Faculdade de Medicina de São José do Rio Preto
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NÍVEIS SÉRICOS E POLIMORFISMOS GENÉTICOS DAS INTERLEUCINAS IL-6 E IL-10 EM INDIVÍDUOS COM SÍNDROME DE DOWN

São José do Rio Preto

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Eixo temático: Medicina e Ciências Correlatas.

Orientadora: Profª. Drª. Érika Cristina Pavarino

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“Todo mundo é um gênio. Mas, se você julgar um peixe por sua capacidade de subir em uma árvore, ela vai passar toda a sua vida acreditando que ele é estúpido”. Albert Einstein.

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Lista de Abreviaturas e Símbolos

AD	*Alzheimer Disease*
Alpha -TNF	*Alpha Tumor Necrosis Factor*
BCL2	*B-Cell Lymphoma 2*
BCL2L1	*B-Cell Lymphoma 2 Like 1*
BDKRB1	*Bradykinin Receptor B1*
Beta-IL1	*Beta Interleukin 1*
CAP	*Community-Acquired Pneumonia*
CCL3	*Chemokine (C-C motif) Ligand 3*
CCR2	*Chemokine Receptor 2*
CCR5	*Chemokine Receptor 2*
CCR7	*Chemokine Receptor 7*
CD19	*Cluster of Differentiation 19*
CD28	*Chemokine Receptor 28*
CD40	*Cluster of Differentiation 40*
CD46	*Cluster of Differentiation 46*
CD80	*Cluster of Differentiation 80*
CD40LG	*Cluster of Differentiation 40 Ligand*
CI	*Confidence interval*
CMV	*Cytomegalovirus*
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
CoQ10	Coenzima Q10
CRP	*C-Reative Protein*
DA	Doença de Alzheimer
DI	Deficiência Intelectual
DNA	*Deoxyribonucleic Acid*

DS	*Down Syndrome*
EDN1	*Endothelin 1*
FAMERP	Faculdade de Medicina de São José do Rio Preto
FAPERP	Fundação de Apoio à Pesquisa e Extensão de São José do Rio Preto.
FAPESP	Fundação de Amparo à Pesquisa do Estado de São Paulo
Gamma-IFN	*Gamma-Interferon*
HWE	*Hardy-Weinberg Equilibrium*
IKBKB	*Inhibitor Of Nuclear Factor Kappa B Kinase Subunit Beta*
IL1	*Interleukin 1*
IL1-β	*Interleukin 1 - Beta*
IL2	*Interleukin 2*
IL4	*Interleukin 4*
IL6	*Interleukin 6*
IL7	*Interleukin 7*
IL8	*Interleukin 8*
IL10	*Interleukin 10*
IL12	*Interleukin 12*
IL13	*Interleukin 13*
IL17	*Interleukin 17*
IFN	*Interferon*
IFN-γ	*Interferon - Gama*
LTA4H	*Leukotriene A4 Hydrolase*
NOS2	*Nitric Oxide Synthase 2*
OR	*Odds Ratio*
PHA	*Phytohemagglutinin*
RANTES	*Regulated on Activation, Normal T Cell Expressed and Secreted*
Real-Time PCR	*Polymerase Chain Reaction*

SD	Síndrome de Down
SNC	Sistema Nervoso Central
SNPs	*Single Nuceotide Polymorphisms*
Taqman Assay ID	*Taqman Assay Identification*
Th1	*T helper cell 1*
Th2	*T helper cell 2*
Th17	*T helper cell 17*
TNF	*Tumor Necrosis Factor*

Resumo

Introdução: A síndrome de Down (SD) é a cromossomopatia humana mais frequente, com incidência aproximada de 1 em 850 nativivos e, em cerca de 90-95% dos casos, é atribuída à trissomia livre do cromossomo 21 resultante da não-disjunção meiótica. Os indivíduos com a síndrome apresentam várias características clínicas, incluindo alterações imunológicas que resultam em resposta inflamatória alterada. A resposta imune é modulada por citocinas pró e anti-inflamatórias cuja expressão pode ser influenciada por polimorfismos genéticos na região codificante ou promotora do gene. **Objetivos**: O presente estudo teve como objetivos avaliar as frequências dos polimorfismos -174G/C, -572G/C e -597G/A na região promotora do gene da interleucina (IL) *6* e dos polimorfismos -592C/A, -1082A/G e -819C/T na região promotora do gene da IL-10 em indivíduos com SD, e em um grupo controle sem a trissomia do cromossomo 21 e investigar o impacto dos genótipos estudados nos respectíveis níveis séricos das interleucinas. **Materiais e Métodos**: Amostras de DNA de 200 indivíduos com SD e 200 controles sem a síndrome foram submetidas à reação em cadeia da polimerase - polimorfismo no comprimento dos fragmentos de restrição (PCR-RFLP) ou PCR em tempo real para avaliação da presença dos polimorfismos IL-6 -174G/C, -572G/C e -597G/A e IL-10 -592C/A, -1082A/G e -819C/T. A dosagem sérica de IL-6 e IL-10 foi realizada em um subgrupo de indivíduos (54 casos e 54 controles) pela técnica de ELISA. A distribuição genotípica entre os grupos foi realizada por regressão logística pelo programa SNPStats, e a avaliação do desequilíbrio de ligação e frequência dos haplótipos foram realizadas pelo programa Haploview. A comparação dos níveis séricos de IL-6 e IL-10 entre os grupos foi realizada pelo teste de Mann Whitney. A análise das concentrações de interleucinas em

relação aos genótipos foi realizada com o teste de Kruskal-Wallis, utilizando o software GraphPad Prism versão 6.0. O erro aceito foi de 5%. **Resultado:** A frequência dos polimorfismos de IL-6 e IL-10 e dos seus haplótipos não mostrou diferenças entre os grupos caso e controle. Também não houve associação entre os níveis séricos de IL-6 e IL-10 e os polimorfismos de IL-6 e IL-10. Os níveis séricos de IL-10 foram aumentados no grupo caso em relação ao grupo controle. **Conclusão:** As frequências dos polimorfismos e haplótipos estudados não diferem entre indivíduos com SD e sem a síndrome e os genótipos não têm efeito nos níveis séricos de IL-6 e IL-10. Os níveis de IL-10 são aumentados em indivíduos com SD, mas os polimorfismos no gene IL-10 não são os principais fatores que influenciam a expressão aumentada da IL-10 na SD.

Palavras-chave: 1. Síndrome de Down; 2. Polimorfismo genético; 3. Interleucina 6; 4. Interleucina 10.

Abstract

Introduction: Down syndrome (DS) is the most frequent human chromosomopathy with approximate incidence of the 1 to 850 live births, nearly 90-95% of these cases are characterized by the presence of three copies of chromosome 21 as a result of the meiotic nondisjunction. DS individuals present many clinic features, including immunological changes that result in altered inflammatory response. The immune response is modulated by pro- and anti-inflammatory cytokines whose expression could be influenced by genetic polymorphisms in the coding or promoter region within the gene. **Objectives:** The study aimed to evaluate the frequencies of the -174G/C, -572G/C e -597G/A polymorphisms in the interleukin (IL) 6 gene promoter region and of the -592C/A, -1082A/G e -819C/T polymorphisms in the IL-10 gene promoter region in individuals with DS, and a control group without 21 trisomy, as well as to investigate the impact of the studied genotypes in the interleukins serum levels. **Material and Methods:** DNA samples of 200 DS individuals and 200 controls without DS were submitted to Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) or real time PCR for evaluate to presence of the -174G>C, -572G>C, and -597G>A IL-6 and -592C>A, -1082A>G, and -819C>T IL-10 polymorphisms. The serum measurement of IL-6 and IL-10 was performed in a subgroup (54 cases and 54 controls) by ELISA essay. The genotypic distribution between groups was performed by multiple logistic regression by SNPStats, program, and the linkage disequilibrium evaluation and haplotype frequency was performed by Haploview program. The comparison of IL-6 and IL-10 serum level between the groups was performed by Mann Whitney test, the interleukins concentrations analyze in relation to genotypes was performed by Kruskal-Wallis test, using the GraphPad Prism version 6.0 software. The

standard error of 5% was accept. **Result**: Either the frequency of IL-6 and IL-10 polymorphisms or their haplotypes did not show differences between the case and control groups. There was no association between the IL-6 and IL-10 serum levels and the IL-6 and IL-10 polymorphisms. IL-10 serum levels were increased in the case group in relation to control group. **Conclusio**n: The frequencies of the polymorphisms and haplotypes evaluated are not different between individuals with and without DS. Genotypes show no effect on the IL-6 and IL-10 serum levels. The IL-10 serum levels are increased in DS individuals, but the IL-10 polymorphisms are not the main factors that influence in higher expression of the IL-10 in DS.

Key words: 1. Down syndrome; 2. Genetic Polymorphism; 3. Interleukin 6; 4. Interleukin 10.

