Genetic Variants in Periodontal Health and Disease

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Dedication

The important thing is not to stop questioning.
Curiosity has its own reason for existing.
Albert Einstein, 1879–1955

Foreword

Periodontal diseases continue to be one of human's most widespread affections and are taking an ever increasing importance in dentistry. Periodontitis is a chronic and complex infectious disease of the supporting tissues of the teeth. Because of the bacterial infection, the periodontal tissues become inflamed and are slowly destroyed by the action of the inflammatory process. Genetic risk factors, lifestyle and environmental factors, including periodontal pathogens, play a role in complex diseases such as periodontitis and peri-implantitis.

Complex diseases are typically polygenic and associated with variations in multiple genes, each having a small overall contribution and relative risk for the disease process. Since the late 1990s, a substantial increase in papers on putative genetic risk factors for susceptibility to and severity of periodontitis has appeared in the field of periodontology. Many papers and reviews have been published on candidate gene approach.

This book, entitled *Genetic Variants in Periodontal Health and Disease*, is different from the existing textbooks in many ways. Indeed, it is a fresh approach in which the latest information has been integrated into already existing knowledge and presented in a highly effective, easy-to-understand manner using innovative diagrams and tables. There has previously not been a comparable publication in the field of periodontology. This book will be useful to undergraduate and postgraduate dental students, teachers, researchers, and periodontists. They will embrace this book.

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Preface

Periodontitis is a complex disease of the tooth supporting structures. It is highly prevalent, affecting 10–15% of the population, and is considered as the most common cause of tooth loss among adults.

Currently, there are two major forms of periodontitis – chronic and aggressive pe-riodontitis. Risk for periodontitis is not shared equally by the population. It is clear that periodontitis severely affects a high-risk group representing around 10–15% of the population, in whom the disease quickly progresses from chronic gingivitis to destructive periodontitis. This differential risk for periodontitis is consistent with heritable elements of susceptibility, but direct evidence for a differential genetic contribution to periodontitis comes from several sources. As recently reveled at the 6th Congress of the European Federation of Periodontology, many genetic factors in periodontitis and some in peri-implantitis have been investigated, but not all are as strong we have hoped for. It should be considered that periodontitis is polygenic (gene-gene interactions) and multifactorial (gene-environment-life style interactions). Several reports results might reflect subpopulation effects and have to be interpreted with care as ethnic differences exist.

This book would be of interest to undergraduate and postgraduate dental students, dental educators, and researchers and provides a comprehensive review on the current knowledge regarding the genetic variants in periodontal health and disease.

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Classification of Periodontal Diseases 1

Classifications systems are necessary in order to provide a framework in which to scientifically study the etiology, pathogenesis, and treatment of diseases in an orderly fashion (Armitage 1999).

Traditionally, periodontal diseases have been divided into two major categories: gingival diseases and periodontal diseases. The former includes diseases that attack only the gingiva, whereas the latter includes diseases that involve the supporting structures of the tooth (Carranza and Newman 1996).

Different classifications of periodontal diseases have been used over the years and have been replaced as new knowledge has improved the understanding of the etiology and pathology of the diseases of the periodontium (Carranza and Newman 1996). They are traditionally based on clinical assessments, such as signs of inflammation, extent and pattern of connective tissue attachment level and of alveolar bone loss, the patient's age at the onset of periodontal destruction, and rate of disease progression or of treatment response.

1.1 Gingival Diseases

Gingivitis is an inflammatory process affecting the soft tissues surrounding the teeth. The inflammatory process does not extend into the alveolar bone, periodontal ligament, or cementum (Suzuki 1988).

Ranney (1993) divided gingivitis into three general groups: one that has as primary cause bacteria that colonize the gingival sulcus and adjacent tooth surfaces, one that additionally is necrotizing, and one that is not plaque-related and does not begin marginally (Ranney 1989) (Table 1.1).

Plaque-associated gingivitis is the most common form of gingivitis, and probably, the most common form of all periodontal diseases. National probability surveys in the United States and Denmark found that gingivitis affected about 60% of teenagers and 40–50% of adults. Only about 5–6% of gingival sites were inflamed (Brown and Löe 1993).

It was shown that, in otherwise healthy people, *gingivitis always develops when plaque accumulates and is always reversed by removing the plaque* (Ranney 1993). This model of *experimental gingivitis* is often used in clinical preventive trials that test the effectiveness of chemotherapeutic agents (Robertson et al. 1989). After a pre-study period to help subjects to

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Table 1.1 Classification of gingivitis (Ranney 1993)

Gingivitis, plaque bacterial	Non-agravated
	Systemically aggravated
	Related to sex hormones
	Related to drugs
	Related to systemic diseases
Necrotizing ulcerative gingivitis	Systemic determinants unknown
	Related to HIV
Gingivitis, nonplaque	Associated with skin disease
	Allergic
	Infectious

establish optimal plaque control and oral health, subjects using a chemotherapeutic agent are compared with subjects using a placebo, while both groups abstain from all other oral hygiene practices. Experimental gingivitis trials are generally limited in duration, because all subjects in the placebo group will develop gingivitis in about 21 days. This model is highly rigorous and clearly differentiates among agents with strong, moderate, and minimal potential to prevent plaque accumulation and the related development of gingivitis (Robertson et al. 1989).

Plaque-associated gingivitis has *clinical features* including some or all of the following: inflammation, edema, bleeding upon probing or spontaneous, gingival sensitivity, and itching (Suzuki 1988). In light-skinned people, the normal pink color changes to red or blue-red. In dark-skinned people, the color changes may not be so obvious, but, depending on the intensity of the normal pigmentation, may be observable as deep blue-red discoloration together with the edema that is detectable by palpation (Ranney 1993) (Fig. 1.1). *No radiographic loss of bone* is associated with gingivitis (Suzuki 1988).

The composition of *subgingival flora* associated with gingivitis is distinctly and significantly different from that of the flora associated with health and that of periodontitis. No differences in the compositions of this flora in the adults or children with naturally-occurring gingivitis were found (Moore et al. 1987). Thus, with increasing degree of severity of the disease, the relative number of motile organisms increases, while coccoid cells and straight rods decrease (Lindhe et al. 1980). It was suggested that the bacteriological diagnosis in gingivitis is unuseful, the diagnosis being basically made clinically (Ranney 1993, 1989).

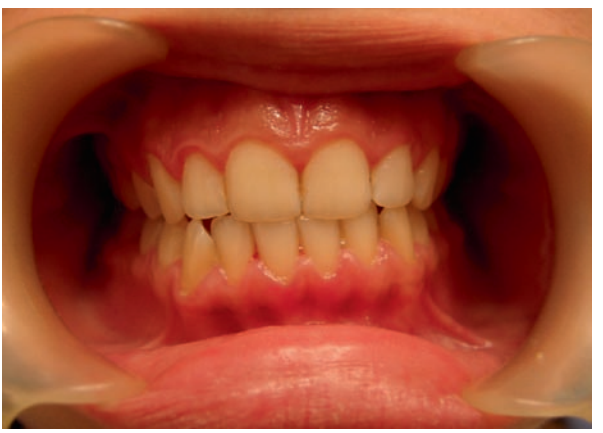

Fig. 1.1 Dental plaque-induced gingival diseases

Morphologic and functional changes in the gingiva during plaque accumulation have been thoroughly investigated, with Page (1986) concluding that the developing lesion followed a series of stages, which by clinical and histopathological criteria could be divided into initial, early, and established stages in clinically apparent gingivitis, and periodontitis has been designated as the advanced stage. In humans the *initial lesion* is seen within about 4 days of the beginning of plaque accumulation. The infiltrated area comprises 5–10% of the marginal gingival connective tissue and in this zone much of the collagen is destroyed. The *early lesion* evolves from the initial lesion within about 1 week following the beginning of plaque accumulation. It is characterized by an infiltrate in which small, medium, and large lymphocytes and macrophages predominate, along with small number of plasma cells located around the periphery of the infiltrate. Lymphocytes account for approximately 75% of the total inflammatory population. The acute inflammation persists as evidenced by vasculitis and the presence of neutrophils, especially in the junctional epithelium. The infiltrated area may occupy from 5 to 15% of the marginal connective tissue and collagen loss in the affected area may reach 60–70%. The resident fibroblasts become pathologically altered as evidenced by electron-lucid nuclei, swollen mitochondria, and vacuolization of the endoplasmic reticulum with rupture of the cell membranes (Table 1.2).

Table 1.2 Histopathogenesis of gingivitis and periodontitis Page & Schroeder, 1982 (reprinted with permission S. Karger AG, Basel)

Histopathologic condition	Initial gingival lesion	Early gingivitis	Established gingivitis
Plaque	Primarily Gram+, aerobic	Primarily Gram+, aerobic	Gram+ and −, in gingival pocket
Junctional epithelium (JE)/ pocket epithelium	Alteration of the most coronal portion of the JE	Initial alteration and lateral proliferation of the JE in coronal region	Lateral proliferation of JE, deepening of sulcus with formation of gingival pocket or pseudopocket
Vessels, inflammatory cells, infiltrate exudate	Classic vasculitis of vessels subjacent to the JE; exudation of fluid from the gingival sulcus; increased migration of leukocytes into the JE and gingival sulcus	Vasculitis, exudation of serum proteins, PMN migration, accumulation of lymphoid cells immediately subjacent to the JE at the site of acute inflammation; very few plasma cells; appearance of immunoglobulins and complement	Acute inflammatory alterations; predominance of plasma cells; presence of immunoglobulins extravascularly in the connective tissue, JE and gingival sulcus; increased sulcus exudate
Fibroblasts, connective tissue, collagen	Loss of perivascular collagen	Cytopathic alterations in resident fibroblasts; collagen loss in infiltrated connective tissue areas	Severe fibroblast injury, further loss of collagen, continued infiltration
Alveolar bone	Normal	Normal	Normal
Course of disease	2–4 days after plaque accumulation	4–7 days after plaque accumulation	1–3 weeks after plaque accumulation

In time, the *established lesion* characterized by a predominance of plasma cells and B lymphocytes evolves. A large number of neutrophils appear in the junctional and pocket epithelium, and macrophages are present in the lamina propria region of the pocket wall. In tissue specimens designated as severe as severe gingivitis, the lymphocytes continue to predominate over the plasma cells, and almost equal numbers of B and T lymphocytes are seen (Page 1986). There is further increase in the proportion of B cells and plasma cells in specimens classified as established lesions (Lindhe et al. 1980). In contrast, the inflammatory infiltrate in the inflamed gingiva of the children was dominated by T lymphocytes (Alcoforado et al. 1990).

It was suggested that gingivitis, at least as detectable histologically, has not been ruled out as a necessary precursor to periodontitis, but it does not invariably progress to periodontitis (Ranney 1986).

1.2 Diseases of the Tooth Supporting Structures

Various textbooks and publications have tried to classify the different types of periodontitis and aid the clinician's diagnostic process (Williams and Paquette 1997). The nomenclature and classification systems we use to describe periodontal disease have changed periodically over the past few decades. This leads to some confusion when one reviews past literature about disease prevalence, treatment, etc. (Oh et al. 2002) (Table 1.3).

Revisions to existing systems have been largely influenced by three dominant paradigms that reflect thinking at the time the classifications were proposed: the Clinical Characteristics paradigm (~1870–1920), the Classical Pathology paradigm (~1920–1970), and the Infection/Host Response paradigm (~1970 to present). Although classification systems for periodontal diseases currently in use are firmly based on, and dominated by, the *Infection/Host Response paradigm*, some features of the older paradigms are still valid and have been retained (Armitage 2002).

According to the *Proceedings of the 1989 World Workshop in Clinical Periodontics*, different forms of periodontitis are classified as follows:

1. Adult periodontitis
2. Early-onset periodontitis
   (a) Prepubertal periodontitis (generalized, localized)
   (b) Juvenile periodontitis (generalized, localized)
   (c) Rapidly progressive periodontitis
3. Periodontitis associated with systemic diseases
4. Necrotizing ulcerative periodontitis
5. Refractory periodontitis

This classification, although soundly based in the Infection/Host Response paradigm, depended heavily on the age of the affected patients and the rates of progression. Other

Table 1.3 Proposed classification schemes for periodontitis

Source	Classification strata
American Academy of Periodontology (1989)	Adult periodontitis
	Early-onset periodontitis
	Periodontitis associated with systemic diseases
	Necrotizing ulcerative periodontitis
	Refractory periodontitis
European Workshop on Periodontics (Attström and der Velden 1994)	Early-onset periodontitis
	Adult periodontitis
	Necrotizing periodontitis
Lindhe and Nyman (1997)	Periodontitis levis
	Periodontitis gravis
	Supplementary diagnosis … et complicata
International Workshop for a Classification of Periodontal Diseases and Conditions (Armitage 1999)	Gingivitis
	Chronic periodontitis
	Aggresive periodontitis
	Periodontitis as a manifestation of systemic diseases
	Necrotizing periodontal diseases
	Abscesses of the periodontium
	Periodontitis associated with endodontic lesions
	Developmental or acquired deformities and conditions

important features included the acknowledgment that some forms of periodontitis could be significantly modified by host factors (i.e., the category of "periodontitis associated with systemic disease") and still other forms did not appear to respond well to conventional therapy (i.e., the "refractory periodontitis" category) (Armitage 2002).

Disadvantages of this classifications (American Academy of Periodontology 1989) include the extensive overlap between the different diagnostic categories, need for assumptions concerning previous disease progression, the necessity of detailed information on the quality of treatment provided previously, the patient's response to this therapy and the apparent lack of a consistent basis for classification (Attström and van der Velden 1994).

Subsequently, a simpler classification was agreed upon at the *First European Workshop in Periodontology in 1993:*

1. Early-onset periodontitis
2. Adult periodontitis
3. Necrotizing periodontitis

Lindhe and Nyman (1997) proposed that the information regarding the condition of the various periodontal structures (the gingiva, the periodontal ligament, the root cementum and the alveolar bone), which has been obtained through the examination procedures, should form the basis for the diagnosis of periodontal disease. They gave to each tooth in the dentition an individual diagnosis:

Periodontitis levis (overt periodontitis) is characterized by an even "horizontal" loss of supporting tissues not exceeding one third of the length of the root. Inflammation must be present, i.e., "bleeding on probing" will occur when the site is probed to the "bottom of the pocket" (Lindhe and Nyman 1997).

Periodontitis gravis (advanced periodontitis) presents an even ("horizontal") loss of supporting tissues exceeding one third of the length of the root, associated with "bleeding on probing" to the "bottom of the pocket"(Lindhe and Nyman 1997) (Fig. 1.2).

Supplementary diagnosis ... et complicata: The supplementary diagnosis *periodontitis complicata* is used when an angular bony defect (infrabony pocket, interdental osseous crater) is present adjacent to a tooth, and for a multi-rooted tooth in which furcation involvements of degree 2 or 3 have been identified (Lindhe and Nyman 1997).

Problems, inconsistencies, and deficiencies associated with the 1989 classification led many clinicians and investigators to call for a revision of the currently used system. This resulted in a 1999 international workshop on the classification of periodontal diseases (Armitage 1999, 2002). One of the goals of this workshop was to correct the problems associated with the 1989 system (Armitage 2002).

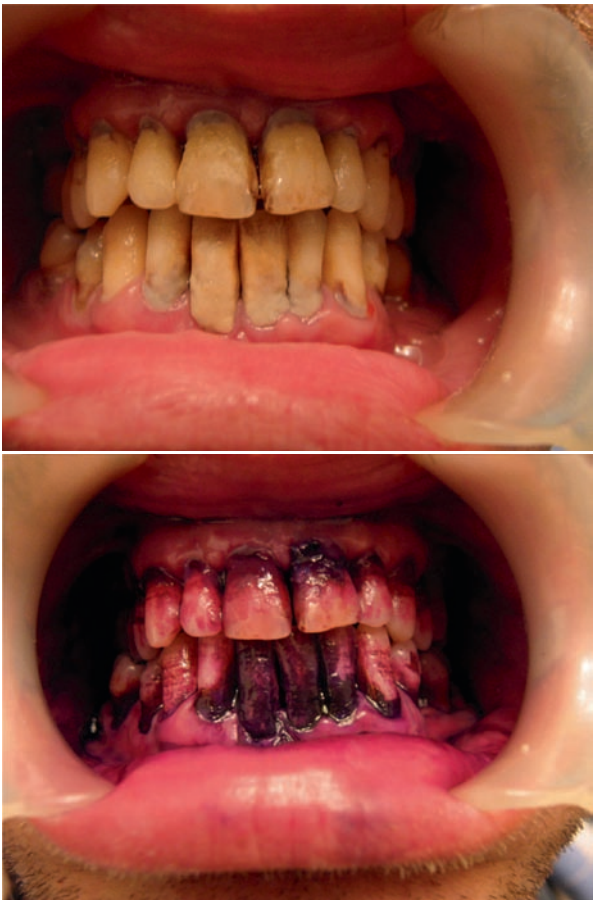

Fig. 1.2 Clinical status of a patient suffering from advanced periodontal disease

There were six major problems with the former 1989 classification that needed to be addressed (Armitage 2002):

- It did not include a gingivitis or gingival disease category.
- The periodontitis categories had nonvalidated age-dependent criteria.
- There was extensive crossover in rates of progression of the different categories of periodontitis.
- "Rapidly progressive periodontitis" was a heterogeneous category.
- There was extensive overlap in the clinical characteristics of the different categories of periodontitis.
- "Refractory periodontitis" was a heterogeneous category.
- "Prepubertal periodontitis" was a heterogeneous category.

What emerged was a classification that was even more firmly based on the *Infection/Host Response paradigm*, but without some of the inherent problems of the 1989 classification. In reality, the changes could be characterized as a "course correction" or "fine-tuning" of the 1989 classification, since no massive alterations were made. A badly needed gingivitis or gingival disease category was added. In addition, the heterogeneous disease categories of prepubertal, refractory, and rapidly progressive periodontitis were eliminated as distinct or stand-alone entities. The "refractory" designation remains in the new classification, but not as a single entity. Conceptually, all forms of periodontitis can be unresponsive to treatment. Furthermore, the troublesome criteria of age and rate of progression were removed as a basis for classifying different forms of periodontitis. The reasons for these changes were not arbitrary, but were based on the available data and the current understanding of the nature of periodontal infections (Armitage 2002).

Eight groups of periodontal diseases and conditions can be distinguished (Armitage 1999):

1. Gingivitis
2. Chronic periodontitis
3. Aggresive periodontitis
4. Periodontitis as a manifestation of systemic diseases
5. Necrotizing periodontal diseases
6. Abscesses of the periodontium
7. Periodontitis associated with endodontic lesions
8. Developmental or acquired deformities and conditions

1.2.1 Chronic Periodontitis

Generally the term "chronic periodontitis" replaces the term "adult periodontitis" (Consensus Report 1999). Chronic periodontitis is defined as an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment, and bone loss. It is characterized by pocket formation and/or gingival recession. It is recognized as the most frequently occurring form of periodontitis. Its onset may be at any age, but is

most common in adults. The prevalence and severity of the disease increase with age. It may affect a variable number of teeth and it has variable rates of progression.

The main characteristics of chronic periodontitis (Classification of Periodontal Diseases 1999) are:

1. Most common in adults, but can occur in children. The prevalence and severity of the disease increase with age.
2. Slow to moderate rate of progression.
3. Amounts of microbial deposits consistent with the severity of periodontal tissue destruction.
4. Subgingival calculus is a frequent finding.
5. Amount of destruction is consistent with the presence of local factors (e.g., tooth-related or iatrogenic).
6. May be modified by and/or associated with systemic disease (e.g., diabetes mellitus).
7. Can be modified by factors other than systemic diseases (e.g., smoking, emotional stress).
8. Variable distribution of periodontal destruction; no discernible pattern.
9. No marked familial aggregation.

Chronic periodontitis is initiated and sustained by bacterial plaque, but host defense mechanisms play an integral role in its pathogenesis. The progressive nature of the disease can only be confirmed by repeated examinations (Consensus Report 1999).

Chronic periodontitis can be further characterized by its extent and severity. *Extent* is the number of sites involved and can be described as localized and generalized. As a general guide, extent can be characterized as localized if <30% of the sites are affected and generalized if >30% of the sites are affected. *Severity* can be described for the entire dentition or for individual teeth and sites. As a general guide, severity can be categorized on the basis of the amount of clinical attachment loss (CAL) as follows: slight = 1–2 mm CAL, moderate = 3–4 mm CAL, and severe ≥ 5 mm CAL (Consensus Report 1999).

1.2.2 Aggressive Periodontitis

In general the new designation of "localised aggressive periodontitis" replaces the older term "localized juvenile periodontitis." Similarly, the new designation of "generalized aggressive periodotitis" replaces the older term "generalized juvenile periodontitis." The common features of localized and generalized forms of aggressive periodontitis are (Consensus Report 1999):

1. Except for the presence of periodontitis, patients are healthy;
2. Rapid attachment loss and bone destruction;
3. Familial aggregation.

Secondary features that are generally, but not universally present, are:

1. Amounts of microbial deposits inconsistent with the severity of periodontal tissue destruction;

2. Elevated proportions o*f A. actinomycetemcomitans*, and in some populations, *Porphyromonas gingivalis* may be elevated;
3. Phagocyte abnormalities;
4. Hyperresponsive macrophage phenotype, including elevated levels of PGE_2 and IL-1β;
5. Progression of attachment loss and bone loss may be self-arresting.

In addition to the features common to all forms of aggressive periodontitis, the following specific features are identified:
Localized aggressive periodontitis

1. Circumpubertal onset;
2. Robust serum antibody response to infecting agents;
3. Localized first molar/incisor presentation with interperoximal attachment loss on at least two permanent teeth, one of which is a first molar, and involving not more that two teeth other than first molars and incisors.

Generalized aggressive periodontitis

1. Usually affecting persons under 30 years, but patients may be older;
2. Poor serum antibody response to infecting agents;
3. Pronounced episodic nature of the destruction of attachment and alveolar bone;
4. Generalized interproximal attachment loss affecting at least three permanent teeth other than first molars and incisors.

1.2.3 Systemic Disease Forms of Periodontitis

The International Workshop agreed that certain systemic conditions (such as smoking, diabetes) can modify periodontitis (chronic or aggressive) and that certain systemic conditions can cause destruction of the periodontium (which may or may not be histopathologically periodontitis), for example neutropenias or leukaemias (Kinane and Lindhe 2005).

1.2.4 Necrotizing Forms of Periodontitis

It was accepted by the International Workshop that "necrotizing ulcerative gingivitis" (NUG) and "necrotizing ulcerative periodontitis" (NUP) be collectively referred to as "necrotizing periodontal diseases." It was agreed that NUG and NUP were likely to be different stages of the same infection and may not be separate disease categories. Both these diseases are associated with diminished systemic resistance to bacterial infection of periodontal tissues. A crucial difference between NUG and NUP is whether the disease is limited to the gingival or also involves the attachment apparatus (Kinane and Lindhe 2005).

1.2.5 Developmental or Acquired Deformities and Conditions

The presence of mucogingival deformities often has an impact on patients in terms of aesthetics and function. It is recommended that mucogingival deformities be divided according to clinical and morphological criteria. In this way a more homogenous group of clinical conditions can be defined before an etiological criterion is applied. The first proposed criterion is clinical and is based on the presence or absence of teeth or implants (Pini Prato 1999). Mucogingival deformities can be divided into three main categories:

1. Soft tissue deformities associated with teeth;
2. Soft tissue deformities associated with implants;
3. Soft tissue deformities associated with edentulous ridges.

The second criterion is morphological, describing the deformity. The third criterion is the severity of the deformity and implies a quantitative (measurement of the deformity) or semiquantitative assessment (assigning a deformity to one or several categories, scores, to classifying its severity) (Pini Prato 1999).

The classification of developmental or acquired deformities and conditions affecting the periodontium (Classification of Periodontal Diseases 1999) is:

1. Localized tooth-related factors that modify or predispose to plaque-induced gingival diseases/periodontitis
   (a) Tooth anatomic factors
   (b) Dental restorations/appliances
   (c) Root fractures
   (d) Cervical root resorption and cemental tears
2. Mucogingival deformities and conditions around teeth
   (a) Gingival/soft tissue recession
      – Facial or lingual surfaces
      – Interproximal (papillary)
   (b) Lack of keratinized gingiva
   (c) Decreased vestibular depth
   (d) Aberrant frenum/muscle position
   (e) Gingival excess
      – Pseudopocket
      – Inconsistent gingival margin
      – Excessive gingival display
      – Gingival enlargement
   (f) Abnormal color
3. Mucogingival deformities and conditions on edentulous ridges
   (a) Vertical and/or horizontal ridge deficiency
   (b) Lack of gingiva/keratinized tissue
   (c) Gingival/soft tissue enlargement
   (d) Aberrant frenum/muscle position
   (e) Decreased vestibular depth
   (f) Abnormal color

4. Occlusal trauma
   (a) Primary occlusal trauma
   (b) Secondary occlusal trauma

1.3 Essentialistic or Nominalistic Disease Classification

The essentialistic idea implies the real existence of a disease caused by a class of agents. However, to date, all indications have been that the causal web for periodontitis is so complex and involves so many factors in so many different constellations that a classification of periodontitis based on etiology is effectively precluded. Since periodontitis has to be regarded as a syndrome, present and future classifications of periodontitis have to be based on the nominalistic concept. Classifications based on this concept should be simple to apply and not susceptible to multiple interpretations. Ideally, such a classification should be determined on the basis of documented differences regarding the consequences of the diagnosis (Van der Velden 2005).

At present, the best option is to classify the periodontitis syndrome in an exhaustive but also exclusive way and use a terminology for the various classes of the disease, which makes it easy to understand the case. A classification that comes closest to these principles was recently published by Van der Velden (2000). This classification was based on four dimensions, i.e., extent, severity, age, and clinical characteristics (Van der Velden 2005).

The following is a presentation of the original classification with a few additions (Van der Velden 2005):

1. Defining when periodontitis is considered to be present. It is suggested to define periodontitis as the presence of inflamed pathological pockets >4 mm deep in conjunction with attachment loss. If present, then the next steps can be taken.
2. Classification based on the extent of the disease, i.e., the number of affected teeth is presented in Table 1.4.
3. Classification based on severity of disease per tooth.

Table 1.4 Classification based on the extent of the disease

	Permanent/mixed dentition (no. of teeth present)		Primary dentition
	$n > 14$	$n < 14$	
Incidental	1 tooth	1 tooth	1 tooth
Localized	2–7 teeth	2–7 teeth	2–4 teeth
Semi-generalized	8–13 teeth	–	5–9 teeth
Generalized	>14 teeth	8–14 teeth	>10 teeth

If teeth are missing, the class description should still reflect the clinical image of the patient. Therefore, it was decided for cases with >14 teeth to omit the class semi-generalized and to change the number of teeth for the generalized class to 8–14 (Van der Velden 2005)

The mean estimated root length based on the literature is approximately 12 mm; in the case of incidental disease, the severity category at that particular tooth is mentioned (Van der Velden 2005). The fact that either attachment loss or bone loss can be used for the classification of severity implies that although it may be important to know the actual root length in a given patient, radiographs are not a prerequisite for the classification of severity.

Classification based on the severity of the disease per tooth:

- Minor: Bone loss <1/3 of the root length or attachment loss <3 mm
- Moderate: Bone loss >1/3 and <1/2 of the root length or attachment loss 4–5 mm
- Severe: Bone loss >1/2 of the root length or attachment loss >6 mm

4. Classification based on age

If in patients classified as adult periodontitis it can be demonstrated on the basis of documentation that they already had moderate or severe periodontitis before the age of 36 years, the disease is classified as early-onset periodontitis (Van der Velden 2005)

(a) Early-onset periodontitis:
   - Prepubertal periodontitis age of onset: <12 years
   - Juvenile periodontitis age of onset: 13–20 years
   - Postadolescent periodontitis age of onset: 21–35 years

(b) Adult periodontitis age of onset: >36 years

5. Classification based on clinical characteristics

Periodontitis associated with systemic diseases, i.e., periodontitis in subjects suffering from general diseases, or periodontitis in subjects using medication, which enhance the rate and severity of periodontal breakdown is not identified as a specific class of periodontitis. However, the association with such a condition should be added to the diagnosis (Van der Velden 2005)

- Necrotizing periodontitis: Interdental gingival necrosis, bleeding, and pain
- Rapidly progressive periodontitis: Documented rapid breakdown (at any age), i.e., rapidly progressive periodontitis patients showing a progression of >1 mm interproximal attachment/bone loss per year at affected sites
- Refractory periodontitis: Documented, no or minimal pocket depth reduction at single rooted teeth after proper initial therapy, and/or further attachment loss despite the proper execution of various treatment modalities

The classification is ascertained in the following way:

- First, the severity category is determined for each tooth.
- Next, the extent category is determined by counting the number of teeth with the most severe condition.

- Diagnosis on the basis of clinical characteristics is added if applicable.
- Diagnosis on the basis of age (Van der Velden 2005).

In the nomenclature, the parameters for the classification are set in the following order: extent, severity, clinical characteristics, and age. Thus, examples for diagnoses are localized minor prepubertal periodontitis, localized severe juvenile periodontitis, semi-generalized minor juvenile periodontitis, generalized severe refractory postadolescent periodontitis, and localized severe adult periodontitis. One could make the diagnosis even more detailed by including two levels of extent and severity when appropriate, e.g., localized severe, semi-generalized moderate adult periodontitis (Van der Velden 2005).

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Evidence for the Role of Genetic Variants in Periodontitis

2

There are estimated to be 25,000–50,000 different kinds of genes in the human genome. Genes can exist in different forms or states. Geneticists refer to the different forms of a gene as *allelic* variants or *alleles*. Allelic variants of a gene differ in their nucleotide sequences. When a specific allele occurs in at least 1% of the population, it is said to be a genetic *polymorphism.* Two or more alleles for a given locus may exist in nature throughout evolution, but may develop at any time. A polymorphic locus is one whose alleles are such that the most common, normal variant (*N-allele*) among them occurs with 99% frequency in the population. Thus, if a locus is, for example, bi-allelic, the rarer allele (designated *R-allele*) must occur with a frequency of 41% in the population. In this way, when different alleles of a given gene coexist in the human population, we speak about genetic polymorphisms (Loos et al. 2005).

Polymorphism arises as a result of mutation. The different types of polymorphisms are typically referred to by the type of mutation that created them. The simplest type of polymorphism results from a *single base* mutation which substitutes one nucleotide for another. The polymorphism at the site harboring such changes has recently been termed a "*single nucleotide polymorphism* (SNP)," although previously, in some instances, such variation was referred to by the particular methods used to detect it. Digestion of a piece of DNA containing the relevant site with an appropriate restriction enzyme could then distinguish alleles or variants based on the resulting fragment sizes via electrophoresis, and this type of polymorphism was thus referred to as "*restriction fragment length polymorphism* (RFLP)" (Loos et al. 2005; Schork et al. 2000).

The SNP may have no effects or may have some important biological effects. For example, if a transition has taken place within the coding region of a gene, it may result in an amino acid substitution and therefore an altered protein structure, which may then alter its function. When such mutations have taken place in the promoter region of the gene, it may alter the gene regulation, for example resulting in (completely) inhibited or reduced gene expression or, alternatively, resulting in over-expression of the gene, perhaps with biological consequences. SNPs occur more frequently than any other type of genetic polymorphism; the frequency of SNPs across the human genome is estimated at every 0.3–1 kb (Loos et al. 2005; Schork et al. 2000).

Other types of genetic polymorphisms result from the insertion or deletion of a section of DNA. The most common type of such "*insertion:deletion*" polymorphism is the existence of variable numbers of repeated base or nucleotide patterns in a genetic region.

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Repeated base patterns range in size from several hundreds of base pairs, known as "*variable number of tandem repeats*" (*VNTRs or "minisatellites"*), to the more common "microsatellites" consisting of two, three, or four nucleotides repeated some variable number of times. Microsatellites are often referred to as "*simple tandem repeats*" (STRs). Repeat polymorphisms often result in many alleles or variants (e.g., several different repeat sizes) within the population and are thus considered "*highly polymorphic*". This can be extremely useful for population genetic studies since the probability that two individuals from different populations (ethnic groups, diseased vs. number of repeats) can be quite low. The genome-wide frequency estimates for STRs are difficult to come by, though a range of figures of one STR every 3–10 kb seems reasonable (Loos et al. 2005; Schork et al. 2000).

Another type of insertion:deletion polymorphism involves the presence or absence of Alu segments at a genetic location. Alu segments are named according to the restriction enzyme that is used to detect them (e.g., AluI), and contain two sequences of approximately 120–150 bases in length, separated by an A base-rich segment. Insertions of this type occur approximately every 3 kb on average. Large insertion:deletion polymorphism such as Alu insertions is easy to identify and genotype given the large differences in resulting amplified fragments (Loos et al. 2005; Schork et al. 2000).

Kinane and Hart (2003) presented the classic relationship among phenotype, environment, and genotype as follows:

Phenotype = Environmental risk factors (smoking status, plaque control, socio-economic status, diabetes, etc.)
+ genotype (includes gene–gene interactions)
+ genotype × environment (that is the interaction between environment and genotype).

Genetic polymorphisms are very useful in the genetic studies of the population. After genotyping individuals and assessing genotype frequencies among groups of interest, one can also calculate the frequency of the N-allele and the R-allele among the groups or populations under study. Frequencies of genotypes and alleles may differ between a diseased group and a healthy group. Subsequently, when a given allele is identified to be associated with a disease, functional studies can be started to investigate the possible role of that gene in the etiology and pathogenesis of the disease (Loos et al. 2005).

Evidence for a genetic predisposition to periodontitis comes from four areas of research: (1) the study of inherited diseases and genetic syndromes, (2) family studies, (3) twin studies, and (4) population studies.

2.1 Study of Inherited Diseases and Genetic Syndromes

Evidence for the role of specific genes in a disease may be gleaned from the study of inherited conditions or genetic disorders, in which the disease is pathognomonic. A number of monogenic syndromes with accompanying severe periodontal disease have been reported

in the literature (acatalasia, hypophosphatasia, Chédiak-Highashi syndrome, chronic neutropenia, leukocyte adhesion deficiency, cyclic neutropenia, Ehlers-Danlos syndrome, Papillon-Lefèvre syndrome) (Hodge and Michalowicz 2001).

