Differential Expression of Inflammation-Related Genes in Children with Down Syndrome

Cláudia Regina Santos Silva, 1 Joice Matos Biselli-Périco, 1 Bruna Lancia Zampieri, 1 Wilson Araujo Silva Jr, 2,3,4 Jorge Estefano Santana de Souza, 5,6 Matheus Carvalho Bürger, 5 Eny Maria Goloni-Bertollo, 1 and Érika Cristina Pavarino 1

1 Unit of Research in Genetics and Molecular Biology (UPGEM), Department of Molecular Biology, Medical School of São José do Rio Preto (FAMERP), 15090-000 São José do Rio Preto, SP, Brazil
2 Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil
3 Center for Medical Genomics at HCFMRP/USP, Ribeirão Preto, SP, Brazil
4 Regional Blood of Ribeirão Preto at HCFMRP/USP, Ribeirão Preto, SP, Brazil
5 Institute of Bioinformatics and Biotechnology (2bio), Ribeirão Preto, SP, Brazil
6 Metrópole Digital Institute (IMD), UFRN, Natal, RN, Brazil

Correspondence should be addressed to Érika Cristina Pavarino; erikapavarino@gmail.com

Received 9 March 2016; Accepted 5 April 2016

Academic Editor: Ulrich Eisel

Copyright © 2016 Cláudia Regina Santos Silva et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Objective. The aim of the study was to investigate the expression patterns of a specific set of genes involved in the inflammation process in children with Down Syndrome (DS) and children without the syndrome (control group) to identify differences that may be related to the immune abnormalities observed in DS individuals. Method. RNA samples were obtained from peripheral blood, and gene expression was quantified using the TaqMan® Array Plate Human Inflammation Kit, which facilitated the investigation into 92 inflammation-related genes and four reference genes using real-time polymerase chain reaction (qPCR). Results. Twenty genes showed differential expression in children with DS; 12 were overexpressed (PLA2G2D, CACNA1D, ALOX12, VCAM1, ICAM1, PLCD1, ADRB1, HTR3A, PDE4C, CASPI, PLA2G5, and PLCB4), and eight were underexpressed (LTA4H, BDKRB1, ADRB2, CD40LG, ITGAM, TNFRSF1B, ITGB1, and TBXAS1). After statistically correcting for the false discovery rate, only the genes BDKRB1 and LTA4H showed differential expression, and both were underexpressed within the DS group. Conclusion. DS children showed differential expression of inflammation-related genes that were not located on chromosome 21 compared with children without DS. The BDKRB1 and LTA4H genes may differentiate the case and control groups based on the inflammatory response, which plays an important role in DS pathogenesis.

1. Introduction

Down Syndrome (DS), which is also referred to as trisomy of chromosome 21 (HSA21), is the most common human aneuploidy (1/660 births) [1], and it is characterized by intellectual deficiency [2], a variety of dysmorphic physical characteristics [3, 4] and a variable spectrum of clinical manifestations [5, 6], including immunodeficiency [7].

Infections, especially in the respiratory tract [8, 9] and autoimmune diseases, including diabetes mellitus [10], primary hypothyroidism [11], and celiac disease [12], occur more frequently in individuals with DS than in individuals without the syndrome [13]. In addition, infectious diseases are a significant cause of hospitalization and mortality in individuals with DS [14, 15].

The aetiology underlying the immunological deficiency has not been fully described. However, changes in the immune system, such as functional and morphological thymus abnormalities [16, 17]; changes in T-cell differentiation, maturation, and activation; and lymphopenia [18–20], suggest
a prematurely senescent phenotype in the immune systems of individuals with DS [21]. Such changes may contribute to increased susceptibility to infections and inflammatory processes.

Gene expression studies in individuals with DS have reported differential expression of genes involved in immunological processes, which may explain the immunodeficiency observed in these individuals [22–27]. In fact, the imbalance due to the extra copies of genes on chromosome 21 and the effects of these gene products of other disomic genes or on specific proteins activities may explain how trisomy 21 results in the DS phenotype [3, 28].

In this study, we analysed the expression patterns of 92 genes involved in immunological processes in children with DS and children without the syndrome (the control group) to identify differences that may be related to the immunological abnormalities observed in individuals with DS.

2. Materials and Methods

2.1. Samples and Groups. Six individuals with DS (four males and two females) with a mean age of 3.2 years (ranging from 2.1 to 6.6 years) were referred to the study by the Genetics Outpatient Service of the Hospital de Base (HB), São José do Rio Preto, SP, Brazil. Of these children, five had free trisomy of HSA21, and one had mosaicism (90% of cells were trisomic). The control group consisted of six children without DS (one male and five females) with a mean age of 5.9 years (ranging from 3.9 to 6.5 years of age) who were undergoing routine care at the Outpatient Pediatric Clinic of the HB. Inclusion criteria were children aged between two and six years for both groups. In the control group, patients were included if they had absence of diseases associated with clinical manifestations of DS. Exclusion criteria for both groups were the presence of clinical signs that suggest acute infection, including cold symptoms, cough, fever, and/or antibiotic use, up to ten days prior to the data collection date, and the absence of chronic infections (bronchitis, asthma, and recurring pneumonias). We performed a C reactive protein analysis using serum samples and a Cobas C 501 Analyzer (Roche) to confirm the absence of infections. All samples included within the study were negative.

