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VALÉRIA KRUCHELSKI HUK

ASSOCIATION OF ORAL CONDITIONS AND POLYMORPHISMS IN THE *TNF* CLUSTER GENES WITH CHRONIC KIDNEY DISEASE AND MORTALITY

Curitiba
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Tese apresentada ao Programa de Pós-Graduação em Odontologia da Pontifícia Universidade Católica do Paraná, como parte dos requisitos para obtenção do título de Doutor em Odontologia, Área de Concentração em Clínica Odontológica Integrada (Ênfase em Estomatologia).

Orientador: Prof.ª Dr.ª Paula Cristina Trevilatto

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TERMO DE APROVAÇÃO

VALÉRIA KRUCHELSKI HUK

ASSOCIAÇÃO DE CONDIÇÃO BUCAL E DE POLIMORFISMOS NOS GENES DO CLUSTER DO TNF COM DOENÇA RENAL CRÔNICA E MORTALIDADE

Tese apresentada ao Programa de Pós-Graduação em Odontologia da Pontifícia Universidade Católica do Paraná, como parte dos requisitos parciais para a obtenção do Título de **Doutor em Odontologia**, Área de Concentração em **Clínica Odontológica Integrada com Ênfase em Estomatologia**.

Orientador (a): Profª Drª Paula Cristina Trevilatto
Programa de Pós-Graduação em Odontologia, PUCPR

Prof. Dr. Paulo Henrique Couto Souza
Programa de Pós-Graduação em Odontologia, PUCPR

Profª Drª Luciana Reis Azevedo Alanis
Programa de Pós-Graduação em Odontologia, PUCPR

Prof. Dr. Eduardo Bauml Campagnoli
Programa de Pós-Graduação em Ciência da Saúde, UEPG

Profª Drª Ana Paula de Souza
Programa de Pós-Graduação em Biologia Buco-Dental, FOP/UNICAMP

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Pai e Mãe
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A melhor faculdade que já estudei. E o melhor
assunto para uma dissertação de mestrado.
Vocês também são a melhor tese de doutorado
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são a morfologia da vida. Vocês são o formato
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SUMÁRIO

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Association of oral conditions and polymorphisms in the *TNF* cluster genes with chronic kidney disease and mortality

Valéria Kruchelski Huk de Andrade[1]; ORCID 0000-0002-6102-7018
Márcia Olandoski[2]; 0000-0002-0979-3604
Miguel Carlos Riella[3]; ORCID 0000-0003-4181-613X
Roberto Pecoits-Filho[2]; ORCID 0000-0002-0255-6710
Cleber Machado de Souza[2]; ORCID 0000-0003-0565-3492
Paula Cristina Trevilatto[1] ORCID 0000-0001-5781-8629

[1] School of Life Sciences, Pontifícia Universidade Católica do Paraná, Curitiba, Brazil.
[2] School of Medicine, Pontifícia Universidade Católica do Paraná, Curitiba, Brazil.
[3] Fundação PRÓ-RENAL, Curitiba, Brazil.

Short title: Oral conditions and *LTA* polymorphisms in CKD

***Corresponding author:**
Paula Cristina Trevilatto
School of Life Sciences
Pontificia Universidade Católica do Paraná (PUCPR)
Imaculada Conceição Street, 1155, Prado Velho
Curitiba-PR. Brazil. CEP: 80.215-901
Telefone +55 (41) 3271-1849
e-mail: paula.trevilatto@pucpr.br

Abstract

Background: Chronic Kidney Disease (CKD) is a complex condition. Exacerbation in the inflammatory mediators is associated with increased morbidity and mortality. The aim was to investigate the association of oral condition and genetic polymorphisms in the TNF cluster genes with risk to chronic kidney disease and the impact of genetic variables on mortality.

Material and Methods: This is a case-control study consisted of 242 subjects divided into 122 patients with CKD undergoing hemodialysis and 120 patients without the disease. Sociodemographic and clinical parameters of oral health were analyzed. Markers for full coverage in the TNF cluster genes were selected from information on the International HapMap Project website and genotyped. The hemodialysis patients' group was followed up for 5 years. For the multivariate analysis, the binary logistic regression model was adjusted and variables with the significance of $p<0.20$ were included. Survival analysis was done using the Kaplan-Meier model and compared using the Log-rank test. Values of $p<0.05$ were considered significant.

Results: Advanced age ($p=0.000$), male sex ($p=0.000$), smoking habit ($p=0.002$), and the G allele of rs2229094 of the LTA gene ($p=0.012$) were associated with CKD. LTA polymorphisms, rs2844482 in the additive and recessive models ($p=0.016$; $p=0.007$ respectively) and rs2229094, in the recessive model ($p=0.029$), were associated with mortality in hemodialysis patients.

Conclusions: An association between sociodemographic and genetic aspects with CKD was found. LTA gene polymorphisms had also an impact on mortality of CKD patients in hemodialysis.

Keywords: Genetic polymorphism; chronic kidney failure, oral health, lymphotoxin-alpha, tumor necrosis factor-alpha.

1. Introduction

Chronic Kidney Disease (CKD) is a complex condition in which environmental and genetic factors are involved[1–4]. The chronic renal patient has physiological alterations due to loss of renal function, leading to an increase in the production of inflammatory cytokines, such as interleukin 1 and 6 (IL-1 and IL-6), tumor necrosis factor (TNF), C-reactive protein, and oxidative stress markers[1,5]. However, the systemic inflammatory burden in individuals with CKD may vary and cannot be explained only by factors such as hemodialysis or clinical procedures[3,6]. Exacerbation in the production of inflammatory mediators has been associated with increased morbidity and mortality in this group of patients[4].

