FABIANO ALVIM PEREIRA

Análise de aspectos clínicos e genéticos associados à perda de implantes dentais osseointegráveis

CURITIBA

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde (PPGCS) do Centro de Ciências Biológicas e da Saúde (CCBS) da Pontifícia Universidade Católica do Paraná (PUCPR), como parte dos requisitos para a obtenção do título de Doutor em Ciências da Saúde, Área de Concentração Medicina.

Orientadora: Profa. Dra. Paula Cristina Trevilatto

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DEDICATÓRIA

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A Pedra

o distraído nela tropeçou
o bruto a usou como projétil
o empreendedor, usando-a, construiu
o camponês, cansado com a lida, dela fez assento
para meninos, foi brinquedo
Drummond a poetizou

já David matou Golias, e Michelangelo
extraiu-lhe a mais bela escultura
em todos estes casos, a diferença não
esteve na pedra, mas no homem!

Não existe "pedra" no seu caminho que
você não possa aproveitá-la para o
seu próprio crescimento

Autor desconhecido

SUMÁRIO

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RESUMO

Resumo A falha de implantes osseointegráveis é um processo complexo e multifatorial. Fatores clínicos sozinhos parecem não explicar totalmente o processo de perda de implantes. Essas observações, juntamente com a tendência de agregação de casos de perda em certos indivíduos, apontam para questões interessantes relacionadas com a susceptibilidade do hospedeiro à falha de implantes dentais. Evidências de influência genética a falhas de implantes dentais vêm sendo demonstradas. Polimorfismos genéticos são classicamente considerados como fatores de risco a diversas patologias, e mais recentemente, à perda de implantes dentais. A vitamina D está envolvida em uma ampla variedade de processos biológicos, como o metabolismo ósseo, e seus efeitos são mediados por seu receptor (VDR). Os objetivos deste estudo são: i) apresentar uma breve descrição das metodologias utilizadas em análises genéticas de doenças complexas, especialmente aplicadas à perda de implantes dentais, seguida de uma revisão crítica da literatura a respeito da suscetibilidade genética à falha de implantes dentais osseointegráveis; ii) investigar fatores clínicos relacionados ao processo de falha de implantes, e iii) analisar a associação entre polimorfismo (rs731236, T*aq*I) do gene do VDR e a perda de implantes dentais osseointegráveis. Duzentos e dezessete (217) pacientes não-aparentados, média de idade 51,7 ± 11,3 anos, foram divididos em dois grupos: *Grupo Controle (C)*, 137 indivíduos com pelo menos um implante osseointegrado em função por pelo menos seis meses e nenhuma perda e *Grupo Estudo (S)*, 80 indivíduos com perda de pelo menos um implante. O perfil socioeconômico, estado médico geral, fumo, parâmetros de higiene bucal e parâmetros clínicos, como número de dentes presentes, condição periodontal, qualidade e quantidade óssea, presença de enxerto, posição do implante, estabilidade primária, comprimento e diâmetro do implante, tipo de plataforma e tempo para carga foram avaliados. Depois da obtenção e purificação do DNA, foi realizada a análise do polimorfismo pela técnica de PCR-RFLP. Diferenças entre grupo controle e estudo e entre implantes perdidos e saudáveis foram avaliadas. Os seguintes parâmetros clínicos estiveram associados à perda de implantes: edentulismo, profundidade de bolsa, posição do implante, estabilidade primária, o comprimento do implante, a técnica cirúrgica e a quantidade óssea. Nenhuma associação foi encontrada entre genótipos/alelos do polimorfismo T*aq*I do VDR e a perda de implantes dentais osseointegráveis. Oservou-se que aspectos clínicos, mas não o polimorfismo estudado, estiveram associados à perda de implantes dentais osseointegráveis.

ABSTRACT

Abstract The observation that clinical factors alone do not explain implant loss in some patients, the understanding of the osseointegrated implant failure as a complex, multifactorial process, and the observed aggregation of repetitive failure in certain individuals raise interesting questions related to host susceptibility to dental implant failure. There has been shown evidence for genetic contribution to implant failure. Genetic polymorphisms have been classically considered risk factors for several diseases and, more recently, for dental implant loss. Vitamin D is a mediator of bone metabolism, which acts bound to its receptor (VDR). The aims of this study were i) to provide a brief description of the current methodology for genetic analysis of complex traits, especially applied to dental implant failure, followed by a comprehensive review of the literature related to genetic susceptibility to dental implant failure; ii) investigate clinical factors related to the failure process, and iii) investigate the association between the rs731236 (T*aq*I) VDR polymorphism and implant loss. Two hundred and seventeen (217) unrelated patients, mean age 51.7 ± 11.3 years, were divided into two groups: *Control group (C)*, 137 individuals presenting at least one implant in function for six months and without any implant failure, and *Study group (S)*, 80 individuals with at least one implant loss. Patients' socioeconomic profile, general medical condition, smoking, hygiene parameters, and clinical measurements such as number of teeth, periodontal status, bone quantity and quality, graft presence, implant position, primary stability, length and diameter, type of platform, and time to load were evaluated. After DNA collection and purification, polymorphism analysis was performed by PCR-RFLP. Differences between control and study groups and between healthy and lost implants were accessed. Regarding clinical aspects, the following parameters were observed to influence implant failure: edentulism, probing pocket depth, implant position, primary stability, implant length, bone quantity, and surgical technique. No association between genotypes or alleles of T*aq*I polymorphism and implant loss was found between the groups. It was observed that clinical aspects, but not the study polymorphism, were associated with dental implant failure.

INTRODUÇÃO

1. INTRODUÇÃO

Implantes dentais osseointegráveis

A reposição de dentes perdidos é de grande importância para a manutenção da saúde geral do paciente. O implante dental é, atualmente, o tratamento de eleição para a reposição de dentes perdidos (Att & Stappert 2003; Henry 2005). A taxa de sucesso dos implantes osseointegráveis varia entre 85 a 100% em estudos longitudinais de até 24 anos (Adell et al. 1990; Bain & Moy 1993; Bain 1996; Lekholm et al. 1999) e para todos os sistemas de implantes (Scolozzi & Jaques 2004; Esposito et al. 2005). Apesar do alto índice de sucesso na colocação de implantes, falhas podem ocorrer, muitas vezes sem a identificação de causa clínica (Ellen 1998; Goodacre et al. 1999; Graziani et al. 2004; Fugazzotto 2005).

A taxa de insucesso é pequena em porcentagem, mas torna-se relevante em número absoluto, devido à grande quantidade de implantes instalados e à demanda crescente de indicações.

As falhas na osseointegração dividem-se em precoces e tardias. As falhas precoces são aquelas ocorridas anteriormente à osseointegração, e as tardias, após o implante ter sido osseointegrado (Esposito et al. 2004). As falhas precoces podem estar relacionadas ao uso do cigarro (Ganeles & Wismeijer 2004), a doenças sistêmicas (Weyant & Burt 1993; Quirynen & Teughels 2003), à qualidade e quantidade óssea (Stanford 2003; Rosenberg et al. 2004; Degidi & Piattelli 2005), ao trauma cirúrgico (Lang et al. 2004) e à contaminação durante a cirurgia (van Steenberghe & Quirynen 1990; Kuttenberger et al. 2005). Já as falhas tardias relacionam-se principalmente à periimplantite (Rosenberg et al. 2004) e à sobrecarga oclusal (Misch et al. 2004).

Estudos controlados são necessários para dar suporte científico ao entendimento dos processos de falhas de implantes osseointegráveis (Graziani et al. 2004; Fugazzotto 2005). Apesar dos diversos estudos realizados sobre falhas de implantes osseointegráveis, várias questões sobre fatores de risco à perda permanecem sem resposta e necessitam de mais investigações (Esposito et al. 2004; Graziani et al. 2004).

A ocorrência de insucesso na colocação de implantes dentais não parece estar distribuída de forma aleatória na população; ou seja, há um pequeno grupo de indivíduos no qual ocorrem inúmeras perdas (Tonetti 1999). Indivíduos com um implante falho são mais propensos a sofrer outras falhas (Weyant & Burt 1993; Hutton et al. 1995), indicando a existência de grupos de risco para a perda de implantes, sugerindo que fatores genéticos podem estar envolvidos nesses insucessos (Esposito et al. 1998).

Os critérios para a avaliação do fracasso de um implante estão baseados em alterações clínicas e radiográficas, que normalmente refletem condições patológicas extensas, nas quais o dano já ocorreu. A identificação de fatores relacionados ou determinantes da perda de implantes pode permitir uma intervenção precoce, minimizando as injúrias ao tecido e aumentando o potencial de sucesso terapêutico (Kao et al. 1995).

Ferramentas de investigação de fatores genéticos associados à perda de implantes dentais osseointegráveis

Análises genéticas aplicadas a implantes dentais começaram a ser realizadas no final dos anos 90 (Wilson & Nunn 1999). Desde então, interesse crescente vem sendo demonstrado em vários estudos (Santos et al. 2004a; Santos et al. 2004b;

Jansson et al. 2005). No entanto, esses estudos são baseados na escolha de genes candidatos em análises de associação. Nesta linha, vem se tentando identificar genótipos/alelos específicos como marcadores de risco à perda de implantes dentais.

As doenças complexas resultam da interação de um ou mais fatores genéticos e ambientais (Schutte & Murray 1999). Quando se estudam doenças complexas é esperada a existência de múltiplos *loci* afetando esta condição (Muhle et al. 2004). Classicamente o primeiro objetivo de estudos genéticos em doenças complexas é a detecção de um componente genético por dados observacionais; porém, a simples identificação do componente genético não revela quais e quantos genes estão envolvidos na determinação da suscetibilidade individual. Usualmente, duas são as principais estratégias utilizadas para a determinação desses genes: i) análise de ligação e ii) análise de associação.

A análise de ligação é uma técnica de determinação de regiões genômicas relacionadas com a doença e seus padrões de segregação em famílias afetadas (Weeks & Lange 1992). As suas limitações mais importantes são: i) a necessidade de obtenção de múltiplos *pedigrees* com indivíduos afetados, o que se torna difícil em doenças de aparecimento tardio, ou muito raras; ii) baixa força em detectar genes de efeito leve a moderado; iii) dificuldades na replicação destes estudos, e iv) baixo poder de determinação exata dos *loci* que estão em ligação com a doença.

