Concordance of Porphyromonas gingivalis Colonization in Families

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Received 17 September 1996/Accepted 12 November 1996

Periodontitis is a widespread disease that appears to be due to a specific bacterial infection. Several species of bacteria have been investigated as potential pathogens, and particularly strong evidence links the presence of Porphyromonas gingivalis with indicators of periodontitis. Information concerning the transmission of P. gingivalis between human contacts may be important in determining risk factors for disease and developing preventive strategies. A few small studies have provided some evidence of transmission between related individuals, but no large-scale study of families that would reflect the typical transmission of this pathogen in the population has been reported. The purpose of this study was to investigate the transmission of P. gingivalis within randomly selected, extended families. The colonization status of 564 members of multigeneration families was determined, and the degree of concordance observed among members of these families was then compared to that expected to occur based on the prevalence of colonization in the population studied. A PCR assay was used for detection of P. gingivalis. Concordance in colonization was more frequently observed within entire families (P = 0.0000) and for spouses (P < 0.001), children and their mothers (P < 0.001), children and their fathers (P < 0.01), adults and their mothers (P < 0.005), and siblings (P < 0.05) than would be expected if P. gingivalis were randomly distributed in the population studied. Results showed that contact with an infected family member substantially increased the relative risk of colonization in these intrafamilial pairs. This indicates that P. gingivalis is commonly transmitted by contact with an infected family member.

Periodontitis is a widespread and often slowly progressing disease that is difficult to detect in its early stages. Evidence of moderate periodontitis occurs in 40% of the population over 12 years of age, and moderate periodontal destruction can be detected in more than 80% of the population over 65 years of age (3). The etiology of periodontitis appears to be a specific bacterial infection, with the immunologic response of the host playing a role in the tissue destruction (15). Several of the hundreds of bacterial species found in the oral cavity have been investigated as potential pathogens. There is particularly strong evidence linking Porphyromonas gingivalis with indicators of periodontitis, such as deeper pockets and attachment loss (7, 14, 22, 26), and in a longitudinal trial, P. gingivalis has been demonstrated to be a risk factor for periodontitis (2).

Information concerning the transmission of P. gingivalis between human contacts and the long-term periodontal health of colonized individuals is important for determination of risk factors for disease and for development of preventive strategies. Routes and frequency of transmission have not been defined, although a few small studies have provided some evidence of transmission between related individuals. In a study of Indonesian families, a significant relationship for colonization among siblings has been observed (23). In another study, concordance of colonization appeared to increase for spouse pairs when one spouse had periodontitis, but the sample size was too small to permit statistical analysis (25). Cases in which strains have been identified within families have also been reported. Isolates of P. gingivalis detected in four families of children with unusual forms of periodontitis were shown to match within those families, suggesting that transmission had occurred (18). In case studies of persons with severe periodontitis, some spouses were found to have identical restriction types (24) or identical ribotypes (21) of P. gingivalis. Cumulatively, these studies suggest that intrafamilial transmission of P. gingivalis can occur, at least with exposure to individuals with the severest forms of periodontitis. However, no large-scale study of randomly selected families that would reflect the typical transmission of this pathogen in the population has been reported.

The purpose of this study was to investigate the transmission of P. gingivalis within extended families. The colonization status of members of multigeneration families was determined, and the degree of concordance observed among members of these families was then compared to that expected to occur based on the prevalence of colonization in the population. A PCR assay was used for detection of P. gingivalis (9). This assay provided the necessary sensitivity for detection of the low levels of bacteria that might be present in children and the efficiency needed to analyze the large number of samples required. A significantly higher concordance of colonization was found among extended families and between spouses, children and their parents, adults and their parents, and siblings than would be expected if P. gingivalis were randomly distributed in the population studied.

MATERIALS AND METHODS

Study population. The study population consisted of 104 extended families recruited from church and community organizations in Columbus, Ohio. Each family unit contained a minimum of a parent and a child, and most (n = 101)
BacteriawereelutedfromthepaperpointsandDNAwasisolatedandpurified.

