The role of *IL18-607C > A* and *IL18-137G > C* promoter polymorphisms in antidepressant treatment phenotypes: A preliminary report

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ABSTRACT

Recent studies suggest that immune activation and cytokines, such as IL-18, are involved in depression. IL-18 is expressed in brain and is increased in patients with moderate to severe depression. In this study we aim to evaluate the role of *IL18-607C > A and IL18-137G > C* promoter polymorphisms in antidepressant treatment phenotypes, specifically relapse and treatment resistant depression (TRD). We genotyped the referred polymorphisms in a subset of 80 MDD patients followed at Hospital Magalhães Lemos, Portugal, within a period of 27 months. Patients carrying *IL18-607* CA or AA genotypes were significantly more prone to relapse after AD treatment and present a significantly lower time to relapse than patients carrying CC genotype. Similarly, patients carrying *IL18-137* GC or CC genotypes have a significantly higher risk of relapse and display relapse significantly earlier than the ones carrying GG genotype. Due to the low number of *IL18-607* CC and *IL18-137 GG* in the relapse subgroup (n = 3 and n = 5, respectively), results were validated by bootstrapping analysis, and remained significant. No association was found between the evaluated genetic polymorphisms and TRD. *IL18* peripheral mRNA levels were upregulated in *IL18-607* CA or AA carriers. This preliminary report indicates that *IL18-607C > A and IL18-137G > C* genetic polymorphisms seem to influence depression relapse after antidepressant treatment in our subset of depressed patients, and may possibly contribute to the disregulated IL-18 levels found in patients with depression.

Keywords: Cytokines Antidepressants; *IL18*; Relapse; Genetic polymorphisms

HIGHLIGHTS

- *IL18-607C > A and IL18-137G > C* polymorphisms were associated with the risk of depression relapse.
- *IL18* peripheral mRNA levels were found upregulated in *IL18-607* CA/AA carriers.
- No association was found between SNPs and the development of TRD.

1. Introduction

Major Depressive Disorder (MDD) is a highly prevalent chronic psychiatric condition associated with a significant morbidity and mortality [1]. Antidepressant drugs (AD) are widely used; however, treatment response is often variable. Only a third of patients treated with AD achieve full remission, and from these many suffer relapse. Additionally, a substantial proportion of patients fail to respond to any antidepressants, displaying a Treatment Resistant Depression (TRD) phenotype [2].

Increasing evidences point towards the involvement of the immune system, and in particular cytokines, in the development and progression of depression [3]. Additionally, several evidences show that pro-inflammatory cytokines, including Interleukin (IL)-18, have been reported to be elevated in patients with MDD [4,5]. IL-18, and its receptors, are expressed in the central nervous system (CNS) where they participate in the neuroinflammatory/neurodegenerative processes and also influence homeostasis and behaviour [6]. *IL18* gene transcripts were identified, by RT-PCR, in a variety of brain regions including the hippocampus, the hypothalamus and the cerebral cortex [7]. Several *in vivo* studies, found IL-18 protein in the pituitary gland, ependymal cells, the neurons of the medial habenula, in Purkinje cells, and in astrocytes in the cerebellum [8]. In addition, it was demonstrated *in vitro* that microglia and astrocytes can produce IL-18 [9].

Moreover, several AD have been shown to suppress pro-inflammatory cytokine production and to release endogenous anti-inflammatory cytokines [10]. Likewise, in healthy rats, chronic antidepressant treatments affected the hypothalamic expression of members of IL-18 system [11]. Taking in consideration these observations, it is plausibly to postulate that the variability of AD therapeutic effect may be modulated by differential expression of cytokines, among these, IL-18. Functional single nucleotide polymorphisms (SNP) in the promoter region of *IL-18* gene, such as rs1946518 and rs187238, are known to alter its transcriptional activity. Rs1946518 polymorphism, involves a change from nucleotide C to nucleotide A at position −607, and it is thought to disrupt a potential cAMP-responsive element binding protein (CREB) site [12]. Similarly, rs187238 polymorphism, located at position −137, involves a change from nucleotide G to nucleotide C, and is recognised to affect the H4TF-1 nuclear factor binding site [12].

IL18 genetic polymorphisms have been associated with distinct clinical conditions, including depressive symptoms in stroke patients [13], and onset of depression in patients previously exposed to stressful-life events [14].