A análise de associação é baseada na comparação de freqüências alélicas e genotípicas entre indivíduos afetados e não-afetados. O alelo/genótipo é dito associado se ocorrer em uma freqüência significativamente maior na população afetada em comparação à não-afetada (Lander & Schork 1994). Geralmente são escolhidos genes candidatos, que tenham algum envolvimento no processo

fisiopatológico do objeto de estudo. Esta análise é mais apropriada para a detecção de genes com baixo a médio efeito (Risch & Merikangas 1996). Por outro lado, em estudos caso-controle pode-se introduzir variáveis não relacionadas com a doença, desta forma obtendo-se associações espúrias (Devlin et al. 2001; Mayeux 2005).

A análise de *scan* genômico caso-controle poderia representar um método alternativo e de maior abrangência na detecção de polimorfismos de base única (SNPs) para o estudo da influência genética na perda de implantes. Dessa forma, aumentariam-se as chances da determinação de marcadores ligados à perda de implantes.

Avanços na elucidação da influência de fatores genéticos na perda de implantes dentais osseointegráveis podem, no futuro, contribuir tanto para a detecção de indivíduos de maior risco à perda de implantes, quanto para o tratamento individualizado e melhor prognóstico.

Receptor da Vitamina D (VDR)

A descoberta da vitamina D ocorreu entre 1919 e 1924 (DeLuca 1988). Ela é considerada um hormônio esteróide multifuncional que modula a homeostasia mineral e a arquitetura esquelética normal (Nagpal et al. 2005), através de ação predominantemente no intestino (Walters et al. 2004; Collins et al. 2005). Ela é produzida pelas células da pele, por ação dos raios ultravioletas, ou pode ser ingerida. Sua forma ativa, 1,25 dihidroxivitamina D_3 [1,25-$(OH)_2D_3$], é obtida após sua metabolização no fígado e posteriormente nos rins (Prosser & Jones 2004; Shinkyo et al. 2004). A vitamina D está envolvida em uma ampla variedade de processos biológicos, como o metabolismo ósseo (Davideau et al. 2004), a modulação da resposta imune (Mathieu et al. 2004) e a regulação da proliferação e

diferenciação celular (Sooy et al. 2005). Análogos sintéticos da vitamina D vêm sendo desenvolvidos para o tratamento de doenças como psoríase, osteoporose, hiperparatireoidismo e câncer (Nagpal et al. 2005), o que denota sua relevância clínica. A maioria dos efeitos já sabidos da vitamina D são mediados via um receptor intracelular de alta afinidade, o receptor da vitamina D (VDR) (van Etten & Mathieu 2005).

O VDR é uma proteína classicamente nuclear, membro da superfamília de receptores de hormônios esteróides (Nezbedova & Brtko 2004). É um fator de transcrição modulado através de um ligante (a vitamina D), formando um complexo capaz de estimular transcrições gênicas, cujo produto pode promover a diferenciação osteoclástica (Kim et al. 2005). O VDR está presente em mais de 30 tecidos-alvo em humanos (Reichel et al. 1989), três deles envolvidos com a homeostasia de cálcio (intestinos, ossos e os rins). Camundongos *knockout* para o gene do VDR mostraram hipocalcemia, hipofosfatemia, hipoparatireoidismo, raquitismo e osteomalácia (Sakai & Demay 2000), demonstrando sua importância na manutenção da homeostasia óssea (Goltzman et al. 2004).

O gene do VDR está localizado no cromossomo 12, na região 12q13.1. Possui 9 éxons, e uma extensa região promotora (Poon et al. 2004), este gene contém 63.454 pb.

Polimorfismos são alterações na seqüência gênica, que geram formas variantes, denominadas alelos, cuja freqüência do mais raro é superior a 1 % (Nussbaum et al. 2002). A abundância e a grande freqüência de polimorfismos no genoma humano os transformam em alvo para explicar a variabilidade genética (Korstanje & Paigen 2002; Thomas & Kejariwal 2004) e sua influência no risco e

progressão de algumas doenças (Morange et al. 2005; Rao et al. 2005; Sun et al. 2005)

Vários alelos polimorficos têm sido descritos no gene do VDR (Faraco et al. 1989; Morrison et al. 1992; Ye et al. 2000) e foram associados a diversas doenças, tais como a osteoporose (Duman et al. 2004), a asma (Poon et al. 2004), o diabetes tipo I (Marti et al. 2004), e diversos tipos de câncer, como o de mama (Guy et al. 2004), o de cólon e reto (Slattery et al. 2004) e o de próstata (Cheteri et al. 2004),

A região não-traduzida 3' (UTR) está envolvida com a regulação de expressão gênica, especialmente pela modificação de estabilidade do mRNA (Decker & Parker 1995; Durrin et al. 1999). Um polimorfismo (T/C) no éxon 9 (rs731236) do gene do VDR, reconhecido pela enzima de restrição T*aq*I, parece estar em desequilíbrio de ligação (LD) com a 3' UTR (Morrison et al. 1992; Morrison et al. 1994). Alelos desse polimorfismo podem estar associados a uma maior ou menor estabilidade do RNAm, modulando sua expressão intracelular. Por regular a disponibilização do VDR, esse polimorfismo é considerado funcional.

O polimorfismo T*aq*I no gene do VDR gene tem sido associado a parâmetros de densidade óssea (Yamagata et al. 1999; Sun et al. 2002) e a doenças complexas, como o câncer (Verbeek et al. 1997; Lundin et al. 1999), a osteoporose (Horst-Sikorska et al. 2005) e a doença periodontal (Sun et al. 2002; de Brito Junior et al. 2004; Park et al. 2006). Além disso, dois blocos de desequilíbrio de ligação (associação de alelos de polimorfismos diferentes que são herdados em bloco) foram estabelecidos para o gene do VDR (Poon et al. 2004), e o polimorfismo T*aq*I pode representar o segundo bloco.

PROPOSIÇÃO

2. PROPOSIÇÃO

Objetivo geral

O objetivo do presente trabalho é investigar a associação de aspectos clínicos e genéticos com a perda de implantes dentais osseointegráveis.

Objetivos específicos

i) Identificar as possíveis metodologias de investigação de fatores genéticos associados à perda de implantes dentais osseointegráveis, seguida de uma revisão crítica da literatura a respeito da suscetibilidade genética à falha de implantes dentais osseointegráveis;

ii) Investigar aspectos clínicos envolvidos com a perda de implantes dentais osseointegráveis;

iii) Analisar a associação entre alelos e genótipos específicos de polimorfismo (rs731236, T*aq*I) no gene do VDR e a perda de implantes dentais osseointegráveis.

ARTIGO 1

3. *Artigo 1*

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Genetic Susceptibility to Dental Implant Failure: a Critical Review

Fabiano Alvim Pereira [1], Claudia C. Montes [1], Marcelo T. Mira, PhD[2], Paula C. Trevilatto, PhD[2]

Authors' affiliations:

[1] PhD Student of the Graduate Program in Health Sciences, Center for Health and Biological Sciences, Pontifical Catholic University of Paraná (PUCPR), Rua Imaculada Conceição, 1155. Curitiba – PR, CEP-80215-901, BRAZIL, Phone: +55 (41) 3271-2618, Fax: +55 (41) 3271-1657. Researcher of Latin-American Dental Research Institute (ILAPEO), Curitiba, PR, Brazil.

[2] Professor of the Graduate Program in Health Sciences, Center for Health and Biological Sciences, Pontifical Catholic University of Paraná (PUCPR), Rua Imaculada Conceição, 1155. Curitiba – PR, CEP-80215-901, BRAZIL, Phone: +55 (41) 3271-2618, Fax: +55 (41) 3271-1657.

Correspondence to: Dr. Paula C. Trevilatto, Center for Health and Biological Sciences, Pontifícia Universidade Católica do Paraná (PUCPR), Rua Imaculada Conceição, 1155. Curitiba – PR, CEP-80215-901, BRAZIL, Phone: +55 (41) 3271-2618, Fax: +55 (41) 3271-1657, e-mail: pctrev@yahoo.com.br

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Abstract The observation that clinical factors alone do not explain why some patients develop implant loss, the understanding of the osseointegrated implant failure as a complex, multifactorial process and the observed aggregation of repetitive failure in certain individuals raise interesting questions related to host susceptibility to dental implant failure. Genetic analysis applied to dental implants began in the late 90's and since then, increased interest in genetic susceptibility to the phenotype has been demonstrated by several studies. These studies, however, have been based on and limited to candidate gene association analysis, intended to find association between specific alleles and/or genotypes of genetic markers and susceptibility to implant failure. The aim of this review is to provide a brief description of the current methodology for genetic analysis of complex traits, followed by a comprehensive review of the literature related to genetic susceptibility to dental implant failure and a discussion of different aspects of the applied methodology. Moreover, it brings up a novel approach of *genome wide, case-control analysis*, as an alternative method to access genetic influence to dental implant failure mechanisms. Advances towards the elucidation of the genetic basis of dental implant loss may contribute to the understanding of why some patients do not respond to currently available treatments while others do, providing potential targets for effective screening, prevention and treatment. For example, clinicians might be able to estimate, before the elective surgical procedure, the risk of a given patient to develop a negative individual host response.

Key Words: dental implant - osseointegration - implant failure – polymorphisms - genetic approaches.

Osseointegrated dental implants are fixtures, commonly of titanium, which are surgically screwed into the jaw bone. After the surgery, a traditional, two-step technique requires a healing phase of 90-180 days without submitting the implant to mechanical, mastigatory forces. Only after the healing phase the prosthesis (crown) is attached to the implant and submitted to occlusal load. In an alternative, one-step, immediate load technique, the healing processes occurs in the presence of mastigatory forces. In both cases, the implant is expected to functionally and structurally connect to the bone in a process known as *osseointegration* [1]. The mechanism of osseointegration of dental implant is very similar to the primary bone healing. First, the inflammatory process promoted by surgical trauma causes circulatory alterations and hematoma. Then, regeneration takes place, with the wound being substituted by bone tissue in a remodeling process that leads to wound maturation [2]. Therefore, successful implant osseointegration is likely to depend on factors such as an appropriate tissue repair mechanism [3] and adequate immunological response [4].

Dental implant is a very predictable procedure that often provides the best result for dental replacement of patients presenting missing teeth [5,6]. In spite of a success rate above 90 %, the absolute number of dental implant failure is significant, given that approximately one million procedures are conducted annually, worldwide [7]. Dental implant osseointegration failure is a complex, multifactorial trait, investigated by several clinical follow-up and retrospective studies [6,8,9]. The process is divided into early and late events: early failure occurs before implant load, and late failure takes place after the implant has received occlusal load [10]. Early failures have been related to smoking [11], aging [12], systemic diseases [13,14], bone quantity and

quality [15-17], surgical trauma [18], and contamination during surgical procedure [19,20]. Late failures have been related to peri-implantitis [17] and occlusal overload [21].

