DOI: 10.4149/BLL_2013_080

Bratisl Lek Listy 2013; 114 (7)
380 – 385

CLINICAL RESEARCH

IL1 cluster gene polymorphisms in macedonian patients with chronic periodontitis

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Abstract: Several studies have investigated the genetic polymorphisms for cytokines as potential genetic markers for periodontitis. The aim of this study was to determine the prevalence of *IL1* cluster genes polymorphisms and their association with chronic periodontitis in the Macedonian population. The group of 114 unrelated Macedonian subjects with chronic periodontitis and 301 periodontitis-free Macedonian subjects were studied. DNA was isolated from peripheral blood leukocytes by phenol-chloroform extraction method. Cytokine genotyping was performed by PCR-SSP. The population genetics analysis package (PyPop) was used for analysis of the cytokine data for this report. Crude odds ratio (OR) was calculated as estimates of the relative risk with 95 % confidence interval (CI). Genotype frequency of *IL1B -511/C:T* was significantly higher in patients with periodontitis than in controls (OR=2.11, 95 % CI=1.35–3.32, p=0.001). *IL1* cluster gene haplotype frequencies of *TTTCT* and *TCTTT* were associated with higher risk for periodontitis (OR=5.06, 95 % CI=1.68–15.26, p<0.0014 and OR=8.35, 95 % CI=1.67–41.69, p<0.002, respectively). No significant association of *IL1* composite genotype (*IL1 -889A:IL1B +3962*) with periodontitis in Macedonians was found. The latter association was found to be significant in genotype *IL1B -511/C:T*, haplotype *TTTCT,* and haplotype *TCTTT*, but without significant association in *IL1* composite genotype *(Tab. 5, Ref. 43)*. Full Text in PDF *www.elis.sk*.
Key words: periodontitis, SSP polymorphism, genetic, interleukine-1, Republic of Macedonia.

Periodontitis is a multifactor disease whereby both environmental and genetic factors contribute to its etiology and/or clinical severity. Cytokines are potent immunomodulating molecules that mediate the inflammation and immune response, and at the same time influence the cellular activation, differentiation, and function. There are many reports showing that a number of cytokine genes are polymorphic and that polymorphisms in gene regulatory regions correlate with the cytokine secretion (1, 2). As these polymorphisms are independently segregated, one individual may have a cytokine expression pattern quite different from that of another (3).

Until recently, the *IL1* ligand family consisted of four members: I*L1A, IL1B, IL1RA*, and *IL18*. Based on conservation of amino acid sequence, identification of gene structure and three dimensional structures, six additional members of this family have been described since. The entire new gene map of the region of chromosome 2 between the *IL1B* and *IL1RN* loci was proposed, suggesting that each of the new *IL1* family members arose from a common ancestral gene that later became duplicated (4, 5). The novel *IL1* family members have been described by several groups using their own nomenclature, thus resulting in a number of different names for the same molecule (6, 7).

The prototypic members of the *IL1* family gene cluster are the genes *IL1A* (MIM 147760), *IL1B* (MIM 147720), and *IL1RN. IL1A* and *IL1B* encode pro inflammatory cytokines involved in host defense against infection. The IL-1 receptor antagonist, encoded by the gene *IL1RN*, is an anti-inflammatory non-signaling molecule that competes for receptor binding with IL-1A and IL-1B (8, 9). However, hundreds of single nucleotide polymorphisms are known for each gene of the *IL1* cluster (10).

The protein encoded by *IL1R1* gene is a cytokine receptor that belongs to the interleukin 1 receptor family. This protein is a receptor for interleukin 1 alpha (IL-1A), interleukin 1 beta (IL-1B), and interleukin 1 receptor, type I (IL-1R1/IL-1RN). It is an important mediator involved in many cytokine-induced immune and inflammatory responses (11). Five cytokine polymorphisms connected with the *IL1* cluster gene on chromosome 2 were included in the cytokine polymorphism component: *IL1A −889, IL1B -511, IL1B +3962, IL1R psti 1970*, and I*L1RN mspa 11100*. Cytokines are the key factors of mediating the inflammatory process during periodontal disease. Functional polymorphisms of the *IL1* genes have been proposed to be a risk factor for periodontitis. Periodontitis is an infective disease, where chronic inflammation develops under the influence of microorganisms forming the dental plaque.

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Acknowledgement: This research is part of the project "Molecular analysis of cytokine gene polymorphisms in the Republic of Macedonia" supported by the Ministry of Education and Science from Republic of Macedonia (Project No. 13-874/3-05). We declare we have no conflict of interest.