2.2. Total RNA Isolation and Quantitative Real-Time PCR (qPCR). Peripheral blood samples (3 mL) were collected into a Tempus Blood RNA tube (Applied Biosystems®) containing 6 mL of RNA Stabilization Solution. Total RNA samples were isolated using the Tempus RNA Isolation Kit (Ambion®). The RNA samples were quantified; their purities (A260/280 nm) were measured using spectrophotometry (Picodrop™ 200, Thermo Scientific), and only samples with intact 18S and 28S fragments were used throughout the gene expression analyses.

Complementary DNA (cDNA) was synthesized using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Carlsbad, California, USA) in accordance with the manufacturer’s instructions. The reverse transcription reaction products (12.5 ng) were analysed in duplicate using the TaqMan Array Human Inflammation 96-well Plate (Applied Biosystems, Carlsbad, California, USA) (catalogue number 4414074) in accordance with the manufacturer’s protocol. The 96-plex gene card included 92 genes related to inflammation and four reference genes (Table 1). The reaction conditions were as follows: 95°C for 10 minutes followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute in a StepOne Plus thermocycler (Applied Biosystems, Carlsbad, California, USA). Raw values throughout the quantification cycle (Cq) were generated using the software StepOne version 2.3 (Applied Biosystems) after we manually adjusted the threshold for each gene analysed.

2.3. Statistical Analysis. Gender and age distributions between groups were compared using Fisher’s exact and Mann-Whitney tests, respectively, with the program GraphPad Prism version 5.01. p values less than 0.05 were considered significant.

Gene expression analyses were performed using the R statistical language [29] and free packages available from the Bioconductor repository [30]. The package HTqPCR [31] was used to preprocess the data and as an interface with other packages using the PCR data as input. A Cq value of 37 was used as the threshold for detectable gene expression because the qPCR data were normally distributed up to this value. The gene expression data were normalized using the quantile normalization method [32, 33]. A normal distribution of data was generated using the mean and standard deviation of the Cq values. Individual Cq values outside the interval set by “quantile” were considered “unreliable” and removed from the analysis.

The criterion for a valid statistical analysis of a given gene was the expression in at least 60% of the biological samples within each group. Differentially expressed genes were identified using linear models for microarray data (LIMMA) [34]. To reduce the risk of false positives, p values were adjusted for multiple testing using the Benjamini-Hochberg False Discovery Rate method [35] with \( \alpha = 0.05 \). Gene’s expression within the case group was expressed relatively to the control group expression (relative quantification, RQ).

2.4. Functional Analyses of Differentially Expressed Genes. The most highly represented biological processes from the differentially expressed genes in children with DS were identified using the software “The Database for Annotation, Visualization, and Integrated Discovery” (DAVID) version 6.7 [36, 37] and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The significant results (\( p < 0.05 \)) after correction for multiple tests are indicated [35].

3. Results

The ages (\( p = 0.093 \)) and genders (\( p = 0.080 \)) of the groups did not differ significantly, which indicates homogeneity between the groups.

3.1. Gene Expression Analyses. Of the 92 inflammation genes tested, 20 genes were differentially expressed between the
### Table 1: Graphical representation of the 96-plex gene card containing 92 genes related to inflammation and four reference genes.

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A | I8S | GAPDH | HPRT | GUSB | A2M | ADRB1 | ADRB2 | ALOX12 | ALOX5 | ANXA1 | ANXA3 | ANXA5 |
| B | LTB4R | MAPK4 | NR3C1 | HPGD | HRH1 | HRH2 | HTR3A | ICAM1 | IL1R1 | IL2RA | IL2RB | IL2RG |
| C | IL13 | ITGAL | ITGAM | ITGB1 | ITGB2 | KLK1 | KLK2 | KLKB1 | KNG1 | LTA4H | LTC4S | MC2R |
| D | NFKB1 | NOS2 | PDE4A | PDE4B | PDE4C | PDE4D | PLA2G1B | PLA2G2A | PLA2G5 | PLCB2 | PLCB3 | PLCB4 |
| E | PLCD1 | PLCG1 | PLCG2 | MAPK1 | MAPK3 | MAPK8 | PTAFR | PTGDR | PTGER2 | PTGER3 | PTGER3 | PTGER3 |
| F | PTGIS | PTGS1 | PTGS2 | TBXAS1 | TNF | TNFRSF1A | TNFRSF1B | VCAM1 | IL1R2 | PLA2G7 | PLA2G10 |    |
| G | PLA2G4C | IL1RL1 | HTR3B | TNFSF13B | CYSLTR1 | HRH3 | PLA2G2D | IL1RAPL2 | KLK14 | PLCE1 | KLK15 | LTB4R2 |

*Reference genes.*
children with DS and control children \((p < 0.05)\) before correcting for multiple tests. Twelve genes showed increased expression \((\text{PLA2G2D, CACAID, ALOXI2, VCAMI, ICAMI, PLCDI, ADRB1, HTR3A, PDE4C, CASPI, PLAG5, and PLCB4})\), and eight genes showed decreasedexpression in children with DS \((\text{LTA4H, BDKRB1, ADRB2, CD40LG, ITGAM, TNFRSF1B, ITGBI, and TBXAS1})\) (Table 2). After a statistical adjustment for multiple tests, only two genes showed significantly different expression levels, Bradykinin receptor B1 \((\text{BDKRB1})\) and Leukotriene A4 hydrolase \((\text{LTA4H})\); the significance value was \(p = 0.02\) for both genes, and both genes showed reduced expression in individuals with DS.