Several factors contribute to the presence of chronic inflammation in CKD patients, including poor oral health[7] and genetic background[2]. Poor oral health status is related to chronic kidney disease as a source of inflammation and contributing to complications that result in morbidity and mortality observed in end-stage patients[8].

In CKD, chronic inflammation is mediated by cytokines[1,5], low molecular weight glycoproteins that participate in numerous immunological processes, including pro and anti-inflammatory activities. The *TNF cluster* is composed of the *LTA*, *TNFA,* and *LTB* genes, located on the short arm of 6 chromosome, which encode the cytokines LT-α, TNF-α, and LT-β, respectively[9]. LT-α and LT-β are secreted by lymphocytes, the first acts locally as a mediator of the acute inflammatory response, recruiting and activating neutrophils, but it is produced in small quantities [9,10]. The second is a transmembrane protein that acts as an anchor for LT-α. Besides, LT-β may be an important regulator of the inflammatory response[9]. TNF-α is secreted by macrophages and participates in numerous processes of the immune-inflammatory response. When produced at high levels it can cause excessive inflammation and tissue damage locally or systemically[11].

It has been suggested that polymorphisms may alter gene expression and/or cytokine production. Moreover, SNPs may influence the phenotype and outcome in CKD patients[3]. The investigation of genetic polymorphisms in the *TNF* cluster genes becomes interesting since those genes play an important role in the pro-inflammatory and immunological response.

Single nucleotide polymorphisms in the *LTA*, *TNFA,* and *LTB* genes have been associated with the development and progression of different diseases, such as cardiopathy associated with Chagas Disease[12], rheumatoid arthritis[13], and tuberculosis[14]. Further, SNPs in the *TNFA* gene were associated with CKD complications [15,16] and death risk [17]*.* This study hypothesized that oral condition and polymorphisms in the *TNF cluster* genes could be related to risk and prognostic in chronic kidney disease.

Thus, this study aimed to investigate the association of oral aspects and polymorphisms in the *TNF cluster* genes with CKD and mortality in hemodialyzed patients.

2. Material and Methods

2.1 Study population

The first part of this study comprised an observational, cross-sectional study with a sample composed of 242 subjects from the Southern region of Brazil of both genders. A sample was divided into case group, with 122 patients with stage 5 CKD undergoing hemodialysis (HD), selected at the Pro-Renal Foundation of Curitiba (Pro-renal) and control group, with 120 subjects without CKD [glomerular filtration rate > 90 mL/min/1.73m^2 estimated according to MDRD (*Modification of Diet in Renal Disease*)], selected at the Dentistry Clinics of the Pontifical Catholic University of Paraná (PUCPR). The data were collected in the period from August 2004 to April 2008 (around sixty months).

Subjects were not included when had chronic use of anti-inflammatory drugs, HIV infection, immunosuppressive chemotherapy, systemic active infection, current or lactating pregnancies, with diseases of hard or soft oral tissues (except caries and periodontal disease), with use of orthodontic appliances, and with the presence of gingivitis and/or necrotizing ulcerative periodontitis.

Informed consent was obtained from all individual participants included in the study, with the protocol approved by the Research Ethics Committee of PUCPR (CAAE 25141813.4.0000.0020).

2.2 Clinical and sociodemographic parameters

All patients answered a questionnaire to inform the baseline parameters such as age, sex, smoking habit, the main cause of CKD, and systemic conditions.

2.3 Oral condition parameters

The following dental health parameters were evaluated: dentist visit frequency, flossing, toothbrushing frequency, plaque index (PI)[18], calculus index (CI)[19], gingival index (GI)[18], presence of periodontal disease (PD), mobility and xerostomia.

Dentist visit frequency was registered as quarterly/semester or annually/rarely. Flossing was answered by the patient as “yes” or “no”. Toothbrushing was answered by the patients as less than three times a day or equal/more than three times a day. For PI, CI, and GI, each tooth received a score ranging from 0 to 3. The scores were recorded, added, and divided by the number of teeth to obtain an individual index for each patient. The average for the indices was carried out within the groups and later dichotomized for better visualization of the results. The diagnosis of chronic periodontal disease (PD) was made based on clinical parameters[20]. Mobility was verified by the evaluator and recorded as “yes” or “no”.

2.4 DNA collection and purification

DNA was obtained from buccal epithelial cells[21]. The participants did a mouthwash with 5 mL of 3% glucose solution for 1 minute. Then, a sterile wood spatula was used to scrape the buccal mucosa. Oral epithelial cells were pelleted by centrifugation at 2000 rpm for 10 minutes. The supernatant was discarded, and the cell pellet resuspended in 1.3 ml of extraction buffer [10 mM Tris–HCl (pH 7.8), 5 mM ethylenediaminetetraacetic acid (EDTA), and 0.5% sodium dodecyl sulfate (SDS)]. Ten microliters of proteinase K (20 mg/ml) were added to the solution, and this was incubated overnight at 65°C. DNA was purified with 10 M ammonium acetate and 1 mM EDTA[22].

2.5 Selection of markers and genotyping

Tag SNP markers, which capture the whole gene information, were selected according to the information available on the *International HapMap Project* website. The release 24/phase 2_Nov08 was used, and the selected markers showed a

minimal allelic frequency of 5% in the African population (YRI). A cutoff of $r^2 \geq 0.8$ of linkage disequilibrium (LD) was used. After applying the above criteria, six tag SNPs for *LTA* (rs2844482, rs2009658, rs2071590, rs2229094, rs2516312, rs3093542), two for *TNFA* (rs1800629 and rs2228088), and one for *LTB* (rs3093553) were selected.