The interaction between the host inflammatory response and the bacterial virulent factors destructs the attachment and increase the osteoclastic resorption of the alveolar bone, which as a final effect results in teeth loss (12). It is well documented that their biological activities *in vivo* are sufficient to produce local inflammation and destruction of the connective tissue and bone (13). IL-1 acts as a signal in many different systems of the body and it strongly affects the progression and severity of periodontitis in adult population. The connection between IL- 1 and periodontitis was extensively described by Offenbacher (14). Plates of individual basis of polymorphism (SNPs) of *IL1* locus, their functional consequences and their connection with receptiveness and severity of various inflammatory diseases are described in literature (15). The presence of the rare allele *T* for this polymorphism is connected with several medical conditions as juvenile rheumatoid arthritis (JPA), systematic lupus erythematosus (SLE) ulcerative colitis, myasthenia gravis, alopecia and some forms of diabetes (16).

The aim of this study was to determine the prevalence of *IL1* cluster genes polymorphisms and their association with chronic periodontitis in the Macedonian population.

Material and methods

Healthy subjects were recruited from the patient pool at the Clinic for Oral Diseases and Periodontology, University Clinical Centre of Stomatology, Skopje, Macedonia (17–19). All subjects were over 20 years of age, had at least 20 teeth present, were from Macedonian ethnic background to the third generation, and were unrelated residents from different geographical regions of the Republic of Macedonia. Subjects were excluded if they had any systemic disease, were pregnant, current smokers, or taking medications known to affect the host immunity (steroids, immunosuppressant, etc.).

The periodontitis group consisted of 114 subjects, age 38.97±10.12 years, previously diagnosed with moderate or severe chronic periodontal disease according to established criteria while the healthy control group consisted of 301 subjects, age 35.20±9.90 years, displaying no sites with a probing depth over 3 mm, no clinical attachment loss (CAL) over 2 mm, gingival Loe-Silnes Index (GI) of 1, and bleeding on probing (BOP) 6.8±6.4 % (20–23). The study was approved by the Committee of the Ministry of Education and Science from the Republic of Macedonia, as well as by the Ethical Committee of the Medical Faculty in Skopje and was part of the Cytokine Polymorphisms and Expression, 15th International Histocompatibility and Immunogenetics Workshop (IHIWS) (24).

Testing Polymorphism on IL1 cluster gene

Genomic DNA was isolated by the phenol-chloroform method from peripheral leukocytes and samples stored in the Macedonian bank for human DNA as previously described (25, 26). *IL1* gene polymorphisms were determined using the PCR-SSP (Heidelberg kit, Cytokine genotyping Tray, *Invitrogen,* GmbH, Karlsruhe, Germany) at the Institute for Immunobiology and Human Genetics at the Faculty of Medicine in Skopje (27). Fourteen cytokine genes with 22 single nucleotide polymorphisms (SNP) were typed: *IL-1alpha -889, IL1beta -511, IL1beta +3962, IL1R pst1 1970, IL1RA mspa 11100, IL4Ralpha +1902, IL12 -1188, IFN*gama*utr5644, TGFbeta1 cdn10, TGFbeta1 cdn25, TNFalpha -308, TNFalpha -238, IL2 -330, IL2 +166, IL4 -1098, IL4 -590, IL4 -33, IL6 -174, IL6 565, IL10 -1082, IL10 -819,* and *IL10 -592*.

Briefly, the PCR-SSP typing Heidelberg kit consists of 48 PCR primer mixes in aliquot amounts within 96-well PCR trays (two typings per tray). Master mix, which was supplied along with the reagents and consisted of MgCl2, buffer, dNTP's, and glycerol was mixed with 1.2–3.0 µg DNA and 20 U Taq polymerase (GE Healthcare) and dispensed in 48 wells. The amplified products were separated by 2 % agarose gel electrophoresis, stained with 0.5 µg/mL ethidium bromide and visualized by ultraviolet exposure.

Statistical analysis

The population genetics analysis package, PyPop, developed by the Biostatistics Core for the Workshop was used for analysis of the *IL1* data for this report (28, 29). Allele frequencies and expected Hardy Weinberg proportions (HWP) for each *IL1* allele were determined (30). The exact test for genotype frequency deviation from HWP was calculated using the Arlequin implementation accessed via PyPop (31, 32). The alleles that did not fit the HWP were evaluated to determine whether there was an excess of homozygotes or heterozygotes, or if any particular genotypes were significantly different from expected frequencies by the chi square test. The Ewens-Watterson homozygozity test of neutrality (EWN) with Slatkin's exact p-values (SEPV) was used to indicate any deviations from the hypothesis of neutral selection for each locus (33). Pearson's p-values, crude Odds Ratio (OR) and Wald 95 % confidence interval (CI) were calculated for associations analysis between *IL1* (alleles, genotypes, haplotypes, and diplotypes) and periodontal disease with Simple Interactive Statistical Analysis (SISA): Two by two table analysis (http://www.quantitativeskills.com/sisa/). Values for p less than 0.05 were taken as significant.