1. INTRODUÇÃO

1. INTRODUÇÃO

A síndrome de Down (SD) (MIM 190685),[1] caracterizada por um cromossomo 21 extra, é a causa mais frequente para malformações congênitas e deficiência intelectual, com ocorrência aproximada de um em cada 850 nativivos.[2, 3]

A maioria dos indivíduos com a síndrome apresenta três cópias completas do cromossomo 21 (trissomia livre) como resultado da não disjunção cromossômica meiótica materna; apenas 10% dos casos são resultantes da não disjunção cromossômica paterna.[4]

A trissomia livre é responsável por aproximadamente 90-95% dos casos da SD, enquanto as translocações cromossômicas, mais frequentemente entre os cromossomos 14 e 21, são observadas em cerca de 4-6% dos indivíduos, seguidas pelo mosaicismo (1-4%), representado por uma proporção de células com 46 cromossomos e outra com trissomia do 21 no mesmo indivíduo.[5, 6]

Os indivíduos com SD apresentam várias desordens físicas e funcionais[7] que incluem sinais dismórficos,[8] deficiência intelectual,[2] defeitos cardíacos congênitos,[9] malformações gastrointestinais e geniturinárias,[10] alterações ortodônticas,[11] problemas oftalmológicos, perda auditiva,[12] obstrução das vias aéreas superiores,[13, 14] disfunção da tireóide,[15] manifestação precoce da doença de Alzheimer,[16] risco aumentado para acidente cérebro vascular,[17] para leucemias específicas[18] e deficiência imunológica.[19]

As disfunções do sistema imune na SD foram atribuídas a anormalidades funcionais e morfológicas do Timo, alterações na diferenciação, maturação e ativação de células T,[20,

21) diminuição do número de linfócitos B, modificação de subclasses de células T, assim como alterações nos níveis de citocinas pró e anti-inflamatória.[22]

Citocinas constituem uma grande variedade de proteínas secretadas por várias células e coordenam diversas atividades celulares da resposta imune inata e adaptativa.[23] Podem ser classificadas segundo suas ações ou propriedades; citocinas que desencadeiam a resposta imune são denominadas pró-inflamatórias e aquelas que atenuam essa resposta restaurando a homeostase do organismo são denominadas citocinas anti-inflamatórias.[24] As citocinas pró-inflamatórias são produzidas principalmente por macrófagos envolvidos nas reações inflamatórias. Dentre as citocinas pró-inflamatórias destacam-se as interleucinas (IL) IL1-β, IL-2, IL-6, IL-7, IL-8, IL-12, interferon gama (IFN-γ) e fator de necrose tumoral alfa (TNF-α). As citocinas anti-inflamatórias são secretadas principalmente por macrófagos, linfócitos T helper de classe 1 e 2 (Th1 e Th2), células dendríticas, linfócitos T citotóxicos, linfócitos B, monócitos, eosinófilo, basófilos e mastócitos, e tem função de inibir ações de células ou citocinas inflamatórias.[24] Dentre as principais citocinas anti-inflamatórias destacam-se IL-4, IL-10, IL-13, interferon alfa (INF-α) e o fator transformador de crescimento beta (TGF-β).[25]

A IL-6 é uma das principais citocinas pró-inflamatórias, secretada por linfócitos T, macrófagos, monócitos, eosinófilos, células endoteliais, fibroblastos, adipócitos e miócitos, e regula a produção de moléculas de adesão para sinalizar a localização da inflamação no endotélio vascular e induzir a secreção de proteína quimiotáctica de monócitos, um importante mediador de liberação de outras citocinas, como TNF e IL-1.[26] Esta citocina aparece em níveis elevados na corrente sanguínea em menos de 24 horas após a exposição a patógenos e também se mostra elevada em pacientes com tumores malignos e metástase.

Além disso, tem a capacidade de induzir a resposta imune contra agentes externos nocivos à saúde.[26, 27]

Em relação às citocinas anti-inflamatórias, destaca-se a IL-10 que é produzida por monócitos, macrófagos, células T helper, linfócitos mastócitos e eosinófilos.[28] Esta citocina desempenha um papel contra doenças inflamatórias e autoimunes, diminuindo a ação de células produtoras de anticorpos e de interleucinas que estimulam a produção de anticorpos.[29] A IL-10 tem efeitos em múltiplas células e atua tanto na inflamação quanto na imunorregulação. Níveis elevados de IL-10 podem prejudicar a resposta a patógenos patogênicos e níveis baixos de IL-10 podem levar ao desenvolvimento de doença autoimune.[29, 30]

Variações nos níveis de IL-6 e IL-10, além de outras interleucinas, têm sido observadas em indivíduos saudáveis com a SD. Em estudo realizado por Trotta et al 2011[31] foi observado que indivíduos com SD apresentaram níveis séricos de IL-10, INF-γ e TNF-α aumentados em comparação com indivíduos com deficiência intelectual (DI) sem SD. Rostami et al. 2012[32] mostraram que níveis séricos de TNF-α e IFN-γ estavam aumentados em indivíduos com SD e em crianças com DI sem a síndrome quando comparados com o grupo controle (sem SD e DI). Entretanto, esses autores observaram que níveis de IL-10 estavam diminuídos em indivíduos com SD em relação aqueles com DI, mas que não diferiu do grupo controle.

Dosagem de citocinas no fluido gengival crevicular de adolescentes com SD mostrou níveis séricos elevados de IL-6, IL-10, IL-1β, IL-4, IL-12, IFN-γ e TNF-α nesses indivíduos quando comparado com aqueles sem SD.[33] Em um estudo realizado por Cetiner

et al. 2010[34] os níveis séricos de IL-4 e IL-10 também mostraram-se elevados em crianças com SD comparados com os de crianças sem a síndrome, enquanto os níveis de IL-6 e TNFα foram menores naquelas com SD. Considerando que as interleucinas anti-inflamatórias IL-4 e IL-10 inibem a síntese das citocinas pró-inflamatórias IL-6 e TNF, esses autores propuseram que os níveis reduzidos dessas citocinas, possivelmente, comprometem a proliferação e função de macrófagos e outros fagócitos, o que poderia explicar a causa das infecções recorrentes observadas nas crianças com SD. De fato, a deficiência imunológica é a principal causa de internação de crianças com SD, e as infecções, em especial do trato respiratório, são a principal causa de internações e umas das principais causas de óbito dos indivíduos com a síndrome.[35, 36]

Estudos com SD em modelos de infecção e inflamação também mostraram variações nos níveis de citocinas quando comparados com grupos controles. Redução da expressão de IL-10 foi observada em indivíduos com SD com periodontite em comparação a indivíduos com periodontite sem SD, atenuando mediadores anti-inflamatórios e estimulando o aumento de mediadores pró-inflamatórios, o que sugere que a via de expressão da IL-10 é um importante modulador da resposta imune.[37]

Cultura de células sanguíneas de indivíduos com SD, estimuladas com vírus *Influenza A*, mostrou aumento da produção de TNF-α, IL-1β, IL-6, e IL-8[38] e, estimuladas com *Streptococcus pneumoniae* apresentou um aumento de IL-10.[39] Esses autores sugerem que a produção aumentada de citocinas pró-inflamatórias pode ser responsável pela inflamação e dano tecidual excessivos resultando em maior gravidade das doenças virais em crianças com SD, enquanto o aumento da IL-10 pode predispor os indivíduos

com SD a um quadro clínico mais grave de pneumonia pneumocócica em função da resposta anti-inflamatória aumentada.

Concentração aumentada de IL-10 também foi observada em células mononucleares do sangue periférico de adolescentes saudáveis com SD, estimuladas por fitohemaglutinina (PHA).[40] Além disso, esses autores observaram um aumento de IFN-γ nas células estimuladas com PHA ou citomegalovírus e IL-7 aumentada no soro desses indivíduos.

Também tem sido sugerido que produção anormal de citocinas pode contribuir com processos degenerativos do sistema nervoso central (SNC) na SD. Sabe-se que concentrações séricas de IL-6 mostram-se elevadas em pacientes com doença de Alzheimer (DA) do tipo esporádica, em um estágio semelhante de demência de indivíduos com SD, quando comparadas com controles saudáveis[41]. Licastro et al. 2005[42] detectaram níveis semelhantes de IL-6 em crianças com SD e em pacientes com DA e sugerem que o fenótipo imune alterado em indivíduos jovens com SD pode representar uma manifestação precoce de alterações do SNC que ao longo dos anos resultaria em declínio cognitivo e demência nesses indivíduos. Griffin et al. 1986[43] observaram imunoatividade aumentada de IL-1 no cérebro de indivíduos com SD e na DA. Franciosi et al., 2005[44] demonstraram que a IL-8 potencializa o efeito do peptídeo beta-amilóide na indução de secreção de citocinas inflamatórias em cultura de células microgliais humana, sugerindo um possível papel no desenvolvimento precoce da neuropatologia de Alzheimer na SD. O IFN-γ também pode causar neurodegeneração e produção de β-amilóide em modelo animal da SD[45] e, possivelmente contribuir para a disfunção cognitiva.

Acredita-se que a proteína solúvel precursora da beta amiloide e várias formas peptídicas da beta amiloide estimulam a ativação da sinalização da resposta imune inata no cérebro[46]. Estudos mostram que o estado pró-inflamatório pode reduzir o acúmulo de proteína beta amiloide em modelo de camundongos atuando beneficamente no combate da DA.[47-52] Entretanto, também tem sido sugerido que expressão exacerbada de citocina pró-inflamatória, como a IL-6, pode causar danos ao tecido saudável[53] e resultar em inflamação exacerbada, levando à disfunção neuronal e consequente deterioração dos neurônio, como observado na progressão da DA.[54] Em relação às citocinas anti-inflamatória, a contribuição para a neurodegeneração não está clara. O estudo de Chakrabarty et al., 2015[55] sugere que altas concentrações de IL-10 contribuem para a redução da fagocitose da beta amiloide pelas micróglias, causando consequente acúmulo, como observado em indivíduos SD.