Although these previous studies have provided an important contribution to the understanding of the implant failure process, in some situations, clinical factors alone do not explain why some patients develop implant loss [22]. Moreover, the occurrence of implant failures is not randomly distributed in treated populations, multiple implant losses are likely to occur in specific high-risk individuals, a phenomenon termed *clusterization* [23], and re-occurrence of implant failure is frequently observed [14,24]. Taken together, these observations strongly suggest the existence of genetic risk factors for dental implant loss [25].

Genetic Analysis of Complex Traits

Complex traits result from an interaction between one or more genetic variants and environmental or non-genetic risk factors [26]. When studying complex traits, it is generally expected the existence of multiple loci affecting the disorder [27]. Classically, the first goal of a genetic study involving a complex trait is to detect a genetic component from observational data. This can be achieved by applying several different strategies, such as the observation of familiar aggregation of cases or the clusterization phenomenon, in cases where access to pedigrees is limited. Another powerful tool is twin studies, in which the concordance rate of a trait is estimated and compared among homozygous and dizygous twins. Finally, complex segregation analysis can be used to describe the mode of inheritance that provides the best fit, given the observed pedigree data. Unfortunately, none of these approaches provide information about the exact nature of the genetic component, such as number, location and identity of the genes involved; therefore further studies are necessary,

typically involving two main strategies: linkage analysis and association analysis. Figure 1 shows the usual pathway one might follow, from detecting a genetic component to the identification of the gene variants responsible for the studied complex phenotype.

Linkage analysis is a genomic region hunting technique that traces patterns of co-segregation of the trait and specific genomic segments in high-risk, multiple affected pedigrees [28]. The goal is to physically locate a disease-causing gene within the narrowest possible genomic interval. Genes are located based solely on their position in the genome. Modern linkage analysis is a powerful approach for studying both mendelian and complex genetic disorders [29], suitable for large candidate region analysis or even genome wide searches [30,31]. Results are usually expressed in LOD scores; it is generally accepted that statistical significance is reached when the LOD score is higher than 3.0 for candidate region analysis, and 3.3 for genome-wide studies [32,33]. The most important limitations of linkage analysis are (i) the need to enroll multiple-affected pedigrees, difficult to obtain in cases of rare or late-onset diseases; (ii) low power to detect genes exerting moderate to low effect over the phenotype [34]; (iii) replication of positive linkage has proven to be a difficult task; and (iv) low power to pinpoint the exact gene/variation causing the linkage effect [35]. In fact, linkage analysis of complex traits often results in the identification of a genomic region several megabases long and containing a large number of genes. In these cases, narrowing down the candidate genomic region is usually attempted through association analysis.

Association analysis is based on the comparison of the allele frequencies of a genetic marker across affected and unaffected individuals. This can be done in a family-based or population-based (case-control) sample. A given allele is considered

to be associated with the disease if that allele occurs at a significantly higher frequency among affected, as compared to unaffected individuals [36]. The strategy is commonly used for candidate gene analysis, with the candidate genes usually defined based on their possible role on disease physiopathology (functional candidates), by previous linkage analysis (positional candidates) or both. Association analysis is more powerful than linkage analysis to detected genetic effects with low to moderate genotypic relative risk [31]. However, since the association effect extends over very short genomic segments, the strategy is not as suitable as linkage analysis for large genomic region or genome-wide screening – several hundreds of thousands of markers would be required for a reliable genome-wide coverage. In addition, the need of large sample sizes, small *P*-values and replication in independent samples have been advocated as reliability parameters for true association [37]. Moreover, in population-based, case-control studies, patients may differ from the control group in their genetic background, introducing variables unrelated to the disease and causing a type of spurious association or confounding named *population stratification* [38,39].

Encouraged by the early success in the identification of genes responsible for monogenic diseases, many investigators have embraced different strategies for the dissection of the genetic component controlling complex diseases [40]. For example, a two-step study using hypothesis-generating, genome-wide linkage analysis followed by association-based, fine mapping of the candidate regions identified has resulted in the first positional cloning of genetic variants associated with an infectious disease [41]. However, the knowledge about the genetic mechanisms controlling complex traits is still fragmented and incomplete, and little is known about genetic susceptibility to most physiopathological processes [4].

Genetic Analysis of Dental Implant Failures

Inter-individual variability to different phenotypes is partially determined by the human genetic code. Specifically, variability is due to the existence of a large number of polymorphisms, gene sequence variations with minimum allele frequency higher than 1 % in the population, distributed evenly throughout the entire genome [42]. Polymorphisms have been shown to modulate host response and susceptibility to numerous diseases [43-46]. A polymorphism is said to be “functional” if modulates gene expression or results in an amino acid change in the polypeptide chain [45]. Alternatively, a polymorphism is defined as “silent” if no obvious, predictable biological impact can be inferred [47]. Independently of their functional impact, polymorphisms can be used as gene markers for genetic analysis, and several cases of association between functional and silent polymorphisms have been observed.

The focus of studies investigating genetic susceptibility to dental implant failure has been limited to candidate gene, association analysis [18,48-51]. In this approach, selected genes are defined as candidates based on available information about the osseointegration process. Incomplete biological knowledge of the involved metabolic pathways, however, limits the search to a fraction of all the correlated genes. In these studies, functional polymorphisms, especially those which modulate the correspondent protein expression rate, are frequently chosen.

A systematic review of the literature was carried out in the electronic database Medline/PubMed. All the studies concerning genetic analysis of human polymorphisms related to dental implants and indexed up to October 2006 were considered.

The most commonly studied functional polymorphisms for dental implant

failure are variations of the interleukin-1 (IL-1) gene cluster, in particular in the IL-1α (*IL1A*) and IL-1β (*IL1B*) genes. Because of IL-1 proinflammatory and bone resorbing properties [52,53], a role has been suggested for this cytokine in controlling the risk of severe chronic periodontitis development [54]. Also, a role for IL-1 on dental implant success was proposed [55]. However, evidence for association has been found between *IL1A* and *IL1B* gene polymorphisms (allele T for both *IL1A*-889 and *IL1B*+3953 polymorphisms, called "positive genotype") and periodontal disease [54], but not with implant failure [56]. A non-statistically significant evidence of an increased risk to implant failure in patients with specific *IL1A* and *IL1B* genotypes was reported for different populations [57,58]. Otherwise, in a partially edentulous group treated for periodontal disease prior to implant treatment, a synergistic effect between the *IL1* positive genotype and smoking was detected [59], characterizing individuals with these two conditions together as a high-risk population for implant failure. Moreover, studies comparing smoking and non-smoking groups detected an increased risk for peri-implant bone loss in a heavy smoking population with *IL1A* and *IL1B* polymorphisms during the after-loading phase [18,49,60].

Interleukin-2 (IL-2) is a cytokine involved in the B-cell activation and stimulates macrophages, natural killer cells and T-cell proliferation, which mediate the cellular immune response, being regarded as a proinflammatory cytokine [61-63]. Interleukin-2 has been also implicated in the stimulation of osteoclast activity in bone resorption [64]. Interleukin-6 (IL-6) plays a role in B-cell differentiation and T-cell proliferation [65]. It also stimulates hematopoiesis [66] and accelerates bone resorption [67]. In spite of the association between *IL2* and *IL6* promoter polymorphisms and periodontal disease [68,69], no significant differences in the distribution of those polymorphisms were found between implant failure and control groups in a Brazilian population [70].

Tumor necrosis factor-*α* (TNF-α) is a potent mediator of immune-inflammatory response [71,72] and also has been reported to induce bone resorption *in vitro* and *in vivo* [73,74]. The *TNFA* (G-308A) gene polymorphism was investigated and showed no association with early implant failure [48].

No association was also found between early osseointegrated implant failure and polymorphisms in the transforming growth factor beta-1 (TGF-β1) gene [51]. This cytokine is a multifunctional protein known to induce the expression of collagen genes, to provoke extracellular matrix fibrosis, and to regulate cell growth, differentiation and function [75].

Matrix metalloproteinases (MMPs) are a family of metal-dependent proteolytic enzymes which mediate the degradation of extracellular matrix and basement membranes in several tissues [76]. MMPs are likely to be involved in the dental implant osseointegration process [77,78]. Polymorphisms that increase transcriptional activity of MMP-1 and MMP-9 were analyzed, and allele and genotype frequencies were compared between the failure and control groups. Results showed that *MMP1* polymorphisms were associated with implant failure, while no association with implant loss was found for the *MMP9* promoter region polymorphism [50].

Polymorphisms in genes involved in bone metabolism have also been investigated. A polymorphism in the bone morphogenetic protein-4 (BMP-4) gene was associated with marginal bone loss before the second stage surgery (implant load) [79]. A positive correlation was also observed between a calcitonin receptor (CTR) gene polymorphism and marginal bone loss at the second stage surgery [80]. A summary of studies investigating the association between genetic polymorphisms and osseointegrated dental implant failure in different populations is shown in table 1. The functional impact of the polymorphisms investigated for susceptibility to

implant failure is shown in table 2.

Despite these promising advances, the exact number, identity and role of regulatory factors that lead to a successful implant osseointegration and its maintenance are still largely unknown, which limits genetic analysis approaches based on functional candidate genes. The challenge then is to map all the involved genes [40], a considerably difficult task given that the human genome is composed of twenty-two pairs of autossomal chromosomes and one pair of sexual chromosomes carrying at least 30,000 genes [81].

Future Perspectives

Although candidate gene, association analysis has proved to be a promising tool for the dissection of the exact nature of the genetic component controlling dental implant failure, the design is limited by the fact that just a small segment of the genome is analyzed. Candidate gene approach is limited in providing a genome-wide perspective on interesting gene regions and gene to gene interactions. In addition, the sample sizes are often small; therefore, findings must be replicated in larger populations. Finally, larger scale studies, such as genome-wide linkage analysis, are made difficult by the need of large samples of multiple affected pedigrees. As a consequence, genetic susceptibility to osseointegrated implant failure remains widely unknown.