3.2. Functional Analyses of Differentially Expressed Genes.

We analysed the metabolic pathways of 20 differentially expressed genes in children with DS. The analysis using the KEGG database classified the genes into three pathways. The calcium signalling pathway ranked first and included six genes. The two other pathways, the cellular adhesion molecule (CAM) and arachidonic acid metabolism pathways, included five genes (Table 3). According to this tool, the \(\text{BDKRB1} \) and \(\text{LTA4H} \) genes that showed statistically significant differential expression after correction for multiple tests are involved in calcium signalling pathways, as well as in the arachidonic acid metabolism.

### Table 2: Significantly different gene expression between children with and without DS.

| Gene      | Gene ID | Gene name                                      | Mean RQ | RQ log 2 | p value |
|-----------|---------|-----------------------------------------------|---------|----------|---------|
| BDKRB1   | 623     | Bradykinin receptor B1                        | 0.0013  | −9.6225  | 0.0003  |
| LTA4H    | 4048    | Leukotriene A4 hydrolase                      | 0.0079  | −6.9899  | 0.0005  |
| ITGAM    | 3684    | Integrin, alpha-M (complement component 3 receptor 3 subunit) | 0.0364  | −4.7811  | 0.0144  |
| ADRB2    | 154     | Adrenoceptor beta 2, surface                  | 0.0543  | −2.4037  | 0.0089  |
| CD40LG   | 959     | CD40 ligand                                   | 0.0737  | −3.7613  | 0.0151  |
| ITGBI    | 3688    | Integrin, beta 1                              | 0.0779  | −3.6822  | 0.0399  |
| TNFRSF1B | 7133    | Tumor necrosis factor receptor superfamily, member IB | 0.0829  | −3.5917  | 0.0258  |
| TBXAS1   | 6916    | Thromboxane A synthase 1 (platelet)           | 0.1032  | −3.2768  | 0.0428  |
| PLCB4    | 5332    | Phospholipase C, beta 4                       | 8.7676  | 3.1322   | 0.0443  |
| ICAMI    | 3383    | Intercellular adhesion molecule 1             | 9.7116  | 3.2797   | 0.0268  |
| HTR3A    | 3359    | 5-Hydroxytryptamine (serotonin) receptor 3A, ionotropic | 9.8319  | 3.2975   | 0.0343  |
| ALOX12   | 239     | Arachidonate 12-lipoxygenase                  | 10.6198 | 3.4087   | 0.0171  |
| PL2G5    | 5322    | Phospholipase A2, group V                     | 12.5688 | 3.6518   | 0.0423  |
| PDE4C    | 5143    | Phosphodiesterase 4C, cAMP-specific           | 19.2927 | 4.2700   | 0.0390  |
| PLCDI    | 5333    | Phospholipase C, delta 1                      | 19.9510 | 4.3184   | 0.0449  |
| PL2G2D   | 26279   | Phospholipase A2, group IID                   | 21.3914 | 4.4190   | 0.0067  |
| ADRB1    | 153     | Adrenoceptor beta 1                           | 24.2235 | 4.5983   | 0.0491  |
| CACNAID  | 776     | Calcium channel, voltage-dependent, L type, alpha 1D subunit | 25.3055 | 4.6614   | 0.0125  |
| CASPI    | 834     | Caspase 1, apoptosis-related cysteine peptidase | 47.1421 | 5.5589   | 0.0479  |
| VCAMI    | 7412    | Vascular cell adhesion molecule 1             | 52.8885 | 5.7249   | 0.0243  |

* Gene Identification second-base gene data, the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/gene).

### Table 3: Metabolic pathways associated with the target genes \((p < 0.05)\) in children with DS based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

| Metabolic pathways | Genes               | \(p value\) |
|--------------------|---------------------|-------------|
| Calcium signalling pathways | ADRB2, ADRB1, PLCB4, PLCDI, BDKRB1, and CACNAID | 0.0075 |
| Cell adhesion molecules (CAMs) | CD40LG, VCAMI, ICAMI, ITGBI, and ITGAM | 0.0179 |
| Arachidonic acid metabolism | TBXAS1, LTA4H, PL2G2D, PL2G5, and ALOX12 | 0.0019 |

*Corrected by the method Benjamini-Hochberg False Discovery Rate.