PCR-basedassaythatdidnotrequirethatthesamplesbecultured.Briefly,regionlocatedbetweenthe16Sand23SrRNAgeneswasfirstamplifiedwith


RESULTS

A total of 564 subjects from 104 families were examined for the presence of P.gingivalis. Results from one family are shown in Fig. 2. Four of the five members of that family were positive for P.gingivalis, while one child was negative. The distribution of family members among generations and by gender is shown in Fig. 1. The study population included 301 females (53.4%) and 263 males (46.6%). P.gingivalis was detected in 39% of females and 35% of males. The difference was not significant by chi-square analysis. The ages ranged from 0.7 to 95.4 years, with a mean of 37.5 (standard deviation, 24.0) years. The racial composition was 98.2% (n = 554) white and 1.8% (n = 10) African-American. P.gingivalis was detected in 37% of white subjects and 40% of black subjects. The sample size for black subjects was too small to allow statistical comparisons based on race. The overall prevalence of P.gingivalis was 37.1%. Among the four generations examined, the prevalence ranged from 50% in the great grandparents to 27.1% among children (Fig. 1). Children were significantly less frequently colonized than
their grandparents, but no statistically significant difference was observed between either children and their parents or parents and grandparents (Fig. 3).

To examine patterns of transmission within families, concordance of colonization was determined for extended families, as well as between pairs of individuals within these families. Table 1 shows the results of a comparison of the observed concordance of colonization for spouses, siblings, children and their parents, and adults and their parents to the expected concordance based on the prevalence in the population. Figure 4 shows a matrix for colonization and the relative risk of colonization with 95% confidence intervals for these pairs.

Husbands and wives were highly significantly more frequently concordant in colonization than would be expected if *P. gingivalis* were randomly distributed in the study population (Table 1). Individuals whose spouses were colonized were 3.78 times more likely to be colonized than those married to persons who were not colonized (Fig. 4A). No relationship was observed between the length of time a couple had been married and their concordance of colonization ($P = 0.61$ by logistic regression). The average durations of marriage were 14.1 years for the parent generation and 39.8 for the grandparent generation.

Concordance in colonization status between siblings was examined. The oldest and second oldest children were significantly more frequently concordant in colonization than would be expected if *P. gingivalis* were randomly distributed among the children in the study (Table 1). The relative risk of colonization is shown in Fig. 4B.

**Table 1. Chi-square test results for observed and expected concordances of colonization of pairs within families**

<table>
<thead>
<tr>
<th>Pair</th>
<th>No. of pairs</th>
<th>% Concordance</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental spouses</td>
<td>102</td>
<td>78</td>
<td>51</td>
</tr>
<tr>
<td>Grandparental spouses</td>
<td>62</td>
<td>73</td>
<td>51</td>
</tr>
<tr>
<td>Oldest, 2nd oldest children</td>
<td>63</td>
<td>71</td>
<td>59</td>
</tr>
<tr>
<td>Mother, oldest child</td>
<td>103</td>
<td>72</td>
<td>54</td>
</tr>
<tr>
<td>Father, oldest child</td>
<td>101</td>
<td>67</td>
<td>54</td>
</tr>
<tr>
<td>Adults, their mothers</td>
<td>98</td>
<td>67</td>
<td>51</td>
</tr>
<tr>
<td>Adults, their fathers</td>
<td>69</td>
<td>64</td>
<td>53</td>
</tr>
</tbody>
</table>

*Calculated based on the prevalence of *P. gingivalis* in the group being considered.

**Table 2. Colonization status of oldest children relative to that of their parents**

<table>
<thead>
<tr>
<th>No. of parents colonized</th>
<th>No. of children colonized (%)</th>
<th>Relative risk of colonization (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 (0%)</td>
<td>0.00 (0.00-0.00)</td>
</tr>
<tr>
<td>1</td>
<td>6/22 (27.3%)</td>
<td>3.3 (1.57-7.15)</td>
</tr>
<tr>
<td>2</td>
<td>15/51 (29.4%)</td>
<td>5.8 (2.3-14.1)</td>
</tr>
</tbody>
</table>

* $P = 0.03$, chi-square test.

* $P < 0.0001$, chi-square test.
Although the difference between having one and two parents colonized was not statistically significant.

Adults and their mothers were also more frequently concordantly colonized than would be expected if P. gingivalis were randomly distributed in the study population. The relationship between colonization of adults and that of their fathers was not significant (Table 1). The relative risks are shown in Fig. 4E and F.

