IL6-174G > C genetic polymorphism influences antidepressant treatment outcome

Serafim Carvalho [a,b], Marlene Santos [c,d,e], Luís Lima [f], Jorge Mota-Pereira [g], Paulo Pimentel [h], Dulce Maia [h], Diana Correia [a], Agostinho Cruz [d], Rui Medeiros [c, i, j, k]

[a]Hospital de Magalhães Lemos, Porto, Portugal; [b]Instituto Superior de Ciências da Saúde, Norte, Portugal; [c]Molecular Oncology & Viral Pathology, IPO-Porto Research Center (CI-IPOP), Portuguese Institute of Oncology, Porto, Portugal; [d]Núcleo de Investigação e Informação em Farmácia-Centrode Investigação em Saúde e Ambiente (CISA), School of Allied Health Technologies, Polytechnic Institute of Porto, Porto, Portugal; [e]Faculty of Medicine, University of Porto, Porto, Portugal; [f]Experimental Pathology and Therapeutics Group, Portuguese Institute of Oncology, Porto, Portugal; [g]Clínica Médico-Psiquiátrica da Ordem, Porto, Portugal; [h]Trás-os-Montes e Alto Douro Hospital Center, Vila Real, Portugal; [i]Department of Pathology and Molecular Immunology, ICBAS, Abel Salazar Biomedical Sciences Institute, University of Porto, Porto, Portugal; [j]Health Faculty of University Fernando Pessoa, CEBIMED, Porto, Portugal; [k]Research Department, Portuguese League Against Cancer (Norte), Porto, Portugal

ABSTRACT

Background: Major depressive disorder is a condition associated with dysregulated cytokine levels; among these, IL6. Furthermore, genetic variations within cytokine genes have been proposed to predict antidepressant treatment outcome.

Objectives: This study aims to evaluate the role of *IL6-174G > C* and *IL6R D358A A > C* functional polymorphisms in antidepressant treatment phenotypes, specifically remission, relapse, and treatment resistant depression (TRD).

Methods: The referred polymorphisms were genotyped in 80 MDD patients followed at Hospital Magalh˜aes Lemos, Portugal, within a period of 27 months.

Results: It was found that patients carrying *IL6-174* GC genotype present a protection towards the development of TRD (OR = 0.242; 95% CI = 0.068–0.869; $p = .038$), when compared with GG genotype. Additionally, carriers of *IL6-174* CC genotype remit earlier than patients with *IL6-174* GG/GC genotypes, with a median time to remission of 6 weeks for CC carriers and 15 weeks for GG or GC carriers ($p = .030$, Log-rank test). No association was found between *IL6R* D358A genetic polymorphism and any of the treatment phenotypes evaluated.

Conclusions: The *IL6-174G > C* polymorphism influences antidepressant treatment outcome in this sub-set of MDD patients, providing a putative mechanistic link for the dysregulated IL-6 levels described in the literature in patients with TRD.

KEYWORDS IL6; genetic polymorphisms; antidepressants; treatment resistant depression

Background

Major Depressive Disorder (MDD) represents, nowadays, one of the major causes of disability. Even though effective antidepressant treatments are available in the clinical setting, approximately one third of all patients fail to respond to antidepressant drugs (AD), contributing to the global burden of this condition (1). Despite its prevalence, the exact framework that contributes to depression and to AD response is far of being totally understood. Emerging studies have been pointing out the inflammatory response as a potential contributor to the pathophysiology of depression. Among these, the increased production of pro-inflammatory cytokines, such as interleukin 6 (IL-6), is one of the most reliable biomarkers (2). Patients with MDD have been shown to have elevated serum and plasma IL-6 levels (3). The same evidence is also found among patients with Treatment Resistant Depression (TRD) (4–7). Modulation of the inflammatory response and cell-mediated immunity has been suggested as one possible mechanism explaining antidepressant mechanism of action (8,9). Additionally, treatment with selective serotonin reuptake inhibitors (SSRI) AD reduced serum IL-6 levels in patients with MDD (10), and ketamine, a fast-acting antidepressant, has proven to reduce IL-6 levels in the rat prefrontal cortex and hippocampus (11). *IL6* gene is located in chromosome 7, and it's organized into five exons and four introns, and its expression might be modulated by polymorphisms, such as rs1800795 in the promoter region of *IL6* (12). However, despite the clear influence of IL-6 in the pathophysiology of depression, and in antidepressant response, studies evaluating the impact of *IL6* genetic polymorphisms on treatment response phenotypes are scarce and only address response, not relapse or TRD phenotypes (13). Additionally, and despite TRD is a clinical important issue, to the best of our

knowledge, no studies have associated the effect of *IL6* and *IL6R* genetic polymorphisms and TRD.