In spite of the fact that several antidepressants drugs are available in the clinical setting, a wide fraction of patients fail to remit to treatment, present relapse or display TRD. Most pharmacogenetic studies regarding antidepressant treatment outcome in MDD patients are typically 8–12 weeks long [2], not exploring remission or relapse phenotypes, which are clinical important outcomes. Identifying patients at risk of relapse, or TRD, using biomarkers could possibly have direct impact on individualizing AD therapy.

Despite the recent implications of IL-18 in depression, *IL18* genetic polymorphisms never been studied in the context of antidepressant treatment phenotypes, including remission and relapse. In this circumstance, we aim to evaluate the role of *IL18-607C > A and IL18-137G > C* functional polymorphisms in antidepressant treatment response phenotypes.

2. Methods

2.1. Patients

The study cohort included 80 participants monitored in a 27 months-follow-up at Hospital Magalhães Lemos (HML), as we previously described [15,16]. Briefly, patients were evaluated for major depression using the Structured Clinical Interview for DSM Axis I Disorders (SCID-I), and for personality disorders using the Axis II Disorders (SCID-II). Severity of the depressive symptoms was evaluated with Beck Depression Inventory (BDI), a cut-off of 20 was used as the point for entry into this study. Exclusion criteria has been described in Santos, et al. (2015) [15]. Patient's treatment followed the Major Depressive Disorder treatment algorithm of Texas Medication Algorithm Project (TMAP) [17]. Initial medication was selected clinically from the medicines included in the first line of approach of the TMAP. The effectiveness of the therapy (administered after at least 6 weeks and at adequate doses according to the TMAP protocol) was assessed as the change in the Beck Depression Inventory (BDI) score. Remission was defined as 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. Antidepressant medication could be changed for nonresponse or intolerably as per the TMAP protocol. 'Treatment resistant depression' (TRD) was recorded 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. Relapse was defined as any depressive episode, upon remission, during the 24-month follow up. Bad Prognosis was considered when patient displayed TRD or relapsed. The study was approved by the ethical committee of Hospital Magalhães Lemos, and written informed consent according to "Declaration of Helsinki" was obtained from each individual after explanation of the study.

2.2. *DNA extraction and* IL18-607C > A and IL18-137G > C *polymorphism analysis*

Peripheral blood samples were collected into ethylenediaminetetraacetic acid (EDTA) containing tubes, and the genomic DNA was isolated according to the manufacturer's instructions, with a commercial kit (E.Z.N.A. – Omega Bio-tek, Norcross, USA), and stored at −20 °C.

The *IL18-607C > A and IL18-137G > C* polymorphisms were determined using a Sequenom MassARRAY system (San Diego, CA, USA), as we previously described [15]. Sequenom Assay Design 3.1 software was used to design the primers for polymerase chain reaction (PCR) amplification and single base extension assays (Sequenom, San Diego, CA, USA). Genotyping data was read blindly to the study endpoint. For quality control, 10% of the samples were randomly selected for a second analysis and 100% of concordance was observed.

2.3. *RNA extraction and IL-18 mRNA expression analysis*

To correlate *IL18* mRNA expression with the different genotype groups, a total of 38 peripheral blood samples were randomly collected after completion of the 27 month follow up. This patient's subgroup included 21 remitters, 9 relapsed and 8 patients with TRD. Age, gender and the last psychotropic drugs prescribed prior to blood recovery were not statistically different among these subgroups of patients (results not shown). Whole blood was collected between 8.30 and 12.30 a.m., and RNA was isolated from blood leucocytes using "Tripure" reagent (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions. RNA's purity and quantity were determined in a "Nanodrop" equipment (ND1000, Nano Drop Technologies Inc. Wilmington, DE, USA), by measuring OD at 260/280 nm and 260 nm respectively. The "High Capacity cDNA Reverse Transcription Kit" (Applied Biosystems, Foster City, CA) was used to reversely transcribed up to 2 μg of total RNA to cDNA, with random primers. TaqMan® Gene Expression Master Mix, primers and probes provided by Applied Biosystems were used for Real-time PCR amplification of cDNA samples, which was performed in a StepOne™ Real-Time PCR System (Applied Biosystems). Expression of *IL18* was measured with TaqMan expression assay (ID: Hs01038788_m1) from Applied

Table 1
Clinical and general characteristics of patients.