To assess the likelihood of entire families being similarly colonized, four individuals from each family were selected for chi-square analysis. A parent and spouse, their oldest child, and a grandparent were considered for this analysis. The grandparent was selected as available based on the following hierarchy: maternal grandmother, maternal grandfather, paternal grandmother, and paternal grandfather. Concordance in colonization status was highly significantly more frequent among family members than would be expected if P. gingivalis were randomly distributed in the population studied (Fig. 5).

Subjects were screened for the presence of periodontally involved teeth. Ninety-five (17%) of the subjects had one or more teeth with a pocket depth or attachment level of ≥5.5 mm. A significantly higher prevalence of P. gingivalis was seen in these subjects than in subjects with a pocket depth or attachment level of <5.5 mm for all teeth (P = 0.04 by chi-square test). Table 3 shows the prevalence of P. gingivalis for subjects with various levels of attachment loss and pocket depths. Subjects with three or more teeth with attachment loss or deep pockets were significantly more frequently colonized than all other subjects. The number of teeth with a pocket depth or attachment level of <5.5 mm was positively related to the likelihood of P. gingivalis detection (P = 0.004 by logistic regression analysis).

The effect of exposure to a family member with attachment loss or deep pockets was investigated. No association was seen between the percentage of family members with more than two teeth with a pocket depth or attachment level of ≥5.5 mm and the percentage of members who were colonized (Fig. 6). Similarly, no relationship was seen between the percentage of family members with any teeth with a pocket depth or attachment level of <5.5 mm and the percentage of colonized family members (R² = 0.007). When families having members with pocket depths or attachment levels of ≥5.5 mm for more than two teeth were compared to those without, the mean percentage of colonized family members was not significantly different (means, 44.5 and 33.2%, respectively; P = 0.09). Also, no significant difference was seen in the percentage of colonized members between families that had members with any periodontally involved teeth (mean, 39.6%) and those that did not (mean, 36.2%; P = 0.62).

**DISCUSSION**

To investigate the transmission of the putative periodontal pathogen P. gingivalis within families, a large number of extended families were sampled. The sample was intended to be a random sample representative of the population of Columbus, Ohio. Families were recruited from 12 churches of various denominations on the basis of their willingness to participate. The gender distribution of subjects was representative of the population. Black subjects were underrepresented in the sample due to a high refusal rate among black churches approached to participate in the study. Only two black families were enrolled.

No attempt was made to select subjects on the basis of periodontal health or disease. The periodontal screening examination showed that 17% of the subjects had at least one site with an attachment level or pocket depth of ≥5.5 mm. This is

**TABLE 3. Periodontal status and prevalence of P. gingivalis**

<table>
<thead>
<tr>
<th>Probing depth or attachment level (mm)</th>
<th>No. of teeth</th>
<th>No. (%) of subjects</th>
<th>Prevalence of P. gingivalis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5.5</td>
<td>All</td>
<td>467 (83)</td>
<td>35*</td>
</tr>
<tr>
<td>≥5.5</td>
<td>1 or 2</td>
<td>48 (9)</td>
<td>31b</td>
</tr>
<tr>
<td>≥5.5</td>
<td>≥2</td>
<td>48 (9)</td>
<td>63</td>
</tr>
<tr>
<td>All subjects</td>
<td>563</td>
<td>37</td>
<td></td>
</tr>
</tbody>
</table>

*P = 0.0002 by chi-square test versus probing depth of ≥5 mm with one or two teeth involved.

bP = 0.002 by chi-square test versus probing depth or attachment level of ≥5.5 mm with more than two teeth involved.
in approximate agreement with the most recent NHANES report, in which the prevalence of pocket depths of 4 mm or less was 29.2% and that of pocket depths of 6 mm or less was 3.9% (3). This suggests that the sample is representative of the general population.

