UK College of Dentistry Research Day
ORAL and POSTER Presentation Abstracts
7th Annual CCTS Spring Conference
March 29, 2012

Oral Presentation

Abstract Title: **Age-related Changes in the Gingival Expression of Inflammasome Genes**

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Abstract:

Although the prevalence of chronic inflammatory disorders (e.g., periodontal disease) increases with aging, the mechanisms underlying this observation remain incompletely understood. Cytosolic multiprotein complexes that involve members of the NOD-like receptors (NLRs) family (i.e., inflammasomes) are emerging innate immune regulators of inflammation and infection; nevertheless their constitutive expression and age-related changes in gingival tissues remain unclear. Objective: To determine the expression of inflammasome genes in healthy and periodontitis gingival tissues across the lifespan Methods: Expression analysis of 16 genes involved in inflammasome pathways was performed in gingival biopsies from 20 healthy nonhuman primates (Macaca mulatta) (5/group) distributed in young (<3 yrs), adolescent (3-7 yrs), adult (12-15 yrs) and aged (18-22 yrs) groups, and 10 animals with periodontitis (5 adults and 5 aged), using the GeneChip® Rhesus Macaque Genome Array. Results: Inflammasome-activating NLRs were differentially expressed in healthy gingival tissue, with high expression of NLRP3 and NLRB/NAIP, intermediate expression of NLRPs 1, 2, 6 and 8; and low expression of NLRPs 4, 5, 7, 10, 11, 14, and NLRC4/IPAF. NLRP2 and 14 were over-expressed in aged compared with young healthy gingival tissue. Although caspase-1 expression did not change with age, there were age-related differences in the expression of its substrates pro-IL1β and pro-IL18. Significant reduction in NLRP1 and NLRP2 expression was related to periodontitis in adult and aged gingival tissues respectively. Finally, pro-IL1β, but not pro-IL-18 expression, increased in both aged and adult periodontitis tissues, and caspase-1 was only up-regulated in adult periodontitis tissues. Conclusion: These results suggest that inflammasome genes are expressed in healthy gingival tissue at different levels, and the expression of a subset of these genes appears to change with age and periodontitis. The mechanisms by which these variations could be related to a higher prevalence and/or severity of periodontal disease with aging will require further research.

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Oral Presentation

Abstract Title: **Oral Epithelial Cell Responses to Multispecies Microbial Biofilms**

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Abstract:

The oral microbial ecology is comprised of hundreds of bacterial species that co-exist as multispecies biofilms. However, little is known concerning the interactions of these complex biofilms with host cells. OBJECTIVE: This study used a novel model of multispecies bacterial biofilms to stimulate oral epithelial cells and profile select cytokines and chemokines that contribute to the local inflammatory environment in the periodontium. METHODS: Three multispecies biofilms comprising Streptococcus gordonii/S. oralis/S. sanguinis, Sg/Fusobacterium nucleatum/Porphyromonas gingivalis and Sg/Actinomyces naeslundii/Fn were grown on rigid gas permeable contact lens and challenged with OKF4 oral epithelial cells for 24 hrs. Controls included incubation of the epithelial cells with or without contact lens. A profile of cytokines/chemokines (IL-1α, IL-6, IL-8, TGFα, Gro-1α, IP-10, RANTES, Fractalkine, MCP-1, Mip-1α) were evaluated in supernatants from the epithelial cultures. RESULTS: The Sg/So/Ss and Sg/Fn/Pg biofilms elicited significantly elevated levels of IL-1α compared to any of the amounts of planktonic challenge and showed synergistic stimulatory activity compared to the composite of monospecies biofilms. Only the Sg/An/Fn multispecies biofilms elicited IL-6 levels above control, although significantly lower than expected from the composite of the monospecies biofilms. IL-8 was a primary response of the cells to the Sg/An/Fn biofilms, albeit the level to the multispecies biofilm was not enhanced compared to a predicted composite levels from the individual monospecies challenges. Both the Sg/So/Ss and Sg/Fn/Pg biofilms inhibited the production of IL-8. CONCLUSIONS: These results represent some of the first data documenting alterations in profiles of oral epithelial cell responses to multispecies biofilms.