### 4. Discussion

Of the 20 differentially expressed genes in children with DS in this study, 12 showed increased expression \((\text{PLA2G2D, CACAID, ALOXI2, VCAMI, ICAMI, PLCDI, ADRB1, HTR3A, PDE4C, CASPI, PLAG5, and PLCB4})\), and eight showed decreased expression \((\text{LTA4H, BDKRB1, ADRB2, CD40LG, ITGAM, TNFRSF1B, ITGBI, and TBXAS1})\) (Table 2). After a statistical adjustment for multiple tests, only two genes showed significantly different expression levels, Bradykinin receptor B1 \((\text{BDKRB1})\) and Leukotriene A4 hydrolase \((\text{LTA4H})\); the significance value was \(p = 0.02\) for both genes, and both genes showed reduced expression in individuals with DS. None of the genes are involved in calcium signalling pathways, as well as in the arachidonic acid metabolism.
gene expression throughout the genome, not only for genes on chromosome 21 [4, 38]. The magnitude of these changes, whether large or small, is likely to be associated with the type of tissue analysed, and the different sensitivities of the various techniques used to analyse gene expression (SAGE, microarray, and qPCR) [39].

Functional analyses of the differentially expressed genes showed that genes BDKRB1 and LTA4H, which showed significantly lower expression in children with DS even after a statistical adjustment for multiple tests, are involved in the calcium signalling and arachidonic acid metabolism pathways, respectively. The gene BDKRB1 is a receptor that specifically binds bradykinin, which is a nine-amino acid peptide generated under pathophysiological conditions, such as inflammation [40]; its activation is involved in producing the proinflammatory cytokines IL-1β and IL-6 [41, 42], neutrophil migration, and activation of various cell pathways [43]. Studies have shown BDKRB1 activation and/or increased expression in several pathological states, including infection [44], allergy [45], arthritis [46], cancer [47], chronic pain and inflammation [48, 42], diabetes mellitus [49], and neurological disorders, such as epilepsy, stroke, multiple sclerosis [50], and Alzheimer’s disease [51]. In Alzheimer’s disease, which appears early in DS individuals [52], BDKRB1 activation likely contributes to neuroinflammation [53].

Individuals with DS exhibit a higher infection frequency, especially in the upper respiratory tract; autoimmune diseases, such as hypothyroidism, celiac disease, and diabetes mellitus; premature neuroinflammatory processes; and an increased mortality rate related to sepsis [54]. Therefore, we expected increased BDKRB1 expression in children with DS. However, the exclusion criteria were the absence of clinical signs of acute infection and absence of chronic infection. Thus, we suggest that individuals with DS may exhibit a lower basal expression level than individuals without the syndrome. In addition, for infections, even with an increase in the BDKRB1 gene expression, the cytokine production pathways, neutrophil migration, and activation levels for several cell-signalling pathways might be compromised. Furthermore, the mechanisms involved in the infection/inflammation processes may be less efficient, which may impair the clinical manifestation of the syndrome.

The gene LTA4H encodes a bifunctional zinc metalloprotein that converts leukotriene A4 (LTA4) into leukotriene B4 (LTB4) [55]. Leukotriene A4 also can yield leukotriene C4 (LTC4) by conjugation with reduced glutathione through the enzyme leukotriene C4 (LTC4) synthase. LTC4 is converted to leukotriene D4 (LTD4) that by sequential amino acid hydrolysis is converted into leukotriene E4 (LTE4) [56]. The leukotrienes (LTs) are powerful eicosanoid lipid mediators involved in acute and chronic inflammatory diseases, such as inflammatory bowel disease [57], chronic pulmonary neutrophilic inflammation [58], arthritis [59] asthma [60], and atherosclerosis [61].

Leukotrienes are the major products of arachidonic acid metabolism and are produced by inflammatory cells, including polymorphonuclear leukocytes, macrophages, and mast cells [56]. In particular, LTB4 is a powerful proinflammatory lipid mediator synthesized by immune cells and stimulates the production of several cytokines implicated in chronic inflammatory diseases and may play a role in recruiting inflammatory cells to the tissue lesion site [56]. Furthermore, LTB4 levels increases were associated with risk for pulmonary complications in multiply traumatized patients [62]. Thus, the altered LTA4H expression observed in this study suggests that individuals with DS may produce leukotrienes less efficiently, which impair the inflammatory response.

The CAM pathway is also worth discussing, which involves the genes CD40LG, ITGB1, ITGAM, VCAM1, and ICAM1. In this study, the gene CD40LG was expressed at lower levels in children with DS, confirming literature data such as the observations by Letourneau et al. [63] and Zampieri et al. [23] in fetal fibroblasts from monozygotic twins and in monocytes from peripheral blood taken from children with DS, respectively. The protein encoded by the CD40LG gene is a ligand for the CD40 receptor and is expressed on the surface of T-cells and regulates B-cell function. The interaction between CD40 and its ligand activates a proinflammatory signalling pathway [64]. Changes in the CD40/CD40LG signalling pathways lead to a type of congenital immunodeficiency characterized by low/absent IgG and IgA, normal circulating B lymphocytes, and increased IgM [64].