The presence of *P. gingivalis* was determined for 564 members of 104 multigeneration families with a sensitive, PCR-based assay (12). This assay was performed directly on plaque samples and did not require that the bacteria be cultured. *P. gingivalis* was significantly more prevalent when periodontitis was present but was detectable even among some apparently periodontally healthy individuals and children. Overall, 37.1% of the population was infected. These data are consistent with a recent report of a random sampling of adults with a sensitive detection method in which the reported prevalence of *P. gingivalis* was 32% (26). In two other studies using less sensitive, culture-based detection methods, the prevalence was found to be only 10 to 14% among randomly selected adults (5, 16). However, in these studies no more than six sites were sampled, and more intensive sampling has been shown to yield a higher prevalence of *P. gingivalis* (8). For the present study, the mesial sulcus of every tooth present was sampled. The relatively higher frequency with which *P. gingivalis* was detected in the current study may be explained both by the more sensitive detection method used and by the number of teeth sampled. The prevalence among the children in the current study was 27.1% (Fig. 1). In a previous study employing the same sampling and detection methods, the prevalence of *P. gingivalis* among randomly selected children was found to be 37% (12). It is possible that the difference may be attributed to the difference in socioeconomic status or dental health between the two samples of children. In the previous study, the children were selected from the patient population of a dental clinic which serves primarily a low-income population, while the current study included subjects from all socioeconomic strata and was not biased towards those seeking dental care.

When the prevalence of *P. gingivalis* was compared among generations by chi-square analysis (Fig. 3), children were significantly less frequently colonized than their grandparents. These data and the concordance of colonization observed among spouses together suggest that although *P. gingivalis* is most commonly acquired during childhood, it may also be acquired later in life.

To assess the frequency of transmission of *P. gingivalis* among family members, the concordance of colonization was examined within extended families. Statistical analysis of the concordance observed in the entire family was obtained by considering a core unit consisting of three generations and including the oldest child, both parents, and a grandparent. Data on 101 families were available for analysis (Fig. 5). A high degree of concordance in *P. gingivalis* colonization was found within these families. As shown in Fig. 5, nearly three times as many families with only uninfected members and five times as many families with only infected members were seen as would be expected if *P. gingivalis* were randomly distributed in the study population. On the other hand, only about half as many mixed-status families were observed as would be expected. This relationship was statistically highly significant. These data indicate that transmission of *P. gingivalis* is common within families. They also suggest that infection from sources outside the family may be unusual.

To further elucidate pathways of transmission, individual relationships were investigated. The concordance of colonization was examined for husbands and wives from both the parent and grandparent generations. Spouses of colonized individuals were almost four times as likely to be colonized as spouses of uninfected individuals. This suggests that transmission between spouses is a common event. In the present study, no relationship between concordance and length of marriage was observed. This suggests, not surprisingly, that transmission probably occurs in the early years of marriage. There is evidence that the periodontal health of spouses is correlated (19, 25). Although common environmental causative factors cannot be ruled out, the results of the current study suggest that transmission of virulent organisms is an important factor.

The likelihood of transmission from parents to children was investigated by examining their concordance of colonization and the relative risk of colonization for children based on the status of parents. When multiple children from a single family had been sampled, the oldest child was selected for statistical analysis. The colonization of children was shown to be highly dependent upon that of their parents. Children were significantly more frequently concordant with both their mothers and their fathers than would be expected if *P. gingivalis* were randomly distributed in the population studied. It appears that exposure to a greater number of colonized parents increased the risk to a child, although the difference between one and two parents did not reach statistical significance. The increase might also be explained by higher levels of *P. gingivalis* in one of the parents, which put both the child and the spouse at greater risk. In any case, fewer than 1 in 10 children of uninfected parents was colonized, and nearly half of the children with two colonized parents were infected. It appears that parents rather than other contacts are the most common source of infection for children and that the presence of *P. gingivalis* in parents is a substantial risk factor for infection among children. The extended families sampled for this study were selected based on their willingness to be sampled together and residence in the same city. Considering this, it seems likely that the grandparents had frequent contact with their grandchildren and could be regarded as potential sources of infection. Of the 101 children for whom data on both parents were available, only 4 were colonized in the absence of *P. gingivalis* in either parent and 3 of these had a colonized grandparent. No information about other potential sources of infection, such as day care providers or other contacts, is available for the children in the study. The presence of *P. gingivalis* is a known risk factor for disease in adults (2), but its effect in children is less clear. It is not known whether infection in childhood is likely to initiate chronic periodontal destruction which becomes evident in adulthood. If it does, having infected parents may be a significant risk factor for later periodontitis.

