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Gene Polymorphisms in Gingivitis

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1. Introduction

Periodontal diseases (gingivitis and periodontitis) are inflammatory processes that involve the supporting tissues of the teeth. According to a currently used classification (Armitage, 1999), gingival diseases can be divided into four major groups: (1) dental plaque-induced gingival diseases, (2) gingival diseases modified by systemic factors, (c) gingival diseases modified by medication, and (4) gingival diseases modified by malnutrition. Plaque-induced gingivitis is the most common form of the periodontal diseases, affecting a significant proportion of the world population. The periodontal tissues react to the presence of bacteria with an inflammatory immune response. In the initial phase, components of the non-specific immune system play a main role while an efficient response of the tissues to the infection depends on a selective but not very specific recognition system. It is mainly based on the detection of chemical structures that are present on the surface of many different microorganisms, but do not occur on the surface of cells of the body. After recognition of these structures, the effector mechanisms of cells (e.g. macrophages, neutrophil granulocytes, etc.) are triggered and humoral systems (such as complement) are activated. The mechanisms of non-specific immunity neutralize and eliminate harmful products generated by pathogenic microorganisms and subsequently help regenerate the damaged tissues (Shapira et al., 2005). In the healthy periodontium, pathogenic bacteria are removed either mechanically (tongue movements, chewing of food, secretion of saliva, separation of epithelial cells) and also by production of gingival crevicular fluid. This fluid plays an important role in the defense mechanisms as it contains a number of proteins and cells whose composition depends on the inflammatory stage. A protection barrier between the plaque and the epithelium formed by neutrophil leukocytes in the sulcus area impedes bacterial penetration into the deeper tissue structures (Ohlrich et al., 2009). If bacteria penetrate subepithelially, due to a chemotactic action of the substances released by the bacteria and activated by cells of the affected tissue, the migration process of further cells

into the junctional epithelium and gingival sulcus increases. Mediators of leukocytes, together with the activated complement components, kinin system and products of arachidonic acid further amplify the inflammatory response leading to a widening of intercellular spaces between the cells of the junctional epithelium, which allows increased diffusion of bacterial products into the gingival tissues. In the early phase, lymphocytes begin to accumulate, at first T lymphocytes and activated macrophages predominate in the inflammatory infiltrate, in the late chronic (or established) phase a change to B lymphocytes and plasma cells is apparent (Seymour et al., 1988). Proliferation of the junctional epithelium spreads apically and laterally. If the process goes on untreated, the periodontal ligament and alveolar bone are destructed and a periodontal pocket forms. However, it must be noted that the pathological process does not always proceed at the same intensity; there are phases of acute exacerbation and chronic stagnation alternate.

In the 1960s experimental gingivitis studies (Loe et al., 1965) demonstrated that gingivitis is the response of the body to the build-up of dental plaque. These studies also showed a substantial variation in this response in different individuals; some people taking longer to manifest disease compared to others. Thus, while it has been known for many years that plaque is the etiological factor, the role of factors contributing to an individual's susceptibility is still not fully understood. Not all individuals with gingivitis will progress to periodontitis, and not all individuals with periodontitis will progress to tooth loss (Ohlrich et al., 2009). Therefore, periodontal diseases, including gingivitis, can be considered as a multifactorial condition influenced by the interaction between microbes and the host genome. Today, the role of external factors (e.g. microorganisms, smoking, stress etc.) is clearer than that of the genes. There are many scientific papers searching for the role of genes and their variants (polymorphisms) in the host response in aggressive and chronic periodontitis and their progression. In contrast, only a few studies examining the role of IL-1, IL-6, IL-10, TNF-α and MMP-9 genetic variability in gingivitis have been published to date. This review will analyze and summarize the literature on putative genetic risk factors predisposing to gingivitis. In addition, new data (still unpublished) on the role of interleukin-18 (IL-18) gene polymorphisms at positions -607C/A (rs1946518), -137G/C (rs187238) and -133C/G (rs360721) and their haplotypes in relation to gingivitis and microbial pathogens in Czech children are presented.

2. Genetic predisposition to gingivitis

For a detailed understanding of the interactions between bacteria and the inflammatory and immune responses of the individual, it is necessary to understand the pathogenesis of gingival disease. It is evident that an individual susceptibility to the presence of microbial pathogens plays an important role in the initiation and development of this disease.

At the end of the last century, the existence of a phenomenon called "*individual susceptibility*" was documented by many studies which proved familial aggregation of the disease and certain ethnic differences in the frequency of the occurrence of the individual forms of periodontal diseases. We assume that the genetic background of an individual plays a role in modulating susceptibility to a disease. Inheritance of susceptibility to periodontal disease varies enormously from nearly 100% in "Mendelian forms of periodontitis" to a minor rate of inheritance and a significant effect of external factors, as e.g. in plaque-induced gingivitis. Although the presence of some bacteria in plaque is a triggering factor, the organism response to this situation is significantly affected by the "genetic background" of the

individual (Hassell & Harris, 1995, Michalowicz, 1994, Takashiba & Naruishi, 2006, Yoshie et al., 2007, Laine et al., 2010). The phase of genetic analyses of the periodontal diseases started in the 1990s; it is usually referred to as the phase of looking for responsible – candidate – genes. Given the important role of inflammation and remodelling in periodontal tissues, the most frequently analyzed candidate genes are still the genes for selected receptors of the immune system, genes for proinflammatory cytokines, chemokines and growth factors, genes for matrix metalloproteinases and other enzymes.

2.1 Methodological aspects of complex disease studies

Research into the genetics of complex diseases represents one of the biggest challenges for scientists. From a methodological point of view, it is necessary to consider the etiopathogenetic character of complex diseases which differs from monogenic diseases in a number of aspects.

The term complex diseases (traditionally referred to as multifactorially conditioned) indicate diseases, the origin and development of which is affected by a "complex" of genetic and external factors. They do not exhibit classical Mendelian (dominant or recessive) inheritance as a result of a change in one locus (so-called diseases caused by one big reason). The genetic component of complex diseases also exhibits familial aggregation (i.e. non-random occurrence in families) but without an explicit type of transmission. Phenotype manifestation is a result of complex, often non-linear interactions, between the involved genes and also between the genes and external factors. Characteristic traits for complex diseases include: 1) incomplete penetration of a pathological phenotype [pathological phenotype in some persons who inherited "unfavorable" genotype does not manifest], 2) the existence of phenocopies [the same phenotype can be present also in persons who are not carriers of the given genotype], 3) genetic heterogeneity [allelic – there can be more "pathogenic" mutations or polymorphisms in the individual genes, loci – clinical manifestation can develop as a consequence of changes in the genes at various loci], 4) polygenic inheritance [predisposition to the disease increases only with the concurrence of a certain set of alleles, which if alone are not significantly pathogenic], 5) high population frequency of the alleles responsible for the development of the pathological phenotype, 6) co-operation of other mechanisms [e.g. imprinting, mitochondrial inheritance, etc.] (Lander & Schork, 1994).

Therefore, study of these diseases present numerous problems that must be solved – besides already mentioned an unclear inheritance model, there is also an unknown number of the genes involved and an ambiguous definition of the clinical phenotype. The concrete disorder which is relevant in the context of the given disease and "closer" to the genetic background than the resulting composed phenotype, is referred to as *intermediary phenotype*. There are principally two approaches for defining pathological phenotype (or disease) – the first approach is based on the *alternative concept of health and disease* (i.e. presence or absence of disease), the other approach corresponds to a so-called *gradation model*, disease and is perceived as a continuous function of a trait. In complex diseases it is rather a continuous transition of physiological values (phenotypes) to pathological ones. "Normality"of certain values and thus their implicitly presumed "unharmfulness" is reflected by so-called reference intervals used in clinical medicine. However, use of statistical methods allows researchers to study the contribution of the genetic variability to the observed phenotype variability while applying both the approaches – alternative concept and continual model.