Recentemente, Zaki et al. 2017[22] encontraram níveis plasmáticos aumentados de IL-6 e TNFα e reduzidos de coenzima Q10 (CoQ10), enzima antioxidante, além da correlação significativamente positiva entre os níveis de CoQ10 e quociente de inteligência e sugerem que IL-6, TNF-α podem representar biomarcadores “chave” no processo neurodegenerativo da SD. Os níveis de INF-γ, TNF-α, IL-6, and IL-10 também foram mais elevados em indivíduos com SD com sintomas clínicos da DA e também naqueles com SD sem declínio cognitivo comparado ao grupo controle.[56]

Os níveis de citocinas podem ser determinados entre outras causas por polimorfismos genéticos na região codificante ou na região promotora do gene. Polimorfismos de nucleotídeos únicos (SNP) são as variações genéticas mais comuns na espécie humana e são caracterizados pela alteração de um único nucleotídeo (A, T, C ou G)

na sequência do genoma. Os SPNs podem acontecer na região promotora de um gene em particular e, nesse caso, são identificados pelo sinal (-) antes do numeral que identifica a posição no gene. Essas alterações genéticas podem causar um potencial efeito regulatório na expressão e síntese proteica do gene.[57]

Três polimorfismos na região promotora do gene *IL-10*, -592 C>A (rs1800872), -819C>T (rs1800871) e -1082A>G (rs1800896) tem sido descritos[58] e foram associados com alterações da expressão de IL-10 e doenças inflamatórias, tais como tuberculose[59], lupus eritromatoso,[60] câncer de colon[61] e artrite reumatoide.[62]

Em relação aos polimorfismos do gene *IL-6*, três SNPs, -597G>A (rs1800797), -572G>C (rs1800796) e -174G>C (rs1800795), foram descritos na região promotora do gene.[63] Esses polimorfismos foram associados com com alterações da resposta inflamatória[27] e suceptibilidade à doenças como diabete melitus tipo 2,[64] lupus eritromatoso[60] e artrite crônica juvenil.[63]

Considerando a importância da IL-6 e IL-10 nas respostas inflamatórias, que polimorfismos genéticos podem resultar em alterações na expressão e síntese destas citocinas e a ausência de estudos sobre o impacto de polimorfismos nos genes *IL-6* e *IL-10* nos níveis destas citocinas em indivíduos com SD, o estudo de variantes genéticas funcionais mostra-se relevante para melhorar o entendimento da resposta imunológica/inflamatória na SD.

1.1 OBJETIVOS

1. Avaliar as frequências dos polimorfismos -174G>C, -572G>C e -597G>A na região promotora do gene da *IL-6* e dos polimorfismos -592C>A, -1082A>G e -819C>T na região promotora do gene da *IL-10* em indivíduos com SD e em um grupo controle sem a trissomia do cromossomo 21.

2. Investigar o impacto dos genótipos estudados nos respectíveis níveis séricos das interleucinas, visando identificar diferenças entre os grupos que possam esclarecer a maior frequência de alterações imunológicas em indivíduos com a síndrome.

2. ARTIGOS CIENTÍFICOS

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ARTIGO I:

Título: Polymorphisms of interleukin 6 in Down syndrome individuals: a case-control study.

Autores: Marlon Fraga Mattos, Lucas Uback, Patrícia Matos Biselli-Chicote, Eny Maria Goloni-Bertollo, Érika Cristina Pavarino.

Periódico: Genetics and Molecular Research, aceito para publicação em julho de 2017 (aprovação no Anexo I).

ARTIGO II:

Título: Interleukin 6 and 10 serum levels and genetic polymorphisms in children with Down syndrome.

Autores: Marlon Fraga Mattos, Patrícia Matos Biselli-Chicote, Joice Matos Biselli, Lucas Uback, Thiago Luís da Silva Assembleia, Eny Maria Goloni-Bertollo, Érika Cristina Pavarino.

Periódico: Mediators of Inflammation, a ser submetido para publicação.

2.1 ARTIGO I:

Polymorphisms of interleukin 6 in Down syndrome individuals: a case-control study

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Polymorphisms of IL-6 in Down syndrome

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ABSTRACT

Down syndrome (DS) individuals present impaired adaptive immune system. However, the etiology of the immunological deficiency in these individuals is not completely understood. This study investigated the frequency of interleukin 6 polymorphisms (rs1800795, rs1800796, and rs1800797) in individuals with DS and individuals without the syndrome. The study included 282 individuals, 94 with DS attended at the General Genetics Outpatient Service of Hospital de Base, São José do Rio Preto, SP, Brazil, and 188 individuals without DS attended at the Pediatric Service of Hospital de Base de São José do Rio Preto, SP, Brazil. Genotyping was performed by allelic discrimination technique by real-time polymerase chain reaction using TaqMan SNP Genotyping Assays (Applied Biosystems). There was no difference in the genotype frequency between individuals with and without DS for the evaluated polymorphisms ($P > 0.05$). The frequency of interleukin 6 polymorphisms did not differ significantly between individuals with and without DS in the casuistic analyzed.

Key words: Down syndrome; Trisomy 21; Polymorphism; Interleukin 6

INTRODUCTION

Down syndrome (DS) or trisomy 21 is a common chromosomal disorder among live-born infants (1:700 live births) (Jorde et al., 2015). Individuals with DS present an impairment of adaptive immune system, with reduced level of lymphocytes (Kusters et al., 2009) and an increase in the susceptibility to infections and autoimmune diseases (Gillespie et al., 2006).

Previous studies reported by our group showed differential expression of genes involved in the immunological and inflammatory processes in DS individuals, which could explain the immunological deficiencies observed in these individuals. Genes with significant high expression include *CD52*, *RANTES*, *CCR2*, *BCL2L1*, *IL10*, and *CCR5*, and genes with reduced expression were *CD46*, *FOS*, *BCL2*, *CCL3*, *IL6*, *EDN1*, *CD40LG*, *CD80*, *CCR7*, *IKBKB*, *CD28*, *NOS2*, *CD19*, *CD40*, *SKI*, *BDKRB1*, and *LTA4H* (Sommer et al., 2008; Zampieri et al., 2014; Silva et al., 2016).

Research in DS has shown that the 21 trisomy is associated with a reduction of T and B cells, alteration in differentiation and maturation of B cells, and activation of the Th1 and Th2 cells (Guazzarotti et al., 2009; Carsetti et al., 2015; Schoch et al., 2017). DS also present functional and morphological alterations of the thymus that is smaller (Kusters et al., 2009; Lorenzo et al., 2013), possibly as a result of the increase of cytokines as gamma-interferon (gamma-IFN), and tumor necrosis factor (alpha-TNF) (Murphy et al., 1992).

The cytokines play a role in the regulation of growth and differentiation of lymphocytes, activation and regulation of inflammatory cells as mast cells, neutrophils and eosinophils, and communication among the immunological system cells (Abbas et al. 2014) . This protein class is classified in interleukins (IL), TNF, chemokines (chemotactic cytokines), IFN, and growth mesenchymal factor (Beaulieu and Pain, 2010). The cytokines IL1, IL2, IL6, IL7, IL8, IL12, IL17, TNF, and gamma-IFN are considered pro-inflammatory, and the cytokines IL4, IL10, IL13, and transforming growth factor-beta play an anti-inflammatory role (Beaulieu and Pain, 2010).

Studies in DS individuals have shown increased serum levels of IL4 and IL10 and decreased serum levels of IL6 and alpha-TNF compared with children without the

syndrome (Cetiner et al., 2010). Furthermore, an increase in the production of alpha-TNF, beta-IL1, -IL6, -IL8, alpha-INF, gamma-INF, and IL10 was observed in peripheral blood culture of DS children, stimulated by influenza A virus and by *Streptococcus pneumoniae* (Broers et al., 2012, 2014). A significant increase in IL7 serum levels in healthy adolescents with DS was observed, and peripheral blood mononuclear cells from these individuals stimulated with phytohemagglutinin (PHA) and cytomegalovirus (CMV) presented an increase of gamma-IFN and IL10 levels (Guazzarotti et al., 2009). The increase of the beta-IL1, -IL4, -IL6, -IL10, -IL12, gamma-IFN, and alpha-TNF levels and cytokines produced by T-helper cells (Th1, Th2, and Th17) was also observed in gingival crevicular fluid of DS adolescents compared with individuals without DS (Tsilingaridis et al., 2012).

