Assessment of the Clinical Validity and Utiltiy of SNPs When Used as Predictors for Periodontitis

BY

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D.D.S., UNIVERSITY OF COSTA RICA, 2008

THESIS

Submitted as partial fulfillment of the requirements for the degree of

Master of Science in Oral Sciences in the Graduate College

of the University of Illinois at Chicago, 2017

Chicago, Illinois

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DEDICATION

To Maria Fernanda, my incredible wife, whose has been the light of my life and the source of my happiness; without her nothing of what and where I am would have been possible.

Also to all my family, for all their support and help during entire journey.

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude and appreciation to Dr. Thomas C. Hart, who has the attitude and substance of a great teacher, an amazing mentor and more over an incredible human being. He transcends the purpose of a great mentor and made me dream bigger and be better. My thanks are total and eternal. “The greatest mentor is not the one who teaches what to see, is the one who make you drop the blindfold so you can start understanding the meaning of a sight” (Julio Obando).

I would like also to thank my committee members, Dr. Seema Ashrafi, Darien Weatherspoon and David Reed for providing indispensable advice, information and support on different aspects of my project and their cooperation in a difficult field assignment.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABREVIATION

SNP	Single Nucleotide Polymorphisms
HGP	Human Genome Project
DNA	Deoxyribonucleic Acid
IL-1α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
IL-1RN	Interleukin-1 antagonist receptor
IL-6	Interleukin-6
MZ	Monozygotic
DZ	Dizygotic
GWA	Genome-Wide Association
LD	Linkage Disequilibrium

CLIA	Clinical Laboratory Improvement Amendments
FDA	Food and Drug Administration
SACGT	Secretary's Advisory Committee on Genetic Testing
DHHS	Department of Health and Human Services
NIH-DOE	National Institutes of Health-Department of Energy
ELSI	Joint Working Group on the Ethical, Legal and Social Implications
AA	Aggregatibacter Actinomycetemcomitans
MMP	Matrix Metalloproteinase
TGF	Transforming Growth Factor
bp	bases pair
DSETC	Diagnosis and Screening Evaluation Test Calculations
PPV	Positive Predictive Value
NPP	Negative Predictive Value
OR	Odds Ratio
RR	Relative Risk
HIV	Human Immunodeficiency Virus

INTRODUCTION

Periodontitis describes the clinical presentation of a group of disease states, characterized by destruction of the periodontal attachment apparatus surrounding the teeth. Etiologically, periodontitis is a multifactorial disease, resulting from the host immune/inflammatory response to microbial biofilm in the periodontium. The disease pathogenesis, which occurs over time, is influenced by local and systemic host factors, environmental and behavioral factors [2-4]. Etiologically, both host genetic and environmental factors are important determinants of disease. From a genetic perspective, periodontitis can be delineated into two general forms, clinically termed aggressive periodontitis and chronic periodontitis [5-7]. The chief genetic distinction between these two forms of periodontitis depends upon how genetic factors contribute to the disease, and can be broadly referred to as Mendelian disease or a common, complex multifactorial disease. While the local anatomical and environmental etiologic factors may be similar in both diseases, the way genes contribute to etiology differs profoundly[8, 9], potentially giving rise to different clinical features, diagnostic and treatment approaches and prognosis.

Genetic studies indicate that forms of aggressive periodontitis demonstrate characteristics of a Simple Mendelian disease form [8, 9]. This means that a mutation in the primary sequence of one gene can be the etiologic cause of the disease [10-13] In contrast to Mendelian diseases, common complex diseases result from the interaction of many (hundreds or thousands) of genetic variants (polymorphisms) that each contribute individually a very small amount to the overall etiologic cause [10]. This is the model that chronic periodontitis tends to fit. When an individual patient is diagnosed with a disease pathology that has a genetic

basis, such as periodontitis, it is important to determine whether they have a Mendelian form of disease or a common complex form of disease. This distinction is important when considering the use of genetic testing for diagnostic or susceptibility testing.

As genes determine qualitative and quantitative aspects of immunological responses, efforts are being directed to identify the genetic differences that can underlie individual differences in disease susceptibility [14-17]. The hope is that genetic variants that are etiologically important for disease risk can be used as the basis for clinically useful genetic tests.

With the development of new technologies to sequence DNA and identify genetic variants, many researchers have reported associations for genetic variants with diseases including periodontitis [10, 18-34]. Companies have also marketed tests to patients and clinicians, claiming these tests can provide clinically useful information to assess individual susceptibility for periodontitis.

A number of different SNPs have been reported to be associated with an increase in different inflammatory systemic diseases [19, 21, 30, 34-37]. IL-1 alpha (-889) rs1800587, IL-1 alpha (+4845) rs17561, IL-1 beta (+3935) rs1143634, IL-1RN (+2018) rs419598 and IL-6 (-174) rs1800795 are SNPs that have been linked with periodontitis, and now they are being used to determine their predictive ability as genetic test to assess the risk of chronic periodontitis.

One of the problems currently facing the dental community relates to lack of existing genetic testing regulations for biomarkers to assess the risk for periodontitis [38, 39]. In this study a thorough analysis was performed for selected

SNPs, when used as genetic tests for periodontitis. The purpose of this thesis is to evaluate factors important in considering genetic testing for periodontitis susceptibility and to evaluate support for the clinical validity and clinical utility of such tests.

CHAPTER ONE

Basic Concepts of Genetics

1.1. Terminology

As it became evident that there is a genetic basis to many if not most human diseases, the idea developed that it is important to identify all human genes to evaluate their role in health and disease [40]. This led to the Human Genome Project (HGP), an international initiative to sequence the human genome to provide basic information to help understand disease etiology and develop better diagnostic tests and etiologic based treatments. Completion of the HGP in 2003 has fostered calls to use genetic information to perform genetic tests to help in diagnostic and susceptibility testing.

The genome that is the DNA sequence of the chromosomes is base on a numerical order of their nucleic acid bases. The locus is the number where a gene can be found based on the sequence previously described [41]. A gene can be defined as a DNA sequence that codes for a specific function, although there are protein-coding genes new evidence has shown that the function of a gene is wider that just coding for proteins and have a broader field of action base on there functionality [41]. Genes could have different or alternative forms within the same the locus; these different genes are called alleles [41]. The alleles that encoded for a trait are referred as the genotype genes. These genes code for different observable characteristics, known as phenotype.

Genetic terminology can be confusing, in part because some genetic terms like mutation were coined before anyone had identified a SNP. The difference between a mutation and a SNP is the frequency in population. Mutations are

generally very rare in the population, SNPs have a minor allele frequency of at least 1% [41], so are far more common than mutations. A mutation and a SNP can both involve a change in a nucleotide, but to note the difference, a rare SNP with a minor allele frequency < 1% is called a SNV (single nucleotide variant)[41]. So a SNP and a SNV can be change in a nucleotide, the difference is minor allele frequency.

1.2. Classification of Genetic Disorders

1.2.1. Simple Gene Disorders

There is grand variety of diseases in which genetics might play an important role in their etiology. Depending on different factors genetic conditions can be classified in three main types; Single gene disorders, common complex disorders and chromosome disorders [42]. A single gene defect or Mendelian disorder is a mutation in a specific gene that relate with the etiology for a disease. These kinds of defects commonly reveal obvious and characteristic pedigree a pattern, much of them are rare with a frequency of 1 in 500 but could be less, usually affects 2 percent of the population as a whole.

1.2.2. Common Complex Gene Disorders

Common complex disease is the genetic disorder in which several genes contribute in the cause of the etiology. These types of pathologies are the results of a combination of small variations in genes that together can predispose to illness, often in conjunction with environmental factors. These disorders do not express the

pedigree patterns of the single gene traits but tend to recur in families. The estimates of this disorder in the whole population are around 60 percent [42].

1.2.3. Chromosome Disorders

There are defects that are due excess or a deficiency of the genes contained in an entire chromosome or in one segment of the chromosome. For instance Down syndrome is the result of an extra copy of one chromosome (chromosome 21). This kind of disorders are very common and they are presence in about 7 per 100-liveborn infants; and they are associated with half of all spontaneous first trimester abortion [42].

1.3. Mutations

All organisms as part of their diversity are exposed to genetic mutation. This process marks the base for evolution [43]. Mutations furnish the raw material of evolutionary change [44]. The way the organisms change their DNA material and pass it to future generations could mark the difference between the continuity of species or their extinction; that is the path for natural evolution [45]. These changes occur at many different levels, and they can have widely differing consequences. When an alteration in a gene changes permanently the DNA sequence a gene mutation is created [46]. Mutations are rarely events that are primarily created by a mistake in the DNA replication machinery [41] The terminology, mutation, is well

used by several authors as a description of a rare allele mean while the term common allele is often interpreted as a polymorphism [41].

1.3.1. Types

A mutation can be heritable if it occurred in the DNA and the cell populates the germline; and that is how changes in the DNA could be passed to future generations. In contrast somatic mutations; defined as the ones that happen by chance in certain cells of specific tissues; are not transmittable mutations.

Mutations can be classified by their amino acids affects. When a nucleotide change in a codon and do not modify the amino acid result a synonymous mutation is encountered [46]. In contrast nonsynonymous mutations are that nucleotide changes that create a new amino acid[46].

1.4. Single Nucleotide Polymorphism

1.4.1. Terminology

Genetic variance is a normal and essential evolutionary part of all biological organisms [47]. At the nucleotide level of the human genome there are many different types of variance. One of the most common forms of genetic variance occurs with single nucleotide polymorphisms (SNPs) [8]. For instance when a specific variation occurs in at least 1% of the population a single nucleotide polymorphism (SNP) is identified [41, 48]. The presence of diseases that related to changes or variations in the DNA sequence in a single base chain; are the type of

alteration called single nucleotide polymorphism (SNP) and these variations seems to be the most common type of genetic variation. All the SNPs are classified with the same two letters "rs" (reference SNP) at the beginning; follow by numbers ID, which designates there order [49].

1.4.2. Structure

SNPs occur approximately once every 300 nucleotides on average [46]. Nucleotides are the alphabet of genetic information, the foundation on which genes are formed. They have three basic parts; a five carbon sugar, a phosphate and a nitrogen rich structure. There are 5 types of nitrogenous bases and they are divided in two different groups. The pyrimidine (cytosine, thymine and uracil) and they are the smaller group [50]. The purine consists in a larger structure and it could be an adenine or a guanine.

This type of single nucleotide variation are found in all parts of the genome, in protein coding regions of genes, in introns and in regulators 5' and 3' regions of genes. More than 20 million SNPs have been identified. The majority of the SNPs have no effect on health or development of diseases. A relatively small proportion of SNPs that have been identified as the etiological factor of an illness [46].

The basic structure of a gene consists of the 5' promoter, exon(s), intron(s) and the 3'regulatory region; each part plays an important role in the functionality and gene expression. The promoter section is the place that acts as a binding site where DNA initiates the transcription process [49]. A mutation in this section of the gene can "turn on or off" the ability of a gene to be transcribed into RNA. Although

previously the intronic part of the gene was thought to be “genetically inactive” newer studies demonstrate that the intronic areas of the gene are functionally important e.g. containing enhancer elements, or transcribe ant-RNA etc [51].

Exons are the parts of the gene that are used as a template that codes for a specific portion of a protein. This site is commonly used to look for SNPs because the possible effect a SNP may have on the resultant amino acid.

1.5. Mutation Analysis

Mutations can be analyzed and could be classified depending on the scale of the potential damage that they can produce. There are several ways to analyze a mutation, and there are different factors that need to be taken into account when the analysis is done. There are numerous programs that predict the functional and expression possibility of a variation; given values are needed to determine if the change in the nucleotide is damaging/deleterious or benign. Factors such as changes in size, molecular weight, physicochemical conditions, hydrophobicity, polarity, pH among others provide information to create parameters that follows the functional scores [52-56].

1.5.1. Programs used to assess biologic effects of a mutation or SNP

A brief description is given of how each program works and ranges the functionality scores.

PROVEAN™: is a software that works by using an algorithm to predict a change in a protein sequence and how it affects functionality. The program can predict several different variants such as substitutions deletions and insertions. A score is given based on a computerized scale. When the score is under a predefined number set by the software, the variation is categorized "deleterious" if the score is over the number the variation is predicted to have a "neutral" effect [52].

PolyPhen-2™: "Most of human genetic variation is represented by SNPs and many of them are believed to cause phenotypic differences between human individuals. PolyPhen-2 is focus on nonsynonymous SNPs, (SNPs located in coding regions and resulting in amino acid variation in protein products of genes)"[53]. It was shown in several studies that impact of amino acid allelic variants on protein structure/function could be reliably predicted via analysis of multiple sequence alignments and protein 3D-structures"[53]. "PolyPhen-2 is an automatic tool for prediction of possible impact of an amino acid substitution on the structure and function of a human protein"[53]. "This prediction is based on a number of features comprising the sequence, phylogenetic and structural information characterizing the substitution"[53]. "For a given amino acid substitution in a protein, PolyPhen-2 extracts various sequence and structure-based features of the substitution site and feeds them to a probabilistic classifier" [53].

Mutation Assessor™: The server predicts the functional impact of amino-acid substitutions in proteins, such as mutations discovered in cancer or missense polymorphisms. The functional impact is assessed based on evolutionary

conservation of the affected amino acid in protein homologs. The server seeks variants from Uniprot [57] and Refseq [58] protein sequences. Both of the variants have to the same sequence on both programs so it could by assess by the program. The prediction system works on based on the impact in function that the variation could generate. The scale is built on a high, medium and low/neutral impact prediction. In which a high results states for an increased risk of a biological impact as a low result means the opposite [54].

Ensembl™: database stores sectors of the genome that vary from individual to individual ("variants") and, relates the conditions phenotype information. “There are different types of variants for several species: single nucleotide polymorphisms, short nucleotide insertions and/or deletions and longer variants classified as structural variants” [56]. The software predicts how the variants interact and regulates features in different species. The same analysis can be run on different data using the Variant Effect Predictor (VEP). The VEP determines the effect of your variants (SNPs, insertions, deletions, CNVs or structural variants) on genes, transcripts, and protein sequence, as well as regulatory regions [56].

1.5.1.1. 1000 Genome Project™

1000 Genomes Project™ is an international collaboration that creates a large public database of human genetic variation in nucleotide sequences of the genome.. “The genomes of about 2,500 unidentified people from about 25 populations around the world have been sequenced using next-generation sequencing technologies”

[55]. Results of Phase 3 are currently available and a database was created to store and share all this genetic information. This has been expanded by other deep sequence projects that have the sequence data of >700,000 people now. These databases can be used to see if a SNP occurs at a specific site and also if other variants including SNV (minor allele frequency< 1%, sometimes called rare SNPs) and mutations.

The main objective of the project is target on locate variants with frequencies of 1 % or more on the studied populations. These are obtained by sequence individuals and compare it to the reference sequence.

Genome-wide association (GWA) studies goal is to find areas of the genome the link with specific diseases or disorders. Properly powered GWAS studies are conducted for a minimum of 1,000 patients and 1,000 controls. The idea is to compare the control variants with the disease/cases variants. Commonly genetic variants are link to others variants in the same region. Linkage disequilibrium (LD) is referred as the association of alleles that are in the same region of a variant that have been link with a disease. [55, 59]. Results of GWAS typically identify 3,000-8,000 or more SNPs with a disease. Also a SNP contributes very little to disease, a large SNP contribution is in the order of approximately 0.5%. Additionally there is redundancy; since SNPs are common in populations. Disease associated SNPs are found in affected and in controls, and are not alone predictive of disease [55]. For instance SNPs have been used for genetic testing for common complex diseases such as prostate cancer (figure1).

GWAS reports from literature: Prostate Cancer Replications

Locus			A Freq		Association		
Chr Reg	SNP		Cntrl	Case	OR	p value	Nearby Genes / Fcn
8q24 (2)	rs16901979	C/A	0.04	0.06	1.52	$1.1x10^{-12}$	Intergenic
8q24 (3)	rs6983267	T/G	0.50	0.56	1.25	$9.4x10^{-13}$	Intergenic
8q24 (1)	rs1447295	C/A	0.10	0.14	1.42	$6.4x10^{-18}$	Intergenic
10q11	rs10993994	C/T	0.38	0.46	1.38	$8.7x10^{-29}$	*MSMB:* suppressor prop.
10q26	rs4962416	T/C	0.27	0.32	1.18	$2.7x10^{-8}$	*CTBP2:* antiapoptotic activity
11q13	rs7931342	T/G	0.51	0.56	1.21	$1.7x10^{-12}$	Intergenic
17q12	rs4430796	G/A	0.49	0.55	1.22	$1.4x10^{-11}$	*HNF1B:* suppressor properties
17q24	rs1859962	T/G	0.46	0.51	1.20	$2.5x10^{-10}$	Intergenic
19q13	rs2735839	A/G	0.83	0.87	1.37	$1.5x10^{-18}$	*KLK2/KLK3:* PSA
Xp11	rs5945619	T/C	0.36	0.41	1.29	$1.5x10^{-9}$	*NUDT10*, *NUDT11:* apoptosis

Witte, Nat Rev Genet 2009

Figure 1. SNPs associations with disease

AliBaba2: Software designed to identify predicted transcription factor and other regulatory region binding sites in anonymous DNA sequences. The program permits evaluation of DNA changes to determine if they result in creation or deletion of potential regulatory motifs in DNA [60].

1.6. Genetic Epidemiology

1.6.1. Definition

Classical epidemiology is the study of disease forms and factors associated with the etiology of diseases having an ultimate aim of preventing the disease [61]. It deals with the analysis of the familial distribution of traits. The measurement of exposures to specific substances and early biological response (that could lead to somatic mutations) is done by molecular epidemiologic. The intention of these types of studies is to assess the host genotype and phenotype against external factors; as well as search to improve disease classifications such as heterogeneity, etiology and prognosis with the use of markers for a specific effect (like gene expression) [61]. Due to the close relation of genetic epidemiology and molecular epidemiology; there is a tendency to overlap. The aim is to identify the heritage section of a particular illness, localize the gene and discover an indicator linked with disease susceptibility [61].

Genetic epidemiology strives for comprehend interactions between the genetic and environmental factors and how they result in different diseases and traits in humans. The studies use large samples. The dynamics of the population group could vary the frequency and distribution of the genetic and environmental influences, causing a novel effect on the study phenotype. As previous stated; the relation gene-gene and gene-environment is commonly analyzed in genetic epidemiology for a specific disease. The most widely accepted definition of genetic epidemiology is “a

science which deals with the etiology, distribution, and control of disease in groups of relatives and with inherited causes of disease in populations" [62].

1.6.2. Categories

As genetic epidemiology studies the role of genetic factors in health and disease; in families and populations all of them co-existing and interacting in a specific environment; different methods to analyze genetic data are available. Each one answering a different question and accessible to studies depending their objectives; either in family studies (segregation, linkage, association) or in population studies (association) [61].

Familial aggregation: Genetic variants are shared among relatives in a greater proportion than with unrelated individuals. A primary characteristics of an illness is that the affected people tend to cluster in families; familial aggregation [42]. A question has to be made; is the disease has a genetic component? One way of trying to answer this question is by looking for the detection and estimation of familial aggregation. Familial aggregation of a trait is a required but not sufficient condition to infer the importance of genetic susceptibility. Environmental and cultural influences can also aggregate in families [61]. Families share not only genes but they might sharing environmental factors, behaviors, diet, nutrition, exposure to pollutants; aspects that have to be taken into consideration when a genetic analysis are done [10].

Twins studies: Another path to assess the genetic contribution of a trait is by using twin studies. An important characteristic of monozygotic (MZ) pairs is that

100% of their genes are shared; in contrast the dizygotic (DZ) twins shared 50% of their genes. It seems that the dissimilarities in health and disease patterns among MZ twins must be created by the environmental factors[10]. On contrary the DZ twins have the environmental features apart from the genetic differences [10]. These types of studies have been used to find associations of a genetic component in the etiology in many diseases [63] including Alzheimer disease [64] , periodontitis [65] and cancer [66, 67].

Segregation analysis: is the study of the pattern of disease transmission in families [10]. As Mendel's laws predicted; genes passed from parents to children in a predictable way [68]. This analysis looks for a model that account for the observed segregation trait throughout families [10].

