transcription repressor activity	3	ID4, LRRFIP1, CBFA2T2
MF	GO:0030528~transcription regulator activity	6	BARX1, ATAD2, ID4, LRRFIP1, RUNX1, CBFA2T2

GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function.

Table IV. Number of differentially expressed genes in Down syndrome samples, compared with control samples.

Expression	Genes (n)	Transcription factors (n)
Upregulated	584	24
Downregulated	0	0

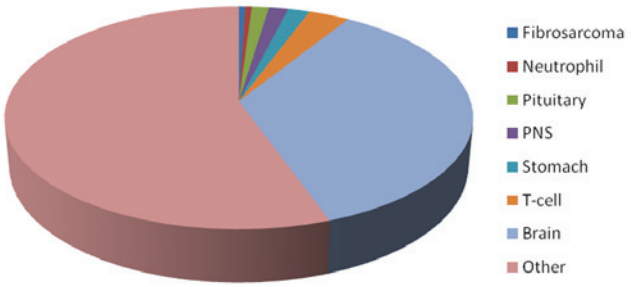

Figure 2. Distribution of tissue-specific differentially expressed genes in Down syndrome samples.

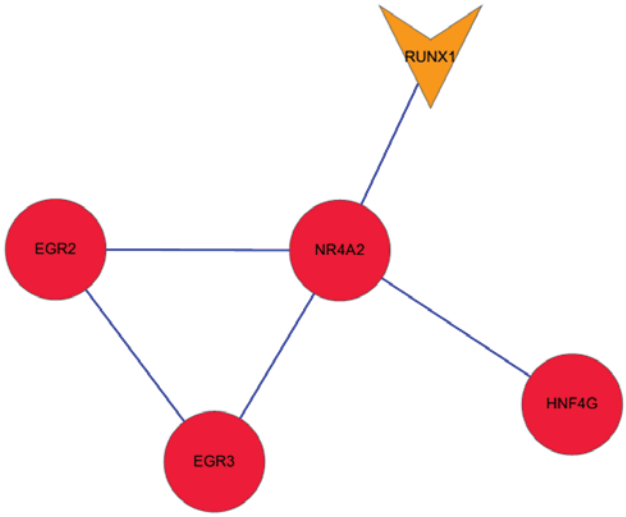

Figure 3. Motif analyses between the genes with demethylated DNA in promoter regions and DEGs in DS samples. Red nodes represent significantly upregulated genes and the orange node represents a moderately upregulated gene. The V-node represents the gene in which the promoter region was significantly demethylated, and circle nodes indicate the genes without significantly altered methylation. The blue lines indicate protein-protein interactions. RUNX1, Runt-related transcription factor 1; NR4A2 nuclear receptor subfamily 4 group A member 2; EGR, early growth response; HNF4G hepatocyte nuclear factor 4, γ.

Discussion

As a genetic disease in which an individual has 47 chromosomes instead of the usual 46 (25), DS affects ~1/730 live births and occurs in all populations equally (26). In the present study, bioinformatics tools were used to determine the genetic fragments associated with DS. A total of 74 CpG regions had significant differential DNA methylation between the DS and normal samples. There were five abnormal DNA demethylated regions in chromosome 21. A total of 43 adjacent genes with demethylation in promoter regions and one adjacent gene with upregulated methylation in promoter regions were identified in the DS samples. In addition, 584 upregulated genes were identified, including 24 genes with transcriptional regulatory function. Only NR4A2, EGR2, EGR3, RUNX1 and HNF4G were involved in the PPI network.

In the present study, upregulated *RUNX1* was located on chromosome 21, and the demethylation of the promoter region of this gene was significant. Functional enrichment analysis

Table V. Differentially expressed genes with transcriptional regulatory function and brain tissue specificity.