The tag SNPs were genotyped using polarized fluorescence (ABI 7500 platform) by real-time PCR technique using the TaqMan™ Genotyping Master Mix (Applied Biosystems 7500 Real-Time PCR System).

2.6 Survival analysis

In the second part of this study, the case group patients (n=117) were followed prospectively for 5 years. During this prospective follow-up data were collected only for the primary endpoint (mortality due to any causes) or end of the study. Five patients had an incomplete dataset and, therefore, they were excluded from the analysis.

2.7 Statistical analysis

The Haploview 4.2 software was used to estimate the Hardy-Weinberg equilibrium, to establish the associated allele or reference allele for each tag SNP, and to analyze the LD between the SNPs of the *LTA*, *TNFA,* and *LTB* genes.

SPSS version 20.0 was used for statistical analysis. The nominal variables were expressed in number and frequency and analyzed by Pearson's chi-square test. Continuous variables were expressed as mean and standard deviation using the t-test for independent samples.

Multivariate analysis was performed using the binary logistic regression model by the *backward conditional* method and variables with the significance of $p<0.20$ were included, the model was adjusted for variables with clinical impact. Univariate survival analysis was done using the Kaplan-Meier model. Survival in different genotype groups was compared using the Log-rank test. Values of $p<0.05$ were considered significant.

2.8 Power Calculation

The calculation of the sample power was performed using the Genetic Power Calculation[23] setting as parameters: prevalence of hemodialysis patients of 0.061% in the Brazilian population[24], effect size of 2.0 for carrying one copy of the risk allele and 4.0 for carrying two copies of the risk allele, D'= 1, frequency of the rare allele of 30%, 1: 1 case and control ratio and level of significance of 0.05, with the power variation being dependent on the relative risk of the rare allele.

3. Results

3.1 Clinical and sociodemographic parameters

The clinical and sociodemographic characteristics of the sample were described in Table 1. In this study, the mean age for the case group was 49.74±12.83 and for the control group, 39.93±9.51. The variables higher age (p=0.000), male sex (p=0.000), and smoking (p=0.000) were associated with CKD in the study population. The ethnic variable showed no significant difference between groups. The main cause of CKD was chronic glomerulonephritis (33.6%), followed by diabetic nephropathy (18.0%), and hypertensive nephropathy (11.5%). About systemic conditions, most patients with CKD were hypertensive (73.0%) and had hepatitis (23.3%), CVD (22.1%), and diabetes (21.3%).

3.2 Oral condition parameters

In the case group, 69 (56.6%) did not use dental floss (p=0.005) and 109 (89.3%) performed brushing less than three times a day (p=0.001). However, about dentist visit frequency, 66 renal patients (54.1%) reported visiting the dentist annually/rarely against 102 (85%) in the control group (p=0.000). The variables PI, CI, GI, and presence of PD did not show significant differences between the groups. In the CKD group, dental mobility was observed in 48 patients (39.3%) (p=0.003) (Table 2).

3.3 Genetic analysis

The genotypic frequencies of all the markers tested were in Hardy-Weinberg equilibrium in the control group. The distribution of the genotypic frequencies in the additive, dominant, and recessive models for the tag SNPs in the *LTA*, *TNFA,* and *LTB* genes is shown in table 3. Some failures in genotyping were observed and the

individuals excluded from the analysis, justifying the variable sample number differently observed in each one of the genotypic models studied.

The LD map showed that rs2844482 and rs2009658 of the *LTA* gene had r^2=1.00, (Fig. 1). For the Brazilian population, only one of them should be genotyped in future analyzes because they are in perfect LD.

3.4 Multivariate analysis

The multivariate analysis by the backward conditional method was performed. The variables: age (*p*=0.000), sex (*p*=0.000), smoking habits (*p*=0.002), and the recessive model for the G allele of rs2229094 (*p*=0.012) of the *LTA* gene remained associated with CKD (Table 4).

3.5 Survival analysis

The survival analysis showed there were 33 (28.2%) death events in the hemodialysis patients during de follow-up period. Overall, nine tag SNPs in the *TNF cluster* were analyzed and after the Log-rank test, only two polymorphisms (rs284482 and rs2229094) in the *LTA* gene were associated with mortality.

Figure 2 illustrates the only significant survival curves for each tag SNPs in the *LTA* gene. The G allele for the rs2229094 (in the recessive model) was associated (*p*=0.029) with mortality in hemodialysis patients during the follow-up. Furthermore, the rs2844482, allele T in the additive and recessive models respectively associated with mortality of CKD patients (*p*=0.016; *p*=0.007).

There was no significant association between *LTB* and *TNFA* polymorphisms and mortality in this group of patients.

3.6 Results of power calculation

The analysis of the genetic power of the sample is directly dependent on the frequency of the risk allele. Thus, for the risk allele frequency of 0.3, the sample power was 95% (detailed in Table 5).

4. Discussion

Chronic kidney disease is a complex condition in which different factors interact for development and progression. Inflammation is a known and well-established risk factor, directly associated with the prognosis of patients[3,4].

In this study, CKD patients were approximately ten years older (49.7) than the control group (39.9) and were mostly male. Although the prevalence of CKD is higher in females, men appear to progress faster to end-stage chronic kidney disease[25]. The influence of sex on the progression of CKD is not well understood yet. However, some differences may be associated with the individual's biological component[26].