The genes ITGB1 and ITGAM also exhibited lower expression levels in children with DS in our study. The ITGB1 gene encodes a membrane receptor involved in cellular adhesion [65], and changes in its expression may impact the pathway that regulates cytoskeletal actin, which is involved in cell growth, survival, and motility [66]. Indeed, previous studies have shown that the number of total lymphocytes is lower in children with DS than in children without the syndrome [20]; in addition, lymphocyte maturation is impaired in these individuals [19]. The ITGAM gene encodes the integrin alpha-M chain; this domain contains integrin alpha and combines with the beta 2 chain (ITGβ2) to form an integrin specific to leukocytes referred to as macrophage-1 receptor (Mac-1) leukocytes. The alpha-M of integrin beta 2 is important for neutrophil and monocyte adhesion to the stimulated endothelium and for phagocytosis of complement-coated particles [67].

The genes VCAM1 and ICAM1 showed increased expression in children with DS in this study and are closely related both structurally and functionally; they also encode cell-surface glycoproteins that are activated by cytokines in endothelial cells. These glycoproteins are ligands for integrins that participate in leukocyte adhesion to the endothelium [68, 69] and play an important role in signal transduction, especially proinflammatory pathways, through promoting the recruitment of inflammatory immune cells, such as macrophages and granulocytes [70]. The signal transduction mechanisms involved in ligation with antigen receptors in B and T-cells require a set of highly coordinated interactions that involve transmembrane and cytosolic proteins. Studies suggest that T-cell signalling changes in response to mitogens may produce abnormal lymphocyte proliferation in individuals with DS [71].

The ICAM1 gene binds CD11 integrins, which are known as lymphocyte function-associated antigen 1 (LFA-1) [69],
a receptor found on leukocytes [72]. When activated by the ICAM-1/LFA-1 pathway, leukocytes bind to endothelial cells and travel through the tissues, which elicits an inflammatory response [73]. Due to its proinflammatory role, the ICAM1 protein has been observed at low concentrations across the membrane of leukocytes and endothelial cells under normal conditions [70].

Lin et al. [74] used a cell adhesion assay with recombinant ICAM-1 and observed increased LFA-1 expression in the lymphocytes of patients with DS as well as lower adhesion of these cells compared with patients without the syndrome. Changes in LFA-1 function may compromise lymphocyte activation and maturation [70]. These results suggest that more generalized pathological processes, such as premature senescence of the immune system or inefficient lymphocyte activation, and subsequent integrin dysfunction may underlie the immunological deficiencies observed in patients with DS.

In conclusion, in our study, we show that children with DS exhibit differential expression of genes related to inflammation that are not located on chromosome 21 compared with children without the syndrome. Altered expression of these genes, especially BDKRBI and LTA4H, may differentiate the case and control groups based on the inflammatory response, which plays an important role in DS pathogenesis.

Ethical Approval

An informed consent form was signed by the parents of the children included in the study, which was approved by the Research Ethics Committee of Medical School of São José do Rio Preto (Faculdade de Medicina de São José do Rio Preto, FAMERP), CAAE number 05843912.8.0000.5415.

Disclosure

Current address is Faculdade de Medicina de São José do Rio Preto (FAMERP), Departamento de Biologia Molecular, Avenida Brigadeiro Faria Lima 5416, 15090-000 São José do Rio Preto, SP, Brasil.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by funding from FAPESP (2010/00153-3). Cláudia Regina Santos Silva was the recipient of fellowships from CAPES. Érika Cristina Pavarino acknowledges support from CNPq for the Research Productivity Fellowship (308551/2014-1). The authors gratefully acknowledge the assistance of Alexandre Lins Werneck in English language.

References

[1] K. L. Jones, Smith Padrões Reconhecíveis de Malformações Congênitas, Elsevier, Rio de Janeiro, Brazil, 6th edition, 2007.

[2] A. Contestabile, F. Benfenati, and L. Gasparini, “Communication breaks-Down: from neurodevelopment defects to cognitive disabilities in Down syndrome,” Progress in Neurobiology, vol. 91, no. 1, pp. 1–22, 2010.

[3] S. E. Antonarakis, R. Lyle, R. Chrast, and H. S. Scott, “Differential gene expression studies to explore the molecular pathophysiology of Down syndrome,” Brain Research Reviews, vol. 36, no. 2-3, pp. 265–274, 2001.

[4] D. R. Fitzpatrick, “Transcriptional consequences of autosomal trisomy: primary gene dosage with complex downstream effects,” Trends in Genetics, vol. 21, no. 5, pp. 249–253, 2005.

[5] I. Ahmed, T. Ghafoor, N. A. Samore, and M. N. Chattha, “Down syndrome: clinical and cytogenetic analysis,” Journal of the College of Physicians and Surgeons Pakistan, vol. 15, no. 7, pp. 426–429, 2005.

[6] C. Y. Chou, L. Y. Liu, C. Y. Chen et al., “Gene expression variation increase in trisomy 21 tissues,” Mammalian Genome, vol. 19, no. 6, pp. 398–405, 2008.

[7] M. A. A. Kusters, R. H. J. Verstegen, E. F. A. Gemen, and E. de Vries, “Intrinsic defect of the immune system in children with Down syndrome: a review,” Clinical and Experimental Immunology, vol. 156, no. 2, pp. 189–193, 2009.

