Interleukin 6 and 10 Serum Levels and Genetic Polymorphisms in Children with Down Syndrome

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1. Introduction

Immunological impairment is a condition that is often observed in individuals with Down syndrome (DS). The immune response is modulated by pro- and anti-inflammatory cytokines whose expressions could be influenced by genetic polymorphisms. The present study was aimed at evaluating the frequencies of -174G>C, -572G>C, and -597G>A polymorphisms in the interleukin 6 (IL-6) gene and -592C>A, -1082A>G, and -819C>T polymorphisms in the IL-10 gene and the IL-6 and IL-10 serum levels in healthy individuals with and without DS. The authors also aimed to investigate the impact of the genotypes on the interleukin concentrations. The genetic polymorphisms were investigated in 200 DS individuals and 200 controls without DS. The serum measurement of IL-6 and IL-10 was performed in a subgroup (54 cases and 54 controls) by enzyme-linked immunosorbent assay (ELISA). The frequencies of the polymorphisms and haplotypes evaluated were not different between individuals with and without DS. IL-10 concentration was higher in DS children but was not influenced by IL-10 gene polymorphisms. IL-6 genotypes had no influence on IL-6 serum levels. The IL-10 serum levels are increased in DS individuals, but IL-10 polymorphisms are not the main factors that influence the IL-10 expression in DS.

1. Introduction

Immunological impairment is a condition often observed in individuals with Down syndrome (DS), which presents an increased susceptibility to bacterial and viral infections and a high frequency of hematologic and autoimmune disorders [1–3]. The immune response is modulated by anti-inflammatory and proinflammatory cytokines, which regulate T cell differentiation. Regulatory cytokines include interleukins (IL), interferons (IFN), tumor necrosis factors (TNF), and growth factors [4].

Interleukin 6 (IL-6) is a proinflammatory cytokine produced by leukocytes, adipocytes, endothelial cells, fibroblasts, and myocytes. IL-6 induces the production of mediators for the release of cytokines such as TNF and IL-1, which drive the inflammatory reaction [5]. The immune system uses anti-inflammatory mechanisms to prevent the exacerbation of inflammatory processes caused by proinflammatory molecules and avoid tissue damage and restore the homeostasis [6]. IL-10 is an important immunoregulatory and anti-inflammatory cytokine secreted by macrophages, monocytes, dendritic cells, T helper 1 (Th1) and Th2 lymphocytes, B lymphocytes, cytotoxic T cells, and mast cells [7]. IL-10 stimulates the activation, proliferation, and differentiation of B cells [6] and participates in the control of the inflammatory response [8]. An imbalance between pro- and anti-inflammatory cytokines avoids the adequate function of the immune system. In DS, alteration of cytokine levels has been observed [9–15], and it can lead to immune deficiency.

The single-nucleotide polymorphisms within the promoter region -597G>A, -572G>C, and -174G>C of the IL-6 gene and -1082G>A, -829C>T, and -592C>A of the IL-10
gene were described [16–18] and can be involved in the modulation of inflammatory responses and susceptibility to inflammatory disorders [17, 19–23].

Considering the immunological impairment in DS individuals, we aimed to determine the prevalence of the polymorphisms -597G>A (rs1800797), -572G>C (rs1800796), and -174G>C (rs1800795) in the IL-6 gene and -1082A>G (rs1800896), -829C>T (rs1800871), and -592C>A (rs1800872) in the IL-10 gene and the serum level of IL-6 and IL-10 in these individuals in comparison with individuals without DS. We also aimed to evaluate the association between these polymorphisms and IL-6 and IL-10 concentrations.

2. Methods

The study was approved by the Research Ethics Committee of Faculdade de Medicina de São José do Rio Preto (FAMERP) (number 427.782).

2.1. Subjects. The study included 200 children with DS (mean age = 4.3 years, 108 males and 92 females) from the General Genetics Outpatient Service of Hospital de Base, São José do Rio Preto, SP, Brazil, and 200 individuals without DS (mean age = 4.3 years, 103 males and 97 females) from the Pediatric Service of the Hospital de Base de São José do Rio Preto, SP, Brazil; all the parents of individuals who participated in the study signed an informed consent. Only individuals who were without leukemia or acute or chronic infection and did not receive medication or immunization within 6 weeks from the serum collection were selected for the interleukin dosage. C-reactive protein (CRP) was quantified by electrochemiluminescence, and only samples with a concentration of ≤0.5 mg/dl were included in the analysis.

2.2. Polymorphism Analysis. DNA was extracted from peripheral blood [24]. Genotyping of IL-10 -1082A>G and -592C>A polymorphisms was performed by polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) analysis. Primer sequences used were as follows: forward 5'-TCTG AAGAAGTCCTGATGTC-3' and reverse 5'-CTCTTACCT ATCCCTACTTCC-3' for the detection of IL-10 -1082A>G polymorphism and forward 5'-GGTGAGCACTACCTGA CTAGC-3' and reverse 5'-CCTAGGTCACAGTGACGTTG-3' for the detection of IL-10 -592C>A polymorphism. PCR products were digested by the restriction enzymes MnII (New England Biolabs, Ipswich, MA) and RsAI (New England Biolabs, Ipswich, MA) for -1082A>G and -592C>A, respectively. The digested products were analyzed on 2.5% agarose gel.

Genotyping of IL-10 -819C>T, IL-6 -174G>C, IL-6 -572G>C, and IL-6 -597G>A was performed using TaqMan SNP Genotyping Assays (C_1747362_10, C_1839695_20, C_11326893_10, and C_1839695_20, Applied Biosystems, Foster City, CA), following the manufacturer’s instructions.

