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Heterogeneity of Human Serum Antibody Responses to *P. gingivalis* in Periodontitis: Effects of Age, Race/Ethnicity, and Sex

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Abstract

Aging humans display an increased prevalence and severity of periodontitis, although the mechanisms underlying these findings remain poorly understood. This report examined antigenic diversity of *P. gingivalis* related to disease presence and patient demographics. Serum IgG antibody to *P. gingivalis* strains ATCC33277, FDC381, W50 (ATCC53978), W83, A7A1–28 (ATCC53977) and A7436 was measured in 426 participants [periodontally healthy (n=61), gingivitis (N=66) or various levels of periodontitis (N=299)]. We hypothesized that antigenic diversity in *P. gingivalis* could contribute to a lack of "immunity" in the chronic infections of periodontal disease. Across the strains, the antibody levels in the oldest age group were lower than in the youngest groups, and severe periodontitis patients did not show higher antibody with aging. While 80% of the periodontitis patients in any age group showed an elevated response to at least one of the *P. gingivalis* strains, the patterns of individual responses in the older group were also substantially different than the other age groups. Significantly greater numbers of older patients showed strain-specific antibody profiles to only 1 strain. The findings support that *P. gingivalis* may demonstrate antigenic diversity/drift within patients and could be one factor to help explain the inefficiency/ineffectiveness of the adaptive immune response in managing the infection.

Keywords

antibody; aging; periodontitis; antigenic drift; adaptive immunity

1.1 INTRODUCTION

Alterations in both innate and adaptive immunity have been universally observed in aging populations, and have led to the concept of such terms as "immune aging" or "immunosenescence" to reflect the deteriorating nature of the immune system [1]. Adaptive immune responses have been consistently reported to be adversely affected by aging, and

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present as increased incidence and severity of a wide array of infectious diseases, as well as the prevalence of development of various cancers [2–5]. Recent reports document the parallel loss of capacity and regulation of normal innate and adaptive immune response with aging that effectively alter immunocompetence and promote the pathogenesis of this diversity of diseases [6, 7]. Nevertheless, the actual cause(s) of immunosenescence and "inflammaging" remain to be established [7, 8].

Aging has been documented to display an increased prevalence and severity of periodontitis, although the underlying causes remain poorly understood. Epidemiological data supports demographic differences in rates of periodontitis the disuse prevalence increased in males, older subjects, and in racial/ethnic minority populations [9]. Among biologically plausible mechanisms for these findings, both innate immune and adaptive immune cells isolated from aged individuals exhibit intrinsic defects that could predispose the elderly to dysregulated immune and inflammatory responses underpinning the exacerbated clinical features of disease with aging [10]. With our current level of knowledge, the true significance and function of the adaptive immune response under normal circumstances remains to be elucidated, let alone how aging specifically modulates the effectiveness of these responses.

The humoral adaptive immune response clearly changes from gingival health to periodontal disease and these responses vary with progressing disease and post-therapy [11, 12]. Measurement of serum IgG antibody from patients with chronic adult periodontitis [13–15] versus healthy controls showed the most consistent elevation to *P. gingivalis* [16–18]. However, the conundrum of existing data is why there appears to be a coincidence of chronic oral infection with accompanying periods of exacerbated disease and an active, often substantial specific local and systemic immune response in periodontitis [11, 19]. Nevertheless, various studies supported the potential for the elevated serum antibody to *P. gingivalis* and other oral pathogens to lower the burden of the overall microbial biofilm challenge or minimize emergence of pathogens and maintain their colonization levels below a threshold that is necessary to induce destructive processes [20–22].

However, phenotypic changes in bacteria are paralleled by the dramatic ability of certain pathogens to alter the surface characteristics that they present to their environment(s). Variation in antigenic composition of pathogens generally comes in two forms, antigenic "variation" (shift) and "diversity" (drift). Antigenic variation is a specific strategy which requires the ability to abruptly replace one type of surface antigen with another. It generally requires multiple nonallelic genes for surface proteins and usually some form of gene rearrangement [23–25]. The antigenic differences within species are termed antigenic diversity (drift) and are often related to surface structures which are exposed to the defense mechanisms of the host [26–31]. Antigenic diversity occurs by the gradual accumulation of mutations in genes that code for the targets of the immune system which in Gram-negative bacteria are frequently the outer membrane components. These mutations result in considerable population heterogeneity and a drift towards selection of an antigenic type that is stable in the particular host-parasite interaction. As such, it has been suggested that lack of protective immunity to reinfection is due to the host immune response being limited to antigens on existing strain(s). This lack of clear "immunity" in periodontal disease may be related to antigenic drift and diversity in the oral opportunistic pathogens that chronically

colonize and are subject to host immune pressure, leading to a selective advantage that accompanies antigenic alterations of the bacteria within the subgingival ecology.

As importantly, description of *P. gingivalis* antigens and measurement of host responses to *P. gingivalis* have essentially only used either type strains or human isolates that have been cultivated over extended time intervals [13, 15, 32–36]. Thus, a critical concept addressed in this report is based upon *P. gingivalis* representing a chronic opportunistic infection of susceptible hosts. Implied in this concept is that characteristics (eg. antigenic diversity/drift) of *P. gingivalis* and/or the susceptible host must be favorable for the maintenance of this colonization and emergence of the pathogen to a threshold level that contributes to eliciting disease symptoms. Thus, the aim of the study was to document the existence of antigenic diversity in *P. gingivalis* that could contribute to a lack of "immunity" in the chronic infections of periodontal disease.

2.1 MATERIALS & METHODS

2.1.1 Samples

The demographics of the biorepository serum samples from 426 patients are depicted in Table 1. Healthy subjects were defined by <10% of sites with bleeding on probing (BOP) ≥1 [37], <5% sites with probing pocket depth (PPD) ≥4mm, and no sites with PPD ≥5mm. Gingivitis was defined by >10% sites with BOP ≥1, <10% sites with PPD ≥4mm, and no sites with PPD ≥5mm. Periodontitis was defined by >10% BOP ≥1, ≥10% of sites with PPD ≥4 mm, and ≥3% sites with clinical attachment loss (CAL) >2 mm. Periodontitis was categorized into mild (mean PPD <3 mm), moderate (mean PPD 3–4 mm), and severe (mean PPD >4 mm) [38, 39]. Inclusion/exclusion criteria for the subjects have been reported previously [40–44].

Sites demonstrating BOP ≥1 and pocket depth ≥5 mm were defined as disease sites and sampled as described previously from 124 participants within the larger cohort (Dawson et al. 2009). All plaque specimens were stored at −80°C and assayed within six months of collection. Bacterial plaque samples in sterile PBS were pelleted, DNA was extracted, and quantified as described previously [45]. Real-time PCR was performed using a LightCycler 2.0. and quantification analysis was performed using LightCycler 4.0 software using sample "crossing points" (CP) to determine the presence and the concentration of the target DNA in known and unknown samples after amplification. DNA isolated from a pure culture of *P. gingivalis* ATCC 33277 was used in generating a standard curve for the universal primers [46, 47]. Quantification of the individual target bacteria and total bacteria from the experimental samples were calculated using the standard curves.

2.1.2 Bacterial cultivation, antigens, and analyses

The strains of *P. gingivalis* included ATCC33277 [48, 49] originally obtained from the ATCC, FDC381 [48, 50], W50 [ATCC53978; https://www.ncbi.nlm.nih.gov/biosample/SAMN00792205/ [48]], and W83 [48, 51], were originally obtained from Dr. A. Tanner (The Forsyth Institute, Boston, MA). A7A1–28 [ATCC53977; [48, 52]] and A7436 [48, 53] were obtained from Dr. Anne Progulske-Fox (University of Florida), and all have been

maintained in our laboratory for over 20 years. The bacteria were cultured in brain heart infusion (Becton Dickinson and Company, Sparks, MD) medium supplemented with 5 μg hemin ml^{-1} and 1 μg menadione ml^{-1} under anaerobic conditions (85% N_2, 10% H2, 5% CO_2) at 37°C [54]. For whole bacterial antigens, the bacteria were harvested by centrifugation, washed with phosphate-buffered saline, and formalin-fixed to detect serum IgG antibody levels using an ELISA [35]. The quantification of the antibody was based upon comparison of antibody levels in the experimental samples to standard curves using purified human IgG incorporated into each plate [55]. All samples were tested in triplicate and re-evaluated if the coefficient of variation was >15%. Interplate comparability was provided by analysis of the standard IgG curves based on determining no statistical difference in the slopes of the curves and reactivity of maximum IgG concentration.