This study reinforces the interaction between smoking and CKD. Smoking accelerates the progression of renal disease and increases the risk for CVD[27]. Evidence shows up to 50% increased the risk for tobacco related CKD[28]. The habit results in renal fibrosis, consequently contributing to a rapid decline in renal function[29]. These changes due to smoking increase the risk of mortality in patients with CKD, due to cardiovascular and other causes[27].

In the univariate analysis, the results showed that there were low rates of tooth brushing and flossing in the studied population. The status of oral health in patients with CKD has already been reported[30]. However, the increased frequency of visits to the dentist observed, different from what the literature shows, is justified because they receive close dental care assistance at the Pro-Renal Foundation in Curitiba/PR, where CKD patients perform their treatment. Frequent dental visits and treatment of oral infectious loci have been reported as fundamental to reduce systemic inflammatory conditions in this particular group of patients[7,31]. Further, the results showed that 39.9% of patients with CKD presented mobility, probably because of PD. The oral inflammatory disease, such as gingivitis and PD, are the most common manifestations of poor oral health and are considered a focus of inflammation[8,32]. The impact of oral health on systemic health has been discussed[33]. Frequent dental visits and treatment of oral infectious loci to reduce systemic inflammatory conditions in this group of patients have been reported as fundamental[7,31]. Despite the lack of association of oral health variables in the multivariate analysis, the results may reinforce the importance that understanding

the interaction between oral health and systemic health is essential to maintain the quality of life of the patient on hemodialysis.

The pathophysiology of chronic kidney disease is well known and studied. Cytokines link to their receptors located in the cell membrane and regulate the transcription of several genes leading to changes in the renal tissues[1]. *LTA* gene encodes LT-α, a proinflammatory cytokine that shares a signaling network with TNF-α[9]. The role of LT-α in the pathophysiology of kidney disease is little discussed in the literature. In this study, the association between the allele G (in the recessive model) of rs2229094 in the *LTA* gene and risk for CKD was found. This same tag SNP was also associated with different conditions[34–37], however, there is no other study in the literature investigating the association of this polymorphism with CKD in other populations. Further, there was no association of *TNF* and *LTB* genes polymorphisms with CKD in the studied population.

It has been suggested that elevated levels in inflammatory markers predict poor prognosis and mortality in dialysis patients[3]. Thus, polymorphisms in the *LTA*, *TNFA,* and *LTB* genes could be associated with mortality in this group of patients. After the survival analysis, the polymorphisms rs2844482 and rs2229094, in the *LTA* gene, are described for the first time as risk factors for mortality in hemodialysis patients.

However, this study has limitations. The frequency of the rarer alleles was low for some tag SNPs. Although the calculation of genetic power shows that the sample size is adequate, the replication of this study in other populations is encouraged to better elucidate the role of these genes influencing the susceptibility to CKD and the outcome in patients on hemodialysis.

It was concluded that sociodemographic aspects, smoking habit, and rs2229094 (allele G, in the recessive model) of the *LTA* gene were associated with CKD in the studied Brazilian population. Also, the polymorphisms rs2844482 and rs2229094 of the *LTA* gene were associated with mortality in hemodialysis patients and might be suggested as markers of bad prognosis for CKD.

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Declarations

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Conflict of interest

The authors declare that they have no conflict of interest.

Availability of data and material

Data will be made available upon request

Ethics approval

Informed consent was obtained from all participants included in the study, with the protocol approved by the Research Ethics Committee of PUCPR (CAAE 25141813.4.0000.0020). The study was performed by the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Consent

Informed consent was obtained from all individual participants included in the study.

Authors' contributions

All authors contributed to the study conception and design. The first author, Valéria Kruchelski Huk de Andrade drafted the article and participated in the interpretation of data. The second author Márcia Olandoski analyzed data and, also, participated in the interpretation of data. The authors Miguel Carlos Riella and Roberto Pecoits-Filho provided intellectual content of critical importance to the work described, especially about renal disease and diagnosis. Cleber Machado de Souza participated in the conception and design of the study and revised the article. The last author, Paula Cristina Trevilatto, provided intellectual content of critical importance to the work and revised the final version. All authors read and approved the final manuscript and are accountable for all aspects of this study.

Tables

Table 1 Clinical and sociodemographic variables for groups case and control.

General Data	Case (n=122)	Control (n=120)	*p-value*	OR (CI 95%)
Age[a]	49.74 ± 12.83	39.93 ± 9.51	0.000*	-
Sex[b]				
Male	81 (66.4)	38 (31.7)	0.000**	2.04 (1.54-2.69)
Female	41 (33.6)	82 (68.3)		
Ethnic Group[b]				
Caucasi	87 (71.3)	94 (78.3)	0.208**	1.45 (0.81-2.61)
Non-caucasian	35 (28.7)	26 (21.7)		
Smoking habits[b]				
Yes	28 (23.0)	8 (6.7)	0.000**	4.17 (1.81-9.58)
No	94 (77.0)	112 (93.3)		
Main cause of CKD[b]				
Chronic glomerulonephritis	41 (33.6)	0 (0.0)	0.000**	-
Diabetic nephropathy	22 (18.0)	0 (0.0)	0.000**	-
Hypertensive Nephropathy	14 (11.5)	0 (0.0)	0.000**	-
Others	40 (16.4)	0 (0.0)	0.000**	-
Systemic condition[b]				
Anemia	41 (33.6)	3 (2.5)	0.000**	
Hypertension	87 (71.3)	2 (1.7)	0.000**	-
Hepatitis	28 (23.0)	2 (1.7)	0.000**	-
Cardiovascular Disease	27 (22.1)	2 (1.7)	0.000**	-
Diabetes	26 (21.3)	2 (1.7)	0.000**	-

[a]Mean ± Standard Deviation
[b]Absolute Number (%)
OR: Odds Ratio
CI (confidence interval)
*p-value for T test for independent samples
**p-value for Pearson *qui*-square test

Table 2 Oral condition variables for groups case and control.