Results

The clinical and demographic data of our Macedonian study populations are shown in Table 1. As predicted, the values of the clinical parameters GI, CAL, and BOP were higher in the periodontitis group than those in healthy controls.

Tab. 1. Demographic and periodontal findings in Macedonian population.

	Chronic periodontitis (n=114)	Control group (n=301)	p
Age (years) Mean±SD	38.97±10.12	35.20±9.90	NS
Female	41.90 %	54.60 %	NS
Male	58.10 %	46.40 %	NS
Loe-Silnes Index (GI)	2.38±0.67	0.5±0	<0.001
BOP %	82.52±8.14	6.8±6.4	<0.001
CAL	5.18±0.716	1.8±0.3	<0.001

SD – standard deviation; n, number of participants; NS – non significant; BOP – bleeding on probing; CAL – clinical attachment loss.

Tab. 2. Association of *IL1* gene cluster allele frequency with periodontitis in Macedonian patients.

IL1 Gene Cluster	Allele	PARO		CONTROL		OR	Wald 95% CI	Pearson's p-value
		N	F	N	F			
IL1A -889	*C*	176	0.793	482	0.814	0.87	0.59–1.28	0.49
	T	46	0.207	110	0.186			
IL1B -511	*C*	144	0.632	404	0.671	0.84	0.61–1.15	0.28
	T	84	0.368	198	0.329			
IL1B +3962	*C*	165	0.737	439	0.729	1.03	0.73–1.46	0.83
	T	59	0.263	163	0.271			
IL1R pstl1970	*C*	154	0.681	399	0.663	1.08	0.78–1.51	0.61
	T	72	0.319	203	0.337			
IL1RN mspa111100	*T*	168	0.737	420	0.698	1.21	0.86–1.70	0.26
	C	60	0.263	182	0.302			

PARO – patients with periodontitis; N – number of alleles; F – frequency of alleles; OR – Odds Ratio; CI – confidence interval.

Tab. 3. Association of *IL1* gene cluster genotype frequency with periodontitis in Macedonian patients.

IL1 Gene Cluster	Genotype	PARO		CONTROL		OR	Wald 95% CI	Pearson's p-value
		N	F	N	F			
IL1A -889	*C:C*	70	63.1	204	68.9	0.77	0.49–1.22	0.26
	C:T	36	32.4	74	25.0	1.44	0.89–2.32	0.13
	T:T	5	4.5	18	6.1	0.73	0.26–2.01	0.54
IL1B -511	*C:C*	42	36.8	143	47.5	0.64	0.41–1.00	0.051
	C:T	60	52.6	118	39.2	2.11	1.35–3.32	0.001*
	T:T	12	10.5	40	13.3	0.77	0.39–1.52	0.45
IL1B +3962	*C:C*	64	57.1	174	57.8	0.97	0.63–1.51	0.90
	C:T	37	33.0	91	30.2	1.14	0.72–1.81	0.58
	T:T	11	9.8	36	12.0	0.80	0.39–1.64	0.54
IL1R pstl1970	*C:C*	54	47.8	133	44.2	1.16	0.75–1.78	0.51
	C:T	46	40.7	133	44.2	0.87	0.56–1.35	0.52
	T:T	13	11.5	35	11.6	0.99	0.50–1.94	0.97
IL1RA mspa11100	*C:C*	6	5.3	30	10.0	0.50	0.20–1.24	0.13
	C:T	48	42.1	122	40.5	1.07	0.69–1.65	0.77
	T:T	60	52.6	149	49.5	1.13	0.74–1.75	0.57

PARO – patients with periodontitis; N – number of genotypes; F – frequency of genotypes; OR – Odds Ratio; CI – confidence interval; * – statistically significant difference.

The association analysis of *IL1* gene cluster allele frequency with periodontitis in Macedonian patients is given in Table 2 but the differences between patients with periodontitis and control subjects were not significant for any allele.

The association analysis of *IL1* gene cluster genotype frequency with periodontitis in Macedonian patients have shown that the genotype *IL1B -511/C:T* was significantly higher in patients with periodontitis than that in controls (OR 2.11, 95 % CI 1.35–3.32, p=0.001). The rest of *IL1* gene cluster genotypes were non-signficantly associated with periodontitis (Tab. 3).