The *IL6* gene is located on the short arm of chromosome 7 (7p21) and presents three important polymorphisms in the promoter region: -174G>C (rs1800795), -572C>G (rs1800796), and -597G>A (rs1800797). The polymorphism -174G>C has an influence on the transcriptional regulation of the *IL6* and is associated with the levels of this cytokine (Wang et al., 2017). The allele *IL6 -572G* was associated with increased IL6 serum levels in patients undergoing post-percutaneous coronary intervention restenosis (Gao et al., 2013) and coronary heart disease susceptibility (Zhang et al., 2017). A study also observed that smokers and non-smokers carrier the *IL6 -572GG* genotype present increased levels of this protein (Shin et al., 2007). Chou et al. (2016) found that the polymorphism *IL6* -597G>A may be associated with susceptibility and severity of community-acquired pneumonia (CAP). The present study investigated the frequency of three genetic polymorphisms in the *IL6* gene (rs15800795, rs15800796, and rs15800797) in DS individuals and without the

syndrome, aiming to identify differences between the groups that could be associated with the clinical conditions of the syndrome.

MATERIAL AND METHODS

Subjects

The Research Ethics Committee of Faculdade de Medicina de São José do Rio Preto - FAMERP (No. 427.782, CAAE 20112313.9.0000.5415) formally approved the study. The study included 282 individuals, 94 DS children with trisomy 21 (case group), from the General Genetics Outpatient Service of Hospital de Base, São José do Rio Preto, SP, Brazil, and 188 individuals without DS (control group) from the Pediatric Service of Hospital de Base de São José do Rio Preto, SP, Brazil.

The group case consisted of 51 males and 43 females with a mean age of 4.3 years (ranging from 1 to 30 years of age) and the control group included 96 males and 92 females with a mean age of 4.4 years (ranging from 1 to 14 years of age).

Genotyping analysis

DNA isolation was performed from peripheral blood (Salazar et al., 1998). The genotyping of the polymorphisms was performed using the TaqMan Allele Discrimination Assay (Applied Biosystems), following manufacturer's instruction. Table 1 presents the specific assays for each polymorphism evaluated. The reactions were performed on

StepOne Plus Real-Time PCR System (Applied Biosystems) and cycled following manufacturer's instructions.

Table 1. Taqman assays (Applied Biosystems) for genotyping of the polymorphisms by allele discrimination.

Polymorphism (rs*)	Substitution	Taqman Assay ID
IL6 rs1800795	*G→C*	C_1839697_20
IL6 rs1800796	*G→C*	C_11326893_10
IL6 rs1800797	*G→A*	C_1839695_20

*http://www.ncbi.nlm.nih.gov/projects/SNP/

Statistical analysis

The allele frequencies of the polymorphisms were evaluated for Hardy-Weinberg (HWE) equilibrium by the chi-square test using the BioEstat software version 5.0. The genotype distribution between the groups was evaluated in the codominant, dominant, recessive, overdominant, and additive model, using the SNPStats software (http://bioinfo.iconcologia.net/SNPstats_web) program. Values of $P \leq 0.05$ were considered significant.

RESULTS

The allele frequencies of the polymorphisms -174G>C (rs1800795), -572C>G (rs1800796), and -597G>A (rs1800797) were in HWE in case and control groups ($P = 0.8$ and $P = 0.57$ for -174G>C; $P = 1$ and $P = 0.49$ for -572C>G; $P = 1$ and $P = 1$ for -597G>A)

(Table 2). There was no difference in the genotype distribution between the groups with DS and without the syndrome ($P > 0.05$) (Table 3).

Table 2. Allele frequencies of -174G>C (rs1800795), -572C>G (rs1800796), and -597G>A (rs1800797) polymorphisms.

Polymorphism	Allele	Case	Control
-174G>C (rs1800795)	*C*	0.27	0.26
-572G>C (rs1800796)	*C*	0.10	0.12
-597G>A (rs1800797)	*A*	0.26	0.27

Table 3. Genotype distribution of -174G>C (rs1800795), -572C>G (rs1800796), and -597G>A (rs1800797) polymorphisms between the groups with DS (case) and without the syndrome (control).

	Genotype	Control	DS	OR (95%CI)	P value
***IL-6* -597G>A**					
Codominant	*GG*	100 (53.2%)	52 (55.3%)	1.00	0.96
	AG	75 (39.9%)	36 (38.3%)	1.07 (0.63-1.80)	
	AA	13 (6.9%)	6 (6.4%)	1.12 (0.40- 3.12)	
Dominant	*GG*	100 (53.2%)	52 (55.3%)	1.00	0.78
	AG - AA	88 (46.8%)	42 (44.7%)	1.07 (0.65-1.77)	
Recessive	*GG - AG*	175 (93.1%)	88 (93.6%)	1.00	0.86
	AA	13 (6.9%)	6 (6.4%)	1.09 (0.40-2.97)	
Overdominant	*GG - AA*	113 (60.1%)	58 (61.7%)	1.00	0.85
	AG	75 (39.9%)	36 (38.3%)	1.05 (0.63-1.76)	
Log-additive	---	---	---	1.06 (0.71-1.59)	0.77
***IL-6* -174G>C**					
Codominant	*GG*	100 (53.2%)	49 (52.1%)	1.00	0.97
	GC	77 (41%)	39 (41.5%)	0.95 (0.57-1.60)	
	CC	11 (5.8%)	6 (6.4%)	0.89 (0.31-2.56)	
Dominant	*GG*	100 (53.2%)	49 (52.1%)	1.00	0.82
	GC-CC	88 (46.8%)	45 (47.9%)	0.94 (0.57-1.56)	
Recessive	*GG-GC*	177 (94.2%)	88 (93.6%)	1.00	0.86
	CC	11 (5.8%)	6 (6.4%)	0.91 (0.33-2.55)	
Overdominant	*GG-CC*	111 (59%)	55 (58.5%)	1.00	0.89
	GC	77 (41%)	39 (41.5%)	0.96 (0.58-1.60)	
Log-additive	---	---	---	0.95 (0.63-1.43)	0.80
***IL-6* -572G>C**					
Codominant	*GG*	146 (77.7%)	76 (80.8%)	1.00	0.69
	GC	38 (20.2%)	17 (18.1%)	1.18 (0.63-2.24)	
	CC	4 (2.1%)	1 (1.1%)	2.12 (0.23-19.29)	
Dominant	*GG*	146 (77.7%)	76 (80.8%)	1.00	0.50
	GC-CC	42 (22.3%)	18 (19.2%)	1.24 (0.66-2.30)	
Recessive	*GG-GC*	184 (97.9%)	93 (98.9%)	1.00	0.50
	CC	4 (2.1%)	1 (1.1%)	2.04 (0.23-18.57)	
Overdominant	*GG-CC*	150 (79.8%)	77 (81.9%)	1.00	0.63
	GC	38 (20.2%)	17 (18.1%)	1.17 (0.62-2.21)	
Log-additive	---	---	---	1.25 (0.71-2.18)	0.43

DISCUSSION

Alteration in the immune system, such as functional and morphological thymus abnormalities (Bloemers et al., 2011; Karl et al., 2012), lymphocytopenia, and alteration in differentiation, maturation, and activation of the T lymphocyte (Guazzarotti et al., 2009; Lorenzo et al., 2013) is frequently observed in DS individuals (Kusters et al., 2009). It can be responsible for increased incidence of infections, mainly in the respiratory tract (Bloemers et al., 2010; Broers et al., 2012), and occurrence of autoimmune disease in DS (Gillespie et al., 2006; Pellegrini et al., 2012).

The physiopathology of various infections involving the immunological system has the inflammation as a common factor (Trotta, 2009). The cytokines belong to a diversified group of protein, which participated in various biological processes, including the mediation of the inflammatory response (Zhang and An, 2007). Changes in the concentration of proinflammatory and anti-inflammatory cytokines in DS individuals demonstrate its association with the syndrome pathogenesis (Tsilingaridis et al., 2003; Guazzarotti et al., 2009; Cetiner et al., 2010; Broers et al., 2012, 2014). The study of Cetiner et al. (2010) observed that the IL6 serum levels were lower in DS children compared with individuals without the syndrome. The authors proposed that the reduced levels of this cytokine possibly difficult the proliferation and function of macrophages and other phagocytes, what could explain the reason for the recurrent infections observed in DS.

Considering that changes in the cytokine concentration may be due to genetic polymorphisms, our study investigated the frequency of -174G>C (rs1800795), -572C>G (rs1800796), and -597G>A (rs1800797) polymorphisms in DS children and without the

syndrome, aiming to identify differences between groups that may be associated with clinical manifestations of the syndrome. Our results showed no difference in genotype distribution between individuals with and without DS for the polymorphisms evaluated.

In our study, the *-174C* allele frequency was 0.27 in the case group and 0.26 in the control group. These frequencies are different from those observed in other ethnic groups, varying of 0.40 in Europeans from the United Kingdom, 0.15 in Gujarati Indian , and 0.05 in the Afro-Caribbean (Fishman et al., 1998). In Brazilians, the study of Vicari et al. (2015) showed a *C* allele frequency of 0.14 in patients with anemia and 0.15 in the control group. Teixeira et al. (2014) observed similar frequencies in individuals with periodontitis (0.15) and the control group (0.17). Interestingly, the allele frequency of this polymorphism was different between three ethnic groups of the Brazilian population presenting a *C* allele frequency of 14.5% in northeast region patients (descendant of Africans and Portuguese), 36.7% in South region (descendant of Germans), and 2.5% in Amerindian from Tiriyó tribe (Gadelha et al., 2005).