Genetic variability, means that for the given gene more variants (i.e. alleles) with different frequency exist in population, counts for phenotype variability of traits. Based on population frequencies, these variants can be split into mutations and polymorphisms. The term *mutation* is usually used to indicate changes where frequency of a less frequent allele in population is lower than 1%. The term *polymorphism* conventionally indicates the existence of several (at least two) alleles; population frequency of the least frequent one is minimally 1%. The term *genetic architecture* of the given phenotype that characterizes how many genes and which of them participate in the genetic determination of a relevant disease, has not been determined for most of complex diseases. It is supposed that population frequency of a certain allele reflects its effect which can be either markedly pathogenic (in this case the relevant alleles are under selection pressure and are eliminated from the population – therefore they have a low population frequency) or in contrast low or neutral (alleles do not subject to selection and thus they are common in population). It has been assumed that these "neutral" variants may be the basis for the genetic determination of common complex civilization diseases (a so-called common disease/common variant hypothesis). According to this hypothesis, the frequency of certain genetic variants determining the susceptibility to a certain trait (frequency of which could have been originally relatively low) increased over the time due to selection neutrality or a certain advantage in the given environment which has disappeared in the present (for example high blood pressure, hypercholesterolemia, etc.). On the contrary, an alternative, less probable hypothesis (a so-called common disease/rare variant hypothesis) assumes that common complex diseases, manifestation of which markedly depends on the environment, are common due to universal exposition to the environment and not because of sharing the same genetic variants.

In complex diseases, the genetic background analysis is further complicated by the presence of interactions of the individual genetic variants (so-called "gene-gene interactions") and their complex impact on the clinical manifestation. It is currently believed that these interactions can be described by a so-called multiplicative model which considers the existence of two (and more) risk loci while only a certain combination increases a risk of the given disease development.

In addition, when studying complex diseases, external factors play an important role by significantly modifying an innate disposition of an individual to some disease and must be taken into consideration. Although there are no doubts about the role environmental factors play in the origin and development of complex diseases, finding the method for their exact definition and quantification still remains a challenge.

2.2 Types of genetic studies

If inheritance is confirmed to play a substantial role in the diseases with characteristics of a complex trait, the next step is mapping and identification of the genes involved. Therefore, genetics of complex diseases has to find answers for these principal questions: a) what genes participate in the development of the given disease, b) which variants play the most important role. A number of methods exist for localization and identification of the genes predisposing to complex diseases, each of them having some advantages and disadvantages. Methodologically there are two basic approaches: *linkage* or *association studies*; in which one or more candidate genes can be studied (a so-called "*candidate gene approach*") or it is possible to work with the whole genome (a so-called "*genome-wide approach*").

Both these approaches differ but principally; while the association approach compares the occurrence of a relevant genetic variant among phenotypically different persons (so-called "case-control" studies), the genome-wide approach investigates transmission of the genetic and phenotypic trait in affected families. Linkage studies can be further split into parametric and non-parametric ("allele sharing methods"), association studies are either retrospective, prospective or in a form of a "transmission disequilibrium test", TDT.

2.2.1 Linkage analysis

Standard linkage analysis (so-called parametric) is a method effective for mapping monogenic diseases, but is rarely usable for study of complex diseases. For the linkage analysis it is necessary to know in advance (or estimate) the inheritance type and then to calculate recombinant and non-recombinant offspring. Then it is possible to: a) find out whether any genetic locus exists which recombines with a disease with frequency θ lower than 50%, this is the frequency expected in the loci that are not in linkage and b) estimate the value θ, which gives the highest lod score (a so-called θmax). It means that this approach is based on the assumption of some inheritance type, which, when applied to the analysis of complex diseases (or traits), encounters a lot of problems.

Methods of linkage analysis (i.e. non-parametric do not consider the model, do not work with the number of loci, external effects and probability of incomplete penetration) were developed. These methods are based only on the assumption that two affected relatives share the alleles predisposing to the disease origin. One of the variants of this method, the *linkage analysis of affected sibpair*, monitors equally affected, so-called concordant sibpairs (eliminating thus the decision whether the affected individual is a non-penetrant carrier of the alleles predisposing to the disease or whether he/she did not inherit these alleles) and the siblings are tested whether they inherited a certain section of the chromosome (marker) more frequently than it can be expected under random segregation. "Maximum likelihood odds ratio" is used to detect whether the allele-sharing deviates significantly from 50% (corresponding to chance), similarly as in the linkage analysis based on models, lod score is used to determine whether the decrease in recombination frequency below 50% is significant. The method of linkage analysis of the affected sibpairs is not burdened by possible false assumptions about the number of the loci involved and how the alleles in these loci interact. However, it is less sensitive and less accurate (Nussbaum et al., 2004). Another interesting method is the *linkage analysis of highly discordant* (differently affected) *sibpairs*. Similarly, this method does not require any assumption about the number of participating loci or the inheritance type. Presumably, these siblings will not share the same alleles at the loci involved in the given trait. DNA analysis is conducted using the polymorphic markers distributed along the whole genome. A decrease in the level of allele-sharing detected in some marker indicates that the given marker is in linkage to the locus whose alleles participate in the studied physiological variable.

2.2.2 Association analysis

Association retrospective studies do not deal with the transmission of a complex trait in families, but compare allele frequency of a certain gene among the groups of affected and control (non-affected) unrelated persons. These studies analyze the alleles of genes whose products are pathophysiologically involved in the given disease (so-called candidate genes). If a certain allele of the given locus in the affected persons occurs in a higher frequency than

in controls, then the association with a disease is considered. The strength of association is expressed as "odds ratio" calculated from the frequency of the relevant allele in patients and controls (OR=ad/bc, where a = number of patients with the given allele, c = number of controls with the given allele, b = number of patients without the given allele and d = number of controls without the given allele). If the frequency of the studied allele in patients is in accordance with that in controls the odds ratio equals 1. Direct association studies deal with concrete substitutions in which a direct, causal effect on a phenotype is presumed. On the contrary, so-called indirect association studies are based on the assumption that the alleles in the vicinity of the causal allele segregate jointly (in a so-called haplotype block) and markers in the area are in complete linkage disequilibrium (LD) with causal substitution. It means that the vicinity of the variant is more important than the effect of the substitution itself.

These association methods are an important tool for seeking the genes involved in the origin of complex diseases and are also used for the analysis of genetic determination of gingivitis. Their advantage is that they can quite easily be conducted (we need only samples from groups of affected individuals and controls). There is no need to work out any laborious pedigree studies and collect samples from relatives. On the other hand, the results obtained must be carefully interpreted because an increase in likelihood ratio for some allele of a certain locus is not evidence that this allele or the whole locus are actually involved in the disease pathogenesis. Firstly, the studied allele can be in linkage disequilibrium with another allele, which really participates in the pathogenesis of the given disease (as explained above). Second, more serious limitation of the association studies is their sensitivity to the consequences of population stratification. If the population is split into several subpopulations (e.g. based on nationalities) and unions between members of different subpopulations are only rare, it can happen that the disease occurring more frequently in one of the subgroups will be falsely associated with some of the alleles that are more frequent in this subpopulation than in the population as a whole. The origin of these "false" associations can be minimized, but not fully eliminated, by a careful selection of the controls.

Prospective association study is based on the defined population sample (cohort), with the determined presence of the studied risk factor, the development of the relevant disease (or detected phenotype) is monitored over a given time horizon. Subsequently, frequencies of alleles (or genotypes, haplotypes, etc.) are compared among symptomless individuals and patients with disease. The advantage of this approach is that it is not necessary to make selection from the control group; the main disadvantage being time and financial costs.

Transmission disequilibrium test, TDT is based on the analysis of genotypes of the affected persons (probands) and their parents who are heterozygotes for the studied polymorphisms (at least one parent must fulfill this condition). The method tests whether the allele of a certain genetic marker from the heterozygous parent is transmitted to the affected offspring so frequently as it could be expected under random Mendelian segregation (when each parental allele is transmitted with the likelihood of 50 %). Transmission with a higher frequency indicates an association of the relevant allele with the given disease. The advantage of this method is the fact that it does not require the analysis of siblings (affected or non-affected by the disease) and that it is not influenced by population stratification.