Linkage analysis: also known as affected pedigree member method; base their studies in the concordant phenotype between siblings or any first blood line members in a family [42]. These studies aim to find the chromosome location for a trait in a gene. These brands of studies are based on the statement that alleles tend to passed from one generation to another [10]. They also try to obtain a crude chromosomal location of the gene or genes associated with a phenotype of interest, e.g. a genetic disease or an important quantitative trait [61]. Sometimes linkage studies get confused with association studies. However, association may result from direct involvement of the gene or linkage disequilibrium (LD) with the disease gene at the population level [61].

Association studies: emphasis on population frequencies, in contrast to LD which concentrate on concordant inheritance [61]. This kind of study as well as linkage analysis were made to follow the genetic mapping of complex traits [42]. Environmental factors working together with several genes may contribute to the disease liability; this situation creates a difficult setting to build a disease model [10]. Association deals with a "susceptibility" locus, that raise the likelihood of getting the illness but is not "necessary" or "sufficient" for the disease to get express [61].

Advantages of association studies vs. linkage: the presence of several affected family members is not required, as it is needed in linkage studies. There are no assumptions about how the inheritance mode of the disease was acquired. Another practical difference is the statistical power used in the association studies; it has the sufficient power to assess weakness genes [61, 62]. Due to the fact that samples come from the affected group and controls; association studies avoid the necessity of gathering families and take samples from each member of a pedigree [42].

HapMap Project™: When sets of genes, alleles and/or SNPs are inherited, they are referred to as a haplotype [69]. International HapMap Project™ was formed to create a haplotype map of the human genome, the HapMap, with the data collected the project will be able to assess common patterns of human DNA sequence variation [70]. The HapMap is expected to work as a resource for researchers to look for genes that could interfere in health, disease, and responses to drugs and environmental factors [70]. The goal of the project is to describe common SNPs in

the human genome, to assess the location of each one and to describe the distributions patterns in specific populations [10]. HapMap was not created to assess relations between polymorphisms and diseases; moreover this project was developed with the intention of producing a catalog of common genetic variants; so clinicians and researchers can join forces and identify the associations between the SNPs and the diseases [10].

GWAS studies: Genome-Wide Associations Studies, were created to determine if specific alleles of a SNP are associated with a disease (case) or control with a frequency that deviates from random possibility[71]. These studies evaluate SNPs across the genome sequence of a population in cases and controls looking for statistically significant associations for a SNP allele with a specific disease [72]. After identification of the SNPs, the biggest challenge is demonstrate the biological functionality, pathways and their mechanism [73]. Moreover the overall goal is the opportunity to formulate novel prevention strategies and treatment plans based on the pathway or biologic function the SNP [72].

1.7. Genetic Testing

1.7.1. Terminology

There are several techniques in which genetic testing can be performed, among them are the analysis of human DNA, RNA and protein [74]. Genetic testing can be define as any medical test that can produce and reproduce results in terms of

variations in chromosomes genes or proteins [75]. More than 3000 of different genetic testing are available for the public.

Genetic testing can be performed by different methods such as gene test, chromosomal genetic test and biochemical tests. Molecular genetic tests or gene tests are the ones that use small parts of the DNA in single genes and identify any variation or disorder that could eventually result in a condition [75]. When the whole chromosome or long parts of the DNA are studied to detect any abnormalities that can be the etiology of a condition; a chromosomal genetic test is conducted [75]. In cases where the measurements of amounts or activities of important proteins are needed to be analyzed the best way to do proceed is by a biochemical genetic test [75, 76].

1.7.2. Types of Genetic Testing

There are a number of different types of genetic tests, which are distinguished based on their goal/reason purpose of the test those include

Newborn screening: this kind of testing is performed just after birth; and its' goal is to identify genetic disorders [75].

Diagnostic testing: the test is use to discard any genetic problem. It does not have a time frame, so it can be performed at any given time. The result can be helpful and can influence a person's choices about their medical treatment and management of a disease or disorder. Is also used when a condition is suspected [75].

Carrier testing: is needed to assess people who have one copy of a gene mutation that in presence of another copy of the mutation can be primary etiology of the condition [75].

Prenatal testing: this testing is a very common tool today; it is used to predict any problems in the fetus's genetic material. However it has its limitations regarding the quantity of diseases or disorders it could identify [75].

Preimplantation testing: when assisted reproductive techniques are performed and the genetic material wants to be review. Only embryos without any changes or condition in their genes and chromosomes are implanted in the uterus [75].

Forensic testing: is DNA collection and analysis testing that enhances the criminal justice field. It is a very helpful tool for convicting the guilty and exonerating the innocent [77]. Furthermore, it can be used for establish biological relationships in cases where needed [75]

1.7.3. Regulation of genetic testing

Due to the fact of the rapid pace of discovery of genetic factors for illness conditions the ability to predict risk of disease in patients without symptoms have increased [78]. These processes have developed areas such as prevention and therapeutic treatment for a few diseases [78]. With new advances in sciences and medical fields novel techniques in genetic testing are been lunched [79].

It is important to realize that the Clinical Laboratory Improvement Amendments (CLIA) program is in charge of regulating the laboratories from a patient testing perspective, with the objective to guarantee accurate and reliable results. “The FDA regulates the fabrication of devices under the Federal Food, Drug, and Cosmetic Act (FFDCA) to make sure that devices, that related to diagnosis, cure treatment or prevention of disease are safe and effective. The main goal of the analytical validity is to evaluate if the test is getting the information it is suppose to get or detect. On the other hand, the way the analytical validity is performed form the FDA’s stand point of view is by review it before to the marketing of the test system. Moreover, the CLIA do not assess the clinical validity as oppose as FDA regulatory scheme.

The FDA defines a Laboratory Developed Test (LDT) “as an *in vitro* diagnostic test that is manufactured by and used within a single laboratory (i.e. a laboratory with a single CLIA certificate)”[49]. LDTs are also sometimes called in-house developed tests, or “home brew” tests. Similar to other *in vitro* diagnostic tests, LDTs are considered “devices,” as defined by the FFDCA, and are therefore subject to regulatory oversight by FDA.

Analytical validity works as an indicator of how well a measurement of a property is being collected in a test. It refers to the accuracy and performance of the test [80]. If the accuracy of a test needs to be measured, clinical validity is needed to fulfill that goal. It predicts the predisposition and the existence or not of a disease [80]. When the usefulness needs to be challenged, clinical utility is one of the required tests. This measurement could be helpful to the user as it provides

important information to the tested person; giving positive or negative results; which can be used to seek treatment or prevention [80].

Clinical validity, clinical utility, analytical validity and social consequences should be the main benchmarks used to assess the benefits and genetics test [80]. Due to the fact of the widely inclusive nature of genetic test; it is fundamental recommendations and new policies to control those genetic tests that are in need of thorough inspection [78].

A genetic test that predicts the phenotype with accuracy and reliability is normally known to have a good clinical validity [81]. Sensitivity, specificity, positive predictive values and negative predictive values form part of the tests that incorporate clinical validity [81]. The test that relates to the proportion of people with a disease, who also show a positive identification for the disease is called sensitivity. Specificity can be defined as the proportion of disease-free people who correctly tested negative for the disease [81]. As for the proportion of a person who tested positive for a disease who actually do have the disease is when a positive predictive value is defined [80]. Although a negative predictive value can be describe as the proportion of a person testing negative for a disease who in fact do not have the disease [80].

Another important term to define is the clinical utility of a genetic test, which is the importance that the use of the test has for decisions that will be taken, in regards to patient management when compared to current treatment modalities without the use of a genetic test [81].

It is relevant to highlight that effectiveness relates to individuals that were diagnosed positive with the disease; and states that the majority of them will improve with the proposed treatment [81]. In contrast, efficacy assesses whether a drug or a treatment will works [81]. Moreover, the measure that assess the risk that a specific circumstance will occur in a group as compared to another group it is called relative risk, and it helps to understand whether there are difference between groups. In regards their interpretation, when the measure is greater than 1 it means that the risk is greater and when it is lesser than 1 it means that the risk is lower [81]. The last term to discussed is the odds ratio, which is described as the odds of an outcome to occurs under the exposure of a particular factor when compare with the odds of the same factor to happen without the presence of the exposure [81].

However, even with the presence of all these tests and knowledge of how genetic testing should be regulated, the lack of a protocol and specific regulations has led to several tests that are available in the market without any independent analysis to verify the claims the product makes [79]. The authority charged of preserve correct conditions and assess the genetic testing is the FDA [80].

There are several principles to follow with the intention of achieving the main goal of genetic testing; which can be defined as the enhancement of the health and well-being [80]. That is why no test should be marketed until the utility and validity have been proven [80].

With the rapid growth in medical genetics, a concern has been raised regarding the control and usefulness of the genetic tests. In 1998 the Secretary's

Advisory Committee on Genetic Testing (SACGT) was given the task of developing advice in the medical, scientific, ethnical, legal, and social areas to the Department of Health and Human Services (DHHS) [38]. After a thorough analysis, the committee concluded that there was a need to create a classification methodology for genetic tests and that there was an existing lack of regulatory control from the FDA [38].

Likewise in 1994 the National Institutes of Health-Department of Energy (NIH-DOE) formed a Joint Work Group on the Ethical, Legal and Social Implications (ELSI) of Human Genome Research launched the Task Force on Genetic Testing [82]. Among other functions the Task Force has the responsibility of supervising, assessment of genetic tests in the United States and creates policies concerning and damaging and benefits of genetic testing [82].

Numerous problems have being postulated regarding the existing policies for genetic testing; among those are regulations on the clinical laboratory quality, clinical validity, transparency, usefulness, effectiveness [83].

At present time there is not a government agency evaluating the clinical utility for most of the genetic tests [39]. Because of the lack of action by the FDA, several companies are marketing questionable claims about their test directly to consumers [39].

Today, the genome can be sequenced both quickly and cheaply. Commercially this can be done for about $500. Getting the DNA sequenced will show many genetic variants for example SNPs, SNV, copy-number variants; however, there is not enough data or evidence regarding what most of the variants do, if

anything. With media-hype people believe that their DNA can be sequenced, and get access to their disease risk and what can be done to treat them; however, this is not the case. Therefore, media-hype creates a problem and unrealistic expectations for genetic testing. These people tend to have a Mendelian bias in understanding genetics; they fail to realize that SNP-based testing is unrealistic. SNPs do not *cause* disease like mutations do. A SNP association is not useful for predicting risk, and should not be expected to do so.

CHAPTER TWO

Periodontal-Genetic Associations

2.1. Periodontal definition

Periodontal disease is a condition that relates the periodontium with the loss of attachment by means of hard and soft tissues destruction [2]. Periodontal pathologies encompass a large number of diseases; periodontitis is one of these periodontal pathologies [2]. Periodontitis has been defined as an inflammatory condition that affects the supporting tissue of the teeth [5, 6]. The attachment apparatus is completely involved during the presence of the disease and the its components are slowly degraded [6]. The main tissues that form the support apparatus of the teeth include the alveolar bone, dental cementum, periodontal ligament and the gingiva [84]. These tissues interact in a dynamic homeostasis that could be affected with the trigger of a threshold on one of the several factors that form the healthy equilibrium [85].

2.2. Classification

The current classification used at U.S. to categorize periodontal pathologies is based on the 1999 International Workshop for Classification of Periodontal Diseases and Conditions [86]. The existing classification describes seven types of pathological entities; chronic and aggressive forms of periodontitis are two of those categories [2].

2.2.1. Chronic Periodontitis

The term chronic periodontitis describes an inflammatory gingival disease which affects the attachment apparatus [6]. Since the condition is non-specific and not related to age the term “chronic” was selected [2]. Depending on the extent of the disease, chronic periodontitis could be further described as localized or generalized [2, 87]. This is the most common form on a population level. Periodontitis is a general clinical term that refers to an inflammatory destruction of the periodontium. Like cancer, it is not telling of the etiology, but a general descriptor.

2.2.1.1. Etiology

During the 1970’s and 1980’s it was hypothesized that the cause of chronic periodontitis was exclusively related to plaque [88, 89]. However, another theory was presented, explaining the etiology from a non-specific stand point of view [90]. The paradigm of an etiology based on a unique bacterial invasion has evolved into an interdisciplinary and comprehensive relationship between the host immune system, epigenetics and the oral microorganisms [3, 91, 92]. Moreover, the “biological system model” integrated with several other etiological factors could play a role in the component of the disease [92].

More evidence is showing the predisposition of periodontal disease due to genetics [10, 58, 93]. Evidence has shown a genetic trace in periodontal patients[65], showing that genetic aspects might significance a 38% to 82% periodontal variations[65]. For chronic periodontitis, the genetic model that best suits it, is the

common complex gene disorder, which explains that hundreds or thousands of genes might be needed to have the phenotypic expression of the disease[42].

2.2.1.2. Clinical presentation

General characteristics of untreated chronic periodontitis can be defined but are not limited to; presence of plaque and calculus supra- and subgingival, edema, erythema, loss of stippling, changes in marginal gingiva, bleeding upon probing, pocketing, loss of the attachment apparatus, bone loss, furcation involvement, tooth loss, mobility, and pathological tooth displacement [94].

2.2.2. Aggressive Periodontitis

Aggressive periodontitis is a term that has been used since the last international workshop for the classification of periodontal disease and conditions[87]. The disease is not related to the quantity of local factors instead is associated with familial aggregation [2, 87, 95]. Although the literature is conflicting on the importance of the bacterial invasion, such as aggregatibacter actinomycetemcomitans (A.A.) microbs, [7], a neutrophil deficiency in the neutrophil function has been suggested [7].

2.2.2.1. Etiology

There are several hypotheses about the cause of the pathology[94]. The current concepts are based on multifactorial etiology with genetics playing an important role but epidemiological studies clearly show other factors such as microbial, smoking [2, 94]. Evidence has shown that the disease falls more into a

simple gene disorder or autosomal dominant inheritance[8]. This is indicative of a direct association with aggressive periodontitis and Mendelian disorder by familial aggregation/ segregation analysis studies [13, 96, 97].

2.2.2.2. Clinical presentation

The condition is characterized by a rapid and aggressive pattern of soft and hard tissue destruction [2, 94]. The clinical characteristics are similar to the ones described for chronic periodontitis, however, within the parameters of a more aggressive and rapid variant[94]. An important pathological characteristic of the disease is the high incidence in young patients, with an age range of less than 30 years old for the generalized form, and circumpubertal age for the localized aggressive form [94].

2.3. Genetic associations with chronic periodontitis

In contrast to Mendelian phenotypes of periodontitis, which are associated with the clinical form of aggressive periodontitis, the majority of the cases of the disease follow the chronic clinical description. For these chronic cases the genetic associations appear to be related to common complex disorders, probably due to their phenotypic differences on a genetic contribution [98] as well as on other environment factors such as microbes, diet, stress, oral hygiene, local factors, demographics and host susceptibility. Common complex diseases are expected to have involvement of many SNPs contributing each one in a small amount to the

etiology. It may influence the immune, inflammatory response, repair, but no one gene contributes to the disease. Additionally, environmental factors likely act through epigenetic, changing the methylsation and histone modifications changing the effect levels of gene expression.

As the genetic field rapidly expands [99], many novel technologies are arriving in the medical arena, including the dental sector; Recently, several companies have been marketing biological and genetics markets to predict the risk for caries and periodontal disease that a patient has depending on their genetic or biological predispositions.

For the periodontitis test the scientific background were developed base on previous studies that have been performed for autoimmune diseases, diabetes and several other conditions with an inflammatory factor [1, 100, 101]. Many of the research was conducted to identify the genes involved in the inflammatory cascade [1, 102]. From different studies, several genes started to show a pattern and a variety of cytokines were identified as possible pathological agents of inflammation [1, 102, 103]. Due to the association of the inflammatory reaction of periodontitis and the inflammatory process of certain autoimmune disease, an extrapolation of the genes found on those studies was conducted, and research using certain cytokines to understand the process of predict the risk periodontitis began [104]. Now there are specific interleukins used as predictors for risk of periodontitis such as IL-1 and IL-6[105, 106].

CHAPTER THREE

Immune System Relations with Periodontitis and Genetics

3.1. Immunology [107]

Immunity is defined as the ability of an organism to oppose disease[107]. The immune system responds to infectious challenges through a complex hierarchical iterative response of cells, proteins and molecules [107]. The science that studies the immune system is called immunology[107].

3.2. Cytokines

Cytokines are large groups of proteins, peptides or glycoproteins secreted by specific cells of the immune system [108, 109]. Cytokines play an important role in the host immune system, specifically as inflammation mediators [104]. These mediators are involved not only in activation of the immune system, but also in regenerative processes and the regulation of metabolism, in the maintenance of bone homeostasis and in many neural functions [110-114].

A number of different cells produce cytokines including: helper T cells, macrophages, mast cells, endothelial cells, and dendrocyte cells [115, 116].

Cytokines are composed of a large family of proteins, composed of mainly of interleukins, interferon, colony-stimulator factors and growth factors.[117].

Due to the associations between cytokines and several inflammatory diseases, these molecules have been studied to understand their relationship in periodontal disease [118]. Studies have shown possible pathways of cytokines and their interactions within the inflammatory cells of the host. Evidence has shown associations between cytokines and matrix metalloproteinase

(MMP)[119, 120], transforming growth factor TGF [121, 122], bone resorption [123, 124] , inflammation, and stress [125, 126].

Cytokines are composed of several different cells that mainly work in cell communication and cell-to-cell effect interactions [1]. The cytokine network involves a large number of cells working together on several pathways [1] as observed in figured 2 [1].

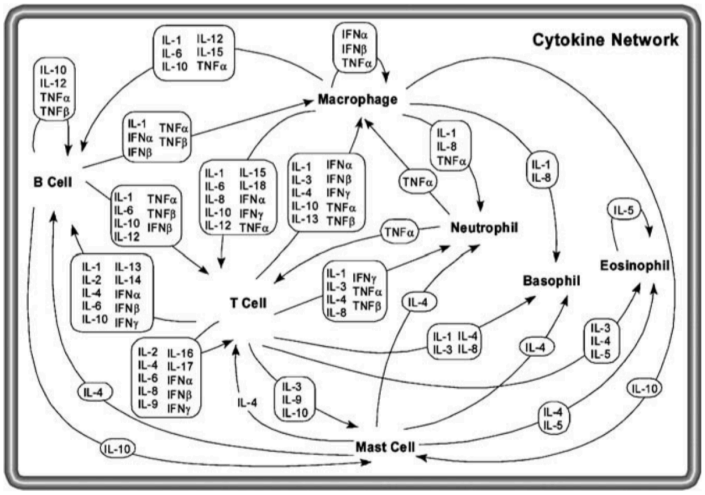

Figure 2. Network showing the possible interaction of immune cells producer and cytokines, taken from Zhang publication [1]

3.3. Interleukin-1

Interleukin-1 is part of the cytokine family within its' multiple functions they could be mentioned as immunity, hematopoiesis and inflammation but not restricted to [116]. Proinflammatory activity has been nexus with IL-1 [23]. IL- 1 beta and alpha are part of the interleukin-1 family. Among their primary activity is

the stimulation of antigen presenting cells and T cells. IL-1α and IL-1β have been elucidated in the activation of proteases for the degradation of the extra cellular matrix and alveolar resorption of the bone [27]. IL-1RN is the receptor antagonist of IL-1 α and β, and it works by regulating their activity by competitively inhibiting the biding at the receptor site, resulting in the down regulation of interleukin-1 [27]. IL-1 is has been marked as a pro-inflammatory cell of the immune system. IL-1α is highly involved in the cutaneous inflammatory reactions and in the initiation of the lesion formation [127]. Evidence has reported that IL-1α and IL-1β increase the amount of prostanglandin E2 and matrix metalloproteinase, creating the loss of the periodontal attachment apparatus [128].

3.3.1. Association with periodontitis

Several studies have attempted to identify associations of a single or a cluster of specific genetic variations with immunologic responses and periodontitis. One of the first associations of a Single Nucleotide Polymorphisms related to an inflammatory response for periodontitis was previously described for rheumatoid disease[104]. The postulated SNPs were mostly cytokines [108, 129-132]. As a result of these previous studies IL-1 has been extensively researched for different pathologies, periodontitis is one of example of the wide spectrum [18, 19, 21, 24, 25, 27, 30-32, 37, 133].

Kornman et al. [133] reported the use of IL-α (-889) (+4845) and IL-1β (+3938) as genetic markers to identify the susceptibility to periodontitis. The previous study

was based on the premise of the chronic inflammatory nature of periodontitis and the associations of some particular cytokines [133]. Several others studies have investigated the possible link of these particular SNPs and periodontitis [18, 22, 24-27].