In our study, the *-174GG* genotype was the most frequent in the case group (52.1%) and the control group (53.2%). The *GC* genotype frequency was 41.5% in the case group and 41% in the control group. The frequency of the *CC* genotype was 6.4% in the case group and 5.8% in the control group. Teixeira et al. (2014) showed a *GG* genotype frequency of 76.1 and 69.4% for the case and control groups, respectively, in patients with periodontitis in the Brazilian population. The frequency of the heterozygous genotype *GC* was 17.2% in the case group and 27.6% in the control group, while the genotype *CC* was 6.7% in the case group and 3.1% in the control group. Vicari et al. (2015) observed in Brazilians with sickle-cell anemia that the genotype *GG* was most frequent in the case

(74%) and the control (75%) groups. The *GC* genotype presented frequency of 24 and 20% in both groups, and the genotype *CC* was present in only 2% of the case individuals and 5% of the control group.

Regarding the -572G>C (rs1800796) polymorphism, a study performed in Sweden with myocardial infarction individuals did not find the *CC* genotype and detected high prevalence of the *GG* genotype (92%), while the heterozygous genotype *GC* was present in 8% of the patients with acute myocardial infarction treated with thrombolysis (Bennermo et al., 2004). Our study also observed higher frequency of the *GG* genotype (80.8% in the case group and 77.7% in the control group) and low frequency of the *CC* genotype (1.1% in the case group and 2.1% in the control group); the frequency of the heterozygous genotype *CG* was 18.1% in the case group and 20.2% in the control group. In the Brazilian population, the *CC* genotype was not present among patients with anemia, and only 3% of the control individuals showed these genotypes (Vicari et al., 2015). These authors also found higher frequency of the *GG* genotype in the case (78%) and control group (68%). On the other hand, a study performed with the Korean population observed prevalence of the genotype *CC* (57%), while the *GC* and *GG* genotypes showed frequencies of 36.2 and 6.8%, respectively (Shin et al., 2007).

Our study showed similar frequencies of the 597G>A (rs1800797) polymorphism in both groups (case and control) for the *G* allele (0.74 and 0.73) and for the *A* allele (0.26 and 0.27). The *G* allele was also most frequent in a Pakistani population with macular degeneration (*G* Allele: 0.96 and *A* allele: 0.04) and in the control group (*G* allele: 0.82 and A allele: 0.18) (Ambreen et al., 2015). A study in a Chinese population showed that the frequency of the *G* allele was 99.48%, and the *A* allele was 0.52% (Gao et al., 2014). The

study performed by Vicari et al. (2015) in the Brazilian population showed that the frequency of the polymorphic *A* allele was 15% in anemia patients and 17% in the control group.

These variations in allele and genotype frequencies among the studies may be due to the genetic origins. The Brazilian population is heterogeneous, and this heterogeneity is a result of crosses between European, African, and Amerindian (Alves-Silva et al., 2000). Allele frequencies vary among the populations probably because of the genetic drift or the adaptation to particular environmental factors (Pena et al., 2011).

In conclusion, in the population evaluated there is no evidence of difference between groups of individuals with DS and without the syndrome for -174G>C (rs1800795), -572C>G (rs1800796), and -597G>A (rs1800797) polymorphisms. However, this may be the result of the reduced sample size, being necessary other studies to better understanding the contribution of these genetic polymorphisms in the modulation of the risk for immunological alterations in DS individuals.

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2.2 ARTIGO II:

Interleukin 6 and 10 serum levels and genetic polymorphisms in children with Down syndrome

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Abstract

Immunological impairment is a condition often observed in individuals with Down syndrome (DS). The immune response is modulated by pro- and anti-inflammatory cytokines whose expression could be influenced by genetic polymorphisms. The study aimed to evaluate the frequencies of the -174G>C, -572G>C and -597G>A polymorphisms in the *interleukin* (*IL*) *6* gene and of the -592C>A, -1082A>G and -819C>T polymorphisms in the *IL-10* gene in healthy individuals with and without DS, as well as to investigate the impact of the genotypes in the interleukins serum levels. Genetic polymorphisms (-174G>C, -572G>C, and -597G>A *IL-6* and -592C>A, -1082A>G, and -819C>T *IL-10*) 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 ELISA assays. The frequencies of the polymorphisms and haplotypes evaluated were not different between individuals with and without DS. Genotypes show no effect on the IL-6 and IL-10 serum levels. The IL-10 serum levels are increased in DS individuals, but the IL-10 polymorphisms are not the main factors that influence in higher expression of the IL-10 in DS.

Key words: Down syndrome; Trisomy 21; Genetic polymorphism; Interleukin 6; Interleukin 10;

Background

Immunological impairment is a condition often observed in individuals with Down syndrome (DS), which present 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 pro-inflammatory cytokines, which regulate T-cell differentiation. Regulatory cytokines include interleukins (IL), interferons (IFN), tumor necrosis factors (TNF), and growth factors [4].

Interleukin (IL) 6 is a pro-inflammatory cytokine produced by leukocytes, adipocytes, endothelial cells, fibroblasts, and myocytes. IL-6 induces the production of mediators to 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 pro-inflammatory molecules and avoid the 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 (Th) 1 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 of the control of inflammatory response [8]. Imbalance between pro- and anti-inflammatory cytokines avoids the adequate function of the immune system.

Variations in the genes encoding cytokines can be involved in the modulation of inflammatory responses and in the susceptibility to inflammatory disorders. *IL-10* gene polymorphisms -1082G>A (rs1800896), -829C>T (rs1800871), and -592C>A (rs1800872) were described [9] and have been associated with alterations in IL-10 expression and

inflammatory diseases, such as tuberculosis [10], systemic lupus erythematosus [11], colon cancer [12], and rheumatoid arthritis [13].

The single-nucleotide polymorphisms -597G>A (rs1800797), -572G>C (rs1800796), and -174G>C (rs1800795) were described within the promoter region of *IL-6* gene [14, 15]. These polymorphisms have been associated with alteration in the inflammatory response [16] and susceptibility to diseases, such as type-2 diabetes mellitus [17], systemic lupus erythematosus [11], and systemic-onset juvenile chronic arthritis [14].

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 in these individuals and compare with individuals without DS, besides to evaluate the association between these polymorphisms and IL-6 and IL-10 serum levels.

Materials and Methods

Subjects

The study included 200 individuals with DS (mean age = 4.3 years, 108 males and 95 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. The study was approved by the Research Ethics Committee of Faculdade de Medicina de São José do Rio Preto - FAMERP (No. 427.782).

Only individuals without leukemia, acute or chronic infection and those which did not receive medication or immunization within 6 weeks from the serum collection were selected for the interleukins dosage. C-reactive protein (CRP) was quantified by electrochemiluminescence and only samples with concentration ≤ 0,5mg/dl were included on analysis.

Polymorphisms analysis

DNA was extracted from peripheral blood [18]. Genotyping of *IL-10* -1082A>G and -592C>A polymorphisms was performed by polymerase chain reaction (PCR) - Restriction Fragment Length Polymorphism (RFLP) analysis, according to Lee et al., (2005), with modifications. Primer sequences used for detection of *IL-10* -1082A>G and Il-*10* -592C>A polymorphisms were: Foward: 5'-TCTGAAGAAGTCCTGATGTC-3'and Reverse: 5'-CTCTTACCTATCCCTACTTCC-3', and Foward: 5'- GGTGAGCACTACCTGACTAGC -3' and Reverse: 5' – CCTAGGTCACAGTGACGTGG, respectively.

PCR products were digested by the restriction enzymes MnlI (New England Biolabs) and RsaI (New England Biolabs) 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 the TaqMan SNP Genotyping Assays (Applied Biosystems, C_1747362_10, C_1839697_20, C_11326893_10 and C_1839695_20), following manufacturer's instructions.

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 with wild-type homozygous genotype, three heterozygous, and three with mutated homozygous genotype for each polymorphism), and 54 individuals without DS (three with wild-type homozygous genotype, three heterozygous, and three with mutated homozygous genotype for each polymorphism). The interleukins concentrations were also evaluated according to the genotype combination and haplotypes. IL-06 and IL-10 quantification was performed using the Novex ELISA kit (Life Technologies), following manufacturer's instructions, and analyzed on Multiskan FC Microplate Photometer (Thermo Scientific) at 450 nm.

Statistical analysis

SNPStats program (http://bioinfo.iconcologia.net/SNPstats_web) was used to analyze the polymorphisms. Allele frequencies were evaluated for Hardy-Weinberg (HWE) equilibrium by the chi-square test using the BioEstat software, version 5.0. Genotype distribution between the groups was evaluated by logistic regression in the codominant, dominant, recessive, overdominant, and additive model. The results were presented as odds ratio (OR) at 95% (CI95%). Haplotype analysis was performed using Haploview software, version 4.2 Comparison of IL-6 and IL-10 serum levels between the groups was performed by Mann Whitney test. Analysis of interleukins concentrations in relation to the genotypes was performed using Kruskal-Wallis test, using GraphPad Prism software version 6.0. The error accepted was 5%.

Results

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) was in accordance with Hardy-Weinberg equilibrium (HWE) in DS (P = 0.68 for -174G>C; P = 0.48 for -572G>C; P = 0.68 for -597G>A) and control (P = 0.71 for -174G>C; P = 0.51 for -572C>G; P = 1 for -597G>A) groups.