2.3. Quantification of IL-10 and IL-6 Serum Levels. The quantification of IL-10 and IL-6 serum levels was performed in a subgroup composed of 54 individuals with DS (three wild-type homozygous, three heterozygous, and three mutated homozygous for each polymorphism) and 54 individuals without DS (three wild-type homozygous, three heterozygous, and three mutated homozygous for each polymorphism). The interleukin concentrations were also evaluated according to the genotype combination and haplotypes. IL-6 and IL-10 quantification was performed using the Novex ELISA kit (Life Technologies, Carlsbad, CA), following the manufacturer’s instructions, and they were analyzed on the Multiskan FC Microplate Photometer (Thermo Scientific, Waltham, MA) at 450 nm.

2.4. Statistical Analysis. Allele frequencies were evaluated for the Hardy-Weinberg (HWE) equilibrium by the chi-square test using the BioEstat software version 5.0. SNPStats program (http://bioinfo.iconcologia.net/SNPstats_web) for the analysis of the genotype distribution between the groups by logistic regression in the codominant, dominant, and recessive models.

The results were presented as odds ratio (OR) at 95% confidence interval (95% CI). Haplotypes analysis was performed using Haplovie software, version 4.2. A comparison of IL-6 and IL-10 serum levels between the groups was performed by the Mann-Whitney test. Analysis of interleukin concentrations in relation to the genotypes was performed using the Kruskal-Wallis or Mann-Whitney test with GraphPad Prism software version 6.0. The error accepted was 5%.

3. Results

3.1. Polymorphisms in DS and Control Groups. The genotype distribution of IL-6 -174G>C (rs1800795), IL-6 -572G>C (rs1800796), and IL-6 -597G>A (rs1800797) polymorphisms was in accordance with the Hardy-Weinberg equilibrium (HWE) in the DS (P = 0.68 for -174G>C, P = 0.48 for -572G>C, and P = 0.68 for -597G>A) and control (P = 0.71 for -174G>C, P = 0.51 for -572G>C, and P = 1 for -597G>A) groups.

The genotype frequencies of IL-10 -592C>A polymorphism did not differ from those that we would expect under HWE in the DS (P = 0.75) and control (P = 0.19) groups. IL-10 -819C>T polymorphism was in accordance with HWE only in the DS group (P = 0.76). In the control group, the genotype frequencies deviated from HWE expectations (P = 0.036). The genotype frequencies of IL-10 -1082A>G presented HWE deviation in DS (P = 0.026) and control (P < 0.0001) groups.

There was no significant difference in genotype distribution between the groups (Table 1). Haplotype analyses were conducted to evaluate the combined effect of the polymorphisms. The IL-6 polymorphisms were in strong linkage disequilibrium, as were the IL-10 polymorphisms. Frequencies of alleles and haplotypes of IL-6 and IL-10 polymorphisms were not statistically different between the groups (P > 0.05).

3.2. IL-6 and IL-10 Serum Levels in DS and Control Groups. IL-10 serum levels were significantly increased in DS children.
### Table 1: Genotype distribution of IL-6 and IL-10 polymorphisms in DS and control groups.

| Models       | Genotype | Control (N (%)) | DS (N (%)) | OR (95% CI) | P value |
|--------------|----------|-----------------|------------|-------------|---------|
|              |          |                 |            |             |         |
| **IL-6 -597G>A** |          |                 |            |             |         |
| Codominant   | GG       | 107 (53.5)      | 122 (61)   | 1.00        |         |
|              | GA       | 79 (39.5)       | 70 (35)    | 1.28 (0.84–1.93) | 0.22    |
|              | AA       | 14 (7)          | 08 (4)     | 1.99 (0.80–4.92) | 0.14    |
| Dominant     | GG       | 107 (53.5)      | 122 (61)   | 1.00        |         |
|              | GA/AA    | 93 (46.5)       | 78 (39)    | 1.35 (0.91–2.01) | 0.44    |
| Recessive    | AA       | 14 (7)          | 08 (4)     | 1.80 (0.74–4.40) | 0.19    |
| **IL-10 -1082A>G** |          |                 |            |             |         |
| Codominant   | GG       | 107 (53.5)      | 122 (61)   | 1.00        |         |
|              | AA       | 63 (31.5)       | 75 (37.5)  | 0.76 (0.49–1.18) | 0.44    |
| Dominant     | GG       | 33 (16.5)       | 30 (15)    | 1.01 (0.57–1.78) | 0.36    |
| Recessive    | GG/GA   | 167 (83.5)      | 170 (85)   | 1.00        |         |

**DS:** Down syndrome; **OR:** odds ratio; **CI:** confidence interval.
compared to those without DS ($P = 0.0019$; Figure 1(a)). IL-6 serum levels did not differ between the DS and control groups ($P > 0.05$; Figure 1(b)).

IL-06 and IL-10 concentrations were evaluated in relation to the genetic polymorphisms; however, IL-6 and IL-10 polymorphisms showed no effect on these interleukin serum levels (Table 2). The polymorphic homozygous genotype for IL-6 -572G>C polymorphism was not detected in this subgroup, and so, the statistical analyses according to the genetic models of the IL-6 were not performed. In this case, the statistic was performed between the wild-type genotype and heterozygous genotype (DS: GG = 13.77 pg/ml versus GC = 10.65 pg/ml ($P = 0.07$); control: GG = 13.67 pg/ml versus GC = 16.73 pg/ml ($P = 0.99$)).