General Data		Case (n=122)	Control (n=120)	*p-value*	OR (CI 95%)
Dentist Visit Frequency[b]					
	Quarterly/Semester	56 (45.9)	18 (15.0)	0.000*	0.20 (0.11-0.39)
	Annualy/rarely	66 (54.1)	102 (85.0)		
Flossing[b]					
	Yes	53 (43.4)	74 (61.7)	0.005*	2.09 (1.25-3.50)
	No	69 (56.6)	46 (38.3)		
Toothbrushing ≤ 3 times/day[b]					
	Yes	109 (89.3)	88 (73.3)	0.001*	3.04 (1.50-6.15)
	No	13 (10.7)	32 (26.7)		
Plaque Index (PI)[b]					
	< 1	34 (27.9)	33 (27.5)	0.949*	0.98 (0.55-1.72)
	≥ 1	88 (72.1)	87 (72.5)		
Calculus Index (CI)[b]					
	=0	96 (78.7)	102 (85.0)	0.203*	1.53 (0.79- 2.97)
	>0	26 (21.3)	18 (15.0)		
Gingival Index (GI)[b]					
	< 1	82 (67.2)	75 (62.5)	0.443*	0.81 (0.47-1.37)
	≥ 1	40 (32.8)	45 (37.5)		
Presence of PD[b]					
	Yes	73 (59.8)	58 (48.3)	0.073*	1.59 (0.95-2.65)
	No	49 (40.2)	62 (51.7)		
Mobility[b]					
	Yes	48 (39.3)	26 (21.7)	0.003[a]	2.34 (1.33-4.13)
	No	74 (60.7)	94 (78.3)		
Xerostomia[b]					
	Yes	55 (45.1)	16 (13.3)	0.000*	2.69 (1.72-4.23)
	No	67 (54.9)	104 (86.7)		
DMF-T Index[a]		34.00 ± 9.26	19.02 ± 6.93	0.121**	-

[a] Mean ± Standard Deviation
[b] Absolute Number (%)
OR: Odds Ratio
CI (confidence interval)
* p-value for Pearson qui-square test
**p-value for T test for independent samples

Table 3. Genetic analysis of *LTA*, *LTB* and *TNFA* genes in the additive, dominant and recessive models

Gene	dbSNP[a]	Genetic Model	Genotypes	Groups - n (%) Case	Control	p-value	OR (CI 95%)
LTA	rs2844482	Additive	CC	79 (64.8)	81 (68.1)	0.251*	-
			CT	36 (29.5)	36 (30.3)		
			TT	7 (5.7)	2 (1.7)		
		Dominant T	TT + CT	43 (35.2)	38 (31.9)	0.586*	1.16 (0.67 -1.98)
			CC	79 (64.8)	81 (68.1)		
		Recessive T	CC + CT	115 (94.3)	117 (98.3)	0.097*	0.28 (0.05 -1.38)
			TT	7 (5.7)	2 (1.7)		
LTA	rs2009658	Additive	GG	79 (66.4)	81 (68.1)	0.353*	-
			CG	34 (28.6)	36 (30.3)		
			CC	6 (5.0)	2 (1.7)		
		Dominant C	CC + CG	40 (33.6)	38 (31.9)	0.782*	1.07 (0.62 -1.85)
			GG	79 (66.4)	81 (68.1)		
		Recessive C	GG + CG	113 (95.0)	117 (98.3)	0.150*	0.32 (0.06 -1.62)
			CC	6 (5.0)	2 (1.7)		
LTA	rs2071590	Additive	CC	61 (50.0)	48 (40.7)	0.347*	-
			CT	51 (41.8)	58 (49.2)		
			TT	10 (8.2)	12 (10.2)		
		Dominant T	TT+CT	61 (50.0)	70 (59.3)	0.147*	0.68 (0.41 -1.14)
			CC	61 (50.0)	48 (40.7)		
		Recessive T	CC + CT	112 (91.8)	106 (89.8)	0.596*	1.26 (0.52 -1.68)
			TT	10 (8.2)	12 (10.2)		
LTA	rs2229094	Additive	AA	60 (49.2)	57 (47.9)	0.113*	-
			AG	46 (37.7)	55 (46.2)		
			GG	16 (13.1)	7 (5.9)		
		Dominant G	GG + AG	62 (50.8)	62 (52.1)	0.842*	0.95 (0.57–1.57)
			AA	60 (49.2)	57 (47.9)		
		Recessive G	AA + AG	106 (86.9)	112 (94.1)	0.056*	1.43 (1.05 – 1.93)
			GG	16 (13.3)	7 (5.9)		
LTA	rs2516312	Additive	AA	114 (94.2)	118 (99.2)	0.066*	-
			AG	7 (5.8)	1 (0.8)		
			GG	-	-		
		Dominant G	GG + AG	7 (5.8)	1 (0.8)	0.066*	0.13 (0.01-1.14)
			AA	114 (94.2)	118 (99.2)		
		Recessive G	AA + AG	121 (100.0)	119 (100.0)	-	-