Association of *IL1* cluster haplotype frequency estimated for loci: *IL1A -889: IL1B -511: IL1B +3962: IL1R psti1079: IL1RN mspa11100* with periodontitis in Macedonian patients have shown that haplotypes *TTTCT* and *TCTTT* were associated with higher risk for periodontitis (OR=5.06, 95 % CI=1.68–15.26, p<0.0014 and OR=8.35, 95 % CI=1.67–41.69, p<0.002, respectively). The rest of *IL1* cluster haplotypes were not significantly associated with periodontitis (Tab. 4).

No significant association of *IL1* composite genotype (*IL1 -889A:IL1B +3962*) with periodontitis in Macedonians was found (Tab. 5).

Discussion

In this report we presented the association of *IL1* cluster gene (alleles, genotypes, haplotypes and composite genotype) with periodontitis in Macedonian patients. We found a significant association of genotype *IL1B -511/C:T*, haplotype *TTTCT,* and haplotype *TCTTT* with periodontitis. For the rest of *IL1* cluster gene alleles, genotypes, haplotypes and composite genotype we did not find a significant association with periodontitis in Macedonian patients.

Pro-inflammatory cytokine interleukin-1 has a very important role in periodontal tissue destruction by stimulating the bone destruction and participating in the production of proteases and arachidonic acid, i.e. in activities directly connected to periodontitis. The level of IL-1 beta is increased in the gingival tissue of patients with periodontitis (34). The association between a composite genotype of allele 2 of *IL1B +3953* gene and allele 2 of *IL1A -889* gene in severe form of adult periodontitis was published (35). Our results of composite *IL1* genotype in Macedonian patients with periodontitis are in agreement with several published papers in which no significant association between composite genotype of *IL1* with periodontitis was found (36–38).

Tab. 4. Association of *IL1* cluster haplotype frequency estimated for loci: *IL1A -889: IL1B -511: IL1B +3962: IL1R psti1079: IL1RN mspa11100* with periodontitis in Macedonian patients.

IL1 Gene Cluster Haplotype	PARO		CONTROL		OR	Wald 95% CI	Pearson's p-value
	N	F	N	F			
CCCCT	46.9	0.215	113.3	0.191	0.91	0.63–1.33	0.64
CTCCT	36.9	0.169	75.0	0.127	1.12	0.73–1.71	0.60
CCCCC	17.7	0.081	50.1	0.085	0.98	0.55–1.71	0.93
CCCTC	14.8	0.068	22.5	0.038	1.83	0.93–3.57	0.07
CCCTT	14.3	0.065	58.3	0.098	0.63	0.34–1.16	0.13
CTCTT	11.0	0.050	48.5	0.082	0.60	0.31–1.18	0.14
CCTCT	6.5	0.030	28.3	0.048	0.67	0.29–1.55	0.35
TCTCT	5.5	0.025	27.7	0.047	0.47	0.18–1.24	0.12
TCTCC	7.4	0.038	22.0	0.037	0.86	0.36–2.04	0.73
CTCCC	0	0	19.4	0.033	&	&	&
TCCCT	4.0	0.018	17.9	0.030	0.59	0.19–1.78	0.35
TCTTC	0	0	15.8	0.027	&	&	&
CCTCC	7.9	0.036	14.3	0.024	1.57	0.65–3.80	0.31
CTCTC	5.2	0.024	12.1	0.020	1.13	0.39–3.26	0.81
CTTCT	3.8	0.017	11.7	0.020	0.90	0.29–2.83	0.86
CCTTC	0	0	10.1	0.017	&	&	&
CCTTT	5.8	0.026	9.1	0.015	1.83	0.64–5.21	0.25
TCCTC	1.2	0.005	6.5	0.011	0.45	0.05–3.76	0.45
CTTTT	0	0	5.8	0.010	&	&	&
TTTCT	8.5	0.039	4.8	0.008	5.06	1.68–15.26	0.0014*
TTCTT	1.9	0.008	4.6	0.008	1.10	0.21–5.64	0.92
TTCCT	3.9	0.018	3.9	0.007	2.75	0.68–11.08	0.14
TTTCC	0	0	2.6	0.004	&	&	&
CTTTC	2.3	0.011	2.5	0.004	2.73	0.38–19.51	0.29
TTTTC	0	0	2.1	0.004	&	&	&
TCTTT	6.1	0.028	2.1	0.003	8.35	1.67–41.69	0.002*
CTTCC	0	0	2.1	0.002	&	&	&
TTTTT	4.0	0.018	0	0	&	&	&
TTCTC	1.5	0.005	0	0	&	&	&

Haplotype frequencies were estimated from unphased data using the expectation-maximization (EM) algorithm (29, 32) reported by PyPop. PARO – patients with periodontitis; N – number of haplotypes; F – frequency of haplotypes; OR – Odds Ratio; CI – confidence interval; * statistically significant difference. &, cannot be calculated because expected value was <5, χ^2 test.