Transcription factor	Description	Brain-specific
ZNF43	Zinc finger protein 43	Yes
EGR3	Early growth response 3	Yes
EGR2	Early growth response 2	No
ZFY	Zinc finger protein, Y-linked	No
MITF	Microphthalmia-associated transcription factor	No
NR4A2	Nuclear receptor subfamily 4, group A, member 2	Yes
NR3C2	Nuclear receptor subfamily 3, group C, member 2	Yes
FOXN1	Forkhead box N1	No
HOXB13	Homeobox B13	No
VAX2	Ventral anterior homeobox 2	No
HNF4G	Hepatocyte nuclear factor 4, γ	No
SIX4	SIX homeobox 4	No
FOXP1	Forkhead box P1	No
HESX1	HESX homeobox 1	No
ERCC8	Excision repair cross-complementing rodent repair deficiency, complementation group 8	No
HOXA1	Homeobox A1	No
HOXB5	Homeobox B5	No
LHX2	LIM homeobox 2	Yes
PAX8	Paired box 8	No
GBX2	Gastrulation brain homeobox 2	Yes
LHX6	LIM homeobox 6	No
PTX3	Pentraxin-related gene, rapidly induced by IL-1β	Yes
NFIA	Nuclear factor I/A	Yes
NFIB	Nuclear factor I/B	No

showed that *RUNX1* was associated with immune system development and possessed the capabilities of transcription factor binding. It is reported that the expression of RUNX1 in megakaryoblasts in children with DS and acute megakaryocytic leukemia is lower, compared with cases of acute megakaryocytic leukemia without DS (27,28). The risk of developing dementia of Alzheimer's disease in individuals with DS is higher, compared with that of the general population, and a variant within *RUNX1* is closely linked with dementia of Alzheimer's disease in DS (29). A previous study reported that *RUNX1* and *NR4A2* can coordinately regulate the differentiation of T cells (30,31). The transcription factor, *NURR1*, which is also known as *NR4A2*, is important in the functional maintenance, development and survival of midbrain dopaminergic neurons (32). As with DS, Parkinson's disease is also a disorder of the central nervous system, and decreased expression levels of NURR1 may contribute to the identification of Parkinson's disease and other neurlogical disorders (33). The transcription factors, *EGR2* and *EGR3*, are members of the Egr family, which is involved in regulating the peripheral immune response, and *EGR2* may serve as a potential target in neuroinflammation therapy for its host defense role in the central nervous system immune response (34). According to the results of the present study, transcription factors *EGR3* and *NR4A2* were identified as brain-tissue specific. In the PPI network, several interactions were identified, including RUNX1-NR4A2, NR4A2-EGR2 and NR4A2-EGR3, indicating that *RUNX1* and *NR4A2* may be involved in DS by coordinately regulating *EGR2* and *EGR3*.

As a member of the ID family, *ID4* inhibits the differentiation or the DNA binding of basic helix-loop-helix transcription factors, regulating genes, which are important in neuronal differentiation (35). A previous study demonstrated that *ID1*, *ID2*, *ID3* and *ID4* are promising primary targets for methyl-CpG binding protein 2-regulated neuronal maturation, which may be responsible for the development of Rett syndrome, a neurodevelopmental disorder (36). In the present study, functional enrichment analysis indicated that *ID4* possessed the capabilities of transcription factor binding, and that *ID4* was involved in neuronal differentiation and transcriptional suppression. Therefore, it was hypothesized that the upregulated expression level of *ID4* may be associated with the symptoms of severe mental retardation and stunting of the nervous system, which are observed in patients with DS.

In conclusion, the present study performed integrated bioinformatics analyses of DNA methylation and RNA-seq data to identify genes, which may be correlated with DS. A total of 43 adjacent genes with demethylation of promoter regions and one adjacent gene with upregulated methylation of its promoter region were identified in the DS samples. In addition, 584 upregulated genes were identified, which included 24 genes with transcriptional regulatory function.

RUNX1, *NR4A2*, *EGR2*, *EGR3* and *ID4* may be correlated with DS. However, their mechanisms of action in DS remain to be fully elucidated and further experimental validation is required.