2.2.3 Study of haplotypes

The term *haplotype* denotes a combination of alleles of the individual polymorphisms and mutations in the gene, or a longer chromosome segment, or a particular nucleotide sequence of one of the pairs of homologous chromosomes that is inherited as a whole. While studying the candidate genes in association studies, attention has been devoted to a haplotype analysis. It is due to the fact that protein production encoded by a relevant candidate gene is a result of summary effects of all variants in the given gene which can affect transcription (or splicing, translation, RNA stability, posttranslational modifications, etc.) and which are difficult to reveal by the analysis of the individual variants. In addition, it has been suggested that genetic variability in a population is unambiguously structured in haplotypes; the haplotype analysis thus reduces the numbers of the analyzed variants, which are not mutually independent, increasing thus statistical power of the test.

A variety of methods exist for haplotype detection but none of them is quite trivial. In linkage studies, haplotypes can be derived if maternal and paternal genotypes are known, suitable programs are for example: GENEHUNTER (Nyholt, 2002), Simwalk2 (Sobel & Lange, 1996) or Merlin (Abecasis et al., 2002). In other cases, haplotypes can be either derived statistically or established through molecular haplotyping. The method for the haplotype inference from genotype data is used most frequently, namely in large association studies. Available statistical programs are based on some of three algorithms: (a) maximum likelihood [SNPHAP, THESIAS (Tregouet et al., 2004), PLEM (Qin et al., 2002)], (b) parsimons [e.g. HAINFREX (Clark et al., 1998)] or (c) Bayesian [HAPLOTYPER (Niu et al., 2002), PHASE (Stephens et al., 2001, Stephens & Donnelly, 2003)]. Besides the algorithm itself, these programs differ in a number of other parameters and initial presumptions such as set size, requirements for maintenance of Hardy-Weinberg equilibrium, requirements for completeness of genotype data, etc. A number of surveys and comparisons of available software packages have been published (Niu, 2004, Salem et al., 2005).

2.2.4 ”High-capacity” techniques

High-capacity “high-throughput“ methods have been intensively developed for a rapid and simultaneous assay of a large number of markers in the scope of the whole genome or its part in vast sets. Other interesting approaches are, for example, “multiplexing“ (for a parallel detection of a large number of markers – e.g. SNPs in one reaction) or “DNA pooling“ (using mixed DNA samples collected from hundreds to thousands of individuals in which allele frequencies are determined). The high-capacity techniques often use the methods of DNA microarrays and techniques based on PCR and their modifications (Shi, 2002, Tsuchihashi & Dracopolli, 2002, Liu B et al., 2004, Brenan et al., 2009).

2.3 Interpretation of results from genetic studies

The aim of genetic research into complex, multifactorially conditioned diseases, such as gingivitis, is to find, by means of proof of positive linkage or association of a particular parameter with the disease, risk or protective variants increasing or on the contrary decreasing the susceptibility to the origin or development of the studied disease. However, the issue of establishing the causality of any relationship found is more complicated; none of the methods given above is able to confirm it directly.

Today association studies are preferred for the analysis of complex diseases (despite the disadvantage mentioned above with the problematic selection of the control group). This is

given by the character of these diseases (first of all polygenic type of inheritance, participation of the alleles with predominantly "smaller" effect, late clinical manifestation that restricts the availability of the analysis of affected relatives) and mainly by an important role of the external environment which can be studied with linkage studies only with difficulties.

Facts about etiopathogenesis of multifactorially conditioned diseases, noted above, suggest that genetic predisposition to gingivitis is affected by a number of loci that may interact with each other in a complex fashion. It means that the genetic variant if studied separately is not necessarily associated with the given phenotype or given disease. This may be the reason why a number of inconsistent results of SNPs analyses from different studies exist. There is a clear trend towards more complex–haplotype or whole-genome studies that can analyze simultaneously thousands to tens of thousands of markers for the given phenotype. This strategy evades one of the disadvantages of candidate-gene association studies as it does not work with an assumption of relevant gene-disease association. On the other hand, it is necessary to analyze a large number of gene variants in sets containing relatively few individuals, which leads to an increased probability of false positive results. The methods commonly used for multiple comparisons correction (e.g. Bonferroni correction, Holm's method, etc.) are more conservative in the situation where high LD between variables are present.

Although as mentioned above molecular-genetic methods which are able to analyze rapidly a huge number of markers are available, "ideal" statistical programs for the evaluation of the acquired data are still missing (eg. how to minimize the likelihood of false positive results at a high number of the analyzed variants in a small number of persons, evaluation of interaction effects between the particular genes and gene variants and environment). Of more recent methods, so-called multilocus methods can be mentioned (Hoh et al., 2001, Hoh & Ott, 2003). They have been used for a relatively short time and it is too early to evaluate them.

3. Strategy of the recovery of published data

A comprehensive literature search on the PubMed database up to January 2011 was conducted using the keyword gingivitis in combination with the words gene mutation or polymorphism. The studies selected for the review (a) were written in English, (b) had a case-control design including patients with gingivitis (G), and (c) reported genotype distribution.

In the present review, the most common variant of the polymorphic locus is nominated as a normal (N) variant (allele). The less frequent allele is designed as a rare (R) variant (allele). Table 1 gives the frequencies of the carriage rate of the R-alleles (frequency of N/R and RR genotypes) among patients and controls. In addition, we showed in this table whether or not the authors of the cited papers have reported statistically significant differences between cases and controls.

4. Candidate genes in relation to gingivitis

Considering the significant role of inflammation in gingivitis, of all the candidate genes, mainly the proinflammatory (IL-1, IL-6, TNF-α and LT-α) and antiinflammatory (IL-10) cytokine genes and genes for matrix metalloproteinases (MMP-9) have been analyzed (Table

1). This review presents all studies investigating association of genetic polymorphisms in relation to plaque-induced gingivitis in the "case-control study" design.

Ethnicity of subjects	Gene	SNP	Controls		Patients with gingivitis		Associated with gingivitis	Reference
			n	R-allele carriage	n	R-allele carriage		
Caucasian	IL-1β	+3953C/T	45	27%	20	45%	-	Galbraith et al., 1999
	TNF-α	-308G/A		24%		35%	-	
Caucasian	IL-10	-1082G/A	86	59%	174	75%	+[1]	Dashash et al., 2005
Caucasian	IL-10	-1082G/A	84	64%	164	77%	- (+[2])	Dashash et al., 2006
		-819C/T		39%		43%	- (+[2])	
		-592C/A		39%		43%	- (+[2])	
Caucasian	IL-1RN	86 repeat	48	35%	98	43%	+[3]	Dashash et al., 2007
Caucasian	IL-6	-174G/C	183	61%	272	68%	+[4]	Izakovicova Holla et al., 2008
		-572G/C		11%		12%	- (+[5])	
		-597A/G		64%		66%	- (+[5])	
Caucasian	IL-18	-607A/C	151	62%	147	64%	-	Vokurka et al., 2009
	MMP-9	-1562C/T		17%		29%	+[6]	

- = association not found, + = association found

+[1]An association with gingivitis was found for R-allele carriage

- (+[2]) Genotype GCC/GCC (-1082/-819/-592) was protective against gingivitis

-[3] R-allele (IL-1 RN*2) was protective against gingivitis, even after correction for plaque

+[4] An association with gingivitis was found for R-allele carriage and R/R genotype carriage even after correction for plaque levels

- (+[5]) Haplotype C(-174)/G(-572)/A(-597) was associated with gingivitis

+[6]An association with gingivitis was found for MMP-8 R-allele carriage (mainly in boys) and for combined genotype: carriage of MMP-9 C/T and IL-18 C/C genotypes

Table 1. Gene polymorphisms and carriage rate of the rare (R) – allele in case-control studies and association with susceptibility to gingivitis

4.1 Polymorphisms in the IL-1 gene cluster

Interleukin-1, one of the main proinflammatory cytokines, is an important mediator of the immune response. IL-1 production may be induced by a number of stimuli, including products of microorganisms or proinflammatory mediators produced by cells during the immune response. Pleiotropic effects of this interleukin include activation of the endothelial cells, which helps migration of neutrophils and monocytes/macrophages to an inflammatory site, proliferation of fibroblasts, activation of osteoclasts and release of enzymes participating in intercellular substance destruction, so-called matrix metalloproteinases. The role of IL-1 in periodontal disease pathogenesis has been confirmed both in animal models (Assuma et al., 1998) and in human medicine; the increased levels of this interleukin are found in tissues and sulcular fluid in patients with gingivitis or periodontitis (Ishihara et al., 1997, Rawlinson et al., 2000).