4. Discussion

Our findings showed an increase in serum levels of the cytokine IL-10 in children with DS. IL-10 is an anti-inflammatory cytokine [25] which participates in the negative feedback control of inflammatory responses [8]. This cytokine plays a crucial role in the prevention of inflammatory and autoimmune pathologies [6]. IL-10 suppresses the gene expression of other cytokines and chemokines by inhibiting the transcription or reducing the levels of mRNA [8]. Increased IL-10 signaling can prevent the maturation of macrophage and dendritic cells and inhibit the production of proinflammatory cytokine [6]. Thus, excessive IL-10 production can inhibit proinflammatory response to several pathogens, resulting in uncontrolled infection and deficient immune response [6], which are characteristics that are often observed in DS.

The first phase of an innate immune response comprises the classical immune activation [26, 27] characterized by the recruitment of Th1 cytokines such as interferon-γ and other proinflammatory cytokines [28, 29]. However, the production of proinflammatory factors can be arrested, and macrophages can produce factors that participate in tissue repair and wound healing, such as anti-inflammatory cytokines [29]. IL-10 and TGF-β are associated with the inhibition of proinflammatory activity [29, 30]. This alternative activation during an immune response provides an anti-inflammatory equilibrium to a proinflammatory acute response. The activated macrophages are immunosuppressive and participate in tissue repair and remodeling of the extracellular matrix [28, 29]. However, repair processes can enhance the fibrosis and contribute to the maintenance of disease [29, 31, 32]. An interrelation between inflammatory and regenerative processes was suggested on neurodegeneration related to the pathogenesis of Alzheimer’s disease (AD) [33].

Overexpression of IL-10 was previously reported in DS [34–36]. The basal levels of the IL-10 gene expression were observed to be upregulated in DS children [37]. The present study evaluated the expression profile of immune-related genes in DS individuals without current infection and concluded that several genes with relevant functions in immune cells are dysregulated in DS [37]. This could explain the increased susceptibility to bacterial and viral infections and inflammatory disorders in these individuals [38, 39].

In addition to the increased basal levels of IL-10 in DS, studies that evaluated the immune response of DS individuals showed increased levels of IL-10 in the presence of pathogens or inflammatory processes [34, 40]. The immune response to antigens is mediated by proinflammatory cytokines that perform the defense of pathogen invasion [6]. However, an excess of inflammation can disrupt the host’s metabolic system. Anti-inflammatory system activation is a mechanism used to avoid tissue damage and restore homeostasis [6]. The inflammatory response to a microbial challenge can be enhanced by down- or overexpression of IL-10. The impairment of IL-10 expression or signaling can result in enhanced removal of pathogens during an acute infection but also can contribute to an exacerbated inflammatory response, resulting in immunopathology and tissue damage [6].

Differential expression of inflammation-related genes was observed in healthy children with DS [41]; IL-10 levels were higher in DS individuals with AD and also in those with DS without a clinically relevant cognitive decline [36]. In a previous study, IL-10 levels from the DS group did not differ from those from the control group; however, it was significantly lower in DS patients than in patients with intellectual
It is believed that soluble amyloid precursor protein and several forms of \( \beta \)-amyloid peptides lead to the activation of the signaling for an innate immune response in the brain [43]. Studies have shown that a proinflammatory state can reduce \( \beta \)-amyloid accumulation in mouse models [44–50], and the high concentrations of IL-10 contribute to reduced amyloid-\( \beta \) phagocytosis by microglia and amyloid-\( \beta \) deposition [51], which is observed in DS individuals.

Interleukin 6 (IL-6) plasma levels were also higher in subjects with DS and AD-related symptoms [36]. A negative correlation was found between IL-6 levels and cognitive decline [36]. Studies have found increased levels of IL-6 in DS [36, 52], although others have observed opposite results [34] or no significant alterations [40]. We did not observe differential concentrations of IL-6 between individuals with DS and those without the syndrome in this study.

IL-6 stimulates T and B cell immune responses upon encountering antigen components, triggering an acute inflammatory response and haematopoiesis [5, 53]. It is important to emphasize that we evaluated individuals with no infection at the moment of sample collection; therefore, we evaluated the basal levels of IL-6. Perhaps, IL-6 is more significantly related to the immune response in these individuals, and its abnormal production occurs after the contact with an antigen. IL-6 concentrations were significantly higher in children with DS upon stimulation with influenza A virus, which reinforces its role in the immune response [54]. Overexpression of proinflammatory cytokines, such as IL-6, could result in injury to healthy tissue [55] and overinflammation, leading to neuronal dysfunction and consequent deterioration of the neurons, as observed in AD progression [56].

The levels of cytokines can be determined by genetic polymorphisms in the promoter region of interleukin genes [7, 17, 57–60]. In this study, we did not find an association between all IL-6 or IL-10 polymorphisms evaluated and the concentrations of these interleukins in both groups of DS individuals and those without the disease.

The polymorphisms -1082 A>G, -592C>A, and 819C>T in the IL-10 gene seem to modulate the IL-10 levels in different disorders, such as cancer [21], tuberculosis [19], systemic lupus erythematosus [20], and rheumatoid arthritis [22], but the results are controversial. To the best of our knowledge, there is no study relating these genetic alterations to IL-10 concentrations in DS until now.

