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Research paper

External apical root resorption and the interleukin-1B gene polymorphism in the Japanese population

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ABSTRACT

External apical root resorption (EARR) is a common consequence of orthodontic treatment. Recently, several studies have reported an association between EARR and an underlying genetic cause. This study investigated whether a single interleukin (IL)-1B gene polymorphism (rs1143634) was associated with EARR in the Japanese population. Genomic DNA, lateral cephalograms, and panoramic radiographs were obtained from 54 Japanese. We measured the EARR in the maxillary central incisors, the mandibular central incisors, and the mesial and distal roots of the mandibular first molar, and analyzed statistically the association between *IL-1B* polymorphism and EARR. We also examined differences in allelic frequency of this *IL-1B* polymorphism in a multi-ethnic study population consisting of Japanese, Han Chinese, African American, European Caucasian, and Hispanic individuals. We found no significant difference in the frequency of the *IL-1B* polymorphism between EARR cases and controls in the Japanese population. We also report marked diversities in the allelic frequencies of the *IL-1B* polymorphism within the multi-ethnic study population. The European Caucasian population carried the T allele at a frequency of 29.2%, whereas the Japanese population carried the T allele at a frequency of 5.6%. The low frequency of the T allele in Japanese population made it difficult to compare population allelic frequencies among different populations. Further studies are required to confirm our findings and to investigate the effect of other single nucleotide polymorphisms in *IL-1B* or other genetic risk factors underlying susceptibility to EARR.

1. Introduction

External apical root resorption (EARR) is a common consequence of orthodontic treatment. Root resorption associated with orthodontic treatment is more apparent in subjects where the applied forces are strong and of extended duration, delivered to the tooth in unfavorable directions, or when the tooth is unable to withstand normal forces due to a weakened support system [1–3].

The effect of orthodontic force depends on the biochemical or physiological makeup of a patient. Risk factors attributed to the adverse effects of orthodontic force in patients include individual susceptibility [4,5], genetic background [3,6–9], and systemic factors [10]. Recently, several studies have reported

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an association between EARR and an underlying genetic cause. In a study of sibling pairs Harris et al. [3] revealed a substantive genetic factor in susceptibility to EARR. Al-Qawasmi et al. [8] reported an association between root resorption and the *IL-1B* gene. The genes *IL-1A* and *IL-1B* encode the proinflammatory cytokine proteins IL-1α and IL-1β, respectively, and *IL-1RN* encodes a related protein, IL-1ra, which acts as a receptor antagonist [11]. Interleukin 1-beta (IL-1β), a potent bone-resorptive cytokine, is a key component of the complex signaling pathways leading to root resorption. A balance between the activities of IL-1β and interleukin receptor antagonist (*IL-ra*) is thought to be crucial in the development of periapical lesions [12]. Al-Qawasmi et al. [8] revealed that an *IL-1B* polymorphism significantly increases the risk of EARR in the Caucasian population.

It is well known that differences in single nucleotide polymorphism (SNP) frequencies among human populations are ethnicity-dependent [13]. Ethnic factors are considered to be a major variable for evaluating the predisposition to disease [13,14]. A race is usually defined as a subdivision of a species formed by individuals who share common biological characteristics. However, races can be distinguished not only biologically but also culturally, though cultural differences used to distinguish race are most likely to be secondary compared with biological and environmental differences. Traditionally, the biological characteristics used to distinguish race include skin pigmentation, facial form, and body build; these characteristics have been shown to be highly heritable. The highly heritable nature of biological characteristics distinguishing one race from another indicates that differences between races are fundamentally of genetic origin [15].

The present study examined the association between a single polymorphism (rs1143634) in the *IL-1B* gene and root resorption in 54 normal Japanese subjects using qualitative and quantitative variables. We further characterized the ethnic dependency of variations at the *IL-1B* locus by examining the allelic frequencies of the *IL-1B* polymorphism in a multi-ethnic study population consisting of Japanese, Han Chinese, African American, European Caucasian, and Hispanic individuals.

2. Materials and methods

2.1. Subjects

Lateral cephalograms, and panoramic radiographs were obtained from 54 Japanese subjects consisting of 18 men (average age, 19 years) and 36 women (average age, 21 years). The average interval between pretreatment and posttreatment records was 3 years and 1 month. Genomic DNA of the subjects were obtained after active orthodontic treatments. The data for the maxillary incisors are summarized (Table 1). The Japanese subjects were patients who had received orthodontic treatment at Showa University Dental Hospitals. Subjects who had congenital disorders, such as cleft palate or general physical disease, were excluded from the study. Additionally, DNA samples from 24 Han Chinese, 24 African Americans, 24 European Americans, and 24 Hispanics with no craniofacial measurements were obtained from the Coriell Cell Repository (Camden, NJ, USA), and used only as reference populations for allelic frequencies of *IL-1B*. The protocol used in this study was approved by the Ethical Committee of Showa University, and all patients gave their written informed consent to participate in the study before samples were taken.

2.2. Measurements

The roots of three types of teeth were measured on pretreatment and posttreatment lateral cephalometric and panoramic radiographs using techniques similar to those described previously [3]. The roots of the maxillary and mandibular central incisors were measured from the pretreatment and posttreatment cephalometric radiographs. The mesial and distal roots of the left and right sides were measured on the pretreatment and posttreatment panoramic radiographs. For each patient, the difference in pretreatment and posttreatment length for the right and left first molar was averaged between the two sides for each root. The data for each of the four variables were considered separately for genetic analyses.

Measurement error was assessed by the random selection of 10 panoramic and cephalometric radiographs on three separate occasions. One-way analysis of variance, used to test the quality of means for the root-length measurements, suggested that this was done in a consistent manner. Mean scores for the root-length measurements did not significantly differ for the three measurements. The measurement error can thus be considered negligible.

2.3. Genotyping

To collect a sample for DNA analysis, the inside of the mouth was scraped with 10 strokes of a brush (MasterAmp™ Buccal Swab DNA Extraction Kit, AR Brown Co. Ltd., Tokyo, Japan). Four samples were collected from each subject, and genomic DNA was obtained from these samples. Polymerase chain reaction (PCR) amplifications were performed according to a standard protocol. To determine the risk genotype of IL-1B, PCR amplification was performed in a 50-μl PCR reaction volume containing 30 ng of genomic DNA, 200 μM of each dNTP, 0.25 units EX Taq (Takara, Otsu, Japan), and 0.1 μM of each primer. The primer sequences for IL-1B (rs1143634) were as follows: forward primer, 5′-CTCAGGTGTCCTCGAAGAAATCAA-3′; and reverse primer, 5′- GCTTTTTTGCTGTGAGTCCCG-3′. Amplifications were performed using a Gene Amp PCR system 9700 (Applied Biosystems, Tokyo, Japan). The PCR cycling conditions were as follows: an initial denaturation at 95 °C for 2 min followed by 40 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. After removal of the remaining primers and dNTPs using ExoSAP-IT (GE Healthcare Life Science, USA), the products were subjected to BigDye v1.1 sequencing on the ABI PRISM 3700 DNA Analyser (Applied Biosystems).

Polymorphisms were identified by means of the Sequencer program (Gene Code Co, Ann Arbor, MI). Polymorphisms were confirmed by sequencing of both DNA strands of each PCR product.

2.4. Statistical analysis

Subjects were classified as unaffected (<2.0 mm) or affected (≥2.0 mm), according to the amount of root resorption. The