The genes encoding for IL-1α and IL-1β (proinflammatory) and IL-1/IL-1-receptor antagonist (IL-1RN, antiinflammatory) cytokine are assigned to chromosome 2q13-21. In all three genes several polymorphic loci have been described. Based on the number of published reports, the two single nucleotide polymorphisms (SNPs) connected with cytosine - thymine interchange (C/T), one within the promoter region of the gene for IL-1α (at position -889) and the other within the fifth exon of the IL-1β gene (at position +3953), appear to be the most studied genetic variant in periodontal diseases. The IL-1α -889 and IL-1β +3953 R-alleles have been shown to increase and the IL-1RN VNTR (a variable number of 86-bp tandem repeat in the second intron) R-alleles, to decrease gene transcription or the protein production levels (Shiroddria et al., 2000, Pociot et al., 1992, Andus et al., 1997) resulting in the R-allele carriers in a more pronounced IL-1 proinflammatory response (Laine et al., 2010).

Results of two case-control studies of IL-1 genes in patients with gingivitis are presented in Table 1. The SNP IL-1β +3954 (+3953) was firstly analyzed as a risk factor for gingivitis (Galbraith et al., 1999). Groups of twenty patients with gingivitis and 20 controls of unknown periodontal health status were compared and no differences in IL-1 +3954 allele or genotype frequencies were found. In contrast, a significant association of this variant with advanced adult periodontitis was confirmed (Galbraith et al., 1999). However, more recent studies have reported a significant association between IL-1RN gene polymorphism and gingivitis in 146 Caucasian children (Dashash et al., 2007). The IL-1 RN*2 allele (A2) was protective against the development of gingivitis. The same allele has been previously associated with an increased production of IL-1 RN (Hurme and Santtila, 1998) and a protective role against rheumatoid arthritis (Lee et al., 2004). Moreover, studies of IL-1 antagonists on periodontitis in animal models have shown that IL-1 Ra is able to inhibit the osteoclast-like cell formation mediated by A. actinomycetemcomitans Y4 capsular polysaccharide in mouse marrow cultures and also to inhibit the differentiation of osteoclasts induced by IL-1 (Nishihara et al., 1995).Taken altogether, the IL-1 gene cluster polymorphisms cannot be considered as general risk factors for gingivitis susceptibility. A very low number of case-control studies have been published so far. The decision whether and how the above given variants can modulate (increase or decrease) risk of gingivitis development will require other studies to be performed and be subject to a meta-analysis.

Another question is the effect of genetic variability on gingival inflammatory parameters. Lang and colleagues (2000) conducted a prospective longitudinal study that investigated the association between the IL-1 complex (IL-1α -889C/T and IL-1β+3954C/T) genotype and gingival inflammation assessed using the bleeding on probing (BOP). The results for 139 non-smoking subjects indicated that IL-1 positive genotype patients were found to have a significantly higher chance of presenting BOP and twice as likely to have increased BOP over a four-appointment recall (Shapira et al., 2005). Using the experimental gingivitis model, Jepsen et al. (2003) investigated the relationship of IL-1 genotype and the susceptibility to develop gingivitis in 10-positive and 10-negative volunteers with healthy gingiva. They did not find any association between parameters of gingival inflammation (such as BOP) and IL-1 genotype after 21 days of no plaque control. Also Goodson and colleagues (2000) found significant differences in BOP between similar groups of subjects (7 IL-1 positive vs. 13 IL-1 negative) in a 10-day experimental gingivitis trial. In contrast, Scapoli et al. (2005) did not find any significant association between IL-1α +4845 (-889), IL-1β (-3953) or combined genotype of both variants and the clinical parameters in the

experimental gingivitis trial in the overall population (N=96) of high and low responder. However, genotype distributions of IL-1RN and IL-1β -511 variants were statistically significantly different between both groups. Müller and Nusair (2007, 2010) described the effect of combined alleles 2 (R-alleles) of both IL-1α -889 and IL-1β +3954 on lower gingival bleeding tendency in plaque-induced gingivitis in a group of fifty subjects of 19-28 years of age. The present studies suggested a subtle influence of the IL-1 gene cluster polymorphisms on gingival inflammation. However, further well-designed larger studies are necessary to confirm these preliminary findings and the role of IL-1 variants in gingivitis susceptibility.

4.2 Polymorphisms in the IL-6 gene

Interleukin-6 is a pleiotropic cytokine produced by a variety of cells, such as gingival fibroblasts, endothelial cells, monocytes and T lymphocytes. It regulates differentiation and/or activation of macrophages and T cells, growth and differentiation of B cells and production of antibodies (Papanicolaou et al., 1998). IL-6 is known to stimulate protein production by hepatocytes during the acute phase of a systemic inflammation. IL-6 expression under the normal conditions is minute. However, it is significantly stimulated by proinflammatory products, such as endotoxin (LPS). Besides affecting inflammation, IL-6 activates osteoclasts thus disturbing the balance between bone formation and degradation (Ota et al., 1999). On the other hand, it can also induce production of the IL-1 antagonist receptor and soluble receptor for TNF-α and thus block effects of these proinflammatory mediators, which suggest possible IL-6 anti-inflammatory effects (Tilg et al., 1994).

The IL-6 gene is localized on chromosome 7 (7p21, Bawcock et al., 1988). The IL-6 5´-region contains numerous polymorphisms that directly influence the expression of the protein. The most frequently studied variants are three single-nucleotide variants (SNPs) at positions -174G/C, -572G/C and -597G/A together with AnTn polymorphism (Osiri et al., 1999, Terry et al., 2000). The first studies dealing with the "functionality"of the given variants underlined an important role of -174G/C polymorphism that involves a binding site for the transcription factor NF-IL-6; the presence of the C allele of this polymorphism leads to a lower basal and by lipopolysaccharide and IL-1 stimulated expression and reduction in plasma IL-6 levels compared to the common G allele (Ferrari et al., 2003). More recently, it has been shown that a number of other promoter polymorphisms, and mainly their mutual interactions, can significantly affect the expression of these genes (Müller-Steinhardt et al. 2007).

There is only one case-control study on the role of IL-6 polymorphisms in gingivitis (Table 1). Data on the relationship of 3 promoter polymorphisms in the IL-6 gene for the development of gingivitis was summarized in our previous study (Izakovicova Holla et al., 2008). The study included 455 children aged 11-13 years. Plaque-induced gingivitis was diagnosed in 272 of them and 183 children were healthy. Differences in allele frequencies or genotype distributions were not statistically significant for IL-6 -572G/C and -597G/A polymorphisms. However, frequency of IL-6 -174 R-allele was significantly higher in children with gingivitis and this allele was associated with gingivitis regardless of the amount of plaque. Boys who carried -174 C allele with the simultaneous presence of plaque were at the highest risk for the gingivitis development. Haplotype analysis proved a significant difference in haplotype frequencies between the healthy and diseased children, risks for the gingivitis development were nearly 1.5-times higher in children with CGA (-174C/-572G/-597A) haplotype and on the contrary reduced in children with GGG variant.