2.1.3 Statistical analysis

Analyses of any differences among inflammatory mediators and IgG antibody levels, was conducted via a Kruskal-Wallis ANOVA with *post hoc* testing of paired groups using a Dunn's method (SigmaStat, Systat Software, Inc., Richmond, CA). Evaluation of the significance of correlation data was performed using the Spearman Correlation test. Results with an alpha of <0.05 (after being adjusted for the multiple comparisons) were accepted as statistically significant. Significant differences in the slopes of the standard curves was determined using the Real Statistics add in for Excel (http://www.real-statistics.com/regression/hypothesis-testing-significance-regresson-line-slope/).

3.1 RESULTS

3.1.1 Characteristics of serum antibody to *P. gingivalis* strains with aging

Serum IgG antibody response levels reacting with various strains of *P. gingivalis* were determined in the population stratified based upon age. This included subjects with periodontitis, as well as in healthy and gingivitis subjects (Fig. 1A–C). First, irrespective of age, antibody levels to all of the strains were low in the health and gingivitis patients with little variation across the strains. Generally, the youngest group of periodontitis patients demonstrated somewhat higher antibody compared to other age groups (increased by 30–260%) to all of the strains, with those individuals exhibiting severe periodontitis having the highest levels. Additionally, the antibody levels to the two more recent clinical isolates (A7A1–28, A7436; [48]) were elevated (increased by 30–200%) in this group compared to any of the more historical laboratory strains. As was noted with the younger group, responses in the 36–50 year old subset were higher to the recent clinical isolates, although the severe periodontitis patients did not show higher antibody. The pattern of responses in the oldest group (>50 yo) was substantially different than the other age groups. First, antibody levels to 2 of the older strains (33277, 381; [48]) were as high as the recent clinical isolates. Also, the levels to these isolates were significantly lower in mild periodontitis patients. Antibody to the older laboratory isolates that were not originally obtained from periodontitis lesions (W50, W83; [48]), were significantly lower than to all other strains. Finally, antibody in the oldest group to the recent clinical isolates was significantly lower in t e severe periodontitis patients. In addition to stratifying the population into specific age groups that provided insights into differences in antibody responses to the various *P.*

gingivalis strains, a correlation analysis was performed to examine the direct relationship of age to antibody response levels (Table 2). In the overall population, generally the antibody levels were negatively correlated with age to each of the strains, consistent with the stratification results; however, within the individual age groups, few statistically significant values were identified.

We also explored the antigenic conservation among the *P. gingivalis* strains when cultivated *in vitro* as antigens. In this study, 3 strains were cultivated on 3 separate occasions approximately 1 month apart under similar conditions. They were used to prepare formalinized bacterial antigens and then sera from 3 patients with varied antibody levels to the strains were examined for the reproducibility of the antibody reactions. As shown in Supplemental Fig. 1, the level of IgG antibody in each serum was reasonably consistent with generally <15% CV suggesting that the antigens displayed on the strains prepared on different occasions were generally stable. Thus, differences in strain reactivities of different samples suggested fundamental antigenic differences across the *P. gingivalis* strains, and supported substantial differences in the *P. gingivalis* antigenic repertoire in patients inducing significantly different antibody responses profiles.

Exploring the characteristics of the population responses to the individual strains showed interesting distributions of elevated antibody to specific strains. Fig. 2A shows that the overall antibody responses to the array of *P. gingivalis* strains was significantly greater in the younger and middle-age group compared to the oldest patients. Fig. 2B demonstrates the results examining the level of response to each strain in individual patients. Antibody levels were stratified into quartiles for each strain across the population. These quartiles were identified for each patient and summarized as the percentage of patients with antibody in the top quartile to 3 or more strains, 2 strains, only 1 strain, and to none of the strains tested. The results demonstrated a significantly different distribution of strain-specific antibody with the oldest patient group showing significantly fewer patients with high levels of antibody to more than 1 strain and nearly 60% having antibody in the top quartile of the population to none of these strains. Additionally, we addressed the strain characteristics of those patients, in whom antibody was highly elevated to only 1 strain. This included 67/297 patients (22.64%) and identified some patients primarily responsive to one of the 6 strains with over 40% of the patients with an elevated response to strain W50 (Fig. 2C).

Finally, the clinical presentation of the patients related to stratification based upon top quartile antibody levels across the *P. gingivalis* strains is presented. No differences were observed in the clinical parameters of mean probing pocket depth or clinical attachment level in the younger or middle-age group (data not shown). However, we observed that in the oldest group of participants (Fig. 2D) the samples with elevated antibody to 1 or none of the strains tended to have lower clinical disease measures than subjects with elevated antibody to multiple *P. gingivalis* strains.

Table 2 provides a summary of correlation analyses across the periodontitis population of antibody to each of the *P. gingivalis* strains. The youngest group of subjects demonstrated significant correlations of the antibody particularly reflected in the recent clinical isolates. In contrast, these relationships were lost in the oldest group, suggesting some alterations in the

antibody repertoire in the oldest patients, in which certain antigenic specificities may have been lost.

3.1.2 Race/ethnicity and aging effects on *P. gingivalis* antibody patterns

Existing studies have generally not integrated aging into evaluation of the characteristics of antibody responses to oral bacteria related to race/ethnicity. Fig. 3A–B displays the results in categorizing the periodontitis patients into various racial/ethnic groups and stratifying according to age to assess antibody to the various *P. gingivalis* strains. Older Caucasian patients had decreased levels of antibody to all of the *P. gingivalis* strains, except the A7A1–28 strain. The Black oldest patient group showed elevated antibody to the 33277 strain, and low antibody to both W50 and W83. Hispanic patients tended to have similar levels of antibody to the various strains across the age groups, albeit the response to W83 was significantly lower in the oldest Hispanic group. Also, a substantial increase in antibody level to the A7436 strain was observed in the middle-age Hispanic group. Finally, the notable pattern in the Asian patients was elevated antibody to multiple strains in the younger group, and significantly decreased antibody to both the W50 and A7436 strains in the oldest group.

3.1.3 Sex and aging effects on *P. gingivalis* antibody patterns

While sex differences have been reported with periodontal disease, the biologic underpinnings of this observation remain to be determined. We examined antibody responses to *P. gingivalis* strains with the periodontitis subjects dichotomized on sex to determine if differences in response characteristics could provide any insight into this clinical observation. No significant differences in antibody to any of the strains based on sex were found (data not shown). However, grouping the patients based on age did reveal some sex differences across the strains. Generally little difference with age or sex was seen in antibody levels to 33277, 381, and A7A1–28 strains. Both female and male oldest patients showed significantly decreased antibody to W50, W83 and A7436 (Supplemental Fig. 2).

3.1.4 Smoking and aging effects on *P. gingivalis* antibody patterns

We also explored the potential impact of smoking with age in the distribution of antibody responses to determine if the antibody diversity appeared to be adversely affected by smoking. Supplemental Fig. 3 demonstrates a rather minimal impact on the antibody levels across the strains in periodontitis subjects under 50 years of age. However, while the extent and severity of periodontitis is significantly increased with aging [56], antibody to 4 of the 6 strains was significantly decreased in the oldest smoking subset of the population.

3.1.5 Antibody diversity to *P. gingivalis* strains

Fig. 4A–C provides examples of the strain specificity of the serum antibody as it related to patient age. The figure includes the antibody distribution to the 6 strains in 10 patients from each age group who demonstrated the highest overall antibody responses as a summation of antibody to all of the strains. While this analysis was carried out for all patients, only the top 10 are depicted, which generally reflects the profile variations within the age grouping. First of note is the difference in the level of antibody to individual strains within the patients.