IL18-607C/A				P	IL18-137G/C		p
Characteristics	Patients (n = 80)	CC (n = 31)	CA/AA (n = 49)		GG (n = 44)	GC/CC (n = 36)	
Age, years mean (SD) range	40.48 (11.06)	43.06 (9.90)	38.84 (11.54)	0.096	42.54 (10.68)	37.94 (11.14)	0.064
	18.0–60.0	24.0–57.0	18.0–60.0		18.0–60.0	19.0–60.0	
Gender, female n (%)	59 (73.8)	20 (64.5)	39 (79.6)	0.135	29 (65.9)	30 (83.3)	0.078
School attendance, years mean (SD) range	9.63 (3.90)	8.90 (3.74)	10.08 (3.97)	0.190	9.27 (3.47)	10.06 (4.38)	0.386
	4.0–20.0	4.0–17.0	4.0–20.0		4.0–17.0	4.0–20.0	
Baseline BDI Score, mean (SD), range	27.81 (7.40)	27.06 (7.60)	28.29 (7.30)	0.476	28.18 (7.94)	27.36 (6.77)	0.625
	20.0–48.0	20.0–48.0	20.0–46.0		20.0–48.0	20.0–45.0	
Last treatment prescribed, n (%)							
SSRIs	43 (53.8)	16 (33.3)	27 (32.9)	0.986	23 (35.4)	20 (30.8)	0.628
SNRIs	8 (10.0)	3 (6.3)	5 (6.1)		6 (9.2)	2 (3.1)	
Tricyclic drugs	36 (45.0)	12 (25.0)	24 (29.3)		15 (23.1)	21 (32.3)	
Benzodiazepines	25 (31.3)	9 (18.8)	16 (19.5)		12 (18.5)	13 (20)	
Mood stabilizers	9 (11.3)	4 (8.3)	5 (6.1)		4 (6.1)	5 (7.7)	
Other drugs	9 (11.3)	4 (8.3)	5 (6.1)		5 (7.7)	4 (6.1)	

SSRIs – Selective serotonin recapture inhibitors. SNRIs—Serotonin and norepinephrine reuptake inhibitors.

Biosystems. Selection of the reference gene for data normalization was performed among a set of 4 housekeeping genes, *ACTB*, *GAPDH*, *HPRT* and *18S*, and *GAPDH* was chosen since it present higher stability among the MDD samples (data not shown). Relative expression values were obtained through the mean cycle threshold deviation of CA+AA genotype group, using the CC genotype group as control for *IL18-607A > C,* normalized by reference transcripts ($2^{-\Delta\Delta Ct}$). The same calculation was performed for *IL18-137G > C* genetic polymorphism comparing the GC+CC group with the control GG group. The REST 2009 Software (ver.2.0.13) was used for the relative expression analysis [18]. The amplification reaction efficiency for each assay was above 95% (as determined by the manufacturer).

2.4. Statistical analysis

Statistical analysis was performed with IBM®SPSS® (ver.22.0) and Epi Info (ver.6.04a). Chi-square ($\chi 2$) analysis was used to compare the categorical variables with a 5% level of significance. The association between genotypes and the risk of developing the evaluated phenotypes was determined by Odds ratio (OR) with a 95% confidence interval (CI). Fisher's Exact Test was used for tables containing cells with less than 5 individuals. Bootstrapping was used to validate the associations, and p values were determined based on 1000 bootstrap samples. Correlation between genotypes and time to relapse was accomplished with Kaplan-Meier survival curves, which were compared by Log-rank statistical test. The linkage disequilibrium (LD) between loci was measured using Lewontin's D' (D') and r2, and the haplotype frequency were determined with SNP & Variation Suite (SVS) 7 *free trial* (Golden Helix) software. In addition, to correct for multiple comparison testing, adjusted P values were determined using the false discovery rate (FDR) method. Regarding gene expression, Pair Wise Fixed Reallocation Randomisation Test©, calculated by REST software, was used to determined the relative gene expression between genotype groups [18]. A posteriori power analysis was performed using Quanto software (ver.1.0;http://hydra.usc.edu/gxe). The magnitude of our sample is appropriate to detected association effects of at least 2.5 OR for the studied SNPs, with a statistical power of 80%, and a maximal 5% experiment-wise type I error.