Table 2: IL-6 and IL-10 serum levels in relation to the genotypes.

| Models     | Genotype | DS (pg/ml) | \( P \) value | Control (pg/ml) | \( P \) value | Genotype | DS (pg/ml) | \( P \) value | Control (pg/ml) | \( P \) value |
|------------|----------|------------|---------------|-----------------|---------------|----------|------------|---------------|-----------------|---------------|
|            |          |            |               |                 |               |          |            |               |                 |               |
| **IL-6 -1082A>G** |          |            |               |                 |               |          |            |               |                 |               |
| Codominant | AA       | 15.07      | 13.60         | 0.82            | 1.60          | GG       | 11.52      | 16.22         | 0.49            | 19.12         |
|            | AG       | 15.02      | 14.33         | 0.69            | 1.33          | GA       | 13.59      | 19.12         | 0.49            | 19.12         |
|            | GG       | 16.84      | 13.71         | 0.91            | 1.60          | GG       | 11.52      | 16.22         | 0.27            | 12.80         |
| Dominant   | AA       | 15.07      | 13.60         | 0.59            | 1.09          | GA       | 13.59      | 19.12         | 0.49            | 19.12         |
|            | AG/GG    | 16.08      | 14.30         | 0.91            | 1.60          | GA/AA    | 13.32      | 16.22         | 0.27            | 12.80         |
|            | AA/AG    | 15.02      | 14.09         | 0.75            | 1.40          | GG/GA    | 13.04      | 16.74         | 0.90            | 11.00         |
|            | GG       | 16.84      | 13.71         | 0.62            | 1.33          | AA       | 12.17      | 11.00         | 0.90            | 11.00         |
| **IL-10 -592C>A** |          |            |               |                 |               |          |            |               |                 |               |
| Codominant | CA       | 15.18      | 14.08         | 0.94            | 1.40          | GC       | 13.59      | 19.12         | 0.49            | 19.12         |
|            | AA       | 14.72      | 14.27         | 0.94            | 1.40          | CC       | 12.17      | 11.00         | 0.94            | 11.00         |
| Dominant   | CC       | 16.84      | 14.03         | 0.94            | 1.40          | GC       | 13.59      | 19.12         | 0.49            | 19.12         |
|            | CA/AA    | 15.02      | 14.08         | 0.94            | 1.40          | GC/CC    | 13.32      | 16.22         | 0.27            | 12.80         |
| Recessive  | CC/CC    | 15.58      | 14.08         | 0.90            | 1.40          | GG/GC    | 13.04      | 16.74         | 0.90            | 11.00         |
|            | AA       | 14.72      | 14.27         | 0.90            | 1.40          | AA       | 12.17      | 11.00         | 0.90            | 11.00         |
| **IL-10 -819C>T** |          |            |               |                 |               |          |            |               |                 |               |
| Codominant | CT       | 15.18      | 14.08         | 0.94            | 1.40          | GC       | 10.65      | 16.73         | 0.94            | 16.73         |
|            | TT       | 14.72      | 14.27         | 0.94            | 1.40          | TT       | 14.27      | 16.73         | 0.94            | 16.73         |
| Dominant   | CC       | 16.84      | 14.03         | 0.94            | 1.40          | GC       | 10.65      | 16.73         | 0.94            | 16.73         |
|            | CT/TT    | 15.02      | 14.08         | 0.94            | 1.40          | GC/TT    | 13.32      | 16.22         | 0.27            | 12.80         |
| Recessive  | CC/CT    | 15.58      | 14.08         | 0.90            | 1.40          | GC/GC    | 13.04      | 16.74         | 0.90            | 11.00         |
|            | TT       | 14.72      | 14.27         | 0.90            | 1.40          | TT       | 14.27      | 16.73         | 0.90            | 11.00         |

*Genotype was not found. DS: Down syndrome.

disability [42]. It is believed that soluble amyloid precursor protein and several forms of \( \beta \)-amyloid peptides lead to the activation of the signaling for an innate immune response in the brain [43]. Studies have shown that a proinflammatory state can reduce \( \beta \)-amyloid accumulation in mouse models [44–50], and the high concentrations of IL-10 contribute to reduced amyloid-\( \beta \) phagocytosis by microglia and amyloid-\( \beta \) deposition [51], which is observed in DS individuals.

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IL-6 -174G>C, -572G>C, and -597G>A polymorphisms have been investigated on the levels of this cytokine in inflammatory diseases such as systemic-onset juvenile chronic arthritis [17], age-related macular degeneration.
The frequencies of the IL-10 and IL-6 polymorphisms evaluated here did not differ between DS individuals and those without the syndrome. To our knowledge, this is the first study to investigate these genetic alterations in DS. Polymorphisms in the IL-10 gene have been associated with some immune-related diseases [54] such as asthma [65, 66], systemic lupus erythematosus [67–69], Crohn’s disease [70, 71], rheumatoid arthritis [22, 72–74], tuberculosis [19, 75], type 2 diabetes [76], and several types of cancer [77–80]. Alterations in the IL-6 gene were also related to inflammatory changes [17, 18, 23, 81–84], including AD [83, 84]. However, IL-6 -572G>C was associated with protection in osteoarthritis [85] and erythematous systemic lupus [86]. As previously mentioned, there are no data in the literature on the role of these polymorphisms in DS.

5. Conclusion

In conclusion, the IL-10 -1082A>G, IL-10 -592C>A, IL-10 -819C>T, IL-06 -597G>A, IL-06 -174G>C, and IL-06 -572G>C polymorphisms have no effect on IL-10 and IL-6 concentrations in DS individuals and individuals without the syndrome evaluated in this study. The levels of IL-10 are increased in DS individuals, but the polymorphisms in the IL-10 gene are not the main factors that drive the overexpression of IL-10 in DS.