A commonality of these conditions is that they are inherited as simple Mendelian traits and are usually due to genetic alterations of a single gene locus. The significance of these conditions is that they clearly demonstrate that a genetic mutation at a single locus can impart susceptibility to periodontitis. Additionally, these conditions illustrate that this genetic susceptibility may segregate by different transmission patterns. The fact that the altered proteins function in different structural and immune pathways indicates that genetic modulation of a variety of different genes can affect a variety of different physiological and cellular pathways, imparting susceptibility to pathological consequences in the periodontium in individuals with appropriate microbial challenges. These conditions illustrate that genetic contributions to periodontitis susceptibility are multifaceted and may potentially involve many different gene loci. However, in contrast to non-syndromic forms of periodontitis, these conditions have periodontal disease manifestations as part of a collection of syndromic manifestations. In most cases of aggressive periodontitis, individuals present with clinical manifestations of periodontitis, but do not appear to have any other clinical disease manifestations (Kinane and Hart 2003).

2.2 Family Studies

There is literature reporting familial aggregation of periodontal diseases, but, due to different terminology, classification systems, and lack of standardized methods of clinical examination, it is difficult to compare reports directly. Although periodontal disease nosology has changed many times over the timeframe of these reports, the most familial reports for periodontitis are of the early-onset forms now called aggressive periodontitis (Stabholz et al. 1998). This aggregation within families strongly suggests a genetic predisposition. It must be borne in mind that familial patterns may reflect exposure to common environmental factors within these families. Thus, it is important to consider the shared environmental and behavioral risk factors in any family. These would include education, socio-economic grouping, oral hygiene, possible transmission of bacteria, diseases such as diabetes, and environmental features such as passive smoking, sanitation, etc. Some of these factors, such as lifestyle and behavior and education, may be under genetic control and may influence the standard of oral hygiene. The complex interactions between genes and the environment must also be considered in the evaluation of familial risk for the periodontal diseases (Kinane and Hart 2003).

In chronic periodontitis, the phenotype or disease characteristics do not present significantly until the third decade of life, whereas in the aggressive forms of periodontal disease, the presentation can occur in the first, second, third, and fourth decades. This variability in presentation of significant signs of the disease makes diagnosis difficult, not only in declaring if a patient suffers from the disease but also in detecting patients who do not suffer from the disease, and differentiating between the adult and aggressive forms of periodontitis (Kinane and Hart 2003).

2.3 Twin Studies

Studying phenotypic characteristics of twins is a method of differentiating variations due to environmental and genetic factors. Monozygous twins arise from a single fertilized ovum and are therefore genetically identical and always the same sex. Dizygous twins arise from the fertilization of two separate ova and share, on average, one half of their descendent genes in the same way as siblings do. Any discordance in disease between monozygous twins must be due to environmental factors. Any discordance between dizygous twins could arise from environmental and/or genetic variance. Therefore, the difference in discordance between monozygous and dizygous twins is a measure of the effects of the excess shared genes in monozygous twins, when the environmental influence is constant (Hodge and Michalowicz 2001).

Based on 110 pairs of adult twins, a significant genetic component was identified, suggesting that 38–82% of the population variance for probing depth (PD), attachment loss (AL) and dental plaque may be attributed to genetic factors (Michalowicz et al. 1991). A study by Corey et al. (1993) of self-reported periodontal health among 4,908 pairs of twins found a history of reported periodontal disease in 420 individuals who were members of 116 monozygotic (MZ) and 233 dizygotic (DZ) twin pairs. The mean age at diagnosis in this sample was 31.4 ± 0.7 years and was significantly earlier in females than males (30.1 vs. 33.0 years, $P < 0.025$). Proband-wise concordance rates were 0.38 for MZ and 0.16 for DZ twins. In a subsequent study on 117 pairs of adult twins (64 MZ and 53 DZ pairs) revealed that approximately half of the variance in disease in the population is attributed to genetic variance. PD, AL, plaque, and gingivitis (GI) were assessed on all teeth by two examiners. Measurements were averaged over all sites, teeth, and examiners. The extent of disease in subjects was defined at four thresholds: the percentage of teeth with AL ≥ 2, AL ≥ 3, PD ≥ 4, or PD ≥ 5 mm. Genetic and environmental variances and heritability were estimated using path models with maximum likelihood estimation techniques. For all clinical measures, MZ twins were more similar than DZ twins. Statistically significant genetic variance was found for both the severity and extent of disease. Adult periodontitis was estimated to have approximately 50% heritability, which was unaltered following adjustments for behavioral variables including smoking. In contrast, while MZ twins were also more similar than DZ twins for GI scores, there was no evidence of heritability for GI after behavioral covariates such as utilization of dental care, and smoking were incorporated into the analyses (Michalowicz et al. 2000).

2.4 Population Studies

Environmental or behavioral risk factors for a disease are often first detected in large epidemiological or population-based studies. In genetic epidemiology, similar approaches can be used to identify genetic risk factors for the disease. The frequencies of polymorphisms

of candidate genes, whose protein products play a role in the inflammatory or immune response, can be compared between cases and controls. A genetic polymorphism is the long-term occurrence in a population of two or more genotypes that could not be maintained by recurrent mutation. A significant difference in the frequency of a specific polymorphism, between a diseased group and a control group, is an evidence that the candidate gene plays some role in determining susceptibility to the disease. An association indicates that either the candidate gene directly affects disease susceptibility or that it is in linkage disequilibrium with (very close to) the disease locus. This method can help to elucidate the pathogenesis of a disease process, identify causal heterogeneity, and ultimately identify individuals most at risk for the disease. In population studies, it is important to clearly define the disease status. Likewise, because of the possibility of racial heterogeneity, it is important to insure that the patient and control groups are racially matched (Hodge and Michalowicz 2001).

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A Gene Mutation of Major Effect on Human Disease and Its Association with Periodontitis

3

Diseases that follow predictable and generally simple patterns of transmission have been called *Mendelian conditions*. The name reflects the fact that these diseases occur in simple patterns in families, and in most cases a single gene locus is the major determinant of the clinical disease phenotype. These diseases follow a classic Mendelian mode of inheritance (autosomal dominant, autosomal recessive, or X-linked). Usually, the prevalence of these Mendelian conditions is rare (typically much less than 0.1%), with the exception of some unique populations that have been isolated from other human populations. When the genetic basis of a Mendelian condition is identified, it is often found that the condition results from the effect of a genetic mutation at a single gene locus. The disease phenotype usually occurs over a broad range of environments, and although environmental factors and other genes can modify them, in many cases they manifest in a remarkably similar way (Kinane and Hart 2003).

Table 3.1 lists a series of genetic syndromes known to be associated with either premature tooth loss due to periodontitis or a phenotype resembling aggressive periodontitis. The significance of these conditions is that they clearly demonstrate that a genetic mutation at a single locus can impart susceptibility to periodontitis. Clinical phenotype of these syndromes suggest that the relationship between periodontitis and the genetic mutation leads to the deficiency of immune system or increased infection, or inflammation. Additionally, these conditions illustrate that this genetic susceptibility may segregate by different transmission patterns. The fact that the altered proteins function in different structural and immune pathways indicates that genetic modulation of a variety of genes can affect a variety of physiological and cellular pathways, imparting susceptibility to pathological consequences in the periodontium in individuals with appropriate microbial challenges. These conditions illustrate that genetic contributions to periodontitis susceptibility are multifaceted and may potentially involve many different gene loci. However, in contrast to nonsyndromic forms of periodontitis, these conditions have periodontal disease manifestations as part of a collection of syndromic manifestations. In most cases of aggressive periodontitis, individuals present with clinical manifestations of periodontitis, but do not appear to have any other clinical disease manifestations. This is not inconsistent with a genetic disease etiology. Expression of genes can vary in different tissues, and mutations of a ubiquitously expressed gene can result in a tissue-specific condition (Kinane and Hart 2003).

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Table 3.1 Examples of syndromic forms of periodontitis in which inheritance is Mendelian and due to a genetic alteration at a single gene locus modified from Kinane and Hart (2003)

Disease	Genetic defect	Phenotype	Inheritance
Papillon-Lefèvre syndrome	Cathepsin C	Prepubertal periodontitis	Autosomal recessive
Haim-Munk syndrome	Cathepsin C	Prepubertal periodontitis	Autosomal recessive
Ehlers-Danlos syndrome	Collagen	Early-onset periodontitis/ localized juvenile periodontitis	Autosomal dominant
Cyclic neutropenia	Neutrophil elastase	Early-onset periodontitis	Autosomal dominant
Chronic familial neutropenia	Defect unknown	Early-onset periodontitis	Autosomal dominant
Chediak-Higashi syndrome	Lysosomal trafficking regulator gene	Severe periodontitis	Autosomal recessive
Leukocyte adhesion deficiency type 1	Leukocyte chain adhesion molecule CD18	Prepubertal periodontitis	Autosomal recessive
Leukocyte adhesion deficiency type II (LAD II)/congenital disorder of glycosylation type IIc	GDP-fucose transporter-1		Autosomal recessive

3.1 Papillon-Lefèvre Syndrome

Papillon-Lefèvre syndrome is a rare autosomal recessive congenital differentiation disorder of chromosome 11p14-q21 and occurs in children from consanguineous marriages. The gene responsible for this syndrome is cathepsin C, lysosomal protease (Toomes et al. 1999). Cathepsin C is suggested to be implicated in a wide variety of immune and inflammatory processes (Toomes et al. 1999). The prevalence in the general population is 1–4 per million, males and females being equally affected with no racial predominance (Hattab et al. 1995).

The two essential features of Papillon-Lefèvre syndrome are hyperkeratosis of the palms and soles (either diffuse or localized) and generalized rapid destruction of the periodontal attachment apparatus resulting in premature loss of both primary and permanent teeth (Deas et al. 2003). The external signs are hyperkeratosis of the palms and soles (Kressin et al. 1995). Changes in the skin observed by electron microscopy revealed the diminution of the tonofibrils, alterations of the keratohialin granules, and acanthosis in the stratum spinosum (Kressin et al. 1995).

Intraorally, periodontal symptoms affect primary and permanent dentitions, with an extensive loss of periodontal attachment accompanied by generalized, severe, and rapid destruction of the alveolar bone, that frequently lead to premature tooth loss (Ahuja et al. 2005; Cagli et al. 2005; Canger et al. 2008; de Freitas et al. 2007; Hattab et al. 1995; Ikeshima 2006; Kothiwale and Mathur 2008; Kressin et al. 1995; Nagaveni et al. 2008). Histologically, the gingiva demonstrates epithelial hyperplasia, increased collagen synthesis, parakeratosis, acanthosis, and focal aggregates of lymphocytes and plasma cells. In addition, reduced osteoblastic activity and reduced thickness of cementum have been described (Ghaffer et al. 1999; Hattab et al. 1995).

Virulent gram-negative anaerobic microbiota has been considered to be an important initiator of the destructive periodontitis observed in these patients. *Aggregatibacter actinomycetemcomitans* has been reported to be the major periodontal pathogen, while *Capnocytophaga gingivalis*, *Eikenella corrodens*, black-pigmented *Bacteroides*, and *Fusobacterium* spp have also been recovered in high numbers in subgingival periodontal lesions in Papillon-Lefèvre syndrome patient (Ishikawa et al. 1994; Lundgren et al. 1998; Rudiger and Berglundh 1999; Velazco et al. 1999).

Papillon-Lefèvre syndrome has been associated with decreased neutrophil chemotaxis, reduced random neutrophil migration, impaired neutrophil phagocytosis, reduced myeloperoxidase activity, and increased superoxide radial neutrophil production, associated with a decreased lymphocyte response to pathogens (Lundgren et al. 1998; Velazco et al. 1999).

The diagnosis of Papillon-Lefèvre syndrome is made by history and clinical findings (Jordan 2004).

3.2 Haim-Munk Syndrome

The Haim-Munk syndrome or keratosis palmoplantaris with periodontopathia and onychogryposis was first described in 1965. In addition to congenital palmoplantar keratosis and progressive early-onset periodontal destruction, other clinical findings shared by these subjects included recurrent pyogenic skin infections, acroosteolysis, atrophic changes of the nails, arachnodactyly, and a peculiar radiographic deformity of the fingers consisting of tapered, pointed phalangeal ends, and a claw-like volar curve. Subsequently, pes planus was reported to be associated with the syndrome. Although the palmoplantar findings and severe periodontitis were suggestive of Papillon-Lefèvre syndrome, the association of other clinical features, particularly nail deformities and arachnodactyly, argued that Haim-Munk syndrome was a distinct disorder (Hart et al. 1997, 2000a, b).

In contrast to Papillon-Lefèvre syndrome, the skin manifestations in Haim-Munk syndrome were reported to be more severe and extensive. In addition to a marked palmoplantar keratosis, affected subjects had scaly, erythematous, and circumscribed patches on the elbows, knees, forearms, shins, and dorsum of the hands. While the periodontium in Haim-Munk syndrome was reported to be less severely affected than in Papillon-Lefèvre syndrome, gingival inflammation and alveolar bone destruction are present and severe (Deas et al. 2003; Hart et al. 1997, 2000a, b; Janjua et al. 2008). However, Hart et al. reported that

the mutated gene of Haim-Muck syndrome is cathepsin C, but this mutation, which changes a highly conserved amino acid in the cathepsin C peptide, is different from that in Papillon-Lefevre syndrome (Hart et al. 2000a, b). This difference may lead to the clinical severity in Haim-Muck syndrome.

3.3 Ehlers-Danlos Syndrome

The term Ehlers-Danlos syndrome, also known as dystrophia mesodermalis and fibrodysplasia elastica generalisatica, covers a heterogenous group of inherited disorders of connective tissue, which may affect the skin, ligaments, joints, eyes, and vascular system (Reichert et al. 1999). EDS is divided into 11 types in accordance with clinical, genetic, and biochemical features (Majorana and Facchetti 1992). The primary cause may be a type I or type II collagen deficiency, a lysyl hydroxylase deficiency, deletion of *N*-telopeptide, or disorders of copper homeostasis and fibronectin defects (Reichert et al. 1999).

The immunological analysis revealed that the concentrations of IgA in saliva and IgA, IgG, and IgM in serum were within normal limits, with unpaired phagocytic capacity of the peripheral blood leukocytes (Reichert et al. 1999). The temporomandibular joint often demonstrates profound laxity in conjunction with generalized joint mobility and dislocation (Fridrich et al. 1990).

Periodontal conditions have been reported with EDS types I, VII, and VIII. Defective dentinogenesis, resulting in aplasia or hypoplasia of root development affecting the mandibular incisors, and predisposition for localized periodontal disease was reported in EDS type I. Radiographic appearance of a bulbous enlargement of the roots together with pulp stones at other teeth were reported. EDS type VII is an autosomal dominant/recessive disease. Poor healing after extractions, caries, a radiographic evidence of pulp stones, and/or malformed teeth have been described. EDS type VIII is an autosomal dominant form characterized by fragile skin, abnormal scarring, and early onset of periodontal disease, with premature loss of the permanent teeth. Fragility of the alveolar mucosa and increased bleeding tendencies have also been suggested (Badauy et al. 2007; Moore et al. 2006; Perez et al. 2002; Sollecito et al. 2005). EDS can also be associated with ligneous periodontitis (generalized membranous gingival enlargement due to an accumulation of fibrin deposits associated with severe alveolar bone loss), and plasminogen deficiency seems to play a central role in its pathogenesis (Pierro et al. 2006).

3.4 Cyclic Neutropenia

Cyclic neutropenia is a rare condition, characterized by cyclical depletion of polymorphonuclear leukocyte numbers, typically in 3-week cycles, although this can be between 2 and 5 weeks. The episode of neutropenia is usually short, but the patient polymorphonuclear

leukocyte count never returns to normal levels, and the differential blood-cell count for polymorphonuclear leukocytes is at least 40% less than normal levels (Kinane 1999).

Periodontal manifestations include inflamed gingival, gingival ulceration, periodontal attachment, and bone loss (Kinane 1999; Rezaei et al. 2004). Unfortunately, even with the best of professional and home care, teeth are often lost because of advancing periodontal disease (Deas et al. 2003).

3.5 Familial Neutropenia

Familial neutropenia is inherited as an autosomal dominant trait, and in these patients, neutrophils are not released properly from the marrow. A slight monocytosis occurs, possibly as compensation, together with the moderate neutropenia. The condition is often diagnosed in patients with a history of recurrent infections. Susceptibility to these infections tends to vary with neutrophil count. The periodontal manifestations include fiery red edematous gingivitis, which is often hyperplastic and accompanied by periodontal bone loss (Kinane 1999).

3.6 Chediak-Highachi Syndrome

Chediak-Higachi syndrome is a rare autosomal recessive disease associated with impaired function of cytoplasmic microtubules or microtubule assembly in PMNs (Oh et al. 2002). The susceptibility to infections, although humoral and cellular immunity are normal, leads to early death (often before 5 years of age) (Steenberghe 1997).

The disease reveals itself periodontally by severe gingivitis and rapid loss of attachment, leading to exfoliation of the teeth (Bailleul-Forestier et al. 2008; Steenberghe 1997).

3.7 Leukocyte Adhesion Deficiency

Two LADs have been described in humans: LAD I and LAD II. Both diseases block a sequence of leukocyte–endothelial-cell interactions, which is generally referred to as the *multistep adhesion cascade*. The cascade is initiated by the tethering and rolling of leukocytes on activated endothelial cells. This first step is mediated by the interaction of the endothelial adhesion molecules E- and P-selectin with their glycoprotein ligands on the leukocyte surface (Fig. 3.1). Subsequently, activation of leukocytes through the action of chemokines leads to the transformation of leukocyte integrins into a high-affinity state. The integrins are now able to arrest the leukocytes, which then migrate to sites where they can finally extravasate into inflamed tissue surrounding the activated blood vessel. Any

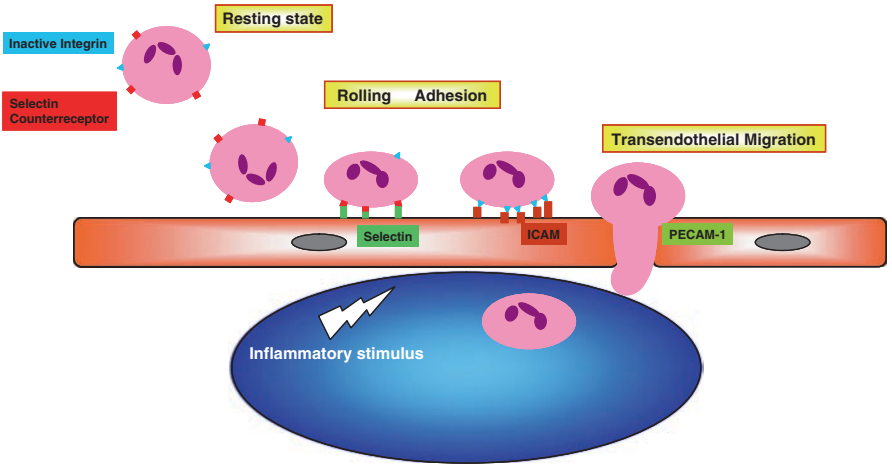

Fig. 3.1 Activated by an inflammatory stimulus, the endothelium expresses selectins, whose binding to their receptors on neutrophils initiates a rolling adhesion of neutrophils to the vessel's luminal wall. The neutrophils activate their integrins, which bind to endothelial ICAMs, permitting a firmer, stationary adhesion. Transepithelial migration may be guided by further adhesive interactions, perhaps involving molecules such as PECAM-1, which endothelial cells express at intercellular junctions (modified and adapted from Kakkar and Lefer 2004) (Reprinted with permission of Elsevier B.V.)

interference with these interactions should therefore reduce the emigration of leukocytes to sites of infection, thus compromising immune responses.

LAD is a rare but well-defined autosomal recessive disease that results in the formation of nonfunctional intracellular adhesion molecule (ICAM receptor). The disease results from mutations in the region on the CD18 gene encoded on chromosome 21q22.3, which codes for the β2 integrin subunit of the leukocyte adhesion molecule. The β2 integrin subunit combines with each of the α subunits (αL, αM, αX) to yield the antigens LFA-1 (αLβ2, CD11a/CD18), Mac-1 (αMβ2, CD11b/CD18), and p150,95 (αXβ2, CD11c/CD18), respectively. These three molecules are expressed exclusively on leukocytes and are therefore referred to as leukocyte integrins. The defective or absent expression of these molecules on the surface of leukocytes decreases their ability to adhere to endothelial cells and to migrate to sites of infection (Cox and Weathers 2008).

Clinical features usually present in infancy or early childhood and consist of recurrent, indolent bacterial infections of the skin, mouth, and the respiratory tract together with delayed separation of the umbilical cord. Skin infections may progress to large chronic ulcers that may become polymicrobial in character. These individuals are susceptible to fungal infections but do not show an increased susceptibility to protozoal or viral infections. In addition, lack of swelling, redness, heat, or pus is noted in the area of the infection (Cox and Weathers 2008).

Severe gingivitis with an early loss of primary teeth, followed by the early loss of secondary teeth, is seen. Individuals with LAD defect in innate host defense display a severe form of periodontitis that does not require specific periodontal pathogens because of entrapment of neutrophils within the blood vessel (Dixon et al. 2004).

A progressive severe periodontitis, alveolar bone loss, periodontal pocket formation, and partial or total premature bone loss of the primary and permanent dentitions were reported in a child with LAD (Cox and Weathers 2008; Dababneh et al. 2008; Majorana et al. 1999). Root surface exhibited cemental and dentinal erosions or "dentinoclastic activity" that may play an important secondary etiologic role in the rapid attachment loss (Waldrop et al. 1995).

3.8 Congenital Disorder of Glycosylation Type IIc CDG-IIc (Also Known as LAD II)

LAD II is much less common than LAD I, although it may show some similar clinical features. LAD II is characterized by a defect in the vascular endothelial receptor, which prevents the normal leukocyte from adhering to the endothelium. It is considered to be a congenital disorder of glycosylation (CDG) and fructose metabolism and a failure to express the ligand for E- and P-selectin, sialyl Lewis-X (CD15s) expressed on the leukocytes. In addition, immunoglobulin M and G (IgM and IgG) heavy chains are also not fucosylated, although they are present in normal amounts (Cox and Weathers 2008).

The summary of LAD II manifestations as presented by Wild et al. (2002):

- Almost absent expression of fucosylated glycoconjugates, including sLe^x, Le^a, Le^b, and H antigen
- Recurrent infections
- Periodontitis
- Neutrophilia
- Reduced neutrophil binding to E- and P-selectin
- Reduced neutrophil rolling
- Reduced neutrophil transmigration under shear
- Reduced DTH reactions
- Severe psychomotor and mental retardation
- Microcephaly and cortical atrophy
- Growth retardation
- Muscular hypotonia
- Facial and skeletal abnormalities

CDGs (formerly carbohydrate-deficient glycoprotein syndrome) are a heterogenous group of autosomal recessive disorders characterized by defects in the N-glycosylation pathway and subsequent aberrant formation of glycoproteins (Enns et al. 2002).

CDG-IIc is caused by mutations in the GDP-fucose (Fuc) transporter (encoded by *FUCT1*). This syndrome is characterized by nonfucosylated oligosaccharides and presents with severe mental retardation and immunodeficiency due to an adhesion defect of the leukocytes (probably due to absence of α-1,3-fucosylated sialyl-Lewis selectin ligands). Diagnosis is based on oligosaccharide and mutational analyses (Eklund and Freeze 2006).

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Modifying Disease Genes in Relation to Periodontitis

4

Most common diseases have a complex genetic etiology. In contrast to the relatively simple monogenetic diseases discussed previously, complex genetic diseases are not due to a single gene defect. In complex diseases, genetic variants at multiple gene loci contribute to overall disease liability. As such, a cause-and-effect relationship between a particular genetic allele and a disease is not possible. In these cases, a genetic allele is found to be statistically associated with disease more than what is found in unaffected individuals. This mathematical association is not necessarily biological or physiological. Studies reporting association vary in design and rigor from reports of an association in only a few individuals in a family (which are not statistically validated to be associated in a general population) to large population-based studies. Association studies ideally evaluate large numbers in population-based studies and thus have power to detect a significant association. Issues of allele frequency in the population studied, case-control design, and population stratification are very important but unfortunately are often omitted from dental studies (Kinane and Hart 2003).

Currently, very little is known about which genes may be involved in periodontitis as disease-modifying genes. Table 4.1 summarizes the candidate gene polymorphisms investigated in relation to periodontitis (Loos et al. 2005).

4.1 Interleukin-1 Gene Polymorphisms

Differences in the expression of cytokines, especially proinflammatory cytokines, are of great interest in periodontal research. Cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) have important roles in bony destruction and inflammatory stomatitis, and attempts have been made to elucidate the association of single nucleotide polymorphisms between patient populations (Nares 2003).

IL-1 family consists of at least three well-studied genes: IL-1α and IL-1β, which have agonist activity, and IL-1Ra, a physiologic antagonist to the other IL-1 cytokines (Table 4.2). The antagonist IL-1Ra exists in two forms. The intracellular form icIL-1Ra functions to modulate IL-1α activity. The extracellular, secreted form sIL-1Ra binds to IL-1 receptors (principally IL-1R1) without inducing signal transduction, thereby acting as an antagonist to IL-1β. Two IL-1 receptors are found on the surface of responsive cells, IL-1RI and IL-1RII.

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Table 4.1 Summary of encoded proteins, for which gene polymorphisms have been investigated as putative risk factors for gingivitis and periodontitis (modified and adapted from Loos et al. 2005. Reprinted with permission of John Wiley & Sons, Inc.)

Angiotensin-converting enzyme	Interleukin-6
Caspase recruitment domain-15 (NOD2)	Interleukin-10
Chemokine receptor-5	Interleukin 12
CD-14	Interleukin-18
Estrogen receptor-2	Lymphotoxin-α
Endothelin-1	Matrix metalloproteinase-1
Fibrinogen	Matrix metalloproteinase-3
Fcγ receptor IIa	Matrix metalloproteinase-9
Fcγ receptor IIb	Myeloperoxidase
Fcγ receptor IIIa	*N*-acetyltransferase-2
Fcγ receptor IIIb	Plasminogen-activator-inhibitor-1
N-formylpeptide receptor-1	RANKL/RANK/OPG
Interferon γ receptor-1	Receptor for advanced glycation end products
Interferon-γ	Transforming growth factor-β
Interleukin-1α	Tissue inhibitor of matrix metalloproteinase
Interleukin-1β	Tumor necrosis factor-α
Interleukin-1 receptor antagonist	Tumor necrosis factor receptor-2
Interleukin-2	Vitamin D receptor
Interleukin-4	

The IL-1 cytokines are made by a variety of cells and, in particular, stimulated monocytes, macrophages, and epithelial cells. These functionally similar molecules are encoded on separate genes in the same region of chromosome 2 (Fig. 4.1). Analysis of the biochemical processes governing IL-1 synthesis, secretion, and biological activity reveals many proven and potential opportunities for regulation. In contrast to IL-1β, IL-1α is generally not found in the circulation or tissue fluids but is retained by the cell in intracellular and membrane-bound forms and is active in both the pro-IL-1α and mature IL-1α forms. Regulation of IL-1 is a critical element of immune responses in health and disease; it is established that low levels of IL-1 are beneficial in host responses to infection, but that the elevated levels can be detrimental and that the margin between beneficial effects of these cytokines and pathologic effects is very small. This is evidenced by data relating circulating levels of IL-1 to disease severity in conditions associated with dysregulated inflammatory responses (Nares 2003).

4.1.1 Interleukin-1 Genotype and Experimental Gingivitis

Development of gingivitis is thought to be a prerequisite for subsequent development of periodontitis. Within 10–20 days of dental plaque accumulation, clinical signs of gingivitis are established in most individuals, although some individuals appear to be more resistant and others more prone to overt gingivitis. No evidence was provided that the IL-1 risk

Table 4.2 Interleukin (IL)-1 cytokine family: nomenclature and function (adapted and modified from Barksby et al. 2007)

Cytokine	Other names	Systematic name	Immunological function
IL-1α	IL-1 and leucocyte activating factor (LAF) (both collectively with IL-1β)	IL-1F1	IL-1R1 agonist with proinflammatory action but mainly acts as an intracellular transcriptional regulator
IL-1β	IL-1 and LAF (both collectively with IL-1α)	IL-1F2	Acts synergistically with TNF-α, activates proinflammatory responses in a wide range of cells, increases expression of adhesion molecules in endothelial cells, and promotes diapedesis and the acute phase response
IL-1Ra		IL-1F3	IL-1RI antagonist, prevents IL-1-dependent signaling
IL-18	IFN-γ inducing factor (IGIF), IL-1γ	IL-1F4	Induces IFN-γ production from T lymphocytes and NK cells and acts synergistically with IL-12 to promote the Th_1 response
IL-1F5	IL-1Hy1, FIL1δ, IL-1L1, IL-1δ, IL-1H3, IL-1RP3	IL-1F5	Possible IL-1Rrp2 receptor antagonist
IL-1F6	FIL1ε	IL-1F6	Agonist via the IL-1Rrp2 receptor. Increases IL-6, IL-8 production in epithelial cells
IL-1F7	FIL1ζ, IL-1H4, IL-1RP1, IL-1H1	IL-1F7	Interacts with IL-18 binding protein to reduce IL-18 activity
IL-1F8	FIL-1η, IL-1H2	IL-1F8	Agonist via the IL-1Rrp2 receptor. Increases IL-6, IL-8 production in epithelial cells. Also upregulates IL-6 and IL-8 production in chondrocytes and synovial fibroblasts
IL-1F9	IL-1H1, IL-1RP2, IL-1ε	IL-1F9	Agonist via the IL-1Rrp2 receptor. Increases IL-6, IL-8 production in epithelial cells
IL-1F10	IL-1Hy2, FKSG75	IL-1F10	Binds soluble IL-1RI, function unknown
IL-33	NF-HEV	IL-1F11	ST2 receptor agonist. Induces Th_2 cytokine expression. Intracellular transcriptional regulator in endothelial cells

IFN interferon; *NK* natural killer; *Th* T helper; *TNF* tumour necrosis factor

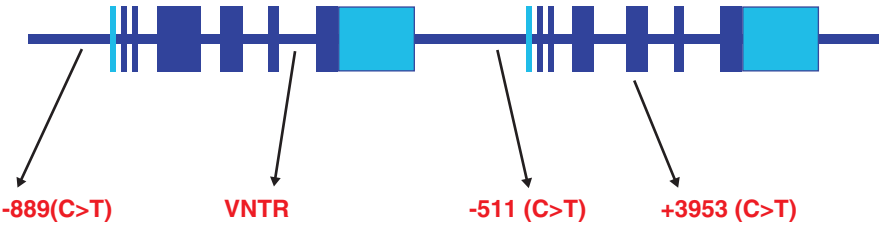

Fig. 4.1 Diagrammatic representation of the human IL1A and IL1B genes. The positions of the following biallelic polymorphisms are illustrated: IL1A −889 (NcoI); IL1B −511 (AvaI); Il1B +3953 (TaqI). In addition, a polymorphism due to a variable number of the 46 bp tandem repeat (VNTR) within intron 6 of IL1A is also illustrated (modified and adapted from Hodge et al. 2001. Reprinted with permission of John Wiley & Sons, Inc.)

genotype was associated with higher gingival crevicular fluid (GCF) volume and percentage bleeding on probing during the development of experimental gingivitis (Jepsen et al. 2003).

Scapoli et al. (2005a) included 96 systemically and periodontally healthy nonsmokers, 46 males (mean age: 23.9 ± 1.7) and 50 females (mean age: 23.3 ± 1.6) in a randomized, split-mouth, localized 21-day experimental gingivitis trial. Plaque index (PI), gingival index (GI), GCF volume, and angulated bleeding score (AngBS) were recorded. The study population was typed for IL-1α (IL-1A +4845), IL-1β (IL-1B +3953, IL-1B −511), and IL-1 receptor antagonist (IL-1RN, intron 2 variable number tandem repeats) gene polymorphisms. It was suggested that an association between IL-1RN polymorphism and subject-based clinical behavior of the gingiva in response to de novo plaque accumulation, as well as a possible association between IL-1B −511 polymorphism and gingivitis susceptibility exists. Similar results were reported by Dashash et al. (2007), Goodson (2000) and Lang et al. (2000).

Recently, Muller and Barrieshi-Nusair (2007) investigated the possible association of a distinct combination of polymorphisms in the IL-1 gene cluster on gingival bleeding tendency in young adult Arabs with plaque-induced gingivitis. A repeated measures two-level (occasion, subject) model of the proportion of sites bleeding on probing, which was adjusted for gender, average PI, probing depth, and calculus, revealed a significantly lower proportion of bleeding sites in genotype-positive subjects (estimate −0.050, standard error 0.025, $P < 0.05$). Biserial correlations of bleeding proportions were high (0.71–0.78), confirming the steady-state plaque environment. It was concluded that inflammatory responses to dental plaque were considerably dampened in genotype-positive, nonsmoking young adults of Arabic heritage.

4.1.2 Interleukin-1 Genotype and Chronic Periodontitis

Several studies provide evidence that polymorphisms in genes of the IL-1 family are associated with severe adult periodontitis in the absence of other risk factors tested in the patient population (Agrawal et al. 2006; Anusaksathein 2003; Armitage et al. 2000; Berdeli

et al. 2006; Brett et al. 2005; Cullinan et al. 2001; Drożdzik et al. 2006; Ferreira et al. 2008; Galbraith et al. 1999; Gore et al. 1998; Goteiner et al. 2008; Guzman et al. 2003; Imamura et al. 2008; Jansson et al. 2006; Kobayashi et al. 2007; Komatsu et al. 2008; Kornman et al. 1997; Kowalski et al. 2006; Kaarthikeyan et al. 2009; Laine et al. 2001; Lopez et al. 2005; Mark et al. 2000; McDevitt et al. 2000; McGuire et al. 1999; Meisel et al. 2002; Meisel et al. 2003; Meisel et al. 2004; Moreira et al. 2005; Moreira et al. 2007; Nastri and Caruso 2003; Papapanou et al. 2001; Sakellari et al. 2003; Sakellari et al. 2006; Socransky et al. 2000; Struch et al. 2008; Trevilatto et al. 2002; Wagner et al. 2007; Yoshie et al. 2007) (Table 4.3).

Kornman et al. (1997) showed that a composite polymorphism of the IL-1A (at position −889) and IL-1B (at position +3953) genes seems to cause an almost sevenfold increased risk for progressive adult periodontitis in nonsmoking patients of Caucasian origin.

In a case–control study (in which132 periodontitis patients were age- and gender-matched with 73 periodontally intact controls), Papapanou et al. (2001) examined polymorphisms at the IL-1 gene in relation to periodontal status, subgingival bacteria, and systemic antibodies to periodontal microbiota. Full-mouth clinical assessments of the periodontal tissues were performed. Subgingival plaque samples (2,440 in total) were analyzed by genomic DNA probes, and serum immunoglobulin G (IgG) antibodies to periodontal microbiota were assessed by an immunoassay. It was revealed that the composite genotype failed to distinguish between periodontitis patients and controls but correlated in patients with the severity of the disease and the antibody responses to periodontal microbiota.