			GG	-	-		
LTA	**rs3093542**	**Additive**	GG	114 (93.4)	109 (93.2)	0.584*	-
			CG	7 (5.7)	8 (6.8)		
			CC	1 (0.8)	-		
		Dominant G	GG + CG	121 (99.2)	117 (100.0)	0.326*	1.96 (0.73-2.22)
			CC	1 (0.8)	-		
		Recessive G	CC + CG	8 (6.6)	8 (6.8)	0.931*	0.95 (0.34-2.63)
			GG	114 (93.4)	109 (93.2)		
LTB	**rs3093553**	**Additive**	AA	106 (87.6)	103 (86.6)	0.809*	-
			CA	15 (12.4)	16 (13.4)		
			CC	-	-		
		Dominant C	CC + CA	15 (12.4)	16 (13.4)	0.809*	1.09 (0.51 – 2.33)
			AA	106 (87.6)	103 (86.6)		
		Recessive C	AA + CA	121 (100.0)	119 (100.0)	-	-
			CC	-	-		
TNFA	**rs2228088**	**Additive**	GG	122 (100.0)	118 (98.3)	0.152*	-
			GT	-	2 (1.7)		
			TT	-	-		
		Dominant T	TT + GT	-	2 (1.7)	0.152*	2.03 (1.78 – 2.31)
			GG	122 (100.0)	118 (98.3)		
		Recessive T	GG + GT	122 (100.0)	120 (100.0)	-	-
			TT	-	-		
TNFA	**rs1800629**	**Additive**	GG	100 (82.0)	98 (82.4)	-	-
			AG	22 (18.0)	21 (17.6)		
			AA	-	-		
		Dominant A	AA + AG	22 (18.0)	21 (17.9)	0.987*	0.97 (0.50 – 1.88)
			GG	100 (82.0)	98 (82.4)		
		Recessive A	GG + AG	119 (100.0)	122 (100.0)	-	-
			AA	-	-		

1 [a] SNP identifier based on NCBI dbSNP
2 * p-value for Pearson chi-square test
3 OR Odds Ratio
4 CI (confidence interval)
5 The difference in n values refer to failure in genotyping
6

Table 4. Results after multivariate analysis using a binary logistic regression model by the backward conditional method.

Variable	*p*-value*	OR (CI 95%)
Age	0.000	1.08 (1.05-1.11)
Sex	0.000	4.14 (2.22-7.72)
Smoking habit	0.002	4.66 (7.99-12.11)
Rs2229094RecG	0.012	3.86 (1.35-11.03)

*_p_-value after multivariate analysis using the binary logistic regression model
OR Odds Ratio
CI (confidence interval)

Table 5. The calculation of the sample power was performed using the Genetic Power Calculation (Purcell et al., 2003) setting as parameters: prevalence of hemodialysis patients of 0.061% in the Brazilian population (Brazilian Census of Nephrology, 2018), effect size from 2.0 for carrying one copy of the risk allele and 4.0 carrying two copies of the risk allele, D'= 1, frequency of the rare allele of 30%, 1:1 case and control ratio and level of significance of 0.05, with the power variation being dependent on the relative risk of the rare allele.

Allele Frequency	Power	N cases for 80% power
0.05	0.12	1526
0.1	0.31	429
0.2	0.76	132
0.3	0.95	70

*****Power of the sample when the rarer allele frequency is 30%
[a] n cases for 80% power when the rarer allele frequency is 30%

Figures

Fig 1 Analysis of linkage disequilibrium (LD) between the selected *LTA*, *TNFA* and *LTB* tag SNPs in the studied population. The LD value was shown inside the squares and the number indicates the ratio in percent (%). The intensity of the color within the squares reflects the LD between two tag SNPs. A darker intensity represents the largest LD among the tag SNPs (1.0), while light gray and white regions represent lower LD (<1.0).