Across all age categories some patients clearly have a predisposition for responding to antigens that are expressed on one strain (*eg.* ID 36, 360, 351, 359, 211, 323), while others show a very limited response to certain individual strains (*eg.* ID 169, 354, 4, 151, 84, 123). While the middle age group showed this similar type of variation, a rather striking feature was the dominance of antibody to the more recent clinical isolates in most of these patients (70%). In the oldest group, it is first notable that the summation of antibodies to these strains is generally lower than the other age groups. Also noted was that in many of the patients, antibody levels to the recent clinical isolates (A7A1–28, A7436) were much less than to the older laboratory strains. The exceptions were patients 129 and 323, whose major responses were to antigens presented by the more recent clinical isolates. A similar patient specific analysis is presented in Fig. 5A–C stratifying the subjects based upon race/ethnicity. Generally, the individual subject level responses in the top 10 sum of antibody responses are lower across the *P. gingivalis* strains in the Hispanic population; however 2 of the individuals (ID 203, 211) showed robust responses to this group of strains. The Caucasian and Black subjects showed the greatest frequency of elevated responses to strain ATCC 33277 and A7A1–28 with Hispanics dominating in responses to strains 381 and A7A1–28. At the individual level, the Caucasian and Black groups showed principal responses to A7A1–28 and A7436 in 50–60% of the patients. In contrast, the dominant responses in 40–50% of Hispanic subjects were to strains A7A1–28 and ATCC 33277. Ranking the level of responses to the various *P. gingivalis* strains across the racial/ethnic background of individual subjects also provided an interesting comparison. As shown in the figure, the distribution of dominant versus lowest strain responses in the Hispanics was significantly different (Caucasian: X^2=45.895, df=5, p<0.0001; Black: X^2=83.086, df=5, p<0.0001), while no difference was noted between the Caucasian and Black subgroups.

3.1.6 Antibody diversity to *P. gingivalis* strains and *P. gingivalis* colonization

The study design also allowed us to explore the relationship between the level of *P. gingivalis* in subgingival plaque from a subse, of the periodontitis patients and the distribution of antibody levels to the various *P. gingivalis* strains. Supplemental Figure 4 summarizes the relationship and demonstrates decreasing antibody levels to all strains except 381 with decreased levels of *P. gingivalis* colonization. Similarly, Supplementary Table 1 provides an estimate of the correlation of the antibody levels across the population and within different age categories to *P. gingivalis* colonization. The results showed a rather limited number of significant correlations of the levels of *P. gingivalis* and antibody to and individual *P. gingivalis* strain.

4.1 DISCUSSION

This report developed data from an extensive cross-sectional analysis demonstrating substantial variations in antigenic makeup of various *P. gingivalis* strains, as reflected by the variation in host antibody reactivity to the strains. Moreover, we demonstrated that antibody to the *P. gingivalis* strains was decreased with aging, and the results reflected not simply a decrease in antibody levels, but a seemingly more limited repertoire of antibody to the range of *P. gingivalis* strains. A conundrum of observations with certain chronic infections is why the pathogen/infection can remain associated with the host in the presence of an acquired

immune response. While this is a feature of many chronic infections, explanations of the continued infection have often been linked to novel intracellular strategies that protect the pathogen from the immune biomolecules/cells, low antigenicity eliciting an ineffective immune response in quantity or quality, structural/chemical characteristics of the pathogen which minimize the impact of immune components, and alterations of the pathogens' antigens, presenting the host immune apparatus an ever changing target and limiting the effectiveness of the responses [57–65]. This last facet of pathogen strategy has been described as antigenic variation, antigenic drift, and antigenic diversity depending upon the pathogenic species [23–28, 30, 66–70].

While the literature has recognized for decades a robust immune (antibody) response to periodontal pathogens in subsets of the affected populations, it also describes clear exacerbations and remissions from active, progressing disease, generally related to the presence of the same species of pathogen [19, 20, 71–73]. This report presents data suggesting the *P. gingivalis* has the ability to alter expression of potentially critical antigens that are recognized by the host and may be important in resulting protection or susceptibility to future disease episodes. Description of diversity of *P. gingivalis* antigens has been rather limited, generally reflected by reports considering antigenic differences in the capsular polysaccharide across various strains [66, 68, 74–77]. Additionally, these capsular antigenic types have been described to have some limited representation in an individual patient, albeit, no reports exist describing longitudinal changes in the capsule antigen type with disease exacerbations [78–80].

Historically, over nearly 4 decades, *P. gingivalis* has been identified as a consistent hallmark microorganism in the microbial biofilms of periodontitis lesions [71, 81–83]. It has been shown to increase in proportion, albeit remaining at an overall low percentage, in the biofilms that transition from health to disease [84, 85]. Importantly, published findings support a wide clonal genetic diversity of *P. gingivalis* strains, and with 19 strains having been sequenced, genome sizes vary by up to 200,000 base pairs, and predicted protein coding genes range from 1,774 to 2,392. Interestingly, Chen et al. [48] just recently published an extensive evaluation of the genomic diversity of *P. gingivalis*, and provided a phylogenetic tree of the existing strains that have been sequenced. A critical component of this analysis was also that across the 19 strains, nearly ½ of the protein coding genes showed sequence heterogeneity with 2000–3000 predicted protein coding genes showing distribution in only one of the these strains. While this report also identified selected proteins from this list that were unique in the various strains, lacking was: (1) any assessment of the impact on antigenic aspects of these genomic differences, and (2) understanding of how these genomic differences could be expressed within a patient overtime, since these strains were individual isolates obtained a one point in time from 19 different patients.

Our results extend these findings by demonstrating either a unique antigenic portfolio expressed by various *P. gingivalis* strains (likely, based on Chen et al. [48]) or similar antigenic composition across all the strains with variations in individual responses that are host genetically regulated [20, 86–88]. While the latter of these options is possible, based upon the variation we noted across this population and the relationship to age, race/ethnicity, and sex, it seems less likely. Importantly, the very unique antibody response patterns that

were noted within individual patients to the array of *P. gingivalis* strains support the likelihood of antigenic diversity of *P. gingivalis*. In a cross-sectional study as this, the data cannot discriminate whether this reflects the strains that originally colonized the individual patient, or antigenic differences arising through genomic alterations that reflect the host-bacterial interactions and immune pressure that would occur with chronic colonization by this opportunistic pathogen. Additionally, within this framework, consideration of genomic modulation within a patient through episodes of disease exacerbations that could explain the novel response patterns remains to be determined.

Beyond this circumstantial evidence antigenic diversity or drift, it was clear that the response repertoire to the *P. gingivalis* strains was affected by aging, more substantially than race/ethnicity or sex. This is an important observation, since clear epidemiologic evidence supports that racial/ethnic minorities [89–91] and males [9, 92] exhibit an elevated prevalence and severity of periodontitis with aging. Nevertheless, novel differences in population antibody levels to the various *P. gingivalis* strains were noted across the racial/ethnic distribution of the population. The findings with somewhat distinctive response patterns in Caucasian, Black, Hispanic, and Asian populations to the various *P. gingivalis* strains may be a reflection of the different interactions of this pathogen with the host immune system within these racial/ethnic groups and could contribute to the variations in expression of periodontitis that has been identified in certain populations [9, 91]. The results suggest that *P. gingivalis* with different antigenic composition may colonize different groups of individuals, resulting in a more pathogenic relationship due to a more limited antibody response, or features of the genetic control of the antibody response repertoire that is affected to a greater degree by aging related to the race/ethnicity of the individual. Sorting out this relationship and understanding if this feature is being driven by targeted antigenic changes in the pathogen, or altered capacity of the host immune system to control the chronic infection is an important facet of future approaches to improved precision in periodontal diagnosis and therapy [73, 93, 94].

We have reported previously that females with periodontitis showed higher levels of antibody to *P. gingivalis* than males affected by this disease [95]. However, those data were limited to examination of responses to only *P. gingivalis* strain 33277. This study expanded the characterization of responses in the disease population related to sex of the individual. Across most strains, older males tended to show lower antibody levels, except to strain A7A1–28. Moreover, comparison of antibody levels between males and females within each strain showed elevated antibody in females to 33277, 381 and W50, with similar antibody levels to strains W83, A7A1–28 and A7436. Generally, the differences were driven by the oldest group of females and males. Epidemiological evidence also clearly documents substantial adverse effects of smoking on many health parameters, including periodontitis [9, 88]. However, a similar analysis of our data related to smoking and age demonstrated a limited impact of strain response differences that only occurred in the oldest periodontitis group to some of the strains. Thus, it is not clear that antigenic diversity of *P. gingivalis* is particularly pronounced related to sex and smoking, compared to the effects noted with age and race/ethnicity.

