Heat Shock 70 Protein Genes and Genetic Susceptibility to Apical Periodontitis

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Abstract

Introduction: Heat shock proteins (HSPs) protect cells under adverse conditions such as infection, inflammation, and disease. The differential expression of HSPs in human periapical granulomas suggests a potential role for these proteins in periapical lesion development, which may contribute to different clinical outcomes. Therefore, we hypothesized that polymorphisms in HSP genes leading to perturbed gene expression and protein function may contribute to an individual's susceptibility to periapical lesion development. **Methods:** Subjects with deep carious lesions with or without periapical lesions (≥3 mm) were recruited at the University of Texas School of Dentistry at Houston and at the University of Pittsburgh. Genomic DNA samples of 400 patients were sorted into 2 groups: 183 cases with deep carious lesions and periapical lesions (cases) and 217 cases with deep carious lesions but without periapical lesions (controls). Eight single nucleotide polymorphisms (SNPs) in *HSPA4, HSPA6, HSPA1L, HSPA4L,* and *HSPA9* genes were selected for genotyping. Genotypes were generated by end point analysis by using Taqman chemistry in a real-time polymerase chain reaction assay. Allele and genotype frequencies were compared among cases and controls by using χ^2 and Fisher exact tests as implemented in PLINK v.1.07. *In silico* analysis of SNP function was performed by using Polymorphism Phenotyping V2 and MirSNP software. **Results:** Overall, SNPs in *HSPA1L* and *HSPA6* showed significant allelic association with cases of deep caries and periapical lesions ($P < .05$). We also observed altered transmission of *HSPA1L* SNP haplotypes ($P = .03$). *In silico* analysis of *HSPA1L* rs2075800 function showed that this SNP results in a glutamine-to-lysine substitution at position 602 of the protein and might affect the stability and function of the final protein. **Conclusions:** Variations in *HSPA1L* and *HSPA6* may be associated with periapical lesion formation in individuals with untreated deep carious lesions. Future studies could help predict host susceptibility to developing apical periodontitis. *(J Endod 2016;■ :1–5)*

Key Words

Apical periodontitis, heat shock proteins, polymorphism

Significance

Heat shock protein 70 genes are plausible candidate genes for apical periodontitis. We showed that *HSPA1L* and *HSPA6* variants were associated with deep caries and periapical lesions and may contribute to development of periapical lesions.

Apical periodontitis is an inflammatory disorder of periapical tissues caused by continuous microbial infections within the root canal system (1). The inflammatory response offers a unique study of many facets of pathogenesis, including bacterial ecology and pathogenicity, innate and acquired immune responses to infection, the regulation of such responses and their effects on host tissues, particularly the periapical region (1). It is viewed as a dynamic encounter between local microbial factors and host defenses at the interface between infected radicular pulp and periodontal ligament that results in local inflammation, resorption of hard tissues, destruction of other periapical tissues, and eventual formation of various histopathologic categories of apical periodontitis (1). Equilibrium of cytokines, endotoxins, and cell-to-cell contacts may influence the defense and inflammatory responses and the balance between bone resorption and regeneration, resulting in lesion expansion or healing of apical periodontitis (2–4). In addition to local factors, genetic predisposition has been suggested as a differential etiologic factor for apical periodontitis development (5–11). Moreover, genetic susceptibility may influence host response to endodontic infection (9).

The most primitive mechanism of cellular protection involves the expression of a polypeptide family called heat shock proteins (HSPs) (12). HSPs have essential roles in the synthesis, transport, and folding of proteins and are often referred to as molecular chaperones. However, HSPs are characteristically induced by stress signals such as elevated temperature, reduced oxygen supply, infectious agents, and inflammatory mediators (13, 14). Stressors that cause protein unfolding, misfolding, or aggregation trigger a protective response that leads to the induction of gene transcription for proteins with the capacity to stabilize and re-fold proteins, thereby reestablishing the

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balance between protein synthesis, assembly, and degradation (15). Therefore, HSPs also exert a protective role against harmful environment (12).

HSPs are subdivided on the basis of their molecular weight (HSPH [HSP110], HSPC [HSP90], HSPA [HSP70], DNAJ [HSP40], HSPD [HSP60], and HSPB [HSP27] and are especially effective in triggering the innate immune response by activating macrophages and macrophage-like cells (16–18). HSPs can increase the cellular response to lipopolysaccharide (LPS) to stimulate the production of prototypic inflammatory cytokines such as tumor necrosis factor-α) (19).