IL-1 α (-889) rs1800587, IL-1 α (+4845) rs17561, IL-1β (+3935) rs1143634 and IL-1RN (+2018) rs419598 are SNPs that have been studied to assess their potential as biological markers to predict the risk for chronic periodontitis.

3.4. Interleukin-6

Interleukin-6 is a cytokine that mediates both pro-inflammatory and anti-inflammatory functions [117]. Endogenous IL-6 functions in an anti-inflammatory role in both local and systemic acute inflammatory responses [134] . Il-6 also plays a central role in the shift from acute to chronic inflammation, where it functions to promote pro-inflammatory responses [135]. This dual effect of IL-6 has resulted in the investigation of its role in a range of autoimmune and inflammatory diseases, including periodontitis.

3.4.1. Association with periodontitis

IL-6 has been reported to be important in the etiology of periodontitis [136, 137]. Increased levels of IL-6 have been reported in chronic periodontitis tissues[138]. While most studies propose that IL-6 drives the destructive process, some propose a protective role [137]. More than 100 genetic polymorphisms are

known to exist in the IL-6 gene and an increasing number of studies have reported associations for a number of these genetic variants with disease states including periodontitis [139].

The single nucleotide polymorphisms rs1800795 is a IL-6 genetic variant, which exists at the 5' regulatory region [140]. Located at -174 in the IL6 gene proximal promoter, the rs1800795 polymorphism has 2 alleles, a more common "G" allele and a less common "C" allele. Secreted levels of IL-6 have been reported to correlate with the presence of a particular rs1800795 allele[141]. Early clinical studies reported the "G" allele was associated with the autoimmune disease rheumatoid arthritis, and increased secretion of IL-6 protein[142]. *In Vitro* studies reported increased LPS induced expression of the "G" allele compared to the "C" allele[138]. Additionally*, in vitro* studies indicated that the "G" allele was associated with greater expression in response to pro-inflammatory stimuli[143]. As a result some investigators concluded that the rs1800795 "G" allele was associated with greater baseline levels of IL-6 and with a greater inflammatory response to pro-inflammatory stimuli [135]. The number of study participants in the previous papers was low and not definitive [129, 130, 136, 144-150]. Nonetheless, investigators proposed this as sufficient evidence for a genetic test

Moreover, IL-6, more specifically the SNP rs 1800795, has been associated with several disorders such as: cardiovascular disease, Kaposi's sarcoma, type-2 diabetes, stroke, obesity, Hodgkin's lymphoma, sudden infant death syndrome, cancer (including breast, gastric, and prostate), hypertension, periodontitis and complications after organ transplantations or grafts [151-158].

The rs1800795 SNP is the basis of a genetic test currently offered to assess an individual's risk for moderate-severe periodontitis [159].

Hypothesis and Objectives

4.1 Hypothesis

Chronic periodontitis is an inflammatory condition, which affects the supporting tissue of the teeth [84, 160]. The disease's pathogenesis is based on the interaction of the biologic constituents, genetics and environmental factors. [92, 118]. Due to the inflammatory components of the disease research has been conducted from a histological and biological basis [129]. Cytokines were proposed to have an important role in the development of periodontitis [104, 118]. Since the pathological paradigm is shifting from a bacterial to a multifactorial etiology[4]; genetic interactions in the etiology have been studied and associated [10, 93, 98, 161].

There is inconclusive evidence showing associations between periodontitis and interleukins [15, 19, 27, 146, 148, 162-166]. These associations have been used as evidence for the use of genetic testing to predict the risk of an individual with chronic periodontitis using several interleukins as genetic markers [24, 37, 133, 167].

The use of SNPs to assess susceptibility of periodontitis is being challenged from a genetic marker point of view because it is not consistent with a common complex disease for a few SNPs to be predictive. Due to this dilemma the hypothesis of this study is that there is a low clinical validity and utility of interleukins polymorphisms (rs1800587, rs17561, rs1143634, rs419598, rs1800795) when utilized as a risk predictor for periodontitis.

4.2 General objective

The purpose of the study is to evaluate the clinical validity and utility of IL-1 alpha (-889) (rs180587), (+4845) (rs17561), beta (+3954) (rs1143634), IL-6 (-174) (1800795) and ILRN (+2018) (rs419598) when used to assess the periodontal risk of a patient.

4.3 Specific objectives

A. To analyze the clinical validity of the 5 interleukin SNPs polymorphisms (rs1800587, rs17561, rs1143634, rs419598, rs1800795).
    a. To assess the pathological status of the 5 interleukin SNPs.
    b. To analyze the genetic penetrance of the synonymous SNPs using Mutation Assessor™ software.
    c. To analyze the genetic functionality of the synonymous SNPs using a tridimensional model of the Mutation Assessor™ software.
    d. To identify reports of the association of rs1800587, rs17561, rs1143634, rs419598, rs1800795 for individuals with periodontitis or healthy periodontium.

B. To analyze the clinical utility and validity of rs1800587, rs17561, rs1143634, rs419598, rs1800795.
    a. To calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), odds ratios (OR) and relative risk (RR) for each SNP in individual studies, populations and summed data.

b. To calculate the allele frequency distributions for each study using Hardy-Weinberg equilibrium.
c. To calculate the allele frequency distributions for each population using Hardy-Weinberg equilibrium.
d. To calculate the specific allele frequency distributions for all population using Hardy-Weinberg equilibrium.

Methodology

5. Methodology

A literature search for reports of IL-1, IL-6 studied genotypes associated with the clinical phenotype was conducted. The information was used to calculate sensitivity, specificity, positive predictive value, negative predictive value, odds ratio and relative risk. These measures were used to determine the clinical validity of these markers as genetic tests.

In addition, an evaluation of the reported population allele frequencies for each SNP was also executed to estimate their expected population frequencies in several specific populations and grouped.

The data collected for the completion of this study was obtained from two processes; a literature review and the 1000 Genome Project™ database. With the information attained, risk factor genotypes were assessed using two genetic models for risk evaluation for each SNP, based on the "high risk" allele proposed by the companies.

Model 1 represents a homozygous (AA) genotype:

Model 1: "high risk" allele = high risk

Model 2 stands for homozygous (AA) and heterozygous (Aa) genotype:

Model 2: "high risk" allele = high risk

Data collected from 1000 Genome Project™ and the scope review was studied and analyzed using the two models previous described.

5.1. Literature review

A scope review through PubMed™ and EMBASE™ was queried to identify reports of the association of the 5 SNPs (*IL-1 α (-889) rs1800587, IL-1 α (+4845) rs17561, IL-1β (+3935) rs1143634, IL-1RN (+2018) rs419598 and IL-6 (-174) rs1800795)* for individuals with chronic periodontitis and healthy controls.

These data were used to calculate sensitivity, specificity, (PPV), (NPV), (OR) and (RR) for each SNP in individual studies and summed data.

5.2. 1000 Genome Project

Data from the 1000 Genomes Project™ were used to estimate the percentage of individuals expected to carry each SNP genotype for African Americans, Han Chinese, Northern Europeans and Caucasian North Americans. Population specific allele frequency distributions were calculated using Hardy-Weinberg equilibrium algorithm.

5.3. SNP analysis software

The analyses of the SNPs were performed by the utilization of 5 genetic software (Provean™, PolyPhen-2™, Esembl™, Mutation Assessor™, 1000 Genome Project™,

AliBaba™). These programs give important information to understand the biological and genetic consequences of the possible allele sequence variation.

Results

6.1. Gene qualitative analysis

The studied SNPs were assessed from a biological and genetic perspective by the use of bioinformatics tools and software.

Table 1

Quantitative description of designated IL -1/ IL-6 genes

SNPs	Genes	Gene size	SNPs in Gene
rs419598	IL-1RN (+2018)	26803 bases	590
rs17561	IL-1 α (+4845)	11480 bases	298
rs1800587	IL-1 α (-889)		
rs1143634	IL-1β (+3935)	7153 bases	257
rs1800795	IL-6 (-174)	6119 bases	107

A total of five single nucleotide polymorphisms were analyzed for this study. Four of the SNPs are part of the IL-1 genes family, which include: 2 SNPs of the IL-1 α family, one SNP of the IL-1β group and one SNP of the antagonist receptor for IL-1α and IL-1β. The last one is part of the IL-6 gene family. When comparing IL-1 with IL-6 the SNPs from IL-1 showed greater number of genetic variations all of them above 250. IL-6 presented 107 genetic variations within their gene. All of the IL-1 genes present a larger chain than the IL-6 gene, which is the smallest. IL-1RN has the largest base pair chain followed by IL-1α, IL-1β and IL-6. The IL-6 polymorphism is the gene that has the lesser amount of SNPs, and IL-1RN is the gene with the greater number of SNPs.

6.2. SNPs Analysis Data

Each SNP was assessed to determine if different alleles were associated with a functional consequence. Assessing different strategies:

1. Consequence of a change in amino acid (for protein coding sequence)
2. Consequence of a change in transcription binding site (for regulatory region)
3. Consequence for altered splicing (ectopic) site (for intron)

Table 2

SNPs qualitative description

Genes	SNPs	Location	Amino Acid Change	Nucleotide Change	Variant Type	Location of the Variant
IL-1 α (+4845)	rs17561	2:113537223	A114S	C/A	Non-Synonymous	Protein-coding region
IL-1 α (-889)	rs1800587	2:113542960	Not aplicable	G/A	Intronic	Noncoding
IL-1β (+3935)	rs1143634	2:113590390	F105F	G/A	Synonymous	Protein-coding region
IL-1RN (+2018)	rs419598	2:113887207	A57A	T/C	Synonymous	Protein-coding region
IL-6 (-174)	rs1800795	7:22766645	Not aplicable	C/G	5'	Noncoding

The four IL-1 SNPs where located at the chromosome 2, IL-6 polymorphism is located at chromosome 7 at a proximal 5' regulatory site of the gene.

The distributions within the gene of the IL-1 SNPs are between a 349984 base pair (bp) range. rs419598 and rs1143634 are 296817 bp apart, rs1143634 and rs1800587 are 47430 bp apart and rs1800587 and rs17561 are 5737 pair bases apart.

The SNPs assessed in this study can be classified depending on the location in which they are found, 3 SNPs are situated in the coding region like rs17561,

rs1143634, rs419598, and the 2 others are not in the coding section (intronic SNPs) such as rs1800587 and rs1800795.

Rs17561 presents an amino acid change (non-synonymous) from an A to an S at the amino acid number 114. On two others SNPs (rs1143634 and rs419598) the nucleotide change did not created a change in the amino acid chain (synonymous changes).

As for IL -6 polymorphism it is located at the chromosome 7. This SNP is far apart from chromosome 2. The variation is located in a non-coding section of the gene.

Depending on where the variations are located, they might interfere with the protein formation process or expression, and this might have biological consequences repercussions on the overall health on an individual. The analysis of the pathologic effect is observed in table 3.

Table 3

Pathological Assessment of the coding-protein SNPs

GENES	SNP	Ensembl	POLY PHEN -2	MUTATION ASSESSOR	PROVEAN
IL-1 α (+4845)	rs17561	Neutral	Damaging	Neutral	Neutral
IL-1β (+3935)	rs1143634	Neutral	Not available	Not available	Neutral
IL-1RN (+2018)	rs419598	Neutral	Not available	Not available	Neutral

For the protein-coding SNPs, the majority of the pathological assessment was found to be neutral; meaning that the SNPs were non-damaging to the genes expression**.** More specifically for the rs17561, three programs estimated non-deleterious changes (neutral), and only one program showed a possibility of a damaging effect (damaging). Concerning rs1143634 and rs419598 two of the software described the variations as a neutral change; the other two were not able to find the variation.

In the case of rs1800587 and rs1800795 different software was needed in order to assess the genetic variations, due to the SNPs location (intronic and 5' regulatory region). The two previous SNPs are in a noncoding region reason why a different software analysis was needed. The AliBaba™ program was used to identify the possible changes of the nucleotides over the genes. For rs1800587 and rs1800795 an additional site for transcription biding is gained.

Figure 1. Prediction for the effect of the ancestral "G" or "C" allele on putative transcription binding sites for rs1800795

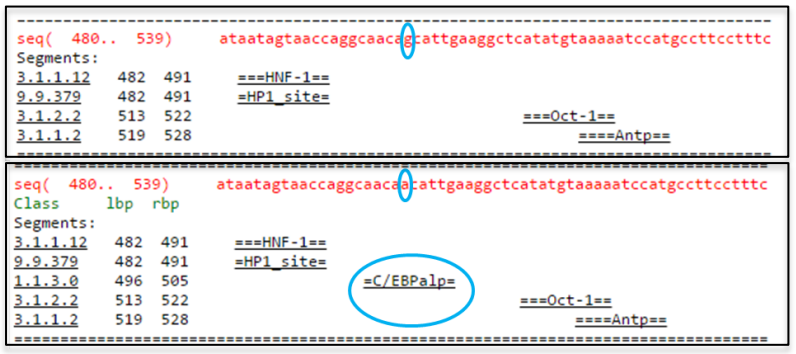

Figure 2. Prediction for the effect of the ancestral "G" or "C" allele on putative transcription binding sites for rs1800587

Based on the AliBaba2 software prediction a new biding site is acquired when the nucleotide change takes place in both of the intronic polymorphisms.

6.3. 1000 Genome Project Data Analysis

Using 1000 Genome software analysis, allele frequencies for each SNP were used, in different populations and grouped as a whole

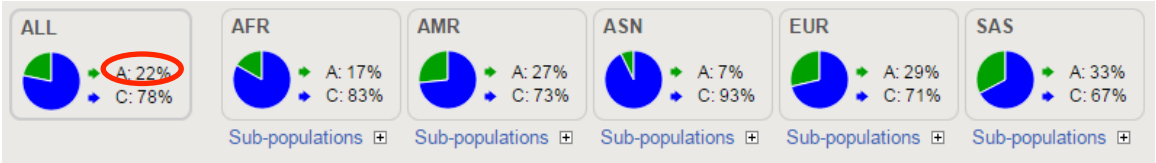

Figure 3. Allele frequencies from 1000 Genomes Project™ for rs17561

Population description, African (AFR), American (AMR), Asian (ASN), European (EUR), South Asian (SAS) and the whole population (ALL). The "risk" allele is marked in a red circle.

The alleles A and C (which are the nucleotides were the SNP is located) are expressed in different percentages in different populations, base on that numbers the frequency of carriers of each genotype for each population can be estimated using a mathematical algorithm (Hardy-Weinberg equilibrium). A mean percentage (22%) of the allele "A" was obtained from the summary of each allele in each population (African, American, Asian, European, South Asian). It is important to illustrate that the mean do not represents the penetration that the allele has for each population. The "risk" allele varies within each population from a 33% in the South Asian to 7% penetrance in the Asian.

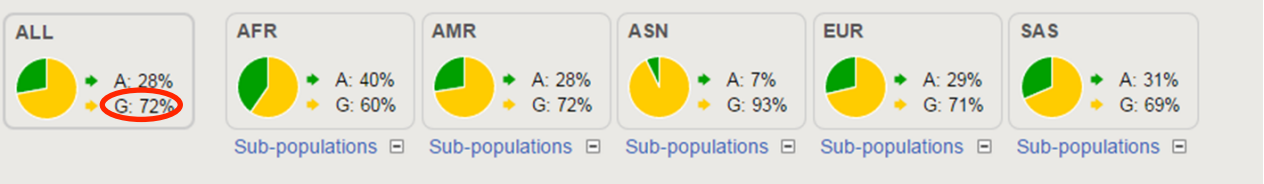

Figure 4. Allele frequencies from 1000 Genomes Project™ for rs1800587

Population description, African (AFR), American (AMR), Asian (ASN), European (EUR), South Asian (SAS) and the whole population (ALL). The "risk" allele is marked in a red circle.

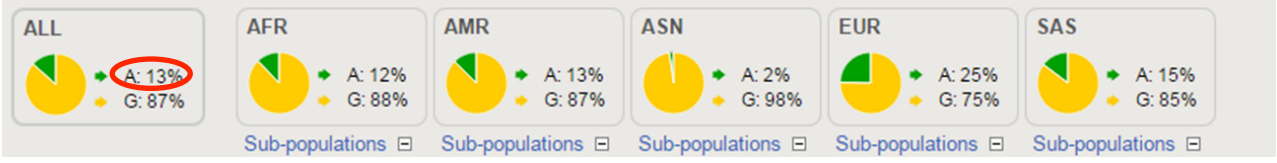

Figure 5. Allele frequencies from 1000 Genomes Project™ for rs1143634

Population description, African (AFR), American (AMR), Asian (ASN), European (EUR), South Asian (SAS) and the whole population (ALL). The "risk" allele is marked in a red circle.

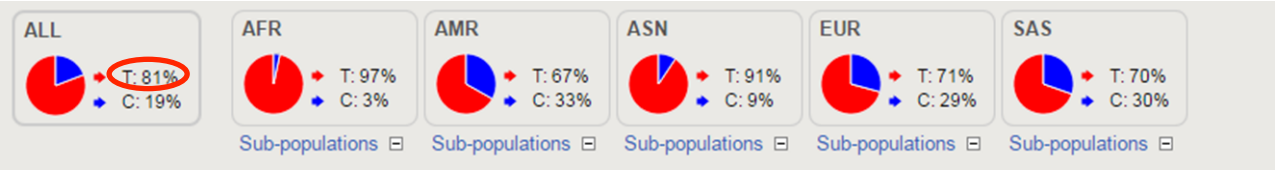

Figure 6. Allele frequencies from 1000 Genomes Project™ for rs419598

Population description, African (AFR), American (AMR), Asian (ASN), European (EUR), South Asian (SAS) and the whole population (ALL). The "risk" allele is marked in a red circle.

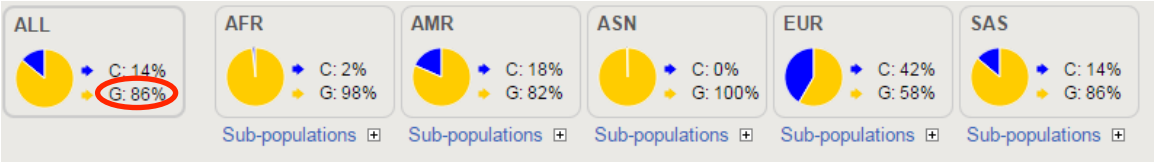

Figure 7. Allele frequencies from 1000 Genomes Project™ for rs1800795

Population description, African (AFR), American (AMR), Asian (ASN), European (EUR), South Asian (SAS) and the whole population (ALL). The "risk" allele is marked in a red circle.

Rs1800795 showed that in some populations such as AFR, they possess a "G" allele frequency of around 98%, followed very closely with the Asian with a 100 % "G" allele penetrance. A wide range the 'risk allele" from 58% to 100% was observed in the IL-6 SNP.

6.3.1. SNPs allele frequency

Using the 1000 Genome Project™ the allele frequency for rs1800587, rs17561, rs1143634, rs419598, rs1800795 was obtained, and the Hardy-Weinberg equilibrium algorithm was used to estimate the genotype frequency in a population.

Hardy-Weinberg equilibrium could be use to predict the possibility of a genotype related to a disease occurs in a population.

Table 4. Proportion of "high risk" genotypes determined for each SNP expected from Hardy Weinberg Allele Distribution (Model 1)

SNPs	IL-1α (-889) (rs1800587)	IL-1α (+4845) (rs17561)	IL-1β (+3954) (rs1143634)	ILRN (+2018) (rs419598)	IL-6 (-174) (rs1800795)
African American	44%	2%	1%	86%	83%
Utah	56%	6%	5%	49%	24%
Han Chinese	88%	0%	0%	86%	100%
South Han Chinese	90%	0%	0%	83%	100%
British	41%	14%	10%	54%	35%

Wide range variations in the genotype frequency distribution between each SNP were found in Model 1; values ranges from 41% - 90% for rs1800587, 0% - 14% for rs17561, 0% - 10% for rs1143634, 54% - 86% for rs419598 and 35% - 100% for rs1800795 for different populations. Additionally, there were high range values within groups of population when using different SNPs to predict the risk for chronic periodontitis; for African America 1% - 86%, Utah 5% - 56%, Han Chinese 0% - 100%, South Han Chinese 0 % - 100%, British 10% - 54%

Table 5. Proportion of "high risk" genotypes determined for each SNP expected from Hardy Weinberg Allele Distribution (Model 2)

SNPs	IL-1α (-889) (rs1800587)	IL-1α (+4845) (rs17561)	IL-1β (+3954) (rs1143634)	ILRN (+2018) (rs419598)	IL-6 (-174) (rs1800795)
African American	89%	26%	23%	99%	99%
Utah	94%	44%	42%	91%	74%
Han Chinese	99%	12%	4%	99%	100%
South Han Chinese	99%	10%	2%	99%	100%
British	46%	60%	53%	93%	84%

In Model 2 wide value ranges in the genotype distribution frequency where found as well between each population when a SNP was tested for risk assessment for periodontitis, for rs1800587 ranges from 46% - 99%, rs17561 from 10% - 60%, rs1143634 from 2% - 53%, rs419598 from 91% - 99% and rs1800795 from 74% - 100%. When comparing the predictability of different SNPs within population groups, a high range is observable, as African American values range from 23%-99%, Utah 44% - 94%, Han Chinese 4% - 100%, South Han Chinese 2% - 100% and British 46% - 93%.