3. Results

3.1. Antidepressant response phenotypes

Demographic and clinical variables of the cohort are presented in Table 1. This cohort was mainly constituted by females (73.8%), with a mean age of 40.48 (S.D. 11.06), a mean of 9 years of school (S.D. 3.90) and a mean BDI baseline of 27.81 (S.D. 7.4). The phenotype remission was reported in 61 patients (76.2%), and 16 remitters (20.0%) presented relapse during the follow-up period. Furthermore, 19 patients (23.8%) presented TRD. Bad Prognosis group, which comprised patients presenting relapse or TRD, represented 43.8% (35 patients) of the sample. In order to identify potential confounders we performed an univariate analysis comparing age, gender, school attendance, baseline BDI and the last treatment prescribed, among the different genotypes of the evaluated polymorphisms. None of the sociodemographic and clinical variables were significantly different in each genotype groups (Table 1).

3.2. Genotype frequencies of IL18 polymorphisms and treatment outcome

The genotype distribution of *IL18-607C > A and IL18-137G > C* polymorphisms were evaluated among the different treatment response phenotypes (Table 2). No significant deviations from the Hardy–Weinberg equilibrium proportions were observed for both SNPs (P > 0.05). We found that patients carrying *IL18-607* CA/AA genotypes are more prone to relapse after AD treatment (OR = 4.145; 95% CI: [1.038–16.555]; Fisher $p = 0.043$; Fisher FDR corrected $p = 0.043$) and present a lower time to relapse than patients carrying CC genotype (Fig. 1A; 69 vs 115 weeks, $p = 0.019$, Log-rank test). We also observed that patients carrying *IL18-137* GC/CC genotypes have a higher risk of relapse (OR = 3.988; 95% CI: [1.176–13.516]; $p = 0.022$; FDR corrected $p = 0.043$) and display relapse earlier than the ones carrying GG genotype (Fig. 1B; 64 vs 112 weeks, $p = 0.006$, Log-rank test). Due to the small sample size, especially the low number of patients in the subgroup of CC and GG genotypes that present relapse, we validated the results by bootstrapping, and these findings remained statistically significant (Table 2). No association was found between the evaluated genetic polymorphisms and TRD. Regarding the phenotype bad prognosis, individuals carrying CA/AA genotype for *IL18-607* present approximately a 3-fold risk of developing this phenotype, when comparing with the ones harboring CC genotype (Table 2).

3.3. IL18 haplotypes and treatment outcome

To estimate the combined influence of *IL18* polymorphisms in the risk of relapse, haplotype analysis was carried out. A total of 3 different haplotypes were identified using the SVS software (Table 3). Haplotype analysis revealed an association