Data Availability

The statistical data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors’ Contributions

All the authors have contributed to the submitted draft of the manuscript and approved it for submission.

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References

[1] D. Chistiakov, “Down syndrome and coexistent autoimmune diseases,” Journal of Applied Biomedicine, vol. 5, p. 71, 2007.
[2] A. C. Xavier, Y. Ge, and J. Taub, “Unique clinical and biological features of leukemia in Down syndrome children,” Expert Review of Hematology, vol. 3, no. 2, pp. 175–186, 2010.
[3] G. Ram and J. Chinen, “Infections and immunodeficiency in Down syndrome,” Clinical & Experimental Immunology, vol. 164, no. 1, pp. 9–16, 2011.
[4] S. E. Broughton, T. R. Hercus, A. F. Lopez, and M. W. Parker, “Cytokine receptor activation at the cell surface,” Current Opinion in Structural Biology, vol. 22, no. 3, pp. 350–359, 2012.
[5] S. Rose-John, K. Winthrop, and L. Calabrese, “The role of IL-6 in host defence against infections: immunobiology and clinical implications,” Nature Reviews Rheumatology, vol. 13, no. 7, pp. 399–409, 2017.
[6] S. S. Iyer and G. Cheng, “Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease,” Critical Reviews™ in Immunology, vol. 32, no. 1, pp. 23–63, 2012.
[7] J. Trifunović, L. Miller, Ž. Debeljak, and V. Horvat, “Pathologic patterns of interleukin 10 expression – a review,” Biochimia Medica, vol. 25, no. 1, pp. 36–48, 2015.
[8] T. A. Hamilton, Y. Ohmori, and J. Tebo, “Regulation of chemokine expression by antiinflammatory cytokines,” Immunologic Research, vol. 25, no. 3, pp. 229–246, 2002.
[9] P. G. Nelson, T. Kuddo, E. Y. Song et al., “Selected neuropeptides, neuropeptides, and cytokines: developmental trajectory and concentrations in neonatal blood of children with autism or Down syndrome,” International Journal of Developmental Neuroscience, vol. 24, no. 1, pp. 73–80, 2006.
[10] 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.
[11] R. Roberson, T. Kuddo, K. Horowitz, M. Caballero, and C. Y. Spong, “Cytokine and chemokine alterations in Down Syndrome,” American Journal of Perinatology, vol. 29, no. 9, pp. 705–713, 2012.
[12] M. B. F. Trottta, J. B. Serro Azul, M. Wajngarten, S. G. Fonseca, A. C. Goldberg, and J. E. Kalil, “Inflammatory and immunological parameters in adults with Down syndrome,” Immunity & Ageing, vol. 8, no. 1, p. 4, 2011.
[13] G. Tsilingaridis, T. Yucel-Lindberg, and T. Modeer, “T-helper-related cytokines in gingival crevicular fluid from adolescents with Down syndrome,” Clinical Oral Investigations, vol. 16, no. 1, pp. 267–273, 2012.
[14] J. Śmigielska-Kuzia, K. Sendrowski, A. Jakubiuk-Tomaszuk et al., “Proinflammatory plasma cytokines in patients with Down syndrome,” Neurologia Dzieci, vol. 21, no. 43, pp. 19–25, 2012.
[15] A. Jakubiuk-Tomaszuk, W. Sobaniec, M. Rusak et al., “Decrease of interleukin (IL) 17A gene expression in leucocytes and in the amount of IL-17A protein in CD4+ T cells in children with Down Syndrome,” Pharmacology Reports, vol. 67, no. 6, pp. 1130–1134, 2015.
[16] M. N. Karimabadi, M. K. Arababadi, E. Hakimizadeh et al., “Is the IL-10 promoter polymorphism at position -592 associated with immune system-related diseases?,” Inflammation, vol. 36, no. 1, pp. 35–41, 2013.
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[17] A. Ansari, N. Talat, B. Jamil et al., “Cytokine gene polymorphisms across tuberculosis clinical spectrum in Pakistani patients,” PloS One, vol. 4, no. 3, article e4778, 2009.

[18] R. M. Talaat, S. A. Alrefaey, I. H. Bassouyuni, M. E. Ashour, and A. A. Raouf, "Genetic polymorphisms of interleukin 6 and interleukin 10 in Egyptian patients with systemic lupus erythematosus," Lupus, vol. 25, no. 3, pp. 255–264, 2016.

[19] B. Ying, Y. Shi, X. Pan et al., "Association of polymorphisms in the human IL-10 and IL-18 genes with rheumatoid arthritis," Molecular Biology Reports, vol. 38, no. 1, pp. 379–385, 2011.

[20] D. Fishman, G. Faulds, R. Jeffery et al., "The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis," The Journal of Clinical Investigation, vol. 102, no. 7, pp. 1369–1376, 1998.

[21] C. F. Terry, V. Loukaci, and F. R. Green, "Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation," Journal of Biological Chemistry, vol. 275, no. 24, pp. 18138–18144, 2000.

[22] M. Bennermo, C. Held, S. Stemme et al., "Interleukin-6 promoter polymorphism is associated with type-2 diabetes mellitus in Native Americans and Caucasians," Human Genetics, vol. 112, no. 4, pp. 409–413, 2003.

[23] L. A. Salazar, M. H. Hirata, S. A. Cavalli, M. O. Machado, and R. D. C. Hirata, "Optimized procedure for DNA isolation from fresh and cryopreserved clotted human blood useful in clinical molecular testing," Clinical Chemistry, vol. 44, no. 8, pp. 1748–1750, 1998.