6.4. Literature Review Data Analysis

A literature review was performed to search for the association between periodontitis and the SNPs proposed by the companies as "high risk" alleles.

Table 6. List of the articles for rs1800587

(IL-1α)(-889)(rs1800587)									
PAPERS		Control				Disease			
		TT	CT	CC	Total cases	TT	CT	CC	Total cases
1	Braosi	7	49	60	116	9	55	66	130
2	Brett	19	37	44	100	38	63	63	164
3	Karasneh	12	30	38	80	15	51	34	100
4	Lopez 2005	2	34	65	101	4	140	186	330
5	Lopez 2009	9	80	119	208	174	119	43	336
6	Rogers	3	24	33	60	6	32	46	84
7	Trevillato	3	31	35	69	3	21	20	44
8	Wagner	29	41	19	89	52	33	10	95
9	Schulz	7	38	44	89	5	31	36	72
10	Laine	8	24	33	65	11	57	37	105
11	Armingohar	2	17	19	38	5	16	15	36
12	Gore	4	8	20	32	18	11	3	32
	Totals	105	413	529	**1047**	340	629	559	**1528**

After the scope review, 12 articles were found that fulfilled the association terms for the SNP rs1800587. A distribution of the genotypes by author is illustrated in table 6. A total of 1047 controls and 1528 disease cases were used for the analyses for this SNP. The CT genotype in the disease cases was the one that showed higher number of cases. Instead in the controls the CC genotype was higher.

Table 7. List of the articles for rs17561

(IL-1α)(+4845)(rs17561)									
PAPERS		Control				Disease			
		TT	GT	GG	Total cases	TT	GT	GG	Total cases
1	Kobayashi 2007	1	15	84	100	2	38	146	186
2	Kobayashi 2009	1	16	91	108	4	32	218	254
3	Sakellari 2003	12	35	63	110	7	17	21	45
4	Sakellari 2006	10	39	51	100	16	38	46	100
5	Gayathri	2	35	15	52	4	31	16	51
	Totals	26	140	304	**470**	33	156	447	**636**

After the literature review for the SNP rs17561, there were 5 papers that were utilized for the analyses. A total of 470 control cases and 636 patients were diagnosed with periodontitis. The genotype distribution per paper was also included, displaying that the GG genotype was higher in both cases and controls.

Table 8. List of the articles for rs1143634

(IL-1β)(+3953/4)(rs1143634)									
PAPERS		Control				Disease			
		TT	CT	CC	Total cases	TT	CT	CC	Total cases
1	Duan	0	4	90	94	0	7	23	30
2	Gore	4	8	20	32	1	13	18	32
3	Galgraith	3	9	33	45	4	7	9	20
4	Laine	4	20	29	53	9	42	54	105
5	Rogers	1	23	36	60	6	12	30	48
6	Zhong	5	17	70	92	5	38	90	133
7	Anusaksathien	0	1	42	43	0	0	54	54
8	Sakellari 2003	9	46	55	110	6	16	23	45
9	Soga	0	5	59	64	0	4	60	64
10	Huang	0	4	85	89	0	31	151	182
11	Lopez 2005	2	11	88	101	7	91	232	330
12	Brett	8	32	58	98	6	17	32	55
13	Moreira	0	7	24	31	3	20	29	52
14	Sakellari 2006	5	39	56	100	9	32	59	100
15	Tian	0	0	36	36	0	4	32	36
16	Gustafsson	0	5	8	13	0	5	8	13
17	Drozdzik	2	19	31	52	0	11	21	32
18	Jansson	1	11	19	31	0	9	11	20
19	Wagner	8	31	53	92	34	35	24	93
20	Ferreira	15	40	120	175	16	30	71	117
21	Goncalves	1	2	14	17	0	8	21	29
22	Kaarthikeyan	1	6	21	28	0	9	24	33
23	Kobayashi 2009	0	6	111	117	0	6	102	108
24	Lopez 2009	5	42	161	208	21	69	134	224
25	Prakash	0	9	39	48	7	30	38	75
26	Shete	1	25	74	100	0	7	93	100
27	Gayathri	0	24	28	52	1	16	34	51
28	Karasneh	6	33	41	80	9	44	47	100
29	Schulz	5	33	50	88	8	20	44	72
30	Trevilatto	2	15	27	44	2	16	51	69
31	Yang	32	62	125	219	19	34	162	215
32	Al-Hebshi	2	28	10	40	10	18	12	40
33	Bascones-Martinez	4	48	48	100	4	32	64	100
34	Garlet	22	44	148	214	30	55	112	197
35	Masamatti	2	7	21	30	7	9	14	30
	Totals	150	716	1930	**2796**	224	797	1983	**3004**

For rs1143634, a greater number of papers were found. 2796 healthy cases and 3004 of periodontal involved patients were the totals for this SNP. The CC genotype was present in greater number for the disease and control cases, and exhibiting a big difference with the number of healthy and disease cases with TT.

Table 9. List of the articles for rs419598

(IL-1RN)(+2018)(rs419598)									
PAPERS		Control				Disease			
		TT	CT	CC	Total Cases	TT	CT	CC	Total Cases
1	Guzman	1	1	15	17	0	13	54	67
2	Kobayashi 2007	0	13	87	100	0	13	173	186
3	Kobayashi 2009	94	13	1	108	235	10	0	245
	Totals	95	27	103	**225**	235	36	227	**498**

Table 9 describes the low number of papers that were located through the scope review. A total of 225 control cases and 498 disease cases met the criteria for the study. The CC control genotype summed a total of 103 cases, the higher in their group. As for the disease group the TT genotype presented the higher number of cases with 235.

Table 10. List of the articles for rs1800795

(IL-6) (-174) (rs1800795)									
PAPERS		Control				Disease			
		GG	GC	CC	Total Cases	GG	GC	CC	Total Cases
1	**Nina Babel**	GG+GC= 84		32	116	GG+GC= 72		52	124
2	**Priscila Ladeira**	29	26	5	60	24	16	3	43
3	**A.M Costa**	12	12	3	27	15	2	0	17
		12	12	3	27	16	5	0	21
4	**Nagaraj B. Kalburgi**	2	4	9	15	10	3	2	15
5	**P.R. Moreira**	30	18	6	54	58	24	6	88
		30	18	6	54	40	24	3	67
6	**P.C. Trevilatto**	12	21	3	36	12	12	0	24
		12	21	3	36	17	3	4	24
7	**Tellervo Tervonen**	37	GC+CC= 141		178	11	GC+CC= 40		51
8	**Luca Scapoli**	75	11	31	117	85	73	19	177
9	**P.M. Brett**	55	19	25	99	22	24	11	57
10	**Fernando Franch-Chillida**	106	28	7	141	79	18	6	103
		151	37	9	197	34	9	4	47
11	**WH Fan**	129	1	0	130	177	1	0	178
12	**L. Nibali**	42	74	28	144	124	142	52	318
		22	6	1	29	68	15	2	85
		38	7	0	45	81	9	0	90
13	**Florenca Abdanur Stefani**	11	8	2	21	12	8	1	21
14	**Lydie Izakovicova Holla**	37	53	17	107	43	71	34	148
15	**Xiao**	132	0	0	132	156	1	0	157
16	**Manuela Lanni**	119	125	34	278	40	25	12	77
17	**Gustavo Garlet**	116	70	18	204	97	69	32	198
18	**Johan Wohlfahrt**	32	36	14	82	48	63	26	137
	Totals	1325	748	256	**2329**	1341	657	269	**2267**

Regarding the findings for the literature review for IL-6 rs1800795, 18 articles were identified that report this Il-6 association. Moreover, the numbers of healthy controls were 2329 cases and 2267 patients that had periodontitis. The genotype GG was present in higher numbers for groups, controls and disease.

6.4.1. Diagnosis and Screening Evaluation Test Calculations (DSETC)

Results from the literature review regarding the studied genotypes associated with the clinical phenotype were calculated using different algorithms. The data was distributed in two genotype models.

Table 11. DSETC Model 1 for all population

GENOTYPES	IL-1α (-889) (rs1800587)	IL-1α (-4845) (rs17561)	IL-1β (+3954) (rs1143634)	ILRN (+2018) (rs419598)	IL-6 (-174) (rs1800795)
SENSITIVITY	22.3%	5.2%	9.8%	47.2%	59.9%
95% CI	20.19 % to 24.42 %	3.60 % to 7.21 %	6.28 % to 14.27 %	42.73 % to 51.68 %	57.71 % to 62.14 %
SPECIFICITY	90.0%	94.5%	93.1%	57.8%	47.3%
95% CI	87.99 % to 91.72 %	92.00 % to 96.35 %	89.28 % to 95.84 %	51.03 % to 64.31 %	44.9 % to 49.6%
PPV	76.4%	55.9%	56.1%	71.2%	55.6%
95% CI	72.18 % to 80.27 %	42.40 % to 68.84 %	39.75 % to 71.52 %	66.00 % to 76.04 %	53.5% to 57.8%
NPV	44.2%	42.4%	53.2%	33.1%	51.6%
95% CI	42.10 % to 46.36 %	39.39 % to 45.47 %	48.48 % to 57.85 %	28.44 % to 37.97 %	49.11 % to 54.08 %
OR	2.5676	0.9346	1.4517	1.2227	1.3
95% CI	2.0300 to 3.2476	0.5510 to 1.5852	0.7627 to 2.7634	0.8899 to 1.6800	1.1219to1.4562
RR	2.2188	0.938	1.2507	1.1176	1.1
95% CI	1.8089 to 2.7215	0.5689 to 1.5463	0.6936 to 2.2552	0.9346 to 1.3365	1.0505 to 1.1759

Table 12. DSETC Model 2 for all population

GENOTYPES	IL-1α (-889) (rs1800587)	IL-1α (-4845) (rs17561)	IL-1β (+3954) (rs1143634)	ILRN (+2018) (rs419598)	IL-6 (-174) (rs1800795)
SENSITIVITY	63.4%	29.7%	43.0%	54.4%	86.9%
95% CI	60.94 % to 65.84 %	26.19 % to 33.44 %	36.56 % to 49.58 %	49.93 % to 58.85 %	85.41% to 88.41%
SPECIFICITY	50.5%	64.7%	73.2%	45.8%	11.8%
95% CI	47.45 % to 53.60 %	60.17 % to 69.00 %	67.69 % to 78.30 %	39.14 % to 52.53 %	10.37% to 13.48%
PPV	65.2%	53.2%	57.1%	69.0%	53.7%
95% CI	62.68 % to 67.59 %	47.90 % to 58.52 %	49.42 % to 64.46 %	64.12 % to 73.50 %	51.64% to 55.09%
NPV	48.6%	40.5%	60.8%	31.2%	43.9%
95% CI	45.61 % to 51.64 %	36.95 % to 44.09 %	55.42 % to 66.02 %	26.25 % to 36.52 %	39.39% to 48.62%
OR	1.7703	0.7743	2.0628	1.0079	0.9
95% CI	1.5089 to 2.0769	0.6004 to 0.9986	1.4272 to 2.9817	0.7348 to 1.3825	0.7382to1.0922
RR	1.2818	0.8414	1.606	1.0036	1.1
95% CI	1.1926 to 1.3776	0.7091 to 0.9983	1.2605 to 2.0464	0.8686 to 1.1596	0.9256 to 1.3045

Tables 11 and 12 provide the DSETC calculations for the whole population for the five SNPs utilized for this study and distributed in Model 1 and Model 2. The percentages for sensitivity for Model 1 ranged from 5.2% to 59.9%, specificity 47.3% - 94.5%, PPV 55.6% - 76.4%, NPV 33.1% - 53.2%, OR 0.93 – 2.57, and RR 0.93 – 2.2. In Model 2, the sensitivity ranged from 29.7% - 86.9%, specificity 11.8% - 73.2%, PPV 53.2% - 69%, NPV 31.2% - 60.8%, OR 0.77 – 2.06, and RR 0.84 – 1.60.

Discussion

Periodontitis describes the clinical presentation of a group of disease states, characterized by destruction of the periodontal attachment apparatus surrounding the teeth. Etiologically, periodontitis is a multifactorial disease; resulting from the host immune/inflammatory response to microbial biofilm in the periodontium, host genetic and environmental factors. From a genetic perspective, disease can be classified into three groups: chromosome disorders, single gene disorders and complex multifactorial disorders [42]. Periodontitis can be delineated into two general forms, aggressive periodontitis and chronic periodontitis [5-7]. Meaning that a mutation or variation in one gene could be the primary etiology for the clinical manifestation of aggressive periodontitis [10-13]. Based on the genetic characteristics and previous evidence, aggressive periodontitis falls into the single gene disorder category [8, 9]. In contrast, chronic periodontitis is a complex multifactorial disease, where hundreds or thousands of genes contribute each in a small part for the overall cause of the disease [10].

As genes determine qualitative and quantitative aspects of immunological responses, efforts are being directed to identify the genetic differences that can underlie individual differences in disease susceptibility [14-17]. The hope is that genetic variants that are etiologically important for disease risk can be used as the basis for clinically useful genetic tests.

With the false premise of health evolution in genetic markers and the lack of control and regulation, several companies are instituting genetic tests to assess the "risk" of a patient to periodontitis. Several companies utilized previous underpowered studies that showed weak associations of interleukins and

periodontal disease [15, 17-20, 29, 35-37, 118, 128, 130, 134-136, 143, 146-148, 150, 151, 164] as the scientific base for the incorporation of a genetic test for periodontitis.

In this study a thorough analysis was performed for selected SNPs, when used as genetic tests for periodontitis. The purpose of this thesis is to evaluate factors important in considering genetic testing for periodontitis susceptibility and to evaluate support for the clinical validity and clinical utility of such tests.

Five SNPs (IL-1 α (-889) rs1800587, IL-1 α (+4845) rs17561, IL-1β (+3935) rs1143634, IL-1RN (+2018) rs419598 and IL-6 (-174) rs1800795) were analyzed to conduct this project. The polymorphisms were selected based on the "risk allele" stipulated by companies that are using these SNPs to predict the risk for periodontitis.

A literature review was performed for IL-1, IL-6 studied genotypes in association with the clinical phenotype. This information was gathered and used to determine the clinical validity of these markers as genetic tests by calculating sensitivity, specificity, PPV, NPV, OR and RR.

In addition, the allele frequencies were obtained using the bio-informatics software for each studied SNP with the goal of estimating the genotype expected population frequencies in several specific populations and grouped. Moreover, each pathological analysis was conducted for each studied polymorphism with the help of biotechnology software. Predicting the possible damaging functionality of the SNP.

Four of the SNPs (rs1800587, rs17561, rs1143634, rs419598) evaluated in the study are located at IL-1 gene. The disparity polymorphism (-174) is located in the IL-6 gene (Table 1). There are a great number of genetic variants present in both of these genes and they are related to their base pair size. Approximately every 130 pair bases a SNP is found[168]; therefore, the number of genetic variations is directly proportional to the number of pair bases. SNPs are genetic variations that usually do not produce a disease or condition[10], therefore in a complex multifactorial disease like chronic periodontitis, hundreds or thousands of SNPs are needed to contribute to create the phenotypic pathology, not only one or a combination of a few SNPs.

The type of genetic variation most commonly known is called single nucleotide polymorphism (SNPs) [10], they can be found in the promoter region, exon or intron areas of the gene[10]. These variations can be classified depending on the action they produce on the amino acid. If the SNP produces no change in the amino acid is called a synonymous variation[46], like in the case of rs1143634 and rs419598. However, if the nucleotide change creates a change in the amino acid, it is designated as a nonsynonymous variant, for instance rs17561. These kinds of variations are more likely to have a damaging effect on the gene due to the change the new amino acid could generate. Regarding the intronic variations such as rs1800795 and 1800587, the amount of information that is currently available is not well understood, meaning that the knowledge of what the SNPs can produce is limited.

Three of IL-1 SNPs are categorized as a protein coding variations, rs1143634 and rs419598 experience a change in the nucleotide that result in a codon variation but did not produce a change in the amino acid. By using bio-informatics it was possible to assess the pathological status (Table 3), all of the software categorized the variations as neutral. What this means is that the variation did not show damaging effects on the functionality, expression, up-regulation or down-regulation of the genes.

In contrast, rs17561 showed a nucleotide change that creates a different codon that produces a change in the amino acid chain. This non-synonymous SNP was classified by the software as neutral in 3 out of the 4 programs. With this result it can be inferred that the damaging value of the variation is not significant.

The variations that are on the protein coding region have been studied the most and there is a better understanding of the significance of their changes, compared with the intronic variations which the knowledge regarding what could be their effect over the gene and in the global health, it is limited.

The amino acid change that will not express a significant variation in size, electronic, hydropath, acid/neutral base, polarity, pH; will result in a new amino acid that probably will has less capabilities to create a damaging effect to the gene expression.

The IL-1 SNPs are closed together separated by a thousand pair chains, its' position could be associated with linkage disequilibrium (LD). LD "refers to the correlations among neighboring alleles, reflecting haplotypes descended from single,

ancestral chromosome"[59]. The parameters of extension that LD presents varies form a few kilobases (kb) to greater than 100 (kb) [59].

On the other side, IL-6 polymorphism and rs1800587 were studied by using AliBaba2 software figure 1 and 2. AliBaba2 is an algorithm that calculates the possibility of having a new biding site. Both of the SNPs gained a new transcription-binding site; the overall functional significance of this is not fully understood.

As previously discussed a few companies are marketing SNPs that claim to assess the individual risk for periodontitis [105, 159, 169]. Moreover, these companies identified their risk alleles, for every SNPs in use. From 1000 Genome Project™ allele distribution estimation was obtained for the five SNPs studied. For rs1800587 the allele market as the risk factor is "G", Figure 4 shows that the allele differs from population to population, for example in the Asians the "G" allele is present in 93 % of the population but for the Africans the percentage change to a 60%. This pattern of diverse allele percentages between populations is not the exception during the analysis of the others SNPs, it is the rule. Assessment of the polymorphism revealed a wide range of percentages of the allele of each SNP within different populations. In Figure 3 the values for the "risk" allele "A" of rs17561 range from 33% to 7% with an average of 22%, for rs1143436 the average is 13% for the "A" risk allele and ranges from 25% to 2% (Figure 5). The "T" allele is the "risk" one for rs419598 (Figure 6) polymorphism with a range of 70% for the south Asian to 97% in the African population. As for Figure 7, the rs1800795 variant has a wide percentage range of the "risk" allele "G", for instance in the European population 58% of people have that allele as compared to 100% of Asians with it.

Using the 1000 Genome Project™ allele distribution and Hardy-Weinberg equilibrium calculation about the possibilities of the "high risk" population was determined for each SNP, using the two Models, Tables 4 and 5. In Model 1 for rs1800587 the "high risk" range from 90% in the south Han Chinese to a 41% in the European (British). When comparing the American population with a South Han Chinese with different SNP such as 1800795 the gap increases, from a 24% to 100%, respectively. Likewise, the SNPs were compared with each other over one population to determine the variation expressed by each polymorphism. The results for Model 1 show that the African American population ranges go from 1% for the rs1800587 to 86% for rs419598, and in a more dramatic example South Han Chinese have percentage from 0 to 100%; demonstrating the lack of consistency and the wide variation of allele presence if one specific SNP is used to predict the risk for periodontitis compare to another within one population (Table 4). In Table 5, the allele distribution is compared for 5 different populations, and the results were similar to the ones obtained in Model 1, representing wide spectrums of percentages of allele frequencies utilizing the same polymorphisms; such as the case of the South Han Chinese with ranges from 2% to 100%. The two Models developed in this study provided the opportunity to cover all of the allele "risk" possibilities in order to assess the frequency distribution and other calculations. The reason this is highlighted is due to the fact that both of the Models showed a high rate of variation during the genotype frequency calculation when using Hardy-Weinberg equilibrium. This indicates that the SNPs that are used to determine the prognosis of the risk for

the disease are not strictly homogenous markers that can be used for an entire population.

