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

M.F. Mattos[1], L. Uback[1], P.M. Biselli-Chicote[1], J.M. Biselli[2], E.M. Goloni-Bertollo[1] and E.C. Pavarino[1]

[1]Unidade de Pesquisa em Genética e Biologia Molecular,
Faculdade de Medicina de São José do Rio Preto,
São José do Rio Preto, SP, Brasil
[2]Instituto de Biociências Letras e Ciências Exatas,
Universidade Estadual Paulista, Câmpus São José do Rio Preto,
São José do Rio Preto, SP, Brasil

Corresponding author: E.C. Pavarino
E-mail: erika@famerp.br

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; Genetic 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 et al., 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 et al., 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), -572G>C

(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 and 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 No. 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), -572G>C (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 -572G>C; 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), -572G>C (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), -572 G>C (rs1800796), and -597G>A (rs1800797) polymorphisms between the groups with DS (case) and without the syndrome (control).

Model	Genotype	Control group	Case group	OR (95%CI)	P value
Interleukin 6 -597G>A (rs1800797)					
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
Interleukin 6 -174G>C (rs1800795)					
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
Interleukin 6 -572 G>C (rs1800796)					
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), -572G>C (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), -572G>C (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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