All the studies mentioned above employ single-nucleotide polymorphisms (SNPs) as gene markers. SNPs are the most frequently observed type of genetic polymorphisms. Catalogued SNPs in public databases have been growing from 1.4 million in 1999 [82] to 2.1 million in 2001 [83] up to approximately 4.1 million markers available in SNP public databases today [84]. Though somewhat less informative than

other types of DNA markers, SNPs are technically easier and less expensive to genotype. As a recent development, DNA microarrays are a new, fully automated technology that allows genotyping hundreds of thousands of SNPs in a single experiment [85]. This new, extremely high throughput SNP genotyping technology is making possible, for the first time, the development of association-based, genome-wide scans using case-control samples, to investigate genes related to complex traits, such as Parkinson disease [86]. These whole-genome association studies, using hundreds of thousands of SNPs covering the entire genome, combine the best features of linkage analysis with the strength of association analysis [87]. In this new approach, classic, family-based linkage analysis would not be necessary, making it possible to study population samples of unrelated subjects. This feature is particularly interesting in the context of dental implant failure, where the difficulty of enrolling multiple-case families poses a major obstacle for the application of family-based linkage tools.

However, some limitations exist. Association-based genome-wide studies are still very expensive and limited to laboratories equipped with cutting-edge genotyping technology [88]. Also, as mentioned before, population-based association analysis always involve the risk of cryptic, undetected population stratification leading to spurious results. Finally, the generation of such tremendous amount of raw data demands the development of new, adequate statistical methods of analysis [38]. Still, due to the large number of tests performed, false-positive results are likely to increase [89]. In this context, replication of the original findings in independent populations becomes mandatory [90].

Despite the difficulties, the motivation to continue to apply traditional and new approaches of genetic analysis to the effort towards a better understanding of dental

implant failure mechanisms is clear. For example, genetic studies may shed new light not only upon the physiopathology of dental implant failure, but also upon broader, related processes, such as bone healing. In addition, a direct result of such studies may be the definition of potential targets for effective screening, prevention and maintenance of dental implants.

Acknowledgments

Latin-American Dental Research Institute (ILAPEO), Curitiba, PR, Brazil.

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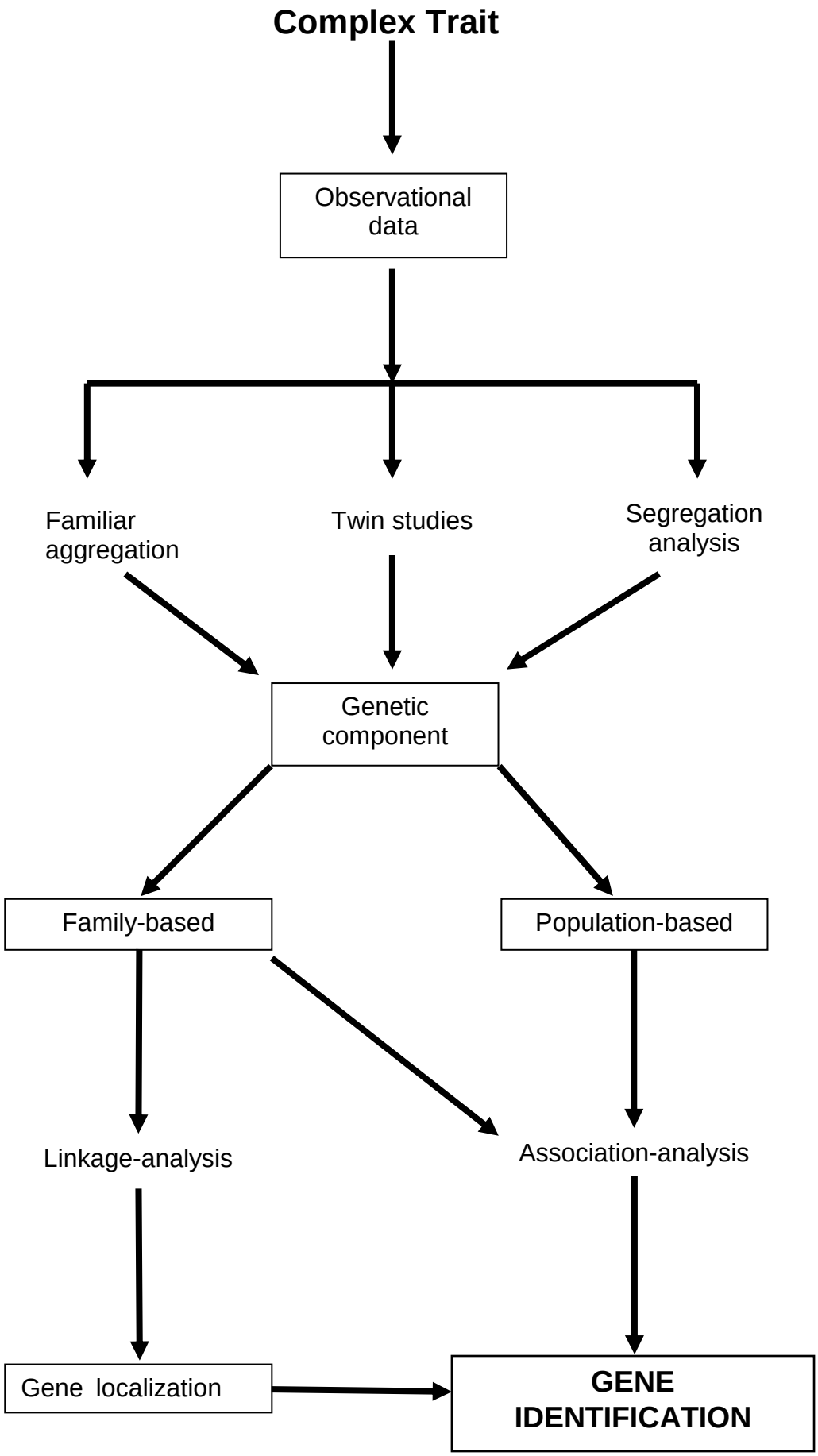
Table 1. A summary of association studies between genetic polymorphisms and osseointegrated dental implant failure in different population.

Authors	Polymorphisms	Case (n)/ Control (n)	Mean age (years)	Smoking Yes/No	Population	Results
(Rogers et al. 2002)	*IL1A* (-889) and *IL1B* (+3953)	19/31	66	?	Australian Caucasian	Not associated with implant failure
(Wilson & Nunn 1999)	*IL1A* (-889) and *IL1B* (+3953)	27/38	57	27/35	?	Not associated with implant failure
(Campos et al. 2005b)	*IL1A* (-889), *IL1B* (-511, +3953), and *IL1RN* (intron 2 - 86 bp repeats)	28/34	47.5	0/62	Brazilian	Not associated with early implant failure
(Feloutzis et al. 2003)	*IL1A* (+4845) and *IL1B* (+3954)	?	59.5	41/39	European Caucasian	Smoking + *IL1* positive genotype associated with marginal bone loss
(Gruica et al. 2004)	*IL1A* (+4845) and *IL1B* (+3954)	34/146	25 to 90	53/127	European Caucasian	Smoking + *IL1* positive genotype associated with late infection
(Jansson et al. 2005)	*IL1A* (-889) *IL1B* (+3954)	6/16	54	10/12	European Caucasian	Smoking + *IL1* positive genotype associated with implant loss
(Shimpuku et al. 2003b)	*IL1A* (-889) and *IL1B* (-511, +3954)	17/22	55.1	14/25	Japanese	Associated with marginal bone loss
(Campos et al. 2005a)	*IL2* (-330) and *IL6* (-174)	34/40	46.3	0/74	Brazilian	Not associated with early implant failure
(Campos et al. 2004)	*TNFA* (-308)	28/38	47.2	0/66	Brazilian	Not associated with early implant failure
(Santos et al. 2004a)	*TGFB1* (-509, -800)	28/40	46	0/68	Brazilian	Not associated with early implant failure
(Santos et al. 2004b)	*MMP1* (-1607) and *MMP9* (-1562)	20/26	45.9	0/46	Brazilian	*MMP1* - associated, and *MMP9* - not associated with implant failure
(Shimpuku et al. 2003a)	*BMP4* (+538)	21/36	52.6	24/38	Japanese	Associated with marginal bone loss
(Nosaka et al. 2002)	*CTR* (+1377)	15/20	54.8	15/20	Japanese	Associated with marginal bone loss in mandible, but not in maxilla.

Table 2. Functional impact of the polymorphisms investigated for susceptibility to implant failure.

Polymorphism	Functional	Type of Functionality	Study
IL1A (C-889T)	yes	Regulation of gene expression	(Dominici et al. 2002)
IL1A (G+4845T)	-	99% linkage disequilibrium with *IL1A* (-889)	(Cox et al. 1998)
IL1B (C+3953/4T)	yes	Regulation of gene expression	(Pociot et al. 1992)
IL1RN (intron 2 – 86 bp repeats)	yes	Regulation of gene expression	(Hu et al. 2005)
IL2 (T-330G)	yes	Regulation of gene expression	(Hoffmann et al. 2001)
IL6 (G-174C)	yes	Regulation of gene expression	(Fishman et al. 1998)
TNFA (G-308A)	yes	Regulation of gene expression	(Hajeer & Hutchinson 2001)
TGFB1 (C-509T)	yes	Regulation of gene expression	(Kim et al. 1989)
TGFB1 (G-800A)	yes	Regulation of gene expression	(Kim et al. 1989)
MMP1 (G-1607GG)	yes	Regulation of gene expression	(Rutter et al. 1998)
MMP9 (C-1562T)	yes	Regulation of gene expression	(Zhang et al. 1999)
BMP4 (T+538C)	yes	Amino acid change Val147Ala	(Mangino et al. 1999)
CTR (C+1377T)	yes	Amino acid change Pro463Leu	(Nakamura et al. 1997)

Figure 1. Suggested flow chart, combining different strategies for genetic analysis of complex traits: from the detection of a genetic component to the identification of the functional gene variants.


Complex Trait
Observational data
Familiar aggregation
Twin studies
Segregation analysis
Genetic component
Family-based
Population-based
Linkage-analysis
Association-analysis
Gene localization
GENE IDENTIFICATION

ARTIGO 2

4. Artigo 2

ANALYSIS OF ASSOCIATION OF CLINICAL ASPECTS AND VDR GENE POLYMORPHISM WITH DENTAL IMPLANT FAILURE

Alvim-Pereira F., Montes C.C., Thomé G., Olandoski M., Trevilatto P.C.

Running Title: Clinical and genetic aspects in implant loss

Marcia Olandoski, Paula Cristina Trevilatto, Center for Health and Biological Sciences, Pontifical Catholic University of Paraná (PUCPR), Curitiba, PR, Brazil.

Fabiano Alvim Pereira, Claudia Cristina Montes, Geninho Thomé, Latin-American Dental Research Institute (ILAPEO), Curitiba, PR, Brazil.