Tab. 5. Association of composite genotype (*IL1 -889A:IL1B +3962*) with periodontitis in Macedonian patients.

IL1 Polymorphism	Composite genotype*	PARO		CONTROL		OR	Wald 95% CI	Pearson's p-value
		N	F	N	F			
IL1A -889:	CC	147	0.672	398	0.644	1.02	0.73–1.43	0.89
IL1B +3962	CT	27	0.120	83	0.140	0.87	0.55–1.39	0.56
	TT	31	0.146	78	0.130	1.10	0.70–1.72	0.68
	TC	12	0.054	73	0.056	0.99	0.50–1.96	0.98

*Cornman et al 1997 (35). PARO – patients with periodontitis; N – number of composite genotypes; F – frequency of composite genotypes; OR – Odds Ratio; CI – confidence interval.

A most recent study has shown that genotype 2/2 of *IL1RN* for the whole Brazilian population and allele *T* of *IL1B (C-511T)* in a subgroup of Afro-Americans and mulattos were suggested to be putative risk indicators for chronic periodontitis (39).

However, a case-control association study on 415 northern European Caucasian patients with aggressive periodontitis and 874 healthy controls was conducted to examine 10 single-nucleotide polymorphisms in the genes of the *IL1* cluster for association with *IL1A, IL1B, CKAP2L* (cytoskeleton-associated protein 2-like), and *IL1RN* (IL-1 receptor antagonist). The results do not support any association between variants in the *IL1* gene cluster and aggressive periodontitis (40).

The association between *IL1* genes and aggressive periodontitis was investigated using 70 markers spanning the 1.1-Mb region where the *IL1* gene family is mapped. The case-control study included 95 patients and 121 control individuals and explored both the linkage disequilibrium (LD) and the haplotype structure. No association between aggressive periodontitis and *IL1A, IL1B,* and *IL1RN* genes was found in either single-point or haplotype analyses, and the study failed to support the existence of a causative variant for generalized aggressive periodontitis within the 2q13-14 region in an Italian Caucasian population (41).

The relationship between specific *IL1* genotypes and level of IL-1β in the gingival crevicular fluid is unclear. Similarly, the ability of the genetic susceptibility test to forecast which patients will develop increased bleeding on probing, periodontitis, or loss of teeth or dental implants is ambiguous. Additional prospective clinical trials are needed to determine the risk of developing peri-

odontitis or peri-implantitis when allele 2 at *IL1A +4845* and *IL1B +3954* loci is present (42).

Recently we published significant associations (after the Bonferroni adjustment) between subjects with periodontitis and the following: (1) cytokine alleles IL4 -1098 and IL4 -33; (2) cytokine genotypes IL4 -1098/G:T; IL4 -1098/T:T, and IL4 -33/T:T, (3) cytokine haplotypes IL4/GCC, IL4/TCC, and IL4/TTC; and (4) cytokine haplotype zygotes IL4/TTC: TCC, IL4/TCT:TTT, and IL4/GCC:TTC. Cytokine polymorphism on IL4 gene appears to be associated with susceptibility to chronic periodontitis in Macedonians (43).

Periodontitis is a complex genetic trait which includes a lot of associated candidate genes. Our results with only one gene cluster (*IL1*) can be only part of the complex investigation of candidate genes for periodontitis in Macedonians. The numbers of patients and controls of our study is small. In association studies, there are possibilities that some positive results might be spurious and some negative findings might be a consequence of low statistical power. It could be due to their small sample size or methodological shortcomings such as selection of an inappropriate control group. Further studies are merited to assess these associations in greater detail (including any gene-gene and gene-environment interactions) and to determine any implications with regard to potential therapies.

In summary, we can conclude that a significant association of genotype *IL1B -511/C:T*, haplotype *TTTCT,* and haplotype *TCTTT* with periodontitis in Macedonian patients was found, however without a significant association of *IL1* composite genotype. Previous reports of the association between *IL1* composite genotype and periodontitis might reflect the subpopulation effects and have to be interpreted with care.

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Received July 18, 2011.
Accepted January 23, 2013.