HSP70 proteins are found in all species and are located in numerous cellular components (ie, mitochondria, the endoplasmic reticulum, lysosomes, cytosol, and nucleus) and are subdivided into *HSPA1A*, *HSPA1B*, *HSPA1L*, *HSPA2*, *HSPA4*, *HSPA5*, *HSPA6*, *HSPA7*, *HSPA8*, *HSPA9*, *HSPA12A*, *HSPA12B*, *HSPA13,* and *HSPA14* (20). These proteins are involved in prevention of aggregation of unfolded polypeptides and the disassembly of multimeric protein complexes by using a mechanism of protein trafficking between cellular compartments, protein folding, and regulation of heat shock response (16, 17). Interestingly, HSPs such as HSP70 can play dual roles in the modulation of host inflammatory immune reaction. It has been shown that the induction of proinflammatory cytokines by HSP70 may contribute to the pathogenesis of autoimmune disease and chronic inflammation (19, 20). Conversely, HSP70 has been shown to downregulate toll-like receptors (19), thus inducing LPS tolerance and preventing augmentation of proinflammatory cytokine levels after LPS stimulation (20).

We have previously shown the differential expression patterns of HSPs in periapical granulomas, with a marked increase in the expression of 4 HSP genes, *DNAJC3*, *HSPA4*, *HSPA6,* and *HSPB1*, when compared with control tissues (21). We observed that the HSP expression in response to LPS was concentration-dependent, and that an increase in LPS concentration was positively correlated with HSP expression. These observations support the hypothesis that HSPs have a role in periapical lesion development, and their expression patterns may be related to different clinical outcomes (21). Therefore, we hypothesized that altered HSP expression that is due to HSP gene polymorphisms may contribute to an individual's susceptibility to periapical lesion development.

Materials and Methods

Sample Population

Two distinct population data sets were used in this study. The first data set was obtained through the University of Pittsburgh Dental Registry and DNA Repository, which gathers clinical information and DNA samples from saliva from patients seeking treatment at the School of Dental Medicine and who agree to participate in the registry. Data are extracted from electronic patient records and linked to the DNA samples for genetic/clinical research and provided to investigators in de-identified format on approved protocols. The second data set was ascertained at the Endodontic Clinic of the University of Texas Health Science Center School of Dentistry at Houston. In brief, all patients receiving treatment in the Endodontic Clinic were invited to participate, and dental/medical history as well as a saliva sample was collected on written informed consent. The University of Pittsburgh Institutional Review Board and the University of Texas Health Science Center at Houston Committee for the Protection of Human Subjects approved this study.

For this study, samples were collected during a period of 5 years. Individuals from both data sets were selected for inclusion on the basis of their radiographic records showing deep carious lesions, involving at least two thirds of the dentin depth, and periapical lesions $\geq$3 mm in diameter (cases) and individuals showing deep carious lesions but no periapical lesions (controls). Endodontic diagnostic tests to assess pulpal and periapical status were performed in all individuals. In brief, pulpal testing was performed by using Endo-Ice (Coltène/Whaledent Inc, Cuyahoga Falls, OH) and the electric pulp tester by using established protocols. Periapical status was assessed by using routine palpation and percussion tests. Individuals with diagnosis of pulp necrosis and apical periodontitis were included in the case group; individuals with diagnosis of vital pulps and normal apical tissues (no apical periodontitis) were included as controls. Patients with any systemic conditions such as diabetes or other hormonal alterations that are related to exacerbated or uncontrolled inflammatory responses and patients with medical conditions requiring the use of systemic modifiers of bone metabolism or other assisted drug therapy (ie, systemic antibiotics, anti-inflammatory, hormonal therapy) during the last 6 months before the study were excluded. At last, our sample population consisted of 400 white individuals with deep carious lesions, 183 case individuals with deep carious lesions and periapical lesions and 217 control individuals with deep carious lesions and no periapical lesions.

Selection of Candidate Genes and Single Nucleotide Polymorphisms

We selected 8 single nucleotide polymorphisms (SNPs) spanning the *HSPA1L, HSPA4, HSPA4L, HSPA6,* and *HSPA9* genes. Some of the SNPs were selected on the basis of published reports and/or their locations within the genes. Additional SNPs were selected on the basis of their likelihood to have functional consequences (ie, located in the promoters, exons, or near exon/intron boundaries) or considered tag SNPs as surrogates for the linkage disequilibrium blocks surrounding the candidate gene. We used information available at the National Center for Biotechnology Information dbSNP (http://www.ncbi.nlm.gov/SNP/) and HapMap Project (http://www.hapmap.org) databases to select polymorphisms. Details of studied genes and polymorphisms are presented in Table 1.

TABLE 1. Details of SNPs Investigated

Gene	SNP ID*	Chromosome: base position*	SNP function	Alleles†
HSPA1L	rs2075800	6:31777946	Missense	**C**/T
	rs2227956	6:31778272		C/**T**
	rs2227955	6:31778077		G/**T**
HSPA4	rs14355	5:132440285	3′ UTR	C/**G**
HSPA4L	rs1380154	4:128723042	Missense	**C**/T
HSPA6	rs1042881	1:161496530	3′ UTR	C/**G**
HSPA9	rs1042665	5:137902339	Missense	C/**T**
	rs10117	5:137892170		**A**/G

*According to National Center for Biotechnology Information GRCh37.p10 assembly.

†Ancestral allele is shown in bold.