Aims

In this study, we aim to evaluate the role of *IL6-174G > C* rs1800795 and *IL6R D358A A > C* rs8192284 functional polymorphisms in antidepressant treatment response phenotypes.

Materials and methods

Patients

For the analysis of the effect of *IL6* and *IL6R* single nucleotide polymorphism (SNPs) in AD treatment response phenotypes, a cohort of 80 MDD patients was recruited at Hospital Magalhães Lemos, Oporto, Portugal, and followed for 27 months (14,15). MDD diagnosis was accomplished with Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) and the severity of the depressive symptoms with Beck Inventory Depression (BDI). Pharmacological treatment was chosen according to the Major Depressive Disorder treatment algorithm of the Texas Medication Algorithm Project (TMAP) (16). The first medicine was selected from the drugs included in the first line of approach of the TMAP. Pharmacotherapeutic effectiveness (administered after at least 6 weeks and at adequate doses according to the TMAP protocol) was determined as the change in the BDI score. When patients present a BDI score less than 10 after 6 weeks of at least one single adequate antidepressant treatment and absence of criteria of MDD according to SCID-I were considered remitters. Antidepressant medication could be changed for non-response or intolerably as per the TMAP protocol. When the patient failed to reach a BDI score less than 10 and present criteria of MDD according to SCID-I, after at least two adequate antidepressant treatments with different drugs within the current episode, they were considered presenting a 'Treatment resistant depression' (TRD) phenotype. Relapse was defined as any depressive episode, upon remission, during the follow-up time. When the patient displayed TRD or relapsed they were considered as being part of a Bad Prognosis phenotype. The evaluated AD treatment response phenotypes were: TRD, relapse, time to remission, and time to relapse. Clinical and sociodemographic characteristics of the patients were described elsewhere (14). The study was approved by the ethical committee of Hospital Magalhães Lemos and written informed consent according to 'The Code of Ethics of the World Medical Association' (Declaration of Helsinki) was obtained from each individual after explanation of the study.

DNA extraction and SNP analysis

Genomic DNA was extracted from the whole blood with a commercial kit (E.Z.N.A. – Omega Bio-tek) according to the manufacturer's instructions, and stored at −20 °C. The *IL6-174G > C* (rs1800795) and *IL6R D358A A > C* (rs8192284) polymorphisms analysis was carried out using Sequenom MassARRAY technology (Sequenom, San Diego, CA). Genotyping data was read blind to the clinical course of illness and, in the case of ambiguous genotypic data, experiments were repeated for determining the genotype of every individual.

Statistical analysis

Data preparation and analysis was carried out using the computer software PAWS Statistics 18 (release 18.0.0). A 5% level of significance was used in the Chi-square (χ^2) analysis to compare the categorical variables. Odds ratio (OR) and 95% confidence interval (CI) were calculated as a measure of association between genotypes and risk of developing a specific phenotype. Kaplan–Meier survival curves were used to evaluate the correlation between genotypes and time to remission and relapse and were compared by log-rank statistical test. The Hardy–Weinberg equilibrium of genotypic frequencies was determined by Chi-square (χ^2).