Finally, we obtained an initial evaluation of the relationship of *P. gingivalis* colonization at disease sites and antibody responses to individual *P. gingivalis* strains. At a population level it did appear that antibody to 5 of the 6 strains decreased with lower proportions of *P. gingivalis* in the subgingival plaque. This type of information has been reported previously, albeit generally those studies only used one *P. gingivalis* strain as the antigen detection system [96–98]. A more patient focused approach showed a rather limited number of correlations between the proportion of *P. gingivalis* and antibody levels of the individual strains. Moreover, we specifically examined this correlation focusing on the individual patient's predominant response across the strains and found few additional relationships (data not shown). These results reinforced the likelihood of more individualized responses to an antigenic repertoire of homologous *P. gingivalis* isolates with each patient, and inferred that further studies linking patient specific isolates parallel with the specific responses to these isolates would confirm antigenic heterogeneity across the disease population and confirming diversity overtime as contributing to the disease process.

5.1 CONCLUSIONS

Our findings support that *P. gingivalis* may, in fact, demonstrate antigenic drift within patients and could be one factor to help explain the inefficiency/ineffectiveness of the adaptive immune response in managing the infection and allowing new antigenic phenotypes to emerge in the biofilms resulting in initiation and progression of disease episodes. This option would also elucidate potential biologic variations of more frequent/rapid occurrences of sufficient antigenic drift that contributes to variations in onset and severity of disease, as suggested by clinical terminology of "rapidly progressive periodontitis" and "refractory periodontitis". These clinical descriptions could incorporate curtailed development of the antibody repertoire having the capacity to recognize this antigenic drift, as an important basis that enables *P. gingivalis* to express its pathogenic properties across decades within individual patients. The results supported a loss of antibody breadth with aging in the periodontitis patients. This could be interpreted as a loss of the fundamental aspects of the B cell repertoire in response to *P. gingivalis* with aging. This type of response alteration has been suggested to occur in other studies of immunity in aging, but data is lacking with responses to chronic antigenic challenge, such as to members of the oral microbiome. Nevertheless a limitation of this study is the lack of a detailed assessment of age effects via isolation of *P. gingivalis* from individual subjects and critical determination of within subject antibody specificity to their, homologous isolates.

A striking component of periodontitis is the extent of variation across the population in age of onset, rate of progression, and extent/severity of the disease. While there remains an age association with these clinical measures, there is minimal capacity to predict any of these at the individual patient level. Moreover, as a chronic disease that is clinically managed, the explanation for the variation in frequency of episodes of disease overtime, generally related to a similar profile of oral pathogen(s) [99] remains enigmatic. These findings support a novel concept regarding aging impacts on the quality of adaptive immune responses to specific oral pathogens and the interplay between antigenic drift of the pathogen, and basis for episodic disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Study of antigenic diversity of *P. gingivalis* in periodontal disease.
- Antibody levels in the oldest age group were lower than in the youngest groups.
- The older group had fewer with high levels of antibody to more than 1 strain.
- *P. gingivalis* may demonstrate antigenic diversity/drift with these chronic infections.

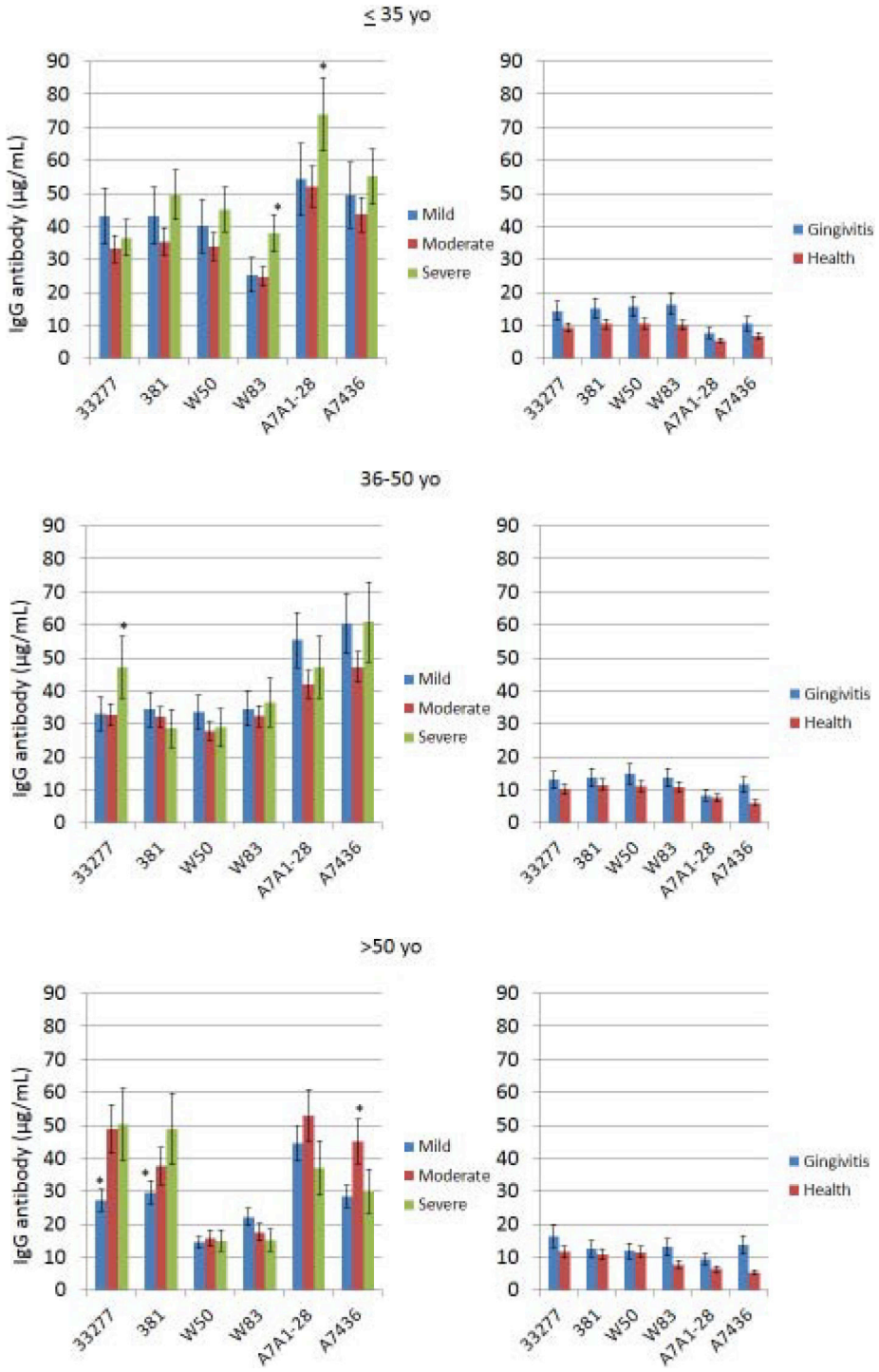


Figure 1A-C:

Serum antibody levels to individual *P. gingivalis* strains in patients in different age categories stratified based upon periodontitis severity. The bars denote group means and the vertical brackets enclose 1 SD. The asterisk (*) denotes statistically different from other disease groups at least at $p<0.05$.

