Influence of IL6-174G>C, IL6-6331T>C and IL6R D358A A>C IL-6 genetic polymorphisms in antidepressant treatment phenotypes

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Introduction

Several studies associated Major Depressive Disorder (MDD) with an increased production of pro-inflammatory cytokines, such as interleukin 6 (IL-6). It has been demonstrated that patients with MDD present elevated levels of serum and plasma IL-6, and disorders involving chronic inflammation are often co-morbilities of MDD. The same evidence was also found in patients with Treatment Resistant Depression (TRD) [1-4]. A meta-analysis performed by Hiles et al. (2012) found that serum or plasma IL-6 levels were elevated in depressed patients compared with controls [5]. Furthermore, according to other meta-analysis, treatment with SSRI antidepressants reduce serum IL-6 levels in patients with MDD [6]. A body of evidence indicates that the therapeutic activity of antidepressants is connected with their modulatory effect on the inflammatory response system and cell-mediated immunity. Moreover, ketamine, a fast-acting antidepressant, has proven to reduce IL-6 levels in rat prefrontal cortex and hippocampus. However, despite the clear influence of IL-6 in the pathophysiology of depression and in antidepressant response, studies evaluating the impact of IL-6 functional genetic single nucleotide polymorphisms (SNPs) on treatment phenotypes are scarce and only address response, not relapse. Additionally, and despite TRD is a clinical important concern, to the best of our knowledge, no studies have evaluated the effect of IL-6 genetic polymorphisms in TRD.

Objectives

We aim to evaluate the role of *IL6-174G>C*, *IL6-6331T>C* and *IL6R D358A A>C* functional polymorphisms in antidepressant treatment phenotypes, specifically remission and relapse and in TRD.

Materials and Methods

Patients

Eighty MDD patients, recruited from Hospital Magalhães Lemos, were followed by a psychiatrist for 18 months. Patients were treated according to the Texas Medication Algorithm [7]. MDD diagnostic was accomplish with Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) and the severity of the depressive symptoms with Beck Inventory Depression (BDI). The study was approved by the ethical committee of Hospital Magalhães Lemos, and written informed consent 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). The *IL6-174G>C* (rs1800795), *IL6-6331T>C* (rs10499563) and *IL6R D358A A>C* (rs8192284) SNPs (Table 1) analysis was carried out using Sequenom MassARRAY technology (Sequenom, San Diego, CA, USA). Genotyping data was read blind to the clinical course of illness.

Table 1 – Characteristics of the Single Nucleotide Polymorphisms (SNPs) selected for the analysis

	Gene	Reference SNP ID	Nucleotide substitution	Genomic location
Interleukin-6 gene	*IL6*	rs1800795	G>C	-174 promoter
	IL6	rs10499563	T>C	-6331 promoter
Interleukin-6 receptor gene	*IL6R*	rs2228145	A>C	Asp358Ala

Statistical analysis

Data preparation and analysis was carried out using the computer software IBM SPSS (v.22). A 5% level of significance was used in the Chi-square ($\chi 2$) 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 correlation between genotypes and time to remission and relapse and were compared by log-rank statistical test.

Results

The genotype frequencies of *IL6-174 G>C* and *IL6-6331 T>C* polymorphisms among MDD patients and its distribution among different treatment response phenotypes are presented in Table 2 (TRD), and Table 3 (Relapse).

IL6-174 G>C polymorphism predicts TRD and time to remission

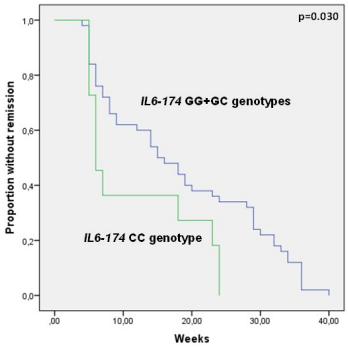
Table 2 shows that patients carrying *IL6-174* GC genotype present an odds ratio of 0.242 when compared with *IL6-174* GG genotype. Taken GG genotype has indicator these results demonstrate that patients carrying *IL6-174* GG genotype are more prone to develop TRD, when compared with the ones carrying GC genotype, having an approximately 4-fold increased risk of TRD (OR=4.125; 95%CI: [1.151-14.786]; p=0.038). Additionally Kaplan-Meier analysis revealed 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 or GC carriers (Figure 1, p=0.030, Log-rank test).

Regarding *IL6-6331 T>C* and *IL6R D358A A>C* polymorphism (results not shown), no association was found between genotype frequencies and TRD development or time to remission.