The genotype frequencies of *IL-10* -592C>A polymorphism did not differ from those we would expect under HWE in DS (P = 0.75) and control (P = 0.19) groups. *IL-10* -819C>T polymorphisms was in accordance with HWE only in 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.

The polymorphic alleles of *IL-6* and *IL-10* were less frequent in DS and control groups, but no significant statistical was observed (P>0,05). The logistic regression did not show statistic difference between the groups on the dominant, recessive, overdominant, codominant and additive genotypic models (P>0,05). (Table 1 and 2). Haplotype analyses were conducted to evaluate the combined effect of the polymorphisms. The *IL-6* polymorphisms were in strong linkage disequilibrium as well as the *IL-10* polymorphisms. The haplotypes frequencies did not differ between the groups (data not shown).

Table 1. Genotype distribution of *IL-6* -597G>A, *IL-6* -174G>C, and *IL-6* -572G>C polymorphisms in DS and control groups.

	Genotype	Control	DS	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)	
Dominant	GG	107(53.5%)	122 (61%)	1.00	0.14
	GA-AA	93(46.5%)	78 (39%)	1.35 (0.91-2.01)	
Recessive	GG-GA	186 (93%)	192 (96%)	1.00	0.19
	AA	14 (7%)	08 (4%)	1.80 (0.74-4.40)	
Overdominant	GG-AA	121 (60.5%)	130 (65%)	1.00	0.38
	GA	79 (39.5%)	70 (35%)	1.20 (0.80-1.81)	
Additive	---	---	---	1.33 (0.96-1.86)	0.09
***IL-6* -174G>C**					
Codominant	GG	108 (54%)	120 (60%)	1.00	
	GC	80 (40%)	72 (36%)	1.22 (0.81-1.85)	0.41
	CC	12 (6%)	08 (4%)	1.66 (0.65-4.21)	
Dominant	GG	108 (54%)	120 (60%)	1.00	0.24
	GC-CC	92 (46%)	80 (40%)	1.27 (0.85-1.89)	
Recessive	GG-GC	188 (94%)	192 (96%)	1.00	0.36
	CC	12 (6%)	08 (4%)	1.53(0.61-3.82)	
Overdominant	GG-CC	120 (60%)	128 (64%)	1.00	0.44
	GC	80 (40%)	72 (36%)	1.18 (0.78-1.76)	
Additive	---	---	---	1.25 (0.9-1.75)	0.19
***IL-06* -572G>C**					
Codominant	GG	155(77.5%)	156 (78%)	1.00	0.37
	GC	41 (20.5%)	43(21.5%)	0.97 (0.6-1.57)	
	CC	04 (2%)	01 (0.5%)	4.07 (0.45-36.78)	
Dominant	GG	155 (77.5%)	156 (78%)	1.00	0.88
	GC-CC	45 (22.5%)	44 (22%)	1.04 (0.65-1.66)	
Recessive	GG-GC	196 (98%)	199(99.5%)	1.00	0.16
	CC	04 (2%)	01 (0.5%)	4.10 (0.45-36.96)	
Overdominant	GG-CC	159 (79.5%)	157 (78.5%)	1.00	0.83
	GC	41 (20.5%)	43 (21.5%)	0.95 (0.59-1.54)	
Additive	---	---	---	1.11 (0.72-1.72)	0.64

Table 2. Genotype distribution of *IL-10* -1082A>G, *IL-10* -592C>A, and *IL-10* -819C>T polymorphisms in DS and control groups

	Genotype	Control	DS	OR (95% CI)	P value
***IL-10* -1082A>G**					
Codominant	AA	104 (52%)	95 (47.5%)	1.00	
	AG	63 (31.5%)	75 (37.5%)	0.76 (0.49-1.18)	0.44
	GG	33(16.5%)	30 (15%)	1.01 (0.57- 1.78)	
Dominant	AA	104 (52%)	95 (47.5%)	1.00	0.36
	AG-GG	96 (48%)	105 (52.5%)	0.83 (0.56-1.23)	
Recessive	AA-AG	167 (83.5%)	170 (85%)	1.00	0.67
	GG	33 (16.5%)	30 (15%)	1.12 (0.66-1.93)	
Overdominant	AA-GG	137 (68.5%)	125 (62.5%)	1.00	0.20
	AG	363 (31.5%)	75(37.5%)	0.76 (0.50-1.15)	
Additive	---	---	---	0.95 (0.72-1.24)	0.68
***IL-10* -592C>A**					
Codominant	CC	98 (49%)	91 (45.5%)	1.00	
	CA	78 (39%)	86 (43%)	0.84 (0.55-1.27)	0.70
	AA	24 (12%)	23 (11.5%)	0.96 (0.51-1.83)	
Dominant	CC	98 (49%)	91 (45.5%)	1.00	0.47
	CA-AA	102 (51%)	109 (54.5%)	0.86 (0.58-1.28)	
Recessive	CC-CA	176 (88%)	177 (88.5%)	1.00	0.88
	AA	24 (12%)	23 (11.5%)	1.05 (0.57-1.93)	
Overdominant	CC-AA	122 (61%)	114 (57%)	1.00	0.41
	CA	78 (39%)	86 (43%)	0.84 (0.57-1.26)	
Additive	---	---	---	0.93 (0.70-1.25)	0.65
***IL-10* -819C>T**					
Codominant	CC	84 (42%)	83 (41.5%)	1.00	0.13
	CT	80 (40%)	94 (47%)	0.84 (0.55-1.28)	
	TT	36 (18%)	23 (11.5%)	1.54 (0.84-2.82)	
Dominant	CC	84 (42%)	83 (41.5%)	1.00	0.91
	CT-TT	116 (58%)	117 (58.5%)	0.98 (0.66-1.45)	
Recessive	CC-CT	164 (82%)	177 (88.5%)	1.00	0.067
	TT	36 (18%)	23 (11.5%)	1.69 (0.96-2.97)	
Overdominant	CC-TT	120 (60%)	106 (53%)	1.00	0.15
	TC	80 (40%)	94 (47%)	0.75 (0.5-1.12)	
Additive	---	---	---	1.14 (0.85-1.49)	0.40

IL-6 and IL-10 serum levels in DS and control groups

IL-10 serum levels were significantly increased in DS individual compared to individuals without DS (P= 0.0019) (Figure 1A). IL-6 serum levels did not differ between DS and control groups (P>0.05) (Figure 1B).

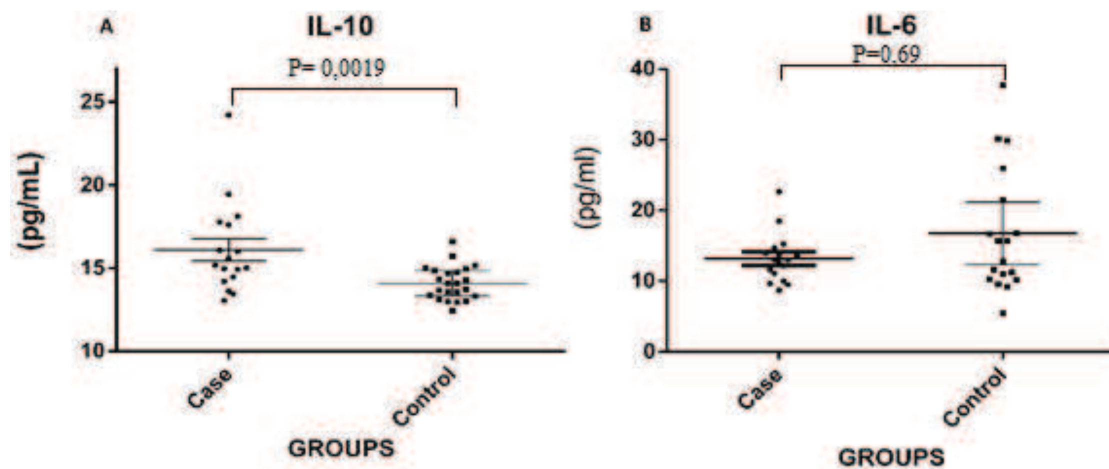

Figure 1. Interleukins concentrations between the groups. (A) IL-10 serum levels in DS (16.10 pg/ml) and control (14.09 pg/ml) groups. Mann Whitney test, P=0.0019. (B) IL-6 serum levels in DS (13.03 pg/ml) and control (15.69 pg/ml) groups. Mann Whitney test, P=0.69. The bars represent median with interquartile variation (25th percentile and 75th percentile).

IL-06 and IL-10 concentrations were evaluated in relation to the genetic polymorphisms; however, *IL-06* and *IL-10* polymorphisms showed no effect on these interleukins serum levels (**Tables** 3).

Table 3. IL-6 and IL-10 serum levels in relation to the *IL-6* and *IL-10* genotypes.

Polymorphism	Genotype	DS	P	Control	P
		IL-6 (pg/ml)			
IL-6 -174G>C	GG	11.52		16.22	
	GC	13.59	0.49	19.12	*0.12*
	CC	12.17		11.00	
IL-6 -572G>C	GG	13.77		13.67	
	GC	10.65	0,07	16.73	0.99
	CC	-*		-*	
IL-6 -597G>A	GG	11.52		16.22	
	GA	13.59	0.49	19.12	0.12
	AA	12.17		11.00	
		IL-10 (pg/ml)			
IL-10 - 819C>T	CC	16.84		14.03	
	CT	15.18	0.57	14.08	0.94
	TT	14.72		14.27	
IL-10 -592C>A	CC	16.84		14.03	
	CA	15.18	0.57	14.08	0.94
	AA	14.72		14.27	
IL-10 - 1082A>G	AA	15.07		13.60	
	AG	15.02	0.82	14.33	0.69
	GG	16.84		13.71	

*Genotype absent.