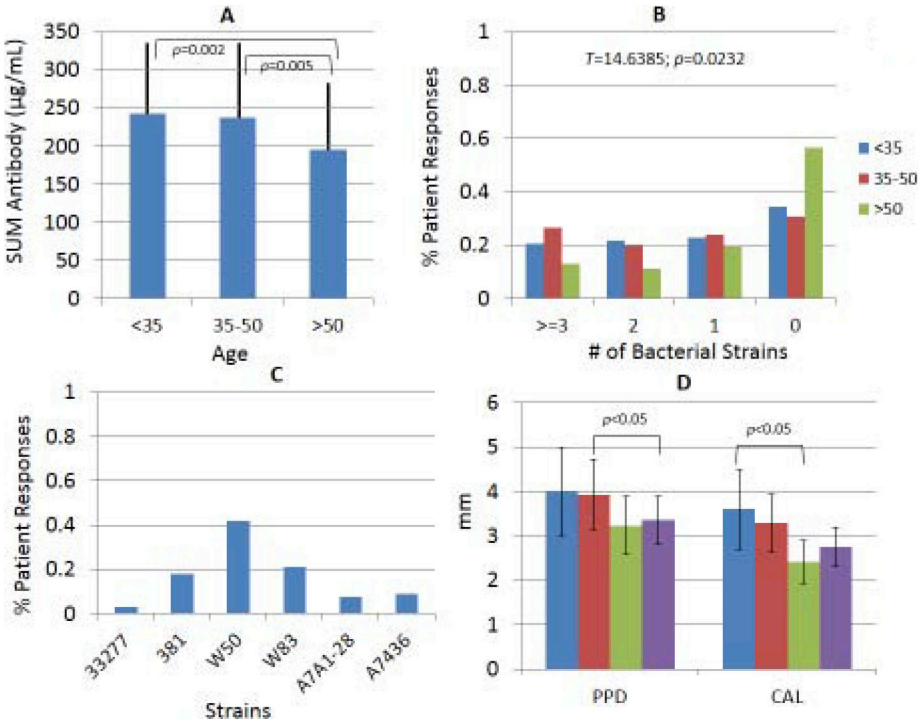


Figure 2A-D:

(A) The overall antibody level to all 6 strains within the different age groups of periodontitis patients. The bars denote the group means and the vertical line is 1 SD. **(B)** Distribution of antibody reactivities in different age groups in which each patient's sample was determined to be in the top tertile of antibody of the entire population to each strain. The oldest group had significantly fewer patients with antibody responses in the top tertile across