These results also showed the lack of consistency of the SNPs to predict within different population the pathology that they are marketing for. If we take for example rs1800795 the chance of having the genotype from one population to another is basically 50 %; more specifically in the Asian population everyone has the "risk" genotype, which would mean that 100% of the Asian population would be at risk of having the disease.

It indicates the SNPs are population specific; they vary the values depending on which population is been tested, as explained before. Additionally, within a specific population there are different percentages of "high risk" genotypes when compare with each SNP, detonating the inconsistency and lack of predictability of the tested cytokines to predict periodontitis.

The analysis of the diagnosis and screening test calculations of Model 1 for rs1800587 revealed a low sensitivity of 22.3% and high 90% of specificity, moderate PPV of 76% and NPV of 44%; ranges that follows the rs17561 and rs1143634 very closely (table 11), these results show low clinical validity. Clinical validity follows the parameter of a test to be able to accurately and reliably predict the disease tested for[170]. Sensitivity, specificity, PPV and NPV are methods that are needed in order to accomplish the calculations of the clinical validity [170]. As for the ILRN and IL-6 polymorphisms, their ranges of sensitivity, specificity, PPV and NPV were found to be more around 50% range; for the exception of PPV and NPV of

ILRN that were 71% and 33% respectively. With these ranges, the balance weighted more to a low value range reflecting again a low clinical validity.

General speaking the ranges of DSETC in Model 1 for sensitivity were found to be 5.2% - 59.9%, specificity from 47.3% - 94.5%, PPV 55.6% - 76.4%, NPV 33.1% - 53.2%, OR 0.9 - 2.5 and RR 0.9 – 2.2. These values show a low clinical validity and utility for the entire test and in all parameters.

When the SNPs were tested for Model 2, (homozygous "risk" genotype plus heterozygous) in general terms the range values are poor as observed in Table 12. A wide range was determined around the 50% of sensitivity, specificity, PPV and NPV. These findings reiterate the ones observed in Table 11, showing a similar pattern of a low clinical validity for the polymorphisms as reliable tools to predict chronic periodontitis.

When considering the OR and the RR of the entire population, neither supports the SNPs allele in either Model 1 or Model 2 as a risk allele for chronic periodontitis. The values range from 0.9 to 2.5; meaning that there is not a high risk and/or high possibility of increasing the chances of getting the disease if the test shows positive for the "risk" allele.

Base on the clinical utility definition and goals and following the line of results observed; there is a tendency of the SNPs tested in these study to do not fulfill the objectives there were create to be accomplished, by showing a low clinical validity and a been a not reliable test to use. Moreover its absent of benefits for the patient in a diagnostic and therapeutic point of view is well proved.

Likewise, the fact that the IL variations are population-specific, the test fails to retain the reliability as the results showed the extent of the inconsistency throughout the different populations. Another factor that needs to be taken into consideration for the analysis the these SNPs is the genetic classification in which aggressive and chronic periodontitis follow; being that aggressive periodontitis is a single gene disorder (autosomal dominant)[8] and chronic periodontitis shows more traits of a common complex disorder. All of these factors add together to produce the result of low clinical validity and utility of the SNPs when used a genetic markers for periodontitis.

Based on biological and genetic points of view for the tested SNPs, there is evidence showing how these interleukins could experience changes in the plasma levels depending on different factors. Starting with IL-1 variations evidence showed positive associations of inflammation and IL-1 plasma levels [171]. IL-α is reported to be present in healthy conditions, and is normally found in keratinocytes of the skin, epithelial cells of the mucosa, platelets, liver, lungs and kidneys, endothelium [172]. On the other hand IL-β is not related to healthy settings, they are a result of the activation of monocytes, macrophages and dendritic cells [172]. Is important to highlight that IL-1 has a biological characteristic of auto activation, seen greatly express in IL-1β, also known as auto-inflammation [1, 172]. In other words, IL-1 could vary its expression depending on the inflammatory response and/or the patient's health status.

In tumor sites, the IL-1 cytokine has been associated with higher expression[173] as well in inflammation environments. Interleukin-1 is considered

an “alarm’ cytokine; inducing the pro-inflammatory signals that propagate and sustain inflammation [173].

IL-β has been related with higher expression when increase production levels of substance P and prostanglandin E_2[1].

IL-1 also function as an anti-inflammatory cytokine[1], IL-1RN is a receptor antagonist proposed to interfered in the activation of IL-1α and IL-1β [1, 18, 127]. Other types of conditions had also been associated with an increase serum of IL-1α more specific (+4845), and a predisposition of acne vulgaris[127], and coronary heart disease [128]; for osteoarthritis and rs1143634 [174]; IL-1α/IL-1β and diabetes type 2, among others.

IL-6 is considered a pleiotropic molecule with anti and pro-inflammatory attributes [137, 142]. Evidence have shown increase levels of IL-6 and IL-1β in sites with periodontal inflammation[137].

Additionally, while most studies reported an association for the G allele with increased serum Il-6, several studies actually reported increased serum Il-6 was associated with the C allele [175]. A review of the original report that there is a significant association between rs1800795 genotype and the presence of specific periodontopathic microbes, is based on a very small sample size, and has not been validated in larger studies and meta-analyses, leading to the conclusion that the effect of this SNP on IL-6 production is minimal or it is not the Il-6 -174 G/C polymorphism that is directly influencing IL-6 production [176].

These initial reports may be over-simplistic and do not take into account that other factors can influence endogenous IL-6 levels, and that there are differences in

tissue and cells location. For example, exercise has been associated with a significant (100-fold) increase in Il-6 expression [35].

These findings are consistent with the current understanding of complex diseases such as chronic periodontitis. It is estimated that thousands of common genetic SNPs influence susceptibility for common complex diseases such as type 2 diabetes, rheumatoid arthritis, human immunodeficiency virus (HIV), and stress[31, 32, 114, 171, 177-179]. Additionally, evidence indicates that these conditions involve a large number of environmental and host genetic interactions. In addition to genetic variants in primary DNA sequence, epigenetic interactions involving methylation and common complex diseases such as chronic periodontitis.

In contrast to the simple Mendelian diseases where a rare mutation of a gene can be largely deterministic of a clinical disease phenotype, common, complex diseases such as chronic periodontitis are quite different in terms of genetic risk. Where many (hundreds) of genetic variants can individually make a small contribution to disease risk [10], in contrast to having one gene or a haplotype variations as the precursors of a genetic diagnostic tool to predict chronic periodontitis. In such a disease models, there is redundancy for disease associated SNPs. Individuals can develop disease with or without any number of individual SNPs. In such a genetic disease model, it is unrealistic to propose that any single SNP type variant can be of diagnostic value. Additionally, it is equally unrealistic to propose that the absence of any individual SNP confers clinically meaningful decreased risk of disease.

Strengths of the study

- The present study complied more than 6000 control cases and more than 5000 for the controls. The high power that was achieved with the number of cases and controls create better and more reliable results.
- The study assessed in depth the biological factors that the single nucleotide polymorphism could affect. This was done in order to create a logical and biological explanation of the possible pathways in which an alteration in the protein sequence could mean. This helps in the overall understanding of how the functionality interrelates with the genetic variations.
- The SNPs that were studied as possible "genetic bio-markers" for periodontitis were exposed to a thorough genetic analysis using bio-informatics software. The use of bio-informatics technology provides a great tool in the assessment of variations giving the possibility to access data bases of thousands of sequences of different populations. Other programs focus in the analysis of the way the SNP behaves. The behavior then translates to numbers and scales showing if the changes will be damaging or benign for the carrier. With this assessment it was possible not only determine the present or not of the variation but also the expected changes in the sequence and the possible results of the changes. This creates an understandable and logic explanation of what could be the biological complications and physiological changes that could happen or not in the presence of certain polymorphisms.

- The results were assessed with the Diagnostic Screening Evaluation Test Calculations (DSETC). As explained before, in order to evaluate the parameters of a genetic test in terms of clinical validity and clinical utility the use of these calculations are a most. Therefore the study was able to evaluate the results with the reliability recommended by the Secretary's Advisory Committee on Genetic Testing (SACGT) for these types of experiments.

Weaknesses of the study

- As part of the difficulties faced while conducting this research was the lack of transparency of the companies that are selling the products. Transparency in terms of making available to the public the data they used as foundations to defend the products. It was difficult to find the genotypes the companies were using as "predictors" for risks of periodontitis.
- When the genotypes were obtained, another complication was that the literature was not descriptive enough in regards of the genotypes. Which means that although some papers could by use because they were relating periodontal disease and genetics, in a grand majority the authors were not adding to the papers the genotypes per cases and controls. This issue produced a decrease of articles that can be used in the current database. Although the power was high this could have been increased if the format of other papers would provide certain characteristics needed to be included in the research.
- In a more detailed analysis, the number of cases and controls that were available in the format needed for the inclusion criteria was low. A total of 225 controls and 498 cases were used for the analysis of the SNP. This problem decreases the power for that specific test. But also illustrates the limited evidence that is available on this variation, creating a more delicate statement of how the genetic test that uses this SNP has a very low scientific support.

- When the SNPs were assessed in regards of the biological considerations the intronic variations revealed a big limitation. Intronic variation have a more difficult and limited analysis and interpretation in the realm of bio-informatics. Consequently the study only speculates of the significance of the variation. Further advances in technology and understanding of genetics need to be gain in order to achieve a reliable and thorough explanation of the changes of these intronic variations.

Conclusions

This study evaluated the clinical validity and clinical utility of 5 interleukin SNPs (IL-1 α (-889) rs1800587, IL-1 α (+4845) rs17561, IL-1β (+3935) rs1143634, IL-1RN (+2018) rs419598 and IL-6 (-174) rs1800795) when used as "risk" predictors for periodontitis. Bio-informatics analyses were conducted for each polymorphism to estimate the genotype frequency distribution within the overall populations and their subgroups. Additionally, a scope review was performed to determine the genotypes and their associations with the phenotypic disease. This was done in order to assess the clinical validity by calculations of sensitivity, specificity, negative predictive value, positive predictive value, odds ratio and relative risk.

Certain companies are using single or multiple polymorphisms to predict the risk of periodontal disease. These companies fail to categorize periodontitis in terms of the clinical phenotype as an aggressive or chronic disorder. Therefore, the failure conducts to another failure on categorizing the phenotypes into a single gene disorder (Mendelian disease) as for aggressive periodontitis, or a complex multifactorial disorder for chronic periodontitis. These issues create skewed results regarding the use of the periodontal tests because they are virtually stating that the SNP or SNPs used, are indicative of a Mendelian trait. With that supposition several problems arrive: the subjective indication that the selected SNP or SNPs are equally distributed in all the populations, and that the studied polymorphisms were major contributors in the etiology of the disease. Then, based on the previous premise,

immediately all the users fall into the Mendelian disorder category when these tests are utilized because they are only using one SNP or haplotypes to test for periodontitis. All of the previous problems raise a red flag regarding the clinical utility, validity and efficacy of the tests.

Based on the genotype frequency distribution results presented in this study, it can be concluded that the "risk" genotypes vary within populations. For example, in Han Chinese and South Han Chinese groups, the genotype frequency is fixed at 100% for model 1 where as in the Utah group was 24%. These ranges were normally observed around both of the models and when compared in different populations. Which means that by using one SNP to predict the risk of the disease, the population makes a notary important factor in the presence of the "risk" allele, leading to an inconsistency in the test efficacy. For instance, if we apply the test to a Han Chinese the result obtained will be 100% "high risk" for periodontitis even if the person is clinically free of the phenotype disease. In contrast, if the same test is given to a Utah person the risk will dramatically change to the lower spectrum 24% (for Model 1). The way the test is given nowadays makes no discrepancy between populations, suggesting that there are no population differences when it is clear that the "risk" genotype is population specific as the present results display.

For the companies that are targeting haplotypes, the way to predict the risk is more complex in the way that the company split the genotypes to gain coverage within populations; overall the parameters follow the same inconsistency showed for IL-6 -174 [180].

The studied polymorphisms showed low parameters in all the Diagnosis and Screening Evaluation Test Calculations (DSETC). This means that the accountability of this SNP as a risk predictor for chronic periodontitis is low. These DSETC calculations should show high percentages when the tests are reliable and efficient. Low parameters in all risk genotypes and in all models, demonstrate the lack of clinical utility these "biomarkers" have to predict the risks for periodontitis.

These findings also highlight the concerns and recommendations of several advisory panels for genetic testing, including the GAO and secretaries advisory committee for genetic testing [38, 78, 80, 83, 181, 182], which highlight the lack of regulation for genetic testing of complex traits using SNP type polymorphisms.

The general public and many clinicians are unaware of the lack or regulation in this area. This study highlights the need to be careful before embracing such testing and supports the development and implementation of methods to evaluate the clinical validity and clinical utility of such tests before they are marketed to clinicians and to the public. This situation emphasizes the lack of evidence required for a research report as a way to accomplish and demonstrate clinical validity for a periodontal test.

In Summary these findings do not support the use of (IL-1 α (-889) rs1800587, IL-1 α (+4845) rs17561, IL-1β (+3935) rs1143634, IL-1RN (+2018) rs419598 and IL-6 (-174) rs1800795) as a genetic test for chronic periodontitis risk or susceptibility.

Measures of clinical validity indicate genotyping are not useful in classifying individuals for risk.

References

1. Zhang, J.M. and J. An, *Cytokines, Inflammation and Pain.* Int Anesthesiol Clin, 2007. **45**(2): p. 27-37.
2. Highfield, J., *Diagnosis and classification of periodontal disease.* Aust Dent J, 2009. **54 Suppl 1**: p. S11-26.
3. Larsson, L., R.M. Castilho, and W.V. Giannobile, *Epigenetics and its Role in Periodontal Diseases - A State-of-the-Art Review.* J Periodontol, 2014: p. 1-18.
4. Baelum, V. and R. Lopez, *Defining and classifying periodontitis: need for a paradigm shift?* Eur J Oral Sci, 2003. **111**(1): p. 2-6.
5. American_Academy_of_Periodontology, *Parameter on chronic periodontitis with advanced loss of periodontal support. American Academy of Periodontology.* J Periodontol, 2000. **71**(5 Suppl): p. 856-8.
6. American_Academy_of_Periodontology, *Parameter on chronic periodontitis with slight to moderate loss of periodontal support. American Academy of Periodontology.* J Periodontol, 2000. **71**(5 Suppl): p. 853-5.
7. American_Academy_of_Periodontology, *Parameter on aggressive periodontitis. American Academy of Periodontology.* J Periodontol, 2000. **71**(5 Suppl): p. 867-9.
8. Marazita, *Evidence for Autosomal Dominant Inheritance and Race-Specific Heterogeneity in Early-Onset Periodontitis.* 1994.
9. Beaty, *A genetic analysis of juvenile periodontitis in families ascertained through an affected proband.* J Hum Genet, 1987. **40**: p. 10.
10. Kinane, D.F.D.F.K., HIDEKI SHIBA & THOMAS C. HART, *The genetic basis of periodontitis Periodontilogy.* Periodontology 2000, 2005. **39**: p. 26.
11. Salvi, G.E., et al., *Influence of risk factors on the pathogenesis of periodontitis.* Periodontol 2000, 1997. **14**: p. 173-201.
12. Hart, T.C. and J.C. Atkinson, *Mendelian forms of periodontitis.* Periodontol 2000, 2007. **45**: p. 95-112.
13. Hart, T.C., et al., *Localisation of a gene for prepubertal periodontitis to chromosome 11q14 and identification of a cathepsin C gene mutation.* J Med Genet, 2000. **37**(2): p. 95-101.
14. Woo, P. and S.E. Humphries, *IL-6 polymorphisms: a useful genetic tool for inflammation research?* J Clin Invest, 2013. **123**(4): p. 1413-4.
15. Brett, P.M., *Functional gene polymorphisms in aggressive and chronic periodontitis.* Journal of Dental Research, 2005. **84**(2005): p. 6.
16. CastroTrindade, S., *Porphyromonas gingivalis HmuY-Induced production of interleukin-6 and IL-6 polymorphism in chronic periodontitis.* **J Periodontol**(2013): p. 6.
17. Nibali, L., *Association between periodontitis and common variants in the promoter of the interleukin-6 gene.* ELSEVIER, 2008. **10**(2008): p. 5.
18. Armingohar, Z., et al., *Polymorphisms in the interleukin-1 gene locus and chronic periodontitis in patients with atherosclerotic and aortic aneurysmal vascular diseases.* Scand J Immunol, 2014. **79**(5): p. 338-45.
19. Braosi, A.P., et al., *Analysis of IL1 gene polymorphisms and transcript levels in periodontal and chronic kidney disease.* Cytokine, 2012. **60**(1): p. 76-82.
20. Brett, e.a., *Functional Gene Polymorphisms in Aggressive and Chronic Periodontitis.* 2005.

21. Karasneh, J.A., et al., *Investigation of the interleukin-1 gene cluster polymorphisms in Jordanian patients with chronic and aggressive periodontitis.* Arch Oral Biol, 2011. **56**(3): p. 269-76.
22. Laine, M.L., B.G. Loos, and W. Crielaard, *Gene polymorphisms in chronic periodontitis.* Int J Dent, 2010. **2010**: p. 324719.
23. Lopez, *Association of Interleukin-1 Polymorphisms With Periodontal Disease.* 2005.
24. Lopez, N.J., C.Y. Valenzuela, and L. Jara, *Interleukin-1 gene cluster polymorphisms associated with periodontal disease in type 2 diabetes.* J Periodontol, 2009. **80**(10): p. 1590-8.
25. Rogers, *Do interleukin-1 polymorphisms predict the development of periodontitis or the success of dental implants?* 2002.
26. Schulz, *Single nucleotide polymorphisms in interleukin-1gene cluster and subgingival colonization with Aggregatibacter actinomycetemcomitans in patients with aggressive periodontitis.* Human Immunology, 2011.
27. Trevilatto, P.C., et al., *Association of IL1 gene polymorphisms with chronic periodontitis in Brazilians.* Arch Oral Biol, 2011. **56**(1): p. 54-62.
28. Wagner, J., et al., *Prevalence of OPG and IL-1 gene polymorphisms in chronic periodontitis.* J Clin Periodontol, 2007. **34**(10): p. 823-7.
29. Gayathri, R., et al., *Allele, genotype, and composite genotype effects of IL-1A +4845 and IL-1B +3954 polymorphisms for chronic periodontitis in an Indian population.* Indian J Dent Res, 2011. **22**(4): p. 612.
30. Goncalves, *Influence of IL-1 gene polymorphism on the periodontal microbiota of HIV-infected Brazilian individuals.* 2009.
31. Kobayashi, T., et al., *The interleukin-1 and Fcgamma receptor gene polymorphisms in Japanese patients with rheumatoid arthritis and periodontitis.* J Periodontol, 2007. **78**(12): p. 2311-8.
32. Kobayashi, T., et al., *Cytokine gene polymorphisms associated with rheumatoid arthritis and periodontitis in Japanese adults.* J Periodontol, 2009. **80**(5): p. 792-9.
33. Sakellari, *Prevalence of IL-1A and IL-1B polymorphisms in a Greek population.* 2003.
34. Sakellari, D., et al., *No correlation of five gene polymorphisms with periodontal conditions in a Greek population.* J Clin Periodontol, 2006. **33**(11): p. 765-70.
35. Fisher, C., *Interleukin-6 in acute exercise and training; what is the biological relevance.* Exerc Immunol Rev, 2006. **12**: p. 27.
36. Fishman, D., et al., *The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis.* J Clin Invest, 1998. **102**(7): p. 1369-76.
37. Agrawal, A.A., et al., *Assessment of single nucleotide polymorphism at IL-1A+4845 and IL-1B+3954 as genetic susceptibility test for chronic periodontitis in Maharashtrian ethnicity.* J Periodontol, 2006. **77**(9): p. 1515-21.
38. Secretary's_Advisory_Commitee, *Conclusions and Recommendations of the Secretary's Advisory Committee on Genetic Testing*. 2001, National Institute of Health. p. 13.