Key Words: dental implant, clinical factors, implant failure, polymorphisms, VDR

Corresponding author:
Paula Cristina Trevilatto
Center for Health and Biological Sciences
Pontifícia Universidade Católica do Paraná (PUCPR)
Rua Imaculada Conceição, 1155
Curitiba – PR 80215-901
BRAZIL
Phone +55 (41) 3271-2618
Fax: +55 (41) 3271-1657
E-mail: pctrev@yahoo.com.br

Abstract

Osseointegration failure is a complex, multifactorial trait shown to concentrate in some treated populations. There has been shown evidence for genetic contribution to dental implant failure. Genetic polymorphisms have been classically considered genetic risk factors for several diseases and, more recently, for dental implant loss. **Objectives:** The purpose of this study was to access clinical factors related to failure process, and to investigate the relationship between a vitamin D receptor (VDR) polymorphism (rs731236, T*aq*I) and dental implant loss. **Material and Methods:** Two hundred and seventeen unrelated patients, mean age 51.7 ± 11.3 years, were divided into two groups: i) *Control group (C)*, 137 individuals presenting at least one osseointegrated implant in function for six months or more and without any implant failure, and ii) *Study group (S)*, 80 individuals presenting at least one implant loss. After DNA collection and purification, VDR T*aq*I polymorphism analysis was performed by PCR-RFLP. Differences between control and study groups and between healthy (H; n=1232) and lost (L; n=135) implants were accessed. **Results:** Positive evidence of association has been detected between implant failure and the following variables: edentulism, implant position, primary stability, and implant length. Cox Regression model showed that primary stability, surgical technique and bone quantity were related to implant survival over time. No association between genotypes or alleles of VDR T*aq*I polymorphism and implant loss was found between the groups. **Conclusion:** It was observed that clinical variables, but not the study polymorphism, were associated with implant dental failure.

The term osseointegration was coined by Branemark (Branemark et al. 1969; Albrektsson et al. 1981) to define a structural and functional contact between titanium surface and bone. Representing the ideal modality of treatment for oral rehabilitation, osseointegrated dental implant has been considered a very predictable procedure that often provides the best results for dental replacement (Davarpanah et al. 2002; Fugazzotto 2005).

Implant osseointegration research has mainly been focusing on the quantification of bone-to-implant contact (BIC) and, often comparing the effects of different implant surface coatings and topographies (Gottlander & Albrektsson 1991; Hallgren et al. 2003). Moreover, the architecture and microenvironment around the implant have been shown to be crucial and determining factors for the healing process (Ohtsu et al. 1997; Fujii et al. 1998; Shirakura et al. 2003; Rammelt et al. 2004). These mechanisms are likely to depend on factors such as an appropriate tissue repair mechanism (Danesh-Meyer 1994) and adequate immunological response (Kronstrom et al. 2001).

In spite of high success rates attributed to dental implants (85 to 100 %) in longitudinal studies up to 24 years (Adell et al. 1990; Bain & Moy 1993; Bain 1996; Lekholm et al. 1999), the absolute number of dental implant failure is significant, considering that around ten millions of people are dental implant rehabilitated annually all over the world (Hospitalar 2007).

Osseointegration failure is a complex, multifactorial trait investigated by several clinical follow-up and retrospective studies (Esposito et al. 2004b; Graziani et al. 2004; Fugazzotto 2005), and has been shown not to occur uniformly distributed in treated populations. Multiple implant loss is often observed in specific high-risk individuals, a phenomenon termed clusterization(Tonetti 1999), and re-occurrence of failure is frequently observed

in this group (Weyant & Burt 1993; Hutton et al. 1995). Moreover, in some cases, even when the patient has appropriate bone quantity and quality, and adequate clinical indication and recommendations are followed, failures in the osseointegration process do still occur. Taken together, these observations suggest the existence of host risk factors for dental implant loss (Esposito et al. 1998a).

Implant failure often occurs at early periods of the healing process (Nevins & Langer 1993; Salonen et al. 1993), what drives us to investigate the regulatory mechanisms modulating bone metabolism, remodeling and turnover.

Bone is one of the classical target tissues for vitamin D action. Vitamin D regulates calcium homeostasis by influencing intestinal calcium absorption, renal calcium reabsorption, and bone calcium resorption (Binkley 2006). Vitamin D is ingested or cutaneously produced upon exposure to ultraviolet B radiation in an inactive form. To be activated, vitamin D is transported in the blood bound to a vitamin D-binding protein, hydroxylated in the liver and the resulting metabolite is further hydroxylated mainly at the kidney, resulting in the active form called 1,25-dihydroxyvitamin D_3 (Panda et al. 2004). In target tissues, 1,25-$(OH)_2D_3$ is believed to exert most of its actions by binding to the vitamin D receptor (VDR), a member of the nuclear steroid hormone receptor superfamily, and by regulating the transcription of vitamin D target genes (Haussler et al. 1998). The VDR also plays a complex role in the control of bone homeostasis and recruits co-regulators, which may have activating or repressing effects. In VDR knockout growing mice, the primary defect of calcium metabolism is at the intestine; loss of VDR causes calcium malabsorption and Rickets that can be prevented by a high calcium diet. Additionally, VDR knockout mice reveal that VDR plays a role in suppression of bone formation (Fleet 2006). Functionally, vitamin D analogs dramatically increase bone mass, size and strength in

rodents (Slatopolsky et al. 2003).

The human VDR is a product of a single gene, which locates on chromosome 12 at 12q13-14 (Labuda et al. 1992). The gene is comprised of 9 exons that, together with intervening introns (Poon et al. 2004) and span approximately 63 kb. Patterns of linkage disequilibrium (LD) in the VDR gene were proposed for a Canadian population (Poon et al. 2004). Block one locates toward the 5' end that spans rougthly 8.4 kb, and block two locates toward the 3' end of VDR that spans approximately 5.8 kb.

It has been demonstrated that mutations in the VDR gene significantly alter its subcellular distribution (Barsony et al. 1997), and that subtle variations in expression and/or function of VDR may contribute to major differences in the regulation of other target genes (Eisman 1999). A genome-wide observation expects over one hundred polymorphisms present in the VDR region (Uitterlinden et al. 2002). Polymorphisms are gene sequence variations whose minimum allele frequency is higher than 1 % in the population, and are distributed throughout the entire genome (Chiba-Falek & Nussbaum 2001).

A restriction fragment length polymorphism (RFLP) recognized by T*aq*I (T/C) in exon 9 (rs731236) of VDR gene may represent the second LD block by the fact it is in strong linkage disequilibrium with other close genetic variations. This way, alleles of different polymorphisms in the same block could be linked and inherited together. Moreover, VDR T*aq*I polymorphism has been shown to have an impact on several complex diseases including prostate (Verbeek et al. 1997), breast cancer (Lundin et al. 1999), and diseases in which bone loss is a clinical sign, like osteoporosis (Horst-Sikorska et al. 2005) and periodontal disease (Sun et al. 2002; de Brito Junior et al. 2004; Brett et al. 2005).

As desirable bone healing response is required to dental osseointegrated implant success, the aim of this study was investigate, together with clinical

parameters, the association between VDR T*aq*I polymorphism (rs731236) and osseointegrated dental implant loss.

Materials and Methods

Study population

A total of 3578 patients were treated with dental implants[1] in *Instituto Latino Americano de Pesquisa e Ensino Odontológico* (ILAPEO), Curitiba - PR, from 1996 to 2006. From these, 126 patients (3.5 %) presented at least one implant loss. Out of these 126 patients, 80 subjects composed the *Study Group (S)*, once the remained patients either were death or were not found. The *Control Group (C)* was composed of 137 individuals presenting at least one osseointegrated implant in function for more than six months and without any implant failure. The study sample was matched by gender, age, and smoking habits (Table 1). A total of 217 unrelated, both gender, mean age 51.7 ± 11.3 (range 25 to 80) years were selected from the ILAPEO Dental Clinics. The patients were from the Southern region of Brazil. Patients signed a consent form within a protocol approved by an Institutional Review Board, after being advised of the nature of the study (approved by the Ethical Committee in Research at PUCPR, protocol 238/05 CEP-PUCPR).

Although the study sample was mostly composed by Caucasian, the Brazilian white population is heterogeneous. Recent articles have not recommended grouping Brazilians into ethnic groups based on color, race and geographical origin because Brazilian individuals classified as white or black have significantly overlapping genotypes, probably due to miscegenation (Parra et al. 2003). According to the Brazilian Government Census 2005, in the Brazilian Southern region, the prevalence of white is 77.8%, black, 2.2%,

[1] NEODENT™ Implante Osteointegrável

mullatto, 18.9%, and japanese, 1.1%. Reporting the white population, there is a predominance of Italian, Spanish, and Portuguese heritage (Campos et al. 2004).

Subjects answered a personal, medical and dental history anamnesis, as well as had their socioeconomic profile assessed according to Brazilian Socioeconomic Classification Criteria (ABEP 2003). Patients socioeconomic profile, general medical condition, current medication, hygiene habits, dental appointment frequency, and clinical measurements such as number of teeth, and implants placed are shown in table 2. Subjects in good general health could not have any of the following exclusion criteria: HIV infection, current pregnancy or lactation, orthodontic appliances; present necrotizing ulcerative gingivitis and periodontitis, signs of aggressive periodontitis.

Periodontal status

Measurements of probing pocket depth (PPD) and clinical attachment loss (CAL) were recorded at four points around each tooth. The following parameters were recorded: the gingival index (GI) (Loe 1967); the plaque index (PI) (Silness & Loe 1964), the calculus index (CI) (Greene & Vermillion 1964), and mobility (absent or present). The periodontal status of all subjects is shown in table 3. Periodontal index were recorded from each site using a conventional U.N.C periodontal probe[2]. All clinical data were collected by one examiner (F. A. P.).

DNA collection and purification

Cells were obtained through a mouthwash with 3 % glucose solution and scraping of the oral mucosa with a sterile spatula (Trevilatto & Line 2000). DNA was extracted from epithelial buccal cells with ammonium acetate 10 M and EDTA 1 mM (Aidar & Line 2007).