References

1. Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, van den Boom D, Bombard AT, Deciu C, Grody WW, *et al*: DNA sequencing of maternal plasma to detect Down syndrome: An international clinical validation study. Genet Med 13: 913-920, 2011.
2. Palomaki GE, Deciu C, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, van den Boom D, Bombard AT, Grody WW, *et al*: DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: An international collaborative study. Genet Med 14: 296-305, 2012.
3. Khocht A, Yaskell T, Janal M, Turner BF, Rams TE, Haffajee AD and Socransky SS: Subgingival microbiota in adult Down syndrome periodontitis. J Periodontal Res 47: 500-507, 2012
4. Rosdi M, Kadir A, Sheikh RS, Hj Murat Z and Kamaruzaman N: The comparison of human body electromagnetic radiation between down syndrome and non down syndrome person for brain, chakra and energy field stability score analysis. In: 2012 IEEE Control and System Graduate Research Colloquium. Malaysia, 370-375, 2012.
5. Ward O: John Langdon Down: The man and the message. Downs Syndr Res Pract 6: 19-24, 1999.
6. Cronk C, Crocker AC, Pueschel SM, Shea AM, Zackai E, Pickens G and Reed RB: Growth charts for children with Down syndrome: 1 month to 18 years of age. Pediatrics 81: 102-110, 1988.
7. Myrelid A, Gustafsson J, Ollars B and Annerén G: Growth charts for Down's syndrome from birth to 18 years of age. Arch Dis Child 87: 97-103, 2002.
8. Lott IT: Neurological phenotypes for Down syndrome across the life span. Prog Brain Res 197: 101, 2012.
9. Roizen NJ and Patterson D: Down's syndrome. Lancet 361: 1281-1289, 2003.
10. Freeman SB, Bean LH, Allen EG, Tinker SW, Locke AE, Druschel C, Hobbs CA, Romitti PA, Royle MH, Torfs CP, *et al*: Ethnicity, sex, and the incidence of congenital heart defects: A report from the national down syndrome project. Genet Med 10: 173-180, 2008.
11. Hernandez D and Fisher EM: Down syndrome genetics: Unravelling a multifactorial disorder. Hum Mol Genet 5: 1411-1416, 1996.
12. Patterson D and Costa AC: Down syndrome and genetics-a case of linked histories. Nat Rev Genet 6: 137-147, 2005.
13. Arron JR, Winslow MM, Polleri A, Chang CP, Wu H, Gao X, Neilson JR, Chen L, Heit JJ, Kim SK, *et al*: NFAT dysregulation by increased dosage of DSCR1 and DYRK1A on chromosome 21. Nature 441: 595-600, 2006.
14. Altafaj X, Dierssen M, Baamonde C, Martí E, Visa J, Guimerà J, Oset M, González JR, Flórez J, Fillat C and Estivill X: Neurodevelopmental delay, motor abnormalities and cognitive deficits in transgenic mice overexpressing Dyrk1A (minibrain), a murine model of Down's syndrome. Hum Mol Genet 10: 1915-1923, 2001.
15. Voronov SV, Frere SG, Giovedi S, Pollina EA, Borel C, Zhang H, Schmidt C, Akeson EC, Wenk MR, Cimasoni L, *et al*: Synaptojanin 1-linked phosphoinositide dyshomeostasis and cognitive deficits in mouse models of Down's syndrome. Proc Natl Acad Sci USA 105: 9415-9420, 2008.
16. Jin S, Lee YK, Lim YC, Zheng Z, Lin XM, Ng DP, Holbrook JD, Law HY, Kwek KY, Yeo GS and Ding C: Global DNA hypermethylation in down syndrome placenta. PLoS Genet 9: e1003515, 2013.
17. Krueger F and Andrews SR: Bismark: A flexible aligner and methylation caller for Bisulfite-Seq applications. Bioinformatics 27: 1571-1572, 2011.
18. Langmead B, Trapnell C, Pop M and Salzberg SL: Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol 10: R25, 2009.
19. Hebestreit K, Dugas M and Klein HU: Detection of significantly differentially methylated regions in targeted bisulfite sequencing data. Bioinformatics 29: 1647-1653, 2013.