[24] K. W. Moore, A. O’Garra, R. W. Malefyt, P. Vieira, and T. R. Mosmann, "Interleukin-10, Annual Review of Immunology, vol. 11, no. 1, pp. 165–190, 1993.

[25] S. Gordon, "Alternative activation of macrophages," Nature Reviews Immunology, vol. 3, no. 1, pp. 23–35, 2003.

[26] C. Yuan, W. X. Chen, J. S. Zhu et al., "IL-10 treatment is associated with prohibitin expression in the Crohn’s disease intestinal fibrosis mouse model," Mediators of Inflammation, vol. 2013, Article ID 617145, 8 pages, 2013.

[27] M. Hesse, M. Modolell, A. C. La Flame et al., "Differential regulation of nitric oxide synthase-2 and arginase-1 by type I/type 2 cytokines in vivo: granulomatous pathology is shaped by the pattern of L-arginine metabolism," The Journal of Immunology, vol. 167, no. 11, pp. 6533–6544, 2001.

[28] P. Eikelenboom and W. A. van Gool, "Neuroinflammatory perspectives on the two faces of Alzheimer’s disease," Journal of Neurotransmission, vol. 111, no. 3, pp. 281–294, 2004.

[29] J. J. M. Hoomezans, R. Veehrus, J. M. Rozemuller, and P. Eikelenboom, Neuroinflammation and regeneration in the early stages of Alzheimer’s disease pathology," International Journal of Developmental Neuroscience, vol. 24, no. 2-3, pp. 157–165, 2006.

[30] L. Guazzaroni, D. Trabattoni, E. Castelletti et al., “Lymphocyte maturation is impaired in healthy young individuals carrying trisomy 21 (Down syndrome),” American Journal on Intellectual and Developmental Disabilities, vol. 114, no. 2, pp. 100–109, 2009.

[31] S. Cetiner, O. Demirhan, T. C. Inal, D. Tastemir, and Y. Sertdemir, "Analysis of peripheral blood T-cell subsets, natural killer cells and serum levels of cytokines in children with Down syndrome," International Journal of Immunogenetics, vol. 37, no. 4, pp. 233–237, 2010.

[32] M. F. Iulita, A. Ower, C. Barone et al., "An inflammatory and trophic disconnect biomarker profile revealed in Down syndrome plasma: relation to cognitive decline and longitudinal evaluation," Alzheimer’s & Dementia, vol. 12, no. 11, pp. 1132–1148, 2016.

[33] 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 e102718, 2014.

[34] A. Tenenbaum, R. N. Hanna, D. Averbuch, I. D. Weaxter, M. Chavkin, and J. Merrick, "Hospitalization of children with Down syndrome," Frontiers in Public Health, vol. 2, p. 22, 2014.

[35] C. Joffre, F. Lesage, O. Bustarret, P. Hubert, and M. Oualha, "Children with Down syndrome: clinical course and mortality-associated factors in a French medical paediatric intensive care unit," Journal of Paediatrics and Child Health, vol. 52, no. 6, pp. 595–599, 2016.

[36] C. J. M. Broers, R. J. B. J. Gemke, S. A. Morré, M. E. Weijerman, and A. M. van Furth, "Increased production of interleukin-10 in children with Down syndrome upon ex vivo stimulation with Streptococcus pneumoniae," Pediatric Research, vol. 75, no. 1, pp. 109–113, 2014.

[37] C. R. S. Silva, J. M. Biselli-Périco, B. L. Zampieri et al., "Differential expression of inflammation-related genes in children with Down syndrome," Mediators of Inflammation, vol. 2016, Article ID 6985903, 8 pages, 2016.

[38] M. Nateghi Rostami, M. Dorraghi, A. Miramin Mohammadi, and B. Nikmanesh, "Altered serum pro-inflammatory cytokines in children with Down’s syndrome," European Cytokine Network, vol. 23, no. 2, pp. 64–67, 2012.

[39] R. E. Mruk and W. S. T. Griffin, "Glia and their cytokines in progression of neurodegeneration," Neurobiology of Aging, vol. 26, no. 3, pp. 349–354, 2005.

[40] V. Boissonneault, M. Filali, M. Lessard, J. Relton, G. Wong, and M. Douraghi, "Increased production of interleukin-10 in children with Down syndrome upon ex vivo stimulation with Streptococcus pneumoniae," Pediatric Research, vol. 75, no. 1, pp. 109–113, 2014.

[41] P. Chakrabarty, C. Ceballos-Díaz, A. Beccard et al., "IFN-γ promotes complement expression and attenuates amyloid plaque deposition in amyloid β precursor protein transgenic mice," The Journal of Immunology, vol. 184, no. 9, pp. 5333–5343, 2010.

[42] P. Chakrabarty, A. Herring, C. Ceballos-Díaz, P. Das, and T. E. Golde, "Hippocampal expression of murine TNFα results in attenuation of amyloid deposition in vivo," Molecular Neurodegeneration, vol. 6, no. 1, p. 16, 2011.
[47] D. L. Herber, M. Mercer, L. M. Roth et al., “Microglial activation is required for Aβ clearance after intracranial injection of lipopolysaccharide in APP transgenic mice,” *Journal of Neuroimmune Pharmacology*, vol. 2, no. 2, pp. 222–231, 2007.