[2] Hu-Friedy - Chicago, IL, USA

VDR TaqI polymorphism

The following primer pair was used for polymerase chain reaction (PCR) amplification of genomic DNA samples: (F - 5' CAG AGC ATG GAC AGG GAG CAA G 3' and R - 5' GGA TGT ACG TCT GCA GTG TG 3'). Reaction conditions and cycling parameters were as follows: 1 µL of the genomic DNA were used for PCR amplification in a reaction mixture containing 22.5 µL PCR Supermix[3] , and 0.5 µL of each primer (25 µM). The reactions were performed in a thermal cycler[4] and consisted of an initial denaturation step of 95℃ for 5 minutes, followed by 35 cycles at 95℃ for 1 minute, 55℃ fo r 1 minute, 72℃ for 1 minute, and a final extension of 72℃ for 7 minutes. RFLP technique was performed in a final reaction volume of 20 µL, using 2 U T*aq*I (T↓CGA)[5], and 10 µL aliquot of PCR products digested at 65℃ overnig ht. The digested products were separated in 1.7 % agarose gel eletrophoresis, visualized by ethidium-bromide-UVB illumination. The genotypes were determined by comparing the restriction length polymorphism band patterns with a 1 kb plus DNA ladder[6]. The RFLP is formed by a single base transition (T/C) at codon 352 in exon 9 of the VDR gene that creates a T*aq*I restriction site. The alleles which result from the cleavage of T*aq*I are designated "C" (T*aq*I site present, with 2 fragments: 293 and 47 bp) or "T" (T*aq*I site absent, with a fragment: 340 bp).

Analysis of implant clinical parameters

A total of 1367 implants were installed in the study patients (control and study groups). Independently on the group, the placed implants were classified as healthy (H; n=1232) or lost (L; n=135).

The following clinical characteristics were accessed and compared

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[3] Invitrogen Life Technologies, Carlsbad, CA, USA

[4] Techne 7-572

[5] Invitrogen Life Technologies, Carlsbad, CA, USA

[6] Invitrogen Life Technologies

between H and L: implant position, primary stability, implant dimensions, design, type of platform, surgical technique, loading aspects, presence of graft, bone quantity and quality.

Statistical analysis

Nominal variables were expressed as frequencies and percents. To assess association between nominal variables, Chi-square (χ^2) test or Fisher's exact test was performed. Continuous variables were expressed as mean and standard deviation and Student's *t*-test was used to compare means between two groups. U-Mann Whitney was used when continuous variables presented non-normal distribution. Differences between two Kaplan-Meier curves were accessed by the Cox-Mantel test. Multivariate analysis was performed by Cox Regression model for survival time of implants and by Logistic Regression model for patients (control and study groups). A p-value < 0.05 was considered statistically significant. Statistical analysis was performed using statistical software[7].

Results

Patient clinical findings

No statistically significant differences (*NS*) were observed in social profile, general medical condition, current use of medication, hygiene habits, clinical appointment frequency, and number of present teeth. Most individuals belong to A1, A2 and B1 socioeconomic class, indicating a social status above the population average, according to government census 2005, for the two groups (Table 2).

There were significantly more edentulous patients in the control (C) group 26/137 (19%) than in the study (S) group 5/80 (6%) (p=0.009). However,

[7] SPSS 10.0 for Windows (SPSS Inc, Chicago, IL).

the average present teeth in the partially edentulous was higher in C (20.6 ± 6.0) than in S (18.56 ± 7.04) (*p*=0.054) (Table 2). The mean number of placed implants was increased in S (5.55 ± 3.57) than in C (4.51 ± 3.23) (*p*=0.013) (Table 2).

Evaluating the periodontal status, statistically significant differences (*SSD*) were only found in the probing pocket depth (PPD) [C (2.68 ± 0.41) *vs.* S (2.52 ± 0.47), *p*=0.011] (Table 3).

VDR (rs731236, TaqI) Genotyping

No significant differences in VDR T*aq*I polymorphism were observed for genotypes (*p*=0.482) and alleles (*p*=0.834) between the groups (Table 4). Considering the study SNP, the genotype distributions were consistent with the assumption of Hardy-Weinberg equilibrium. The genotype frequencies and the allele distribution for the T*aq*I polymorphism is shown in figure 1. In the same figure, genotype and allele distributions of the study polymorphism among different populations can be compared.

Using the Logistic Regression model, there was still a lack of association between T*aq*I polymorphism and implant loss, in the presence of the variables such as age, sex, smoking, diabetes, rheumatoid diseases, AINES medication, number of present teeth, PPD and tooth mobility.

Implant clinical findings

Out of the total number of placed implants (n=1367), when comparing healthy (H; n=1232) and lost (L; n=135) implants, some clinical parameters were observed to contribute to implant failure. Significant differences were found in the position of implant placement between maxilla and jaw (*p*=0.003), and between posterior and anterior region (*p*=0.037). In jaw, there was higher

prevalence of lost (85/135, 63%) than healthy implants (605/1232, 49%). Posterior region showed more losses (88/135, 65%) than healthy (682/1232, 55%) implants. Primary stability > 40 N was shown to be greater in H (652/947, 69%) than in L (49/93, 53%) (p=0.001). The mean implant diameter did not show *SSD*, but the mean implant length was greater in H (13.16 ± 2.55) than in L (12.35 ± 2.75) (p=0.001). Taken into consideration implant design, platform types, and surgical technique, it was not found *SSD* between the groups. There was no evidence of *SSD* in time to load between the groups. Implants that received load were more frequent in H (1178/1232, 96%) than in L (25/134, 19%) (p=0.001). In relation to graft presence and bone quantity/quality, *NS* was observed between H and L (Table 5).

Considering each implant independently (n=1367), a Kaplan-Meier survival curve was estimated. Fifty percent of the failures occurred before 20 weeks (range 0 to 237 weeks) (Fig. 2). Differences between two curves were assessed by Cox-Mantel test, considering the following variables: maxilla/mandible, anterior/posterior region, primary stability (>40N/≤40N), immediate load (Y/N), surgical technique (one/two steps), implant design (cylindrical/conical), graft (Y/N), bone quantity (good/poor) and quality (good/poor). Cox Regression model was used to analyze the mentioned variables together in relation to the implant survival time. Implant diameter and length were also included in the model. Primary stability (p<0.001), surgical technique (p=0.016) and bone quantity (p=0.049) were related to implant survival over time.

Discussion

Several parameters may influence implant failure such as age (Piattelli et al. 2003), gender (Mau 1993), medical condition (el Askary et al. 1999), smoking

(Jemt & Hager 2006), hygiene habits like brushing, dental floss use, and mouthwash (Botero et al. 2005), follow-up maintenance therapy (Silverstein & Kurtzman 2006), grafts (Sennerby & Roos 1998), bone quality and quantity (Kronstrom et al. 2001; Stanford 2003; Rosenberg et al. 2004), and implant design (Lee et al. 2005).

Sex, age and smoking habits were matched between control and study groups to minimize their recognized influence in the results.

In the present study, social profile, general medical condition, current use of medication, hygiene habits, clinical appointment frequency, and number of present teeth did not seem to influence implant loss.

Higher percentage of edentulism was found in pacients that did not present implant failure. The bacterial reservoir in the remaining teeth may be a risk factor for implant failure (Apse et al. 1989; Ellen 1998). In fact, a major percentage of edentulism was found in pacients that did not present implant failure. In addition, in dentate subjects it is difficult to achieve ideal mechanical position considering the aesthetic pattern of the remaining teeth. Considering only partially edentulous subjects, the number of present teeth was higher in the control group. A higher number of present teeth could lead to an improvement of anatomical conditions and partially preserves alveolar bone. This situation, together with a more favorable distribution of mastigatory forces, makes the implant procedure more predictable (Esposito et al. 1998b).

More needs for oral rehabilitation were present in the study group; thus a higher mean number of implants were installed in those patients.

Although higher plaque (PI), gingival (GI), and calculus index (CI), increased clinical attachment loss (CAL), and tooth mobility may indicate implant morbidity, they did not seem to contribute to implant loss in this study. In spite of the significance shown between the groups, the difference of 0.16 mm

in probing pocket depth (PPD) can not be considered clinically relevant.

Recently, reports have provided evidence for genetic contribution to dental implant failure (Nosaka et al. 2002; Feloutzis et al. 2003; Shimpuku et al. 2003b; a; Santos et al. 2004; Jansson et al. 2005). Single nucleotide polymorphisms (SNPs) have been shown to modulate host response and susceptibility to several diseases (Knight 2005; Lao et al. 2005; Min-Oo & Gros 2005; Matheson et al. 2006). Some SNPs have been identified in the VDR gene, but their influence on VDR protein function is still largely unknown (Uitterlinden et al. 2004). The 3' untranslated region (UTR) is known to be involved in regulation of gene expression, especially through modification of mRNA stability (Decker & Parker 1995; Durrin et al. 1999). Alleles of VDR T*aq*I polymorphism seem to be in linkage disequilibrium (LD) with 3' regulatory region containing the UTR (Morrison et al. 1992; Morrison et al. 1994).

Polymorphisms in the VDR gene have been reported to account for much of the heritable component of bone density (Yamagata et al. 1999; Sun et al. 2002). The homozygous genotype CC for T*aq*I polymorphism was associated with low bone mineral density (BMD) at both lumbar spine and femoral neck in different populations (Yamagata et al. 1999; Sun et al. 2002).

A lack of association between T*aq*I polymorphism of VDR (that represents the second LD block) and implant failure was observed by univariate and multivariate analyses. To our knowledge, this is the first study investigating the association of polymorphisms in the VDR gene with implant failure. Other polymorphisms in the VDR gene and/or in other genes of the host bone metabolism response may also be involved in the determination of susceptibility to implant failure. Recently, an independent polymorphism (rs2228570, F*ok*I) in the start codon of VDR was associated with periodontal disease (Park et al. 2006). A physical mapping considering linkage disequilibrium blocks with a

number of polymorphisms representing the whole gene could help understand the real involvement of VDR and other genes of the host bone metabolism in the determination of susceptibility to implant failure.

Concerning clinical aspects of individual implants, the jaw cortical layer generally is dense and thick and tends to become narrower and porous in the posterior region; the same occurs with the cancellous portion. Moreover, the presence of the mandibular canal limits the available bone volume in the posterior jaw region (Esposito et al. 1998b; das Neves et al. 2006). Maxilla bone topography tends to present major cancellous bone than cortical, enhancing bone healing and remodeling (Rubin & McLeod 1994; Huja et al. 2006). Primary stability has been considered adequate when higher than 40 N (Hui et al. 2001), and seems to be related to implant success prediction (Meredith 1998; Lioubavina-Hack et al. 2006), in agreement with what was observed in this study.

Increase in diameter seems to be more favorable to distribution load (Misch 1999), but could be a problem if promotes an excessive bone condensation (de Oliveira et al. 2007). Although in this study *NS* was found between the groups, some controversy exists in the literature (Lee et al. 2005). Mean implant length was greater in H. Even though a linear relationship between length and success rate has not been proven, studies have shown that shorter implants have statistically lower success rates (Winkler et al. 2000).