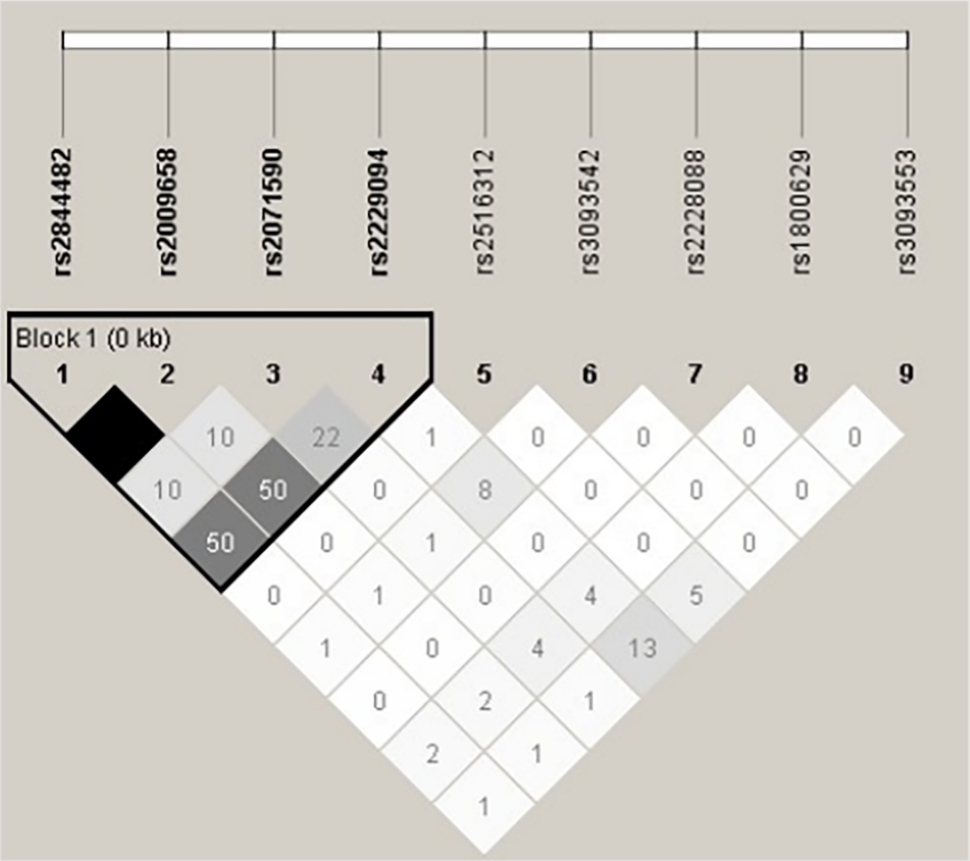
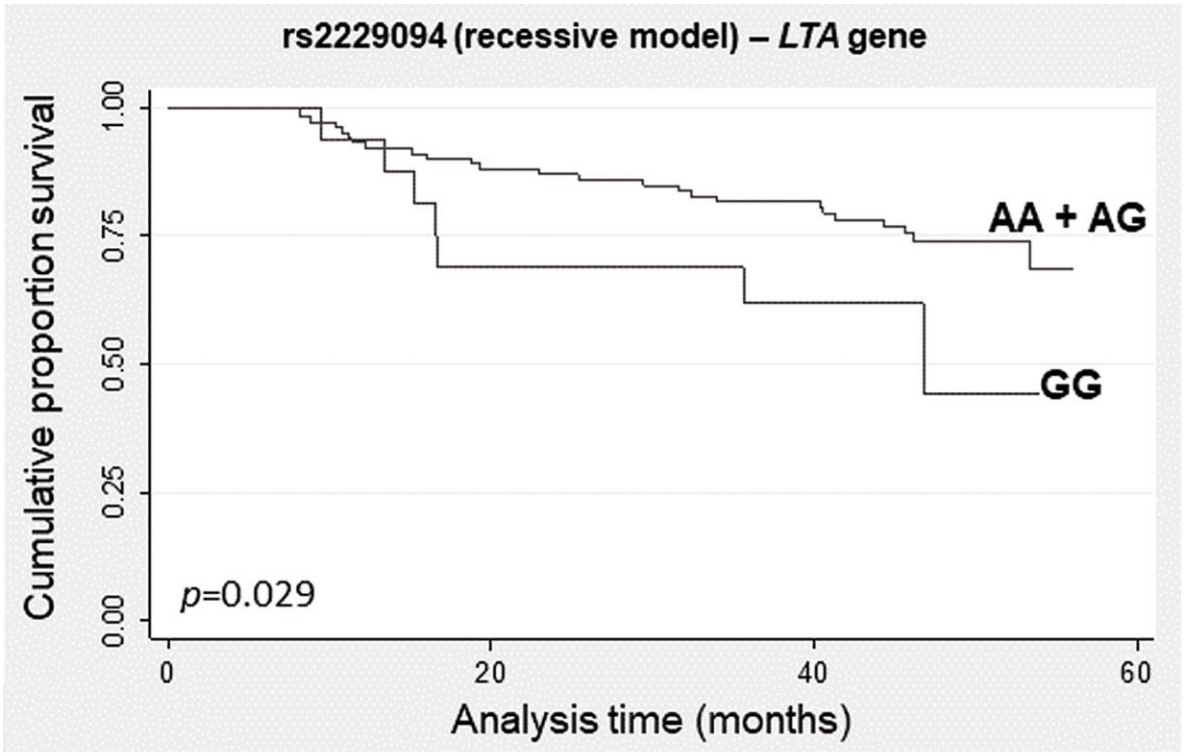
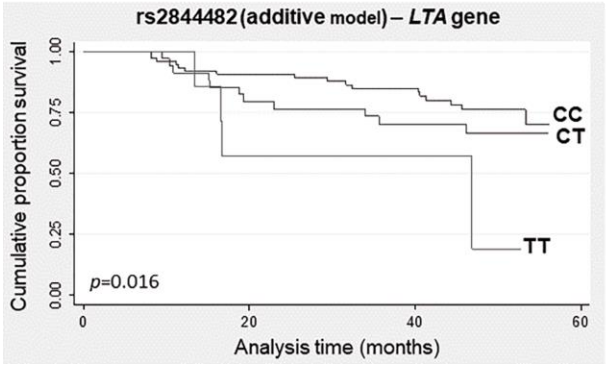
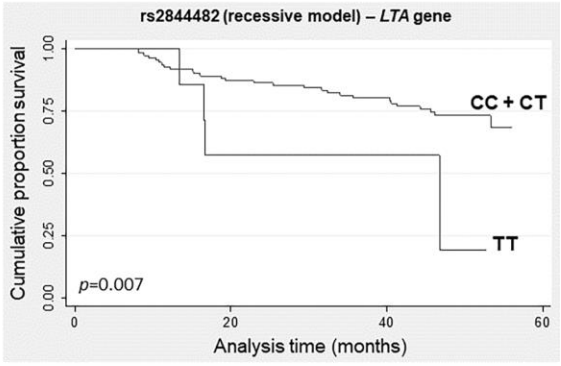


Fig 2 The probability of survival in hemodialysis patients with respect to *LTA* rs2229094 (allele G recessive model), rs2844482 (additive model) and rs2844482 (allele T recessive model) polymorphic variants.