Table 2 – Genotype frequencies and risk estimates for TRD development

		Resistance (TRD)						
		No		Yes				
		N	%	N	%	OR	95% CI	p-value
IL6-174 G/C	GG	20	32.8	11	57.9	1.0	Referent	-
	GC	**30**	**49.2**	**4**	**21.1**	**0.242**	**[0.068-0.869]**	**0.038***
	CC	11	18.0	4	21.1	0.661	[0.170-2.577]	0.740*
	C carrier	41	67.2	8	42.2	0.355	[0.123-1.020]	0.050
IL6-6331 T/C	TT	30	49.2	10	52.6	1.0	Referent	-
	TC	27	44.3	9	47.4	1.000	[0.354-2.829]	1.000
	CC	4	6.6	0	0.0	**	**	0.559*
	C carrier	31	50.9	9	47.4	0.871	[0.311-2.442]	0.793

OR: Odds ratio. CI: Confidence interval. *One cell count is less than 5, Fisher exact test was used. **One cell count is 0, unable to calculate OR



IL6-6331T>C polymorphism predicts treatment time to relapse

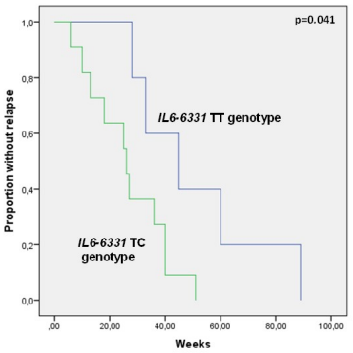
We also observed that patients carrying *IL6-6331* TC genotype have a higher risk of relapse (OR=3.988; 95%CI: [1.176-13.516]; p=0.022), when compared with TT genotype carriers. Kaplan-Meier analysis revealed that carriers of the *IL6-6331* TC genotype seem to relapse earlier, after 26 weeks, than patients carrying the *IL6-6331* TT genotype , which relapse after 45 weeks (Figure 2, p=0.041, Log-rank test).

Regarding *IL6-174 G>C* and *IL6R D358A A>C* polymorphism (results not shown), no association was found between genotype frequencies and relapse.

Table 3 – Genotype frequencies and risk estimates for relapse development

		Relapse						
		No		Yes				
		N	%	N	%	OR	CI 95%	p-value
IL6-174 G/C	GG	14	31.1	6	37.5	1.0	Referent	-
	GC	22	48.9	8	50.0	0.848	[0.242-2.970]	0.797
	CC	9	20.0	2	12.5	0.519	[0.085-3.156]	0.676*
	C carrier	31	68.9	10	62.5	0.753	[0.228-2.481]	0.640
IL6-6331 T/C	TT	25	55.6	5	31.3	1.0	Referent	-
	TC	**16**	**35.6**	**11**	**68.8**	**3.988**	**[1.176-13.516]**	**0.022**
	CC	4	8.9	0	0.0	**	**	1.000*
	C carrier	20	43.5	11	68.8	2.750	[0.820-9.219]	0.095

OR: Odds ratio. CI: Confidence interval. *One cell count is less than 5, Fisher exact test was used. **One cell count is 0, unable to calculate OR



Discussion and Conclusions

Among the most repeatedly validated biomarkers of MDD and TRD are the elevated levels of IL-6, however there is no valid explanation for the reasons of this increase.

We identified patients carrying *IL-6 174* GG genotype as having a higher risk of developing TRD, than GC genotype. Data from several studies have demonstrated that the *IL6-174G>C* SNP results in a functional alteration and individuals who harbor the homozygous GG genotype produce higher IL-6 concentrations than GC or CC genotype in healthy volunteers. Therefore, we hypothesized that the presence GG genotype of this polymorphism may be a risk factor of TRD by modulation of IL-6 expression. Conversely, we observed that carriers of CC genotype remit earlier that CG or GG genotype corroborating the putative influence of this polymorphism in the AD response. This results are in accordance with the evidences of other studies which have shown that TRD is associated with higher levels of IL-6 and that suppression of proinflammatory IL-6 cytokines does not occur in depressed patients who fail to respond to AD, contrary to responder patients [3].

Regarding the risk of relapse and time do relapse we identified that patients caring *IL6-6331* TC genotype have a higher risk of relapse, and tend to relapse earlier that TT genotype. Only one study evaluated the functionality of this polymorphism in vitro and concluded that the presence of C allele of *IL6-6331* affects transcription, reducing IL-6 expression. Since in our sample C allele is underrepresented, is it not possible to conclude about its effects in homozygosis [8].

Although apparently these are non-concordant results, in which the elevated level of IL-6 are associated with TDR and MDD but not with relapse, little is known about the interaction of G and C allele of *IL6-6331* in heterozygous individuals, and authors have demonstrated that both *IL6-174* and *-6331* variants may act in concert to affect gene expression. To fully elucidate these findings is necessary to determine plasma and mRNA IL-6 expression in our subset of MDD patients.

In this study we identify *IL6-174* promoter SNP as a prognostic risk factor of Treatment Resistant Depression 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 between the elevated levels of IL-6 found among Treatment Resistant Depression patients, possibly due to its correlation with IL-6 production. Functionality studies evaluating IL-6 expression in MDD and TRD patients are needed to fully disclose the role of *IL6- 174* and *IL6-6331* polymorphisms.

References

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