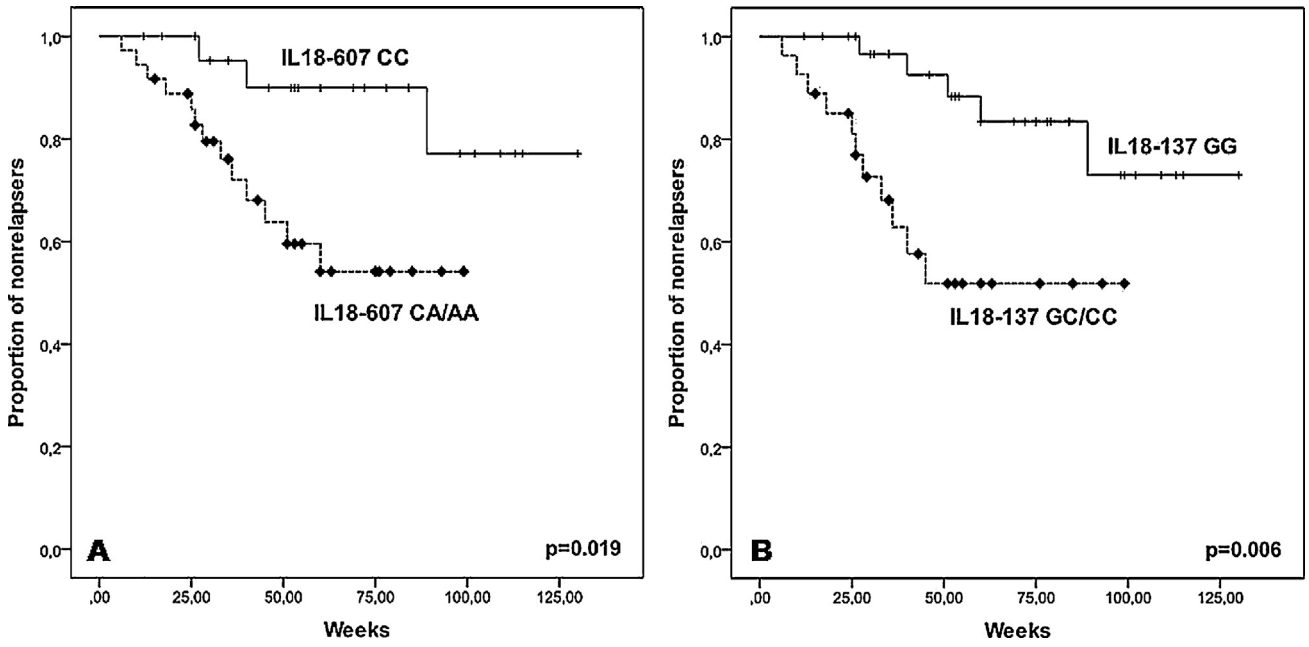

Fig. 1. Effect of *IL18* polymorphisms in time to relapse in MDD patients. Kaplan-Meier analysis was used to evaluate time to relapse between (A) *IL18-607* CC and CA+AA carriers and (B) *IL18-137* GG and GC+CC carriers. Comparison performed by Log-rank test ((A) p = 0.019; (B) p = 0.006). +censored "*IL18-607* CC carriers" or "*IL18-137* GG carriers"; ♦ censored "*IL18-607* CA or AA carriers" or "*IL18-137* GC or CC carriers".

Table 2
Overview of association between genotypes under investigation and outcomes.

	Genotypes Frequency															
	IL18-607 Rs1946518C > A								IL18-137 rs187238G > C							
		No		Yes		OR	CI 95%	P value	P value*		No		Yes		OR	CI 95%	P value	P value*
		N	%	N	%						N	%	N	%				
Relapsed	CC	22	48.9	3	18.7	1.0	Referent	–	–	GG	29	64.4	5	31.2	1.0	Referent	–	–
	CA	18	40.0	11	68.8	4.481	[1.083–18.549]	0.060[a]	**0.038**	GC	13	28.9	9	56.3	4.015	[1.123–14.356]	**0.027**	**0.032**
	AA	5	11.1	2	12.5	2.933	[0.383–22.463]	0.296[a]	0.198	CC	3	6.70	2	12.5	3.867	[0.510–29.304]	0.213[a]	0.094
	A carrier	23	51.1	13	81.3	4.145	[1.038–16.550]	**0.043**[a]	**0.023**	C carrier	16	35.6	11	68.8	3.992	[1.176–13.916]	**0.022**	**0.019**
TRD	CC	25	41.0	6	31.5	1.0	Referent	–	–	GG	34	55.7	10	52.7	1.0	Referent	–	–
	CA	29	47.5	9	47.4	1.293	[0.404–4.138]	0.665	0.664	GC	22	36.1	7	36.8	1.082	[0.358–3.266]	0.889	0.907
	AA	7	11.5	4	21.1	2.381	[0.522–10.860]	0.410[a]	0.253	CC	5	8.20	2	10.5	1.36	[0.228–8.105]	0.662[a]	0.616
	A carrier	36	59.0	13	68.5	1.505	[0.504–4.492]	0.462	0.463	C carrier	27	44.3	9	47.3	1.133	[0.404–3.183]	0.812	0.795
Bad Prognosis	CC	22	48.9	9	25.8	1.0	Referent	–	–	GG	29	64.4	15	42.9	1.0	Referent	–	–
	CA	18	40.0	20	57.1	2.716	[0.996–7.409]	**0.048**	**0.045**	GC	13	28.9	16	45.7	2.379	[0.910–6.223]	0.075	0.082
	AA	5	11.1	6	17.1	2.933	[0.711–12.108]	0.129	0.13	CC	3	6.70	4	11.4	2.578	[0.509–13.046]	0.402[a]	0.211
	A carrier	23	51.1	26	74.2	2.763	[1.061–7.197]	**0.035**	**0.032**	C carrier	16	35.6	20	57.1	2.417	[0.977–5.979]	0.054	0.060

TRD: Treatment Resistant Depression. Bad Prognosis: Relapse and TRD. OR: odds ratio. CI: confidence interval. Significant P values in bold.
[a] Fisher exact test.
* p value based on 1000 bootstrap samples.