[8] B. L. P. Bloemers, C. J. M. Broers, L. Bont, M. E. Weijerman, R. J. B. J. Gemke, and A. M. van Furth, “Increased risk of respiratory tract infections in children with Down syndrome: the consequence of an altered immune system,” Microbes and Infection, vol. 12, no. 11, pp. 799–808, 2010.

[9] C. J. M. Broers, R. J. B. J. Gemke, M. E. Weijerman, D.-J. Kuik, I. M. W. van Hoogstraten, and A. M. van Furth, “Frequency of lower respiratory tract infections in relation to adaptive immunity in children with Down syndrome compared to their healthy siblings,” Acta Paediatrica, vol. 101, no. 8, pp. 862–867, 2012.

[10] K. M. Gillespie, R. J. Dix, A. J. K. Williams et al., “Islet autoimmunity in children with Down’s syndrome,” Diabetes, vol. 55, no. 11, pp. 3185–3188, 2006.

[11] I. B. Purdy, N. Singh, W. L. Brown, S. Vangala, and U. P. Devaskar, “Revisiting early hypothyroidism screening in infants with Down syndrome,” Journal of Perinatology, vol. 34, no. 12, pp. 936–940, 2014.

[12] O. Uibo, K. Teesalu, K. Metskül et al., “Screening for celiac disease in Down’s syndrome patients revealed cases of subtotal villous atrophy without typical for celiac disease HLA-DQ and tissue transglutaminase antibodies,” World Journal of Gastroenterology, vol. 12, no. 9, pp. 1430–1434, 2006.

[13] F. P. Pellegrini, M. Marinoni, V. Frangione et al., “Down syndrome, autoimmunity and T regulatory cells,” Clinical and Experimental Immunology, vol. 169, no. 3, pp. 238–243, 2012.

[14] A. Englund, B. Jonsson, C. S. Zander, J. Gustafsson, and G. Annerén, “Changes in mortality and causes of death in the Swedish Down syndrome population,” American Journal of Medical Genetics, Part A, vol. 161, no. 4, pp. 642–649, 2013.

[15] P. Fitzgerald, H. Leonard, T. J. Pikora, J. Bourke, and G. Hammond, “Hospital admissions in children with down syndrome: experience of a population-based cohort followed from birth,” PLoS ONE, vol. 8, no. 8, Article ID e70401, 2013.

[16] B. L. P. Bloemers, L. Bont, R. A. de Weger, S. A. Otto, J. A. Borghans, and K. Tesselaar, “Decreased thymic output accounts for decreased naive T cell numbers in children with Down syndrome,” Journal of Immunology, vol. 186, no. 7, pp. 4500–4507, 2011.
Mediators of Inflammation

[17] K. Karl, K.-S. Heling, A. Sarut Lopez, G. Thié, and R. Chaouki, "Thymic-thoracic ratio in fetuses with trisomy 21, 18 or 13," Ultrasound in Obstetrics & Gynecology, vol. 40, no. 4, pp. 412–417, 2012.

[18] E. Roat, N. Prada, E. Lugli et al., "Homeostatic cytokines and expansion of regulatory T cells accompany thymic impairment in children with Down syndrome," Rejuvenation Research, vol. 11, no. 3, pp. 573–583, 2008.

[19] L. Guazzarotti, D. Trabattoni, E. Castelletti et al., "T lymphocyte maturation is impaired in healthy young individuals carrying trisomy 21 (Down syndrome)," The American Journal on Intellectual and Developmental Disabilities, vol. 114, no. 2, pp. 100–109, 2005.

[20] E. F. A. Gemen, R. H. J. Verstegen, J. Leuvenink, and E. De Vries, "Increased circulating apoptotic lymphocytes in children with Down syndrome," Pediatric Blood & Cancer, vol. 59, no. 7, pp. 1300–1312, 2012.

[21] L. P. E. Lorenzo, K. E. Shatynski, S. Clark, P. J. Yarowsky, and M. S. Williams, "Defective thymic progenitor development and mature T-cell responses in a mouse model for Down syndrome," Immunology, vol. 139, no. 4, pp. 447–458, 2013.

[22] F. A. Lima, C. A. Moreira-Filho, P. L. Ramos et al., "Decreased AIRE expression and global thymic hypofunction in Down syndrome," The Journal of Immunology, vol. 187, no. 6, pp. 3422–3430, 2011.

[23] B. L. Zampieri, J. M. Biselli-Périco, J. E. S. de Souza et al., "Altered expression of immune-related genes in children with Down syndrome," PLoS ONE, vol. 9, no. 9, Article ID e70728, 2014.

[24] W. Malagó Jr., C. A. Sommer, C. Del Cistia Andrade et al., "Gene expression profile of human Down syndrome leucocytes," Croatian Medical Journal, vol. 46, no. 4, pp. 647–656, 2005.