One hundred and ninety-four individuals (97 CP patients, 97 controls) were genotyped for the IL-1 polymorphisms IL-1A (−889C > T) and IL-1B (+3953C > T). Both IL-1 polymorphisms were statistically significant. The heterozygous variant for IL-1A was present in 32% of the CP patients and in 20% of the controls (homozygosity (patients/controls) CC: 10/21% and TT: 55/33%). Heterozygosity for IL-1B was observed in 37% of the CP patients vs. 34% in the controls (homozygosity (patients/controls) CC: 26/57% and TT: 37/9%) (Wagner et al. 2007).

When microbiological parameters in IL-1 genotype-negative and -positive adult subjects with a range of periodontitis severities were compared, it was revealed that genotype-positive subjects more frequently had higher levels of "red" and "orange" complex species that are known to be strongly associated with measures of periodontal inflammation: *Bacteroides forsythus, Treponema denticola, Fusobacterium nucleatum* subspecies, *F. Fusobacterium periodonticum, Campylobacter gracilis, Campylobacter showae, Streptococcus constellatus*, *Streptococcus intermedius, S. gordonii,* and three *Capnocytophaga* species. Significantly higher mean counts of *B. forsythus, Porphyromonas gingivalis, T. denticola*, the *F. nucleatum* subspecies, *F. periodonticum*, *Campylobacter rectus*, *C. showae*, *Eubacterium nodatum*, *S. constellatus*, *S. gordonii*, and *S. intermedius* were detected at periodontal pockets >6 mm in subjects who were genotype-positive when compared with genotype-negative subjects. The increase was due to increased numbers of cells of these species rather than a major shift in proportion (Socransky et al. 2000).

In a cross-sectional health survey in northeast Germany, Struch et al. (2008) genotyped 1515 subjects aged 40 to 60 years for the IL-1 genotype, examined their periodontal status, and assessed diabetes, including the history of diagnosed diabetes, the use of antidiabetic

Table 4.3 Interleukin-1 gene polymorphisms in aggressive and chronic periodontitis (selection of studies)

References	Periodontits	Subjects	Ethnicity	Smoking status	Association
Diehl et al. (1999)	Aggressive	35 families with early-onset periodontitis	African American and Caucasian American families with two or more affected members	Smokers and nonsmokers	S
Hodge et al. (2001)	Aggressive	56 patients with generalized early-onset periodontitis, 56 controls	Caucasian population	Smokers and nonsmokers	NS
Li et al. (2004)	Aggressive	122 generalized aggressive periodontitis patients, and 95 healthy controls	Chinese population	Smokers and nonsmokers	S
Parkhill et al. (2000)	Aggressive	70 early onset periodontitis (EOP) patients, including a subgroup of 21 localized. patients with early onset periodontal diseases and 72 healthy controls	Caucasian population	Smokers and nonsmokers	NS
Quappe et al. (2004)	Aggressive	36 patients with AgP, 75 healthy controls, and 75 subjects of unknown periodontal status (reference population)	Chilean population	Smokers and nonsmokers	S
Scapoli et al. (2005 b)	Aggressive	40 patients with generalized aggressive periodontitis and 96 healthy controls	Caucasian population	Smokers and nonsmokers	NS
Tai et al. (2002)	Aggressive	47 patients with generalized EOP (G-EOP) and 97 healthy controls	Japanese population	Nonsmokers	S
Thomson et al. (2001)	Aggressive	980 participants at the Dunedin Multi-disciplinary Health and Development Study (DMHDS)	New Zealand population	Smokers and nonsmokers	S
Anusaksathien (2003)	Chronic	123 Thai subjects that were clinically and radio-graphically assessed for their periodontal status.	Thai population	Smokers and nonsmokers	NS
Armitage et al. (2000)	Chronic	300 volunteers of Chinese heritage (ages 21–69 years)	Chinese population	Smokers and nonsmokers	NS
Cullinan et al. (2001)	Chronic	295 subjects with chronic periodontitis recruited on the basis of availability and consent to undergo genetic testing	Caucasian population	Smokers and nonsmokers	S
Gore et al. (1998)	Chronic	32 adult patients with periodontitis individually matched for sex and age with 32 healthy controls	Caucasian population	Smokers and nonsmokers	S

(*continued*)

Table 4.3 (continued)

References	Periodontits	Subjects	Ethnicity	Smoking status	Association
Kornman et al. (1997)	Chronic	49 subjects with mild to no periodontitis, 42 subjects with moderate periodontitis and 43 subjects with generalized severe periodontitis	Caucasian population	Nonsmokers	S
Laine et al. (2001)	Chronic	105 patients with severe adult periodontitis and 53 healthy controls	Caucasian population	Smokers and nonsmokers	S
Lopez et al. (2005)	Chronic	330 patients with periodontitis and 101 healthy controls	Chilean population	Smokers and nonsmokers	S
McDevitt et al. (2000)	Chronic	90 healthy to diseased patients in a population that is typically encountered in a dental practice setting	Mixed population	Nonsmokers or former smokers with less than 10 pack-year (pk/yr) history	S
Meisel et al. (2002)	Chronic	154 subjects	Caucasian population	Smokers and nonsmokers	S
Meisel et al. (2004)	Chronic	Randomly selected population-based study (1,085 test persons)	Caucasian population	Smokers and nonsmokers	S
Papapanou et al. (2001)	Chronic	132 patients with periodontitis age- and gender-matched with 73 healthy controls	Caucasian population	Smokers and nonsmokers	NS
Sakellari et al. (2003)	Chronic	110 healthy subjects of unknown periodontal status and 45 patients with chronic periodontitis (CP)	Greek Caucasian population	Nonsmokers	NS
Wagner et al. (2007)	Chronic	194 individuals (97 patients with CP, 97 controls)	Caucasian population	Nonsmokers	S
Ferreira et al. (2008)	Chronic	292 individuals (117 patients with CP, 175 controls)	Brazilian population	Nonsmokers	S
Fiebig et al. (2008)	Aggressive	415 patients with aggressive periodontitis and 874 healthy controls	Caucasian population	Smokers and nonsmokers	NS
Guzeldemir et al. (2008)	Aggressive	31 patients with localized aggressive periodontitis and 31 healthy controls	Turkish population	Nonsmokers	S

S: Significant; NS: Non-significant

medications, and hemoglobin A(1c) (HbA(1c)) measures. It was showed that subjects with increased levels of HbA(1c) had more widespread and severe periodontal disease than normoglycemic subjects. There is a gene-environmental interaction because diabetic subjects bearing a variant IL-1 genotype C/T or T/T had an enhanced risk for periodontal disease in comparison with their IL-1 wild-type counterparts. Bleeding on probing (P = 0.007), attachment loss (P = 0.009), and number of teeth (P <0.001) were associated significantly with diabetes and the IL-1 genotype.

4.1.3 Interleukin-1 Genotype and Aggressive Periodontitis

Patients with aggressive periodontitis (formerly termed juvenile periodontitis, early onset periodontitis (EOP), or rapidly progressive periodontitis) are characterized by a rapid and severe periodontal destruction in mainly younger individuals. With regard to the relationship between IL-1 genotype and aggressive periodontitis susceptibility, conflicting results have been presented in different ethnic populations, including Caucasian American, African American, European Caucasian, and Asian Populations (Berdeli et al. 2006; Brett et al. 2005; Diehl et al. 1999; Drożdzik et al. 2006; Fiebig et al. 2008; Gonzales et al. 2003; Havemose-Poulsen et al. 2007; Hodge et al. 2001; Krátká et al. 2007; Li et al. 2004, 2005; Maria de Freitas et al. 2007; Meng et al. 2007; Papapanou et al. 2001; Parkhill et al. 2000; Quappe et al. 2004; Ren et al. 2008; Sakellari et al. 2006; Scapoli et al. 2005; Shimomura-Kuroki et al. 2009; Tai et al. 2002; Thomson et al. 2001; Walker et al. 2000) (Table 4.3).

A high powered study should be mentioned. Fiebig et al. (2008) conducted a case-control association study on 415 northern European Caucasian patients with aggressive periodontitis and 874 healthy controls to examine 10 single-nucleotide polymorphisms (SNPs) in the genes of the IL1 cluster for association with IL1A, IL1B, CKAP2L (cytoskeleton-associated protein 2-like), and IL1RN (IL-1 receptor antagonist). It was concluded that the results do not support an association between variants in the IL1 gene cluster and aggressive periodontitis. This case-control study had at least 95% power to detect genuine associations with variants carrying relative risks of at least 1.5 for heterozygous carriers and 2.25 for homozygous carriers.

4.1.4 Interleukin-1 Genotype and Smoking

In most of the research studies evaluating role for IL-1 gene cluster polymorphisms in the risk assessment for periodontal diseases, smokers were excluded (Cattabriga et al. 2001; McDevitt et al. 2000), and it was hypothesized that smoking could obscure the association of the genetic factor to the disease (Meisel et al. 2002). Since smoking is one of the main environmental risk factors for periodontal disease, biasing of the results could not be excluded. Thus, there are contradictory results regarding the IL-1 genotype-associated risk of periodontitis with respect to the impact of smoking as an environmental factor. Several studies performed by Meisel et al. (2001, 2003, 2004) were conducted aiming at elucidating the gene-environment interaction between the risk factors in smoking and IL-1 polymorphism.

In 154 Caucasian subjects clinically and radiographically assessed for their periodontal status, Meisel et al. (2001) reported a possible interaction among the IL-1 genotype, the periodontal phenotype of alveolar bone loss and smoking. This study revealed an obvious over-representation of genotype-positive subjects among patients with severe forms of periodontitis in smokers, but not in nonsmokers.

Later, in the Study of Health in Pomerania (SHIP), 3,148 subjects were randomly selected from the population and assessed for a broad range of diseases and environmental/

behavioral risk factors. From the complete study group in the age 40–60 years, n = 1,085 subjects were genotyped for the IL-1 genotype composite polymorphism in relation to periodontal parameters. An increased risk of periodontal disease was found for IL-1 genotype-positive smokers: odds ratio (OR) adjusted for age, sex, education, and plaque OR = 2.50 (95% C.I. 1.21–5.13; P = 0.013). This was not the case with subjects who never smoked: OR = 1.09 (95% CI: 0.73–1.62; P = 0.676) (Meisel et al. 2003). Similar results were obtained in a randomly selected population-based study, when 1,085 test persons were genotyped for the IL-1 genotype, examined for their periodontal status, and assessed for their smoking behavior including present and past quality and quantity of smoking. A significant dose–effect relationship between the exposure to tobacco smoke and the extent of periodontal disease assessed as attachment loss and tooth loss was reported (Meisel et al. 2004). Moreover, there was a gene-environment interaction. Subjects bearing at least one copy of the variant allele 2 (−889T and +3953T) at the position IL-1A −889 and IL-1B +3953 (genotype positive) had an enhanced smoking-associated periodontitis risk as compared to their IL-1 genotype-negative counterparts. The IL-1 genotype has no influence in nonsmokers.

The interaction of environmental factors with periodontal diseases is poorly understood. With respect to smoking, direct local effects as well as systemic effects can be distinguished. Complex interactions are to be taken into consideration between circulatory and immunological effects exerted by nicotine and toxic effects by arylamines. A synergistic risk pattern may explain the genetic–environmental interaction. Phenotypic differences exist in IL levels corresponding to the genotype, and these differences are related to periodontal disease. The positive IL-1 genotype results in enhanced levels of proinflammatory cytokines, and an increased formation of ILs is known to be induced by nicotine and/or bacterial lipopolysaccharides (LPSs). These changes may serve as predisposing factors in periodontal disease. Moreover, the IL-1 polymorphisms are highly related to plasma levels of C-reactive protein (CRP) and fibrinogen. These and other markers of systemic inflammation are influenced by tobacco smoking, possibly by a smoking-induced tissue inflammation. In conclusion, synergistic actions of smoking and IL-1-related genetic factors may explain the association of risk factors shown (Meisel et al. 2003).

4.1.5 Interleukin-1 Genotype and IL-1 Levels

In vitro, monocytes from patients showing this mutation at the IL-1B gene secrete two- to fourfold more IL-1β in response to bacterial LPS than patients without this mutation (Pociot et al. 1992). Increased IL-1 levels were also detected in inflamed periodontal tissues of IL-1 genotype-positive patients (Engebretson et al. 1999).

Investigations regarding the correlation between IL-1 genotype status and GCF levels of IL-1α and IL-1β have provided evidence of a genetic influence on the levels of these inflammatory mediators in GCF (Nares 2003). In a population with severe periodontal disease, there was at least a threefold increase in the mean level of IL-1α protein in GCF of patients who carried one or two copies of allele 2 of the IL-1A gene (−889C/T or −889T/T) when compared with those who were homozygous for allele 1 (−889C/C). In

nonsmokers, carriers of allele 2 (−889T) showed a nearly fourfold mean increase in the IL-1α protein in GCF. There was an approximately 10-fold higher mean concentration of IL-α protein in GCF from diseased patients compared to that of healthy individuals. In smokers, carriers of allele 2 (−889T) showed a 2.8-fold mean increase in the IL-1α protein in GCF (Shirodaria et al. 2000). In adults with moderate to advanced periodontal disease, it was shown that in shallow sites (<4 mm), total IL-1β in GCF was 2.5 times higher for periodontitis-associated genotype PAG(+) patients prior to conservative periodontal treatment, and 2.2 times higher after treatment, while differences were less apparent in deeper sites. Following treatment, a reduction in IL-1β concentration in GCF was seen for PAG(−) but not for PAG(+) patients (Engebretson et al. 1999).

4.1.6 Interleukin-1 Genotype and Periodontal Treatment

The association of the composite IL-1 genotype with periodontitis treatment outcomes was recently reviewed by Huynh-Ba et al. (2007). Ehmke et al. (1999) assessed the prognostic value of the IL-1 haplotype on the progression of periodontal disease following nonsurgical therapy. Forty-eight adult patients with untreated periodontitis harboring *Aggregatibacter actinomycetemcomitans* and/or *Porphyromonas gingivalis* were randomly assigned to receive full-mouth scaling alone (control) or in combination with systemic metronidazole plus amoxicillin and supragingival irrigation with chlorhexidine digluconate (test). All patients received supportive periodontal therapy at 3–6 months intervals. In 33 patients, lymphocyte DNA was analyzed for polymorphism in the IL-1A gene at position −889 and IL-1B gene at position +3953. Two years following initial periodontal therapy, no differences in the survival rates of sites or teeth not exhibiting probing attachment loss of 2 mm or more compared to baseline were found between patients who tested positive (85% sites, 53% teeth) and patients who tested negative (89% sites, 56% teeth) for the IL-1 haplotype. Engebretson et al. (1999) tested whether gingival crevicular fluid (GCF) levels of IL-1β and TNF-α, and gingival tissue levels of IL-1α, IL-1β, and TNF-α correlate with polymorphism of the composite periodontitis-associated genotype, and examined the effect of conservative periodontal therapy on these levels in 22 adults with moderate to advanced periodontal disease. Measurements were performed at baseline and 3 weeks following conservative treatment. 7 patients were genotype-positive. In shallow sites (probing depth (PD)<4 mm), total IL-1β in GCF was 2.5 times higher for genotype-positive patients prior to treatment ($P = 0.03$), and 2.2 times higher after treatment ($P = 0.04$), while differences were less apparent in deeper sites. Following treatment, a reduction in IL-1β concentration in GCF was seen for genotype-negative but not for genotype-positive patients. It was suggested that genotype-positive patients may demonstrate phenotypic differences as indicated by elevated levels of IL-1β in GCF.

Cattabriga et al. (2001) evaluated the role of the IL-1 (IL-1) polymorphism on the rate of bone and tooth loss in nonsmoking, periodontally treated patients during maintenance. Sixty consecutive nonsmoking patients (mean age 46.8 ± 5.0) with moderate to severe periodontitis treated and maintained for over 10 years were selected. IL-1

negative patients who showed minimal initial bone loss responded to the therapy better than the IL-1 positive patients. IL-1 positive patients with severe initial BL showed a better response to the therapy than IL-1 negative patients, suggesting that on an individual patient basis, the IL-1 genotype, in combination with the initial bone level, seems useful at the beginning of therapy for predicting bone level variation. When patients with severe periodontal disease in maintenance care were evaluated, it was reported that both IL-1 genotype and heavy smoking were significantly related to tooth loss. A positive IL-1 genotype increased the risk of tooth loss by 2.7 times, and heavy smoking by 2.9 times. The combined effect of IL-1 genotype and heavy smoking increased the risk of tooth loss by 7.7 times. It was suggested that knowledge of IL-1 genotype status would be important in developing a treatment plan and predicting tooth survival for a new patient who smokes and presents with periodontal disease, especially if restorative care is needed (McGuire and Nunn 1999). As reviewed by Huynh-Ba et al. (2007), the impact of IL-1 genotype status on the clinical outcomes of supportive periodontal therapy was also evaluated by McGuire and Nunn (1999), Lang et al. (2000), Nieri et al. (2002) and König et al. (2005).

The impact of IL-1 genotype status on the clinical outcomes of guided tissue regeneration (GTR) in deep intrabony defects was investigated in subjects diagnosed with chronic periodontitis (Huynh-Ba et al. 2007, De Sanctis and Zucchelli 2000; Christgau et al. 2003; Cortellini and Tonetti 2004). Christgau et al. (2003) evaluated in a controlled retrospective study (12 month long) the influence of an IL-1 gene polymorphism on the clinical and radiographic healing outcomes of guided tissue regeneration (GTR) therapy. The study included 47 adult periodontitis patients with 94 deep intrabony defects treated by GTR using different membrane materials. Bone changes in the defect regions due to GTR therapy were quantitatively evaluated using digital subtraction radiography. Polymorphisms of the IL-1A gene at position −889 and of the IL-1B gene at position +3953 were analyzed by polymerse chain reaction (PCR). Neither the clinical nor the radiographic healing parameters revealed any statistically significant differences in the GTR healing outcome between IL-1+ and IL-1− patients.

One year after GTR therapy with nonresorbable polytetrafluoroethylene (ePTFE) barriers in intrabony defects of 14 IL-1 genotype-positive and 26 IL-1 genotype-negative patients, the IL-1 genotype did not seem to have a significant influence on the gain of clinical attachment level. However, after 4 years, IL-1 genotype-positive patients had lost significantly more clinical attachment than genotype-negative patients (De Sanctis and Zucchelli 2000).

It was shown that the mean response to mucogingival surgery to cover localized gingival recessions is similar irrespective of the IL-1 periodontal genotype; however, full coverage was achieved more frequently in genotype-negative subjects (Caffesse et al. 2002a).

As Huynh-Ba et al. (2007) concluded, controversial associations between the positive composite IL-1 genotype and periodontal disease progression and/or influence on treatment outcomes emerged. As a clinical consequence, screening for IL-1 composite genotype to determine the risk for periodontitis does not seem to be justified. Results from commercially available genetic tests should be interpreted with caution and factors such as smoking status, systemic conditions, specific microbiological profiles and genetic confounders should be incorporated in a multilevel risk-assessment model.

4.1.7 Interleukin-1 Genotype and Ethnicity

Striking differences were noted in the distribution of cytokine polymorphisms among differing ethnic populations (Nares 2003). It was reported that prevalences of both IL-1A and IL-1B polymorphisms are dramatically lower in Chinese (2.3–3.3%) than in Europeans (Armitage et al. 2000). In contrast, the prevalence of genotype-positive subjects in a Mexican Hispanic population was 26%, similar to the prevalence found among ethnic populations from or descended from Northern, Central, and Southern Europe (30%) (Caffesse et al. 2002b). In a Thai population, only 1.6% (2 out of 123) of the subjects were genotype positive, which was too low to determine the association between the composite genotype of IL-1β(+3954) and IL-1α(−889) and severe forms of periodontal disease (Anusaksathien et al. 2003). Studies in Chinese, Greek, Japanese, and Thai populations did not find a relationship between IL-1 genotypes and CP disease susceptibility or severity (Table 4.3).

Investigators have failed to identify an association of IL-1 polymorphism in European Caucasians with generalized EOP (Nares 2003). In adult forms of periodontitis, nonsmokers of northern European heritage carrying the "2" allele of the IL-1α −889 and the IL-1β +3953 restriction fragment length polymorphisms (RFLPs) in either the heterozygous or the homozygous state (IL-1A −889C/T or −889T/T; IL-1B +3953C/T or +3953T/T), both loci were observed to have a greater risk for developing severe periodontitis. However, highly significant evidence of linkage disequilibrium for both African American and Caucasian subjects with generalized EOP and juvenile periodontitis has been reported. IL-1α and IL-1β polymorphisms were in strong disequilibrium with each other in Caucasians, but not in African Americans (Diehl et al. 1999). The prevalence of the IL-1α and IL-1β genotype polymorphisms in an African American population was evaluated by Walker et al. (2000). The IL-1B +3953 allelle "1" (+3953C) was carried by >99% of the African American control population and by 100% of the African American LJP (localized juvenile periodontitis) group, with most individuals being homozygous 1:1. The prevalence of the composite genotype with at least one allele "2" (+3953T and +4845T) at each of the IL-1B +3953 and IL-1A +4845 loci was 14% (African American control group) and 8% (African American LJP group). Given the high frequency of the IL-1β allele "1" in the African American population, it would appear that knowledge of this +3953 polymorphism would provide little diagnostic or predictive information for this population (Walker et al. 2000).

4.1.8 Interleukin-1 Genotype and Oral Implants

Implants are now an integral part of periodontal and restorative dentistry. However, owing to the recent introduction of predictable therapies in implant dentistry, very few studies have been reported addressing the influence of genetics on implant survival (Nares 2003) (reviewed by Andreiotelli et al. 2008 and Huynh-Ba et al. 2008) (Montes et al. 2009; Laine et al. 2006; Jansson et al. 2005; Gruica et al. 2004; Feloutzis et al. 2003; Shimpuku et al. 2003). Factors that increase the risk of periodontitis may also contribute to the success of

dental implants. Rogers et al. (2002) examined IL-1A −889 and IL-1B +3953 alleles in Caucasian patients with adult and early-onset periodontitis, patients with dental implants, and healthy individuals. IL-1A −889, IL-1B +3953, and the composite genotype (L-1A −889 allele 2 (T) plus IL-1B +3953 allele 2 (T)) showed no association with failure of dental implants. Similar results were reported by Wilson and Nunn (1999), which failed to demonstrate a relationship between implant failure and the IL-1A −889, IL-1B +3953 composite genotype in Caucasian patients.

In contrast, Shimpuku et al. (2003) revealed that the IL-1B −511 2/2 genotype (T/T) has a significant association with the incidence of early marginal BL around endosseous implants. In a retrospective investigation (5.6 ± 2.5 years) of a sample of 90 consecutive Caucasian patients (aged 33–88 years), treated with at least one ITI implant, it was reported that in heavy cigarette smokers, carriage of a functionally significant IL-1 gene complex polymorphism is associated with an increased risk for peri-implant BL following prosthetic reconstruction and during the supportive periodontal care phase of the treatment (Feloutzis et al. 2003). Laine et al. (2006) demonstrated significant differences in the carriage rate of allele 2 in the IL-1RN gene polymorphism (a penta-allelic variable number of an 86-bp tandem repeat polymorphism in the intron 2) between peri-implantitis patients and healthy controls (56.5 vs. 33.3%, respectively; ORs 2.6; 95% CI: 1.2–5.6; $P = 0.015$).

Elevated levels of the inflammatory cytokine IL-1 (IL-1) in the crevicular fluid around diseased implants seem to play an important role in the pathogenesis and severity of peri-implantitis. Recently, Andreiotelli et al. (2008) critically reviewed the genetic associations regarding IL-1 genotype claimed for peri-implant disease and revealed that the diagnostic value of both IL-1 genotyping and genetic tests for peri-implantitis should be reconsidered before altering treatment planning, regimens, and maintenance in implant dentistry.

Summary of the findings on the IL-1 composite genotype in periodontitis as presented by Kinane and Hart (2003) were:

- it is unlikely to be relevant in aggressive periodontitis;
- it is, at best, in linkage disequilibrium with the gene contributing susceptibility to CP;
- it confers risk independent of that attributable to smoking;
- the polymorphism is at best one of several involved in the genetic risk to CP, which is likely to be a disease in which multiple genes may confer risk;
- the polymorphism is a useful marker in only defined populations, is relatively absent in some (Armitage et al. 2000), and is too prevalent (Walker et al. 2000) in others to be a genetic marker with utility;
- demonstration of the functional significance of this gene polymorphism has yet to be confirmed; and
- clinical utilization of these composite polymorphisms for risk assessment and prognostic determination is currently premature.

Recently, Nikolopoulos et al. (2008) conducted a systematic review and a meta-analysis, in order to investigate the potential association of cytokine gene polymorphisms (IL-1A G[4845]T, IL-1A C[-889]T, IL-1B C[3953/4]T, IL-1B T[-511]C, IL-6 G[-174]C and TNFA G[-308]A.) with either aggressive or chronic periodontal disease. Using random effect methods we found statistically significant association of IL-1A C[-889]T and IL-1B C[3953/4]T polymorphisms with chronic periodontal disease without any evidence of

publication bias or significant statistical heterogeneity. A weak positive association was also found concerning IL-1B T[-511]C and chronic periodontal disease. No association was found for all the cytokines examined as far as the aggressive form of the disease is concerned (Nikolopoulos et al. 2008).

As Huynh-Ba et al. (2007) concluded, controversial associations between the positive composite IL-1 genotype and periodontal disease progression and/or influence on treatment outcomes emerged. As a clinical consequence, screening for IL-1 composite genotype to determine the risk for periodontitis does not seem to be justified. Results from commercially available genetic tests should be interpreted with caution and factors such as smoking status, systemic conditions, specific microbiological profiles and genetic confounders should be incorporated in a multilevel risk-assessment model.

4.2 Interleukin-2 Gene Polymorphisms

IL-2 (IL-2) is a proinflammatory cytokine derived from Th_1 cells (helper T cells of Th_1 subtype). This cytokine is involved in B-cell activation and stimulates macrophages, natural killer (NK) cells, T-cell proliferation, and osteoclast activity. IL-2 has been also implicated in the stimulation of osteoclast activity in bone resorption (Scarel-Caminaga et al. 2002). IL-2 is produced by lymphocytes isolated from chronically inflamed periodontal tissues (Seymour et al. 1985), but it was also found that gingival mononuclear cell (GMC) culture supernatants from inflamed tissues of patients with CP contained no detectable IL-2 at both mRNA and protein levels (Fujihashi et al. 1993). However, the mean levels of IL-2 were significantly elevated in the sera of patients with periodontal disease (88.5%) compared to control values (10%), being suggested that the measurement of IL-2 could provide a sensitive laboratory test for assessing periodontal disease activity (Cutando-Soriano et al. 1998; McFarlane and Meikle 1991).

The IL-2 gene is located in chromosome 4q26. John et al. (1998) detected two novel single nucleotide polymorphisms in the IL-2 gene at positions −330(T > G) and +166(G > T) relative to the transcription start site. The +166 change occurs within the leader peptide and does not affect amino acid sequence. The −330 polymorphism has two common alleles, making it an ideal marker for genetic association studies (John et al. 1998). The relationship between the −330 polymorphism in the IL-2 gene and severity of chronic periodontal disease susceptibility to inflammatory diseases was investigated in a convenience sample of 113 unrelated, nonsmoking subjects >25 years of age. It was shown that individuals with the T allele seem to be approximately half as likely to develop severe periodontal disease (OR = 1.99; 95% CI: 1.07–3.7). The frequency of genotype TT in the control/moderate group was also significantly different compared to the group formed by patients with severe periodontal disease (P = 0.019). Individuals with the TT genotype seem to be 2.5 times less likely to develop the severe periodontal disease than individuals who are heterozygous or GG homozygous (OR = 2.57; 95% CI: 1.15–5.73). It was suggested that there is active participation of IL-2 in the pathogenesis of periodontal disease (Scarel-Caminaga et al. 2002).

4.3 Interleukin-4 Gene Polymorphisms

This cytokine is released by T helper cells of the Th_2 subtype and is particularly active on resting and active B cells. It was originally called the B-cell stimulating factor. On resting B cells, as well as on macrophages, IL-4 increases major histocompatability complex (MHC) II expression. On activated B cells, proliferation and differentiation is stimulated and an antibody class switch is induced. A B cell stimulated with IL-4 alone becomes a plasma cell secreting IgE and other allergy-related antibodies. IL-4 acts with IL-10 in an immunoregulatory manner to decrease the activity of activated macrophages (http://pim.medicine.dal.ca/il4.htm). Several studies have investigated IL-4 at both mRNA and protein levels in periodontal lesions (Yamamoto et al. 1996; Yamazaki et al. 1994), GCF (Giannopoulou et al. 2003; Tsai et al. 2007), and sera of patients with periodontal disease (McFarlane and Meikle 1991). A high TNF-α/IL-4 ratio found in tissue biopsies from patients with periodontitis strongly correlated with the severity of periodontitis (Gorska et al. 2003). It was shown that initial nonsurgical periodontal therapy (NSPT) resulted in decreased total amount of IFN-γ, increased concentration of IL-4, and increased ratio of IL-4 to IFN-γ levels in GCF. It was suggested that a low ratio of IL-4 to IFN-γ levels might be involved in the destruction (diseased sites) of periodontal tissue, whereas an increased ratio of IL-4 to IFN-γ levels could be related to the improvement of clinical periodontal health (Tsai et al. 2007).

The gene for IL-4 is found in chromosome 5q31.1 (Sutherland et al. 1988). There are the polymorphisms of IL-4 at −590(C > T) and −34(C > T) in the promoter region. Controversial results were obtained, as several studies relating to aggressive periodontitis and CP have failed to establish a connection between IL-4 polymorphism loci and periodontal disease in Caucasian, Korean, Japanese, and African American Brazilian populations (Gonzales et al. 2004; Hooshmand et al. 2008; Kang et al. 2003; Kara et al. 2008; Michel et al. 2001; Pontes et al. 2004; Scarel-Caminaga et al. 2003). However, Gonzales et al. (2007) have demonstrated an association between the IL-4 −590T/T and IL-4 −34T/T genotypes and aggressive periodontitis, while Holla et al. (2008) suggested that the high-production IL-4 haplotype was associated with an increased risk for CP in the Czech population.

4.4 Interleukin-6 Gene Polymorphisms

Monocytes, macrophages, and bone marrow cells secrete this cytokine, but the major producer is the Th_2 type of T helper cells. IL-6 acts on proliferating B cells to promote differentiation into plasma cells and it stimulates antibody secretion. IL-6 helps myeloid stem cells to differentiate. IL-6 has been described as “hepatocyte stimulating factor” and strongly stimulates hepatocytes to make acute phase proteins in response to inflammation. This cytokine is always found in increased levels in sites of inflammation and is likely very important in a number of undescribed ways in inflammatory regulation (http://pim.medicine.dal.ca/il6.htm). IL-6 as a pleiotropic cytokine is responsible for the synthesis of CRP and

other acute phase proteins in order to contain the local infection. The data indicated, that in patients with severe periodontal infections, increased serum IL-6 was associated with carriage of allele 2 for IL-1A (−899), TNF-α (−308), and IL-6 (−174), while serum CRP was associated with allele 2 for IL-1A (−889) and IL-6 (−174). Serum concentrations of both the inflammatory markers have also assumed a significant role as predictors for future cardiovascular events in healthy populations (D'Aiuto et al. 2004b, 2005).

IL-6 has been thought to play a crucial role in the pathogenesis of periodontal disease, especially in bone loss (Irwin and Myrillas 1998). IL-6 is present at higher levels in inflamed tissue (Takahashi et al. 1994), GCF (Lee et al. 1995), and plasma (Buhlin et al. 2003) of periodontitis patients. However, significant reduction of serum IL-6 was observed after periodontal therapy (D'Aiuto et al. 2004a). The IL-6 gene was demonstrated to be localized in chromosome 7p21 (Bowcock et al. 1988). In the IL-6 gene there are presently four known promoter polymorphisms: −597 (G/A), −572 (C/G), −174 (G/C) single nucleotide polymorphisms (SNPs), and −373 A_nT_m polymorphism (Komatsu et al. 2005). IL-6 receptor polymorphisms strongly influenced the serum levels of soluble IL-6 receptor (Galicia et al. 2004). Several studies have evaluated the relationship between IL-6 polymorphism and periodontal disease (Table 4.4). It has been revealed that the IL-6 −373 A_9T_{11} allele could be associated with reduced susceptibility to CP among Japanese subjects and decreased serum IL-6 level (Komatsu et al. 2005).

In a recent meta-analysis, investigating the possible differential risk for chronic periodontitis of the IL-6 174C allele carriers, the pooled OR of the C allele versus the G allele was 1.083 (95% CI: 0.814–1.442) with corresponding results in the subgroup analyses. The genotype contrasts showed insignificant findings (Nikolopoulos et al. 2008).

4.5 Interleukin-10 Gene Polymorphisms

This interesting cytokine was originally described as "cytokine synthesis inhibitory factor" because of its important inhibitory role. It acts on macrophages to inhibit cytokine production in order to downregulate the Th_1 type of T helper cell. It is released by Th_2 cells and also downregulates MHC II expression on antigen-presenting cells. It has been shown to act with IL-4 to decrease macrophage inflammatory activity and may be an important cytokine in immune regulation (http://pim.medicine.dal.ca/il10.htm). It has been shown that stimulation of peripheral blood mononuclear cells with *P. gingivalis* outer membrane induced significantly higher IL-10 mRNA expression in periodontitis patients than in healthy controls ($P < 0.05$) (Aoyagi et al. 2000). The gene encoding IL-10 was mapped to chromosome 1q31–32. Three promoter single nucleotide polymorphisms have been described in this gene: (−1087) G/A; (−819) C/T; and (−592) C/A (Yoshie et al. 2007) (Fig. 4.2). A limited number of studies have investigated genetic variations at three positions in the *IL-10* promoter region. For all three positions some significant differences in the allele carriage rates between patients and controls have been reported (Table 4.5).