Table 3
Haplotype frequencies derived from *IL18* −137/-607 polymorphisms in remitters and relapsed patients with MDD.

Haplotype	Remitter's Frequency	Relapse's Frequency	OR	95% CI	*p* value
GC vs Non GC	0.688	0.531	0.512	0.224–1.168	0.109
CA vs Non CA	0.211	0.406	2.557	1.073–6.093	**0.032**
GA vs Non GA	0.100	0.065	0.600	0.123–2.938	0.525

OR: Odds ratio. CI: Confidence interval.

between relapse and the presence of IL18 −137/-607CA haplotype (OR = 2.557, 95% CI [1.073–6.093], $p = 0.032$). However, after correcting for multiple comparisons, the association did not retained significance (FDR $p = 0.093$). Regarding TRD, no association was found between the evaluated haplotypes and this phenotype.

3.4. Peripheral IL-18 mRNA expression

In order to identify whether the different *IL18-607C > A* and *IL18-137G > C* genotypes modulate the peripheral expression of *IL18* transcripts; we evaluated mRNA expression by quantitative real-time PCR in the peripheral blood of 38 MDD patients. *IL18-607C > A*

Table 4
Gene Expression Pattern in the *IL18-607C > A* and *IL18-137G > C* Genotypes.

SNP	Expression	CI (95%)	*P* value*	Result
IL18-607C > A, CC vs CA + AA	**1.855**	**0.097–89.536**	**0.029**	**UP**
IL18-137G > C, GG vs GC + CC	1.625	0.081–70.274	0.117	NS

CI, confidence interval; UP, upregulated; NS, sample group is not different than the control group.

* Pair Wise Fixed Reallocation Randomization test.

was associated with a 1.855 fold increase in *IL18* expression for CA/AA carriers (1.855; *p = 0.029;* 95% CI [0.097–89.536]), when compared to CC carriers (Table 4). Regarding *IL18-137G > C*, despite *IL18* mRNA levels were more elevated in patients carrying *IL18-137* GC/CC genotypes than in GG genotypes, results were not statistically significant (1.625; $p = 0.117$; 95% CI [0.081–70.274]). Additionally, peripheral mRNA expression was not associated with the last treatment prescribed or treatment response ($p > 0.05$).

4. Discussion

Among pro-inflammatory cytokines involved in the etiopathogenesis of depression, the potential role of IL-18 relies both on animal models and clinical studies [19]. Dysregulated IL-18 levels have been attributed to several causes, such as neurogenic stimulation or stress [6], but it is still not clear whether IL-18 elevation contributes to neuropsychiatric pathologies or whether it is a consequence of these disorders [6]. Taken this in consideration, and given the fact the *IL18* genetic polymorphisms have never been evaluated in AD treatment outcome, we evaluated the role of *IL18* promoter SNPs in antidepressant treatment phenotypes.

The results reported herein suggest an association of *IL18-607C > A and IL18-137G > C* genetic polymorphisms and the risk of depression relapse upon AD treatment. Moreover, we also observed an association of these polymorphisms with time to relapse. Regarding the combined effect of the presence of both variants, haplotype analysis showed an association between relapse and the presence of *IL18-137/-607* CA haplotype, although it loses significance after multiple correction. We also observed a tendency for Bad Prognosis in patients carrying IL18-607CA/AA, although is likely to occur do to the contribution of the relapse phenotype, since we haven't found an association between any of the genotypes and TRD. These results point towards a putative connection between *IL18* genetic polymorphisms, and a higher risk of depression relapse.

The idea that IL-18 dysregulation may contribute to depressive symptoms has been supported by other authors. In the study of Kroes et al., *IL18* was one of the twenty-two transcripts differentially expressed in the neocortex between dominant and subordinate mice, undergoing stressful social loss and victory [19]. Authors suggested that this molecule might help submissive animals to better cope with the social stress. This hypothesis was further supported by another study, in which these two *IL18* genetic polymorphisms, rs187238 and rs1946518, were associated with increase susceptibility to depression in patients previously exposed to stressful life events [14]. Additionally, it has been observed that IL-18 serum levels were significantly higher in MDD patients [20]. In that study, authors reported that even when patients were treated with TCAs (*e.g.*, amitriptyline), the levels of IL-18 were still higher than those in the control group. This indicates that in some patients, despite undergoing AD treatment, still exhibit a severe immunological stimulation, where IL-18 plays its part. It is possible that this IL-18 immunological dysregulation could be due to the contribution of genetic polymorphisms, as we describe herein.