Results

The genotype frequencies of *IL6-174G > C* (rs1800795) and *IL6R D358A A > C* (rs8192284) polymorphisms among MDD patients and its distribution among different treatment response phenotypes are presented in Table 1. The distribution of *IL6* rs1800795 and *IL6R* rs8192284 gene polymorphisms were consistent with the Hardy–Weinberg equilibrium ($p > .05$, results not shown). Patients carrying *IL6-174*GC genotype presented approximately a 75% a reduced risk to develop TRD, when compared with the ones carrying GG genotype (OR = 0.242; 95% CI = 0.068–0.869; $p = .038$). No association between this polymorphism and relapse was observed. Kaplan–Meier survival curves were used to evaluate the correlation between genotypes and time to remission and time to relapse and were compared by Log-rank statistical test. It was observed that carriers of the *IL6-174* CC genotype seem to remit earlier than patients carrying the *IL6-174* GG and GC genotypes, with a median time to remission of 6 weeks for CC carriers and 15 weeks for GG/GC carriers (Figure 1, $p = .030$, Log-rank test). No association between this polymorphism and time relapse was observed. Regarding *IL6R D358A* (rs8192284) polymorphism, no statistically significant association was found between genotype frequencies and antidepressant treatment phenotypes, namely TRD and relapse, as well as time to remission or relapse.

Discussion

Recent evidence suggests that MDD is a systemic condition, where central and peripheral inflammatory changes seem to have a main role in its pathophysiology. Among these, elevated IL-6 plasma or serum levels are one of the most consistent MDD biomarkers, and a top indicator of suicide risk (17,18). Further research has indicated that genetic variations within cytokine genes may also predict the AD

Table 1. Overview of the association between genotypes or alleles under investigation and outcomes.

		IL6-174 rs1800795 G/C								*IL6R* rs2228145 A/C						
		No		Yes						No		Yes				
		n	%	*n*	%	OR	95% CI	*p*-value		*n*	%	*n*	%	OR	95% CI	*p*-value
Releapsed	GG	14	31.1	6	37.5	1.0	Referent	—	AA	17	37.8	5	31.3	1.0	Referent	—
	GC	22	48.9	8	50.0	0.848	0.242–2.970	.797	AC	18	40.0	10	62.5	1.889	0.535–6.670	.320
	CC	9	20.0	2	12.5	0.519	0.085–3.156	.676*	CC	10	22.2	1	6.2	0.340	0.035–3.340	.637
	C carrier	31	68.9	10	62.5	0.753	0.228–2.481	.640	C carrier	28	62.2	11	68.8	1.336	0.396–4.510	.640
Resistant (TRD)	GG	20	32.8	11	57.9	1.0	Referent	—	AA	22	36.1	8	42.1	1.0	Referent	—
	GC	30	49.2	4	21.1	0.242	0.068–0.869	.038†*	AC	28	45.9	9	47.4	0.884	0.293–2.666	.827
	CC	11	18.0	4	21.1	0.661	0.170–2.577	.740*	CC	11	18.0	2	10.5	0.500	0.090–2.765	.696*
	C carrier	41	67.2	8	42.2	0.355	0.123–1.020	.050	C carrier	39	63.9	11	57.9	0.776	0.271–2.217	.635
Bad prognosis (relapse or TRD)	GG	14	31.1	17	48.6	1.0	Referent	—	AA	17	37.8	13	37.1	1.0	Referent	—
	GC	22	48.9	12	34.3	0.449	0.116–1.218	.113	AC	18	40.0	19	54.3	1.380	0.524–3.634	.514
	CC	9	20.0	6	17.1	0.549	0.157–1.920	.345	CC	10	22.2	3	8.6	0.392	0.089–1.721	.307*
	C carrier	31	68.9	18	51.4	0.478	0.191–1.194	.112	C carrier	28	62.2	22	62.9	1.027	0.412–2.560	.954

TRD: Treatment Resistant Depression; OR: odds ratio; CI: confidence interval.
†Significant *p*-value.
*Fisher exact test.