Discussion

Our findings showed an increase of serum levels of the cytokine IL-10 in children with DS. IL-10 is an anti-inflammatory cytokine [19], which participate of 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 acts suppressing 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 pro-inflammatory cytokine [6]. Thus, the excessive IL-10 production can inhibit pro-inflammatory response to several pathogens,

resulting in uncontrolled infection and deficient immune response [6], characteristics often observed in DS.

The first phase of an innate immune response comprises the classical immune activation [20, 21] characterized by the recruitment of Th-1 cytokines such as interferon-γ and other pro-inflammatory cytokines [22, 23]. However, the production of pro-inflammatory factors can be arrested and macrophages can produce factors that participate in tissue repair and wound healing, such as anti-inflammatory cytokines [23]. IL-10 and TGF-β are associated with the inhibition of the pro-inflammatory activity [23]. This alternative activation during an immune response provides an anti-inflammatory equilibrium to a pro-inflammatory acute response. Alternatively, activated macrophages are immunosuppressive and participate of tissue repair and remodeling of the extracellular matrix [22, 23]. However, repair processes can enhance the fibrosis and contribute for maintenance of disease [23-25]. An interrelation between inflammatory and regenerative processes was suggested on neurodegeneration related to the pathogenesis of Alzheimer's disease (AD) [26].

Overexpression of IL-10 was previously reported in DS [27-29]. The basal levels of IL-10 gene expression were observed up-regulated in DS children [30]. The 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 [30]. This could explain the increased susceptibility to bacterial and viral infections and inflammatory disorders in these individuals.

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 [27, 31]. The immune response to antigens is

mediated by pro-inflammatory cytokines that perform the defense of pathogens invasion [6]. However, the excess of inflammation can disrupt the metabolic system of the host. The activation of the anti-inflammatory system is a mechanism that the organism use to avoid the tissue damage and restore the 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 resulted in enhanced removal of pathogens during an acute infection, but also can contribute for an exacerbated inflammatory response, resulting in immunopathology and tissue damage [6].

The plasma levels of inflammatory molecules were investigated in DS with and without dementia in a recent study [29]. IL-10 levels were higher in DS individuals with AD and also in those with DS without clinically relevant cognitive decline. It is believed that soluble amyloid precursor protein and several forms of β-amyloid peptides lead to an activation of the signaling for an innate immune response in the brain [32]. Studies have shown that a pro-inflammatory state can reduce β-amyloid accumulation in mouse models [33-39] and the high concentrations of IL-10 contribute for reduced amyloid- β phagocytosis by microglia and amyloid-β deposition [40], which is observed in DS individuals.

Interleukin 6 (IL-6) plasma levels were also higher in subjects with DS and AD-related symptoms [29]. A negative correlation was found between IL-6 levels and cognitive decline at 2 years [29]. Studies have found increased levels of IL-6 in DS [29, 41], although others have observed oposite results [28] or no significant alterations [31]. We did not observe differential concentrations of IL-6 between individuals with DS and without the syndrome in this study.

IL-6 is a proinflammatory cytokine which participate of antibody and autoantibody production, T cells activation, B cell differentiation, and hematopoiesis [42]. This cytokine are produced by macrophages, T and B cells, and stimulate T- and B-cell immune responses upon encountering antigen components triggering an acute inflammatory response [5]. It is important to emphasize that we evaluated individuals with no infection at the moment of the samples collection, therefore we evaluated the basal levels of IL-6. Maybe 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, reinforce its role in the immune response [43]. Overexpression of the proinflammatory cytokine, like IL-6, could cause to injuries in health tissue too [44] and result in over inflammation, leading to neuronal dysfunction and consequent deterioration of the neurons, like observed in AD progression [45].

The levels of cytokines can be determined by genetic polymorphisms in the promoter region of interleukin genes [7, 14, 46-49]. In this study, we did not find association between *IL-6* or *IL-10* polymorphisms and the concentrations of these interleukins in both groups of DS individuals and those without he disease. As in our study, -1082 A>G polymorphism in *IL-10* gene was not associated with alteration of the IL-10 levels in cancer [50]. On the other hand, the presence of homozygous ancestral genotype for *IL-10* -1082 A>G was related to increased levels of IL-10 in tuberculosis patients [10] and systemic lupus erythematosus [11].

The polymorphisms -592C>A and 819C>T in the promoter region of *IL-10* gene have been associated with altered concentration of IL-10 [12, 13]. In inflammatory diseases, as

rheumatoid arthritis and colon cancer, the *IL-10* -592A allele and -592AA genotype have been shown to reduce IL-10 mRNA and protein levels [12, 13]. The mutated genotype *IL-10* -819TT was also related to reduced mRNA levels in colon cancer [12]. In our study, we did not observe an influence of *IL-10* -592C>A and 819C>T polymorphisms in the serum concentration of this interleukin. Similarly, other studies have shown no association of *IL-10* polymorphisms with the quantification of IL-10 in inflammatory states such as basal-cell carcinoma [50] and systemic lupus erythematosus [11]. By our knowledge, there is no study relating these genetic alterations to IL-10 concentrations in DS until now.

Regarding the *IL-6* gene polymorphisms, the major allele of *IL-6* -174G>C polymorphism was associated with increased levels of this cytokine in diseases such as systemic-onset juvenile chronic arthritis [14, 49] and age-related macular degeneration [51]. On the other hand, the association between this polymorphism and IL-6 concentration has been questioned, and negative results have been reported [47, 52, 53]. We also found no difference in IL-6 plasma levels according to the genotypes for *IL-6* -174G>C polymorphism, corroborating a study in cognitive impairment [52] and dementia [53]. In addition, this polymorphisms was not associated to the IL-6 levels in systemic lupus erythematosus [11].

We did not observe influence of the polymorphisms *IL-6* -572G>C on the serum levels if IL-6 in our study. This polymorphisms was also not associated with serum levels in study of age-related macular degeneration [51]. However, individuals with idiopathic pulmonary arterial hypertension carrying the *IL-6* -572GG or GC genotype showed significantly lower IL-6 levels compared to the -572CC genotype [54]. In osteoarthritis, it was observed a

reduction in IL-6 serum levels in individuals with -572GC and -572CC genotypes in relation to the -572GG genotype [55].

Few studies have evaluated the effect of polymorphism *IL-6* -597G>A on the IL-6 concentration. Similarly our results, no significant result was found between this genetic alteration and IL-6 levels in type 2 diabetes patients [56], age-related macular degeneration [51], and healthy Chinese [47].

The frequencies of the *IL-10* and *IL-6* polymorphisms evaluated here did not differ between DS individuals and those without the syndrome. According to our knowledge, this is the first study to investigate these genetic alterations in DS. These polymorphisms have been associated with some immune-related diseases [9]. *IL-10* -1082A>G polymorphism was associated with asthma[57, 58], systemic lupus erythematosus [59-61], Crohn's disease [62, 63], rheumatoid arthritis [64-66], and tuberculosis [10, 67].

Regarding the polymorphism *IL-10* -592C>A, the results are divergent. The frequency of the wild-type allele *IL-10* -592A was found increased in patients with asthma [57, 58], and rheumatoid arthritis [13, 66]. On the other hand, the -592CC genotype was prevalent in tuberculosis patients [67]. The -592C allele and CC genotype were more prevalent in patients with type 2 diabetes when compared to healthy individuals [68]. In colon cancer the -592AA genotype was more frequent [12], while in non-small cell lung cancer the allele -592C presented association with the disease [69].

The polymorphism *IL-10* -819C>T was associated with systemic lupus erythematosus [60], tuberculosis [67], and Crohn's disease and ulcerative colitis [62]. The wild-type allele -819C was more frequent on these diseases. However, the genotype distribution varies according to the cancer types. In non-small cell lung cancer [69], the allele -819C was more

prevalent, while in prostate [70] and colon cancer[12], the -819TT genotype presented increased frequency.

Alterations in IL-6 gene were also related to inflammatory diseases. The -174G>C polymorphism was associated with an increased inflammatory response [71] and was related to a variety of disease states such as AD [72, 73], atherosclerosis [74], cardiovascular disease [75], cancer [76], Hodgkin lymphoma [77], type-2 diabetes mellitus [17], sepsis [78], systemic lupus erythematosus [11], and systemic-onset juvenile chronic arthritis [14].

The polymorphism *IL-6* -572G>C was considered a protective factor for hip and knee osteoarthritis [55]. The presence of the polymorphic allele -572C was associated with protection to erythematosus systemic lupus [79]. The allele -572C could result in a low expression of the *IL-6* gene in response to a stimulus and reduce the inflammatory response. The alteration -597G>A of *IL-6* gene was related to a susceptibility and severity of pneumonia [80]. On the other hand, alleles and haplotypes for -174G>C, -572G>C, and -597G>A were not associated with rheumatoid arthritis susceptibility and therapy response [81].