All evidences corroborate our findings, since we observed that rs187238 and rs1946518 were associated with the relapse of depressive symptoms and, moreover, we've detected that *IL18* was upregulated in patients harboring CA or AA genotypes of *IL18-607C > A.* Giedraitis et al. postulated that two SNPs at positions −607 C > A and −137 G > C within the *IL-18* promoter region alter the promoter activity [12]. However we have not observed any statistically significant influence of −137 G > C polymorphism on IL-18 expression, probably because spontaneous and cell line transcripts are different as suggested by Liang *et al.,* [21]. Our results are in agreement with the evidences shown by Khripko and colleagues [22], which reported a higher frequency of A-allele carriers among persons with a high level of spontaneous IL-18 production. Moreover, they also found that the IL-18 production by LPS-stimulated peripheral blood mononuclear cells (PBMC) was significantly greater in healthy donors carrying the CA genotype than in those with the CC genotype at the *IL18-607* [22]. This is also in accordance with our findings since the in our A allele carriers (CA+AA) group, CA genotype represents approximately 85% of all group.

The association of *IL18-607C > A and IL18-137G > C* genetic polymorphisms with higher risk of relapse and a lower time to relapse could be due to influence of IL-18 in stress induced depressive symptoms [6]. IL-18 is one of the cytokines (as well as IL-1β) activated by the inflammasome complex, a structure recognised to be a central mediator by which psychological and physical stressors contribute to the development of depression [23]. So, it is likely that *IL18* polymorphisms, by affecting IL-18 levels, would consequently interfere with downstream events such as the processing of pro-IL-18 to IL-18, by the inflammasome component caspase 1 [24], contributing to depression relapse. Likewise, recent findings suggest that sustained sad mood, results in a substantial increase in plasma IL-18 concentration, via μ-opioid neurotransmission, potentially in response to perceived emotional stress [25], and additionally, IL-18 is thought to be involved in the regulation of the stress response Hypothalamic–Pituitary–Adrenal (HPA) axis, known to be dysregulated in depression [6,26]. Besides these hypotheses, recent literature suggests an alternative possibility, by which IL-18 would influence synaptic plasticity and neurogenesis, because it is recognised as an activator of JAK–STAT pathway, through STAT3, in hippocampal cells [27].

To the best of our knowledge, this is the first study to evaluate the role of *IL18* functional polymorphisms in antidepressant treatment outcome. We recognize that despite its novelty, this preliminary study holds some limitations. One limitation concerns sample size, particularly when considering the low number of patients in the subgroup of CC and GG genotypes that present relapse. Taken this in account, bootstrapping analysis was performed and validated our results. Additionally, this study is, as far as we know, the largest one studding the influence of genetic variants in antidepressant treatment phenotypes in Portuguese population, with a long follow up time. Albeit, the statistical power is still sufficient to detect the magnitude of the effect reported regarding individual polymorphisms. However and since haplotype analysis loses its significance after multiple correction, probably due the small sample size, we recognize that our findings should be replicated and validated in larger independent cohorts. A further limitation stays in the fact that *IL18* genotypes have been correlated with peripheral transcripts levels not with central IL-18 levels, and we have not controlled patients' exposure to stressors, a condition which could influence mRNA analysis. However, the literature is consensual about the central role of IL-18 [11], and regarding the possible link between IL-18 and physical or psychological stressors, future studies may include of the measure of self-perception of stress, using Perceived Stress Scale or Life Events and Difficulties Schedule.

The findings reported herein may corroborate the immunoinflammatory mechanisms underlying depression; suggesting that

IL-18 polymorphisms may play a role in a relapse predisposed phenotype. Still, it is likely that other molecules may influence this phenotype, thus this marker should be integrated with others to detect the susceptibility of relapse upon AD treatment.

Funding

Funding for this study was partially provided by AstraZeneca Foundation; the AstraZeneca Foundation had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication. MS holds a grant from Politécnico do Porto (IPP/P-067/2015).

Contributors

MS, SC, RM were responsible for the study design. SC, JMP, PP, DM, DC, and SG collected the data. MS and LL performed the experiments. MS, LL, SC and RM analysed the data. MS wrote the first draft of the manuscript. LL, RM, SC and AC provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved the final version submitted.

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