Localized juvenile periodontitis affects otherwise healthy teenagers and destroys supportive periodontal tissues around first molars and incisors. *Actinobacillus actinomycetemcomitans*, a gram-negative, facultatively anaerobic rod, is the major pathogen in localized juvenile periodontitis and some forms of adult periodontitis. Certain serotype b strains of *A. actinomycetemcomitans*, including strains JP2 and Y4, have been reported to exert especially high periodontal pathogenicity, possibly related to leukotoxin activity [1, 2].

Our previous results also suggest that certain *A. actinomycetemcomitans* serotype b clones are closely related to severe periodontitis [3]. A further interesting observation was that *A. actinomycetemcomitans* isolates of a given arbitrarily primed PCR (AP-PCR) genotype belong to the same serotype, which indicates a systematic genetic dissimilarity between *A. actinomycetemcomitans* serotypes [3]. Thus, grouping clinical *A. actinomycetemcomitans* isolates according to serotype seems to constitute a reasonable first approach to determining an association between certain *A. actinomycetemcomitans* clones and periodontal disease status.

All *A. actinomycetemcomitans* isolates contain the gene operon coding for leukotoxin, but isolates vary in their ability to produce leukotoxin [2]. *A. actinomycetemcomitans* strains with a deletion in the promoter region of the *ltx* operon seem to express high leukotoxin activity [2]. In the present study we determined the *A. actinomycetemcomitans* serotypes, genotypes, and *ltx* promoter types for isolates from juveniles and adults with severe periodontitis to identify *A. actinomycetemcomitans* strains that may display increased periodontal pathogenicity.

**Materials and Methods**

The study material comprised one *A. actinomycetemcomitans* isolate from each of 112 periodontally diseased Finnish individuals, including 54 patients with localized juvenile periodontitis (17 patients <18 years of age, 18 patients 18–22 years of age, 14 patients 22–26 years of age, and 5 patients >26 years of age; mean age, 20 years [SD, 4.3]) and 58 patients with adult periodontitis (mean age, 47 years [SD, 11.6]). The subjects had been referred for periodontal treatment in the Department of Periodontology, University of Helsinki, Helsinki. An orthopantomogram and, when indicated, periapical radiograms were obtained for each patient.

The diagnosis of localized juvenile periodontitis was based on the criteria of Baer [4]. The severity and extent of periodontal destruction in localized juvenile periodontitis varied from initial attachment loss (the cervical third of the root length) to advanced lesions (extending to the apical third of the root length), mainly around the first molars and/or incisors. The adult periodontitis patients (age >35 years) exhibited advanced adult periodontitis. They each had at least 20 natural teeth, including at least two molars. Advanced attachment loss was measured around at least one tooth in each sextant, and at least two furcation lesions extended to the middle furcation area.

The study also included *A. actinomycetemcomitans* isolates from 51 Americans with periodontitis (mean age, 43 years [SD, 17.4]) who were referred for microbial analysis at the Oral Microbiology Testing Laboratory, School of Dentistry, University of Southern California, Los Angeles. Fourteen subjects had periodontitis of early onset, and the remaining 37 patients had adult periodontitis.

Subgingival *A. actinomycetemcomitans* was recovered on selective tryptic soy-bacitracin-vancymycin agar in 10% CO2/90% air and identified as previously described [5]. One isolate per subject was examined, since most *A. actinomycetemcomitans*-infected periodontitis patients harbor only one serotype and genotype of the species [6, 7]. The *A. actinomycetemcomitans* isolates were serotyped in an immunodiffusion assay using serotype (a–e)–specific rabbit antiserum and cellular antigens prepared by autoclaving [6]. AP-PCR was used for genotyping [3, 8] and thus to verify the presence of isolates with JP2-like and Y4-like genotypes. The AP-PCR genotypes were designated according to the system of Asikainen et al. [3].

The oligonucleotide 5′-CAGCACCACAC-3′ primer was used in AP-PCR for all isolates, and two additional random primers, 5′-GAAAACGGGTG-3′ and 5′-AGTCAAGCCAC-3′, were used for further discrimination of isolates when indicated. *A. actinomycetemcomitans* DNA for AP-PCR amplification was extracted by a rapid detergent/proteinase method or by phenol/chloroform purification [3]. Two μL of supernatant or 0.1 μg of DNA was used as template in a 50-μL PCR reaction volume consisting of 0.2 mM of dNTP (Pharmacia Biotechnology, Piscataway, NJ), 0.4 μM of primer, 10 mM of Tris-HCl (pH, 8.3), 50 mM of KCl, 4 mM of MgCl2, and 2.5 U of NativeTaq (Perkin Elmer Cetus, Norwalk, CT).