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 *IL-10* gene are not the main factors that drive the overexpression of IL-10 in DS.

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Availability of data and materials

All data and materials are available.

Authors' contributions

Not applicable

Author's information

Not applicable

Ethical Approval and consent to participate

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 20112313. 9. 0000. 5415.

Consent for publication

All the authors have consented for publication.

Competing Interests

The authors declare that there is no conflict of interests.

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3. CONCLUSÕES

3. CONCLUSÕES

1. As frequências dos polimorfismos IL-6 -597G>A (rs1800797), -572G>C (rs1800796) e -174G>C (rs1800795) e IL-10 -592 C>A (rs1800872), -819C>T (rs1800871) e -1082A>G (rs1800896) e seus haplótipos não diferem entre indivíduos com SD e sem a síndrome.
2. Os polimorfimos estudados não têm efeito nos níveis séricos de IL-6 e IL-10, assim, os polimorfismos no gene *IL-10* não representam os principais fatores que influenciam a expressão aumentada da IL-10 na SD observada nesta casuística.

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5. APÊNDICES

FAMERP – FACULDADE DE MEDICINA DE SÃO JOSÉ DO RIO PRETO
AUTARQUIA ESTADUAL
TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

(Obrigatório para Pesquisas Científicas em Seres Humanos – Resolução n.º 466/12 – CNS)

I. Dados de identificação do sujeito da pesquisa e responsável legal:

- Nome do sujeito da pesquisa:
- Data de nascimento: Sexo:
- Nome do responsável legal:
- Grau de parentesco:
- Endereço:
  Bairro: Cidade: CEP:

  Telefone:

II. Dados sobre a pesquisa científica/pesquisador:

- Título do Projeto: Avaliação do impacto de polimorfismos genéticos das interleucinas IL-6 e IL-10 em indivíduos com síndrome de Down
- Pesquisador Responsável: Érika Cristina Pavarino
- Inscrição no Conselho Regional: Conselho Regional de Biologia (CRB-1) n° 18306/01-D
- Cargo/Função: Professor Adjunto
- Instituição: Faculdade de Medicina de São José do Rio Preto - FAMERP
- Endereço: Avenida Brigadeiro Faria Lima, 5416
  Bairro: Vila São Pedro

  CEP: 15090-000 Fone: (17)3201-5904

III. Avaliação do risco da pesquisa:

(X) risco mínimo ( ) risco médio ( ) risco maior

Consequência imediata do estudo: Risco da coleta de sangue que inclui vermelhidão local transitória, e raramente a formação de pequenos hematomas e inflamação local.

IV. Esclarecimentos sobre a pesquisa científica:

- **Objetivo da pesquisa:** Investigar variações nos genes IL-6 e IL-10 (material genético) e quantificar no soro os níveis destas interleucinas (proteínas) em indivíduos com síndrome de Down e em um grupo controle sem a síndrome.
- **Método empregado para colheita de material biológico (sangue periférico):** O sangue será colhido com seringa descartável por profissionais habilitados.

- **Desconfortos e riscos esperados:** O risco da coleta inclui vermelhidão local transitória, e raramente a formação de pequenos hematomas e inflamação local.
- **Benefícios que poderão ser obtidos:** Este estudo é importante, pois contribuirá para o conhecimento das alterações imunológicas em indivíduos com síndrome de Down.
- O sujeito da pesquisa/responsável legal consente ao pesquisador utilizar os resultados advindos da pesquisa apenas para divulgação em reuniões de caráter científico e/ou publicações em meios especializados, sendo, portanto, mantido sigilo das informações.
- O sujeito da pesquisa/responsável legal pode consultar a pesquisadora responsável pelo telefone (17) 32015904 ou a secretaria do Comitê de Ética em Pesquisa da Faculdade de Medicina de São José do Rio Preto, telefone: (17) 32015813, para esclarecimento de qualquer dúvida.
- O sujeito da pesquisa/responsável legal está livre para, a qualquer momento, deixar de participar da pesquisa e não precisa apresentar justificativas para isso.
- O sujeito da pesquisa/responsável legal autoriza o armazenamento do material coletado e será contatado(a) para conceder ou não a autorização para o uso deste material em futuros projetos.
- O sujeito da pesquisa/responsável legal que concordar em participar desta pesquisa e com a retirada e uso do material, do modo descrito acima, não terá quaisquer benefícios ou direitos financeiros sobre os eventuais resultados decorrentes desta pesquisa e também não terá qualquer tipo de despesa para participar do estudo.
- Caso necessário, o sujeito da pesquisa será convocado para uma nova coleta de sangue periférico para quantificação das interleucinas no soro.

V. Consentimento pós-esclarecimento:

Declaro que, após ter sido convenientemente esclarecido pelo pesquisador, consinto em participar na amostragem do projeto de pesquisa em questão, por livre vontade sem que tenha sido submetido a qualquer tipo de pressão.

São José do Rio Preto, ______ de ________________,_______.

______________________________ ______________________________

Responsável legal

Érika Cristina Pavarino
Pesquisadora Responsável

Nota: Este termo foi elaborado em duas vias, ficando uma via em poder do paciente ou seu representante legal e outra com o pesquisador responsável pelo projeto.

MINISTÉRIO DA SAÚDE
Conselho Nacional de Saúde
Comissão Nacional de Ética em Pesquisa - CONEP

PARECER Nº2400/2004

Registro CONEP: 10618 (Este nº deve ser citado nas correspondências referentes a este projeto)

Registro CEP: 3340/04 **Processo nº 25000.106488/2004-41**
Projeto de Pesquisa: *"Avaliação genético clínica e molecular em Síndrome de Down."*
Pesquisador Responsável: Dra. Érika Cristina Pavarino Bertelli
Instituição: Faculdade de Medicina de São José do Rio Preto - FAMERP
Área Temática Especial: Genética Humana

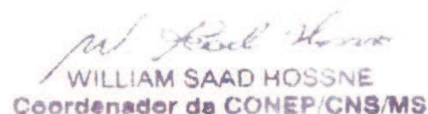
Ao se proceder à análise das respostas ao parecer CONEP nº 2001/2004, relativo ao projeto em questão, considerou-se que:

1) tendo em vista a afirmação da pesquisadora responsável que será estabelecido um banco de material biológico, solicita-se que seja feito um banco de dados junto ao CEP da instituição, informando: quem será o responsável pelo banco, condições de armazenamento, segurança do banco, como será o acesso pelos pesquisadores a esse banco, de que forma será garantida a confidencialidade dos indivíduos que doarem o material para a formação desse banco;
2) as informações enviadas atendem aos aspectos fundamentais da Res. CNS 196/96 sobre diretrizes e normas regulamentadoras de pesquisas envolvendo seres humanos;
3) o projeto foi aprovado pelo Comitê de Ética em Pesquisa – CEP da instituição supracitada.

Diante do exposto, a Comissão Nacional de Ética em Pesquisa - CONEP, de acordo com as atribuições definidas na Resolução CNS 196/96, manifesta-se pela aprovação do projeto de pesquisa proposto com a recomendação 1. acima citada, devendo esta ser acompanhada pelo CEP, para posterior início da pesquisa.

Situação: Projeto aprovado com recomendação

Brasília, 29 de Novembro de 2004



WILLIAM SAAD HOSSNE
Coordenador da CONEP/CNS/MS

Parecer n.º 427.782

COMITÊ DE ÉTICA EM PESQUISA

O projeto de pesquisa CAAE **20112313.9.0000.5415** sob a responsabilidade de **Érika Cristina Pavarino** com o título "Avaliação do impacto de polimorfismos genéticos das interleucinas IL-6 e IL-10 em indivíduos com síndrome de Down" está de acordo com a resolução do CNS 466/12 e foi **aprovado por esse CEP.**

Lembramos ao senhor(a) pesquisador(a) que, no cumprimento da Resolução 251/97, o Comitê de Ética em Pesquisa em Seres Humanos (CEP) **deverá receber relatórios semestrais sobre o andamento do Estudo,** bem como a qualquer tempo e a critério do pesquisador nos casos de relevância, além do envio dos relatos de eventos adversos, com certeza para conhecimento deste Comitê. **Salientamos ainda, a necessidade de relatório completo ao final do Estudo.**

São José do Rio Preto, 17 de outubro de 2013.

Profª. Drª. Maria Rita Rodrigues Vieira
Vice-Presidente do CEP/FAMERP

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1. ANEXOS

5. *ANEXOS*

ANEXO 1

Ribeirão Preto, 21 de Julho de 2017

Prezados autores,

Informamos que o artigo "Polymorphisms of interleukin 6 in Down syndrome individuals: a case-control study

" GMR9738, de autoria M.F. Mattos, L. Uback, P.M. Biselli-Chicote, J.M. Biselli, E.M. Goloni-Bertollo and E.C. Pavarino, foi aceito para publicação na Genetics and Molecular Research (GMR).

Aproveitamos a oportunidade para informar que a GMR está indexada em 63 bases de dados, entre elas: Index Medicus, PubMed, Medline e ISI. E tem fator de impacto 0,768, segundo JCR - junho 2016.

Atenciosamente,

Francine Muniz
Coordenadora editorial (Mtb 44.300)
Genetics and Molecular Research
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