all the *P. gingivalis* strains. **(C)** Depiction of the *%* of patients whose antibody levels were in the top tertile of only 1 strain. **(D)** Relationship of patients with antibody levels in the top tertile to 0, 1, 2, or ≥3 strains. The bars denote the group means of probing pocket depth and clinical attachment level within the response categories. The vertical brackets denote 1 SD.

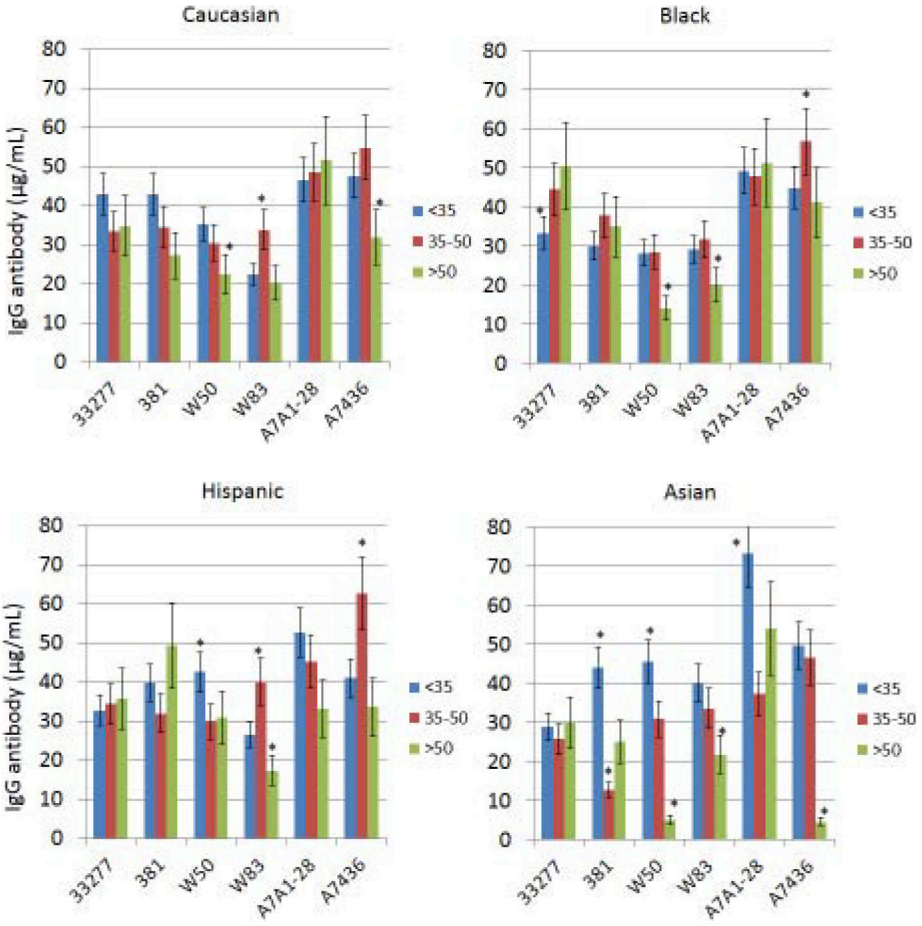
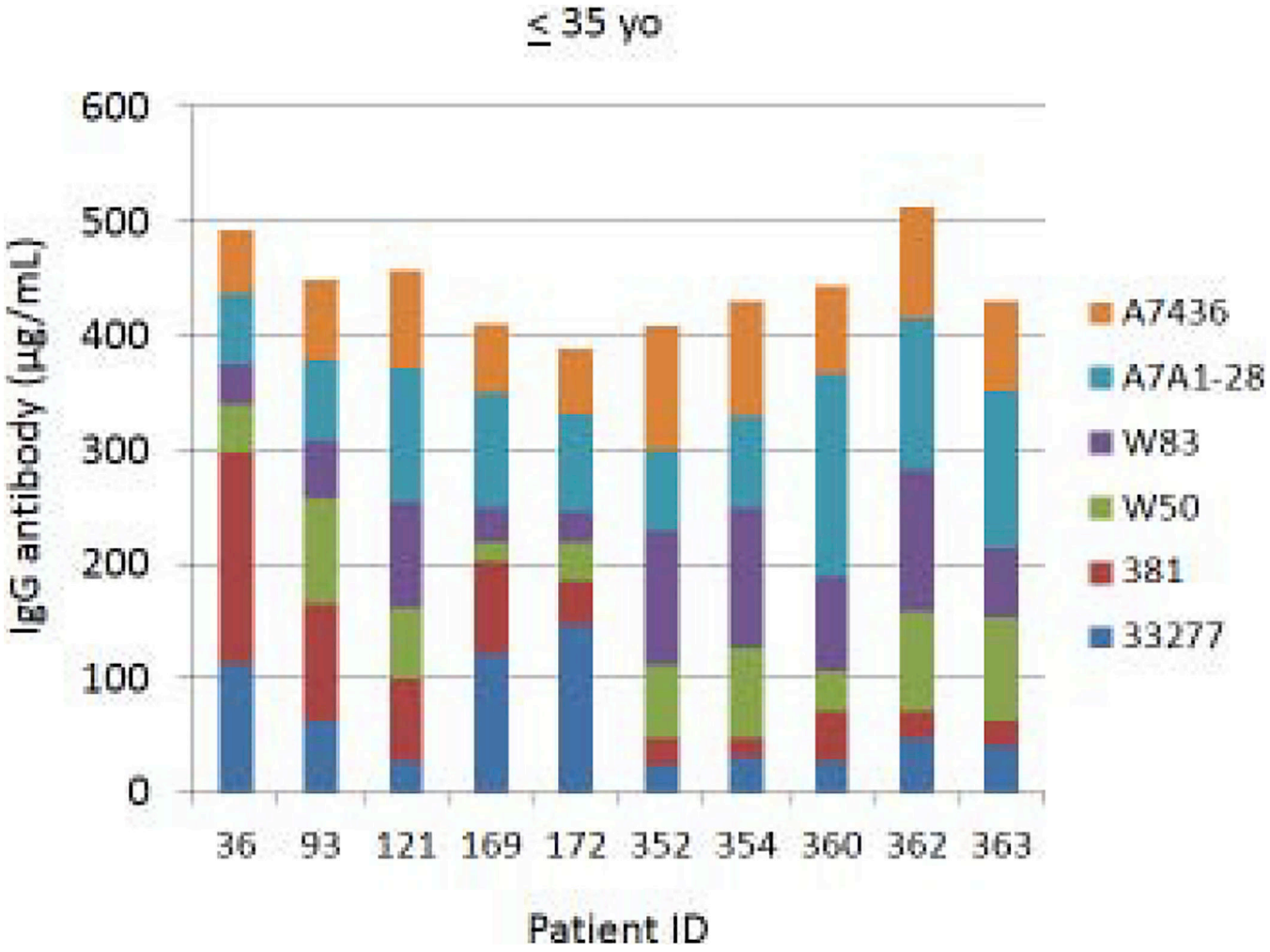
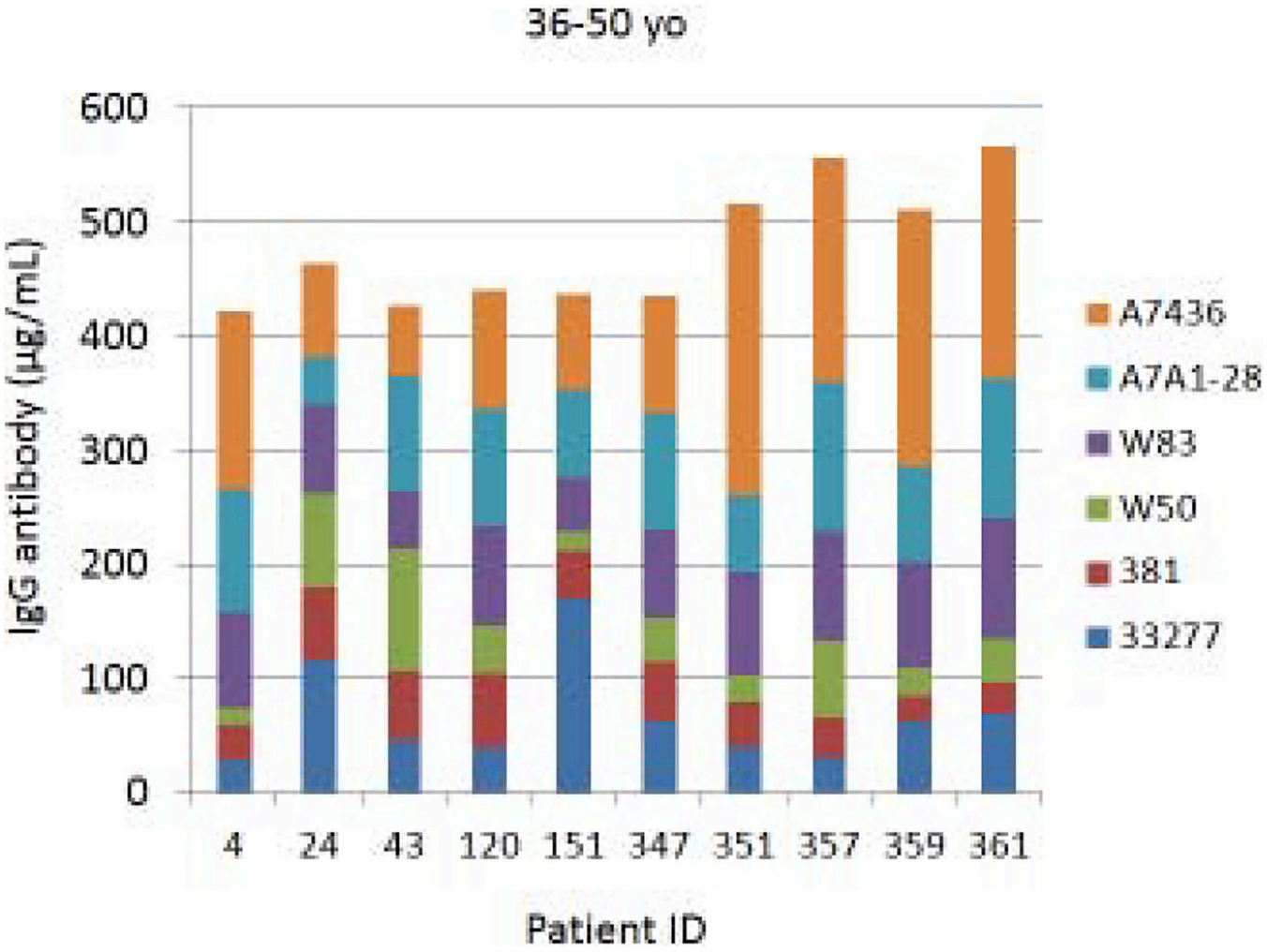