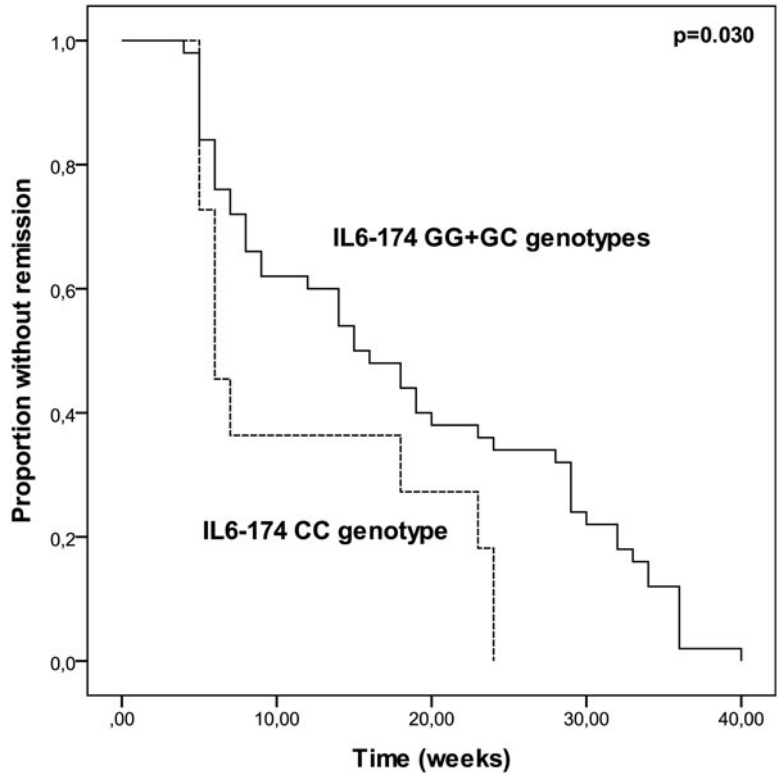

Figure 1. Effect of *IL6* genotypes in time to remission in MDD patients. Kaplan–Meier analysis was used to evaluate time to relapse between *IL6-174* CC and GG + GC carriers. Comparison performed by Log-rank test ($p = .030$).

treatment outcome (19). However, the deregulatory mechanism of IL-6 in MDD, and its normalization upon AD treatment, is poorly understood. This way, we have determined *IL6-174G > C* (rs1800795), and *IL6R D358A A > C* (rs8192284) genotype and allele carrier frequencies in a cohort of patients with MDD, treated according to the Texas Medication Algorithm.

We observed that patients carrying *IL6-174* GC genotype presented a protection for the development of TRD, when compared with GG genotype. According to the literature, *IL6-174G > C* SNP (rs1800795) results in a functional alteration, and healthy individuals, who harbour the homozygous GG polymorphism, have been described to have higher IL-6 concentrations than G/C or CC genotype (20). We hypothesized that the presence of GG genotype may turn out to be a risk factor of TRD, through its ability of modulating IL-6 expression. Conversely, we observed that carriers of CC genotype remit earlier, corroborating the putative influence of this polymorphism in the AD treatment response. This functional *IL6* (rs1800795) polymorphism is located in the promoter region of the IL6 gene and has been previously associated with an increased risk of IFN-induced depression (21). Interestingly, in patients undergoing antiviral treatment, subjects carrying CC genotype

presented significantly lower changes from baseline in IFN-induced depression (21). This possibility is in accordance with the evidences from other studies, which have shown that TRD was associated with a higher level of IL-6 and that suppression of pro-inflammatory IL-6 cytokine does not occur in depressed patients who fail to respond to AD, contrary to responder patients (6). Additionally, serum IL-6 has recently been found to be a predictive biomarker for ketamine's antidepressant effect in TRD patients (22). Furthermore, in a GWAS study, an association with response to escitalopram was observed for the rs7801617 polymorphism of *IL6* gene (13).

The effects of IL-6 in antidepressant response are likely to be explained by its control of SERT levels, and, consequently, from the serotonin re-uptake. In fact, IL-6 has recently been recognized to regulate the SERT gene through a STAT3-dependent regulation region (23). The serotonin transporter (SERT, SLC6A4) is the main site of action of the most commonly prescribed antidepressant drugs (e.g. SSRIs), and SERT activity is known to affect serotonergic neurotransmission. Giving this possibility, it is likely that IL6-*174G > C* genetic polymorphism, by affecting IL-6 levels/functionality, may have a consequent impact on SERT, and therefore the serotonin re-uptake (due to the control of SERT by IL-6) might affect antidepressant response.

Although preliminary, this study demonstrates *IL6-174* promoter SNP was associated with TRD development. The relevance of this finding relies on the fact that this SNP has never been studied in TRD patients and provides a putative mechanistic link for the dysregulated IL-6 levels described in the literature in patients with TRD. Functionality studies evaluating IL-6 expression in MDD and TRD patients are needed to fully disclose the role of *IL6-174*G > C polymorphism.

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