39. Javitt, G., *FDA Regulation of Genetic Tests*, in *Genetic & Public Policy*. 2008.
40. Wright, J.T. and T.C. Hart, *The genome projects: implications for dental practice and education.* J Dent Educ, 2002. **66**(5): p. 659-71.
41. Elston, R.C., J.M. Satagopan, and S. Sun, *Genetic terminology.* Methods Mol Biol, 2012. **850**: p. 1-9.
42. Nussbaum, R.L., et al., *Thompson & Thompson genetics in medicine*. Seventh Edition. / ed. 2007, Philadelphia: Saunders/Elsevier. xi, 585 page.
43. Theissen, G., *Saltational evolution: hopeful monsters are here to stay.* Theory Biosci, 2009. **128**(1): p. 43-51.
44. Lange, K., *Statistics for Biology and Health*. 2nd ed. 2003: Springer.
45. Avise, J., *Molecular Markers, Natural History and Evolution: Natural History and Evolution*. 1994: Chapman and Hall.
46. Genetics-Home-References. Available from: http://ghr.nlm.nih.gov/.
47. Lange, K., *Stistics for Biology and Health*. 2003.
48. International_Hap_Map_Project. *Genetic variation and use of the HapMap*. 2006; Available from: http://hapmap.ncbi.nlm.nih.gov/abouthapmap.html.
49. National-Center-for-Biotecnology-Information. Available from: http://www.ncbi.nlm.nih.gov/.
50. Nelson, D.L., and Michael M. Cox. , *"Nucleotides and Nucleic Acids." In Lehninger Principles of Biochemistry*. 3rd ed. 2000, New York: Worth Publishers.
51. Rose, A.B., *Intron-mediated regulation of gene expression.* Curr Top Microbiol Immunol, 2008. **326**: p. 277-90.
52. PROVEAN. Available from: http://provean.jcvi.org/genome_submit_2.php.
53. PolyPhen-2. Available from: http://genetics.bwh.harvard.edu/pph2/.
54. Assesor, M.; Available from: http://mutationassessor.org/.
55. 1000-Genomes-Project. Available from: http://www.1000genomes.org/.
56. ENSEMBL. *ENSEMBL*. Available from: http://useast.ensembl.org/index.html.
57. UniProt. Available from: http://www.uniprot.org/.
58. NCBI-Reference-Sequence-Database. Available from: http://www.ncbi.nlm.nih.gov/refseq/.
59. Reich, D.E., *Linkage disequilibrium in the human genome.* Nature, 2001. **411**: p. 5.
60. AliBaba2. *Documentation*. 2014; Available from: http://www.gene-regulation.com/pub/programs/alibaba2/intro.html.
61. Dorak, M.T. *GENETIC EPIDEMIOLOGY*. Available from: http://www.dorak.info/epi/genetepi.html.
62. Morton, N.E., *Outline of genetic epidemiology*. 1982, Basel ; New York: Karger. vii, 252 p.
63. MacGregor, A.J., et al., *Twins. Novel uses to study complex traits and genetic diseases.* Trends Genet, 2000. **16**(3): p. 131-4.
64. Raiha, I., et al., *Alzheimer's disease in Finnish twins.* Lancet, 1996. **347**(9001): p. 573-8.
65. Michalowicz, *Periodontal findings in adults twins.* J Periodontol, 1991. **62**: p. 7.

66. Lichtenstein, P., et al., *Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland.* N Engl J Med, 2000. **343**(2): p. 78-85.
67. Risch, N., *The genetic epidemiology of cancer: interpreting family and twin studies and their implications for molecular genetic approaches.* Cancer Epidemiol Biomarkers Prev, 2001. **10**(7): p. 733-41.
68. Monaghan, F. and A. Corcos, *On the origins of the Mendelian laws.* J Hered, 1984. **75**(1): p. 67-9.
69. Consortium, T.I.H., *A haplotype map of the human genome.* Nature, 2005. **437**(7063): p. 1299-320.
70. Hap-Map-Project. Available from: http://hapmap.ncbi.nlm.nih.gov/index.html.en.
71. Norrgard, K., *Genetic Variation and Disease: GWAS.* Nature Education, 2008. **1**.
72. National-Human-Genome-Research-Institute. *Genome-Wide Associations Studies*. 2015 [cited 2015; Available from: https://www.genome.gov/20019523.
73. Huang, Q., *Genetic Study of Complex Diseases in the Post-GWAS Era.* J Genet Genomics, 2015. **42**(3): p. 87-98.
74. National_Human_Genome_Research_Institute. *Overview of Genetic Testing*. 2015; Available from: http://www.genome.gov/10002335.
75. Genetics-Home-References. *Genetic Testing: Benefits, costs, risks, and limitations of genetic testing*. 2015; Available from: http://ghr.nlm.nih.gov/handbook/testing/genetictesting.
76. National-Human-Genome-Research-Institute. Available from: https://www.genome.gov/.
77. National_Institute_of_Justice. *DNA Evidence Basics*. 2012 2012; Available from: http://nij.gov/topics/forensics/evidence/dna/basics/pages/welcome.aspx.
78. National_Human_Genome_Research_Institute. *Promoting Safe and Effective Genetic Testing in the United States: Final Report of the Task Force on Genetic Testing*. 2006; Available from: http://www.genome.gov/10001733.
79. National_Human_Genome_Research_Institute. *Regulation of Genetic Tests*. 2015 2015; Available from: http://www.genome.gov/10002335.
80. Advisory_Committee_on_Genetic_Testing, S.s., *Enhancing the Oversight of Genetic Tests: Recommendations of the SACGT*. 2000, National Institutes of Health.
81. Teutsch, S.M., et al., *The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Initiative: methods of the EGAPP Working Group.* Genet Med, 2009. **11**(1): p. 3-14.
82. National_Human_Genome_Research_Institute, *Task Force Makes Final Recommendations on U.S. Genetic Testing*. 1997.
83. Secretary's_Advisory_Commitee, *The Integration of Genetic Technologies Into Health Care and Public Health: A Progress Report and Future Directions of the Secretary's Advisory Committee on Genetics, Health, and Society*. 2009.

84. Polimeni, G., A.V. Xiropaidis, and U.M. Wikesjo, *Biology and principles of periodontal wound healing/regeneration.* Periodontol 2000, 2006. **41**: p. 30-47.
85. Bartold, P.M. and T.E. Van Dyke, *Periodontitis: a host-mediated disruption of microbial homeostasis. Unlearning learned concepts.* Periodontol 2000, 2013. **62**(1): p. 203-17.
86. American_Academy_of_Periodontology, *Diagnosis of Periodontal Diseases.* J Periodontol, 2003. **74**: p. 10.
87. Armitage, G.C., *Development of a classification system for periodontal diseases and conditions.* Ann Periodontol, 1999. **4**(1): p. 1-6.
88. Loesche, W.J., *The antimicrobial treatment of periodontal disease: changing the treatment paradigm.* Crit Rev Oral Biol Med, 1999. **10**(3): p. 245-75.
89. Loesche, W.J. and J.R. Giordano, *Treatment paradigms in periodontal disease.* Compend Contin Educ Dent, 1997. **18**(3): p. 221-6, 228-30, 232 passim; quiz 234.
90. Moore, W.E. and L.V. Moore, *The bacteria of periodontal diseases.* Periodontol 2000, 1994. **5**: p. 66-77.
91. Fenesy, K.E., *Periodontal disease: an overview for physicians.* Mt Sinai J Med, 1998. **65**(5-6): p. 362-9.
92. Offenbacher, S., S.P. Barros, and J.D. Beck, *Rethinking periodontal inflammation.* J Periodontol, 2008. **79**(8 Suppl): p. 1577-84.
93. Khalili, J., *Genetic test and periodontal disease.* Lik Sprava, 2010(1-2): p. 91-5.
94. Takei, N., *CARRANZA'S CLINICAL PERIODONTOLOGY 12TH EDITION*. 12 th ed. 2014. 853.
95. Lindhe, J., <*Clinical periodontology and implant dentistry 2003.pdf*>, ed. Fourth. 2003: Blackwell.
96. Marazita, *Evidence for autosomal dominant inheritance and race-specific heterogenetity in early onset periodontitis.* J periodontol, 1994. **65**: p. 8.
97. Saxen, L., *Juvenile periodontitis in Finland.* Proc Finn Dent Soc, 1987. **83**(3): p. 143-9.
98. Michalowicz, *Periodontal findings in adult twins.* J Periodontol, 1991. **62**: p. 6.
99. Childs, B., *Medical genetics to genomic medicine.* Med Secoli, 2002. **14**(3): p. 707-21.
100. Stastny, P., et al., *Lymphokines in the rheumatoid joint.* Adv Clin Pharmacol, 1974. **6**: p. 71-6.
101. Nouri, A.M., G.S. Panayi, and S.M. Goodman, *Cytokines and the chronic inflammation of rheumatic disease. II. The presence of interleukin-2 in synovial fluids.* Clin Exp Immunol, 1984. **58**(2): p. 402-9.
102. Cohen, S. and S. Winkler, *Cellular immunity and the inflammatory response.* J Periodontol, 1974. **45**(5): p. 348-50.
103. Ryan, G.B., *Inflammation. Mediators of inflammation.* Beitr Pathol, 1974. **152**(3): p. 272-91.
104. Reinhardt, *Gingival fluid IL-1 and IL-6 levels in refractory periodontitis.* J Clinical Periodontol, 1993. **20**(6): p. 8.

105. *Interleukin Genetics, Inc. and the University of Michigan to assess predictive value of new genetic test for periodontal disease.* Dent Assist, 2010. **79**(5): p. 6, 8.
106. Scapoli, L., et al., *IL6 and IL10 are genetic susceptibility factors of periodontal disease.* Dent Res J (Isfahan), 2012. **9**(Suppl 2): p. S197-201.
107. Abbas, A.K., *Basic Immunology. Fuctions and disorders of immune system*. Fourth ed. 2014: ElSevier. 307.
108. M, W., *Cytokine-inducing components of periodontopathogenic bacteria.* J Periodont Res, 1996. **31**: p. 16.
109. Belibasakis, G.N., *cytokine responses of human gingival fibroblast to actinobacillus actinomycetemcomitans cytolethal distending toxin.* ELSEVIER, 2004. **30**(1): p. 8.
110. Ferrari, S.L., et al., *A functional polymorphic variant in the interleukin-6 gene promoter associated with low bone resorption in postmenopausal women.* Arthritis Rheum, 2001. **44**(1): p. 196-201.
111. Munoz-Canoves, P., et al., *Interleukin-6 myokine signaling in skeletal muscle: a double-edged sword?* FEBS J, 2013. **280**(17): p. 4131-48.
112. Petersen, A.M. and B.K. Pedersen, *The anti-inflammatory effect of exercise.* J Appl Physiol (1985), 2005. **98**(4): p. 1154-62.
113. Febbrario, M., *Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction.* Diabetes, 2004. **53**: p. 6.
114. Yudkin, J., *Inflammation, obesity, stress and coronary heart disease; is interleukin-6 the link?* Atherosclerosis, 2000. **148**: p. 6.
115. Elsik, C.G., et al., *Community annotation: procedures, protocols, and supporting tools.* Genome Res, 2006. **16**(11): p. 1329-33.
116. Akira, S., *Biology of multifuntional cytokines: IL 6 and related molecules (IL 1and TNF).* The FASEB Journal, 1990. **4**(1990): p. 8.
117. Scheller, J., *The pro- and anti-inflammatory properties of the cytokine interleukin-6.* ELSEVIER, 2011. **1813**(1): p. 11.
118. Bickel, M., et al., *Cytokine gene expression in chronic periodontitis.* J Clin Periodontol, 2001. **28**(9): p. 840-7.
119. Pettigrew, D.W., et al., *Inhibitors of collagenolytic enzymes synthesized by fibroblasts and epithelial cells from porcine and macaque periodontal tissues.* Arch Oral Biol, 1980. **25**(4): p. 269-74.
120. Surlin, P., et al., *Matrix metalloproteinase -7, -8, -9 and -13 in gingival tissue of patients with type 1 diabetes and periodontitis.* Rom J Morphol Embryol, 2014. **55**(3 Suppl): p. 1137-41.
121. Astrom, P., et al., *Matrix metalloproteinase-8 regulates transforming growth factor-beta1 levels in mouse tongue wounds and fibroblasts in vitro.* Exp Cell Res, 2014. **328**(1): p. 217-27.
122. Witte, M.B., et al., *Metalloproteinase inhibitors and wound healing: a novel enhancer of wound strength.* Surgery, 1998. **124**(2): p. 464-70.
123. Delaisse, J.M., et al., *(Pro)collagenase (matrix metalloproteinase-1) is present in rodent osteoclasts and in the underlying bone-resorbing compartment.* J Cell Sci, 1993. **106 (Pt 4)**: p. 1071-82.

124. Roodman, G.D., *Interleukin-6: an osteotropic factor?* J Bone Miner Res, 1992. **7**(5): p. 475-8.
125. Genco, R.J., et al., *Relationship of stress, distress and inadequate coping behaviors to periodontal disease.* J Periodontol, 1999. **70**(7): p. 711-23.
126. Lu, H., et al., *Chronic stress enhances progression of periodontitis via alpha1-adrenergic signaling: a potential target for periodontal disease therapy.* Exp Mol Med, 2014. **46**: p. e118.
127. Szabo, K., et al., *Interleukin-1A +4845(G> T) polymorphism is a factor predisposing to acne vulgaris.* Tissue Antigens, 2010. **76**(5): p. 411-5.
128. Bashour, L., R. Khattab, and E. Harfoush, *The Role of Interleukin-1 Genotype in the Association between Coronary Heart Disease and Periodontitis in a Syrian Population.* ISRN Dent, 2013. **2013**: p. 195678.
129. Kinane, D.F., *Clinical, pathological and immunological aspects of periodontal disease.* Acta Odontol, 2001. **59**(1): p. 7.
130. Azuma, M.M., *The role of IL-6 on apical periodontitis; a systematic review.* International Endodontic Journal, 2013. **10**(2013): p. 7.
131. Nakajima, *Periodontitis-associated up-regulation of systemic inflammatory mediator level may increase the risk of coronary heart disease.* Journal of Periodontal Research, 2010. **45**(1): p. 7.
132. Van-de-Zee, *Cytokines modulate routes of collagen breakdown.* J Clin Periodontology, 1997. **24**(1): p. 10.
133. Kornman, K.S., et al., *The interleukin-1 genotype as a severity factor in adult periodontal disease.* J Clin Periodontol, 1997. **24**(1): p. 72-7.
134. Xing, Z., *IL-6 is an antiflammatory cytokine required for controlling local systemic acute inflammatory responses.* J Clin Invest, 1998. **101**(2): p. 10.
135. Gabay, C., *Interleukin-6 and chronic inflammation.* Arthritis Research & Therapy, 2006. **8**(2): p. 6.
136. Chillida, F., *Association between interleukin-6 polymorphisms and periodontitis in Indian non-smokers.* Journal of Clinical Periodontology, 2010. **37**(2010): p. 8.
137. Irwin, C., *The role of Il-6 in the pathogenesis of periodontal disease.* Oral Diseases, 1998. **4**(1): p. 5.
138. Wilson, *Cytokine-inducing components of periodontopathogenic bacteria.* Journal of Periodontal Research, 1996. **31**: p. 16.
139. Malacards. *Human disease database*. 2014; Available from: http://www.malacards.org/search/results/il-6.
140. dbSNP. *Short Gentic Variations*. 2014; Available from: http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?searchType=adhoc_search&type=rs&rs=rs1800795.
141. Mandal, S., F. Abebe, and J. Chaudhary, *-174G/C polymorphism in the interleukin-6 promoter is differently associated with prostate cancer incidence depending on race.* Genet Mol Res, 2014. **13**(1): p. 139-151.
142. Hirano, T., *Biological and clinical aspect of interleukin 6.* Immunology Today, 1990. **11**(12): p. 7.
143. Nibali, L., *Interleukin-6 Polymorphisms are associated with pathogenic bacteria in subjects with periodontitis.* J Periodontol, 2008. **79**(4): p. 7.

144. Geivelis, M., *Measurements of interleukin-6 in gingival crevicular fluid from adults with destructive periodontal disease.* J Periodontol, 1993. **64**(10): p. 4.
145. Shao, M.Y., et al., *Interleukin-6 polymorphisms modify the risk of periodontitis: a systematic review and meta-analysis.* J Zhejiang Univ Sci B, 2009. **10**(12): p. 920-7.
146. Ladeira, P., *Peri-implant disease and chronic periodontitis is interleukin-6 gene promoter polymorphism the common risk factor in a Brazilian population.* The International Journal of Oral & Maxilofacial Implants, 2013. **28**(2013): p. 10.
147. Tervonen, T., *Polymorphisms in the CD14 and Il-6 associated with periodontal disease.* Journal of Clinical Periodontology, 2007. **34**(2007): p. 7.
148. Trevilatto, P.C., *Polymorphism at position -174 of IL-6 is associated with susceptibility to chronic periodontitis in a Caucasian Brazilian population.* J Clin Periodontal, 2003. **30**(5): p. 5.
149. Wohlfahrt, J.C., *No Association Between Selected Candidate Gene Polymorphisms and Severe Chronic Periodontitis.* J Periodontol, 2006. **77**(2006): p. 11.
150. Xiao, L., *Association among interleukin-6 gene polymorphism, diabetes and periodontitis in a Chinese population.* Oral Diseases, 2009. **15**(2009): p. 7.
151. Antonicelli, R., et al., *The interleukin-6 -174 G>C promoter polymorphism is associated with a higher risk of death after an acute coronary syndrome in male elderly patients.* Int J Cardiol, 2005. **103**(3): p. 266-71.
152. Foster, C.B., et al., *An IL6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in men infected with human immunodeficiency virus.* Blood, 2000. **96**(7): p. 2562-7.
153. Gatti, L.L., et al., *Interleukin-6 polymorphisms, Helicobacter pylori infection in adult Brazilian patients with chronic gastritis and gastric adenocarcinoma.* Arch Med Res, 2007. **38**(5): p. 551-5.
154. Greisenegger, S., et al., *The (-174) G/C polymorphism in the interleukin-6 gene is associated with the severity of acute cerebrovascular events.* Thromb Res, 2003. **110**(4): p. 181-6.
155. Hohaus, S., et al., *Polymorphism in cytokine genes as prognostic markers in Hodgkin's lymphoma.* Ann Oncol, 2007. **18**(8): p. 1376-81.
156. Jeng, J.R., et al., *Association of interleukin-6 gene G-174C polymorphism and plasma plasminogen activator inhibitor-1 level in Chinese patients with and without hypertension.* Am J Hypertens, 2005. **18**(4 Pt 1): p. 517-22.
157. Lu, K.C., et al., *Interleukin-6 and interferon-gamma gene polymorphisms in the development of bronchiolitis obliterans syndrome after lung transplantation.* Transplantation, 2002. **74**(9): p. 1297-302.
158. Sawczenko, A., et al., *Intestinal inflammation-induced growth retardation acts through IL-6 in rats and depends on the -174 IL-6 G/C polymorphism in children.* Proc Natl Acad Sci U S A, 2005. **102**(37): p. 13260-5.
159. Lab, O.D. *Periodontal Testing*. Available from: http://www.oraldna.com/myPerioID.html.
160. Scherp, H.W., *CURRENT CONCEPTS IN PERIODONTAL DISEASE RESEARCH: EPIDEMIOLOGICAL CONTRIBUTIONS.* J Am Dent Assoc, 1964. **68**: p. 667-75.