[48] G. Naert and S. Rivest, “CC chemokine receptor 2 deficiency aggravates cognitive impairments and amyloid pathology in a transgenic mouse model of Alzheimer’s disease,” *The Journal of Neuroscience*, vol. 31, no. 16, pp. 6208–6220, 2011.

[49] S. S. Shaftel, S. Kyrkanides, J. A. Olschowka, J. N. H. Miller, R. E. Johnson, and M. K. O’Banion, “Sustained hippocampal IL-1β overexpression mediates chronic neuroinflammation and ameliorates Alzheimer plaque pathology,” *The Journal of Clinical Investigation*, vol. 117, no. 6, pp. 1595–1604, 2007.

[50] D. Lobo-Silva, G. M. Carriche, A. G. Castro, S. Roque, and M. Saraiva, “Balancing the immune response in the brain: IL-10 and its regulation,” *Journal of Neuroinflammation*, vol. 13, no. 1, p. 297, 2016.

[51] P. Chakrabarty, A. Li, C. Ceballos-Diaz et al., “IL-10 alters immunoproteostasis in APP mice, increasing plaque burden and worsening cognitive behavior,” *Neuron*, vol. 85, no. 3, pp. 519–533, 2015.

[52] M. E. Zaki, H. T. El-Bassyouni, A. M. S. Tosson, E. Youness, and J. Hussein, “Coenzyme Q10 and pro-inflammatory markers in children with Down syndrome: clinical and biochemical aspects,” *Jornal de Pediatria*, vol. 93, no. 1, pp. 100–104, 2017.

[53] J. Scheller, A. Chalaris, D. Schmidt-Arras, and S. Rose-John, “The pro- and anti-inflammatory properties of the cytokine interleukin-6,” *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, vol. 1813, no. 5, pp. 878–888, 2011.

[54] C. J. M. Broers, R. J. B. J. Gemke, M. E. Weijerman, K. F. van der Sluijs, and A. M. van Furth, “Increased pro-inflammatory cytokine production in Down syndrome children upon stimulation with live influenza A virus,” *Journal of Clinical Immunology*, vol. 32, no. 2, pp. 323–329, 2012.

[55] C. A. Colton and D. M. Wilcock, “Assessing activation states in microglia,” *CNS & Neurological Disorders - Drug Targets*, vol. 9, no. 2, pp. 174–191, 2010.

[56] P. Grammas and R. Ovase, “Inflammatory factors are elevated in brain microvessels in Alzheimer’s disease,” *Neurobiology of Aging*, vol. 22, no. 6, pp. 837–842, 2001.

[57] D. Lio, F. Licastro, L. Scola et al., “Interleukin-10 promoter polymorphism in sporadic Alzheimer’s disease,” *Genes & Immunity*, vol. 4, no. 3, pp. 234–238, 2003.

[58] S. Y. Gao, S. Liang, M. Pan et al., “Interleukin-6 genotypes and serum levels in Chinese Hui population,” *International Journal of Clinical and Experimental Medicine*, vol. 7, no. 9, pp. 2851–2857, 2014.

[59] F. Su, F. Bai, and Z. Zhang, “Inflammatory cytokines and Alzheimer’s disease: a review from the perspective of genetic polymorphisms,” *Neuroscience Bulletin*, vol. 32, no. 5, pp. 469–480, 2016.

[60] X. M. Han, C. H. Wang, X. Sima, and S. Y. Liu, “Interleukin-6–174G/C polymorphism and the risk of Alzheimer’s disease in Caucasians: a meta-analysis,” *Neuroscience Letters*, vol. 504, no. 1, pp. 4–8, 2011.

[61] F. Ambreen, M. Ismail, and I. Z. Qureshi, “Association of gene polymorphism with serum levels of inflammatory and angiogenic factors in Pakistani patients with age-related macular degeneration,” *Molecular Vision*, vol. 21, pp. 985–999, 2015.

[62] L. Qi, R. M. van Dam, J. B. Meigs, J. A. E. Manson, D. Hunter, and F. B. Hu, “Genetic variation in IL6 gene and type 2 diabetes: tagging-SNP haplotype analysis in large-scale case–control study and meta-analysis,” *Human Molecular Genetics*, vol. 15, no. 11, pp. 1914–1920, 2006.

[63] J. W. Chae, T. Ng, H. L. Yeo et al., “Impact of TNF-α (rs1800629) and IL-6 (rs1800795) polymorphisms on cognitive impairment in Asian breast cancer patients,” *PLoS One*, vol. 11, no. 10, article e0164204, 2016.

[64] M. van Oijen, P. P. Arp, F. J. de Jong et al., “Polymorphisms in the interleukin 6 and transforming growth factor β1 gene and risk of dementia: the Rotterdam Study,” *Neuroscience Letters*, vol. 402, no. 1-2, pp. 113–117, 2006.

[65] X. Y. Zheng, W. J. Guan, C. Mao et al., “Interleukin-10 promoter 1082/-819/-592 polymorphisms are associated with asthma susceptibility in Asians and atopic asthma: a meta-analysis,” *Lung*, vol. 192, no. 1, pp. 65–73, 2014.

[66] W. Nie, Z. Fang, B. Li, and Q. Y. Xiu, “Interleukin-10 promoter polymorphisms and asthma risk: a meta-analysis,” *Cytokine*, vol. 60, no. 3, pp. 849–855, 2012.

[67] P. Liu, J. Song, H. Su et al., “IL-10 gene polymorphisms and susceptibility to systemic lupus erythematosus: a meta-analysis,” *PLoS One*, vol. 8, no. 7, article e69547, 2013.