However, Scapoli et al. (2007) did not find any significant association between -174G/C and -597G/A variants and the clinical parameters in the experimental gingivitis trial in the overall population (N=96) of high and low responder. In addition, genotype distributions of both IL-6 polymorphisms did not differ between both groups statistically significantly.
We concluded that the IL-6 polymorphisms may be associated with plaque-induced gingivitis susceptibility, similarly as with CP susceptibility (Laine et al., 2010). However, other studies and meta-analysis are needed before the final conclusion is made.

4.3 Polymorphisms in the IL-10 gene

Interleukin-10 (IL-10) is an antiinflammatory cytokine synthesized by activated monocytes and T-lymphocytes in response to inflammation (Kobayashi et al., 2011). It inhibits production of several cytokines such as IL-1α, IL-1β, IL-6, TNF-α, and IL-10 itself. IL-10 production is partially genetically determined (Westendorp et al., 1997). The gene encoding for IL-10 is located on chromosome 1q31-q32, in a cluster with other interleukin genes, such as IL-19, IL-20 and IL-24. Several polymorphic sites in the IL-10 promoter region have been identified at positions -1082, -819, -627, -592, and -590. SNP at position -1082 lies within a putative Ets transcription factor binding site (Kube et al., 1995), variant at position -819 may affect an estrogen responsive element (Scarel-Caminaga et al., 2004) and polymorphism at -592 can have a negative regulatory function (Kube et al., 1995). The IL-10 -1082G/A, -819C/T and -592C/A variants showed strong linkage disequilibrium and form two common haplotypes. The R-allele of the -592 polymorphism has been associated with decreased synthesis of IL-10 *in vivo* and *in vitro* (Koss et al., 2000, Crawley et al., 1999). IL-10 plays a protective role in periodontal destruction due to inhibition of matrix metalloproteinases (MMPs) and receptor activator for nuclear factor-κB (RANK) system. Therefore, the individuals with decreased production of IL-10 can be less protected against bacterial pathogens.
In Table 1, results from two published studies investigating IL-10 gene polymorphisms in gingivitis susceptibility are presented. Dashash and colleagues in the first study (2005) analyzed the relationship between IL-10 -1082G/A variant and susceptibility to gingivitis among 260 Caucasian children (86 controls and 174 patients), aged 8 to 12 years from the UK. An increased risk of having gingivitis was found in allele A positive children, regardless of plaque or age. This allele was previously associated with low production of this cytokine (Turner et al., 1997). One year later, the same authors published haplotype analysis of three IL-10 gene variants at positions -1082, -819 and -592 in the same group of subjects and found that GCC haplotype was protective and ACC and ATA haplotypes were associated with gingivitis manifestation.
In conclusion, IL-10 alleles, genotypes and haplotypes have been associated with gingivitis susceptibility. However, both studies were published by the same authors using the same subjects and the results have not yet been replicated in another population. Therefore, the IL-10 gene may be one of the "candidate" genes for gingivitis but these positive associations must be confirmed in other ethnic groups.

4.4 Polymorphisms in the TNF genes

Tumor necrosis factor α (TNF-α) and tumor necrosis factor β (TNF-β, newly lymphotoxin α - LT-α) are key pleiotrophic proinflammatory cytokines. TNF-α is released primarily by monocytes and macrophages and triggers a cascade of other mediators of inflammation

(Beutler & Grau, 1993). LT-α which plays an important role in tumor cell destruction and viral infections is released mainly by T-lymphocytes.
Besides its local para- , juxta- and autocrine effects within the proinflammatory activities, TNF-α stimulates expression of some enzymes important in the remodelling of extracellular matrix; so-called matrix metalloproteinases - e.g. MMP-1, MMP-8 and MMP-13 (Panagakos & Kumar, 1995; Johansson et al., 1997) participating in degradation of periodontal ligaments (Birkedal-Hansen H, 1993). A great number of studies have found elevated levels of these cytokines in sulcular fluid and higher expression of TNF-α in the inflamed periodontal tissues in patients (Roberts et al., 1997). Besides the effects on the soft tissues of the periodontium, TNF-α is one of key cytokines that promotes bone resorption.
The genes for TNF-α and LT-α lie in the MHC class III region on the long arm of chromosome 6 (6p.21) between HLA-B and DR (Carroll et al., 1987). Many polymorphisms have been found in the TNF gene cluster, the G/A variant at position –308 in the promoter region of the TNF-α gene being the most profoundly studied. The less frequent allele of this polymorphism (-308A, R-allele) has been associated with increased production of TNF *in vitro* (Braun et al., 1996) and an increased risk of developing many diseases, including periodontitis (Galbraith et al., 1999). Only one study about the role of TNF-α polymorphism in gingivitis has been published so far (Table 1).
Galbraith and colleagues (1999) studied 20 subjects with plaque-associated gingivitis and 20 patients with adult periodontitis aged 35 and 65 years. Referent population consisted of 45 unrelated Caucasian subjects of unknown periodontal health status. For the TNF-α -308 variant, the frequencies of the R-allele were comparable between the reference subjects and patients with gingivitis and varied between 22.5-26.7%. The second, but not case-control study was afterwards published by Scapoli et al. (2007) on the group of 96 systemically and periodontally healthy individuals as a 21-day experimental gingivitis clinical trial. No relationship between the TNF-α -308 or LT-α +252 polymorphism and susceptibility to gingivitis was demonstrated.
To date there are very limited data analyzing possible effects of TNF gene variants in plaque-induced gingivitis; none, so far, has proven an association of the TNF gene(s) with this disease.

4.5 Polymorphisms in the MMP-9 gene

Enzymes denoted as matrix metalloproteinases (MMPs) are important mediators of tissue destruction in periodontal disease. Matrix metalloproteinases are secreted by various cells - polymorphonuclear leukocytes, macrophages, fibroblasts, epithelial and endothelial cells. (Birkedal-Hansen H, 1993). MMPs expression and activity are regulated by different mechanisms, including modulation of transcription, activation of latent pro-forms and inhibition by tissue inhibitors of metalloproteinases – TIMPs (Brew et al., 2000).
MMP-9 (also known as gelatinase B or 92-kDa type IV collagenase) is one of the MMPs active against denaturated collagen (gelatin) and collagen types IV, V and XI together with proteoglycans and elastin. The coding gene is located on chromosome 20q11.2-13.1 and several polymorphisms have been detected in the MMP-9 gene, some of them have been found to be "functional" (Zhang et al., 1999). The C to T substitution in position -1562 increases transcriptional activity of the MMP-9 in macrophages, as confirmed by *in vitro* studies (Zhang et al., 1999). In agreement with these findings from the *in vitro* experiments it was found that persons carrying the T allele have increased MMP-9 levels in plasma (Zhang

et al., 1999). Higher levels of the MMP-9 in patients with periodontitits in comparison to the healthy population were also observed (Ingman et al., 1996). However, in later studies no associations of MMP-9 functional polymorphisms with plasma MMP-9 levels in healthy were found (Demacq et al., 2008). No correlation between MMP-9 -1562C/T variant and salivary MMP-9 levels was found. However, significantly higher levels of MMP-9 were detected in patients with CP and correlated with clinical parameters of periodontal destruction (Isaza-Guzman et al., 2011).

Data on frequencies of polymorphism in the gene for MMP-9 (-1562C/T) together with polymorphism in the gene for IL-18 (–607A/C) in children with gingivitis have been published only in one study so far (Table 1). In the association case-control study in Czech adolescents aged 11-13 years, the authors demonstrated differences in frequencies of MMP-9 –1562C/T polymorphism between children with healthy gingiva and those with plaque-induced gingivitis. Boys with gingivitis carried the T allele (and genotypes with this allele) significantly more frequently compared to children with healthy gingiva (15.9% vs. 6.5%). Patients with combined genotype CT (of MMP-9 -1562C/T) and CC (of IL18 -607A/C) had 5.6 times higher risk of the gingivitis development compared to persons without this genotype (Vokurka et al., 2009).