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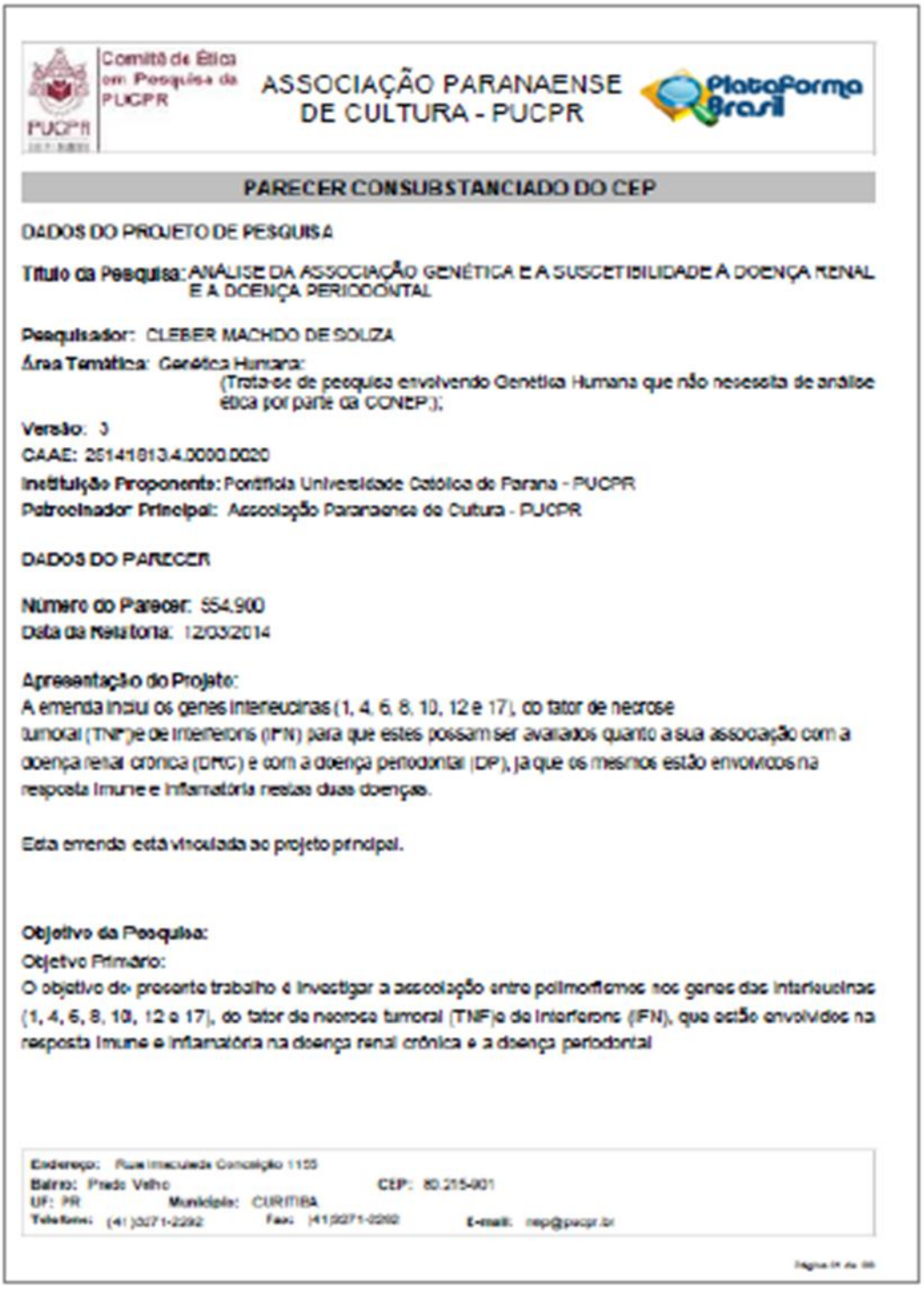
Parecer do Comitê de Ética em Pesquisa



Comitê de Ética em Pesquisa da PUCPR

ASSOCIAÇÃO PARANAENSE DE CULTURA - PUCPR

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ANÁLISE DA ASSOCIAÇÃO GENÉTICA E A SUSCETIBILIDADE À DOENÇA RENAL E A DOENÇA PERIODONTAL

Pesquisador: CLEBER MACHDO DE SOUZA

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP);

Versão: 3

CAAE: 25141613.4.0000.0020

Instituição Proponente: Pontifícia Universidade Católica do Paraná - PUCPR

Patrocinador Principal: Associação Paranaense de Cultura - PUCPR

DADOS DO PARECER

Número do Parecer: 554.900

Data da Relatoria: 12/03/2014

Apresentação do Projeto:

A emenda inclui os genes interleucinas (1, 4, 6, 8, 10, 12 e 17), do fator de necrose tumoral (TNF)e de interferons (IFN) para que estes possam ser avaliados quanto a sua associação com a doença renal crônica (DRC) e com a doença periodontal (DP), já que os mesmos estão envolvidos na resposta imune e inflamatória nestas duas doenças.

Esta emenda está vinculada ao projeto principal.

Objetivo da Pesquisa:

Objetivo Primário:

O objetivo do presente trabalho é investigar a associação entre polimorfismos nos genes das interleucinas (1, 4, 6, 8, 10, 12 e 17), do fator de necrose tumoral (TNF)e de interferons (IFN), que estão envolvidos na resposta imune e inflamatória na doença renal crônica e a doença periodontal

Endereço: Rua Imaculada Conceição 1155
Bairro: Prado Velho CEP: 80.215-901
UF: PR Município: CURITIBA
Telefone: (41)3271-2292 Fax: (41)3271-2292 E-mail: nep@pucpr.br

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