[25] C. A. Sommer, E. C. Pavarino-Bertelli, E. M. Goloni-Bertollo, and F. Henrique-Silva, "Identification of disregulated genes in lymphocytes from children with Down syndrome," Genome, vol. 51, no. 1, pp. 19–29, 2008.

[26] G. Skogberg, V. Lundberg, S. Lindgren et al., "Altered expression of autoimmune regulator in infant down syndrome thymus, a possible contributor to an autoimmune phenotype," Journal of Immunology, vol. 193, no. 5, pp. 2187–2195, 2014.

[27] C. Li, L. Jin, Y. Bai et al., "Genome-wide expression analysis in Down syndrome: insight into immunodeficiency," PLoS ONE, vol. 7, no. 11, Article ID e49130, 2012.

[28] R. H. Reeves, L. L. Baxter, and J. T. Richtsmeier, "Too much of a good thing: mechanisms of gene action in Down syndrome," Trends in Genetics, vol. 17, no. 2, pp. 83–88, 2001.

[29] R. Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2013, http://www.R-project.org/.

[30] R. C. Gentleman, V. J. Carey, D. M. Bates et al., "Biocoductor: open software development for computational bio and bioinformatics," Genome Biology, vol. 5, no. 10, article R80, 2004.

[31] H. Dvinge and P. Bertone, "HTqPCR: high-throughput analysis and visualization of quantitative real-time PCR data in R," Bioinformatics, vol. 25, no. 24, pp. 3235–3236, 2009.

[32] B. M. Bolstad, R. A. Irizarry, M. Aastrand, and T. P. Speed, "A comparison of normalization methods for high density oligonucleotide array data based on variance and bias," Bioinformatics, vol. 19, no. 2, pp. 185–193, 2003.

[33] J. C. Mar, Y. Kimura, K. Schroder et al., "Data-driven normalization strategies for high-throughput quantitative RT-PCR," BMC Bioinformatics, vol. 10, article N10, 2009.

[34] G. K. Smyth, "Linear models and empirical Bayes methods for assessing differential expression in microarray experiments," Statistical Applications in Genetics and Molecular Biology, vol. 3, no. 1, pp. 1–25, 2004.

[35] Y. Benjamini and Y. Hochberg, "Controlling the false discovery rate: a practical and powerful approach to multiple testing," Journal of the Royal Statistical Society—Series B: Methodological, vol. 57, no. 1, pp. 289–300, 1995.

[36] D. W. Huang, B. T. Sherman, and R. A. Lempicki, "Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources," Nature Protocols, vol. 4, no. 1, pp. 44–57, 2009.

[37] D. W. Huang, B. T. Sherman, and R. A. Lempicki, "Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists," Nucleic Acids Research, vol. 37, no. 1, pp. 1–13, 2009.

[38] D. R. FitzPatrick, J. Ramsay, N. I. McGill, M. Shade, A. D. Carothers, and N. D. Haste, "Transcriptome analysis of human autosomal trisomy," Human Molecular Genetics, vol. 11, no. 26, pp. 3249–3256, 2002.

[39] R. Lyle, C. Gehrig, C. Neergaard-Henrichsen, S. Deutsch, and S. E. Antonarakis, "Gene expression from the aneuploid chromosome in a trisomy mouse model of Down syndrome," Genome Research, vol. 14, no. 7, pp. 1268–1274, 2004.

[40] The National Center for Biotechnology Information—NCBI, BDdRBI Bradykinin Receptor B1 (Homo Sapiens (Human)), NCBI, Bethesda, Md, USA, 2011, http://www.ncbi.nlm.nih.gov/ gene/623.

[41] S. Talbot and R. Couture, "Emerging role of microglial kinin B1 receptor in diabetic pain neuropathy," Experimental Neurology, vol. 234, no. 2, pp. 373–381, 2012.

[42] R. Couture, M. Harrison, R. M. Vianna, and F. Cloutier, "Kinin receptors in pain and inflammation," European Journal of Pharmacology, vol. 429, no. 1–3, pp. 161–176, 2001.

[43] R. Medeiros, G. F. Passos, C. E. Vitor et al., "Effect of two active compounds obtained from the essential oil of Cordia verbenacea on the acute inflammatory responses elicited by LPS in the rat paw," British Journal of Pharmacology, vol. 151, no. 5, pp. 618–627, 2007.

[44] A. G. Todorov, D. Andrade, J. B. Pesquero et al., "Trypanosoma cruzi induces edematogenic responses in mice and invades cardiomyocytes and endothelial cells in vitro by activating distinct kinin receptor (B1/B2) subtypes," The FASEB Journal, vol. 17, no. 1, pp. 73–75, 2003.

[45] S. C. Christiansen, J. Eddleston, K. M. Woessner et al., "Up-regulation of functional kinin B1 receptors in allergic airway inflammation," The Journal of Immunology, vol. 169, no. 4, pp. 2054–2060, 2002.

[46] H. C. Seegers, P. S. Avery, D. F. McWilliams, L. Haywood, and D. A. Walsh, "Combined effect of bradykinin B2 and neurokinin-1 receptor activation on endothelial cell proliferation in acute synovitis," The FASEB Journal, vol. 18, no. 6, pp. 762–764, 2004.