Although the matrix metalloproteinase genes seem good candidates for their association with periodontal inflammation and destruction, investigations have not yet yielded sufficient data for conclusions about the role of these genes in plaque-induced gingivitis.

5. Interleukin-18 (IL-18) gene polymorphism in relation to gingivitis and microbial pathogens in Czech population

5.1 Introduction

Interleukin-18 (IL-18) belongs to possible factors that can play a role in the development of periodontal disease. It is a pro-inflammatory cytokine, a member of the IL-1 family that was originally described as INF-γ inducing factor that modulates both innate and adaptive immunity (Okamura et al., 1995). It is generally considered to be involved in the T-helper type 1 (Th1)-mediated immune response. IL-18 directly increases the production of TNF-α and subsequent release of IL-1β and IL-8 from monocytes. On the other hand, IL-18 might initiate Th2 responses with production of IgE via the stimulation of IL-4 and IL-13 synthesis in mast cells and basophils and eosinophil recruitment. The local production of IL-1β and IL-18 in the gingival tissue samples (Johnson & Serio, 2005) or gingival crevicular fluid (Orozco et al., 2006) increases with increasing inflammation and IL-18 was the predominant cytokine at both gingivitis and periodontitis sites. Among other functions, regulation of expression of MMPs and stimulation of MMP-9 active form can be mentioned (Delaleu et al., 2004).

The gene for human IL-18 is located at chromosome 11q22.2-22.3. It is composed of 6 exons (Kalina et al., 2000) and several polymorphisms have been identified in the *IL-18* gene (Zhang et al., 1999). Among them, -607A/C (rs1946518), -137C/G (rs187238) in promoter and -133C/G (rs4988359) in intron 1 have been studied in more detail with respect to transcriptional activity or IL-18 production by monocytes (Liang et al., 2008; Arimitsu et al., 2006). A change from C to A at position -607 disrupts a potential binding site for cAMP-responsive element binding protein, while allele C at -137C/G has been shown experimentally to disrupt a confirmed H4TF1-binding site (Giedraitis et al., 2001). The -

133C/G SNP in intron 1 is situated in an NF-1 binding site that is supposed to activate the transcription of a number of immune proteins, such as transforming growth factor (TGF)-β1, tumor necrosis factor (TNF) receptor 2, and IL-1β (Krohn et al., 1999).
There are only three studies of the IL-18 gene polymorphisms in chronic periodontitis (Folwaczny et al., 2005, Noack et al., 2008, 2009). Only one previous study on the potential association between IL-18 -607A/C variant and risk of gingivitis has been published so far by our group (Vokurka et al., 2009). With respect to the key role of interleukins in inflammatory response in gingivitis, the IL-18 gene is an obvious functional candidate for this disease. We analyzed the distributions of the IL-18 -607A/C, -137C/G and -133 C/G alleles, genotypes, and haplotypes in Czech children aged 11 to 13 years with plaque-induced gingivitis and with healthy gingiva.

5.2 Materials and methods

5.2.1 Subjects

A total of 572 Caucasian adolescents (311 boys and 261 girls) of exclusively Czech nationality, aged 11-13 years, selected from the ELSPAC (European Longitudinal Study of Pregnancy and Childhood) Brno group, which comprises over 5000 children and their families, was examined to assess gingival health in this case-control study. Children underwent a dental examination at the Clinic of Stomatology, St. Anne´s University Hospital and Faculty of Medicine, Masaryk University. Inclusion criteria consisted of a simple informed consent of the respective children and their families and of their willingness to participate. Thus, the randomness of the set is ensured; although a slight drift towards families with mothers with higher education can be expected. The study group comprised children with clinical evidence of plaque induced gingivitis (N=307, 197 boys and 110 girls). The healthy group (N=265, 114 boys and 151 girls) included children who had healthy gingiva with no clinical signs of inflammation (GI index = 0).
The clinical assessment was carried out by one investigator and the following parameters were assessed: DMFT (decayed/missing/filled teeth) score, gingival index (GI), plaque index (PI) and calculus index (CSI) as previously described (Izakovicova Holla et al., 2008). The radiograph examination was not performed as it was not part of routine care for these adolescents and would therefore have been deemed unethical. Phenotype status was assigned without knowledge of genotype by independent investigators.
The study was performed with the approval of the Committee for Ethics of the Medical Faculty, Masaryk University Brno and written informed consent was obtained from all parents (in case of children), in line with the Helsinki declaration before inclusion in the study.

5.2.2 Molecular assessment of periodontopathic bacteria

Microbial samples were taken from the subgroups of randomly selected subjects (N=207). Microbial samples were collected from gingival sulcus of each quadrant by inserting a sterile paper point into a base of the pocket for 20 seconds. Bacterial plaque samples from each individual were pooled in one tube. The detection of periodontal bacteria was performed using a commercially available microarray system (ParoCheck®; Greiner Bio-One GmbH, Frickenhausen, Germany), which allows the simultaneous detection of up to 20 different oral bacterial species based on species-specific highly conserved regions from the 16S rRNA gene. The ParoCheck® chip is a coated glass slide with a total of 86 DNA

measuring points, which can be evaluated by all commercially available microarray scanners. In this study, dental paper points soaked with gingival fluid were shaken out into deionized water and 1 µl aliquot was put in a 20- µl reaction mixture containing 0.2 µl Taq DNA polymerase and 18.8 µl Master Mix supplied with the Parocheck® kit (containing buffers, $MgCl_2$, dNTPs, DNase-free water and fluorophore-labelled primers). PCR was performed to amplify the target sequences and the cycling conditions used were as follows: 94°C for 1 min followed by 45 cycles at 95°C for 20 s, annealing at 60°C for 20 s, 72°C for 30 s and final elongation at 72°C for 1 min. Next, the labelled amplified products were hybridized to pathogen-specific oligomers according to the manufacturer's instructions. This step was first performed at 60°C in a steam-saturated atmosphere for 5 min. Next, 30 µl hybridization buffer was mixed with 5 µl PCR product at room temperature and incubated for 2 min at 95°C using a heating block. Then, 25 µl hybridization mix was transferred into each well of the chip and incubated for 10 min at 60°C. After washing and then drying using an air spray, the chip was read using a scanner (Axon 4100 A; Axon Instruments Inc., Union City, CA) and the software Paroreport 20. The bacterial counts were semi-quantitatively analyzed on a graduated scale ranging from 0 to 4 according to the dot intensity measured and were calibrated to serial dilutions of the relevant microorganisms.

5.2.3 Genotyping

Genomic DNA was extracted from peripheral blood leukocytes by a standard method using the proteinase K digestion of cells. For detection of single nucleotide polymorphisms (SNP) in promoter at positions -607C/A and -137C/G allele-specific PCR according to Giedraitis et al. (Giedraitis et al., 2001) was slightly adopted. The original PCR-RFLP method according to Kruse et al. (Kruse et al., 2003) was used for the analysis of SNP in intron 1 at position -133C/G). The PCR products were then digested by the restriction enzyme SmaI. The primers, PCR conditions, and restriction enzymes used are listed in Table 2. The genotyping was performed by one investigator unaware of the phenotype. 10% subjects were genotyped twice for each polymorphism to verify correctness of the analysis.