161. Taba, M., Jr., S.L. Souza, and V.C. Mariguela, *Periodontal disease: a genetic perspective.* Braz Oral Res, 2012. **26 Suppl 1**: p. 32-8.
162. Costa, A.M., *Interleukin-6 (G-174C) and tumor necrosis factor-alpha (G-308A) gene polymorphisms in geriatric patients with chronic periodontitis.* Gerodontology, 2010. **27**(2010): p. 6.
163. Babel, N., *Analysis of tumor necrosis factor-alpha, transforming growth factor-beta, interleukin-10, IL-6, and interferon-gamma gene polymorphisms in patients eith chronic periodontitis.* J Periodontol, 2006. **77**(2006): p. 6.
164. Fan, W., *Coronary heart disease and chronic periodontitis: is polymorphism of interleukin-6 gene the common risk factor in a Chinese population.* Oral Diseases, 2010. **17**(2011): p. 270-276.
165. Garlet, G.P., *The use of chronic gingivitis as reference status increases the power and odds of periodontitis genetic studies.* Journal of Clinical Periodontology, 2012. **39**(2012): p. 10.
166. Rogers, *Do interleukin-1 polymorphisms predict the development of periodontitis or the success of dental implants?* 2002, (IL-1A -889) .pdf>.
167. Kornman, K., G. Duff, and P. Reilly, *Re: A critical assessment of interleukin-1 (IL-1) genotyping when used in a genetic susceptibility test for severe chronic periodontitis. Greenstein G, Hart TC (2002;73:231-247).* J Periodontol, 2002. **73**(12): p. 1553-6; author reply 1556-8.
168. Schneider, K., et al., *SNP frequency and allelic haplotype structure of Beta vulgaris expressed genes.* Molecular Breeding, 2001. **8**(1): p. 63-74.
169. Genetics, I. *PerioPredict*. Available from: http://www.periopredict.com/professional/index.php.
170. Deverka, P., *Evaluation of Clinical Validity and Clinical Utility of Actionable Molecular Diagnostic Tests in Adult Oncology*, in *Center for Medical Technology Policy*. 2013.
171. Lo Gullo, A., et al., *Toll-like receptor 3 and interleukin 1beta expression in CD34+ cells from patients with rheumatoid arthritis: association with inflammation and vascular involvement.* Clin Exp Rheumatol, 2014. **32**(6): p. 922-9.
172. Dinarello, C.A. and J.W. van der Meer, *Treating inflammation by blocking interleukin-1 in humans.* Semin Immunol, 2013. **25**(6): p. 469-84.
173. Apte, R.N., et al., *Effects of micro-environment- and malignant cell-derived interleukin-1 in carcinogenesis, tumour invasiveness and tumour–host interactions.* European Journal of Cancer, 2006. **42**(6): p. 751-759.
174. Kaarvatn, M.H., et al., *Associations of the interleukin-1 gene locus polymorphisms with risk to hip and knee osteoarthritis: gender and subpopulation differences.* Scand J Immunol, 2013. **77**(2): p. 151-61.
175. Humphries, S.E., et al., *The interleukin-6 -174 G/C promoter polymorphism is associated with risk of coronary heart disease and systolic blood pressure in healthy men.* Eur Heart J, 2001. **22**(24): p. 2243-52.
176. Huang, M., et al., *Lack of an association between interleukin-6 -174G/C polymorphism and circulating interleukin-6 levels in normal population: a meta-analysis.* DNA Cell Biol, 2013. **32**(11): p. 654-64.

177. Zhou, D., *Exposure to physical and psychological stressors elevates plasma interleukin 6 relationship to the activation of hypothalamic-pituitary-adrenal axis.* Endocrinology, 1993. **133**(6).
178. Havemose-Poulsen, A., et al., *Polymorphisms within the IL-1 gene cluster: effects on cytokine profiles in peripheral blood and whole blood cell cultures of patients with aggressive periodontitis, juvenile idiopathic arthritis, and rheumatoid arthritis.* J Periodontol, 2007. **78**(3): p. 475-92.
179. Batool, A., et al., *Genetic association of IDE, POU2F1, PON1, IL1alpha and IL1beta with type 2 diabetes in Pakistani population.* Mol Biol Rep, 2014. **41**(5): p. 3063-9.
180. Diehl, S.R., F. Kuo, and T.C. Hart, *Interleukin 1 genetic tests provide no support for reduction of preventive dental care.* J Am Dent Assoc, 2015. **146**(3): p. 164-173.e4.
181. Burke, W., L.E. Pinsky, and N.A. Press, *Categorizing genetic tests to identify their ethical, legal, and social implications.* Am J Med Genet, 2001. **106**(3): p. 233-40.
182. Burke, W. and R.L. Zimmern, *Ensuring the appropriate use of genetic tests.* Nat Rev Genet, 2004. **5**(12): p. 955-9.

Appendices

Appendix A. Diagnosis and Screening Evaluation Test Calculations rs1800587

Model 1 Homozygous (IL-1A)(-889)(rs1800587)								
PAPERS	**SENSITIVITY**	95% CI	**SPECIFICITY**	95% CI	**PPV**	95% CI	**NPV**	95% CI
Braosi	**6.92%**	3.22 % to 12.74 %	**93.97%**	87.96 % to 97.53 %	**56.25%**	29.92 % to 80.17 %	**47.39%**	40.79 % to 54.06 %
Brett	**19.00%**	11.85 % to 28.07 %	**76.83%**	69.61 % to 83.05 %	**33.33%**	21.41 % to 47.07 %	**60.87%**	53.86 % to 67.56 %
Karasneh	**9.00%**	4.21 % to 16.40 %	**92.50%**	84.38 % to 97.18 %	**60.00%**	32.33 % to 83.57 %	**44.85%**	37.11 % to 52.77 %
Lopez 2005	**1.21%**	1.21 %0.34 % to 3.08 %	**98.02%**	93.01 % to 99.70 %	**66.67%**	22.68 % to 94.67 %	23.29%	19.36 % to 27.61 %
Lopez 2009	**87.20%**	83.15 % to 90.58 %	**95.67%**	91.94 % to 98.00 %	**97.02%**	94.42 % to 98.63 %	82.23%	76.82 % to 86.83 %
Rogers	**5.00%**	1.10 % to 13.94 %	**92.86%**	85.09 % to 97.32 %	**33.33%**	7.88 % to 69.93 %	57.78%	48.98 % to 66.22 %
Trevillato	**6.82%**	1.51 % to 18.68 %	**95.65%**	87.80 % to 99.04 %	**50.00%**	12.42 % to 87.58 %	**61.68%**	51.78 % to 70.92 %
Wagner	**54.74%**	44.19 % to 64.98 %	**67.42%**	56.66 % to 76.97 %	**64.20%**	52.77 % to 74.55 %	**58.25%**	48.12 % to 67.89 %
Schulz	**6.94%**	2.32 % to 15.48 %	**92.13%**	84.46 % to 96.77 %	**41.67%**	15.32 % to 72.25 %	**55.03%**	46.68 % to 63.18 %
Laine	**15.09%**	6.77 % to 27.60 %	**89.52%**	82.02 % to 94.65 %	**42.11%**	20.30 % to 66.47 %	**67.63%**	59.17 % to 75.31 %
Armingohar	**13.89%**	4.72 % to 29.51 %	**94.74%**	82.22 % to 99.20 %	**71.43%**	29.27 % to 95.48 %	**53.73%**	41.12 % to 66.00 %
Gore	**56.25%**	37.67 % to 73.62 %	**87.50%**	70.99 % to 96.41 %	**81.82%**	59.70 % to 94.70 %	**66.67%**	50.45 % to 80.42 %

Appendix B. Diagnosis and Screening Evaluation Test Calculations rs17561

Model 1 Homozygous (IL-1A)(+4845)(rs17561)								
PAPERS	**SENSITIVITY**	95% CI	**SPECIFICITY**	95% CI	**PPV**	95% CI	**NPV**	95% CI
Golcalvez	**29.31%**	18.10 % to 42.73 %	**76.60%**	61.97 % to 87.68 %	**60.71%**	40.58 % to 78.47 %	**46.75%**	35.29 % to 58.48 %
Kobayashi 2007	**1.08%**	0.16 % to 3.84 %	**99.00%**	94.53 % to 99.83 %	**66.67%**	11.55 % to 94.53 %	**34.98%**	29.43 % to 40.85 %
Kobayashi 2009	**1.57%**	0.44 % to 3.99 %	**99.07%**	94.93 % to 99.85 %	**80.00%**	28.81 % to 96.70 %	**29.97%**	25.26 % to 35.02 %
Sakellari 2003	**15.56%**	6.52 % to 29.46 %	**68.18%**	58.62 % to 76.74 %	**16.67%**	7.00 % to 31.37 %	**66.37%**	56.88 % to 74.98 %
Sakellari 2006	**22.86%**	13.67 % to 34.45 %	**90.00%**	82.37 % to 95.09 %	**61.54%**	40.58 % to 79.75 %	**62.50%**	54.05 % to 70.42 %
Gayathri	**7.84%**	2.23 % to 18.90 %	**96.15%**	86.76 % to 99.42 %	**66.67%**	22.68 % to 94.67 %	**51.55%**	41.18 % to 61.82 %

Appendix C. Diagnosis and Screening Evaluation Test Calculations rs1143634

Model 1 (Homozygous) (IL-1B)(+3953/4)(rs1143634)								
PAPERS	**SENSITIVITY**	95% CI	**SPECIFICITY**	95% CI	**PPV**	95% CI	**NPV**	95% CI
Duan	**0.00%**	0.00 % to 11.68 %	**100.00%**	96.11 % to 100.00 %	*	*	**75.81%**	67.30 % to 83.04 %
Gore	**3.03%**	0.51 % to 15.82 %	**87.50%**	70.99 % to 96.41 %	**20.00%**	3.30 % to 71.19 %	**46.67%**	3.67 % to 60.00 %
Galgraith	**65.38%**	44.34 % to 82.75 %	**48.00%**	27.82 % to 68.68 %	**56.67%**	37.44 % to 74.52 %	**57.14%**	34.04 % to 78.14 %
Laine	**8.57%**	4.00 % to 15.65 %	**92.45%**	81.77 % to 97.86 %	**69.23%**	38.61 % to 90.72 %	**33.79%**	26.16 % to 42.11 %
Rogers	**12.50%**	4.76 % to 25.26 %	**98.33%**	91.03 % to 99.72 %	**85.71%**	42.23 % to 97.63 %	**58.42%**	48.18 % to 68.14 %
Zong	**3.76%**	1.25 % to 8.56 %	**94.57%**	87.76 % to 98.19 %	**50.00%**	18.89 % to 81.11 %	**40.47%**	33.84 % to 47.35 %
Anusaksathien	**0.00%**	0.00 % to 6.67 %	**100.00%**	91.70 % to 100.00 %	*	*	**44.33%**	34.24 % to 54.77 %
Sakellari 2003	**13.33%**	5.09 % to 26.80 %	**91.82%**	85.03 % to 96.18 %	**40.00%**	16.43 % to 67.67 %	**72.14%**	63.94 % to 79.38 %
Soga	**0.00%**	0.00 % to 5.66 %	**100.00%**	94.34 % to 100.00 %	*	*	**50.00%**	41.04 % to 58.96 %
Huang	**0.00%**	0.00 % to 2.03 %	**100.00%**	95.90 % to 100.00 %	*	*	**32.84%**	27.28 % to 38.78 %
Lopez 2005	**2.12%**	0.86 % to 4.32 %	**98.02%**	93.01 % to 99.70 %	**77.78%**	40.06 % to 96.53 %	**23.46%**	19.50 % to 27.80 %
Brett	**10.91%**	.14 % to 22.26 %	**91.84%**	84.54 % to 96.40 %	**42.86%**	17.76 % to 71.08 %	**64.75%**	56.20 % to 72.66 %
Moreira	**5.77%**	1.27 % to 15.97 %	**100.00%**	88.68 % to 100.00 %	**100.00%**	30.48 % to 100.00 %	**38.75%**	28.06 % to 50.30 %
Sakellari 2006	**9.00%**	4.21 % to 16.40 %	**95.00%**	88.71 % to 98.34 %	**64.29%**	35.18 % to 87.11 %	**51.08%**	43.66 % to 58.46 %
Tian	**0.00%**	0.00 % to 9.83 %	**100.00%**	90.17 % to 100.00 %	*	*	**50.00%**	37.98 % to 62.02 %
Gustafsson	**0.00%**	0.00 % to 24.88 %	**100.00%**	75.12 % to 100.00 %	*	*	**50.00%**	29.94 % to 70.06 %
Drozdzik	**0.00%**	0.00 % to 10.99 %	**96.15%**	86.76 % to 99.42 %	**0.00%**	0.00 % to 80.71 %	**60.98%**	49.57 % to 71.56 %
Jansson	**0.00%**	0.00 % to 16.99 %	**96.77%**	83.24 % to 99.46 %	**0.00%**	.00 % to 83.45 %	**60.00%**	45.18 % to 73.59 %
Wagner	**36.56%**	26.81 % to 47.19 %	**91.30%**	83.58 % to 96.16 %	**80.95%**	65.87 % to 91.37 %	**58.74%**	50.21 % to 66.90 %
Ferreira	**13.68%**	8.03 % to 21.26 %	**91.43%**	86.26 % to 95.12 %	**51.61%**	33.07 % to 69.83 %	**61.30%**	55.10 % to 67.24
Goncalves	**0.00%**	0.00 % to 12.06 %	**94.12%**	71.24 % to 99.02 %	**0.00%**	0.00 % to 83.45 %	**35.56%**	21.88 % to 51.22 %
Kaarthikeyan	**0.00%**	0.00 % to 11.68 %	**96.77%**	77 %83.24 % to 99.4(	**0.00%**	0.00 % to 83.45 %	**50.00%**	36.81 % to 63.19 %
Kobayashi 2009	**0.00%**	0.00 % to 3.14 %	**100.00%**	96.61 % to 100.00 %	*	*	**48.00%**	41.31 % to 54.74 %
Lopez 2009	**9.38%**	5.90 % to 13.97 %	**97.60%**	94.48 % to 99.21 %	**80.77%**	0.64 % to 93.37 %	**50.00%**	45.03 % to 54.97 %
Prakash	**9.33%**	3.85 % to 18.30 %	**100.00%**	92.53 % to 100.00 %	**100.00%**	58.93 % to 100.00 %	**41.38%**	32.31 % to 50.90 %
Shete	**0.00%**	0.00 % to 3.66 %	**99.00%**	94.53 % to 99.83 %	**0.00%**	0.00 % to 83.45 %	**49.75%**	42.60 % to 56.90 %
Gayathri	**1.96%**	0.33 % to 10.49 %	**100.00%**	93.08 % to 100.00 %	**100.00%**	16.55 % to 100.00 %	**50.98%**	40.89 % to 61.01 %
Karasneh	**9.00%**	4.21 % to 16.40 %	**92.50%**	84.38 % to 97.18 %	**60.00%**	32.33 % to 83.57 %	**44.85%**	37.11 % to 52.77 %
Schulz	**11.11%**	4.94 % to 20.73 %	**94.32%**	87.23 % to 98.11 %	**61.54%**	31.64 % to 86.00 %	**56.46%**	48.05 % to 64.61 %
Trevilatto	**2.90%**	0.44 % to 10.10 %	**95.45%**	84.50 % to 99.31 %	**50.00%**	8.30 % to 91.70 %	**38.53%**	29.37 % to 48.34 %
Yang	**8.84%**	5.41 % to 13.46 %	**85.39%**	80.00 % to 89.78 %	**37.25%**	24.13 % to 51.92 %	**48.83%**	43.71 % to 53.95 %
Al-Hebshi	**25.00%**	12.71 % to 41.20 %	**95.00%**	83.05 % to 99.24 %	**83.33%**	51.58 % to 97.42 %	**55.88%**	43.32 % to 67.92 %
Bascones-Martinez	**4.00%**	1.12 % to 9.94 %	**96.00%**	90.06 % to 98.88 %	**50.00%**	16.01 % to 83.99 %	**50.00%**	42.72 % to 57.28 %
Garlet	**15.23%**	10.52 % to 21.02 %	**89.72%**	84.85 % to 93.44 %	**57.69%**	43.21 % to 71.27 %	**53.48%**	48.17 % to 58.73 %
Masamatti	**23.33%**	9.98 % to 42.29 %	**93.33%**	77.89 % to 98.99 %	**77.78%**	40.06 % to 96.53 %	**54.90%**	40.34 % to 68.87 %

Appendix D. Diagnosis and Screening Evaluation Test Calculations rs419598

Model 1 (Homozygous) (IL-1RN)(+2018)(rs419598)								
PAPERS	**SENSITIVITY**	95% CI	**SPECIFICITY**	95% CI	**PPV**	95% CI	**NPV**	95% CI
Guzman	**0.00%**	0.00 % to 5.41 %	**94.12%**	71.24 % to 99.02 %	**0.00%**	0.00 % to 83.45 %	**19.28%**	11.44 % to 29.41 %
Kobayashi 2007	**0.00%**	0.00 % to 1.98 %	**100.00%**	96.34 % to 100.00 %	*	*	**34.97%**	29.45 % to 40.80 %
Kobayashi 2009	**4.08%**	1.98 % to 7.38 %	**12.96%**	7.28 % to 20.80 %	**9.62%**	4.72 % to 16.98 %	**5.62%**	3.11 % to 9.26 %

Appendix E. Diagnosis and Screening Evaluation Test Calculations rs1800587

Model 2 Heterozygous (IL-1A)(-889) (rs1800587)								
PAPERS	**SENSITIVITY**	95% CI	**SPECIFICITY**	95% CI	**PPV**	95% CI	**NPV**	95% CI
Braosi	**49.23%**	40.36 % to 58.14 %	**51.72%**	42.26 % to 61.10 %	**53.33%**	44.01 % to 62.49 %	**47.62%**	38.65 % to 56.70 %
Brett	**56.00%**	45.72 % to 65.92 %	**38.41%**	30.94 % to 46.32 %	**35.67%**	28.19 % to 43.70 %	**58.88%**	48.95 % to 68.30 %
Karasneh	**53.00%**	42.76 % to 63.06 %	**51.25%**	39.81 % to 62.59 %	**57.61%**	46.86 % to 67.85 %	**46.59%**	35.88 % to 57.54 %
Lopez 2005	**43.64%**	38.21 % to 49.18 %	**64.36%**	4.21 % to 73.64 %	**80.00%**	73.40 % to 85.58 %	**25.90%**	20.59 % to 31.78 %
Lopez 2009	**51.79%**	46.30 % to 57.24 %	**57.21%**	50.18 % to 64.03 %	**66.16%**	60.09 % to 71.86 %	**42.35%**	36.50 % to 48.36 %
Rogers	**45.00%**	32.13 % to 58.39 %	**54.76%**	43.52 % to 65.66 %	**41.54%**	29.44 % to 54.43 %	**58.23%**	46.59 % to 69.23 %
Trevillato	**54.55%**	38.85 % to 69.60 %	**50.72%**	38.41 % to 62.98 %	**41.38%**	28.60 % to 55.07 %	**63.64%**	49.56 % to 76.18 %
Wagner	**89.47%**	81.49 % to 94.83 %	**21.35%**	13.37 % to 31.32 %	**54.84%**	46.65 % to 62.83 %	**65.52%**	45.67 % to 82.04 %
Schulz	**50.00%**	37.98 % to 62.02 %	**49.44%**	38.67 % to 60.25 %	**44.44%**	33.40 % to 55.91 %	**55.00%**	43.47 % to 66.15 %
Laine	**60.38%**	46.01 % to 73.54 %	**35.24%**	26.17 % to 45.17 %	**32.00%**	23.02 % to 42.08 %	**63.79%**	50.12 % to 76.00 %
Armingohar	**58.33%**	40.76 % to 74.47 %	**50.00%**	33.39 % to 66.61 %	**52.50%**	36.13 % to 68.48 %	**55.88%**	37.89 % to 72.80 %
Gore	**90.62%**	74.95 % to 97.91 %	**62.50%**	43.70 % to 78.88 %	**70.73%**	54.46 % to 83.85 %	**86.96%**	66.38 % to 97.07 %

Appendix F. Diagnosis and Screening Evaluation Test Calculations rs17561

Model 2 Heterozygous (IL-1A)(+4845)(rs17561)								
Golcalvez	**29.31%**	18.10 % to 42.73 %	**59.46%**	42.10 % to 75.23 %	**53.12%**	34.75 % to 70.89 %	**34.92%**	23.34 % to 47.97 %
Kobayashi 2007	**21.51%**	15.83 % to 28.11 %	**84.00%**	75.32 % to 90.56 %	**71.43%**	57.79 % to 82.70 %	**36.52%**	30.29 % to 43.10 %
Kobayashi 2009	**14.17%**	10.13 % to 19.08 %	**84.26%**	76.00 % to 90.55 %	**67.92%**	53.68 % to 80.07 %	**29.45%**	24.42 % to 34.87 %
Sakellari 2003	**53.33%**	37.88 % to 68.33 %	**57.27%**	47.48 % to 66.66 %	**33.80%**	23.00 % to 46.01 %	**75.00%**	64.36 % to 83.81 %
Sakellari 2006	**54.00%**	43.74 % to 64.01 %	**51.00%**	40.80 % to 61.13 %	**52.43%**	42.35 % to 62.36 %	**52.58%**	42.18 % to 62.81 %
Gayathri	**68.63%**	54.11 % to 80.88 %	**28.85%**	17.14 % to 43.08 %	**48.61%**	36.65 % to 60.69 %	**48.39%**	30.17 % to 66.93 %