Figure 3A-B:

Levels of serum antibody to the *P. gingivalis* strains (Pg277 – 33277, PgA7 – A7A1–28, Pg7436 – A7436) with patients categorized into racial/ethnic groups and stratified by age. The bars denote group means and the vertical brackets enclose 1 SD. The asterisk (*) denotes statistically different from other age groups at least at $p<0.05$.





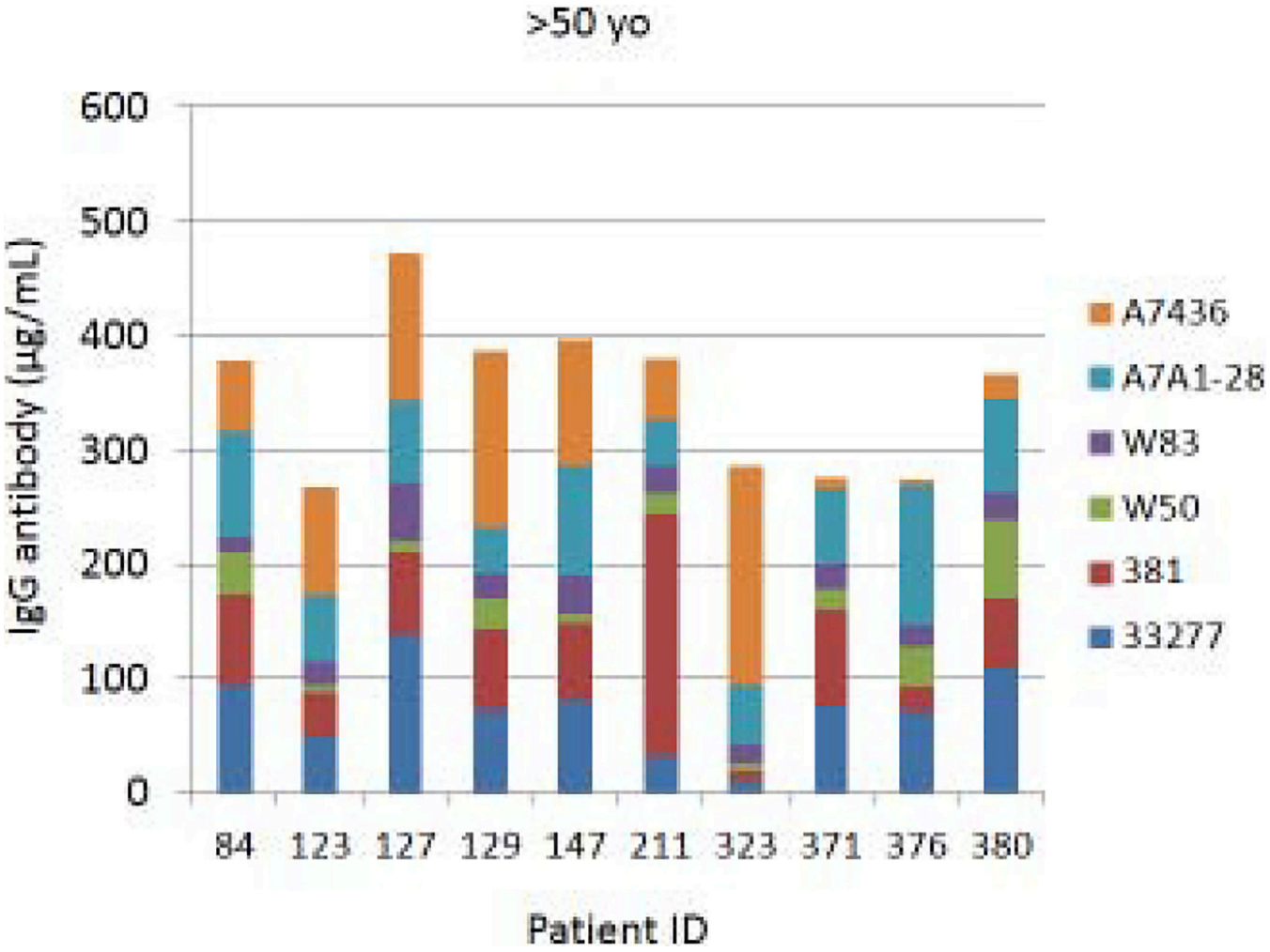
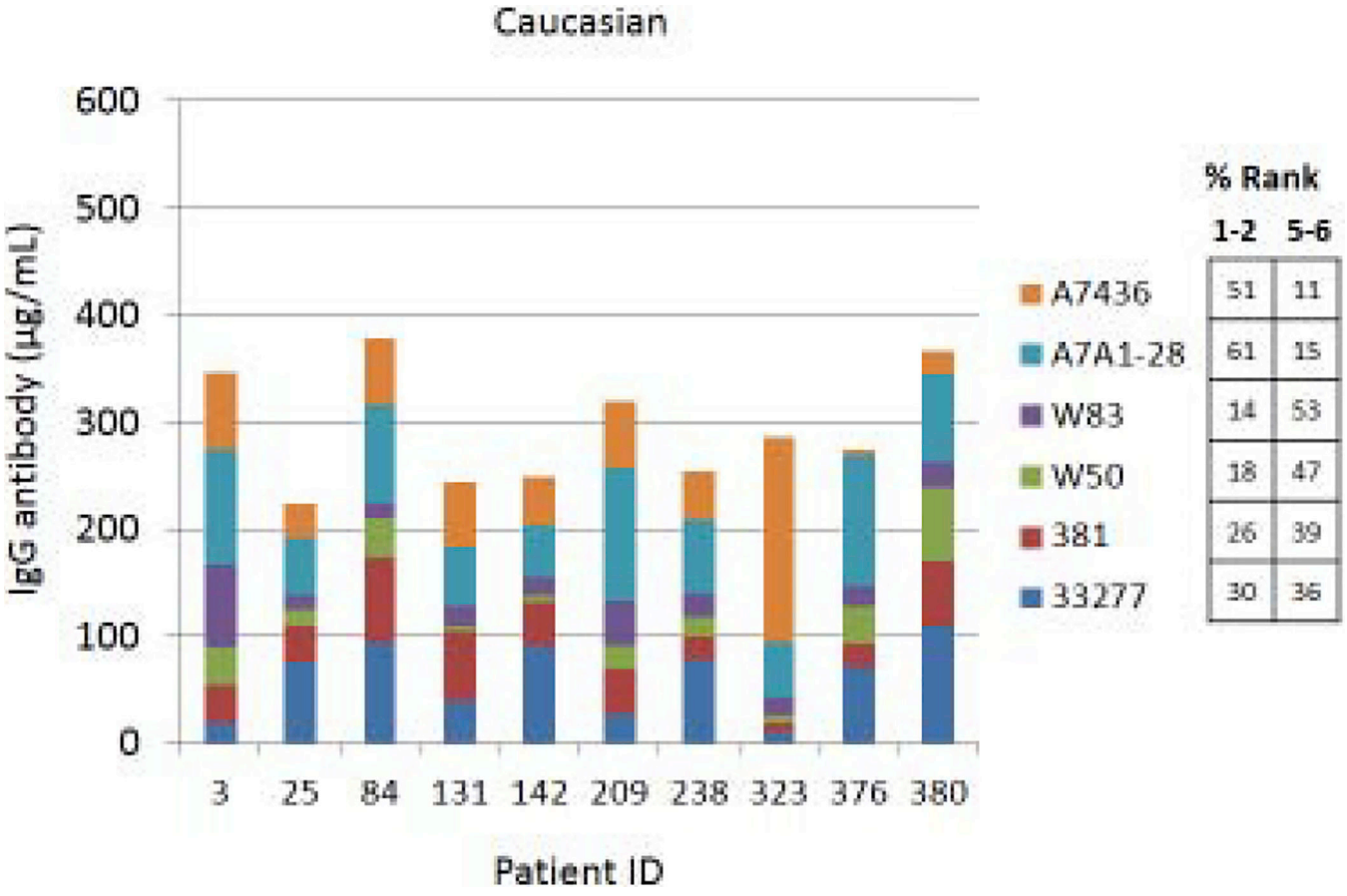
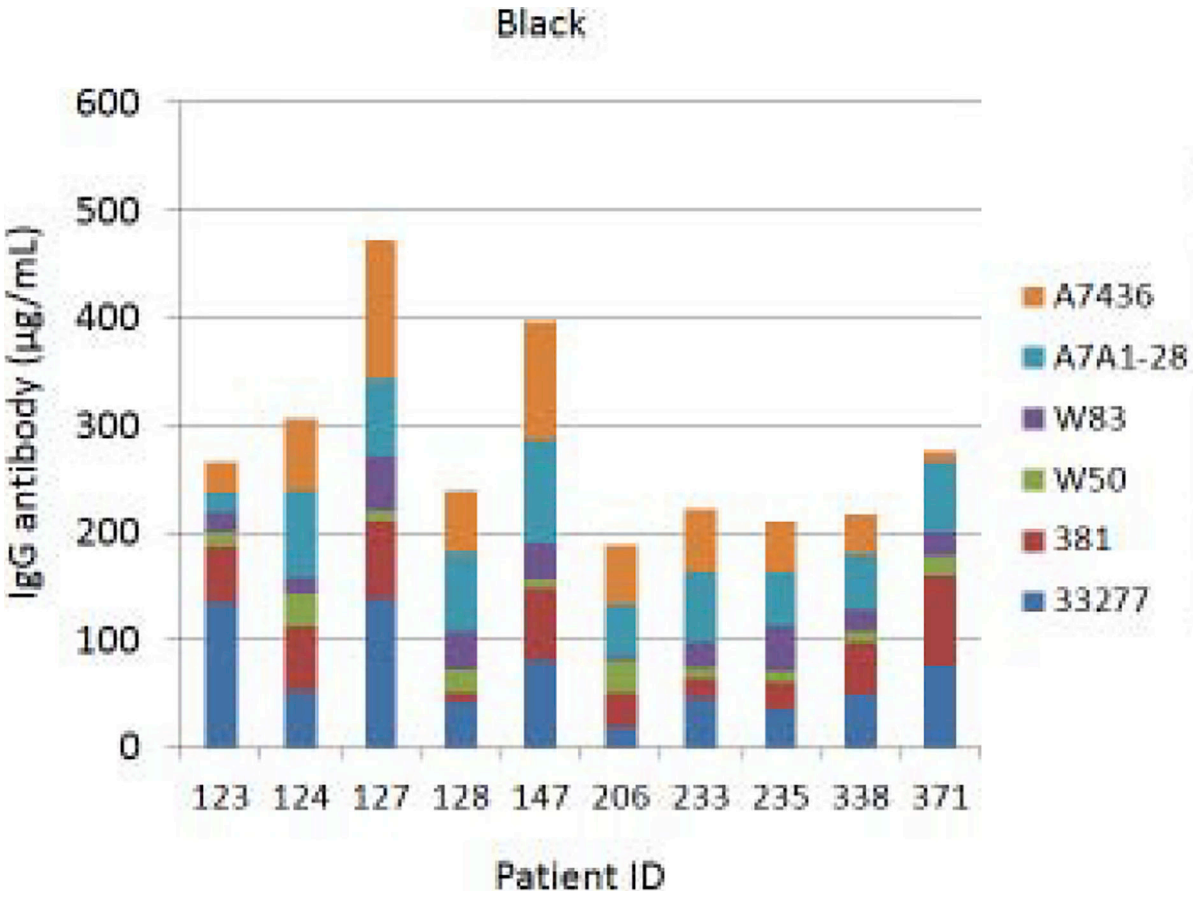


Figure 4A-C:
Serum antibody levels to the 6 *P. gingivalis* strains in individual patients based upon age category. The top 10 patients with summed antibody to all the strains are presented in each age grouping.