SNP	Primers	Annealing temperature	Enzyme
-607A/C (rs1946518)	5´-TAACCTCATTCAGGACTTCC- 3´; 5´-CTTTGCTATCATTCCAGGAA- 3´; 5´-GTTGCAGAAAGTGTAAAAATTATTAA-3´; 5´- GTTGCAGAAAGTGTAAAAATTATAC- 3´	64°C, 57°C	-
-137C/G (rs187238)	5´-AGGAGGGCAAAATGCACTGG-3´; 5´- CCAATAGGACTGATTA-3´ 5´-CCCCAACTTTTACGGAAGAAAAG-3´; 5´CCCCAACTTTTACGGAAGAAAAC-3´	68°C, 62°C	-
-133C/G (rs4988359)	5´-GTATTCATAAGCTGAAACTCCCCG-3´; 5´-TGTTCTATGGCATTAGCCTTAC-3´	53°C	*SmaI*

Table 2. PCR-RFLP analysis

5.2.4 Statistical analyses

Comparisons were made between allelic and genotype frequencies in the cases and controls. The allele frequencies were calculated from the observed numbers of genotypes. The significance of differences in the allele frequencies among groups was determined by Fischer's exact test. χ^2 analysis was used to test for deviation of genotype distribution from Hardy-Weinberg equilibrium and for comparison of differences in genotype combinations among groups. Variations in the quantity of subgingival bacteria corresponding to the particular genotypes were tested by Kruskal-Wallis ANOVA and/or Fisher´s exact test. Only the values of P less than 0.05 were considered significant. To examine the LD between all SNPs, pairwise LD coefficients [D´] and haplotype frequencies were calculated using the SNPAnalyzer program (http://snp.istech.info/istech/board/login_form.jsp). Contingency table analysis, odds ratio (OR), 95% confidence intervals and significance values were estimated with the use of the Statistica ver. 9.0 (Statsoft Inc., Tulsa, Oklahoma, USA) program package.

5.2.5 Results

The genotype and allele frequencies of the IL-18 gene polymorphisms in healthy children and those with gingivitis are presented in Table 3. The frequencies of -607A/C, -137C/G and -133C/G genotypes in the healthy controls and plaque-induced gingivitis group were in accord with those expected by the Hardy-Weinberg equilibrium (P>0.05). There were no significant differences in the allele and genotype frequencies between children with gingivitis and healthy controls (p = 0.35 and p = 0.90 for -607A/C, p=0.35 and p=0.89 for -137C/G, and p=0.17 and p=0.57 for -133C/G variant, respectively, Table 3).

		Genotypes of IL-18 -607A/C			Alleles		
	N	A/A (%)	A/C (%)	C/C (%)	A	C	P
Healthy gingiva	265	41 (15.5)	138 (52.1)	86 (32.4)	0.415	0.585	
Gingivitis	307	45 (14.7)	157 (51.1)	105 (34.2)	0.402	0.598	P = NS
		Genotypes of IL-18 -137C/G			Alleles		
	N	C/C (%)	C/G (%)	G/G (%)	C	G	P
Healthy gingiva	265	28 (10.6)	112 (42.3)	125 (47.2)	0.317	0.683	
Gingivitis	307	29 (9.4)	129 (42.0)	149 (48.5)	0.305	0.695	P=NS
		Genotypes of IL-18 -133C/G			Alleles		
	N	C/C (%)	C/G (%)	G/G (%)	C	G	P
Healthy gingiva	265	120 (45.3)	119 (44.9)	26 (9.8)	0.677	0.323	
Gingivitis	307	150 (48.9)	133 (43.3)	24 (7.8)	0.705	0.295	P=NS

P - statistical significance for the comparison of genotype or allele frequencies between the two groups by χ2-test (for genotypes) or Fisher's exact test (for alleles).

NS = non-significant differences

Table 3. Genotype and allele frequencies of the IL-18 gene polymorphisms in controls and patients with gingivitis

As haplotype analyses may be of a higher informative value for drawing associations between phenotypes and genetic variation than SNPs, we also assessed haplotype frequencies using the SNPAnalyzer 2 program. Of eight haplotype combinations found, only three had frequency higher than 10% because all three variants in the IL-18 gene were in tight linkage disequilibrium with each other to various degrees (D´= from 0.656 to 0.786 in controls and D´= from 0.726 to 0.790 in patients with plaque-induced gingivitis). We found no significant differences in frequency of the IL-18 haplotypes between children with gingivitis and control subjects (Table 4).

Haplotypes			Healthy gingiva	Gingivitis	OR (95%CI)
-607A/C	-137C/G	-133 C/G			
C	G	C	0.510	0.521	1.05 (0.83-1.32)
A	C	G	0.250	0.239	0.91 (0.69-1.19)
A	G	C	0.117	0.119	1.00 (0.70-1.46)
C	G	G	0.034	0.040	1.08 (0.55-2.10)
A	C	C	0.027	0.037	1.37 (0.66-2.86)
C	C	C	0.230	0.029	1.27 (0.62-2.59)
A	G	G	0.021	0.016	0.86 (0.38-1.92)
C	C	G	0.017	0.000	0.17 (0.02-1.47)
				P = NS	

NS = non-significant differences

Table 4. The frequencies of the IL-18 haplotypes in both groups

Possible links between genetic variants of IL-18 and microbiological colonization (occurrence of bacteria in gingival sulcus, including *A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. forsythia, T. denticola, P. micros,* F. *nucleatum, E. corrodens, A. viscosus, C. rectus, S.mitis, S. gordonii, P. nigrescens, S. constellatus, V. parvula, C. gingivalis, A. odontolyticus, C. concisus, E.nodatum, C. gracilis*) were assessed in the subgroups of subjects (N=215). Although we found a significant difference in the occurrence of periodontal bacteria between the children with gingivitis and healthy controls (P=0.00002 for *P. gingivalis*, P=0.0001 for *A. actinomycetemcomitans*, P=0.000001 for *P. intermedia*, P=0.000004 for *F. nucleatum*, P=0.0002 for *A. odontolyticus*, P=0.0009 for *P. micros*, P=0.007 for *C. gingivalis*, P=0.008 for *C. gracilis*, P=0.01 for *S. constellatus,* P=0.002 for *S. mitis*, and P=0.01 for *C. rectus*) (Table 5), no relationships between IL-18 variants and microbial pathogens were observed in any group (data not shown).

Bacteria	Healthy gingiva	Gingivitis	P level	OR (95% CI)
	occurrence of bacteria in gingival sulcus (%)			
P. gingivalis	2.94	25.2	0.00002	11.11 (2.58-47.72)
A. actinomycetemcomitans	35.3	64.0	0.0001	3.26 (1.78-5.98)
P. intermedia	19.1	61.9	0.000001	6.87 (3.43-13.75)
T. forsythia	1.5	5.0	NS	3.55 (0.43-29.48)
T. denticola	2.9	7.9	NS	2.84 (0.61-13.17)
P. micros	27.9	51.8	0.0009	2.77 (1.48-5.18)
F. nucleatum	22.1	55.4	0.000004	4.39 (2.26-8.52)
E. corrodens	77.9	82.7	NS	1.36 (0.66-2.79)
A.viscosus	50.0	58.3	NS	1.40 (0.78-2.50)
C. rectus	11.8	25.9	0.01	2.62 (1.14-6.01)
S. mitis	76.5	92.1	0.002	3.58 (1.56-8.23)
S. gordonii	80.9	86.3	NS	1.49 (0.69-3.24)
P. nigrescens	4.4	9.4	NS	2.24 (0.61-8.13)
S. constellatus	29.4	15.1	0.01	0.43 (0.21-0.86)
V. parvula	38.2	49.6	NS	1.59 (0.88-2.88)
C. gingivalis	16.2	33.1	0.007	2.56 (1.23-5.35)
A.odontolyticus	29.4	56.1	0.0002	3.07 (1.65-5.70)
C. concisus	0.0	3.6	NS	*
E. nodatum	0.0	1.4	NS	*
C. gracilis	1.5	11.5	0.008	8.72 (1.13-67.17)

NS = non-significant difference
*not applicable (small numbers)

Table 5. Occurrence of bacteria in children with healthy gingiva and patients with gingivitis