[68] G. G. Song, S. J. Choi, J. D. Ji, and Y. H. Lee, “Associations between interleukin-10 polymorphisms and susceptibility to systemic lupus erythematosus: a meta-analysis,” *Human Immunology*, vol. 74, no. 3, pp. 364–370, 2013.

[69] M. Zhou, L. Ding, H. Peng et al., “Association of the interleukin-10 gene polymorphism (–1082A/G) with systemic lupus erythematosus: a meta-analysis,” *Lupus*, vol. 22, no. 2, pp. 128–135, 2013.

[70] H. Lv, Y. Jiang, J. Li et al., “Association between polymorphisms in the promoter region of interleukin-10 and susceptibility to inflammatory bowel disease,” *Molecular Biology Reports*, vol. 41, no. 3, pp. 1299–1310, 2014.

[71] H. Zhu, X. Lei, Q. Liu, and Y. Wang, “Interleukin-10 -1082A/G polymorphism and inflammatory bowel disease susceptibility: a meta-analysis based on 17,585 subjects,” *Cytokine*, vol. 61, no. 1, pp. 146–153, 2013.

[72] B. de Paz, M. Alperi-López, F. J. Ballina-García et al., “Interleukin-10 and tumor necrosis factor-α genotypes in rheumatoid arthritis — association with clinical response to glucocorticoids,” *The Journal of Rheumatology*, vol. 37, no. 3, pp. 503–511, 2010.

[73] J. Zhang, Y. Zhang, J. Jin et al., “The –1082A/G polymorphism in the interleukin-10 gene and the risk of rheumatoid arthritis: a meta-analysis,” *Cytokine*, vol. 56, no. 2, pp. 351–355, 2011.

[74] A. Paradrowska-Gorycka, J. Treffer, J. Maciejewska-Stelmach, and J. K. Lacki, “Interleukin-10 gene promoter polymorphism in Polish rheumatoid arthritis patients,” *International Journal of Immunogenetics*, vol. 37, no. 4, pp. 225–231, 2010.

[75] B. Liang, Y. Guo, Y. Li, and H. Kong, “Association between IL-10 gene polymorphisms and susceptibility of tuberculosis: evidence based on a meta-analysis,” *PLoS One*, vol. 9, no. 2, article e88448, 2014.

[76] M. K. Arababadi, M. R. Mirzaei, S. M. A. Sajadi et al., “Interleukin (IL)-10 gene polymorphisms are associated with type 2 diabetes with and without nephropathy: a study of patients from the southeast region of Iran,” *Inflammation*, vol. 35, no. 3, pp. 797–802, 2012.
[77] C. M. Shih, Y. L. Lee, H. L. Chiou et al., “The involvement of
genetic polymorphism of IL-10 promoter in non-small cell
lung cancer,” Lung Cancer, vol. 50, no. 3, pp. 291–297, 2005.

[78] J. M. Faupel-Badger, L. C. R. Kidd, D. Albanes, J. Virtamo,
K. Wooldson, and J. A. Tangrea, “Association of IL-10 poly-
morphisms with prostate cancer risk and grade of disease,”
Cancer Causes & Control, vol. 19, no. 2, pp. 119–124, 2008.

[79] A. DeMichele, A. M. Martin, R. Mick et al., “Interleukin-6
−174G→C polymorphism is associated with improved out-
come in high-risk breast cancer,” Cancer Research, vol. 63,
no. 22, pp. 8051–8056, 2003.

[80] W. Cozen, P. S. Gill, S. A. Ingles et al., “IL-6 levels and
genotype are associated with risk of young adult Hodgkin
lymphoma,” Blood, vol. 103, no. 8, pp. 3216–3221, 2004.

[81] M. Bennermo, C. Held, F. Green et al., “Prognostic value of
plasma interleukin-6 concentrations and the -174 G > C and
-572 G > C promoter polymorphisms of the interleukin-6
gene in patients with acute myocardial infarction treated
with thrombolysis,” Atherosclerosis, vol. 174, no. 1, pp. 157–
163, 2004.

[82] C. M. Chapman, J. P. Beilby, S. E. Humphries, L. J. Palmer,
P. L. Thompson, and J. Hung, “Association of an allelic variant
of interleukin-6 with subclinical carotid atherosclerosis in an
Australian community population,” European Heart Journal,
vol. 24, no. 16, pp. 1494–1499, 2003.

[83] A. Flex, E. Gaetani, R. Pola et al., “The -174 G/C polymor-
phism of the interleukin-6 gene promoter is associated with
peripheral artery occlusive disease,” European Journal of
Vascular and Endovascular Surgery, vol. 24, no. 3, pp. 264–
268, 2002.

[84] B. Schlüter, C. Raufhake, M. Erren et al., “Effect of the
interleukin-6 promoter polymorphism (-174 G/C) on the
incidence and outcome of sepsis,” Critical Care Medicine,
vol. 30, no. 1, pp. 32–37, 2002.

[85] M. T. P. Fernandes, K. B. P. Fernandes, A. S. Marquez et al.,
“Association of interleukin-6 gene polymorphism (rs1800796)
with severity and functional status of osteoarthritis in elderly
individuals,” Cytokine, vol. 75, no. 2, pp. 316–320, 2015.

[86] K. H. Chua, B. P. Kee, S. Y. Tan, and L. H. Lian, “Interleukin-6
promoter polymorphisms (-174 G/C) in Malaysian patients
with systemic lupus erythematosus,” Brazilian Journal of Med-
tical and Biological Research, vol. 42, no. 6, pp. 551–555, 2009.