20. Mortazavi A, Williams BA, McCue K, Schaeffer L and Wold B: Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods 5: 621-628, 2008.
21. Hsu H and Lachenbruch PA: Paired *t* test. Wiley Encyclopedia of Clinical Trials, 1-3, 2008.
22. Huang DW, Sherman BT, Tan Q, Kir J, Liu D, Bryant D, Guo Y, Stephens R, Baseler MW, Lane HC and Lempicki RA: DAVID bioinformatics resources: Expanded annotation database and novel algorithms to better extract biology from large gene lists. Nucleic Acids Res 35 (Web Server issue): W169-W175, 2007.
23. Jensen LJ, Kuhn M, Stark M, Chaffron S, Creevey C, Muller J, Doerks T, Julien P, Roth A, Simonovic M, *et al*: STRING 8-a global view on proteins and their functional interactions in 630 organisms. Nucleic Acids Res 37 (Database issue): D412-D416, 2009.
24. Saito R, Smoot ME, Ono K, Ruscheinski J, Wang PL, Lotia S, Pico AR, Bader GD and Ideker T: A travel guide to Cytoscape plugins. Nat Methods 9: 1069-1076, 2012.
25. Jiang Y, Mullaney KA, Peterhoff CM, Che S, Schmidt SD, Boyer-Boiteau A, Ginsberg SD, Cataldo AM, Mathews PM and Nixon RA: Alzheimer's-related endosome dysfunction in Down syndrome is Abeta-independent but requires APP and is reversed by BACE-1 inhibition. Proc Natl Acad Sci USA 107: 1630-1635, 2010.
26. van Gameren-Oosterom H, Fekkes M, van Wouwe JP, Detmar SB, Oudesluys-Murphy AM and Verkerk PH: Problem behavior of individuals with Down syndrome in a nationwide cohort assessed in late adolescence. J Pediatr 163: 1396-1401, 2013.
27. Bourquin JP, Subramanian A, Langebrake C, Reinhardt D, Bernard O, Ballerini P, Baruchel A, Cavé H, Dastugue N, Hasle H, *et al*: Identification of distinct molecular phenotypes in acute megakaryoblastic leukemia by gene expression profiling. Proc Natl Acad Sci USA 103: 3339-3344, 2006.
28. Edwards H, Xie C, LaFiura KM, Dombkowski AA, Buck SA, Boerner JL, Taub JW, Matherly LH and Ge Y: RUNX1 regulates phosphoinositide 3-kinase/AKT pathway: Role in chemotherapy sensitivity in acute megakaryocytic leukemia. Blood 114: 2744-2752, 2009.
29. Patel A, Rees SD, Kelly MA, Bain SC, Barnett AH, Thalitaya D and Prasher VP: Association of variants within APOE, SORL1, RUNX1, BACE1 and ALDH18A1 with dementia in Alzheimer's disease in subjects with Down syndrome. Neurosci Lett 487: 144-148, 2011.
30. Sekiya T, Kashiwagi I, Inoue N, Morita R, Hori S, Waldmann H, Rudensky AY, Ichinose H, Metzger D, Chambon P and Yoshimura A: The nuclear orphan receptor Nr4a2 induces Foxp3 and regulates differentiation of CD4+ T cells. Nat Commun 2: 269, 2011.
31. Okada M, Hibino S, Someya K and Yoshmura A: Regulation of regulatory T cells: Epigenetics and plasticity. Adv Immunol 124: 249-273, 2014.
32. Jankovic J, Chen S and Le WD: The role of Nurr1 in the development of dopaminergic neurons and Parkinson's disease. Prog Neurobiol 77: 128-138, 2005.
33. Le W, Pan T, Huang M, Xu P, Xie W, Zhu W, Zhang X, Deng H and Jankovic J: Decreased NURR1 gene expression in patients with Parkinson's disease. J Neurol Sci 273: 29-33, 2008.
34. Yan Y, Tan X, Wu X, Shao B, Wu X, Cao J, Xu J, Jin W, Li L, Xu W, *et al*: Involvement of early growth response-2 (Egr-2) in lipopolysaccharide-induced neuroinflammation. J Mol Histol 44: 249-257, 2013.
35. Umetani N, Mori T, Koyanagi K, Shinozaki M, Kim J, Giuliano AE and Hoon DS: Aberrant hypermethylation of ID4 gene promoter region increases risk of lymph node metastasis in T1 breast cancer. Oncogene 24: 4721-4727, 2005.
36. Peddada S, Yasui DH and LaSalle JM: Inhibitors of differentiation (ID1, ID2, ID3 and ID4) genes are neuronal targets of MeCP2 that are elevated in Rett syndrome. Hum Mol Genet 15: 2003-2014, 2006.