5.2.6 Discussion

Plaque-induced gingivitis affects most children and adults. It is characterized by inflammation of the gingiva without loss of connective tissue attachment, alveolar bone or teeth. Given the multifactorial nature of inflammation and the major role of IL-18 in modulating immune functions, the possible association of polymorphisms in the IL-18 gene with plaque-induced gingivitis has been investigated. Previously we demonstrated no significant association of IL-18 -607A/C variant within the IL-18 gene with gingivitis (Vokurka et al., 2009). In a much larger (twice as large) study, we screened three IL-18 SNPs (at positions -607A/C, -137C/G, and -133C/G) alone and in combination (i.e. haplotypes) in Czech adolescents with and without plaque-induced gingivitis. We again failed to find any association of the three SNPs alone with gingivitis. In addition, no differences in haplotype frequencies were found between children with healthy gingiva and gingivitis and no

significant relationships between microbial pathogens, IL-18 polymorphisms and gingivitis were determined. Only three other studies have analyzed IL-18 gene variant in periodontal diseases. Folwaczny et al. (2005) did not find an association between several IL-18 polymorphisms at positions -656, -607, -137, +113, +127 and codon 35/3 and periodontitis in Germany. The frequencies of the alleles and genotypes for -607A/C and -137C/G SNPs observed in their study were similar to our data both in healthy subjects and in children with gingivitis compared to patients with periodontitis. Indeed, some race/ethnicity-based differences exist in the relative frequencies of the IL-18 genotypes in non-Caucasian subjects (summary from Innate Immunity Programs for Genomic Applications, IIPGA database is available at http://www.innateimmunity.net). Two more recent studies (Noack et al. 2008, 2009) analyzed associations of IL-18 polymorphisms at positions -368G/T and -838C/A (plus two TLR4 variants) with aggressive and chronic periodontitis and failed to find an association between IL-18 polymorphisms and periodontal destruction. However, IL-18 production is regulated on different levels of transcription, translation and post-translation. This may be the reason why, although the IL-18 gene is a plausible "candidate" gene for periodontal inflammation, simple phenotype-genotype association may be difficult to find.

In conclusion, all to date published results reject the hypothesis that functionally relevant IL-18 gene variants have a major effect in periodontal disease – gingivitis, as well as chronic or aggressive periodontitis. Assuming that inflammation of gingival and periodontal destruction are multifactorial conditions, it would be interesting to study the impact of IL-18 variants in a more complex genetic background of bacterial recognition and host response in gingivitis.

6. General conclusions

Inflammatory diseases of periodontium are one of the most common disorders in populations and together with dental caries are a major cause of tooth loss. Based on severity, intensity and location of the inflammatory process, it is possible to distinguish between gingivitis, and periodontitis. Inflammation of periodontal tissues may be associated not only with oral health but also with general state (mainly diabetes mellitus, cardio-vascular diseases, pulmonary diseases, etc.), (Herzberg & Meyer, 1996, Taylor et al., 1996, Grossi & Genco, 1998, Persson GR & Persson RE, 2008, Williams et al., 2008).

The origin of periodontal disease depends on the interaction of numerous endogenous and exogenous factors. The presence of a microbial plaque on the tooth surfaces initiates inflammation of the periodontal tissues. Periodontal pathogens are mainly anaerobic bacteria whose pathogenicity and virulence correlates with their quantity, biochemical and physical conditions within the sulcus or periodontal pocket and individual host's responsiveness. The exact mechanisms of the disease development and progression have not been fully clarified yet. Gingivitis may, or may not, progress to periodontitis (Brown & Löe, 1993). Today the great majority of these diseases are considered a multifactorial problem (a so-called complex disease) initiated and maintained by bacteria but significantly affected by a response of the individual (Kornman et al., 2000). Unlike monogenic diseases, gene variants participating in the development of complex diseases are not rare, distinctly pathogenic mutations. These are mostly "common" variants of "normal" genes (it means polymorphisms), often the most frequent alleles in population whose original (assumed) evolution advantage was lost under the condition of the current civilization.

Molecular and genetic research has not devoted much attention to periodontal diseases Although these diseases do not lead to premature death, study of their etiopathogenesis is very important as the high prevalence of periodontal diseases in population has very important social and economic consequences. Furthermore, we are not able to prevent the onset of these diseases in all individuals as primary preventive intervention is possible only after a detailed clarification of etiopathogenesis (thus also genetic determination of these diseases), which makes secondary preventive interventions even more urgent. This involves earlier diagnosis, mainly in the asymptomatic or incipient stages, efficient therapy and, last but not least, possibilities of determination of the individual's risk for the development of more serious forms of the disease.

The association between plaque-induced gingivitis and genetic polymorphisms has been studied on only seven genes in several studies, therefore it is difficult to conclude whether the analyzed polymorphisms can be involved in etiopathogenesis of gingivitis. Furthermore, there are several reasons for discrepancies in the findings reported in these studies:

1. One of the main problems is very low "power of study" due to small sample sizes. Most associations refer to small odds ratios and relatively large confidence intervals which contributes to the risk for false positive or negative results.
2. So far the most common case-control association study design is suitable for a lot of potential methodological problems connected with different definitions of clinical disease phenotype, different selections and definitions of control subjects, diversity of ethnic backgrounds of study samples etc. (Ioannidis, 2003).
3. Because of the complexity of plaque-induced gingivitis and the large number of host derived and environmental/external factors involved in disease pathogenesis, it is logical to assume that multiple genetic variants (SNPs) on different genes may contribute to overall disease susceptibility. As such, a simple cause and effect relationship between a particular genetic allele and a disease is not possible. Therefore, rather than associations of the individual polymorphisms, combinations of polymorphisms in connection with other factors should be investigated by multivariate analyses.

In conclusion, research on genetic polymorphisms in plaque-induced gingivitis has had limited success in unravelling significant and reproducible genetic factors for susceptibility to these diseases. Taken together the data published so far on gene variants in gingivitis, we can conclude that there is an insufficient number of studies to draw relevant conclusions. Nevertheless, the preliminary evidence suggests that polymorphisms in the IL-10, IL-1RN, IL-6 and MMP-9 may be associated with plaque-induced susceptibility. Results of genetic research, if verified in larger study cohorts, it could also lead to new diagnostic possibilities and help improve therapy of this disease or find parameters determining the risk of progression to more serious forms of periodontal disease. These findings could thus be a stimulus for an individual-based treatment approach and its optimization. Further clinical and genetic studies verifying the importance of these findings and their usability for monitoring of this disease activity still will have to be conducted.

7. Acknowledgement

This publication was supported by the project MSMT CR 1M0528 and by grant IGA NT11405-6.

8. References

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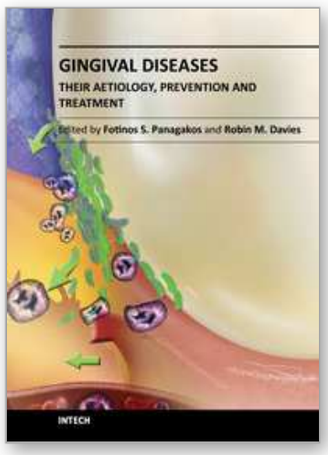
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Gingival Diseases - Their Aetiology, Prevention and Treatment

Edited by Dr. Fotinos Panagakos

ISBN 978-953-307-376-7
Hard cover, 230 pages
Publisher InTech
Published online 22, September, 2011
Published in print edition September, 2011

Gingival diseases are a family of distinct pathological entities that involve the gingival tissues. These signs and symptoms of these diseases are so prevalent in populations around the world that they are often considered to be â€œnormalâ€ features. The diseases are now classified into two main groups namely: Plaque-Induced and Non-Plaque Induced Gingival Diseases. This book provides dentists, dental hygienists, dental therapists and students with a comprehensive review of gingival diseases, their aetiology and treatment.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Lydie Izakovicova Holla, Kristina Musilova, Jan Vokurka, Pavla Pantuckova, Lubomir Kukla, Martina Kukletova and Zdenek Broukal (2011). Gene Polymorphisms in Gingivitis, Gingival Diseases - Their Aetiology, Prevention and Treatment, Dr. Fotinos Panagakos (Ed.), ISBN: 978-953-307-376-7, InTech, Available from: http://www.intechopen.com/books/gingival-diseases-their-aetiology-prevention-and-treatment/gene-polymorphisms-in-gingivitis

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