Implant design (cylindrical or conical) modifies compressing and stress levels to the bone. Implant design needs to fit in each particular situation to transmit adequate mechanical strength to the bone (Cehreli et al. 2004). In the study sample no interference of implant design was noted related to implant failure. A lack of association was identified for platform types, maybe by the fact that the sample is almost totally composed of early failure. In a recent

study, implant platforms were similar clinically and radiographically, but might represent an influence in future bone loss surrounding implants (Machtei et al. 2006). It was also observed no influence of one or two-step surgery on implant success, similar to what has been reported in the literature (Becker et al. 1997; Petersson et al. 2001).

The ideal time between implant placement and submission to load forces (time to load) has not been yet well established (Esposito et al. 2004a). Immediate load was more frequent in H, which reinforces that bone stimuli forces are a predictive factor for implant success (Piattelli et al. 1997). In this study, *SSD* were not found in the mean time to load between the groups either in jaw or in maxilla. Implants that received load were more frequent in H (96%). These results reflect that most failures occurred before loading (82%). After osseointegration, 18% of the implant failures occurred post loading, and only 4% in immediately loaded implants. This observation points to a host response role on the individual healing process (Kao et al. 1995; Scaglioni & Deliga 1996; Bianchi et al. 2003; Piattelli et al. 2003).

Adequate bone quantity and quality are necessary to obtain successful osseointegration (Santos et al. 2002). Insufficient bone does not permit a secure anchorage for endosteal implants. To solve this problem, various augmentation procedures are made for rehabilitation of deficient edentulous ridge (Esposito et al. 2006). Bone grafting seems to negatively influence implant success rates (Esposito et al. 1998a), but detailed outcome of this procedure is beyond the scope of this article. Graft presence and bone quantity/quality do not seem to be related to implant loss in this study.

Using Cox Regression model, this study demonstrated a relationship among three variables: primary stability, surgical technique, and bone quantity. Primary stability is augmented when adequate bone characteristics are found,

permitting an adequate bone–implant contact amount (Misch et al. 2004). A use of a desirable surgical technique augments a BIC and is followed by an increasing in primary stability (Esposito et al. 2004a). All these factors are important in the choice of the surgical approach (one or two steps).

Although some studies do provide an important contribution to the understanding of the implant failure process, yet in some situations clinical factors alone do not explain why some patients develop implant loss (Deas et al. 2002). In this context, studies that consider together clinical parameters and factors related the host response are desirable to understand the whole piece of information regarding implant failure.

Conclusions

In summary, the association of clinical parameters and T*aq*I polymorphism in the VDR gene with implant loss was investigated. Regarding clinical aspects, the following parameters were observed to influence implant failure in the univariate analysis: edentulism, implant position, primary stability, and implant length. Primary stability, type of surgery and bone quantity showed interference with implant failure in Cox Regression model analysis. No association between genotypes or alleles of T*aq*I polymorphism and implant loss was found in the study population. More studies considering other polymorphic regions of VDR gene might be performed to clarify its importance in the physiopathology of implant loss.

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Table 1. Baseline characteristics of all sampled subjects (n=217).

	Control Group (n=137)		Study Group (n=80)		
	n	%	n	%	*p* value
Ethnic Group					
Caucasoid	131	95.6	79	98.8	*0.261
Non-caucasian	6	4.4	1	1.3	
Age years†	51.64 ± 11.12		52.81 ± 11.03		**0.414
Gender					
Female	87	63.5	48	60.0	*0.664
Male	50	36.5	32	40.0	
Smoking					
Yes	25	18.2	18	22.5	*0.483
No	112	81.8	62	77.5	

†Mean±Standart Deviation;
*Chi-square test;
**Student's t-test.

Table 2. Patients' clinical findings (n=217).

	Control Group (n=137)		Study Group (n=80)		
	n	%	n	%	*p* value
Social profile					
A1/A2/B1	77	56.2	40	50.0	*0.399
B2/C/D	60	43.8	40	50.0	
General medical condition					
Systemic disease	41	29.9	23	28.8	*0.879
Diabetis	9	6.6	1	1.3	*0.096
Rheumatoid diseases	27	19.7	20	25.0	*0.395
Osteoporosis	3	2.2	1	1.3	*1.000
HAS[a]	27	19.7	21	26.3	*0.310
Cardiovascular diseases	9	6.6	8	10.0	*0.434
Hipotireoidism	12	8.8	9	11.3	*0.636
Under medical treatment					
Yes	55	40.1	37	46.3	*0.396
No	82	59.9	43	53.8	
Current medication					
No drugs use	79	57.7	44	55.0	*0.777
Antihypertension	24	17.5	18	22.5	*0.379
Antimicrobials	11	8.0	7	8.8	*0.999
AINES[b]	5	3.6	6	7.5	*0.219
AIES[c]	4	2.9	3	3.8	*0.710
Hormony Reposition	29	21.2	17	21.3	*1.000
Estatinas	6	4.4	4	5.0	*0.999
Higine Habits					
Brushing daily					
1 to 3 times	102	74.5	62	77.5	*0.744
More than 3 times	35	25.5	18	22.5	
Dental floss daily					
Yes	110	80.3	61	76.3	*0.495
No	27	19.7	19	23.8	
Mouth washing daily					
Yes	71	51.8	38	47.5	*0.575
No	66	48.2	42	52.5	
Clinical appointments[†]		6.12 ± 4.38		6.25 ± 4.76	**0.868
Clinical measurements					
Edentulous	26	19.0	5	6.3	*0.009
Presents teeth[†]		20.59 ± 6.01[††]		18.56 ± 7.04[†††]	**0.054
Placed implants[†]		4.51± 3.23		5.55 ± 3.57	**0.013

*Fisher's exact test;
**U-Mann-Whitney's test;
[†]Mean±Standart Deviation; [††]n=111, [†††]n=75;
[a]High blood pressure; [b]Anti-inflammatory nonsteroidal drug; [c]Anti-inflamatory steroidal drug.

Table 3. Periodontal status of partially edentulous patients (n=186).

Periodontal Status	Control Group (n=111)	Study Group (n=75)	*p* value
Gingival Index [†]	0.64 ± 0.38	0.65 ± 0.55	*0.343
Plaque Index [†]	0.14 ± 0.26	0.25 ± 0.42	*0.755
Calculus Index [†]	0.08 ± 0.13	0.14 ± 0.25	*0.265
PPD [a] (mm) [†]	2.68 ± 0.41	2.52 ± 0.47	**0.011
CAL [b] (mm) [†]	3.61 ± 0.76	3.66 ± 1.10	**0.723
Mobility (absence/presence)	98/13	60/15	[‡]0.145

[a]Probing pocket depth; [b]Clinical attachment level;
[†]Mean±Standart Deviation;
*U-Mann-Whitney's test;
**Student's t-test;
[‡]Fisher's exact test.

Table 4. Genotype and allele frequencies of T*aq*I polymorphism (rs731236).

	Control Group (n=137)		**Study Group (n=80)**		
	n	%	n	%	*p* value
Genotypes					
T T	63	46.0	33	41.3	
T C	54	39.4	38	47.5	*0.482
C C	20	14.6	9	11.3	
Alleles[†]					
T	180	65.7	107	66.9	**0.834
C	94	34.3	53	33.1	

*Chi-square test;
**Fisher's exact test;
[†]Control Group n=274, Study Group n=160.

Table 5. Clinical findings (n=1367) for all implants.

	Healthy Implants (n=1232)		Lost Implants (n=135)		
	n	%	n	%	*p* value
Position					
Maxilla	627	50.9	50	37.5	*0.003
Madible	605	49.1	85	62.5	
Anterior region	550	44.6	47	35.3	*0.037
Posterial region	682	55.4	88	64.7	
Primary stability					
Good (>40)	652	68.8	49	52.7	*0.001
Poor (≤40)	295	31.2	44	47.3	
Dimention					
Diameter†	3.99 ± 0.41 (1232)		4.03 ± 0.45 (135)		‡0.348
Length†	13.16 ± 2.55 (1232)		12.35 ± 2.75 (135)		‡0.001
Design					
Cylinder	231	18.8	24	17.8	*0.783
Conical	1001	81.3	111	82.2	
Plataform					
Internal Hex	353	28.7	37	27.4	
External Hex	832	67.7	95	70.4	*0.536
GT¥	18	1.5	0	0.0	
CM¥	27	2.2	3	2.2	
Surgical technique					
One-step surgery	456	46.2	44	40.4	*0.250
Two-step surgery	532	53.8	65	59.6	
Loading Time					
Immediate load					
Yes	150	12.2	9	6.7	*0.056
No	1075	87.8	126	93.3	
Time to load (weeks)					
Maxilla†	43.39 ± 37.08 (608)		43.93 ± 52.79 (10)		‡‡0.964
Mandible†	40.50 ± 46.88 (570)		29.44 ± 56.07 (15)		‡‡0.370
Load					
Load implants	1178	95.6	25	18.7	*0.001
Unload implants	54	4.4	109	81.3	
Graft Procedure					
Yes	235	19.1	22	16.3	*0.433
No	997	80.9	113	83.7	
Bone quantity					
Good (II/III)	1096	89.0	123	91.1	*0.445
Poor (I/IV)	136	11.0	12	8.9	
Bone quality					
Good (B/C)	1092	88.6	118	87.4	*0.671
Poor (A/D)	140	11.4	17	12.6	

†Mean±Standart Deviation (n);
*Chi-square test;
‡Student's t-test;
‡‡ U-Mann-Whitney's test;
¥CM and GT platforms were considering together for the test.

Figure 1. Genotype/alelle frequencies of VDR T*aq*I polymorphism in different populations.