The primer pair 5′-ATA TTA AAT CTC CTT GT-3′ and 5′-ACC TGA TAA CAG TAT T-3′ was used to amplify the 470-bp or 1,000-bp product characteristic of, respectively, the *ltx* promoter region of *A. actinomycetemcomitans* strain JP2 and the minimally toxic *A. actinomycetemcomitans* reference strain 652 [2]. Two μL of boiling lysate supernatant or 0.1 μg of purified DNA of *A. actinomycetemcomitans* isolates was used as DNA template.

The reaction mixture consisted of 0.2 mM of dNTP, 0.4 mM of primer, 10 mM of Tris-HCl (pH, 8.3), 50 mM of KCl, 3 mM of MgCl2, and 1.25 U of polymerase (Promega, Madison, WI). The temperature profile in a thermocycler was 35 cycles at 94°C for 1 minute, 45°C for 1 minute, and 72°C for 1 minute, with an initial denaturation at 94°C and a final extension at 72°C, each for 5 minutes. A positive control (*A. actinomycetemcomitans* JP2 lysate or purified JP2 DNA) and a negative control (reaction mixture without template) were included in each amplification reaction.

The repeatability of the methods has been extensively studied in our laboratory. The serotype and AP-PCR characteristics have consistently been the same when multiple colonies from the single-
A. actinomycetemcomitans±positive American subjects. The boy whose primary molars were affected by periodontitis [14].

Serotype b was found in 48 (43%) of 112 A. actinomycetemcomitans—positive Finnish subjects (23 with localized juvenile periodontitis and 25 with adult periodontitis) and in 16 (31%) of 51 A. actinomycetemcomitans—positive American subjects. The oligonucleotide 5′-CAGCACCCAC-3′ primer distinguished a total of 16 AP-PCR types.

Results

Table 1 shows that the serotype distribution of A. actinomycetemcomitans strains did not differ significantly between the isolates from Finland and the United States (χ² test: χ² = 7.5, P = .19). Serotype b was found in 48 (43%) of 112 A. actinomycetemcomitans—positive Finnish subjects (23 with localized juvenile periodontitis and 25 with adult periodontitis) and in 16 (31%) of 51 A. actinomycetemcomitans—positive American subjects. The oligonucleotide 5′-CAGCACCCAC-3′ primer distinguished a total of 16 AP-PCR types.

The genotype distribution pattern of serotype b isolates was further analyzed in the patient population from Finland. Five AP-PCR genotypes were identified among the serotype b isolates. The distribution of the serotype b AP-PCR genotypes differed significantly between localized juvenile periodontitis and adult periodontitis (χ² test: χ² = 19.2, P < .001) (table 2). In localized juvenile periodontitis, 83% of A. actinomycetemcomitans serotype b isolates belonged to the AP-PCR genotypes 8 and 9, which represent the AP-PCR genotypes of strains JP2 and Y4, respectively. In adult periodontitis, 72% of the serotype b isolates belonged to AP-PCR genotype 2. The AP-PCR genotype 2 differed from the AP-PCR genotypes of the A. actinomycetemcomitans reference strains American Type Culture Collection (ATCC) 29522, ATCC 29524, ATCC 29523, and National Collection of Type Cultures (NCTC) 9710.

No Finnish A. actinomycetemcomitans isolates and only three American A. actinomycetemcomitans isolates displayed the 470-bp amplicon characteristic of the ltx promoter of strain JP2; the 470-bp amplicon was recovered from an 8-year-old black patient, a 13-year-old black patient, and a 33-year-old patient of unknown race. These three strains also revealed a JP2-like AP-PCR genotype. The remaining study strains all showed a 1,000-bp ltx promoter amplicon.