% Rank	
1-2	5-6
62	14
51	16
14	52
13	57
23	32
39	29

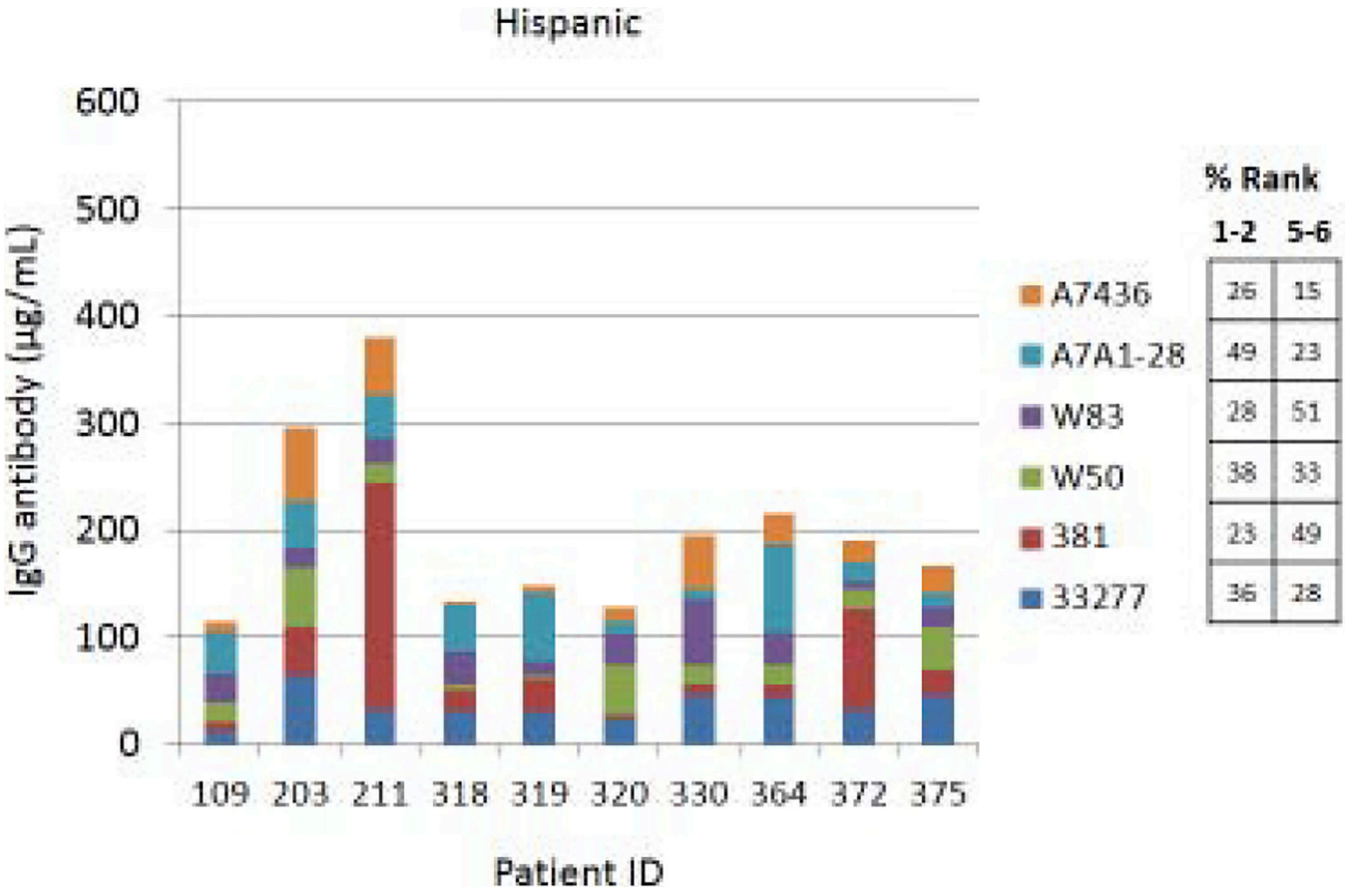


Figure 5A-C:

Serum antibody levels to the 6 *P. gingivalis* strains in individual patients based upon race/ethnicity category. The top 10 patients with summed antibody to all the strains are presented in each grouping. The table describes the frequency distribution across all subjects within the racial/ethnic category, with strain responses ranked 1 through 6 for each individual. The numbers denotes percentage of subjects with strain ranked 1 or 2 (highest level) versus the percentage ranked 5 or 6 (lowest level).

Table 1:

Demographics of population

Group	Age Mean (range)	Age Group (Y:M:O) %	Gender M:F	Race/Ethnicity (C:B:H:A) %	% Smokers	% Sites BOP Mean (range)	Mean PPD mm (range)	% Sites PPD ≥4 mm (range)	% Sites PPD ≥5 mm (range)
Health (n=61)	39.5 (21–65)	43:36:21	26:35	66:12:23:0	8.2	1.2 (0–2.9)	2.1 (1.7–2.7)	1.8 (0–3.2)	0
Gingivitis (n=66)	36.9 (24–60)	45:41:14	30:36	65:12:18:5	19. ‘	27.2 (9.3–71.4)	2.2 (1.6–2.3)	4.4 (1.9–8.1)	0
Periodontitis/Total (299)	40.4 (21–74)	30:54:16	187:112	51:21:20:8	28.1	41.1 (5.4–100)	3.5 (2.2–5.9)	29.2 (1.1–86.8)	13.6 (0–67.3)
Mild (n=85)	39.1 (22–74)	48:44:8	40:45	72:13:9:8	18.8	21.1 (10.1–78.6)	2.8 (2.2–3.0)	15.6 (1.1–86.8)	3.1 (0–13.7)
Moderate (n=152)	41.0 (21–62)	18:66:16	94:58	48:21:22:9	27.0	43.0 (5.4–96.2)	3.5 (3.0–4.0)	29.2 (2.4–84.3)	12.0 (0–40.5)
Severe (n=62)	40.8 (23–66)	34:37:29	45:17	29:36:30:5	43.5	62.8 (12.9–100)	4.5 (4.0–5.9)	47.2 (3.2–81.3)	31.6 (2.3–67.3)

Age group identified as Y=≤35; M=36–50; O=>50 years of age

Race/ethnicity identified as Caucasian:Black:Hispanic:Asian

Table 2:

Correlation analysis of antibody responses with age to the various *P. gingivalis* strains. Correlation coefficients in bold are significantly correlated at least at $p<0.05$ using a Spearman Rank analysis. SUM denotes correlation of age and antibody as a summation of levels to all strains in each subject.

Group (yrs.)	33277	381	W50	W83	A7A1–28	A7436	SUM
Total	0.0292	**−0.1469**	**−0.2730**	−0.0134	−0.0312	**−0.1186**	**−0.1382**
≤35	0.0756	−0.1007	−0.0194	0.0741	−0.0372	−0.1728	−0.0530
36–50	0.0339	−0.0748	−0.0439	0.0075	**−0.1795**	−0.1194	−0.1187
>50	−0.0357	0.0343	**0.2773**	0.1104	0.1421	−0.0584	0.0762

Table 3:

Correlation analysis of antibody responses in different age groups to antigens presented by the various *P. gingivalis* strains. Correlation coefficients in bold are significantly correlated at least at $p<0.05$ using a Spearman Rank analysis.

Age Group	Pg Strain	Pg277	Pg381	PgW50	PgW83	PgA7	Pg7436
≤35 years	33277	1	**0.466**	−0.101	0.055	0.161	0.244
	381		1	0.090	0.052	0.080	0.197
	W50			1	**0.420**	**0.298**	**0.412**
	W83				1	**0.472**	**0.748**
	A7A1–28					1	**0.668**
	A7436						1
36–50 years	33277	1	**0.383**	0.084	0.207	0.192	**0.292**
	381		1	0.131	**0.320**	0.160	**0.256**
	W50			1	0.209	−0.002	0.098
	W83				1	**0.292**	**0.566**
	A7A1–28					1	**0.491**
	A7436						1
>50 years	33277	1	0.307	0.324	**0.411**	**0.431**	0.234
	381		1	0.158	0.180	0.064	0.200
	W50			1	0.023	0.356	−0.056
	W83				1	0.327	0.208
	A7A1–28					1	0.169
	A7436						1