The IL10 −1087 polymorphism may be an interesting polymorphism for future studies, as Berglundh et al. (2003) have shown that the G-allele is more abundant in periodontitis, in particular in nonsmokers. These observations have led the authors to speculate that the G-allele prevalence in periodontitis patients may result in higher levels of auto-antibodies,

Table 4.4 Interleukin-6 gene polymorphisms in aggressive and chronic periodontitis

References	Periodontits	Subjects	Ethnicity	Smoking status	Association
Komatsu et al. (2005)	Chronic	112 subjects with CP and 77 subjects with non-CP	Japanese population	Nonsmokers	S
Galicia et al. (2006)	Aggressive and chronic	422 subjects (169 with CP; 70 healthy controls for CP; 43 with aggressive periodontitis; 140 healthy controls	Japanese population	Nonsmokers	S
Tervonen et al. (2007)	Chronic	51 subjects with moderate to severe CP and 178 reference group	Caucasian population	Smokers and nonsmokers	S
Babel et al. (2006)	Chronic	122 adult patients with CP and 114 healthy controls	Caucasian population	Smokers and nonsmokers	S
Holla et al. (2004b)	Chronic	148 patients with CP and 107 unrelated controls	Caucasian population	Smokers and nonsmokers	S
Trevilatto et al. (2003)		36 healthy individuals (control group), 24 subjects with moderate and 24 with severe periodontitis	Brazilian population	Nonsmoking	S
Moreira et al. (2007)	Chronic	209 Brazilian individuals with and without periodontitis	Brazilian population	Nonsmoking	S
D'Aiuto et al. (2004b)	Chronic	94 subjects with severe generalized periodontitis	65% of subjects were European Caucasians, 20% Asians, and 15% African or Afro-Caribbeans	Smokers and nonsmokers	S
D'Aiuto et al. (2005)	Chronic	55 individuals with severe (probing pocket depths greater than 6 mm and marginal alveolar bone loss >30%, and generalized (at least 50% of teeth affected) periodontitis	Caucasian population	Smokers and nonsmokers	S
Nibali et al. (2008c)	Aggressive	107 subjects diagnosed with severe forms of aggressive periodontitis	Mixed population	Smokers and non-smokers	S
Nibali et al. (2008a)	Aggressive	224 patients with aggressive periodontitis and 231 healthy controls	Mixed population	Smokers and nonsmokers	S
Nibali et al. (2007)	Aggressive	45 young adults diagnosed with generalized aggressive periodontitis	Mixed population	Smokers and nonsmokers	S
Nibali et al. (2009)	Aggressive and chronic	765 subjects (167 generalized aggressive periodontitis, 57 localized aggressive, 310 chronic periodontitis and 231 controls)	Mixed population	Smokers and nonsmokers	S
Guan et al. (2008)	Chronic	93 patients with periodontitis and 96 controls	Chinese population	Smokers and nonsmokers	S

S: Significant; NS: Non-significant

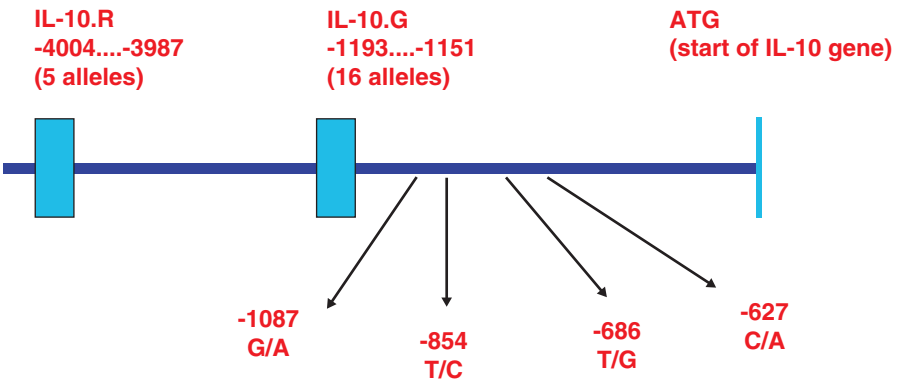

Fig. 4.2 Schematic representation of the polymorphic elements in the human interleukin-10 promoter region. Both IL-10.R and IL-10.G microsatellites lie within the promoter region of the IL-10 gene on chromosome 1 (Kinane et al. 1999) (Reprinted with permission of John Wiley & Sons, Inc.)

which may lead to increased periodontal destruction. Further studies on *IL-10* as candidate gene seem justified (Loos et al. 2005).

4.6 Interleukin-12 Gene Polymorphisms

Inflammatory cell infiltrate containing lymphocytes and macrophages is often observed in periodontal lesions. T-lymphocytes are predominant in stable lesions, whereas B-lymphocytes and plasma cells are increased in progressive lesions. This suggests that Th_1 cytokine expression T-cells are the main regulators of inflammation in the early/stable lesions. If the immune regulation is inadequate, reduced level of IL-12 often leads to low expression of Th_1 and uncontrolled infection (Tsai et al. 2007). Higher levels of interleukin-12 (IL-12) have been found in gingival crevicular fluid from chronic periodontitis sites than gingivitis and healthy one, while the gene expression of IL-12 p35 was significantly higher in periodontitis compared with gingivitis, suggesting that Th_1 plays a potential role in the progression of periodontitis (Tsai et al. 2005; Yücel et al. 2008; Honda et al. 2008; Orozco et al. 2006).

Reichert et al. (2008) revealed an association between the genotype IL-12 1188 A/C and gingivitis as assessed by bleeding on probing. Although a relationship between the investigated IL-12 polymorphism and certain periodontopathic bacteria or the bleeding index was shown, this polymorphism was not significantly related to the presence of aggressive periodontitis or chronic periodontitis (Reichert et al. 2008).

4.7 Interleukin-18 Gene Polymorphisms

Interleukin-18 (IL-18) is a member of the IL-1 family, and, together with their receptors, it is also a member of the IL-IR/TLR superfamily involved in the regulation of both the innate as well as the acquired immune response (Noack et al. 2008). It was showed that the

Table 4.5 Interleukin-10 gene polymorphisms in aggressive and chronic periodontitis

References	Periodontits	Subjects	Ethnicity	Smoking status	Association
Sumer et al. (2007)	Chronic	75 patients with severe generalized CP and 73 healthy subjects	Turkish population	Nonsmokers	S
Savarrio et al. (2007)	Chronic	106 patients with generalized, severe CP and 69 controls periodontally healthy subjects	Caucasian population	Smokers and nonsmokers	NS
Babel et al. (2006)	Chronic	122 adult patients with CP and 114 healthy controls	Caucasian population	Smokers and nonsmokers	S
Brett et al. (2005)	Chronic and aggressive	51 patients with aggressive periodontitis, 57 CP patients, 100 unrelated healthy individuals of unknown periodontal status as controls	Caucasian population	Smokers and nonsmokers	NS
Scarel-Caminaga et al. (2004)	Chronic	567 patients with CP and 543 control subjects	Brazilian population	Nonsmokers	S
Berglundh et al. (2003)	Chronic	60 patients with severe and generalized CP; 39 healthy controls	Caucasian population	Smokers and nonsmokers	S
Gonzales et al. (2002)	Chronic and aggressive	23 patients with CP, 18 patients with aggressive periodontitis, 21 healthy controls	Caucasian population	Nonsmokers	NS
Yamazaki et al. (2001)	Chronic	34 adult patients with periodontitis, 18 patients with generalized early-onset periodontitis and 52 controls	Caucasian population	Smokers and nonsmokers	NS
Kinane et al. (1999)	Aggressive	77 patients with generalized EOP	Caucasian population	Not given	NS
Cullinan et al. (2008)	Chronic	252 adults who were part of a prospective longitudinal study on the progression of periodontal disease	Australian population	Smokers and nonsmokers	S
Donati et al. (2008)	Chronic	53 subjects with generalized and severe CP	Caucasian population	Nonsmokers	S
Reichert et al. (2008)	Chronic aggressive	27 patients with generalized CP, 32 with generalized aggressive periodontitis and 34 healthy controls	Caucasian population	Smokers and nonsmokers	S
Mellati et al. (2007)	Aggressive	52 subjects suffering from generalized aggressive periodontitis compared to 61 healthy controls	Iranian population	Nonsmokers	NS
Claudino et al. (2008)	Chronic	116 chronic periodontitis patients and 173 controls	Brazilian population	Nonsmokers	S
Hu et al. (2009)	Chronic	145 chronic periodontitis, 65 generalized aggressive periodontitis patients, 126 controls	Taiwanese population	Smokers and nonsmokers	S

S: Significant; NS: Non-significant

Table 4.6 IL-18 gene polymorphisms in aggressive and chronic periodontitis

References	Periodontitis	Subjects	Ethnicity	Smoking status	Association
Noack et al. 2008	Aggressive	111 patients with aggressive periodonititis and 80 healthy controls	Caucasian population	Smokers and nonsmokers	NS
Folwaczny et al. 2005	Aggressive and chronic	123 patients with periodontitis and 121 healthy controls	Caucasian population	Not given	NS

S: Significant; NS: Non-significant

Table 4.7 Transforming growth factor-β gene polymorphisms in aggressive and chronic periodontitis

References	Periodontits	Subjects	Ethnicity	Smoking status	Association
de Souza et al. (2003a)	Chronic	26 patients with severe periodontitis 24 patients with moderate periodontitis 37 health controls	Caucasian population	Nonsmokers	S
Holla et al. (2002b)	Chronic	90 patients with adult periodontitis and 108 health controls	Caucasian population	Smokers and nonsmokers	NS
Attila et al. 2006	Aggressive and chronic	51 patients with chronic periodontitis, 43 with generalized aggressive periodontitis and 40 controls	Turkish population	Nonsmokers	S for Chronic periodontitis NS for Aggressive periodontitis
Babel et al. 2006	Chronic	122 patients with chronic periodontitis and 114 controls	Caucasian population	Smokers and nonsmokers	S

S: Significant; NS: Non-significant

different IL-18 gene polymorphisms were not associated with periodontal disease (Noack et al. 2008; Folwaczny et al. 2005) (Table 4.6).

4.8 Transforming Growth Factor-β (TGF-β) Gene Polymorphisms

TGF-β is secreted by platelets, macrophages, and lymphocytes. It has many functions which include increasing IL-1 production by activated macrophages, inducing a class switch to IgA by proliferating B cells, acting as a chemo-attractant for monocytes and macrophages, and inhibiting the proliferation of cells required for the inflammatory process. This means that TGF-β actually aids in wound healing because it limits the inflammation caused by injury. TGF-β is a very powerful inhibitor of lymphocyte function but stimulates other cells (http://pim.medicine.dal.ca/tgfb.htm). The gene for TGF-β is located in chromosome 19q13.1 (Yoshie et al. 2007).

There are limited data investigating the role of TGF-β gene polymorphisms in the pathogenesis of Chronic periodontitis (CP) (Table 4.7). Holla et al. 2002b) evaluated TGF-β gene polymorphisms in CP. They could not find any association between −988(C/A), −800(G/A), −509(C/T), codon 10(Leu10Pro in exon1), and codon 25 (Arg25Pro in exon

1) polymorphisms of TGF-β1 and susceptibility to CP in a Czech population. In another population, de Souza et al. (2003a) found that −509(C/T) polymorphism of TGF-β1 gene has a limited effect on gingival inflammation in a Brazilian population with CP. Babel et al. (2006) showed that the TGF-β1 (codon 25) GG (Arg25/Arg25) genotype was detected more frequently in control subjects than in periodontitis patients (OR = 0.459; 95% CI: 0.230–0.920; $P = 0.0421$). Atilla et al. (2006) investigated the TGF-β1 gene polymorphisms in a Turkish population with CP and generalized aggressive periodontitis (GAP) as well as the association between TGF-β1 genotype and clinical periodontal parameters. Patients with CP (72.5%) had elevated +915C allele positivity compared to patients with GAP (53.5%) and healthy controls (52.5%). It was shown that +915G/C heterozygote subjects are common in Turkish population. In addition, patients with GAP showed a trend towards an increase in 263Ile (major allele in this site is Thr) positive genotype (Atilla et al. 2006).

4.9 Fc Receptor Polymorphisms

Leukocyte IgG receptors (FcγR) serve as a link between the humoral and cellular branches of the immune system. They confer potent leukocyte effector functions to the specificity of IgG (Fig. 4.3). Binding of the constant region of IgG to FcγR induces a plethora of cell type-specific pro- and counterinflammatory functions. FcγR polymorphisms influence the efficacy of cellular responses, and have been associated with inflammatory disease and disease severity. Leukocyte FcγR belong to the Ig superfamily and are divided into three classes, FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16), encompassing at least 12 isoforms (Fig. 4.4). FcγR classes contain structurally and biochemically distinct molecules, and differ in cell distribution and affinity for IgG subclasses. FcγR induce leukocyte effector functions, such as phagocytosis, cytotoxicity, cytokine production, degranulation, antigen presentation, and regulation of antibody production upon crosslinking, e.g., after binding to immune complexes (Meisel et al. 2001; Nimmerjahn and Ravetch 2006).

Leukocyte FcγR are encoded by eight genes on the long arm of chromosome 1. In humans, all three FcγR groups present genetically determined polymorphism. The most studied polymorphic forms of FcγR involve changes in the extracellular domains, containing

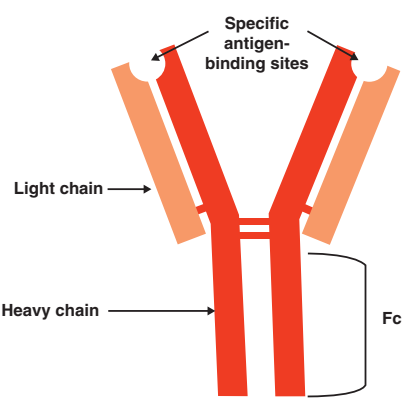

Fig. 4.3 Immunoglobulin structure

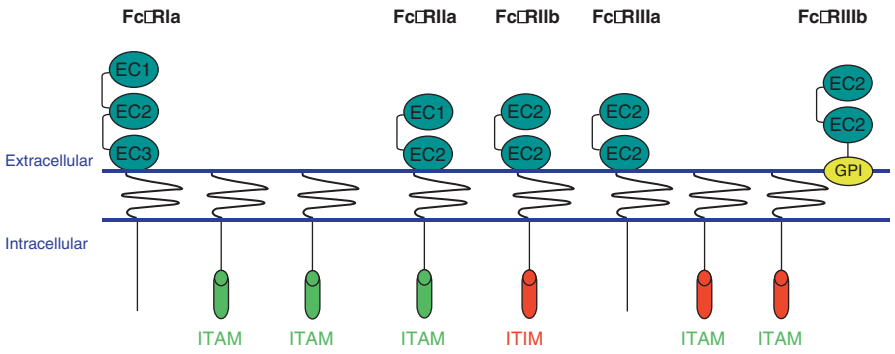

Fig. 4.4 Schematic model for activating and inhibitory FcγR. All receptors belong to the Ig super family, with their ECs composed of disulfide (S–S)-bonded domains. FcγRI, FcγRIIa, and FcγRIIIa are activating receptors, characterized by the presence of an immunoreceptor tyrosine-based activation motif (ITAM) in the cytoplasmic domain of the receptor (FcγRIIa) or associated with the receptor as an accessory signaling subunit (γ and/or ζ chains associated with FcγRI and FcγRIIIa). FcγRIIb is an inhibitory receptor containing ITIM in its cytoplasmic domain. FcγRIIIb is linked to the plasma membrane by a glycosyl phosphatidylinositol (GPI) anchor (modified and adapted from Dijstelbloem et al. 2001 and Ivan and Colovai 2006) (Reprinted with permission of Elsevier B.V.)

Table 4.8 Characteristics of human leukocyte IgG receptors (van Sorge et al. 2003) (Reprinted with permission of John Wiley & Sons, Inc.)

	FcγRI	FcγRII	FcγRIII
Genes	FcγRIA, -B and -C	FcγRIIA, -B and -C	FcγRIIIA and -B
Chromosome	1q21	1q23–24	1q23–24
Isoforms	Ia; Ib1; Ib2; Ic	IIa1; IIa2; IIb1; IIb2; IIb3; IIc	IIIa; IIIb
Molecular weight (kDa)	72	40	50–80
Affinity for IgG	High (10^8–10^9/M)	Low (<10^7/M)	IIIa: Intermediate (3–10^7/M) IIIb: Low (<10^7/M)

the ligand binding sites, which affect the affinity for immune complexes. The frequency of these polymorphic forms varies among ethnic groups (Ivan and Colovai 2006; Meisel et al. 2001; van Sorge et al. 2003) (Table 4.8).

FcγRIIa was shown to have two allelic forms: HR (high responder) and LR (low responder). These names are based on the capacity of T cells from normal individuals to proliferate in response to the monoclonal antibody (IgG1) anti-CD3 in the presence of HR or LR monocytes. The HR and LR alleles are codominant. FcγIIa in HRs has an arginine (R) at position 131 and has low affinity for all human IgG subclasses, particularly IgG2. The LR allotype has a histidine (H) at 131 and binds IgG2 and IgG3 with higher affinity. Eastern Asians have a higher frequency of H131/H131 homozygotes compared to Caucasians (Ivan and Colovai 2006; Meisel et al. 2001; van Sorge et al. 2003). It has been shown that FcγRIIa-H/H131 subjects exhibit a higher percentage of IL-1β-producing cells than FcγRIIa-R/H131 and -R/R131 subjects (Yamamoto et al. 2007).

A single-nucleotide polymorphism for the FcγRIIB gene results in the substitution of isoleucine (I) with threonine (T) at position 232 in the transmembrane region. FcγRIIB-232T

has a higher capacity for inhibiting B-cell receptor signaling compared to FcγRIIB-232I (Ivan and Colovai 2006; Meisel et al. 2001; van Sorge et al. 2003). The polymorphism of FcγRIIA by 494G >A results in the substitution of arginine (R) or histidine (H) at amino acid 131 of the FcγRIIa protein. The frequency of FcγRIIa-131R/R homozygotes is about 23% in the Caucasian population, 31% in Asian Indians, and 6% in the Japanese and Chinese populations (Osborne et al. 1994).

FcγRIIIa has two allelic forms that differ in the amino acid at position 158: FcγRIIIa-V158 has a valine (V), and FcγIIIa-F158, a phenylalanine (F). The 158V allelic variant of FcγRIIIa has higher affinity for IgG1, IgG3, and IgG4 than the 158F-type receptor (Ivan and Colovai 2006; Meisel et al. 2001; van Sorge et al. 2003) (Table 4.9).

Table 4.9 Leukocyte FcγR subclass distribution and ligand specificity (Ivan and Colovai 2006) (Reprinted with permission of Elsevier B.V.)

Receptor	Affinity for immunoglobulin	Cellular expression	GenBank accession #
FcγRIa (CD64)	10^8–10^9/M to IgG1>3>4>>2	Macrophage, monocyte, neutrophil (induced by G-CSF and IFNγ), eosinophil, DC	α: X14356
FcγRIIa (CD32a)	<10^7/M to IgG3>1, 2>>4	Macrophage, neutrophil, eosinophil, platelet, DC, LC	α: M31932
FcγRIIb (CD32b)	<10^7/M to IgG3>1>4>2	IIb1:Bcell, IIb2: mast cell, basophil, DC, LC, macrophage, neutrophil, eosinophil	α: X52473
FcγRIIIa (CD16a)	2×10^7/M to IgG1, 3>>2, 4	Macrophage, monocyte, neutrophil, NK cell, mast cell, eosinophil (induced by IFNγ), γ/δ T cells, DC, LC	X52645
FcRIIIb (CD16b)	<10^7/M to IgG1, 3>>2, 4	Neutrophil, eosinophil	X07934 AB025256
FcγRIV	3×10^7/M to IgG2a, 2b (mouse)	Myeloid cells	NM 010188
FcαRI (CD89)	2×10^7/M to IgA1, IgA2	Macrophage, neutrophil, eosinophil	X92558-62
Fcα/μR	10^8/M to IgA, IgM	B cell, macrophage	E15470
FcRn	2×10^8/M to IgG	Small intestine, placenta, monocyte, DC, endothelial cells	α: U12255
PIgR	10^9/M to IgA 10^8/M to IgM	Epithelium, small intestine, liver, lung, urogenital tracts	X73079
Fc∈RI	>10^{10}/M to IgE	Mast cell, eosinophil, basophil, DC, LC	α: X06948 β: M89796
Fc∈RII (CD23)	10^6/M to IgE	Ubiquitous, platelet	X04772, A10542, M14766
FcRH	ND	B cell	AY043464, AF397452

Table 4.10 Fc gene polymorphisms in aggressive and chronic periodontitis

References	Receptor	Periodontits	Subjects	Ethnicity	Smoking status	Association
de Souza and Colombo (2006)	FcγRIIa FcγRIIIb	Aggressive	31 patients with generalized aggressive periodontitis and 49 healthy controls	Brazilian population	Nonsmokers	S
Fu et al. (2002)	FcγRIIa FcγRIIIa	Aggressive	48 patients with localized aggressive periodontitis, 67 healthy controls	African American population	Not given	S
Loos et al. (2003)	FcγRIIa FcγRIIIa FcγRIIIb	Aggressive	12 subjects with aggressive periodontitis, 56 individuals with CP, 61 controls	Caucasian population	Smokers and nonsmokers	S
Kobayashi et al. (1997)	FcγRIIa FcγRIIIb	Chronic	100 patients with adult periodontitis and 105 healthy controls	Japanese population	Smokers and nonsmokers	
Yamamoto et al. (2004)	FcγRIIa	Chronic	213 subjects with CP and 209 controls	Caucasian population	Smokers and nonsmokers	S
Yoshihara et al. (2001)	FcγRIIIb	Chronic	42 subjects with generalized EOP, 52 subjects with adult periodontitis and 55 healthy controls	Japanese population	Smokers and nonsmokers	S
Chung et al. (2003)	FcγRIIa FcγRIIIb	Chronic and aggressive	50 patients with CP, 30 patients with generalized aggressive periodontitis, and 74 healthy controls	Taiwanese population	Nonsmokers	NS
Kobayashi et al. (2000a)	FcγRIIIa FcγRIIIb	Chronic and aggressive	38 patients with generalized EOP, 83 patients with adult periodontitis, 104 healthy controls	Japanese population	Nonsmokers	S
Kobayashi et al. (2000b)	FcγRIIIb	Recurrent	15 patients with adult periodontits and 18 healthy controls	Japanese population	Smokers and nonsmokers	S
Sugita et al. (2001)	FcγRIIIb	Refractory	46 periodontitis-resistant individuals and 73 periodontitis-susceptible patients	Japanese population	Nonsmokers	S
Colombo et al. (1998)	FcγRIIa FcγRIIIb	Refractory	32 refractory, 54 successfully treated, and 27 controls	Caucasian population	Smokers and nonsmokers	NS
Kobayashi et al. (2001)	FcγRIIa FcγRIIIa FcγRIIIb	Severe chronic	50 patients with severe CP and 39 patients with moderate CP, 64 controls	Japanese population	Nonsmokers	S
Meisel et al. (2001)	FcγRIIa FcγRIIIa FcγIIIb	Severe chronic	154 subjects exhibiting all stages of periodontal disease	Caucasian population	Smokers and nonsmokers	S

(*continued*)

Table 4.10 (continued)

References	Receptor	Periodontits	Subjects	Ethnicity	Smoking status	Association
Kobayashi et al. (2007)	FcγRIIa FcγRIIIa FcγRIIIb	Chronic	100 rheumatoid arthritis group; 100 periodontitis only group; 100 healthy controls	Japanese population	Smokers and nonsmokers	NS
Komatsu et al. (2008)	FcγR FcαR	Chronic	113 patients with CP and 108 healthy controls	Japanese population	Smokers and nonsmokers	S
An et al. 2009		Aggressive and chronic	30 aggressive periodontitis patients, 131 chronic periodontitis patients and 47 healthy controls	Chinese population	Not given	Significant for aggressive Nonsignificant for chronic
Nibali et al. 2006		Aggressive	224 patients with AgP and 231 healthy controls	Mixed population	Smokers and nonsmokers	S
Yasuda et al. 2003		Aggressive and chronic	32 aggressive periodontitis patients, 72 chronic periodontitis patients and 72 healthy controls	Japanese population	Smokers and nonsmokers	S

CP: chronic periodontitis; EOP: early onset periodontitis; S: Significant; NS: Non-significant

The FcγRIIIB locus has three alleles: NA1, NA2, and SH. NA1 and NA2 were initially described as neutrophil alloantigens that may cause blood transfusion reactions and alloimmune anemia. Both FcγRllIb allotypes differ by several amino acids, with changes at amino acid positions 65 and 82 resulting in two extra glycosylation sites (six instead of four) in the IIIb-NA2 allotypic form. This allotypes difference can be detectable by the differential electrophoretic mobility of these protein or the sensitive PCR-based methods using allele-specific oligonucleotides (de Haas et al. 1995; Rascu et al. 1997). Another allotypes, termed SH, showed the substitution of C to A at position 266, which results in an Ala78Asp amino acid substitution (Bux et al. 1997).

The NA1 type accounts for more efficient phagocytosis of IgG1- and IgG3-opsonized particles compared to the NA2 variant. The NA2 allele is more frequent in French, German, and U.S. populations, whereas NA1 is more common in Asian populations (Ivan and Colovai 2006; Meisel et al. 2001; Rascu et al. 1997; van Sorge et al. 2003).

Several invesitigators have evaluated the Fcγ polymorphism in chronic, aggressive, and refractory periodontal disease in different ethnic population (Table 4.10).

de Souza and Colombo (2006) determined the frequency of FcγRIIa and FcγRIIIb alleles/genotypes in patients with GAP. It was suggested that the FcγRIIIb-NA2 allele and/or FcγRIIIb-NA2/NA2 genotype and the composite genotype FcγRIIIb-NA2/NA2 plus FcγRIIa-H/H131 may be associated with GAP, whereas FcγRIIIb-NA1 and/or FcγRIIIb-NA1/NA1 may be related to periodontal health in a sample of the Brazilian population. Similar results were obtained by Loos et al. (2003) who suggested that FcγRIIa-H/H131 genotype may

be a putative susceptibility and severity factor, and the FcγRIIIa-V158 allele a putative susceptibility factor for periodontitis in Northern European Caucasians. The allele carriage rate of FcγRIIa-H131 was 79% in aggressive periodontitis, compared to 51% for the control group. Also, the genotype frequency of FcγRIIa-H/H131 was significantly higher in aggressive periodontitis compared with controls (58 vs. 25%). The carriage rate of the FcγRIIIa-V158 allele in aggressive periodontitis was 63%, and therefore higher than the corresponding frequency in the controls. Eleven of the 12 aggressive periodontitis patients (92%) were of the FcγRIIIa-V+ (V/F and V/V158) genotype, while this genotype occurred in only 59% of the controls. The frequency of patients with aggressive periodontitis with both FcγRIIa- H/H131 and IIIa-V+ genotypes (H/H & V+ haplotype) was higher than among controls; 7 of 12 patients with aggressive periodontitis (58%) were genotyped as H/H & V+. It was observed that patients with the FcγRIIa-H/H131 genotype had more teeth with severe periodontal breakdown than patients with the R+ genotype (R/H and R/R131) (Loos et al. 2003).

In patients with CP, Kobayashi et al. (1997) assessed the distribution of FcγRIIa and FcγRIIIb genotypes in Japanese patients with adult periodontitis and race-matched healthy controls. A significant over-representation of the FcγRIIIb-NA2 allotype was found in patients with disease recurrence. Homozygosity for the FcγRIIIb-NA2 allele was found in 22.3% of the 85 patients with, compared to 6.7% in the 15 patients without, recurrence. Moreover, the annual rate of recurrence was significantly higher in patients with the FcγRIIIb-NA2/NA2 and FcγRIIIb-NA1/NA2 genotypes than in FcγRIIIb-NA1/NA1 individuals. It was suggested that the FcγRIIIb-NA2 allotype represents a risk factor for recurrence of adult periodontitis. In an elderly Japanese population, the FcγRIIIb-NA1 allotype was over-represented in the periodontitis-resistant group, compared with the periodontitis group (Sugita et al.1999), while in Caucasian population a significant over-representation of FcγRIIa-H/H131 in the CP patient group compared to the control group (patients vs. controls: 35.1 vs. 19.0%) was observed. Additionally, smokers with FcγRIIa-H/H131 exhibited significantly greater mean clinical attachment loss (3.44 ± 0.16 mm) than those with FcγRIIa-R/H131 (2.91 ± 0.14 mm) and R/R131 (2.82 ± 0.16 mm).

In severe periodontitis cases between 40 and 60 years of age, an over-representation of the FcγRIIIa-V/V158 genotype was observed. The FcγRIIIb genotype distribution showed a tendency towards an over-representation of the FcγRIIIb-NA2/NA2 genotype among individuals with severe BL. The BL, either assessed in terms of severity or extent, was highest among the FcγRIIIa-V/V158 homozygotes, medium in F/V158 heterozygotes, and lowest in F/F158 homozygotes. Even when more stringent limits to distinguish between severe forms of the disease and minor forms were applied, these relationships were still significant for the FcγRIIIa polymorphism (Meisel et al. 2001). A significant over-representation of FcγRIIIa-158V allele in Japanese patients with severe CP compared to patients with moderate CP was reported by Kobayashi et al. (2001). In addition, a strong association between CP severity and FcγR composite genotype comprising FcγRIIIa-158V plus FcγRIIIb-NA2 was found. Patients with CP positive for the composite genotype exhibited more severe signs of periodontitis than composite genotype-negative individuals (Kobayashi et al. 2001).

Efficient clearance of periodontopathic bacteria by neutrophils via IgG1 and IgG3-FcγRIIIb interactions may be crucial for the prevention of periodontitis. FcγRIIIb is the predominant FcγR on neutrophils, which are present in high numbers in GCF and subjacent to the apical part of the pocket epithelium. Serum samples from patients with periodontitis and gingivitis contain significant levels of IgG1 and IgG3 specific for periodontopathic bacteria.

In inflamed human gingiva and crevicular fluid, IgG1 represents the predominant subclass (Sugita et al. 2001). Refractory periodontitis subjects are individuals who fail to respond to a series of periodontal therapies, including mechanical debridement, periodontal surgery, administration of systemic antibiotics and proper maintenance care. A number of possible factors could lead to a subject being refractory to conventional therapy. These include host factors such as an impaired or exacerbated humoral and cellular host response, or environmental factors such as smoking and/or genetic predisposition (Colombo et al. 1998). Previous studies showed the genetically determined polymorphisms of Fc-γRIIIb, NAI and NA2 to be associated with the recurrence of adult periodontitis (OR = 4.3, 95% CI: 1.19–16.24) (Colombo et al. 1998; Kobayashi et al. 1997; Sugita et al. 2001). When the relevance of the FcγRII1b-NA1 allele to resistance to periodontitis was assessed, there were significant overrepresentations of the NAI carrier and the FcγRIIIb-NA1 allele in the periodontitis-resistant group compared to the periodontitis-susceptible group (Sugita et al. 2001).

4.10 Tumor Necrosis Factor-α

Chronic inflammatory bone diseases, such as rheumatoid arthritis, periodontal disease, and aseptic periprosthetic osteolysis, are characterized by BL around affected joints and teeth caused by increased osteoclastic bone resorption. This resorption is mediated largely by the increased local production of proinflammatory cytokines, such as TNF-α. These cytokines may induce resorption indirectly by affecting the production of the essential osteoclast differentiation factor, receptor activator of NF-κB ligand (RANKL), and/or its soluble decoy receptor, osteoprotegerin (OPG), by osteoblast/stromal cells, or directly by enhancing proliferation and/or activity of cells in the osteoclast lineage (Fig. 4.5) (Boyce et al. 2005).

Polymorphisms in the promoter region of the TNF-α gene at positions −238 (G to A) and −308 (G to A) have been reported. In general a lack of association between TNF-α polymorphism and periodontitis has been consistently reported (Craandijk et al. 2002; Folwaczny et al. 2004a; Maria de Freitas et al. 2007) (Table 4.11). However, Soga et al. (2003) reported higher frequency of subjects who carried at least one variant allele in TNF-α −1031, −863, or −857 single-nucleotide polymorphisms in Japanese patients with severe periodontitis than in healthy subjects. Fassmann et al. (2003) have shown that the TNF-α (−308G/A) polymorphism itself showed no association with CP, whereas significant differences existed in the frequencies of the combined genotypes (TNF-α and lymphotoxin α) between the control and the patient groups. Shimada et al. (2004) investigated whether TNF receptor type2 (TNFR2) +587T/G gene polymorphisms were associated with CP. It was found that in 196 nonsmoking Japanese with different severities of CP, the frequency and the positivity of the +587G allele were significantly higher in severe CP than in controls ($P = 0.009$; OR = 2.61, $P = 0.007$; OR = 3.06).

In a recent meta-analysis Nikolopoulos et al. (2008) investigated the possible differential risk for periodontal disease of the TNF-α -308G/A allele. The per-allele relative risk for chronic periodontitis (CP) of the mutant (A) allele was 0.878 (95% CI: 0.635–1.213). Generally, all summary estimates indicated the lack of association between the TNF -α-308G/A polymorphism and the susceptibility to CP. Similar non-significant results were

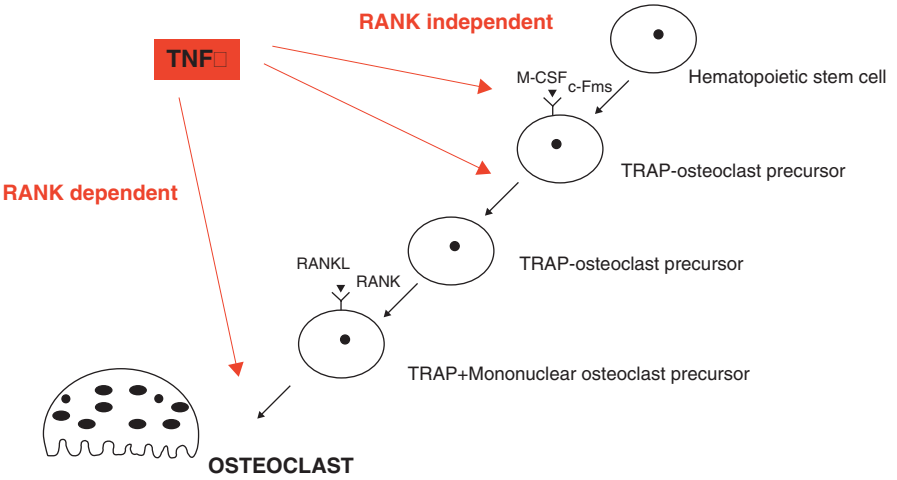

Fig. 4.5 RANK dependent and independent mechanisms of TNF-mediated osteoclastogenesis. During the early stages of osteoclastogenesis, TNF increases the pool size of marrow osteoclast precursors by promoting their proliferation and differentiation in response to M-CSF and by stimulating c-Fms expression, which is independent of the RANK pathway. These osteoclast precursors then differentiate into mature osteoclasts in the presence of RANKL, and this process is accelerated by TNF. The role of TNF at this later stage of osteoclast differentiation is RANKL/RANK dependent (modified and adapted from Boyce et al. 2005) (Reprinted with permission of Keio Journal of Medicine)

observed in the meta-analysis of four studies that evaluated the role of the afore mentioned TNF-α polymorphism on the occurrence of aggressive periodontitis. The pooled OR for the allele comparison was 1.044 with a 95% CI: 0.704–1.550 (Nikolopoulos et al. 2008).