[47] L. Barki-Harrington, A. L. Bookout, G. Wang, M. E. Lamb, L. M. F. Leeb-Lundberg, and Y. Daaka, "Requirement for distinct kinin receptor (B1/B2) subtypes," The FASEB Journal, vol. 11, no. 26, pp. 1507–1509, 2003.

[48] J. B. Calixto, D. A. Cabrini, J. Ferreira, and M. M. Campos, "Defective thymic progenitor development and maturation is impaired in healthy young individuals carrying trisomy 21 (Down syndrome)," Frontiers in Pharmacology, vol. 3, no. 1, pp. 50–64, 2012.

[49] M. M. Campos, B. Ongali, H. De Souza Buck et al., "Expression and distribution of kinin B1 receptor in the rat brain and..."
alterations induced by diabetes in the model of streptozocin," *Synapse*, vol. 57, no. 1, pp. 29–37, 2005.

[50] D. Rodi, R. Couture, B. Ongali, and M. Simonato, “Targeting kinin receptors for the treatment of neurological diseases,” Current Pharmaceutical Design, vol. 11, no. 10, pp. 1313–1326, 2005.

[51] B. Lacoste, X.-K. Tong, K. Lahjouji, R. Couture, and E. Hamel, “Cognitive and cerebrovascular improvements following kinin B1 receptor blockade in Alzheimer’s disease mice,” *Journal of Neuroinflammation*, vol. 10, article 57, 2013.

[52] M. Wilcock and W. S. T. Griffin, “Down’s syndrome, neuroinflammation, and Alzheimer neuropathogenesis,” *Journal of Neuroinflammation*, vol. 10, article 84, 2013.

[53] T. A. Viel and H. S. Buck, “Kallikrein-kinin system mediated inflammation in Alzheimer’s disease in vivo,” *Current Alzheimer Research*, vol. 8, no. 1, pp. 59–66, 2011.

[54] M. M. Garrison, H. Jeffries, and D. A. Christakis, “Risk of death for children with Down syndrome and sepsis,” *The Journal of Pediatrics*, vol. 147, no. 6, pp. 748–752, 2005.

[55] P. C. Rudberg, F. Tholander, M. Andberg, M. M. G. M. Thunnissen, and J. Z. Haeggstroem, “Leukotriene A4 hydrolase: identification of a common carboxylate recognition site for the epoxide hydrolase and aminopeptidase substrates,” *The Journal of Biological Chemistry*, vol. 279, no. 26, pp. 27376–27382, 2004.

[56] M. Peters-Golden and W. R. Henderson Jr., “Leukotrienes,” *The New England Journal of Medicine*, vol. 357, no. 18, pp. 1841–1854, 2007.

[57] B. J. R. Whittle, C. Varga, A. Berko et al., “Attenuation of inflammation and cytokine production in rat colitis by a novel selective inhibitor of leukotriene A4 hydrolase,” *British Journal of Pharmacology*, vol. 153, no. 5, pp. 983–991, 2008.

[58] R. J. Snegrove, P. L. Jackson, M. T. Hardison et al., “A critical role for LTA4H in limiting chronic pulmonary neutrophilic inflammation,” *Science*, vol. 330, no. 6000, pp. 90–94, 2010.

[59] M. T. Mendes and P. F. Silveira, “The interrelationship between leukotriene B4 and leukotriene-a4-hydrolase in collagen/adjuvant-induced arthritis in rats,” *BioMed Research International*, vol. 2014, Article ID 730421, 9 pages, 2014.

[60] M. Liu and T. Yokomizo, “The role of leukotrienes in allergic diseases,” *Allergology International*, vol. 64, no. 1, pp. 17–26, 2015.

[61] G. Riccioni, A. Zanasi, N. Vitulano, B. Mancini, and N. D’Orazio, “Leukotrienes in atherosclerosis: new target insights and future therapy perspectives,” *Mediators of Inflammation*, vol. 2009, Article ID 737282, 6 pages, 2009.

[62] B. Auner, E. V. Geiger, D. Henrich, M. Lehnert, I. Marzi, and B. Relja, “Circulating leukotriene B4 identifies respiratory complications after trauma,” *Mediators of Inflammation*, vol. 2012, Article ID 536156, 8 pages, 2012.

[63] A. Letourneau, F. A. Santoni, X. Bonilla et al., “Domains of genome-wide gene expression dysregulation in Down’s syndrome,” *Nature*, vol. 508, no. 7496, pp. 345–350, 2014.

[64] S. Ferrari and A. Plebani, “Cross-talk between CD40 and CD40L: lessons from primary immune deficiencies,” *Current Opinion in Allergy and Clinical Immunology*, vol. 2, no. 6, pp. 489–494, 2002.

[65] The National Center for Biotechnology Information—NCBI, “ITGBI integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) [Homo sapiens (human)],” 2014, http://www.ncbi.nlm.nih.gov/gene/3688.

[66] A. Mammoto and D. E. Ingber, “Cytoskeletal control of growth and cell fate switching,” *Current Opinion in Cell Biology*, vol. 21, no. 6, pp. 864–870, 2009.