Other oral bacteria have been shown to be transmitted between close family contacts. Studies of *Streptococcus mutans* have suggested that transmission of this organism occurs directly from mothers but not from fathers, to children (10, 11). In contrast, genotypes of *Actinobacillus actinomycetemcomitans* have been shown to be shared by either mothers or fathers and their children (1, 4, 17, 20). In the present study, no difference was observed in *P. gingivalis* colonization concordance between children and their mothers compared to fathers, suggesting that transmission of *P. gingivalis* from either parent may occur.

Sibling pairs were also more frequently concordant than would be expected if *P. gingivalis* were randomly distributed among the children examined for this study (Table 1). Since siblings were exposed to the same colonized adults, it is not possible to determine if the concordance was due to common exposure or if transmission occurs between children. Genetic determinants of susceptibility to infection must also be considered as possible factors contributing to concordance of colonization. Previous investigators have shown that several indi-
cators of periodontal disease, including plaque and attachment loss, were found to be higher among monzygous twins than among dizygous twins (13). Genetic factors may play some role in the concordances observed in the current study between siblings, between adults and their parents, and between parents and their children.

It is somewhat surprising that a significantly higher concordance of colonization was found between adults and their mothers than would be expected if P. gingivalis were randomly distributed in the study population (Table 1). Having a colonized mother gave an adult more than twice as great a risk of colonization as the offspring of uninfected mothers. One possible explanation is that colonization is relatively stable over long periods of time. Alternatively, the bacteria may be repeatedly transmitted through ordinary contact via eating utensils or more direct routes. This may be particularly likely in this sample of families, since they were all frequently in contact. It is also possible that common environmental factors such as oral hygiene habits or genetic factors play an important role. Investigation of the concordance of related adults who are no longer in contact would be interesting. The difference in concordance of colonization observed for adults and their fathers was not statistically significant. The sample size for males from the grandparent generation was smaller, and this may account for the fact that significance was observed for mothers but not fathers. Alternatively, it may reflect less frequent or intimate contact between the males of the grandparent generation and their offspring, either in the early years of child rearing or in adult life.

To determine whether the disease status of individuals influenced the frequency of transmission of P. gingivalis, the effect of the presence of family members with indicators of periodontitis on the prevalence of P. gingivalis among other family members was investigated. There was a clear association between the presence of P. gingivalis and the presence of multiple deep pockets or attachment loss for individual subjects. The strong association of disease with the presence of P. gingivalis could be accounted for by subjects with more than two teeth with deep pockets or attachment loss. Table 3 shows that the prevalence of P. gingivalis was nearly doubled for subjects with more than two teeth with deep pockets or attachment loss and that the prevalence of P. gingivalis among subjects with one or two involved teeth was comparable to that found in the apparently healthy group. Since it is possible that the scoring of two teeth with attachment loss could be due to factors other than periodontitis, such as toothbrush abrasion, statistical analyses to determine the effect of the presence of disease indicators on transmission were performed both with more than two involved teeth as the threshold for disease and more conservatively with any involved teeth as the threshold. No analyses showed any relationship between the presence of family members with disease indicators and the prevalence of P. gingivalis in other family members. Considering these findings, it does not appear that the presence of more advanced disease confers additional risk for transmission of P. gingivalis. This is somewhat surprising, since other investigators have demonstrated high levels of P. gingivalis at sites with deep pockets and attachment loss (22). It may indicate that a large inoculum is not required to transmit P. gingivalis. However, the number of individuals in the sample with periodontitis, and particularly severe periodontitis, was not large. Further study is indicated to elucidate the role of diseased family members as a risk factor for transmission of periodontopathogenic bacteria.

Although the data indicate that transmission of P. gingivalis is a common occurrence within families, further study to identify strains is planned to confirm pathways of transmission. Preliminary analysis indicates that within families, clonal types are nearly always shared (6). In conclusion, contact with an infected family member substantially increased the relative risk of colonization for spouses, children and their parents, adults and their mothers, and siblings. These same pairs, as well as the entire extended family group, were all more frequently concordant in colonization than would be expected if P. gingivalis were randomly distributed in the study population. This indicates that P. gingivalis is commonly transmitted by contact with an infected family member.

ACKNOWLEDGMENTS

This work was supported by NIH grant DE10467. We thank Gerard McDonnell for assistance in recruiting and sampling subjects.

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