4.11 Human Leukocyte Antigen Polymorphisms

Human leukocyte antigens (HLA) (also called MHC) are involved in genetically predetermined humoral immune response via recognition of foreign antigens. The HLA complex plays an important role in immune responsiveness and may be involved in antigen recognition of periodontal pathogens. In humans, the "classical" MHC Class I molecules (HLA-A, -B, and -C) are expressed on most nucleated cells, while MHC Class II molecules (HLA-DP, -DQ, -DR) are expressed on cells that immunosurvey host cells including B and T cells, macrophages, and accessory cells for the presence of foreign peptides (Nares 2003; Takashiba and Naruishi 2006).

HLA-A, -B, -C and DR antigen frequencies were determined in a group of patients with juvenile periodontitis and rapidly progressive periodontitis by Firatli et al. (1996). In patients with juvenile periodontitis, HLA-A24 and DR4 were found at a significantly higher level than in the control group, and in rapidly progressive periodontitispatients, A9 and DR4 were found at a significantly higher level than the controls. Similar results were obtained by Amer et al. (1988) who reported the presence of HLA-A9 in 36.7% of the

Table 4.11 TNF-α gene polymorphisms in aggressive and chronic periodontitis

References	Periodontits	Subjects	Ethnicity	Smoking status	Association
Soga et al. (2003)	Severe chronic	64 patients with severe adult periodontitis and 64 healthy subjects	Japanese population	Nonsmokers	S
Folwaczny et al. (2004a)	Chronic	81 patients with generalized CP and 80 healthy controls	Caucasian population	Not given	NS
Craandijk et al. (2002)	Chronic	90 patients with periodontitis and 264 reference control subjects	Caucasian and non-Caucasian population	Smokers and nonsmokers	NS
Fassmann et al. (2003)	Chronic	132 patients with CP together with 114 healthy controls	Caucasian population	Smokers and nonsmokers	S
Maria de Freitas et al. (2007)	Aggressive	30 patients with generalized aggressive periodontitis and 70 without periodontal disease	Brazilian population	Nonsmokers	NS
Shimada et al. (2004)	Chronic	58 patients with severe chronic periodontits, 72 with moderate chronic periodontits, 52 controls	Japanese population	Nonsmokers	S
Guzeldemir et al. (2008)	Aggresive	31 patients with localized aggressive periodontitis and 31 healthy controls	Turkish population	Nonsmokers	NS
Trombone et al. (2008)	Chronic	127 patients with CP and 177 control subjects	Brazilian population	Nonsmokers	S
Schulz et al. (2008)	Aggressive chronic	54 patients with CP, 69 with aggressive periodontitis, 52 healthy controls without periodontitis	Caucasian population	Smokers and nonsmokers	NS
Babel et al. (2006)	Chronic	122 patients with chronic periodontitis and 114 controls	Caucasian population	Smokers and nonsmokers	NS
D'Aiuto et al. (2004)	Chronic	94 subjects with periodontitis	Mixed population	Smokers and nonsmokers	S
Menezes and Colombo (2008)	Agressive and chronic	74 patients with chronic periodontitis, 38 with generalized aggressive periodontitis, 51 controls	Brazilian population	Smokers and nonsmokers	NS
Pérez et al. (2004)	Aggresive	27 individuals with aggressive periodontitis, 27 individuals with AP combined with diabetes mellitus, and 27 individuals with diabetes mellitus, 30 controls	Chilean population	Nonsmokers	S
Zhu et al. (2007)	Aggresive	64 agressive periodontitis patients, and 78 controls	Chinese population	Not known	NS

S: Significant; NS: Non-significant

patients with rapidly progressive periodontitis and 2.5% of the elderly subjects with minimal disease (considered as a resistant group). The relative risk for HLA-A9 (previously reported to be associated with periodontal disease) was 15.5. HLA-A10 showed a significantly increased incidence in the resistant group (30.0%) compared to a non-periodontally diagnosed control population (9.0%), and was absent from the patient group (Amer et al. 1988). Reichert et al. (2002) revealed that gender is a confounding variable, which should be considered in further studies of HLA and periodontitis.

The expression of various HLA antigens was investigated by several investigators (Alley et al. 1993; Amer et al. 1988; Bonfil et al. 1999; Machulla et al. 2002; Ohyama et al. 1996; Reichert et al. 2007; Reichert et al. 2008; Stein et al. 2003; Takashiba et al. 1994) (Table 4.12). In a Japanese population with EOP, Ohyaama et al. (1996) has found that DRB1*1401,

Table 4.12 HLA gene polymorphisms in aggressive and chronic periodontitis

References	Periodontits	Subjects	Ethnicity	Smoking status	Association
Alley et al. (1993)	Chronic	Four groups (n = 15) of age- (young adult) and sex-matched Caucasian subjects with or without type 1 diabetes and moderate to severe periodontitis: Group DP = diabetics with periodontitis; Group DnP = diabetics without periodontitis; Group nDP = nondiabetics with periodontitis; and Group nDnP = nondiabetics without periodontitis	Caucasian population	Not known	S
Bonfil et al. (1999)	Aggressive	48 patients with severe periodontitis ("rapidly progressive periodontitis" subgroup) and 55 "controls"	Caucasian population	Smokers and nonsmokers	S
Hodge et al. (1999)	Aggressive	68 patients with EOP and 67 controls	Caucasian population	Smokers and nonsmokers	NS
Machulla et al. (2002)	Chronic and aggressive	50 unrelated German Caucasian patients with RPP and a group of 102 unrelated German Caucasian patients with adult periodontitis are compared with a group of 102 healthy controls	Caucasian population	Not given	S
Ohyama et al. (1996)	Aggressive	24 Japanese patients with EOP	Japanese population	Not known	S
Reichert et al. (2007)	Chronic and aggressive	110 patients with juvenile idiopathic arthritis, 50 patients with generalized aggressive periodontitis, 102 patients with CP, 102 healthy controls	Caucasian population	Smokers and nonsmokers	S
Stein et al. (2003)	Chronic and aggressive	50 patients with generalized aggressive and 102 with CP, 102 healthy controls	Caucasian population	Smokers and nonsmokers	S
Takashiba et al. (1994)	Aggressive	70 patients with EOP periodontitis and 26 healthy controls	Japanese population	Smokers and nonsmokers	S
Roshna et al. (2006)	Aggressive	40 patients with generalized aggressive periodontitis and 80 controls	Indian population	Smokers and nonsmokers	S

S: Significant; NS: Non-significant

DRB1*1501, DQB1*0503, and DQB1*0602 HLA class II alleles were more frequently ("susceptible") found in the EOP patients than in healthy controls. In contrast, DRB1*0405 and DQB1*0401 were found less frequently ("resistant") in EOP patients (Ohyama et al. 1996). Stein et al. (2003) reported that certain HLA markers were significantly positively (HLA-A*11, -A*29, -B*14, -Cw*08, -DRB1*13) and significantly negatively (HLA-A*03, -A*30, -A*30/31 and -DRBblank* (non-DRB3/4/5)) associated with chronic or aggressive periodontitis, respectively (Machulla et al. 2002). Lately, Stein et al. (2003) reported a significantly lower frequency of HLA-DRBblank* homozygosity (non-DRB3*/DRB4*/DRB5*) in CP, whereas HLA-DRB1*15: DRB5*(DR51): DQB1*06 showed a slightly higher homozygosity rate in all patients. As the combination HLA-A*02, A*03 was significantly decreased in aggressive periodontitis, HLA-A*01, A*03 heterozygosity was significantly lowered in CP. Among others, the known positive associations for HLA-A*68/69 (A28) and HLA-DRB1*04 were confirmed by the haplotypes HLA-A*68/69: Cw*07: B*18 in aggressive periodontitis and HLA-Cw*08: B*14: DRB1*04 in CP (Stein et al. 2003). In contrast, Hodge et al. (1999) found no association with regard to the occurrence of EOP and the presence of the *Bam*HI site in the HLA-DQB1 in European Caucasians.

Reichart et al. (2007) evaluated the common HLA associations in patients with juvenile idiopathic arthritis ($n = 110$), in patients with GAP ($n = 50$), and in patients with CP ($n = 102$) in comparison to healthy controls (no periodontitis, no arthritis ($n = 102$)). It was found that, in comparison with the controls, HLA-DRB3n occurred more frequently in both females suffering from juvenile idiopathic arthritis (74.58 vs. 54.54%, p50.024) and females suffering from CP (73.02 vs. 54.54%, p50.035). Furthermore, among patients with juvenile idiopathic arthritis, an increased OR for attachment loss was found in subjects who expressed HLA-An01 (OR = 4.6, $P = 0.014$) or HLA-An01: DRB3n (O = 54.3, $P = 0.031$).

A recent meta-analysis performed by Stein et al. (2008) in Caucasian population showed no significant HLA associations in patients with chronic periodontitis. Patients with aggressive periodontitis showed a positive association with HLA-A9 (OR = 2.59, 95% CI: 1.36-4.83, P = 0.004) and HLA-B15 (OR = 1.90, 95% CI: 1.15-3.16, $P = 0.01$) as well as a negative association with HLA-A2 (OR = 0.72, 95% CI: 0.56-0.94, $P = 0.01$) and -B5 (OR = 0.49, 95% CI: 0.30-0.79, $P = 0.004$). No significant associations were found with HLA class II antigens (Stein et al. 2008).

4.12 CD14 Gene Polymorphisms

CD14, a glycoprotein localized on the cell surfaces of myeloid cells, functions as a pattern recognition receptor for various bacterial products, such as LPS. It has been reported to be expressed on neutrophils, monocytes/macrophages, and fibroblasts, all of which are present in periodontitis lesions. CD14 interacts exclusively with bacterial LPS, which is bound with high affinity to the systemically circulating LPS-binding protein (Fig. 4.6). The signal transduction of the LPS/LBP (LPS-binding protein)/CD14 ternary complex on effector cells is then transferred via the toll-like receptor (TLR)4/MD-2. Upon stimulation, the TLR4/MD-2 complex leads to the activation of innate host defense mechanisms via the nuclear factor κB pathway and the release of proinflammatory cytokines, i.e., TNF-α,

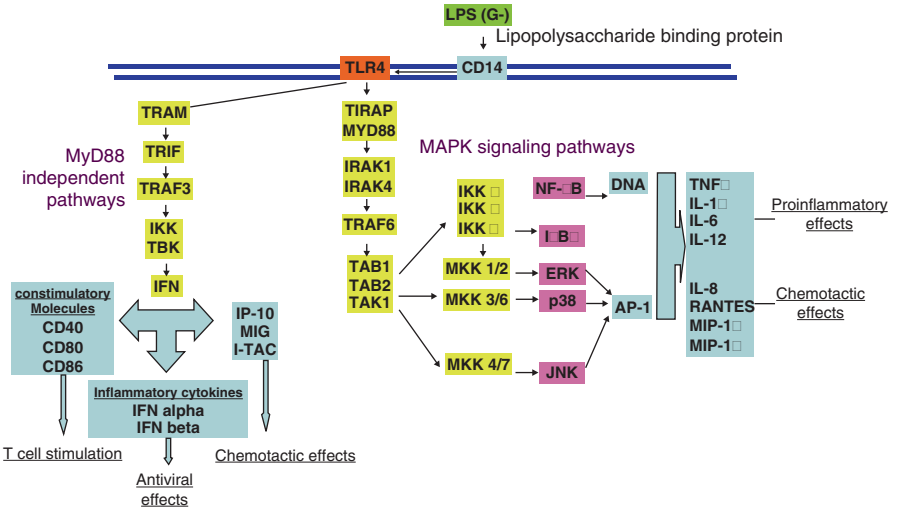

Fig. 4.6 Toll-like receptor signaling pathway (adapted from http://www.genome.ad.jp). All TLRs share a common cytoplasmic signaling domain, the Toll-interleukin 1 (IL-1) receptor domain (TIR domain). This domain mediates association between TLRs and a family of TIR domain-containing adaptors. The best characterized of these is MyD88, a cytoplasmic protein comprising an N-terminal death domain and a C-terminal TIR domain. MyD88 is used by most, if not all, TLRs. The death domain of MyD88 recruits members of the IL-1 receptor-associated kinases IRAK-1 and IRAK-4. After being recruited, these kinases are autophosphorylated, leading to association with TRAF6, an E3 ligase. Through an undefined mechanism, TRAF6 mediates the activation of MAP kinases as well as the IκB kinases IKKα and IKKβ. The result is the activation of AP-1 and NF-κB transcription factors and expression of a wide variety of proinflammatory genes. In addition, a MyD88-independent pathway activates the transcription factor IRF-3 and expression of type I interferons (Barton and Medzhitov 2004). *TRAM* toll-like receptor adaptor molecule; *TRAF* TNF receptor-associated factor; *TRAF3* TNF receptor-associated factor 3; *IBKK* inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon; *TBK* TANK-binding kinase 1; *IRF* interferon regulatory factor; *TIRAP* toll-interleukin 1 receptor (TIR) domain containing adaptor protein; *MYD88* myeloid differentiation primary response gene (88); *IRAK1* interleukin-1 receptor-associated kinase 1; *TRAF6* TNF receptor-associated factor 6; *IKK* inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta; *MKK* mitogen-activated protein kinase kinase 3; *IkBa* nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; *NKkB* nuclear factor of kappa light polypeptide gene enhancer in B-cells 1; *TNF* tumor necrosis factor; *IL* interleukin; *RANTES* regulated upon activation, normal T-cell expressed, and secreted.

IL-1β, IL-6, and IFN-γ (Folwaczny et al. 2004c; Yamazaki et al. 2003). The gene for the CD14 receptor is on chromosome 5 (region q23–21), consists of ≈3,900 bp organized in two exons, and encodes a protein of 375 amino acids (Ferrero and Goyert 1988).

Previous data have shown the strong relevance of the CD14 receptor activity in the clinical manifestation and development of periodontitis (Folwaczny et al. 2004c) (Table 4.13). The systemic level of the soluble form of CD14 (sCD14) is significantly increased in patients with periodontal disease (Hayashi et al. 1999), while in contrast the CD14 expression within the periodontal tissue was found to be negatively correlated with the amount of attachment loss (Jin and Darveau 2001). Significantly lower levels

Table 4.13 CD14 gene polymorphisms in aggressive and chronic periodontitis

References	Periodontits	Subjects	Ethnicity	Smoking status	Association
Holla et al. (2002a)	Chronic	135 patients with CP and 207 unrelated randomly selected white subjects who did not have a clinical history of periodontal disease	Czech population	Smokers and nonsmokers	S
Yamazaki et al. (2003)	Chronic	163 subjects with periodontitis and in 104 healthy controls	Japanese population	Nonsmokers	NS
Folwaczny et al. (2004c)	Chronic	70 patients with periodontal disease and 75 healthy controls	Caucasian population	Not given	S
Donati et al. (2005)	Chronic	60 patients with severe and generalized CP and 39 healthy controls	Caucasian population	Smokers and nonsmokers	S
Tervonen et al. (2007)		51 subjects with moderate to severe CP and 178 healthy controls	Caucasian population	Smokers and nonsmokers	S
James et al. (2007)	Chronic and aggressive	73 subjects with aggressive periodontitis, 95 subjects with CP, 95 healthy controls	Caucasian population	Smokers and nonsmokers	NS
Laine et al. (2005)	Chronic	100 patients with severe periodontitis and from 99 controls	Caucasian population	Smokers and nonsmokers	S
Donati et al. (2008)	Chronic	53 e subjects with generalized and severe chronic periodontitis	Caucasian population	Smokers and nonsmokers	NS
Schulz et al. (2008)	Aggressive and chronic	133 periodontitis patients (60 chronic; 73 aggressive) and 80 controls	Caucasian population	Smokers and nonsmokers	NS

S: Significant; NS: Non-significant

of the sCD14 protein were observed at sites with advanced attachment loss, indicating a protective effect for CD14. Moreover, a reduced expression of CD14 on monocytes was suggested to be linked to an increasing susceptibility for EOP (Buduneli et al. 2001; Folwaczny et al. 2004c).

Several studies have investigated the CD14 polymorphism in chronic and aggressive periodontitis (Folwaczny et al. 2004c; Holla et al. 2002a; Yamazaki et al. 2003). In a Caucasian population, Holla et al. (2002a) revealed that the frequency of the G allele in the −1359G > T polymorphism was higher in patients with severe disease than moderate disease. The homozygous genotype of this polymorphism was found to be significantly increased in the severe patient group. Allele and/or genotype frequencies of the −159C > T polymorphism did not differ significantly between severe and moderate periodontitis patients. However, they detected a tendency for an increased frequency of the −159T/T homozygotes in patients with severe periodontitis (19.2%) compared to the moderate subgroup (8.3%). In a Japanese study, no association was found between the CD14 −159C > T polymorphism and periodontitis. In contrast, there was a statistically significant difference in genotype distribution between young (under 35 years of age) and older (over 35 years of age) subjects and between young subjects and older subjects with advanced disease (Yamazaki et al. 2003).

In a Caucasian population, the CD14 −159T allele was prevalent in 39.3% of periodontitis patients and in 48.0% of the healthy control subjects. The overall allele frequencies were not significantly different between both study populations. However, after stratification of

the cohort according to gender, the prevalence of the CD14 −159T allele among females was 33.3% for periodontitis patients and 55.6% for control individuals. Among male subjects, the prevalence of the CD14 −159T allele was 45.6% as compared to 43.7% for healthy controls (Folwaczny et al. 2004c). Donati et al. (2005) demonstrated that the CD14 −159C > T gene polymorphism, however, revealed significant differences between test and control groups. Thus, the proportion of subjects that exhibited the TT genotype was significantly smaller in the group with severe periodontitis than in periodontal healthy group. Further, the C allele carriage was 90% in the periodontitis group and significantly higher than in the healthy control group (72%). It was therefore suggested that the CD14 −159C > T gene polymorphism is associated with CP in Caucasian subjects of a north European origin.

More recently, Tervonen et al. (2007) showed that the extent of periodontal disease was higher in subjects with the T-containing genotype (TT and CT) of CD14 −260C > T and the GG genotype of IL-6 −174C > G when compared to the extent in the rest of the group. Subjects carrying the composite genotype of the above two were most severely affected by periodontal disease.

Recently, Stein et al. (2008) performed a systematic literature review and a meta-analysis focusing on the distribution of HLA class I and II phenotypes in Caucasian patients with chronic periodontitis (CP) and aggressive periodontitis (AP). In patients with CP, no significant HLA associations were found. Patients with AP showed a positive association with HLA-A9 (OR = 2.59, 95% CI: 1.36–4.83, $P = 0.004$] and HLA-B15 (OR = 1.90, 95% CI: 1.15–3.16, $P = 0.01$) as well as a negative association with HLA-A2 (OR = 0.72, 95% CI: 0.56–0.94, $P = 0.01$) and -B5 (OR = 0.49, 95% CI: 0.30–0.79, $P = 0.004$). On grouping all patients into one periodontitis group (AP1CP), the same deviations were confirmed with higher statistical significance. For HLA-A9 and -B15, significant heterogeneity was found between the studies. No significant associations were found with HLA class II antigens. It was concluded that HLA-A9 and -B15 seem to represent susceptibility factors for AP whereas HLA-A2 and -B5 are potential protective factors against periodontitis among Caucasian.

4.13 Toll like receptor, TLR2 and TLR4 Gene Polymorphisms

Toll was first discovered as a Drosophila gene. The gene product is a transmembrane protein, functioning as a signal transduction receptor. TLRs are mammalian homologs of Toll. TLRs are members of the superfamily of Toll/IL-1 receptors (TIR) with two important domains: leucine-rich repeats (LRR) and TIR domain. The intracellular TIR domain, which contains 200 amino acids, has an important role in downstream signaling box; the extracellular LRR, which contains 24–29 amino acids repeats, has an important role in ligand recognition (Barton and Medzhitov 2004; Huang et al. 2008).

TLRs are important initiators of innate immunity, recognizing diverse microbial products which are collectively known as pathogen-associated molecular patterns (PAMPs). Signaling pathways are then activated that culminate in the induction of proinflammatory proteins that trigger both innate and adaptive immunity (Fig. 4.6) (Doyle and O'Neill 2006).

There are 10 TLRs, named TLRs 1–10, known in mammals, and each of these receptors recognizes molecules derived from a unique class of microbial agents. TLR4 recognizes

LPS, which is unique to Gram-negative bacteria; TLR2 recognizes peptidoglycan, which is abundant in Gram-positive bacteria; and TLR3 recognizes double-stranded RNA from double-stranded and negative-stranded viruses. TLR7 and 8 recognize RNA from single-stranded viruses, and TLR9 recognizes unmethylated CpG DNA found abundantly in prokaryotic genomes and DNA viruses (Pasare and Medzhitov 2003, 2004a, b). Ligation of TLRs by their PAMPs results in different effector responses depending on the cell type involved. Four adapter molecules are known to be involved in signaling. These proteins are known as MyD88, Tirap (also called Mal), Trif, and Tram. The adapters activate other molecules within the cell, including certain protein kinases (IRAK1, IRAK4, TBK1, and IKKi) that amplify the signal, and ultimately lead to the induction or suppression of genes that orchestrate the inflammatory response. In terms of signaling, one thing all TLRs have in common is that every TLR tested so far leads to NF-κB activation. Once activated by a PAMP, a TLR4 triggers a cascade of cellular signals, culminating in the eventual activation of NF-κB which binds to a discrete nucleotide sequence in the upstream regions of genes that produce proinflammatory cytokines, such as TNF-α, IL-1 and IL-2, thereby regulating their expression. The release of these cytokines and cytokines such as IFN-γ is the hallmark of the cellular response to the activation of the innate immune system (Doyle and O'Neill 2006).

Enhanced expression of CD14 and TLR4 on inflammatory human gingival fibroblasts was revealed (Wang et al. 2000, 2001, 2003). The variance in human epithelial cell TLRs, linked with Asp299Gly carriage, which results in a hyporesponsive epithelial cell phenotype less susceptible to Gram-negative diseases and associated systemic conditions, was emphasized (Kinane et al. 2006).

Several studies have attempted to associate the TLR polymorphisms with periodontitis, but controversial results were obtained (Table 4.14). Schröder et al. (2005) reported that TLR-4 single-nucleotide polymorphisms (Asp299Gly and Thr399Ile) were correlated with CP (OR = 5.56, 95% CI: 2.19–14.04, $P < 0.0001$), but not with aggressive periodontitis. In a Japanse population case–control study, Fukusaki et al. (2007) revealed that the frequency of the C/C genotype in TLR4 3725G > C polymorphism was significantly higher in both the moderate and the severe periodontitis patient group than in the control group. James et al. (2007) demonstrated a significant association between the carriage of the TLR4 Asp299Gly polymorphism and aggressive periodontitis in young Caucasian adults, of exclusively Western European origin, living in the United Kingdom. Subjects with aggressive periodontitis carried this allele Gly299 significantly less frequently than a population-based control group. The carriage of this allele Gly299 was in some way protective against the development of aggressive periodontitis. Brett et al. (2005) has found that, for the TLR-4 (−399) gene polymorphism, the only statistically significant difference was between total subjects with periodontal disease (aggressive/chronic) and controls.

4.14 CARD15/NOD2 Gene Polymorphisms

Nucleotide-binding oligomerization domain (NOD) receptors, including NOD1, NOD2, and ICE protease activating factor (IPAF), are cytoplasmic receptors for recognition of microbial products. These receptors either detect organisms that enter the cytoplasm of the

Table 4.14 TLR2 and TLR4 gene polymorphisms in aggressive and chronic periodontitis

References	Periodontits	Subjects	Ethnicity	Smoking status	Association
Ilzakovicová Hollá et al. (2007)	Chronic	171 patients with CP and 218 unrelated controls	Caucasian population	Smokers and nonsmokers	NS
Laine et al. (2005)	Chronic	100 adult patients with severe periodontitis and 99 periodontally healthy controls	Caucasian population	Smokers and nonsmokers	NS
Fukusaki et al. (2007)	Chronic	97 patients with CP and 100 control subjects	Japanese population	Not given	S
James et al. (2007)	Chronic and aggressive	73 subjects with aggressive periodontitis, 95 subjects with CP, 95 healthy controls	Caucasian population	Smokers and nonsmokers	NS chronic S aggressive
Brett et al. (2005)	Chronic and aggressive	51 aggressive periodontitis patients, 57 CP patients, 100 unrelated healthy individuals of unknown periodontal status as controls	Caucasian population	Smokers and nonsmokers	S chronic/ aggressive
Schröder et al. (2005)	Chronic and aggressive	197 individuals suffering from generalized periodontitis	Caucasian population	Smokers and nonsmokers	S chronic NS aggressive
D'Aiuto et al. (2004b)	Chronic	94 subjects with severe generalized periodontitis	Mixed population	Smokers and nonsmokers	NS
Berdeli et al. (2007)	Chronic	83 patients with CP and 106 periodontally healthy subjects	Caucasian population	Smokers and nonsmokers	NS
Emingil et al. (2007)	Aggressive	90 patients with generalized aggressive periodontitis and 155 periodontally healthy subjects	Turkish population	Smokers and nonsmokers	NS
Zhu et al. (2008)	Chronic and aggressive	40 patients with generalized aggressive periodontitis, 50 patients with CP, and 100 periodontally healthy controls	Chinese population	Not known	NS
Imamura et al. (2008)	Chronic	43 patients with periodontitis and 49 healthy controls	Japanese population	Not known	NS
Folwaczny et al. (2004d)	Chronic	122 patients with periodontitis and 122 healthy controls	Caucasian population	Not known	NS

S: Significant; NS: Non-significant

cell, such as the intracellular bacterium *Shigella flexneri*, or detect products that may be released or transported into the cytoplasm by processes such as phagocytosis and degradation of microbes. NOD1 and NOD2 recognize peptidoglycan from bacterial cell walls. A conserved structure within peptidoglycan called muramyl dipeptide (MDP) is the specific activating ligand for NOD2. Ligand binding by NOD1 or NOD2 leads to NACHT [NAIP (neuronal apoptosis inhibitory protein), CIITA (MHC class II transcription activator), HET-E (incompatibility locus protein from *Podospora anserina*), and TP1 (telomerase-associated protein)-conserved] domain-mediated homo-oligomerization of the proteins, leading to recruitment of caspase recruitment domain (CARD)-containing adapter

molecules that are responsible for signaling. NOD1 and NOD2 interact with receptor-interacting protein 2 (RIP2), also called RICK, a protein consisting of a kinase domain and a CARD. RIP2 directly interacts with inhibitor of NF-κB kinase γ (IKKγ), leading to ubiquitinylation of IKKγ and activation of the kinase activities of IKKα and IKKβ. This results in activation of proinflammatory responses mediated by NF-κB (Ferrero-Miliani et al. 2007; Underhill 2007) (Fig. 4.7).

The NOD2 gene is linked to inflammatory diseases such as Crohn's disease (one of inflammatory bowel disease), Blau syndrome, and early onset sarcoidosis (Franchi et al. 2008). The NOD2 protein encoded by the CARD15 gene (originally NOD2 gene) is located on chromosome 16, and is predominantly expressed in peripheral monocytes (Fig. 4.8). The gene is characterized by two amino-terminal CARDs, a nucleotide (ATP or GTP)-binding domain, and a distal LRR region (Ferrero-Miliani et al. 2007).

A clear mRNA expression of NOD1 and NOD2 was revealed in gingival fibroblasts (Uehara and Takada 2007) and in oral epithelial cells (Sugawara et al. 2006). However, despite the fact that thses genes seem good candidates for their association with

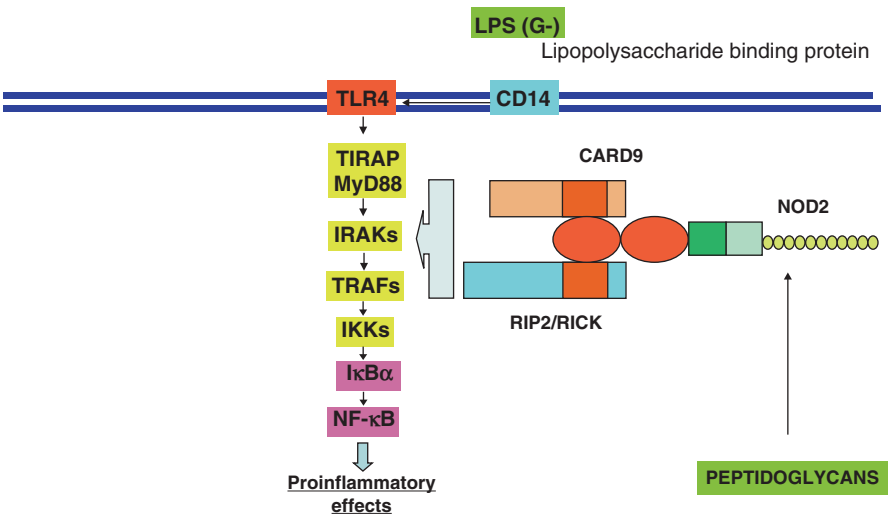

Fig. 4.7 Model for activation of NOD2 signaling pathways (modified and adapted from Underhill 2007) (Reprinted with permission of John Wiley & Sons, Inc.)

Fig. 4.8 Structure of NOD2. NOD2 is comprised of two successive N-terminal caspase recruitment domain (CARD) domains; a central NOD-like receptor (NACHT) (NOD) domain, followed by a NACHT association domain (NAD) and C-terminal leucine-rich repeats (LRRs) (modified and adapted from Underhill 2007) (Reprinted with permission of John Wiley & Sons, Inc.)

Table 4.15 CARD15/NOD2 gene polymorphisms in aggressive and chronic periodontitis

References	Periodontits	Subjects	Ethnicity	Smoking status	Association
Laine et al. (2004)	Chronic	104 Dutch Caucasian patients with severe adult periodontitis and in 97 healthy controls	Caucasian population	Smokers and nonsmokers	NS
Folwaczny et al. (2004b)	Chronic	80 patients with chronic periodontal disease and 122 healthy controls	Caucasian population	Not given	NS
Noack et al. (2006)	Aggressive	86 generalized aggressive periodontitis patients in comparison with 67 healthy controls	Caucasian population	Smokers and nonsmokers	NS

S: Significant; NS: Non-significant

periodontitis, the *CARD15* polymorphisms (3020insC and C2104T), which were reported in patients with Crohn's disease have not been associated with periodontitis (Laine et al. 2004; Folwaczny et al. 2004b; Noack et al. 2006) (Table 4.15).

4.15 *N*-Formyl-L-Methionyl-L-Leucyl-L-Phenylalanine Receptor Polymorphisms

Human neutrophils play a key role in host defense against bacterial infections. Neutrophils are activated by the bacterial formyl peptide *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP). FMLP binds to a specific formyl peptide receptor (FPR). These receptors are seven transmembrane pertussis toxin-sensitive G protein-coupled receptors. Ligand binding to FPR activates a number of downstream effector enzymes including phospholipase C, catalyzing the cleavage of phosphatidylinositol 4,5-biphosphate into secondary messengers inositol 1,4,5-triphosphate and diacylglycerol leading to calcium mobilization and activation of protein kinase C. FPR ligation has also been shown to signal through the small G protein Cdc42 to activate Rac- and ARP2/3-dependent pathways leading to actin nucleation. The culmination of these signaling events triggers morphological and biochemical alterations including polarization of the actin cytoskeleton, activation of various integrins, and directed migration. FPR signaling also initiates production of superoxide and arachidonic acid metabolites and induces degranulation. These events allow phagocytic leukocytes to locate, sequester, and destroy invading microorganisms (Wenzel-Seifert et al. 1998; VanCompernolle et al. 2003).

FPR was identified in 1990 by Boulay et al. from a differentiated HL-60 myeloid leukaemia-cell cDNA library. Two additional human genes, designated FPRL1 and FPRL2 (FPR like-2), were subsequently isolated by low-stringency hybridization using FPR cDNA as a probe and shown to cluster with FPR on human chromosome 19q13.3. It is generally

accepted that FPRL1 shares signal transduction features with FPR, since both receptors are sensitive to pertussis toxin (PTX) and possess a high degree of amino acid identity in the cytoplasmic signaling domains. As FPR, or an antigenically similar receptor, is widely localized in different human tissues and organs, including the thyroid, adrenals, liver, and nervous system, it can be speculated that FPR may have additional functions to host defence against bacterial infection. FPRL1 is expressed in an even greater variety of cell types in addition to phagocytic leukocytes, including hepatocytes, epithelial cells as well as T and microvascular endothelial cells (Selvatici et al. 2006).

Localized aggressive periodontitis is a disease characterized by rapid loss of alveolar bone in teeth of otherwise healthy patients. Neutrophils from patients with localized aggressive periodontitis have been shown to exhibit diminished chemotaxis and low levels of FPR surface expression.

Severe functional defects in FPR-110Phe–>Ser and FPR-126Cys–>Trp were associated with LJP (Perez et al. 1991; Seifert and Wenzel-Seifert 2001). Jones et al. (2003) transfected Chinese hamster ovary cells with wtFPR, FPR-110Phe–>Ser, FPR-126Cys–>Trp, or FPR-110Phe–>Ala mutants and determined their surface expression of FPR, their ligand binding affinity, their G-protein coupling, and their chemotaxis toward FMLP. FPR-110Phe–>Ser mutants failed to show any significant surface expression or chemotaxis toward FMLP. FPR-126Cys–>Trp mutants exhibited slightly lower than normal binding affinity, markedly lower G-protein coupling response, and markedly lower chemotaxis toward FMLP than that observed with wtFPR. The FPR-110Phe–>Ala mutant demonstrated markedly lower surface expression, normal ligand binding affinity, markedly lower G-protein coupling, and markedly lower chemotaxis toward FMLP. It was concluded that the FPR-110Phe–>Ser polymorphism appears to be more defective than the FPR-126Cys–>Trp polymorphism, indicating that patients with the former polymorphism might be expected to exhibit a more severe form of aggressive periodontitis.