Appendix G. Diagnosis and Screening Evaluation Test Calculations rs1143634

Model 2 (IL-1B) (3953/4)(rs1143634)								
	SENSITIVITY	95% CI	**SPECIFICITY**	95% CI	**PPV**	95% CI	**NPV**	95% CI
Duan	**23.33%**	9.98 % to 42.29 %	**95.74%**	89.45 % to 98.80 %	**63.64%**	30.88 % to 88.85 %	**79.65%**	71.04 % to 86.64 %
Gore	**43.75%**	26.38 % to 62.33 %	**62.50%**	43.70 % to 78.88 %	**53.85%**	33.39 % to 73.39 %	**52.63%**	35.82 % to 69.01 %
Galgraith	**55.00%**	31.55 % to 76.90 %	**73.33%**	58.05 % to 85.38 %	**47.83%**	26.85 % to 69.39 %	**78.57%**	63.18 % to 89.68 %
Laine	**48.57%**	38.70 % to 58.53 %	**54.72%**	40.45 % to 68.43 %	**68.00%**	56.22 % to 78.30 %	**34.94%**	24.80 % to 46.19 %
Rogers	**37.50%**	23.96 % to 52.65 %	**60.00%**	46.54 % to 72.43 %	**42.86%**	27.73 % to 59.04 %	**54.55%**	41.81 % to 66.85 %
Zong	**32.33%**	24.48 % to 40.99 %	**76.09%**	66.06 % to 84.36 %	**66.15%**	53.35 % to 77.43 %	**43.75%**	35.93 % to 51.80 %
Anusaksathien	**0.00%**	0.00 % to 6.67 %	**97.67%**	87.67 % to 99.61 %	**0.00%**	0.00 % to 83.45 %	**43.75%**	33.64 % to 54.25 %
Sakellari 2003	**48.89%**	33.71 % to 64.22 %	**50.00%**	40.32 % to 59.68 %	**28.57%**	18.85 % to 40.00 %	**70.51%**	59.11 % to 80.30 %
Soga	**6.25%**	1.77 % to 15.25 %	**92.19%**	82.69 % to 97.39 %	**44.44%**	13.97 % to 78.60 %	**49.58%**	40.29 % to 58.89 %
Huang	**17.03%**	11.88 % to 23.30 %	**95.51%**	88.88 % to 98.73 %	**88.57%**	73.24 % to 96.73 %	**36.02%**	29.89 % to 42.50 %
Lopez 2005	**29.70%**	24.82 % to 34.95 %	**87.13%**	78.99 % to 92.96 %	**88.29%**	80.80 % to 93.61 %	**27.50%**	22.68 % to 32.74 %
Brett	**41.82%**	28.66 % to 55.89 %	**59.18%**	48.79 % to 69.01 %	**36.51%**	24.74 % to 49.60 %	**64.44%**	53.65 % to 74.25 %
Moreira	**44.23%**	30.47 % to 58.67 %	**77.42%**	58.90 % to 90.37 %	**76.67%**	57.71 % to 90.02 %	**45.28%**	31.57 % to 59.55 %
Sakellari 2006	**41.00%**	31.26 % to 51.29 %	**56.00%**	45.72 % to 65.92 %	**48.24%**	37.26 % to 59.34 %	**48.70%**	39.27 % to 58.19 %
Tian	**11.11%**	3.18 % to 26.08 %	**100.00%**	90.17 % to 100.00 %	**100.00%**	40.23 % to 100.00 %	**52.94%**	40.45 % to 65.17 %
Gustafsson	**38.46%**	14.00 % to 68.36 %	**61.54%**	31.64 % to 86.00 %	**50.00%**	18.89 % to 81.11 %	**50.00%**	24.71 % to 75.29 %
Drozdzik	**34.38%**	18.59 % to 53.19 %	**59.62%**	45.10 % to 72.99 %	**34.38%**	18.59 % to 53.19 %	**59.62%**	45.10 % to 72.99 %
Jansson	**45.00%**	23.10 % to 68.45 %	**70.73%**	54.46 % to 83.85 %	**42.86%**	21.86 % to 65.96 %	**72.50%**	56.11 % to 85.38 %
Wagner	**74.19%**	64.08 % to 82.71 %	**57.61%**	46.86 % to 67.85 %	**63.89%**	54.08 % to 72.91 %	**68.83%**	57.26 % to 78.90 %
Ferreira	**39.32%**	30.42 % to 48.77 %	**68.57%**	61.13 % to 75.37 %	**45.54%**	35.60 % to 55.76 %	**62.83%**	55.55 % to 69.69 %
Goncalves	**27.59%**	12.77 % to 47.24 %	**82.35%**	56.55 % to 95.99 %	**72.73%**	39.08 % to 93.65	**40.00%**	23.88 % to 57.88
Kaarthikeyan	**30.00%**	14.76 % to 49.40 %	**77.42%**	58.90 % to 90.37 %	**56.25%**	29.92 % to 80.17 %	**53.33%**	37.88 % to 68.33 %
Kobayashi 2009	**5.13%**	1.92 % to 10.83 %	**94.44%**	88.29 % to 97.92 %	**50.00%**	21.21 % to 78.79 %	**47.89%**	41.01 % to 54.82 %
Lopez 2009	**40.18%**	33.70 % to 46.92 %	**77.40%**	71.11 % to 82.90 %	**65.69%**	57.10 % to 73.59 %	**54.58%**	48.70 % to 60.36 %
Prakash	**49.33%**	37.59 % to 61.13 %	**81.25%**	67.36 % to 91.03 %	**80.43%**	66.08 % to 90.62 %	**50.65%**	39.01 % to 62.24 %
Shete	**7.00%**	2.87 % to 13.90 %	**74.00%**	64.27 % to 82.26 %	**21.21%**	9.02 % to 38.92 %	**44.31%**	36.64 % to 52.19 %
Gayathri	**33.33%**	20.77 % to 47.92 %	**53.85%**	39.47 % to 67.76 %	**41.46%**	26.33 % to 57.89 %	**45.16%**	32.48 % to 58.32 %
Karasneh	**53.00%**	42.76 % to 63.06 %	**51.25%**	39.81 % to 62.59 %	**57.61%**	46.86 % to 67.85 %	**46.59%**	35.88 % to 57.54 %
Schulz	**38.89%**	27.62 % to 51.11 %	**6.82%**	45.82 % to 67.34 %	**42.42%**	30.34 % to 55.21 %	**53.19%**	42.61 % to 63.56 %
Trevilatto	**26.09%**	16.26 % to 38.06 %	**61.36%**	45.50 % to 75.63 %	**51.43%**	34.00 % to 68.61 %	**34.62%**	24.20 % to 46.24 %
Yang	**24.65%**	19.04 % to 30.97 %	**57.08%**	50.24 % to 63.73 %	**36.05%**	28.31 % to 44.38 %	**43.55%**	37.74 % to 49.51 %
Al-Hebshi	**70.00%**	53.47 % to 83.42 %	**25.00%**	12.71 % to 41.20 %	**48.28%**	34.95 % to 61.78 %	**45.45%**	24.42 % to 67.77 %
Bascones-Martinez	**36.00%**	26.64 % to 46.21 %	**48.00%**	37.90 % to 58.22 %	**40.91%**	30.54 % to 51.91 %	**42.86%**	33.55 % to 52.55 %
Garlet	**43.15%**	36.13 % to 50.38 %	**69.16%**	62.50 % to 75.28 %	**56.29%**	47.99 % to 64.34 %	**56.92%**	50.66 % to 63.02 %
Masamatti	**53.33%**	34.34 % to 71.64 %	**70.00%**	50.60 % to 85.24 %	**64.00%**	42.53 % to 81.99 %	**60.00%**	42.12 % to 76.12 %

Appendix H. Diagnosis and Screening Evaluation Test Calculations rs419598

Model 2 Heterozygous (IL-1RN)(+2018)(rs419598)								
PAPERS	**SENSITIVITY**	95% CI	**SPECIFICITY**	95% CI	**PPV**	95% CI	**NPV**	95% CI
Guzman	**19.40%**	10.76 % to 30.89 %	**88.24%**	63.52 % to 98.20 %	**86.67%**	59.51 % to 97.95 %	**21.74%**	12.72 % to 33.31 %
Kobayashi 2007	**6.99%**	3.78 % to 11.66 %	**87.00%**	78.79 % to 92.89 %	**50.00%**	29.94 % to 70.06 %	**33.46%**	27.75 % to 39.55 %
Kobayashi 2009	**100.00%**	98.49 % to 100.00 %	**0.93%**	0.15 % to 5.07 %	**69.60%**	64.50 % to 74.37 %	**100.00%**	16.55 % to 100.00 %

Appendix I. Diagnosis and Screening Evaluation Test Calculations rs1800795

Model 1 Homozygous (IL-6)(-174)(rs1800795)								
PAPERS	**SENSITIVITY**	**95% CI**	**SPECIFICITY**	**95% CI**	**PPV**	**95% CI**	**NPV**	**95% CI**
Brett	**38%**	25.52 % to 51.63 %	**44%**	34.46 % to 54.78 %	**29%**	18.85 % to 40.00 %	**55%**	43.47 % to 66.15 %
Chillida	**77%**	67.34 % to 84.46 %	**25%**	34.46 % to 54.78 %	**43%**	35.47 % to 50.17 %	**59%**	45.75 % to 71.93 %
Fan	**99%**	96.90 % to 99.91 %	**0.8%**	0.13 % to 4.23 %	**58%**	52.09 % to 63.44 %	**50%**	8.17 % to 91.83 %
Garlet	**49%**	41.84 % to 56.17 %	**43%**	36.24 % to 50.24 %	**46%**	38.72 % to 52.48 %	**47%**	39.29 % to 53.94 %
Holla	**29%**	21.89 % to 37.08 %	**65%**	55.61 % to 74.35 %	**54%**	42.24 % to 64.97 %	**40%**	32.68 % to 47.66 %
Kalburgi	**67%**	38.41 % to 88.05 %	**87%**	59.51 % to 97.95 %	**83%**	51.58 % to 97.42 %	**72%**	46.53 % to 90.20 %
Ladeira	**58%**	42.15 % to 72.34 %	**52%**	38.40 % to 64.77 %	**47%**	33.66 % to 61.19 %	**62%**	47.18 % to 75.34 %
Lanni	**52%**	40.26 % to 63.48 %	**57%**	51.15 % to 63.09 %	**25%**	18.62 % to 32.64 %	**81%**	74.93 % to 86.35 %
Scapolli	**48%**	40.47 % to 55.64 %	**36%**	27.24 % to 45.29 %	**53%**	45.09 % to 61.05 %	**31%**	23.61 % to 39.92 %
Stefani	**57%**	34.04 % to 78.14 %	**48%**	25.75 % to 70.19 %	**52%**	30.61 % to 73.15 %	**53%**	28.90 % to 75.51 %
Tervonen	**22%**	11.30 % to 35.33 %	**79%**	72.50 % to 84.92 %	**23%**	12.05 % to 37.32 %	**78%**	71.15 % to 83.72 %
Worhlfahrt	**35%**	27.09 % to 43.65 %	**61%**	49.57 % to 71.56 %	**60%**	48.44 % to 70.80 %	**36%**	28.01 % to 44.54 %
Xioa	**99%**	96.49 % to 99.89 %	**0%**	0.00 % to 2.78 %	**54%**	48.22 % to 60.03 %	**0%**	0.00 % to 83.45 %
Trevillato	**50%**	29.15 % to 70.85 %	**67%**	49.03 % to 81.43 %	**59%**	29.15 % to 70.85 %	**77%**	49.03 % to 81.43 %
Trevillato	**70%**	48.91 % to 87.33 %	**67%**	49.03 % to 81.43 %	**59%**	38.94 % to 76.46 %	**77%**	58.90 % to 90.37 %
Moreira	**66%**	55.03 % to 75.68 %	**44%**	30.92 % to 58.60 %	**66%**	55.03 % to 75.68 %	**44%**	30.92 % to 58.60 %
Moreira	**60%**	47.00 % to 71.51 %	**44%**	30.92 % to 58.60 %	**57%**	44.75 % to 68.91 %	**50%**	32.93 % to 61.54 %
Costa	**88%**	63.52% to 98.20	**56%**	35.34% to 74.50%	**56%**	35.34% to 74.50%	**88%**	63.52% to 98.20%
Costa	**76%**	52.83% to 91.69%	**56%**	35.34% to 74.50%	**57%**	37.1 % to 75.52 %	**75%**	50.89% to 91.25%
Nibali	**39%**	33.60 %to44.59 %	**71%**	62.68 %to78.10 %	**75%**	67.38 %to81.12 %	**34%**	29.06 %to40.18 %
Nibali	**90%**	81.86 %to95.31 %	**16%**	6.52 % to 29.46 %	**68%**	58.90 %to76.31 %	**44%**	19.83 %to70.08 %
Nibali	**80%**	69.92 %to87.89 %	**24%**	10.34 %to43.55 %	**76%**	65.36 %to84.00 %	**29%**	12.67 %to51.09 %

Appendix J. Diagnosis and Screening Evaluation Test Calculations rs1800795

Model 2 Homozygous (IL-6)(-174)(rs1800795)								
PAPERS	**SENSITIVITY**	**95% CI**	**SPECIFICITY**	**95% CI**	**PPV**	**95% CI**	**NPV**	**95% CI**
Brett	**57%**	43.23 % to 69.83 %	**25%**	17.06 % to 34.98 %	**31%**	22.27 % to 40.50 %	**50%**	35.53 % to 64.47 %
Chillida	**94%**	87.74 % to 97.82 %	**5%**	2.03 % to 9.97 %	**42%**	35.94 % to 48.68 %	**54%**	25.22 % to 80.67 %
Fan	**100%**	97.93 % to 100.00 %	**0%**	0.00 % to 2.83 %	**58%**	52.06 % to 63.37 %	Cannot be estimated	
Garlet	**84%**	77.96 % to 88.67 %	**8.8%**	5.32 % to 13.59 %	**47%**	41.85 % to 52.52 %	**36%**	22.92 % to 50.81 %
Holla	**77%**	69.40 % to 83.53 %	**16%**	9.54 % to 24.22 %	**56%**	48.78 % to 62.81 %	**33%**	20.77 % to 47.92 %
Kalburgi	**87%**	59.51 % to 97.95 %	**60%**	32.33 % to 83.57 %	**68%**	43.46 % to 87.35 %	**82%**	48.24 % to 97.18 %
Ladeira	**93%**	81.71 % to 98.53 %	**8%**	2.79 % to 18.40 %	**43%**	33.27 % to 53.75 %	**63%**	24.70 % to 91.03 %
Lanni	**84%**	74.36 % to 91.67 %	**12%**	8.62 % to 16.67 %	**21%**	16.63 % to 26.01 %	**74%**	58.87 % to 85.72 %
Scapolli	**89%**	83.74 % to 93.41 %	**27%**	18.77 % to 35.45 %	**65%**	58.40 % to 70.74 %	**62%**	47.18 % to 75.34 %
Stefani	**95%**	76.11 % to 99.21 %	**9.5%**	1.45 % to 30.42 %	**51%**	34.79 % to 67.58 %	**67%**	11.55 % to 94.53 %
Babel	**58%**	48.87 % to 66.86 %	**28%**	19.70 % to 36.66 %	**46%**	38.15 % to 54.31 %	**38%**	27.71 % to 49.34 %
Worhlfahrt	**81%**	73.44 % to 87.21 %	**17%**	9.67 % to 26.99 %	**62%**	54.47 % to 69.15 %	**35%**	20.64 % to 51.68 %
Xioa	**100%**	97.65 % to 100.00 %	**0%**	0.00 % to 2.78 %	**54%**	48.39 % to 60.17 %	Cannot be estimated	
Trevillato	**100%**	85.62 % to 100.00 %	**8.3%**	1.85 % to 22.49 %	**42%**	29.15 % to 55.92 %	**100%**	30.48 % to 100.00 %
Trevillato	**83%**	62.60 % to 95.16 %	**8.3%**	1.85 % to 22.49 %	**38%**	24.79 % to 52.11 %	**43%**	10.42 % to 81.25 %
Moreira	**93%**	85.74% to 97.44%	**11%**	4.22% to 22.64%	**63%**	54.17% to 71.37%	**50%**	21.21% to 78.79%
Moreira	**96%**	87.45% to 99.02%	**11%**	4.22% to 22.64%	**57%**	47.45% to 66.45%	**67%**	30.07% to 92.12%
Costa	**100%**	80.33 % to 100.00 %	**11%**	2.48 % to 29.19 %	**41%**	26.33 % to 57.89 %	**100%**	30.48 % to 100.00%
Costa	**100%**	83.75% to 100.00%	**11%**	2.48% to 29.19%	**47%**	31.67% to 62.12%	**100%**	30.48 % to 100.00 %
Nibali	**84%**	79.12%to87.54%	**19%**	13.33%to26.86%	**70%**	64.75%to74.21%	**35%**	24.67%to46.48%
Nibali	**100%**	95.94%to100.00%	**0%**	0.00 % to7.95%	**67%**	58.04 % to74.54%	Cannot be estimated	
Nibali	**98%**	91.74%to99.65%	**3.4%**	0.58%to17.83%	**75%**	65.64%to82.54%	**33%**	5.47%to88.45 %

VITA

Education

2014-2017 (expected)	Periodontal Residency	University of Illinois at Chicago College of Dentistry
2014-2017 (expected)	Master in Oral Sciences	University of Illinois at Chicago College of Dentistry
2013-2014	Fellow in Research of Genetics Basis in Gingival and Periodontal Pathologies	University of Illinois at Chicago College of Dentistry
2012-2013	Periodontal Postdoctoral Special Program	University of Illinois at Chicago College of Dentistry
2002-2008	Licentiate in Dentistry	University of Costa Rica School of Dentistry
2002-2008	Doctor in Dental Surgery	University of Costa Rica School of Dentistry
2003-2004	Business Administration Studies	University Hispanoamericana School of Business

Teaching

2014-2017 (expected)	Clinical instructor for pre-doctoral Students	University of Illinois at Chicago Periodontal Dept.
2016-2017(expected)	TA for PG students head and neck Anatomy	University of Illinois at Chicago School of Medicine

HONORS AND AWARDS

2015	Assessment of the Clinical Validity and Clinical Utility of IL SNPs as Predictors for Periodontitis	Clinic and Research Day, UIC College of Dentistry 2nd Place
2006-2007	President of Dental Student Association	University of Costa Rica School of Dentistry
2003-2007	Assembly Member of the Student Representation	University of Costa Rica School of Dentistry
2003-2007	Member of the Dental Student Association	University of Costa Rica School of Dentistry
2004-2006	Teacher Assistant Scholarship	University of Costa Rica School of Medicine
2003	Academic Excellence Scholarship	University of Costa Rica School of Dentistry

CLINICAL EXPERIENCE

2009-2012	General Dentistry Practice	Private Clinic Dr. Obando, San Jose, Costa Rica
2009-2012	Business Administration Practice	Private Clinic Dr. Obando, San Jose, Costa Rica
2009-2012	General Dentistry Practice	Private Clinic Dr. Gazel, San Jose, Costa Rica
2008	Internship in Periodontics	University of Costa Rica School of Dentistry
2008	Externship in Psychiatric Health	National Psychiatric Hospital
2008	Externship Disability Dental Care	Learning & Disability Special Center "Centeno Guell"
2008	Cleft and Lip Palate Observational Surgical Training	National Children Hospital, San Jose, Costa Rica

RESEARCH PRESENTATIONS

2015	Assessment of the Clinical Validity and Clinical Utility of IL SNPs as Predictors for Periodontitis	Clinic and Research Day, UIC College of Dentistry Poster Presentation
2015	Efficacy and Efficiency of SNPs as a Genetic Test for Periodontitis	IADR 44th Annual Meeting, Boston, MA Poster Presentation
2014	IL-6 SNP rs1800795 Is A Poor Genetic Test For Periodontitis	IADR 43th Annual Meeting, Boston, MA Poster Accepted
2014	Assessment of the Clinical utility of IL-6 rs1800795 as Predictors for Periodontitis	Clinic and Research Day, UIC College of Dentistry Poster Presentation
2013	Assessment of Damaging SNPs in Aggressive Periodontitis associated Genes	Penn Periodontal Research Conference, University of Pennsylvania Poster Presentation
2008	Anterior-Posterior Dimensional Changes of Surgically Treated Cleft and Lip Palate Patients	University of Costa Rica, San Jose, Costa Rica