Table 1. Distribution of serotypes in A. actinomycetemcomitans isolates from Finland and the United States.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Finland (n = 112)</th>
<th>United States (n = 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>32 (29)</td>
<td>14 (28)</td>
</tr>
<tr>
<td>b</td>
<td>48 (43)</td>
<td>16 (31)</td>
</tr>
<tr>
<td>c</td>
<td>16 (14)</td>
<td>14 (28)</td>
</tr>
<tr>
<td>d</td>
<td>5 (5)</td>
<td>0</td>
</tr>
<tr>
<td>e</td>
<td>6 (5)</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Untypeable</td>
<td>5 (5)</td>
<td>4 (8)</td>
</tr>
</tbody>
</table>

Discussion

Little or no data are available on the occurrence of the five A. actinomycetemcomitans serotypes in geographically distinct isolates. We found a similar distribution of A. actinomycetemcomitans serotypes among Finnish and American periodontitis patients. In addition, as reported earlier [9, 10], the most frequent A. actinomycetemcomitans serotype in both populations was serotype b. The distinct difference in the distribution of AP-PCR genotypes of serotype b isolates between juvenile and adult forms of periodontitis suggests that different A. actinomycetemcomitans clones may play different roles in these diseases. Similar findings have been reported earlier [3].

Although isolates with a JP2-like AP-PCR genotype were found in 11 of the 112 Finnish patients with localized juvenile periodontitis or severe adult periodontitis, none exhibited a JP2-like ltx promoter structure. The lack of the JP2-like ltx promoter has been reported previously with regard to A. actinomycetemcomitans strains from white Europeans [11, 12]. However, a recent report found JP2-like ltx promoter in A. actinomycetemcomitans strains from 11 of 17 juvenile periodontitis patients of African origin, living in Scandinavia [13].

Localized juvenile periodontitis first appears in the circumbiberal years and affects the first permanent molars and incisors in odontally healthy adolescents [15, 16] suggests an ability of juvenile periodontitis, 83% of A. actinomycetemcomitans isolates displayed the 470-48 bp amplicon characteristic of the ltx promoter of strain JP2; the patients with localized juvenile periodontitis strongly argues against its unique significance in the pathogenesis of the disease. No Finnish A. actinomycetemcomitans isolates and only three American A. actinomycetemcomitans isolates displayed the 470-bp amplicon characteristic of the ltx promoter of strain JP2; the 470-bp amplicon was recovered from an 8-year-old black patient, a 13-year-old black patient, and a 33-year-old patient of unknown race. These three strains also revealed a JP2-like AP-PCR genotype. The remaining study strains all showed a 1,000-bp ltx promoter amplicon.

Discussion

Little or no data are available on the occurrence of the five A. actinomycetemcomitans serotypes in geographically distinct isolates. We found a similar distribution of A. actinomycetemcomitans serotypes among Finnish and American periodontitis patients. In addition, as reported earlier [9, 10], the most frequent A. actinomycetemcomitans serotype in both populations was serotype b. The distinct difference in the distribution of AP-PCR genotypes of serotype b isolates between juvenile and adult forms of periodontitis suggests that different A. actinomycetemcomitans clones may play different roles in these diseases. Similar findings have been reported earlier [3].

Although isolates with a JP2-like AP-PCR genotype were found in 11 of the 112 Finnish patients with localized juvenile periodontitis or severe adult periodontitis, none exhibited a JP2-like ltx promoter structure. The lack of the JP2-like ltx promoter has been reported previously with regard to A. actinomycetemcomitans strains from white Europeans [11, 12]. However, a recent report found JP2-like ltx promoter in A. actinomycetemcomitans strains from 11 of 17 juvenile periodontitis patients of African origin, living in Scandinavia [13]. In the present study two of the three subjects with A. actinomycetemcomitans strains with a JP2-like promoter structure were also black. The absence of this promoter structure in the present A. actinomycetemcomitans isolates from the white patients with localized juvenile periodontitis strongly argues against its unique significance in the pathogenesis of the disease.

Table 2. Distribution of arbitrarily primed PCR (AP-PCR) types of 48 A. actinomycetemcomitans serotype b isolates from Finnish patients with localized juvenile periodontitis and adult periodontitis.

<table>
<thead>
<tr>
<th>AP-PCR designation</th>
<th>Localized juvenile periodontitis</th>
<th>Adult periodontitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 2</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>Type 8</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Type 9</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Type 12</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Type 16</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>25</td>
</tr>
</tbody>
</table>
tans periodontitis, since determinants other than the JP2-like promoter structure may be involved in controlling ltx expression.

Our study suggests that the search for highly virulent *A. actinomycetemcomitans* strains might include those with AP-PCR genotype 9 (Y4-like) and AP-PCR genotype 2. Studies on the significance of the various *A. actinomycetemcomitans* serotypes in periodontitis seem relevant, since the majority of our patients with severe periodontitis harbored *A. actinomycetemcomitans* isolates of serotypes other than b.

References