Only three investigators have evaluated the association between FMLP receptor polymorphisms and aggressive periodontitis. Gwinn et al. (1999) has reported an association between localized aggressive periodontitis with two polymorphisms of FRP (Phe110Ser and Cys126Trp). On contrary, Zhang et al. (2003) could not demonstrate in African American patients that two FPR gene polymorphisms (c.329T > C [Phe110Ser] and +378C > G [Cys126Trp]) play an etiologic role in aggressive periodontitis, but it was suggested that single-nucleotide polymorphisms in the second extracellular loop may be etiologically important. In a Japanese population, Gunji et al. (2007) performed an association study with 49 patients with aggressive periodontitis and 373 controls using 30 variations identified by sequencing the 21.1 kb gene region. Five polymorphisms (−12915C > T, −10056T > C, −8430A > G, 301G > C, and 546C > A) showed significant association with aggressive periodontitis. Polymorphonuclear neutrophils from subjects carrying the −12915T allele expressed significantly lower levels of FPR1 transcripts than those homozygous for the −12915C allele. Furthermore, the −12915T allele decreased activity of transcriptional regulation in a luciferase assay. Haplotype association analysis with three SNPs (−12915C > T, 301G > C, and 546C > A) revealed that one haplotype (−12915T-301G-546C) was significantly represented in patients with aggressive periodontitis ($P < 0.0001$).

4.16 Matrix Metalloproteinase Polymorphisms

Matrix metalloproteinases (MMPs) are a large family of calcium-dependent zinc-containing endopeptidases, which are responsible for the tissue remodeling and degradation of the extracellular matrix (ECM). There are 23 human MMP genes (Table 4.16) (Somerville et al. 2003). Before this genetic diversity was known, it was customary to divide the MMPs into collagenases, gelatinases, stromelysins, elastases, and others on the basis of the known substrates, but as some MMPs have overlapping substrate specificities, the boundary between the previously used enzyme classes is now blurred. Nevertheless, the trivial names are often useful, particularly if they reflect a function or a distinct structural feature or location, and they have therefore been retained (Somerville et al. 2003).

MMPs are excreted by a variety of connective tissue and proinflammatory cells including fibroblasts, osteoblasts, endothelial cells, macrophages, neutrophils, and lymphocytes. These enzymes are expressed as zymogens, which are subsequently processed by other proteolytic enzymes (such as serine proteases, furin, plasmin, and others) to generate the active forms. Under normal physiological conditions, the proteolytic activity of the MMPs is controlled at any of the following three known stages: activation of the zymogens, transcription, and inhibition of the active forms by various tissue inhibitors of MMPs (TIMPs). In pathological conditions this equilibrium is shifted toward increased MMP activity leading to tissue degradation (Verma and Hansch 2007).

Most of the MMPs consist of four distinct domains, which are N-terminal pro-domain, catalytic domain, hinge region, and C-terminal hemopexin-like domain. This may be responsible for the macromolecular substrate recognition as well as for interaction with TIMPs. The membrane-type MMPs (MT-MMPs) contain an additional transmembrane domain that anchors them in the cell surface. The active site in catalytic domain consists of two distinct regions: a groove in the protein surface centered on the catalytic zinc ion and an S1′ specificity site that varies considerably among members of the family. Bound inhibitors adopt extended conformations within the groove, make several β-structure-like hydrogen bonds with the enzyme, and provide the fourth ligand for the catalytic zinc ion (Verma and Hansch 2007).

In keeping with their potential for tissue destruction, MMPs are stringently regulated at multiple levels, including transcription, activation of the zymogen forms, extracellular inhibitors, location inside or outside the cell, and internalization by endocytosis. Four classes of metalloproteinase inhibitors are found in extracellular spaces and body fluids, which have broad inhibitory activity against many MMPs. One class is the TIMPs, which are disulfide-bonded proteins of 20–30 kDa that directly interact with the MMP active site through a small number of their amino acids (Somerville et al. 2003). At present, the selectivity of the most known MMP inhibitors relies on the two dominant molecular features: (a) chelating moiety that interacts with the catalytic zinc ion and (b) hydrophobic extensions protruding from the catalytic site into the large and hydrophobic S1′ pocket (P1′ group). Since the structural differences between MMP families occur mainly in the S1′ subsite, modifications of the P1′ group have been utilized to introduce inhibitor selectivity have been utilized (Li and Anderson 2003; Verma and Hansch 2007). An unrelated small

Table 4.16 Classification of matrix metalloproteinase enzymes (modified from Somerville et al. 2003)

MMP No.	Class	Enzyme	Substrates
MMP-1	Collagenases	Collagenase-1	Collagen types I, II, III, VII, VIII, X, gelatin Aggrecan, casein, nidogen, serpins, versican, perlecan, proteoglycan link, protein, and tenascin
MMP-8		Collagenase-2	Collagen types I, II, III, V, VII, VIII, X, and gelatin Aggrecan, laminin, and nidogen
MMP-13		Collagenase-3	Collagen types I, II, III, IV, V, IX, X, XI, and gelatin Aggrecan, fibronectin, laminin, perlecan, and tenascin
MMP-18			
MMP-2	Gelatinases	Gelatinase-A	Collagen types I, IV, V, VII, X, XI, XIV, and gelatin Aggrecan, elastin, fibronectin, laminin, nidogen, proteoglycan link protein, and versican
MMP-9		Gelatinase-B	Collagen types IV, V, VII, X, and XIV Fibronectin, laminin, nidogen, proteoglycan link protein, and versican
MMP-3	Stromelysins	Stromelysin-1	Collagen types II, IV, IX, X, and gelatin Aggrecan, casein, decorin, elastin, fibronectin, laminin, nidogen, perlecan, proteoglycan, proteoglycan link protein, and versican
MMP-10		Stromelysin-2	Collagen types III, IV, V, and gelatin Fibronectin, laminin, and nidogen
MMP-11		Stromelysin-3	Laminin
MMP-7	Matrilysins	Matrilysin-1	Collagen types I, II, III, V, IV, and X Aggrecan, casein, elastin, enactin, laminin, and proteoglycan link
MMP-26		Matrilysin-2, endometase	Collagen type IV and gelatin Casein, fibrinogen, and fibronectin
MMP-14	MT-MMP (membrane type)	MT1-MMP	Collagen types I, II, III, and gelatin Aggrecan, dermatan sulphate, proteoglycan, fibrin, fibronectin, laminin, nidogen, perlecan, tenascin, transglutaminase and vitronectin
MMP-15		MT2-MMP	Collagen types I, II, III, and gelatin Aggrecan, fibronectin, laminin, nidogen, perlecan, tenascin, and vitronectin
MMP-16		MT3-MMP	Collagen types I, III, and gelatin Aggrecan, casein, fibronectin, laminin, and vitronectin

(*continued*)

Table 4.16 (continued)

MMP No.	Class	Enzyme	Substrates
MMP-17		MT4-MMP	Gelatin Fibrin and fibronectin
MMP-24		MT5-MMP	Gelatin Chondroitin sulfate, dermatin sulfate, and fibronectin
MMP-25		Leukolysin, MT6-MMP	Collagen type IV and gelatin
MMP-12	Other enzymes	Macrophage metalloelastase	Elastin
MMP-19		RASI-1	Collagens types I, IV, and gelatin Aggrecan, casein, fibronectin, laminin, nidogen, and tenascin
MMP-20		Enamelysin	Aggrecan, amelogenin, and cartilage oligomeric protein
MMP-21		X-MMP	
MMP-23		CA-MMP	Gelatin
MMP-28		Epilysin	Casein

inhibitor derived by proteolysis of the procollagen C-proteinase enhancer has structural similarity to TIMPs and may inhibit MMPs through a similar mechanism. Recently, a membrane-anchored molecule, reversion-inducing cysteine-rich protein with Kazal motifs (RECK), has been discovered that appears to regulate MMP-2, MMP-9, and MMP-14 post-transcriptionally by affecting secretion and activation as well as by inhibition of the active site. In the circulation, the protease inhibitor -2-macroglobulin inactivates active MMPs by a "bait and trap" mechanism: when protease-sensitive sites within the inhibitor are cleaved, it springs closed around the proteinase and isolates it from potential substrates (Somerville et al. 2003).

MMPs are usually minimally expressed in normal physiological conditions and thus homeostasis is maintained: angiogenesis, apoptosis, bone remodeling, cervical dilation, embryonic development, hair follicle cycling, immune response, inflammation, nerve growth, organ morphogenesis, ovulation, postpartum uterine involution, wound healing. Overexpression of MMPs results in an imbalance between the activity of MMPs and TIMPs, which can lead to a variety of pathological disorders: arthritis, multiple sclerosis, Alzheimer's disease, nephritis, atherosclerosis, neurological disease, breakdown of blood-brain barrier, osteoarthritis, cancer, periodontal disease, cardiovascular disease, rheumatoid, endometrial cycling, central nervous system disorders, skin ulceration, corneal ulceration, Sorby's fundus disease, emphysema, vascular disease, fibrotic lung disease, gastric ulcer, Guillian-Barre disease, liver cirrhosis, liver fibrosis, and metastasis (Mart 2002; Verma and Hansch 2007).

MMP genes are structurally similar to each other, indicating that they evolved by duplication of a common ancestral gene followed by divergent evolution. A number of MMP genes (MMP1, MMP3, MMP7, MMP8, MMP10, MMP12, MMP13, MMP20, and MMP26) are found in a cluster on human chromosome 11 (11q21–23) (Somerville et al. 2003).

MMPs are one of the most important groups of enzymes involved in periodontal connective tissue destruction (Reynolds et al. 1994; Uitto et al. 2003). It was demonstrated that the MMPs collagenase, gelatinase A, stromelysin-1, and their specific inhibitor TIMP-1 can all be detected in gingival tissues by immunological assays, both from patients with periodontitis and from patients undergoing crown-lengthening procedures. Cells secreting MMPs and TIMP-1 were often observed at sites that histologically showed signs of connective tissue remodeling. Stromelysin-1 synthesis was observed by cells subjacent to the oral epithelium and could sometimes be found bound to the matrix within central connective tissue adjacent to strongly positive cells, within epithelial cells (not shown), and adjacent to inflammatory foci. Cells positive for collagenase were seen next to inflammatory cell foci, subjacent to the oral epithelium, and within oral epithelial cells. Gelatinase A was also seen subjacent to oral epithelium as well as in other zones. Although MMPs could be identified in inflammatory cell foci from hematoxylin- and eosin-stained sections, no gelatinase B (92-kDa gelatinase) was seen within intracellular granules, in either cultured or uncultured tissues (Reynolds et al. 1994).

Periodontitis, an inflammatory disease of the tooth-supporting tissues involving complex host-bacteria interactions, is initiated by an overgrowth of specific Gram-negative anaerobic bacteria. This mixed bacterial infection leads to gingival connective tissue destruction and irreversible alveolar bone resorption. The continuous high secretion of MMPs such as MMP-2, MMP-3, MMP-8, and MMP-9 by host cells following stimulation by periodontopathogens contributes to periodontal tissue destruction (Romanelli et al. 1999; Zhou and Windsor 2006). Investigators have detected several of the 25 types of MMPs at elevated levels in inflamed human gingivae (Dahan et al. 2001; Uitto et al. 1998), saliva (Miller et al. 2006), and GCF (Alpagot et al. 2001; Chen et al. 2000; Figueredo et al. 2004; Ilgenli et al. 2006; Kiili et al. 2002; Kinane et al. 2003; Mäntylä et al. 2003, 2006; Okuda et al. 2001; Pozo et al. 2005; Söder et al. 2002, 2006; Tüter et al. 2005) of subjects with periodontitis than in healthy subjects

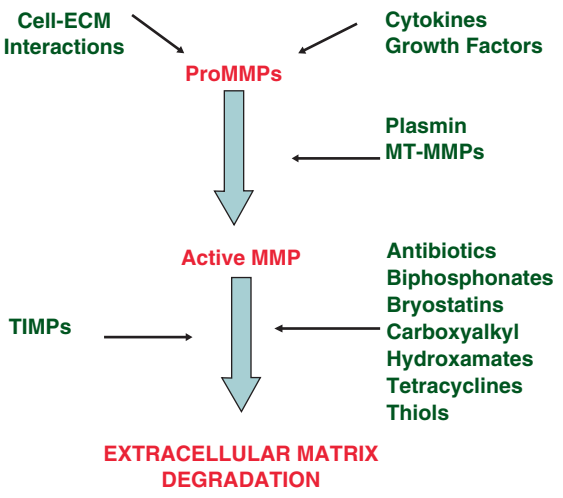

Fig. 4.9 Schematic illustration of representative inhibitors of MMP synthesis and activity

Table 4.17 MMPs gene polymorphisms in aggressive and chronic periodontitis

References	MMP	Periodontits	Subjects	Ethnicity	Smoking status	Association
Holla et al. (2004a)	MMP-1	Chronic	133 patients with mild to severe CP and 196 unrelated control subjects	Caucasian population	Smokers and nonsmokers	S
Astolfi et al. (2006)	MMP-1	Chronic	114 subjects exhibiting sites >5 mm clinical attachment and 109 healthy controls	Brazilian population	Nonsmokers	NS
de Souza et al. (2003b)	MMP-1	Chronic	26 subjects with severe periodontitis, 24 subjects with moderate periodontitis and 37 healthy controls	Brazilian population	Nonsmokers	S
Cao et al. (2005)	MMP-1	Aggressive	40 subjects with generalized aggressive periodontitis and 52 healthy controls	Chinese population	Smokers and nonsmokers	S
Itagaki et al. (2004)	MMP-1	Chronic and aggressive	37 generalised aggressive, 205 slight-to-severe generalised chronic-periodontitis patients and 142 healthy subjects.	Japanese population	Nonsmokers	NS
Pirhan et al. (2008)	MMP-1	Chronic	102 patients with severe CP and 98 periodontally healthy subjects	Turkish population	Nonsmokers	S
Chen et al. (2007)	MMP-2	Aggressive	79 patients with generalized aggressive periodontitis and 128 healthy controls.	Chinese population	Smokers and nonsmokers	NS
Holla et al. (2005)	MMP-2	Chronic	149 patients with mild to severe CP and 127 healthy controls	Caucasian population	Smokers and nonsmokers	NS
Gürkan et al. (2008a)	MMP-2	Chronic	87 severe CP patients and 107 healthy controls	Turkish population	Smokers and nonsmokers	NS
Gürkan et al. (2008b)	MMP-2	Aggressive	92 subjects with generalized aggressive periodontitis and 157 healthy controls	Turkish population	Smokers and nonsmokers	NS
Astolfi et al. (2006)	MMP-3	Chronic	114 subjects exhibiting sites >5 mm clinical attachment and 109 healthy controls	Brazilian population	Nonsmokers	S
Itagaki et al. (2004)	MMP-3	Chronic and aggressive	37 generalised aggressive, 205 slight-to-severe generalised chronic-periodontitis patients and 142 healthy subjects	Japanese population	Nonsmokers	NS
Cao et al. (2006)		Chronic	60 subjects with chronic periodontitis and 50 controls	Chinese population	Smokers and nonsmokers	S
Keles et al. (2006)		Chronic	70 severe generalized chronic periodontitis patients and 70 healthy subjects	Turkish population	Nonsmokers	S
Ustun et al. (2008)		Chronic	42 patients with mild periodontitis, 84 patients with moderate to severe periodontitis and 54 controls	Turkish population	Smokers and nonsmokers	S

(*continued*)

Table 4.17 (continued)

References	MMP	Periodontits	Subjects	Ethnicity	Smoking status	Association
Chen et al. (2007)	MMP-9	Aggressive	79 patients with generalized aggressive periodontitis and 128 healthy controls	Chinese population	Smokers and nonsmokers	NS
Holla et al. (2006)	MMP-9	Chronic	169 patients with CP (76 with a mild to moderate form and 93 with severe generalized CP) and 135 control subjects	Caucasian population	Smokers and nonsmokers	NS
De Souza et al. (2005)	MMP-9	Chronic	35 subjects with severe periodontitis, 27 subjects with moderate periodontitis and 38 healthy controls	Brazilian population	Nonsmokers	NS
Gürkan et al. (2008a)	MMP-9	Chronic	87 severe CP patients and 107 healthy controls	Turkish population	Smokers and nonsmokers	S
Gürkan et al. (2008b)	MMP-9	Aggressive	92 subjects with generalized aggressive periodontitis and 157 healthy controls	Turkish population	Smokers and nonsmokers	S
Gürkan et al. (2008a)	MMP-12	Chronic	87 severe CP patients and 107 healthy controls	Turkish population	Smokers and nonsmokers	NS
Gürkan et al. (2008b)	MMP-12	Aggressive	92 subjects with generalized aggressive periodontitis and 157 healthy controls	Turkish population	Smokers and nonsmokers	NS
Chen et al. (2007)	TIMP-2	Aggressive	79 Chinese patients with generalized aggressive periodontitis and 128 healthy controls	Chinese population	Smokers and nonsmokers	S
De Souza et al. (2005)	TIMP-2	Chronic	35 subjects with severe periodontitis, 27 subjects with moderate periodontitis and 38 healthy controls	Brazilian population	Nonsmokers	The high frequency of GG genotype in the TIMP-2 gene promoter in the population studied did not allow any conclusion regarding its effect on CP

S: Significant; NS: Non-significant

(Hernandez et al. 2007). Similar results were reported in patients with peri-implantitis (Borsani et al. 2005).

MMPs are produced by the major cell types in human periodontal tissue, including neutrophils, macrophages, epithelial cells, and fibroblasts (Uitto et al. 2003). Under normal conditions, MMPs are involved in the remodeling and turnover of periodontal tissue and are under the strict control of TIMPs, which bind specifically to the active site of the enzyme and maintain equilibrium between degradation and regeneration of the ECM. Proteinases produced by periodontopathogens may enhance the MMP activities by converting latent MMPs to their active forms as well as by degrading TIMPs. Plasminogen activators, including urokinase-type plasminogen activator (uPA) and tissue plasminogen

activator (tPA), are able to convert plasminogen into plasmin, a serine protease with a broad activity spectrum, that degrades fibrin and converts latent MMPs into their active forms (Bodet et al. 2007; Restaino et al. 2007) (Fig. 4.9).

Several investigators have evaluated in the recent years the relationshiop between polymorphisms of genes for MMPs and periodontitis (Table 4.17). Owing to the limited number of studies carried out to date, it is difficult to relate single-nucleotide polymorphisms of MMP genes with periodontitis.

4.17 Cathepsin C Polymorphisms

Aggressive periodontitis in prepubertal children is often associated with genetic disorders such as Papillon–Lefèvre syndrome (PLS). This syndrome is associated with mutations in the cathepsin C gene (Hart et al. 1999; Pham et al. 2004). PLS involves severe prepubertal periodontitis and palmoplantar hyperkeratosis. The incidence of the disease is estimated at 1–4 per million. In patients with PLS, the gingiva becomes severely inflamed upon eruption of the teeth. The inflammation progresses rapidly and results in the destruction of the tissues supporting the roots of the teeth, often leading to premature loss of the deciduous dentition. After a brief edentulous period, the process resumes during the early stages of the permanent dentition. The hyperkeratosis involves dry and scaly hand palms and foot soles during the first years of life. The extent of hyperkeratotic plaques varies considerably between patients and often involves, besides the palmoplantar regions, the knees and elbows as well (de Haar et al. 2006).

Cathepsin C (dipeptidyl peptidase I (DPPI); EC 3.4.14.1) is a 200-kDa proteinase that belongs to the papain family of endopeptidases subfamily C1A. Activity of the cysteine proteinase cathepsin C is found in many cell types, including fibroblasts, but is particularly abundant (up to 20 times more per milligram of protein) in cells of the immune system. Cathepsin C gene consists of seven exons and is located on chromosome 11q14-q21 t al. (McGuire et al. 1997). Cathepsin C deficiency does not lead to major changes in the structure of the mice periodontium. The overall structure of the gingiva, periodontal ligament, alveolar process, and cementum layer is normal in cathepsin C-deficient mice, with one exception, namely that epithelial rests of Malassez in the periodontal ligament of the cathepsin-deficient mice are slightly enlarged (de Haar et al. 2006).

Several studies have evaluated whether the pathogenetic role of cathepsin C gene variants relates to periodontitis other than syndrome-associated periodontitis (Hewitt et al. 2004; Hart et al. 2000; Noack et al. 2004; Toomes et al. 1999). In a case study it was reported a novel *CTSC* mutation (a deletion of seven nucleotides in exon 4 (c.566–572del)) in an otherwise healthy child with aggressive periodontitis (Noack et al. 2004). A gene mutation for nonsyndromic periodontitis was reported, and it was shown that nonsyndromic prepubertal periodontitis is an allelic variant of the type IV palmoplantar ectodermal dysplasias (Hart et al. 1999). Fourteen members of a consanguineous Jordanian family segregating prepubertal periodontitis were evaluated. No family members showed nor reported signs of palmoplantar type lesions, their histories, or an increased susceptibility

to infections. Of the 14 members of this extended consanguineous family who were clinically examined, 4 were found to be affected by generalized prepubertal periodontitis. The parents of the affected subjects are first cousins. A gene of major effect for prepubertal periodontitis was localized in this kindred to a 14 cM genetic interval on chromosome 11q14 flanked by D11S916 and D11S1367. This prepubertal periodontitis candidate interval overlaps the region of chromosome 11q14, which contains the cathepsin C gene responsible for PLS and the Haim–Munk syndrome. Sequence analysis of the cathepsin C gene from prepubertal periodontitis-affected subjects from this Jordanian family indicated that all were homozygous for a missense mutation that changes a tyrosine to a cysteine. All four parents were heterozygous carriers of this Tyr347Cys cathepsin C mutation [missense mutation (1040A–>G)]. None of the family members who were heterozygous carriers for this mutation showed any clinical findings of prepubertal periodontitis.

Noack et al. (2008) analyzed the Cathepsin C genotype in 110 persons with GAP in comparison with 78 control individuals. The carrier frequency of the missense variant p.I453V was significantly increased in persons with disease compared to healthy control individuals (17.3 vs. 6.4%, $P < 0.05$). Cathepsin C activity in leukocytes from individuals harboring this variant was significantly reduced (119.8 Delta OD/min*10(5) cells, 95% CI: 17.4–174.9, $P = 0.018$). No influence of promoter variants was found on mRNA expression. It was concluded that Cathepsin C gene variants contribute to increased susceptibility in GAP.

4.18 Vitamin D Receptor Polymorphisms

Bone metabolism and polymorphism in the vitamin D receptor gene have been associated with osteocalcin levels and bone mineral density. Alveolar bone destruction results from the periodontitis disease process. If left untreated, consequences of periodontitis are tooth mobility and eventually tooth exfoliation. Therefore, it is conceivable that mediators of bone metabolism play a role in the pathophysiology of periodontitis.

The vitamin D3 receptor (VDR) is an intracellular hormone receptor that specifically binds the active form of vitamin D (1,25-dihydroxyvitamin D3 or calcitriol) and interacts with target-cell nuclei to produce a variety of biologic effects (Yamada et al. 2003) (Fig. 4.10). In addition to its central role in calcium and bone metabolism, 1,25-(OH)2D3 has potent immunomodulatory effects on many immune cell types, including both innate and adaptive immune cells. Consistent with these effects, the VDR is widely expressed in most immune cell types such as antigen-presenting cells (APCs; monocyte/macrophage, DCs), NK cells, T cells, and B cells. Furthermore, the intriguing effects of 1,25-(OH)2D3 have been demonstrated in several autoimmune diseases, namely, systemic lupus erythematosus, type I diabetes, collagen-induced arthritis, experimental allergic encephalomyelitis, multiple sclerosis, cancer, and inflammatory bowel disease (Cantorna 2006; Dusso et al. 2005; Nagpal et al. 2005; Welsh et al. 2003).

By studying rat/human somatic cell hybrids, Szpirer et al. (1991) showed that the VDR gene is located on 12q in the human and chromosome 7 in the rat. Labuda et al. (1991) assigned the VDR gene to 12q12-q14 by in situ hybridization. Several polymorphisms

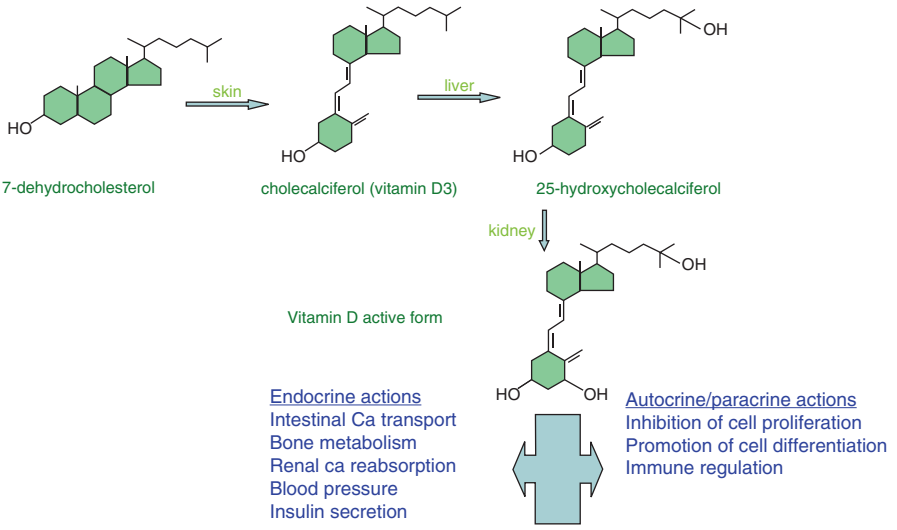

Fig. 4.10 Renal and extrarenal $1,25(OH)_2D_3$ production serving endocrine, autocrine, and paracrine functions

have been identified in the VDR gene, most of which are identified by biallelic variation in restriction enzyme sites (Fig. 4.11). Examples of RFLPs in the VDR gene include *Tru*9I, *Taq*I, *Bsm*I, *Eco*RV, *Apa*I, and *Fok*I. All these RFLPs, except *Fok*I in exon 2, are located between exons 8 and 9. By using the sequencing technique, new polymorphisms such as *Cdx2* (taranscription regulator)-binding site in the promoter region have also been detected (Naito et al. 2007). Many allelic variants (polymorphisms) of the VDR gene occur naturally in the human population, with substantial differences between races and ethnic groups. Their polymorphisms are associated with decreased bone density, propensity to hyperparathyroidism, chronic renal failure, nephrolithiasis, resistance to vitamin D therapy, susceptibility to infections, cancer, as well as autoimmune diseases such as lupus, cirrhosis, hepatitis, Crohn's and Graves' disease, and multiple sclerosis (Dusso et al. 2005; Uitterlinden et al. 2002, 2004; Valdivielso and Fernandez 2006).

Several studies have evaluated the correlations between VDR gene polymorphism and periodontal disease (de Brito Junior et al. 2004; de Souza et al. 2007; Henning et al. 1999; Inagaki et al. 2003; Li et al. 2008; Naito et al. 2007; Nibali et al. 2008; Park et al. 2006; Sun et al. 2002; Tachi et al. 2001; Tachi et al. 2003; Wang and Pan 2008; Wang et al. 2008; Wang et al. 2009; Yoshihara et al. 2001; Zhang et al. 2005) (Table 4.18). In a Japanese population, Naito et al. (2007) revealed that F-carriers (*Fok*I-cut allele) of *Fok*I VDR polymorphisms were less likely to develop severe CP than non-F-carriers (*Fok*I-uncut allele). The *Apa*I and *Bsm*I VDR polymorphisms did not show significant differences in the alleles or genotypes between the subjects with or without severe CP. The Abf (*Apa*I-cut allele[A]/ *BsmI*-uncut allele[b]/ *Fok*I-uncut allele[f]) homozygous individuals had a significantly higher prevalence of severe CP than the others, and adjustments for age, smoking status, number of teeth present, and prevalence of diabetes did not change this association

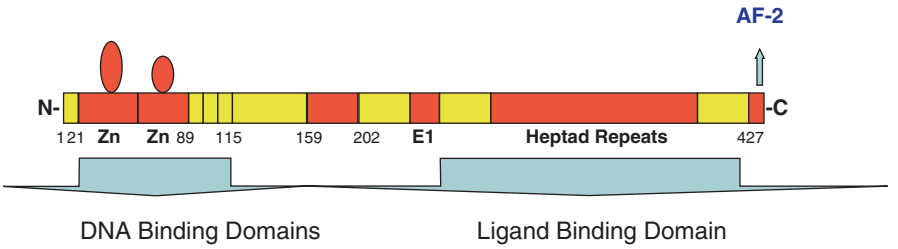

Fig. 4.11 Schematic representation of the domains of the human vitamin D receptor (VDR) molecule mediating ligand and DNA binding, nuclear localization, heterodimerization with the retinoid X receptor (RXR), and transactivation, as well as the two major phosphorylation sites. *AF-2*, activation function-2. These functional domains of the vitamin D receptor are involved in the major steps for VDR control of gene transcription: (1) ligand binding, (2) heterodimerization with RXR, (3) binding of the heterodimer to vitamin D response elements (VDREs) in the promoter of $1,25(OH)_2D$-responsive genes, and (4) recruitment of VDR-interacting nuclear proteins (coregulators) into the transcriptional preinitiation complex, which markedly enhance or suppress the rate of gene transcription by the VDR. The ligand binding domain (LBD), located in the COOH terminal portion of the VDR molecule, is responsible for the high-affinity binding of $1,25(OH)_2D_3$. $25(OH)D_3$ and $24,25(OH)_2D_3$ bind nearly 100 times less avidly. Upon ligand binding, repositioning of helix 12 in the COOH terminus of the VDR ligand binding domain, known as ligand dependent activation function 2 (AF2), imparts a major conformational change in the three-dimensional structure of the VDR. This activation step appears to be required for the recruitment by the VDR of motor proteins, responsible for a rapid translocation of cytoplasmic VDR to the nucleus along microtubules. The selective association between the VDR and its protein partner, the RXR, involves dimerization surfaces in three different domains of the VDR molecule and induces a VDR conformation that is essential for VDR transactivating function. The DNA-binding domain (DBD) of the VDR is highly conserved among nuclear steroid receptors. The DBD is organized into two zinc-nucleated modules, the zinc finger DNA binding motifs, that are responsible for high-affinity interaction with specific DNA sequences in the promoter region of $1,25(OH)_2D_3$ target genes, called vitamin D-responsive elements (VDREs). The natural mutations in the zinc finger region of the human VDR result in defective DNA binding (modified from Dusso et al. 2005) (Reprinted with permission of American Physiological Society)

(OR = 7.5; 95% CI:1.6–34.4; $P = 0.01$). In a Korean study group it was reported that the C/C genotype at start codon 27823 (C > T; *Fok*I-RFLP) of the VDR gene was associated with an increased risk for GAP, but the 60890G > A (*BsmI*-RFLP) in intron 8 and 61968T > C (*Taq*I-RFLP) in exon 9 polymorphisms were not associated with GAP. The VDR haplotype ht1(VDR 27823C–60890G–61968T) carrying 27823C/C genotype was associated with a 1.8-fold increased risk of GAP (Park et al. 2006). Inagaki et al. (2003) demonstrated an association between the *Apa*I VDR polymorphism and the progress of periodontal disease in adult men who participated in the Dental Longitudinal Study in the United States. Specifically, they reported that the loss of alveolar bone and clinical attachment occurred at the highest rate in the AA genotype of the *Apa*I VDR polymorphism (A: ApaI- cut allele, a: ApaI-uncut allele) compared to the Aa or aa genotypes. de Brito Junior et al. (2004) observed that *Taq*I and *BsmI* polymorphisms of the VDR gene are associated with clinical attachment loss due to periodontal disease in a Brazilian population.

Table 4.18 VDR gene polymorphisms in aggressive and chronic periodontitis

References	Periodontits	Subjects	Ethnicity	Smoking status	Association
Sun et al. (2002)	Chronic and aggressive	24 cases of adult periodontitis, 37 cases of early-onset periodontitis EOP and 39 healthy controls	Chinese population	Smokers and nonsmokers	NS chronic S aggressive
Naito et al. (2007)	Chronic	97 unrelated healthy Japanese men	Japanese population	Smokers and nonsmokers	S
Yoshihara et al. (2001)	Chronic and aggressive	42 with generalized EOP, 52 with adult periodontitis, 55 healthy controls	Japanese population	Smokers and nonsmokers	S
Park et al. (2006)	Aggressive	93 generalized aggressive periodontitis patients and 143 healthy controls	Korean population	Not given	S
Hennig et al. (1999)	Aggressive	69 EOP patients, including 20 patients with unequivocal evidence of localized disease and 72 healthy controls	Caucasian population	Not given	S
Inagaki et al. (2003)	Chronic	125 medically healthy, middle-aged men	Caucasian population	Smokers and nonsmokers	S
Tachi et al. (2003)	Chronic	74 subjects with CP, 94 healthy controls	Japanese population	Smokers and nonsmokers	S
Tachi et al. (2001)	Chronic and aggressive	37 with generalized EOP, 72 with adult periodontitis, 39 healthy controls	Japanese and Chinese population	Not given	S chronic S aggressive
de Brito Junior et al. (2004)	Chronic	44 healthy individuals (control group) and 69 subjects with CP	Mixed population	Nonsmokers	S
Nibali et al. (2008b)	Chronic and aggressive	231 subjects with healthy periodontium, 224 aggressive periodontitis and 79 CP patients	42% Caucasian population Mixed population	Smokers and nonsmokers	S
Li et al. (2008)	Aggressive	51 patients with Generalized aggressive periodontitis and 53 healthy controls	Chinese population	Nonsmokers	S
Gunes et al. (2008)	Chronic	72 patients with severe generalized CP and 102 healthy controls	Turkish population	Nonsmokers	NS
Zhang et al. (2005)	Chronic	166 patients with severe, moderate and mild CP respectively and 80 matched control individuals	Chinese population	Smokers and nonsmokers	S
de Souza et al. (2007)	Chronic	222 subjects with and without end-stage renal disease (in hemodialysis) were divided into groups with and without periodontitis	Brazilian population	Not known	NS
Wang et al. (2009)	Chronic	107 patients with severe chronic periodontitis and 121 control subjects	Chinese population	Nonsmokers	S

S: Significant; NS: Non-significant

Vitamin D receptor *Taq*-I TT polymorphism was moderately associated with both the presence and the progression of periodontitis in smokers, while no association was detected in nonsmoking individuals (Nibali et al. 2008b).

4.19 Calcitonin Gene Polymorphism

Calcitonin is a hormone produced in the thyroid that causes a reduction of calcium ions in the blood.

Suzuki et al. (2004) investigated the genomic markers for periodontitis, using large scale single-nucleotide polymorphism association studies comparing healthy volunteers and patients with periodontitis. Genomic DNA was obtained from 19 healthy volunteers and 22 patients with severe periodontitis, all of whom were Japanese. Statistically significant differences between the healthy volunteers and patients with severe periodontitis were found in calcitonin receptor (*CALCR*) (chromosome location: 7, chromosome position: 92691285, SNP position: Intron 3, Allele: A/B C/T) (OR = 2.69, 95% CI: 1.03–6.98, $P = 0.0039$).