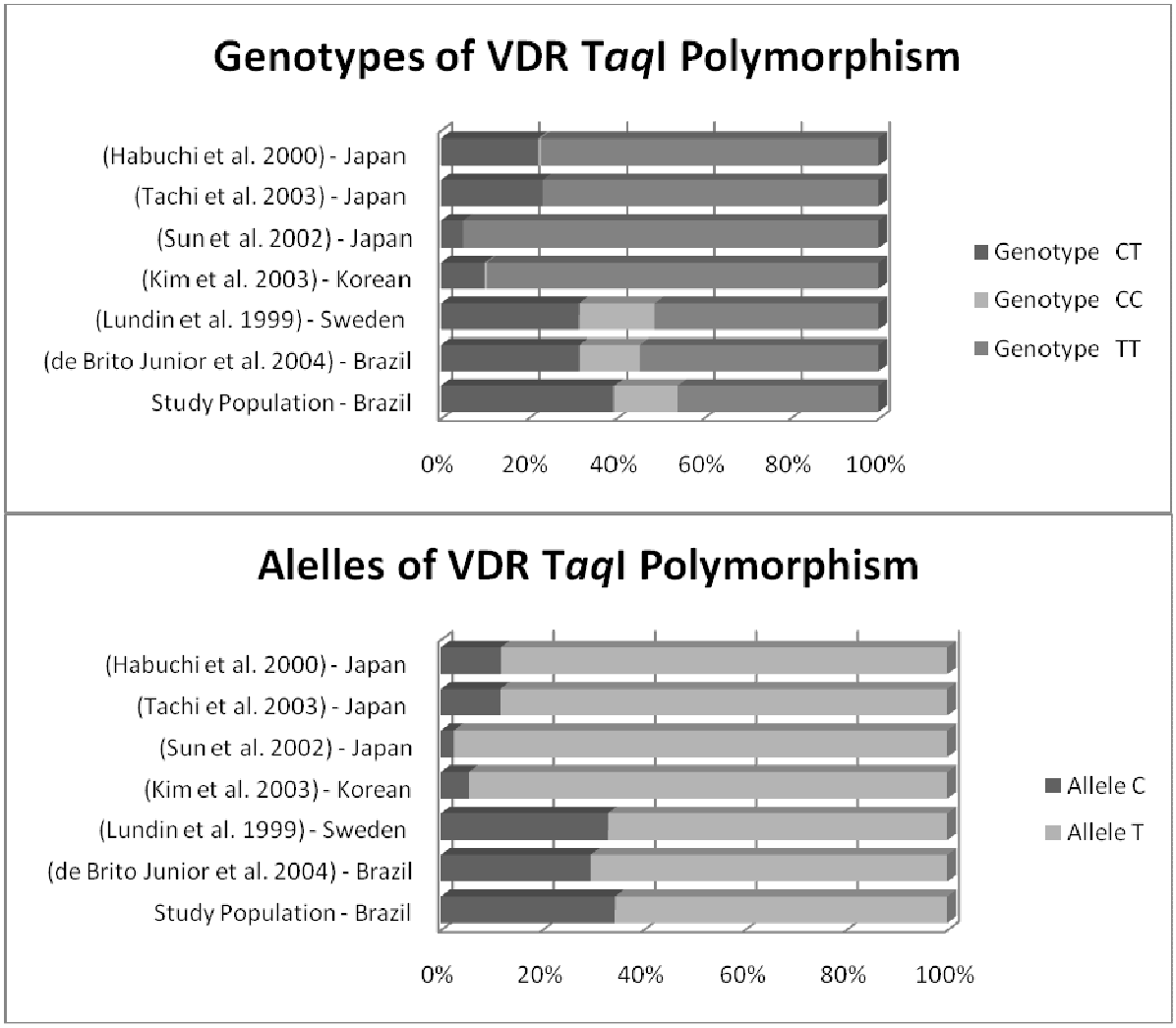
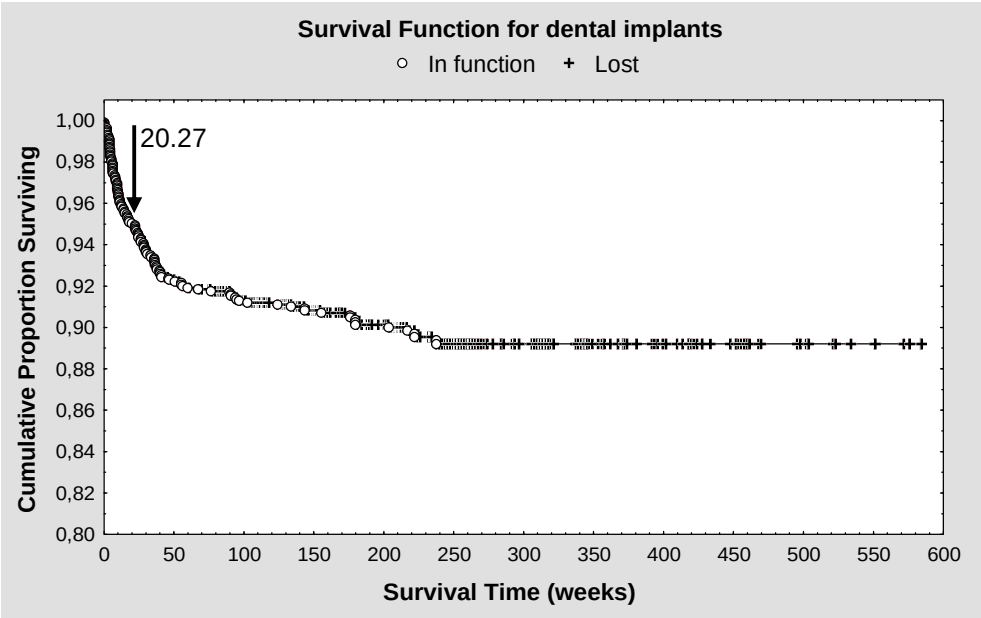


Figure 2. Kaplan-Meier curve showing cumulative proportion of implant survival over time.



CONCLUSÃO

5. CONCLUSÃO

i) Até o momento, a abordagem genética utilizada em estudos considerando falhas de implantes dentais osseointegráveis foi a análise de polimorfismos em genes candidatos. Nesse âmbito, o *scan* genômico caso-controle pode ser uma ferramenta genética promissora, pela capacidade de análise de polimorfismos genéticos em larga escala.

ii) Os seguintes parâmetros clínicos estiveram associados à perda de implantes dentais osseointegráveis: edentulismo, profundidade de bolsa, posição do implante, estabilidade primária, comprimento do implante, quantidade de osso e técnica cirúrgica.

iii) Alelos e genótipos do polimorfismo T*aq*I no gene do VDR (rs731236) não estiveram associados à perda de implantes dentais osseointegráveis.

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5. REFERÊNCIAS

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ANEXO

7. *ANEXO*

Outros artigos relacionados à tese, submetidos à publicação:

Failing Factors Associated with Osseointegrated Dental Implant Loss

Claudia Cristina Montes, * Fabiano Alvim Pereira, DDS, * Geninho Thomé, DDS, MS, † Edson Durval Menezes Alves, DDS, MS, † Rogéria Vieira Acedo, DDS, † José Renato de Souza, DDS, MS, † Ana Cláudia Moreira Melo, DDS, PhD, † Paula Cristina Trevilatto, DDS, PhD*

Analysis of the Relationship between *IL1B* (+3954) and *IL1RN* (intron 2) Polymorphism and Osseointegrated Implant Failure in a Caucasian Brazilian Population

Montes C.C., Alvim-Pereira F., Thomé G., Menezes-Alves E.D., Olandoski M.,Trevilatto P.C.

Failing Factors Associated with Osseointegrated Dental Implant Loss

Claudia Cristina Montes, * Fabiano Alvim Pereira, DDS, * Geninho Thomé, DDS, MS, † Edson Durval Menezes Alves, DDS, MS, † Rogéria Vieira Acedo, DDS, † José Renato de Souza, DDS, MS, † Ana Cláudia Moreira Melo, DDS, PhD, † Paula Cristina Trevilatto, DDS, PhD*

ABSTRACT

Dental implants are currently the esthetic and functional alternative of choice for tooth absence. Despite the high success rate shown by longitudinal studies, failures do occur, even in patients who present appropriate clinical conditions. The aim of the present study was to identify factors related to or determinant of dental implant loss in patients of the Latin-American Dental Research Institute (ILAPEO), Curitiba, PR. Retrospective analysis of 3,578 records of patients who had placed implants in this institute during the period of 1996 to 2006 was performed. Beyond records, panoramic and periapical radiographies were analyzed. Out of 3,578 individuals implant treated, failures occurred in 126 (3.5%) patients (mean age 52.2 ± 10.6 years). Men lost more implants (4.5%) than women (3.1%) (p=0.05). Early failure represented the majority of failure cases (88.2%). The main detectable causes of implant loss were evaluated. Most implant losses (75%) did not present apparent clinical cause. Identified causes were: 17.5% iatrogenic conditions (surgical technique, contamination and/or occlusal trauma); poor bone quality and quantity (3%); peri-implantitis (1%), and 3.5% were misregistrations. Failure was more frequent when the implant was installed in the posterior jaw. The results obtained in this study suggest that host factors can be contributing for the implant failure.

Analysis of the Relationship between *IL1B* (+3954) and *IL1RN* (intron 2) Polymorphism and Osseointegrated Implant Failure in a Caucasian Brazilian Population

Montes C.C., Alvim-Pereira F., Thomé G., Menezes-Alves E.D., Olandoski M.,Trevilatto P.C.

ABSTRACT Dental implants have become an important therapeutical modality for tooth replacement. Although the high success rate showed in longitudinal studies, failures occur, even in patients who present appropriate clinical conditions. Together with the clusterization phenomenon, which is the presence of multiple implant losses in the same group of individuals, this fact suggests that host response aspects may influence the implant failure process. However, little is known about the influence of genetic susceptibility on implant loss. IL-1*β* and IL-1ra are believed to play a key role in immunological reactions and inflammation. Polymorphisms *IL1B* (C+3954T) *and IL1RN* (intron 2 – 86 bp variable number of tandem repeats) are evidenced to alter the expression of the coding proteins. Higher levels of IL-1*β* were found in diseased implant sites, which could be a molecular indicator of implant failure. The aim of this study was investigate the association between failure of dental implants and the functional polymorphisms *IL1B* (+3954) *and IL1RN* (intron 2). The study population (n=266) was obtained from the patient pool treated in the Latin American Dental Research Institute (ILAPEO), and divided into: *Test group (T)* - 90 subjects with implant loss, and *Control group (C)* - 176 subjects with at least one implant in function for at least six months. The patients were matched by sex, age and smoking status. Patients' socioeconomic profile, general medical condition, hygiene parameters, and clinical measurements such as number of teeth and periodontal status were analyzed. Genomic DNA from oral mucosa was amplified by polymerase chain reaction (PCR). The fragments were submitted to agarose gel electrophoresis, followed by ethidium-bromide staining method. Polymorphisms *IL1B* (+3954) and *IL1RN* (intron 2) were in Hardy-Weinberg equilibrium. Regarding clinical aspects, the following parameters were observed to influence implant failure: edentulism, probing pocket depth, and number of present teeth. No differences in genotype distribution and allele frequencies between C and T groups were found for *IL1B* (+3954) and *IL1RN* (intron 2) polymorphisms in the study population. It was concluded that clinical parameters, but not the study polymorphisms, were associated to implant loss.