The relationship between the calcitonin receptor genotype (at position 1377 (C > T) of cDNA) and mandibular buccal marginal BL at stage II surgery for endosseous implants was evaluated in 237 implants that were placed in 35 patients. It was observed patients with the TC genotype were 20 times more likely to suffer buccal marginal BL in the mandible than patients with the CC genotype. Furthermore, there were no significant differences in the distribution of age, smoking status, postmenopausal women, and bone quality between patients with and without BL in either jaw. It was suggested that the known risk factor for BL cannot explain the early marginal BL around the implants and that calcitonin receptor genetic test could become a useful tool in the planning of treatment before implant surgery and lead to more predictable implant treatment (Nosaka et al. 2002).

4.20 RANK/Osteoprotegerin Gene Polymorphism

Bone remodeling in adults occurs by removal of old bone (resorption) by osteoclasts, followed by new bone formation by osteoblasts. RANKL, its receptor RANK, and a decoy receptor, OPG, are three key molecules that regulate osteoclast recruitment and function (Fig. 4.12). RANKL-binding to its receptor, RANK, expressed on osteoclast precursor c, elicits their differentiation and activation. On the other hand, OPG-binding to RANKL interrupts RANKL–RANK ligation; consequently, OPG inhibits the ability of RANKL to induce osteoclastogenesis. RANKL is produced as a 317-amino acid, 45-kDa membrane-associated protein (35.5 kDa before glycosylation), as a 31-kDa soluble protein by proteolytic cleavage, or as a 39.5-kDa soluble protein following expression of the hRANKL3 isoform: the soluble forms also exhibit biological activity. RANKL is not only been

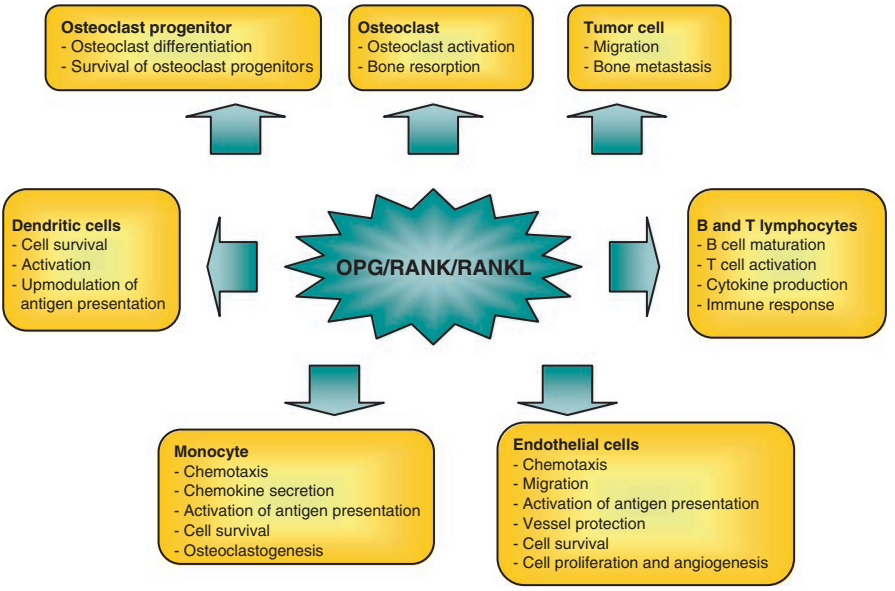

Fig. 4.12 OPG/RANK/RANKL: key partners in osteoimmunology and vascular diseases (modified and adapted from Baud'huin et al. 2007) (Reprinted with permission of Birkhäuser Verlag AG)

reported to be involved in physiological osteoclastogenesis, but also in pathological BL: osteoporosis, rheumatoid arthritis, tumor-induced bone diseases, periodontal diseases, periprosthetic implant loosening, Paget's disease and inherited bone diseases, and vascular diseases (Blair et al. 2007; Haynes et al. 2004; Nagasawa et al. 2007; Tanaka et al. 2005). Therefore, RANKL inhibition offers the therapeutic possibility to treat periodontal bone resorption (Taubman et al. 2007).

The RANKL locus on human chromosome 13q14 spans approximately 36 kb of genomic DNA and comprises six exons. For the canonical RANKL gene (hRANKL1; GenBankTM accession number AF053712), both 5′ and 3′ ends of the mRNA transcript contain untranslated regions, resulting in a transcript of 2,271 bp. Two other isoforms, hRANKL2 and hRANKL3, are translated at downstream in-frame start codons. Both isoforms lack the N-terminal intracellular domain; hRANKL3 also lacks the transmembrane domain, producing a soluble form. The C-terminal extracellular domains of all three isoforms are identical (Blair et al. 2007).

There is now considerable evidence to support the findings that periodontitis patients exhibit higher RANKL expression in GCF (Bostanci et al. 2007; Lu et al. 2006; Mogi and Otogoto 2007; Mogi et al. 2004; Vernal et al. 2004) or gingival tissues (Bostanci et al. 2007; Crotti et al. 2003; Liu et al. 2003) than periodontally healthy subjects. Bostanci et al. (2007) demonstrated that RANKL and OPG were oppositely regulated in GCF from periodontitispatients. Thus, OPG (which antagonizes RANKL-mediated bone resorption) levels were higher in healthy subjects than in those patients with periodontitis. Importantly, RANKL/OPG ratios were significantly elevated in the GCF of three forms of periodontitis.

It was suggested that the relative RANKL/OPG ratio in GCF is indicative of periodontitis and, as such, may provide clinical diagnostic potential.

Cigarette smoking may favor bone resorption through increased ratios of IL-6: IL-10 and RANKL: OPG in periodontal tissues (Cesar-Neto et al. 2007) and through suppression of serum OPG production (Lappin et al. 2007).

The association of RANK/RANKL/OPG gene polymorphisms with aggressive periodontitis was evaluated by Soedarsono et al. (2006) in a Japanese population. An association analysis with allelotypes showed that single-nucleotide polymorphisms (RANK: 27SNPs, RANKL: 7SNPs, OPG: 7SNPs) identified in the RANK/RANKL/OPG genes have no significant association with aggressive periodontitis (Fig. 4.13).

Polymorphisms in the OPG gene have a potential impact on the structural and functional properties of the protein and, therefore, may change the OPG/RANKL ratio as well. The following allelic variants were investigated: OPG-Lys3Asn (in exon 1, G > C) and OPG-Met256Val (in exon 4, T > C) in 194 unrelated, nonsmoking Caucasian individuals 35–77 years of age. Genotyping of the patient and control groups revealed patients with more CP displaying homozygosity for the wild-type Lys3 allele in the OPG gene in comparison with the healthy individuals. Asn3 homozygosity was present in patients with less CP compared with the healthy subjects. The genotype frequencies for OPG-Lys3 heterozygous (Lys/Asn) individuals were marginally higher in the test group than in the control group. Homozygosity for the Met256 allele (Met/Met) in OPG tended to occur in patients with less CP than in periodontically healthy patients. The 256Val/Val genotype of OPG was found in two individuals of the CP cohort and in one of the healthy controls. OPG-Met256 heterozygotism (Met/Val) was more frequent in the patient group than in the control population. No statistically significant differences were revealed in the genotype frequencies for the both OPG Lys3Asn and Met256Val polymorphisms between patients with CP and healthy individuals (Lys3Asn: $P = 0.352$ and Met256Val: $P = 0.146$) (Wagner et al. 2007).

No association between polymorphism OPG-223 (C/T), 245 T>G and chronic periodontal disease was reported recently by Wohlfahrt et al. (2006) and Baioni et al. (2008).

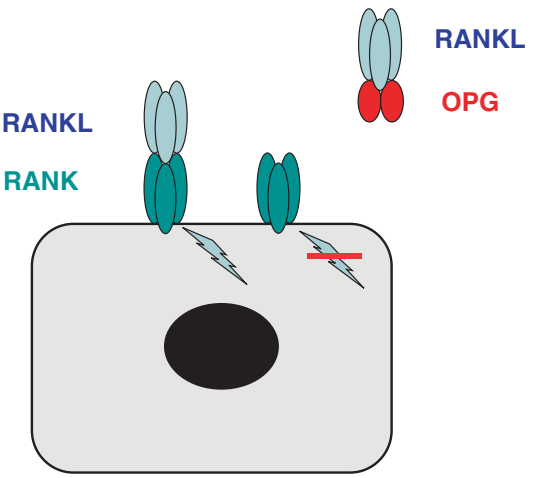

Fig. 4.13 Characteristics of OPG/RANK/RANKL interactions. RANK is expressed on the osteoclast membrane and binds RANKL produced by osteoblast/bone marrow stromal cells. OPG acts as a decoy receptor and blocks the interaction between RANKL and RANK (modified and adapted from Baud'huin et al. 2007) (Reprinted with permission of Birkhäuser Verlag AG)

Park et al. (2008) revealed that the TG haplotype of T950C and G1181C polymorphisms in the OPG gene may be useful genetic markers for the prediction of aggressive periodontitis

4.21 Plasminogen Activator Gene Polymorphism

In periodontitispatients, similar as in cardiovascular diseases, abnormal regulation in the coagulation and fibrinolytic systems plays an important role (Fig. 4.14). Plasminogen-activator-inhibitor-1 (PAI-1), a member of the serpin (serine protease inhibitor) family, is the main inhibitor of tissue- and urokinase-type plasminogen activators and one of the major determinants of fibrinolysis. This protein is an acutephase reactant; its concentrations are increased by inflammatory stimuli such as IL-1β and TNF (Izakovicová Hollá et al. 2002).

Several investigators have evaluated the effect of tissue plasminogen activator (t-PA) and PAI-1 genes on periodontal status (Table 4.19).

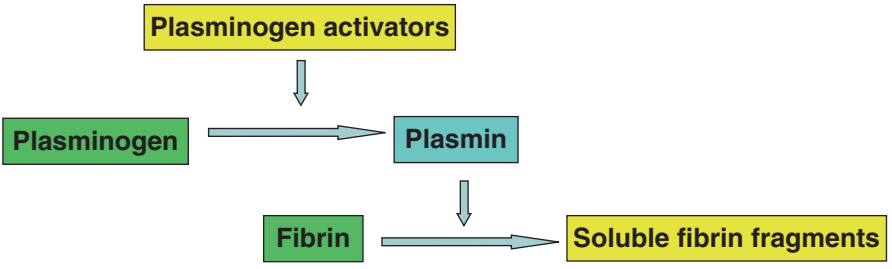

Fig. 4.14 Basic fibrinolytic system

Table 4.19 Plasminogen-activator-inhibitor-1 gene polymorphisms in aggressive and chronic periodontitis

References	Periodontits	Subjects	Ethnicity	Smoking status	Association
Izakovicová Hollá et al. (2002)	Chronic	116 subjects with chronic periodontitis and 188 healthy controls	Caucasian population	Smokers and nonsmokers	S
Emingil et al. (2007)	Aggressive	90 generalized aggressive periodontitis patients and 154 periodontally healthy subjects	Caucasian population	Smokers and nonsmokers	S
Gürkan et al. (2007)	Chronic	84 patients with chronic periodontits and 105 periodontally healthy subjects	Caucasian population	Smokers and nonsmokers	NS

S: Significant; NS: Non-significant

Izakovicová Hollá et al. (2002) investigated relationships among adult periodontitis, smoking, and a variation in the deletion/insertion (4G/5G) promoter polymorphism (G_4 or G_5 repeat at position −675) of PAI-1 gene in 304 Caucasian subjects. When only the non-smoking subjects were included in the analysis, differences between the allelic and genotype frequencies of the 4G/5G polymorphism in the adult patients with periodontitis and normal population became highly significant. No significant differences were detected in the allelic and genotype distributions between smoking periodontitis patients and smoking controls. In contrast, Gürkan et al. (2007) reported that tissue t-PA or PAI-1 genotypes (Alu-repeat insertion (I)/deletion (D) polymorphism in intron 8) were not associated with susceptibility to CP in Turkish subjects. The genotype distributions and allele frequencies of t-PA polymorphism were not different between patients with CP and healthy subjects (24.7% I/I, 45.7% I/D, and 29.6% D/D; 30.3% I/I, 45.5% I/D, and 24.2% D/D, respectively; $P > 0.05$).The t-PA D allele frequency was similar in patients with CP (52.4%) and healthy subjects (46.5%). PAI-1 genotype distribution in patients with CP (30.9% 4G/4G, 35.8% 4G/5G, and 33.3% 5G/5G) and healthy subjects (36.2% 4G/4G, 41.9% 4G/5G, and 21.9% 5G/5G) was also similar. The 4G allele frequency was not different between patients with CP (48.8%) and healthy subjects (57.1%).

In a total of 244 unrelated Caucasians of Turkish descent, the genotype distributions of t-PA and PAI-1 genes were evaluated. Among the GAP subjects, 33.4% were homozygous for the for the Alu deletion allele (D/D), 44.4% were heterozygous carriers of the Alu insertion (I/D), and 22.2% were homozygous for the I allele (I/I). The D allele was found in 55.6% of the G-AgP patients as compared with 46.1% in the healthy group. There was a significant difference among the study groups in D allele. These data suggest that the D polymorphic allele of t-PA gene polymorphism could be associated with susceptibility to GAP (Emingil et al. 2007).

DeCarlo et al. (2007) reported a significant relationship between levels of alveolar BL in periodontal disease and HindIII restriction fragment length polymorphism in the 3′ end of PAI-1 gene, and a BamHI restriction fragment length polymorphism at the 3′ end of the urokinase plasminogen activator gene (uPA) in a convenience sample of adult patients (>47 years of age).

4.22 NAT2 (*N*-acetyltransferase) Gene Polymorphism

N-acetyltransferase 2 (NAT2) metabolizes arylamine and hydrazine moieties and determines the individual susceptibility to toxicity from certain therapeutic drugs, industrial or occupational chemicals, or cancerogenic heterocyclic amines. The gene encoding NAT2 is polymorphic, thus resulting in rapid or slow acetylator phenotypes. In general, slow acetylators are more susceptible to adverse drug effects than rapid acetylators towing to delayed drug elimination with the consequence of increased concentrations of isoniazid, sulphamethazine, or procain amide. The acetylator status may, therefore, predispose drug-induced toxicities and cancer risks, such as bladder, colon, and lung cancer (Borlak and Reamon-Buettner 2006; Hein et al. 2000).

The NAT2 acetylation polymorphism is very important in clinical pharmacology and toxicology because of its primary role in the activation and/or deactivation of a large and diverse number of aromatic amine and hydrazine drugs used in clinical medicine. Although there is a wide variation in acetylation capacities, there exists a bimodal frequency distribution showing high concordance between acetylator phenotype and NAT2 gene mutations. Previous studies on the possible role of the acetylation polymorphism in immune and/or inflammatory responses gave ambiguous results. Thus, associations were suggested between acetylation polymorphism and rheumatoid arthritis, systemic lupus erythematodes, human immunodeficiency syndrome, and diabetes (Borlak and Reamon-Buettner 2006; Hein et al. 2000).

As smoking is one of the risk factors for periodontal disease, and the risk may be influenced by the polymorphism of NAT2 via metabolism of smoke-derived xenobiotics. Subjects are to be classified as "rapid" (wild-type allele) or "slow" (carrying two mutant alleles among three) acetylator phenotypes according to how fast their bodies metabolize such xenobiotics. Differences in their ability to detoxify these substances may contribute to an increased risk for periodontitis in subjects exposed to cigarette smoke or other xenobiotics. It was therefore hypothesized that a NAT2 genotype would be a risk factor for periodontal disease. A total of 154 Caucasian subjects were assigned to one of two groups: (1) no or mild and (2) severe periodontal disease, based on radiographic (bone destruction) and clinical criteria (probing depth, attachment loss) and the number of teeth. In the less-affected group, genotyping showed a fraction of predicted slow and rapid acetylators (53.6 and 46.4%, respectively) corresponding to the normal distribution in Caucasians. Severely affected patients were predominantly slow acetylators, the ORs being between 2.38 and 5.02 for the NAT2-related risk depending on the outcome parameters chosen (Meisel et al. 2000) (Table 4.20).

In a subsequent study, in 154 Caucasian subjects with no moderate, and severe periodontal disease based on bone and attachment loss, a tendency to over-representation of slow acetylators with severe disease was reported. When using BL as measure of periodontitis, this over-representation shows a significant association with the disease (OR = 2.13). In the logistic regression analysis, adjusted for age and smoking, NAT2 slow phenotype was significantly associated with the severity of BL, the OR being 2.09 (95% CI: 1.02–4.26). In a case-control analysis (controlled for smoking, gender, and age) mean BL showed a significant difference between the two NAT2-type groups (Kocher et al. 2002).

Table 4.20 NAT2 gene polymorphisms in aggressive and chronic periodontitis

References	Periodontits	Subjects	Ethnicity	Smoking status	Association
Meisel et al. (2000)	Chronic	154 subjects with (1) no or mild and (2) severe periodontal disease	Caucasian population	Smokers and nonsmokers	S
Kocher et al. (2002)	Chronic	154 Caucasian subjects with no, moderate, and severe periodontal disease	Caucasian population	Smokers and nonsmokers	NS

S: Significant; NS: Non-significant

4.23 Fibrinogen Gene Polymorphism

Fibrinogen is one of the acute-phase proteins whose levels are elevated during periodontal disease. Recent studies suggest that excessive fibrinogen production might play a role in upregulating host immune responses. In addition, there is a relationship between the −455G/A polymorphism (HaeIII-RFLP) in the 5′ flanking region of the beta-fibrinogen gene promoter and increased fibrinogen levels (Sahingur et al. 2003).

In order to assess the −455G/A polymorphism, restriction fragment length polymorphism (RFLP) analysis with HaeIII enzyme (H1: HaeIII-cut allele, H2: HaeIII-uncut allele) was performed in the promoter region of the beta-fibrinogen gene by Sahingur et al. (2003) (Table 4.21). This was carried out on 79 patients with CP as compared to 75 periodontally healthy subjects, matched to age, gender, and race. The frequency of homozygocity for the rare allele of the beta-fibrinogen gene (H2H2) was 13% for the periodontitis patients and 3% for the control group. The distributions of H1H1 and H1H2 genotypes were 48 and 39% in the patient group and 70 and 27% in the control group, respectively. Furthermore, periodontitis patients have significantly higher fibrinogen levels compared to healthy individuals. It was suggested that H1H2 or H2H2 genotypes as well as elevated fibrinogen levels, in conjunction with other factors, may put individuals at higher risk of having periodontal disease, or may result from periodontal infection–genetic interactions.

Suzuki et al. (2004) found statistically significant differences between healthy volunteers and patients with severe periodontitis in the fibrinogen-like 2 (FGL2) gene. It was suggested that H1H2 or H2H2 genotypes as well as elevated fibrinogen levels, in conjunction with other factors, may put individuals at higher risk of having periodontal disease, or may result from periodontal infection–genetic interactions.

Table 4.21 Fibrinogen gene polymorphisms in aggressive and chronic periodontitis

References	Periodontits	Subjects	Ethnicity	Smoking status	Association
Sahingur et al. (2003)	Chronic	79 patients with CP as compared to 75 periodontally healthy subjects,	Caucasian population	Smokers and nonsmokers	S
Suzuki et al. (2004)	Chronic	22 patients with severe periodontitis and 9 healthy volunteers	Japanese population	Nonsmokers	S
Ge et al (2008)	Chronic	121 patients with moderate to severe periodontitis and periodontally healthy and gingivitis controls	Chinese population	Not given	S

S: Significant; NS: Non-significant

4.24
Estrogen Receptor-α Gene Polymorphism

Periodontitis is an inflammatory disease and also one of the most familiar bone diseases, characterized by loss of connective tissue and alveolar bone. Estrogen is a hormone that regulates bone metabolism (Fig. 4.15). Its special receptor is estrogen receptor (ER). In the absence of hormone, ER resides in either the cytoplasm or nucleus of the target cells associated with a large heatshock protein-chaperone complex that maintains the receptor in a transcriptionally inactive form. Upon binding a ligand, the receptor undergoes a conformational change, leading to its displacement from the chaperone complex and subsequent dimerization. In this biochemical state, the receptor can interact with target gene promoters in a direct manner through specific estrogen-response elements (EREs) or indirectly through interactions with proteins associated with the promoter. The DNA-bound receptor then nucleates the assembly of a large multiprotein coactivator complex that serves to remodel local chromatin structure, stabilize the preinitiation complex, and enhance transcriptional output (Stein and McDonnell 2006). There are two isoforms of the human ER,

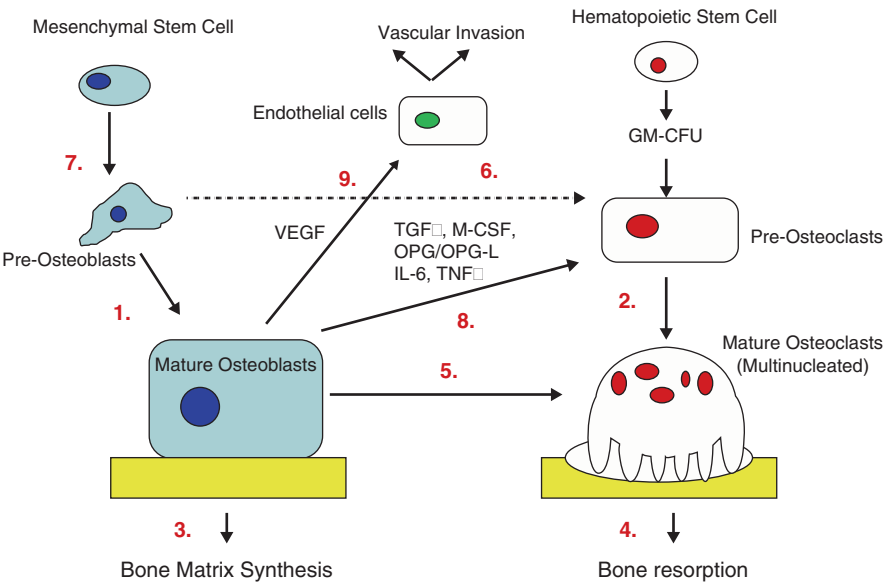

Fig. 4.15 Known and potential sites of estrogen action within the bone microenvironment. Estrogen may control each of the following processes: proliferation/differentiation of preosteoblasts (*1*) and preosteoclasts (*2*); the activity of mature osteoblasts (*3*) and osteoclasts (*4*); the "coupling" between mature osteoblasts and osteoclasts (*5*) and between preosteoblasts and preosteoclasts (*6*); the lineage commitment of mesenchymal progenitor cells to either osteoblasts and adipocytes (*7*); the production of bone-resorbing cytokines by osteoblasts (*8*) and osteoclast/monocyte/macrophage lineage cells (not shown for clarity); and the production of angiogenic factors and hence vascularization by osteoblasts (*9*) (modified and adapted from Rickard et al. 1999) (Reprinted with permission of John Wiley & Sons, Inc.)

named ER-α and ER-β, each with distinct tissue and cell patterns of expression. ER-α has been found expressed in osteoblasts, osteoblast-like cells, osteoclasts, and bone cells. It has been proven that *Pvu*II (in intron1) and *Xba*I (between exon 2 and 5′-flanking intron) RFLPs of the ER-α gene were associated with altered bone mineral density and osteoporosis (Guise 2000; Rickard et al. 1999; Vanderschueren et al. 2004; Zhang et al. 2004).

The relationship between ER-α gene polymorphisms and periodontitis was evaluated in 90 patients with aggressive periodontitis, 34 patients with CP, and 91 healthy controls. All these subjects belonged to the Han Chinese race. It was found that the detection frequency of XX genotype (X: XbaI-cut allele; x: XbaI-uncut allele) was significantly higher in patients with CP than in the healthy controls (11.8 vs. 4.4%). The difference between the female patients with CP and healthy controls (26.7 vs. 2.1%, $P < 0.01$) was statistically significant, but no difference was found between the male patients and controls. As a consequence, it was suggested that in female Han Chinese population, the XX genotype may be a risk indicator for CP (Zhang et al. 2004).

The relationship between ER gene polymorphisms and tooth loss, oral bone loss, and postcranial bone mineral density in Japanese postmenopausal women was investigated by Taguchi et al. (2003) in 149 Japanese postmenopausal women. It was revelead that ER *PvuII* polymorphism was associated with tooth loss, but not with oral bone mass and postcranial bone mineral density in the studied population.

Wang et al. (2008) investigated the relationship between CP and the genetic polymorphisms of ER gene in a Chinese population. PCR-RFLP was applied to examine the *Xba I* and *Pvu II* polymorphisms of the ER genes. It was revevealed that 39.6% of patients with CP took ER XX genotype, whereas the rate in healthy controls was 20.0%. The people who took BBXX genotype had the worst periodontal conditions among all CP patients.

4.25 Cytochrome P450 Gene Polymorphism

Cytochrome P450 (CYP) enzymes, CYP1A1 and CYP2E1, are considered to play important roles in the activation of xenobiotics, especially tobacco-derived substances, such as polycyclic aromatic hydrocarbons and nitrosamines. Polymorphism of CYP1A1 and CYP2E1 are associated with enhanced catalytic activities of these enzymes (Kim et al. 2004). CYP1A1, CYP2E1, and GSTM1 (glutathione *S*-transferases1) enzymes may also contribute to an increased risk for periodontitis. The genetic polymorphisms of these enzymes have been linked with an increased risk for tobacco-related diseases such as oral cavity cancer (Anantharaman et al. 2007; Cha et al. 2007; Gattas et al. 2006; Leichsenring et al. 2006; Walle and Walle 2007).

Kim et al. (2004) investigated the prevalence of the polymorphisms of polymorphisms of CYP1A1 (m1 in the 3′-flanking region (6235T > C) and m2 within exon 7 (4889A > G)) and CYP2E1 (two effective polymorphism combinations (c1 allele: PstI−, RsaI+; c2 allele: PstI+, RsaI−) in the 5′-flanking region on its transcription regulation) genes in 115 patients with periodontitis as well as in 126 control subjects. Significantly increased risk for periodontitis was observed for subjects with the polymorphic CYP1A1 m2 allele (OR = 2.3).

However, no association was observed between the CYP2E1 Pst1 polymorphism and risk for periodontitis (OR = 1.3). The risk was not changed among individuals with CYP2E1 c2 allele regardless of smoking history, although data suggested the potential of a risk increase among smokers (OR = 3.3). In contrast, a risk change for CYP1A1 polymorphisms between eversmokers and nonsmokers was observed. Study subjects with CYP1A1 m2 alleles had a significant increase in periodontitis risk among nonsmokers (OR = 2.8), but no risk increase was observed among eversmokers (OR = 1.6). These data suggested that the CYP1A1 enzymes might play a role in the pathway for developing periodontal disease in nonsmokers and that CYP2E1 enzyme might increase the risk for smoking-related periodontitis (OR = 3.3). The presence of an interaction between smoking and genotypes implies that the two variables are independent of risk factors for periodontal disease. This suggests that individuals are affected by both risk factors: smoking and genotypes, separately. Higher expression of metabolic enzymes determined by genetic factors may lead to an increased damage by oxidative molecules in periodontal tissues and, as a result, the rate of tissue damage increases in association with a higher estimated relative risk of disease. In smokers, tobacco smoke induces the tissue damage. Consequently, the estimated relative risk of periodontal disease in smokers increases with tobacco use. In smokers who have increased metabolic enzyme expression, tissue damages may accumulate faster than in smokers with low-enzyme expression or in nonsmokers with low-enzyme expression, leading to an overall higher estimated relative risk (Kim et al. 2004).

4.26 RAGE (Receptor of Advanced Glycation End-Products) Gene Polymorphism

The RAGE is a multiligand member of the Ig superfamily of cell surface molecules. RAGE is composed of an extracellular region (EC) containing one Variable ("V"-type) Ig domain and two Constant ("C"-type) Ig domains. The extracellular portion of the receptor is followed by a hydrophobic transmembrane-spanning domain and then by a highly charged short cytoplasmic domain that is essential for post-RAGE signaling (Yonekura et al. 2005).

Its ability to recognize multiple classes of ligands, such as advanced glycation end products (AGEs), S100/calgranulins, amphoterin, amyloid-β peptide and β-sheet fibrils, and MAC-1, suggests that the repertoire of RAGE-dependent effects in the tissues may be diverse. In this context, the function of this receptor does not appear to be to degrade/detoxify ligand but, rather by RAGE cytosolic domain-triggered signal transduction, to propagate immune/inflammatory responses (Naka et al. 2004). Binding of AGEs to RAGE in endothelial cells has been shown to induce drastic alterations in many endothelial functions representing a critical and initiating factor in the development of diabetic vascular disease (Rojas and Morales 2004). (Fig. 4.16)

A recent study suggested that RAGE polymorphisms could be involved in the pathophysiology of periodontitis. Caucasian patients (101) with CP together with 162 orally healthy subjects were studied. Three polymorphisms, one in intron 7 (1704G > T), second in intron 8 (2184A > G), and the third in exon 3 (G82S) of the RAGE gene, were investigated. A statistically significant difference in allele frequencies between patients and the reference

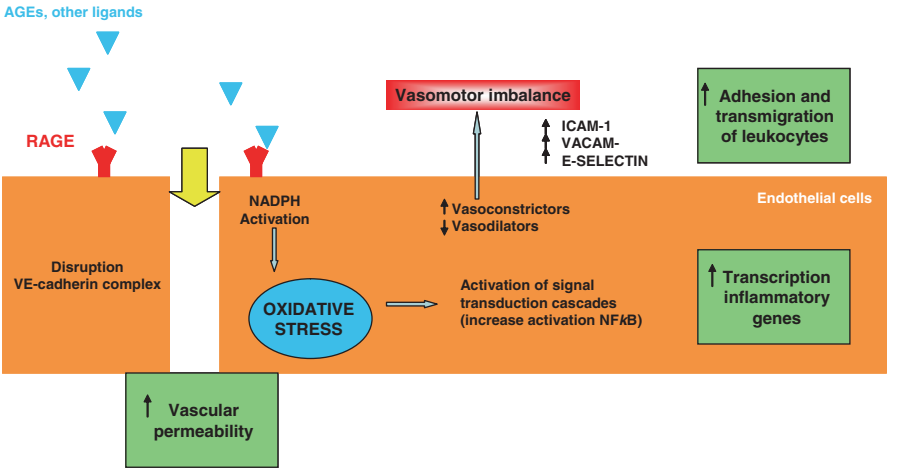

Fig. 4.16 Schematic depiction of main AGE-induced alterations in endothelial cells, resulting in a marked imbalance of vascular homeostasis (modified and adapted from Rojas and Morales 2004) (Reprinted with permission of Elsevier B.V.)

group was found for intron variant1704G > T. There was no significant difference in genotype or allele frequency distributions between groups for intron variant 2184A > G or for the exon variant exchanging amino acid Gly for Ser at position 82 (G82S) (Hollá et al. 2001).

4.27 CCR5 (Crotonyl Coenzyme Reductase 5) Gene Polymorphism

Chemokines comprise a family of structurally and functionally related cytokines that show chemotactic activity for different types of leukocyte populations. Commonly, chemokines are produced by an array of immune and nonimmune cells, i.e., periodontal fibroblasts, endothelial cells, and keratinocytes. Based on structural and genetic criteria, the chemokines are divided into four different subfamilies: CXC (α chemokine), CC (β chemokine), C (lymphotactin), and CX3C (fractalkine). The CC chemokine subfamily includes the macrophage inflammatory proteins (MIP-1α and MIP-1β), monocyte chemoattractant protein-1, and CCL5 (RANTES; regulated on activation normal T cell expressed and secreted), which are highly expressed also in periodontal disease. However, among others the CC chemokines appear to specifically interact with chemokine receptor5 (CCR5) (Savarrio et al. 2007; Kim et al. 2006) (Table 4.22).

According to previous reports, mutations in the CCR5 gene cause considerable changes in the expression and function of the protein product. A 32-bp deletion has been identified as one of the most important mutations of the CCR5 gene (CCR5-Δ32) that lead to a frame shift at amino acid 185. The 32-nucleotide deletion in the CCR5 coding region produces a truncated receptor protein that cannot reach the cell surface. Consequently, cells of

Table 4.22 CCR5 gene polymorphisms in chronic periodontitis

References	Periodontits	Subjects	Ethnicity	Smoking status	Association
Savarrio et al. (2007)	Chronic	106 patients with generalized, severe CP and from 69 periodontally healthy subjects	Caucasian population	Smokers and nonsmokers	NS
Folwaczny et al. (2003)	Chronic	81 patients with generalized periodontitis and 121 healthy controls	Caucasian population	Not given	NS
Wohlfahrt et al. (2006)	Chronic	137 patients with eight or more teeth having ≥5 mm of proximal clinical attachment loss; 82 healthy controls	Caucasian population	Smokers and nonsmokers	NS

S: Significant; NS: Non-significant

CCR5-Δ32 homozygous individuals completely fail to express a functional CCR5 protein. Previous reports have attributed to the CCR5-Δ32 mutation a considerable impact on the susceptibility to and severity of various inflammatory and infectious diseases: HIV infection and on monocytes and/or T lymphocytes, that may play a key role in the pathogenesis of periodontal disease. CCR5 downregulation has been shown to be important in oral wound healing. CCR5 was more intensely expressed ($P < 0.05$) in gingival tissues of patients with periodontitis than in control subjects (Garlet et al. 2003). It was also demonstrated that the development of chronic adult periodontitis is related to the expression of CCR5(+)cells in inflamed gingival tissue, as determined by immunohistochemistry. Periodontal treatment, which consisted of the removal of the bacterial plaque, reduced the degree of inflammation and the total amount of cytokines present in the GCF (Gamonal et al. 2001).

However, there was no report revealing the association between the CCR5 polymorphism and susceptibility to periodontal disease (Folwaczny et al. 2003; Savarrio et al. 2007; Wohlfahrt et al. 2006).

4.28 Lactoferrin Gene Polymorphism

Lactoferrin is an is an iron-binding protein stored within the specific granules of polymorphonuclear leukocytes, which plays an important role in regulating bacteria that are associated with aggressive periodontitis. Lactoferrin kills directly (via its strongly cationic N-terminal region) and indirectly, through sequestering the iron that bacteria require for growth (Jordan et al. 2005). Wei et al. (2004) showed that GCF from periodontitis sites exhibited significantly greater total amount of lactoferrin than gingivitis and healthy sites (Table 4.23). Moreover, total amount of lactoferrin was positively correlated with PI, GI, probing depth, and probing attachment level (Wei et al. 2004). Fourteen days after surgical periodontal treatment by a minimal invasive flap technique, the lactoferrin concentrations

Table 4.23 Lactoferrin gene polymorphisms in aggressiveperiodontitis

References	Periodontits	Subjects	Ethnicity	Smoking status	Association
Jordan et al. (2005)	Aggressive	46 patients with and 78 controls	African American population	Smokers and nonsmokers	S
Velliyagounder et al. (2003)	Aggressive	9 patients with localized juvenile periodontitis and 17 healthy subjects	African American population	Not given	S
Wu et al. (2009)	Aggressive and chronic	65 patients with aggressive periodontitis, 278 with chronic periodontitis and 88 controls	Taiwanese population	Smokers and nonsmokers	Significant for aggressive Non-significant for chronic periodontitis

S: Significant; NS: Non-significant

decreased significantly in the crevicular fluid and in saliva, suggesting that the defense factor lactoferrin is suitable for monitoring of periodontal treatment results (Jentsch et al. 2004).

It was hypothesized that genetic variation (single nucleotide polymorphism (A > G) causing a threonine/alanine substitution at position 11 (Thr11Ala)) within the lactoferrin gene may play a role in susceptibility to periodontal disease. In a pilot case-controlled study of aggressive periodontitis, analysis of 46 African American patients and 78 controls showed that patients were twice as likely to express the G nucleotide (alanine) allele over controls (60.3 vs 30.4%; OR = 2.564). A Caucasian population of 77 patients and 131 controls showed no such association (OR = 0.862) (Jordan et al. 2005). Similar results were obtained by Velliyagounder et al. (2003) who showed that the Lys and Arg alleles (at position 29 in the N-terminal region in lactoferrin) had frequencies of 24 and 76%, respectively, among 17 healthy human subjects, and 72 and 28%, respectively, among 9 patients with LJP.

Recently, Wu et al. (2008) showed that the lactoferrin polymorphism is related to the development of periodontitis. The risk allele, G (arginine), was more frequent in the aggressive periodontitis group (76.2%), followed by the CP group (69.6%), and then the healthy control group (60.2%).

4.29 Interferon-γ Gene Polymorphisms

IFN-γ has multiple immunoregulatory effects, mediates host defense against infection, and is a potent activator of mononuclear phagocytes. IFN-γ, released during the early and late stages of the immune response by natural killer cells and activated T cells, respectively, regulates several aspects of the immune response. IFN-γ also acts upon uncommitted myeloid immature dendritic cells (DCs) to polarize them into Th1 cell–promoting effector cells that produce high levels of IL-12 upon stimulation. Data suggest that antigen-presenting cells, including DCs and macrophages, also produce large amounts of IFN-γ (Table 4.24).

Table 4.24 IFN-γ gene polymorphisms in aggressive and chronic periodontitis

References	Periodontitis	Subjects	Ethnicity	Smoking status	Association
Babel et al. 2006	Chronic	122 patients with chronic periodonitis and 114 controls	Caucasian population	Smokers and nonsmokers	NS
Reichert et al. 2008	Aggressive and chronic	72 patients with aggressive periodontitis and 52 patients with chronic periodontitis, and 74 healthy controls	Caucasian population	Smokers and nonsmokers	NS
Hooshmand et al. 2008	Aggressive and chronic	27 patients with aggressive periodontitis and 26 patients with chronic periodontitis, and 56 healthy	Iranian population	Nonsmokers	NS

S: Significant; NS: Non-significant

During the generation of a primary Th1 response, IFN-γ acts as a positive regulator by selectively inducing Th1 differentiation through the increased transcription of T-bet, which results in enhanced IL-12 responsiveness, and by suppressing Th2 lineage commitment. It was also revealed that the total amount and concentration of cytokine IFN-γ in gingival crevicular fluid samples and transcription factor T-bet expression were increased in progressive periodontal lesions in patients with chronic periodontitis (Dutzan et al. 2009). It was also showed that IFN-γ positively modulates *Actinobacillus actinomycetemcomitans*-specific RANKL+ CD4+ Th-cell-mediated alveolar bone destruction in vivo (Teng et al. 2005), while IFN-γ deficiency attenuates local *P. gingivalis*-induced inflammation (Houri-Haddad et al. 2002). Tsai et al. (2007) compared the ratios of gingival crevicular fluid IL-4 to IFN-γ in the baseline and post- nonsurgical periodontal therapy sites and found that IL-4 to IFN-γ ratios were increased after treatment. It was suggested an association of the increased IL-4 to IFN-γ ratio with the improvement of periodontal status.

The results of several studies (Babel et al. 2006; Reichert et al. 2008; Hooshmand et al. 2008) suggest that the IFN-γ gene polymorphisms may not be associated with the susceptibility of Caucasian and Iranian individuals to periodontitis.

Mutations in the gene coding for the ligand binding chain (alpha, R1) of the IFN-γ receptor (IFNGR1) confer suseptibility on infections caused by poorly virulent mycobacteria. Using an intronic (CA)n polymorphic microsatellite marker within the IFNGR1 gene Fraser et al. (2003) investigated whether genetic polymorphisms are associated with periodontitis. In 62 periodontitis patients and 56 healthy controls were found a total of 13 polymorphisms, 11 of which were found in the periodontitis patients and 9 in the controls. Although a trend towards an association with disease for allele 192 was observed, there were no significant differences in allele frequency between patients and controls. Therefore it was not possible to find any evidence to suggest that IFNGR1, as a single dominant gene, contributes to susceptibility to periodontitis. However, in combination with the environmental risk factor, smoking, the same allelic marker was significantly associated (OR = 5.56, $P = 0.014$) with periodontitis (Fraser et al. 2003).

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Heritability of Chronic Periodontitis 5

Most patients with plaque-induced periodontitis will have the chronic form. The main clinical features and characteristics of chronic periodontitis are (Armitage 2004):

- Most prevalent in adults, but can occur in children and adolescents.
- Amount of destruction is consistent with the presence of local factors.
- Subgingival calculus is a frequent finding.
- Associated with a variable microbial pattern.
- Slow to moderate rate of progression, but may have periods of rapid progression.
- Can be associated with local predisposing factors (e.g., tooth-related or iatrogenic factors).
- May be modified by and/or associated with systemic diseases (e.g., diabetes mellitus).
- Can be modified by factors other than systemic disease, such as cigarette smoking and emotional stress.

The typical patient is over 30 years, with substantial deposits of plaque and calculus associated with the presence of gingival inflammation, periodontal pockets, and attachment loss. In most cases, the disease is slowly progressing, but short periods of rapid attachment loss can occur. Chronic periodontitis was once called "*adult periodontitis*," since it was believed that only adults developed the disease. However, epidemiologic data clearly show that the disease can also be found in children and adolescents. Although chronic periodontitis can occur in localized or generalized patterns, the two forms appear to be identical with regard to their etiology and pathogenesis (Armitage 2004).

Genes have also been implicated to play a role in chronic periodontitis, but in contrast to aggressive periodontitis, chronic periodontitis does not typically follow a simple pattern of familial transmission or distribution. The twin study is probably the most popular method that supports the genetic aspects of chronic periodontitis. This study substantiates the contribution that genes make vs. the environment in a phenotypic expression. Monozygous twins, in contrast to dizygous twins, come from a single ovum and therefore share exactly the same genes. Discordance in the disease experience of monozygous twins must be caused by environmental determinants as seen in twins reared apart. In dizygous twins, differences could be a result of both genetic and environmental differences (Yoshie et al. 2007). Studies conducted on twins provide us with interesting points on the genetic influences in diseases to what extent this is influenced by the environment (Yoshie et al. 2005).

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Table 5.1 Genes associated with chronic periodontitis risk (modified and adapted from Yoshie et al. 2005)

Gene	Locus(genotype)	Negative reports	Positive reports
Interleukin-1 cluster	IL-1A +4845G/T, IL-1A −889C/T IL-1B 3953(3954)C/T, IL-1B −511T/C) Composite IL-1A +4845G/T and IL-1B 3953(3954)C/T Composite IL-1A −889C/T and IL-1B 3953(3954)C/T IL-1 receptor antagonist (RN) +2018 T/C, +2028, variable number tandem repeats in intron 2 [IL-1RN *2 allele (A2)]	13	24
Interleukin-2	−330 T/G	0	1
Interleukin-4	IL-4 −590C/T, IL-4 70bp repeat in intron2, IL-4 RA Q551R	4	2
Interleukin-6	IL-6 -174G/C, IL-6R 48892A/C, IL-6R −183G/A	0	12
Interleukin-10	−592C/A, −597C/A, −627C/A, −819C/T, −824C/T, −1082G/A, −1087G/A	4	8
Interleukin-12	1188 A/C	1	0
Interleukin-16	−295T/C	1	0
Interleukin-18	−656G/T, -607C/A, -137G/C, +113T/G, +127C/T, codon 35A/C (third position of codon 35)	1	0
Fc gamma receptor	**FcγRIIA**: 494 G/A results in R(Arg) 131H (His) **FcγRIIB**: I232T, 646-184A/G (intron4) **FcγRIIIA**: V158F **FcγRIIIB**: 141 G-C (NA antigen), 266 C-A (SH antigen): NA1, NA2, SH	5	8
Tumor necrosis factor	−238G/A, −1031T/C, −863C/A, −857C/T, −308G/A, −376G/A, +489G/A	5	7
Transforming Growth Factor beta TGFβ	−988C/A, −800 G/A, −509C/T, codons 10 (L10P) and 25 (R25P) of exon 1, + 915G/C, Thr263Ile and 713-8delC	1	3
Vitamin D receptor	RFLP: *Bsm*I, *Taq*I, *Apa*I, and *Fok*I	4	8
Estrogen receptor-α	RFLP: *Xba* I and *Pvu* II	0	2
RANK/RANKL/OPG	OPG: −223C/T, Lys3Asn and Met256Val, +245T/G, A163G, T245G, T950C, G1181C, C4441T, A6890G	3	1
Lactoferrin	Lys29Arg	1	0
MMP matrix metalloproteinases −1, −2, −3, −9, −13	**MMP-1:** 1G/2G at position −1607, −519A/G, and −422A/T **MMP-2:** −1575G/A, −1306C/T, −790T/G, and −735C/T **MMP-3:** 5A/6A at position −1171 **MMP-9:** −1562C/T and R279Q **MMP-13:** −77A/G	7	8

(*continued*)

Table 5.1 (continued)

Gene	Locus(genotype)	Negative reports	Positive reports
Human leukocyte antigen	**HLA-A** : A1, A2, A3, A9, A10, A11, A29 (A19), A28 **HLA-B**: B15, B18, B5 **HLA-DR**: DR1, DR2, DR3, DR4, DR5, DR6, DR7, DR8, DR9, DR10 **HLA-DQ**: DQ1, DQ6 (DQ1), DQ2, DQ3	0	5
CD 14	159C/T, −260C/T	3	6
Toll-like receptor: TLR-2, −4	**TLR2**: Arg753Gln, Arg677Trp, −183 A/G, −148 C/T, −146 T/G, +1350 T/C, +2343 G/A **TLR4**: Asp299Gly, Thr399Ile, +3725 G/C, +3528 C/G, +4022 C/G, +4529 G/C	9	3
CARD15	CARD15: 3020insC and 2104T mutations	2	0
Tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1)	4G/5G polymorphism in the promoter region of the PAI-1 gene and the Alu-repeat insertion (I)/deletion (D) polymorphism in intron 8 of the t-PA gene	1	1
NAT2 *N*-acetyltransferase	191G/A, 282C/T, 481C/T, 590G/A, 803A/G, 857G/A	1	1
Fibrinogen	**fibrinogen-b**: 455 G/A, **fibrinogen-like 2 (*FGL2*) gene**: SNP (A/G) in CDS3	0	3
Cytochrome*P*450	**CYP1A1:**6235 T>C, 488 PA>G, **CYP2E1:***Pst1*-RFLP	0	1
Calcitonin gene receptor	CALCR: SNP (C/T) in intron 3	0	1
RAGE receptor of advanced glycation end-products	1704G/T, +2184A/G, Gly82Ser	0	1
CCR5 crotonyl co-enzyme reductase 5	59653 C/T, Delta32 (32 base pair deletion in CCR5 gene)	3	0
Interferon IFNγ	874A/T, 5644G/A	3	0

Michalowicz et al. (1991) examined the relative contribution of environmental and host genetic factors to clinical measures of periodontal disease through the study of twins reared together and monozygous twins reared apart. Probing depth, clinical attachment loss, gingivitis, and plaque were assessed from the Ramfjord teeth in 110 pairs of adult twins (mean age, 40.3 years), including 63 monozygous (MZ) and 33 dizygous (DZ) twin pairs reared together and 14 monozygous twin pairs reared apart. Heritability estimates indicated that between 38 and 82% of the population variance for these periodontal measures of disease may be attributed to genetic factors. Genetic and environmental variances and heritability for gingivitis and adult periodontitis using data from twins reared together were evaluated using path models with maximum likelihood estimation techniques in 117 pairs of adult twins (64 MZ and 53 DZ pairs). Adult periodontitis was estimated to have approximately

50% heritability, which was unaltered following adjustments for behavioral variables, including smoking (Michalowicz et al. 2000). Corey et al. (1993) revealed that approximately half of the variance in disease in the population is attributed to genetic variance.

Genetic polymorphisms thus far found to be associated with chronic (adult) periodontitis are listed in Table 5.1. Interleukin-1, 2, 4, 6, 10, Fcγ receptor, TNF, and vitamin D receptor are possible candidate genes for susceptibility of chronic periodontitis.

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Heritability of Aggressive Periodontitis 6

Aggressive periodontitis is less common than chronic periodontitis and principally affects young patients. It occurs in localized and generalized forms that differ in many respects with regard to their etiology and pathogenesis. Localized aggressive periodontitis (LAP) and generalized aggressive periodontitis (GAP) were previously called "*localized and generalized juvenile periodontitis*" or "*early-onset periodontitis*" respectively. Features of aggressive periodontitis that are common to both the localized and generalized forms of the disease are (Armitage 2004):

- *Primary features:* Except for the presence of periodontitis, patients are clinically healthy; rapid attachment loss and bone destruction; familial aggregation.
- *Secondary features (often present):* Amounts of microbial deposits are inconsistent with the severity of periodontal tissue destruction; elevated proportions of *Aggregatibacter actinomycetemcomitans* and, in some populations, *Porphyromonas gingivalis* may be elevated; phagocyte abnormalities; hyperresponsive macrophage phenotype, including elevated levels of prostaglandin E_2 (PGE_2) and interleukin-1β (IL-1β); progression of attachment loss and bone loss may be self-arresting (Armitage 2004).

Some types of aggressive periodontitis seem to be inherited in a Mendelian manner, and both autosomal modes and X-linked transmission have been proposed. Genetic segregation analyses have been carried out using family pedigrees and the distribution of family members with aggressive periodontitis. Most of the evidence for a genetic predisposition to aggressive periodontitis comes from segregation analyses of families with affected individuals in two or more generations, and the results in different sets of families are consistent with both autosomal-dominant and autosomal-recessive inheritance, as well as X-linked dominant inheritance, but no single inheritance mode that would include all families has been established (Meng et al. 2007).

In a family study, 39 sibships (116 individuals, aged 13-48) were evaluated for clinical indices, neutrophil chemotaxis, and serum antibodies to *A. actinomycetemcomitans*. In 14 sibships, all affected persons had localized form of juvenile periodontitis; 14 other sibships had affected individuals with generalized form of juvenile periodontitis; and 11 had at least one sib with each form. For probands with decreased chemotaxis, 71% of affected sibs and 36% of clinically healthy sibs had decreased chemotaxis. The associations of disease with these risk factors were stronger in localized form of juvenile periodontitis-only sibships.

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Table 6.1 Genes associated with aggressive periodontitis risk (modified and adapted from Yoshie et al. 2005)

Gene	Locus (genotype)	Negative reports	Positive reports
Interleukin-1 cluster	IL-1A +4845G/T, IL-1A −889C/T IL-1B 3953(3954)C/T, IL-1B −511T/C) Composite IL-1A +4845G/T and IL-1B 3953(3954)C/T Composite IL-1A −889C/T and IL-1B 3953(3954)C/T IL-1RN: variable number tandem repeats in intron 2 [IL-1RN *2 allele (A2)]	11	12
Interleukin-4	IL-4 −590C/T, IL-4 70bp repeat in intron2	3	1
Interleukin-6	IL-6 −174G/C, IL-6R 48892A/C, IL-6R −183G/A	0	5
Interleukin-10	IL-10 −627C/A, IL-10 −1082G/A	4	2
Interleukin-12	1188 A/C	1	0
Interleukin-13	IL-13 −1112C/T, IL-13 −1512A/C	1	0
Interleukin-18	promoter region: c. −368G/C, c. −838C/A −656G/T, −607C/A, −137G/C, +113T/G, +127C/T, codon35A/C (third position of codon 35	2	0
Fc gamma receptor	**FcγRIIA**: +494 G/A results in R(Arg)131H(His) **FcγRIIB**: +695 T/C **FcγRIIIA**: +559 G/T, V158F **FcγRIIIB**: 141 G-C (NA antigen), 266 C-A (SH antigen): NA1, NA2, SH	1	6
Tumor necrosis factor α	−238G/A, −1031T/C, −863C/A, −857C/T	4	2
Transforming Growth Factor beta TGFβ	+915G/C, Thr263Ile, 713-8delC	1	0
Vitamin D receptor	RFLP: *Bsm*I, *Apa*I, *Taq*I, and *Fok*I	1	6
Estrogen receptor	RFLP: *Xba* I and *Pvu* II	2	0
RANK/RANKL/OPG	OPG: A163G, T245G, T950C, G1181C, C4441T, A6890G	1	1
Lactoferrin	Lys29Arg, Thr11Ala	1	3
MMP matrix metalloproteinases −1, −2, −3, −9, −12, TIMP	**MMP-1:** 1G/2G at position −1607 **MMP-2:** −1306C/T, −735C/T **MMP-3:** 5A/6A at position −1171 **MMP-9:** −1562C/T **MMP-12:**Asn357Ser **TIMP-2:**−418-G/C	4	2
Human leukocyte antigen	**HLA-A** : A1, A2, A3, A9, A23 (A9), A24 (A9), A10, A11, A29 (A19), A30 (A19), A31 (A19), A28 **HLA-B**: B51 (B5), B52 (B5), B12, B44 (B12), B45 (B12), B13, B14, B15, B18, B27, B35, B40 **HLA-Cw** : Cw1, Cw2, Cw3, Cw4, Cw5, Cw6, Cw7, Cw8 **HLA-DR**: DR1, DR2, DR3, DR4, DR5, DR6, DR7, DR8, DR9, DR10	1	9

(*continued*)

Table 6.1 (continued)

Gene	Locus (genotype)	Negative reports	Positive reports
CD 14	−159C/T, −1359G/T	2	0
Toll-like receptor, TLR-2, -4	**TLR2:** Arg677Trp, Arg753Gln **TLR4:** Asp299Gly, Thr399Ile	3	2
CARD15	c.2104 C>T, c.2722 G>C, c.3020insC (p. R702W	1	0
Cathepsin C	−1209_ −1219 del, −932 G>A, −18 T>C, 458 C>T, 1357 A>G, 386T>A, 935A>G, 1235A>G, 1040A>G	0	4
FMLP formyl–methionyl–leucyl–phenylalaine receptor	FPR1: 329T/C and 378C/G	1	1
Tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1)	4G/5G polymorphism in the promoter region of the PAI-1 gene and Alu-repeat insertion/deletion (I/D) polymorphism in intron 8 of the TPA gene.	0	1
Interferon IFNγ	874A/ T, 5644G/A	2	0

Some affected sibs had neither risk factor, while many currently healthy sibs had one or both (Boughman et al. 1992). Mixed model segregation analyses of 100 families, ascertained through 104 probands with early-onset periodontitis, were carried out by Marazita et al. (1994) to test major locus and multifactorial hypotheses for the etiology of early-onset periodontitis. The segregation analysis results were consistent with an autosomal major locus being sufficient to explain the family patterns of early-onset periodontitis in the entire dataset. A dominant mode of transmission was most likely, with penetrance of about 70%.

The advent of more advanced molecular techniques enabled scientists to investigate genetic polymorphisms in population, case–control, and functional studies. Reports on the association between gene polymorphisms and AP are summarized in Table 6.1. Seven out of 11 published studies on four IL-1 gene loci, namely A−889, A+4845, B+3954, and RN had positive association. Polymorphisms in genes of cell-surface receptors for immunoglobulins (Fc), formyl-methionyl-leucyl-phenylalanine (FMLP), human leukocytic antigen (HLA), and vitamin D are promising candidates for susceptibility assessment of aggressive peridontitis (Yoshie et al. 2007).

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Heritability of Gingivitis

7

Periodontal diseases (gingivitis and periodontitis) are inflammatory processes of the gingival and supporting structures of the teeth induced by a microbial biofilm, but individual differences in the host immune response to infection may affect the susceptibility and severity of the disease. Gingivitis is mainly related to plaque and calculus and leads to a local inflammatory response, which, however, is unable to eliminate the microbial products completely, and chronic progression may turn into periodontitis (Vokurka et al. 2009).

Growing evidence suggests that gingival inflammation may represent a true risk factor for attachment loss and tooth loss, and that an association may exist between susceptibility to gingivitis and susceptibility to periodontitis. In this context, it is feasible that genes implicated in the regulation of inflammatory process of periodontal tissues associated with plaque accumulation may play a role in explaining the individual variability in the severity of both plaque-induced gingivitis and destructive periodontitis (Dashash et al. 2007).

The degradation of collagen fibers and extracellular matrix components results from the activity of matrix metalloproteinases (MMPs). MMPs, structural and functional family of proteolytic enzymes, may play an important role in tissue remodeling and repair associated with development of inflammatory response. Periodontal disease development and progression can be caused by MMPs produced by both infiltrating and resident cells of the periodontium. One of the most important MMPs, MMP-9 (also known as gelatinase B or 92-kD type IV collagenase), is active against collagens and proteoglycans. The coding gene is located on chromosome 20q11.2-q13.1, and several polymorphisms have been detected in the MMP-9 gene (Vokurka et al. 2009). In a study group of 298 Caucasian children aged 11–13, Vokurka et al. (2009) found significant differences in −1562T allele frequencies for MMP-9 polymorphism (1562C > T) that were not significant for IL-18 variant (−607A > C). Furthermore, a highly significant association of the composite genotype (formed by the variants of both the genes) with gingivitis was found ($P = 0.004$, $P_{corr} < 0.05$).

Because of the reported inter-individual differences in the gingival response to plaque accumulation, several investigators tested the association between IL-1 genotype and the severity of gingival inflammation, but contrasting results were obtained (Cullinan et al. 2001; Goodson et al. 2000; Jepsen et al. 2003; Müller and Barrieshi-Nusair 2007; Scapoli et al. 2005).

Interleukin-1 receptor antagonist (IL-1Ra) is an endogenous receptor and an anti-inflammatory cytokine, which is able to block the action of IL-1α and IL-1β by modulating their biological effects and preventing signal transduction. A significant association

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Table 7.1 Genes associated with gingivitis risk

Gene	Locus (genotype)	Negative reports	Positive reports
Interleukin-1 cluster	ILA +4845G/T, IL-1B +3953(3954)C/T, IL-1B −511T/C Combination of alleles 2 of interleukin: IL-1A(-889) and IL-1B(+3954); IL-1RN: variable number tandem repeats in intron 2 [IL-1RN *2 allele (A2)]	1	5
Interleukin-6	−174G/C, −572C/G, and −597G/A	1	1
Interleukin-10	−1087 G/A, −819 C/T, −592 C/A	0	2
Interleukin-12	1188 A/C	0	1
Interleukin-18	−607 A/C	1	0
MMP-9	−1562 C/T	0	1
Tumor necrosis factor	−308G/A	1	0
Lymphotoxin alpha (LT-A)	+252A/G	1	0
Fibrinogen	−455 G/A	0	1

was observed between IL-1Ra gene polymorphism (a variable number of 86 bp tandem repeat (VNTR) located in the second intron of IL-1Ra gene) and gingivitis in children (Dashash et al. 2007). The IL-1RN*2 allele (A2; two repeats of VNTR) was significantly more frequent in controls (37 vs. 22% in children with gingivitis). In addition, the carriage of A2 seemed to be protective against gingivitis, and it was more frequent in controls (60 vs. 40% in children with gingivitis, $P = 0.008$). Moreover, multiple logistic regression analysis showed that the association between IL-1Ra gene polymorphism of VNTR and gingivitis in children remained significant ($P = 0.014$) regardless of the significant influence of plaque ($P = 0.013$).

Genetic polymorphisms thus far studied with gingivitis are listed in Table 7.1. (Dashash et al. 2005; Dashash et al. 2007; Dashash et al. 2006; Goodson 2000; Holla et al. 2008; Jepsen et al. 2003; Lang et al. 2000; Müller and Barrieshi-Nusair 2007; Scapoli et al. 2005; Scapoli et al. 2007; Vokurka et al. 2009)

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Heritability of Peri-Implantitis

8

Periodontal diseases are classified into different forms of gingivitis and periodontitis. The term "gingivitis" refers to a gingival inflammation with no signs of loss of supporting tissues, while periodontitis in addition to gingival inflammation is characterized by loss of attachment and bone. The inflammatory lesions that develop in the tissues around implants are collectively recognized as peri-implant diseases. In accordance with the classification of periodontal disease at teeth, peri-implant disease includes two entities: peri-implant mucositis that corresponds to gingivitis and peri-implantitis that corresponds to periodontitis. While peri-implant mucositis was defined as a reversible inflammatory reaction in the soft tissues surrounding a functioning implant, peri-implantitis described inflammatory reactions associated with loss of supporting bone around an implant in function (Zitzmann and Berglundh 2008).

Crosssectional analyses have investigated potential risk indicators for peri-implant disease, including poor oral hygiene, smoking, history of periodontitis, diabetes, genetic traits, alcohol consumption and implant surface (Heitz-Mayfield 2008). As reviewed by Andreiotelli et al. (2008) and Huynh-Ba et al. (2008), several studies revealed that the diagnostic value of both interleukin-1 (IL-1) genotyping and genetic tests for early implant failures or peri-implantitis should be reconsidered before altering treatment planning, regimens, and maintenance in implant dentistry (Montes et al. 2009; Laine et al. 2006; Jansson et al. 2005; Gruica et al. 2004; Feloutzis et al. 2003; Shimpuku et al. 2003). In a partially edentulous group treated for periodontal disease before implant treatment, a synergistic effect between the *IL-1* genotypes and smoking was detected (Jansson et al. 2005), characterizing individuals with these two conditions together as a high-risk population for implant failure (Montes et al. 2009). Negative results were reported as well, suggesting that the IL-1 polymorphism exerted only little influence on the peri-implant crevicular immune response, and this influence appeared to be of limited impact in sites with established peri-implantitis lesions (Lachmann et al. 2007; Rogers et al. 2002; Campos et al. 2005; Wilson and Nunn 1999).

Huynh-Ba et al. (2008) summarized, after adjusting for smoking status, that no definitive conclusions could be made with respect to the extent and validity of the association between the IL-1 genotype status and marginal bone loss as a surrogate marker of peri-implantitis. There is no evidence to support or refute an association between IL-1 genotype status and development of peri-implantitis. Clinically, a systematic genetic screening for the assessment of the risk of peri-implantitis in conjunction with cigarette smoking would

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be premature and further research is needed before definitive recommendation can be made. Similar to the patient-based risk assessment after active periodontal therapy, the genetically determined predisposition to peri-implantitis represents only one of several components (Huynh-Ba et al. 2008).

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Common Guidelines for Association Studies 9

The candidate gene approach tries to identify one allele of a gene that is more frequently seen in subjects with the disease than in subjects without the disease. Candidate genes are chosen on the basis of their known or presumed functions that are thought to have some plausible role in the disease. There are three types of candidate genes: functional candidate genes, positional candidate genes, and expressional candidate genes. Functional candidate genes are derived from an existing knowledge of the phenotype and the potential function of the gene involved after clinical or physiological studies of affected individuals. Positional candidate genes are based on the involvement of the gene to a marked location after genetic linkage analyses. Expressional candidate genes are determined through differences in gene expression using microarrays (Hodge 1993; Yoshie et al. 2007).

For several genes, which have been individually sequenced for association with periodontitis, a scattered picture was observed from different studies of varied populations and ethnicities. To produce scientifically sound and meaningful disease-association studies, some issues and concerns should be addressed (Yoshie et al. 2007).

9.1 Ethnic Heterogeneity

In designing a case-control study, subjects should be carefully matched by ethnogeographic origin in addition to other potential confounding factors in order to avoid systematic differences in genetic composition between the two groups. Failing to do so could result in different frequencies of single nucleotide polymorphism alleles and the unsuspecting investigator might then draw unwarranted conclusions about localizations of susceptibility genes. There is also a clear statement that in the presence of large biological and environmental variability, genetic effects can differ across different populations, or even among generations within the population. Variation in genotype frequencies across diverse populations may affect the number of individuals at increased risk for a disease, and population substructure imbalances may create spurious differences in genotype frequencies of the compared groups in gene disease association studies. Considering the issues mentioned, it was suggested to select a more homogenous population (age- and race-matched), and to study, with caution, the applicability of a certain gene marker before

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commencing with any attempts to replicate the same study in the population under investigation (Yoshie et al. 2007).

9.2 Clinical Classification

Classifying periodontal diseases has been a longstanding dilemma largely influenced by paradigms that reflect the understanding of the nature of periodontal diseases during a given historical period. As a result of its familial tendency, aggressive periodontitis generally appears in individuals before the age of 35, but age alone is not sufficient to establish diagnosis. On the other hand, chronic periodontitis is quite complex and much more dependent on environmental factors that confront the patient during his lifetime. In addition, microbial plaque deposition, smoking, and systemic diseases largely influence the phenotypic expression of the disease. For these combined reasons, chronic periodontitis is considered to appear later in life. The periodontist is therefore challenged regarding which classification a patient would properly fall. Therefore, investigators should strictly adhere to the classification set during the American Academy of Periodontology workshop in 1999. Moreover, subjects falling into the gray zone between aggressive and chronic periodontitis should be excluded in the study (Yoshie et al. 2005, 2007).

9.3 Functional Polymorphisms

Structural gene defects can affect the qualitative response, and regulatory polymorphisms can alter the response quantitatively. However, many studies fail to provide functional evidence for gene polymorphisms and periodontal diseases. The majority only statistically demonstrated the association between polymorphisms and periodontitis (Yoshie et al. 2007). Kinane and Hart (2003) outlined the requirements in providing a disease-polymorphism association:

- The polymorphism must influence the gene product.
- Biases in the study population should be recognized and controlled for.
- Confounders such as smoking and socio-economic class must be sorted out.
- Affected gene product should be part of the disease etiopathology.

9.4 Sample Size of the Study Subjects

Owing to limited number of samples, most sample sizes in genetics are small. This scenario describes very well the small number of cases in aggressive periodontitis association studies. The number of subjects in studies of chronic periodontitis tends to be larger, but

variations do occur. The size of subjects clearly contributes to the differences in statistical power of the results, especially in a complex disease like periodontitis (Yoshie et al. 2007).

9.5 Choice of Controls

Defining the appropriate controls for a case-control study in periodontitis still lacks clarity. Some reports generally described their control as healthy, while others specifically characterized controls as patients with gingivitis or slight periodontitis (Yoshie et al. 2007).

9.6 Data Presentation

Expressing the results only in *P*-values is extremely popular in all types of studies in periodontitis. It was suggested that the data presented should be evaluated using confidence intervals (CIs) and relative risk (RR) values, as these portray the effect size with a description of its precision. This is in contrast to the *P*-value, which tests against the null hypothesis of no association and could provide false-positive conclusions. Furthermore, RR and 95% CIs provide readers with more useful information that does use hypothesis testing (Yoshie et al. 2007).

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Clinical Utility of Genetic Knowledge

10

With the background information previously presented, which reveals the likely complexity of genetic risk for periodontitis, it is worth examining an existing prognostic test for periodontitis. Kornman et al. (1997) described a study demonstrating an association between a variant, or polymorphism, in a gene, and risk for periodontitis in adults. They demonstrated that variants in genes found in the population (not mutations, but rather normal variations in genes) that regulated IL-1 production by monocytes were associated with the severity of periodontitis. This association was biologically reasonable because of the role played by IL-1 in collagen and bone destruction and other inflammatory processes. The study provoked a great deal of interest in the research and clinical community both for its scientific value and because it provided the basis for a commercially marketed laboratory test for periodontal disease risk. The concept that risk assessment for periodontal disease in adults might some day include determination of genetic risk was not new, but the unexpected availability of such a tangible diagnostic tool inspired the profession to ponder both the utility of such information and the ethical application of the technology (Schenkein 2002).

The discovery of the association between polymorphisms in IL-1 genes and severity of periodontitis has been described as a major breakthrough in clinical practice, and the observed increase in risk due to this single genetic factor has been confirmed in a population of "typical" dental practice patients. The clinical test for such a genetic risk has been proposed as a component of the risk assessment profile for chronic periodontitis that can be used to provide a rationale for explaining individual patient susceptibility, providing early preventive or therapeutic intervention, and allowing superior prognostic capabilities (Schenkein 2002).

The conceptually simplest application of genetic information would be the development of prognostic tests to answer the question, what is the likelihood that a patient will develop disease or experience disease progression? This is the basic issue addressed by the currently available genetic test for the IL-1 genetic polymorphisms associated with periodontitis severity. In principal, when administered to an individual with little or no disease, this test putatively provides an estimate of the likelihood that a patient will ultimately develop severe periodontitis (Schenkein 2002).

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However, the practical clinical utility of this test of susceptibility must be questioned for a number of reasons (Schenkein 2002):

- The data demonstrate either cross-sectional associations with disease severity or retrospective examination of disease outcomes. Neither of these models provide the necessary prospective data required to demonstrate that predetermination of the genotype leads to the disease or, more importantly, to the clinician that knowledge of the genotype gives the therapist the ability to modify the course of the disease or its treatment.
- The polymorphisms used in this test have either not been evaluated in certain populations (e.g., African Americans) or have been shown to be too rare in some populations to be of general utility. If the test had clinical value, it could only be used in select populations.
- The test, applied on an individual basis, has limited sensitivity and specificity, that is, a significant percentage of individuals who demonstrate the at-risk genotype do not have periodontitis and a significant percentage of individuals with periodontitis (who do not smoke) do not demonstrate the genotype.
- The genes in question determine a relatively small, but significant, component of the overall risk for the disease. Other polymorphisms also appear to contribute a small but significant amount of risk for the disease (e.g., FcγRIIIb polymorphisms), so that the IL-1 polymorphisms alone are inadequate to provide a sufficient risk profile.

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