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Oral Presentation

Abstract Title: **Analysis of Single Nucleotide Polymorphisms (SNPs) in the FOXE1 gene and Implications in the Development of Familial Non-Medullary Thyroid Cancer (FNMTC)**

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Abstract:

Background: Thyroid cancer is a life-threatening condition that affects many Americans each year, with the rate of occurrence rising in recent decades. Thyroid cancer can be divided into 4 subdivisions: papillary, follicular, medullary and undifferentiated. Non-medullary makes up the largest subdivision, accounting for nearly 94% of cases, in which 3-6% of these cases are FNMTC. SNP rs965513, located on chromosome 9q22.33 near FOXE1/TTF2, was associated with papillary and follicular thyroid cancer in a large Icelandic study. The aim of this study was to determine whether rs965513 was associated with a FNMTC cohort from the US. Methods: Peripheral blood was collected from 36 FNMTC patients (26 Papillary, 10 Follicular; 22 kindreds), 53 sporadic NMTC patients (43 Papillary, 10 Follicular) and 24 healthy volunteers. DNA was isolated from white blood cells. The SNP rs965513 was analyzed by TaqMan® genotyping on the Roche Lightcycler ®480. Both Hardy-Weinberg Equilibrium (HWE) and Chi-square association analyses (with co-dominance m.o.i.) were performed with significance set at p=0.05. Results and Conclusions: SNP rs96513 did not deviate from HWE. Although no association was observed in this study, there appears to be a trend with the allele A being present more frequently in FNMTC than in healthy subjects (p=0.109) and sporadic thyroid cancer cases (p=0.206). This is the same allele reported to be associated with thyroid cancer in the Icelandic study. Increased study size will be necessary to determine if this trend is significant in our US population and the A allele plays a role in the development of FNMTC.

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Oral Presentation

Abstract Title: **Association Analysis of Single Nucleotide Polymorphisms (SNPs) rs10054203 and rs944289 in Familial Non-Medullary Thyroid Cancer (FNMTC) Patients**

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Abstract:

Background: FNMTC represents 3-6% of all thyroid cancers. When compared to the sporadic counterpart, FNMTC is associated with more aggressive tumors, increased rates of extra-thyroid extension, lymph node metastasis and a younger age of onset. Currently, no gene mutations have been identified as a causative agent, however, the genetic variations, rs10054203 (in an intron of the hTERT gene) and rs944289 (near the NKX2-1/TTF1gene) may have a role in either FNMTC and/or sporadic cases. The aim of this project was to determine whether these genetic variations play a role in our cohort of FNMTC and sporadic cancer patients. Methods: Blood samples were collected from 36 FNMTC (26 Papillary, 10 Follicular; 22 kindreds), 53 sporadic NMTC cases (43 Papillary, 10 Follicular) and 24 healthy volunteers. DNA was isolated from mononuclear cells and SNPs genotyping was assessed using TaqMan® methodology. Hardy-Weinberg Equilibrium (HWE) and association analyses were performed using Chi-square statistical test (co-dominant m.o.i. and significance at p=0.05). Results and Conclusion: While rs10054203 maintained HWE in the controls, there was a slight deviation from HWE with rs944289, possibly due to our small cohort. No association was observed between FNMTC and rs10054203 (p=0.407) or rs944289 (p=0.266) compared to the control/sporadic cancer subjects in our study. The SNPs rs10054203 and rs944289 do not appear to play a major role in the development of FNMTC in our cohort. Further studies in a larger cohort are required to determine if these genetic variations may play a minor role in the development FNMTC in an American population.

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Oral Presentation

Abstract Title: **Aging Effects on Gingival Antigen Presentation Pathways in Health/Disease**

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Abstract:

A paradigm in Periodontology is that periodontal disease increases in prevalence and severity with age. However, whether periodontitis is a “disease of aging” or an “aging-associated disease” remains to be delineated through more robust molecular studies of the disease during aging. Objectives: To determine the transcriptome of genes related to antigen presentation and adaptive immunity in health and periodontitis gingival tissues from Macaca mulatta across the lifespan Methods: 20 periodontally healthy M. mulatta from 3-25 years of age (5/group: young (Y) <3 yo; adolescent (AL): 3-7 yo; adult (AD): 12-15 yo; aged (AG): >17 yo) and 10 animals with periodontitis (5/group adult and aged) provided gingival tissue specimens. Gene expression was measured using the GeneChip® Rhesus Macaque Genome Array. The transcriptome was mapped to the antigen presentation pathways KEGG database for ontology comparison across the different age groups. Results: In healthy tissues, MHC I-related genes were over-expressed in both AL and AG groups (AL>AG) compared with Y and AD groups, and MHC II pathway genes were up-regulated in the AG with respect to Y and AL animals. Of note, few changes were observed in gene expression related to CD4 and CD8 T cell markers with age; although expression of the killer cell immunoglobulin receptor (KIR) related to increasing NK cell regulatory potential in these tissues was observed. In general, the over-expression of antigen presentation-related genes during periodontitis appeared fairly similar in both adult and aged animals. Interestingly, genes related to both CD8 and NK cell antigen recognition were only elevated in the aged animals with periodontitis. Conclusions: These results suggest that altered gene expression profiles related to antigen presentation pathways were noted in adolescent and aged periodontitis tissues that could reflect a susceptibility to a more aggressive gingival inflammatory response and potential for tissue destruction at these ages.

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Oral Presentation

Abstract Title: **Antenatal and Intrapartum Risk Factors for Dental Caries: A Population-Based Study, Kentucky 2000-2005**

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Abstract:

Purpose: To investigate the relationship between antenatal/intrapartum factors on subsequent use of Medicaid dental services. Methods: A retrospective cohort study was done including children who were enrolled continuously in Medicaid from 2000-2005. Three Kentucky administrative databases were used: 1) Composite birth records from 2000, 2) Individual Medicaid eligibility files, and 3) Medicaid dental claims data covering 2000-2005. Mode of delivery, mother's race, newborn weight, and age at first dental visit were variables included in the analysis. The outcome variables were Medicaid dental claims (restorative/emergency). Unadjusted/adjusted odds ratios were calculated. Results: There were 55,978 live births in Kentucky in 2000. Of the 26,456 Medicaid-enrolled children, 5,342 were continuously enrolled and generate a claim. The bivariate analysis revealed children of Caucasian mothers were 34% more likely to have more than 1 restorative dental claim than children of non-Caucasian mothers (OR=1.34, CI 1.10-1.65, P=.0046). Children born with low-birth weight were 37% more likely to have emergency dental claims (OR= 1.37 CI 1.02-1.83, P=.03). The logistic regression analysis found that the likelihood of a restorative/emergency claim was 33% and 56% respectively higher in children of Caucasian mothers compared with children of non-Caucasian mothers (P<.001). Finally, 2 interesting trends were found; the likelihood of an emergency dental claim was 42% higher for children born with low birth weight and 25% higher for children born by c-section. Conclusions: This study found an association between children of Caucasian mothers and the likelihood of experiencing dental claims. Also a relationship was found between children born with low birth weight and c-section and the likelihood of use of Medicaid dental services.

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191 **Abstract Title:** **Parental Oral Health Literacy and its Effects on Pediatric Oral Health: A Rural Vs. Urban Comparison**

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Abstract:
Purpose: This pilot study investigated oral health literacy differences between parents of pediatric dental patients from urban and rural areas in Kentucky and assessed the impact of parental oral health literacy on pediatric oral health outcomes. Methods: Parents of dental patients aged 1-5 years old were recruited from an urban university pediatric dental clinic and two rural-based pediatric dental practices. Parents completed a 24-item survey assessing sociodemographics and oral health knowledge. The Rapid Estimate of Adult Literacy in Dentistry (REALD-30) measured oral health literacy. Pediatric oral health outcomes were assessed using the Basic Screening Survey, completed by the dentist that examined the patient. Results: Forty-five parent/child dyads participated: 38% were from rural areas and 62% from urban areas. The majority of parents were female (73%) and married (69%). Rural parents are more likely to drive 20+ miles to the dentist compared to urban parents (47% vs. 7%). Urban children were more likely to have caries than rural children (P=.02). The average REALD-30 score was higher for urban participants than rural (23.0 vs. 21.2, respectively); the difference was not statistically significant (P=.2). The correct pronunciation of 'dentition, halitosis, periodontal, and analgesia' was significantly associated with urban residence (P=.017, .04, .04, and .002, respectively). No significant correlation was found between literacy scores and oral health outcomes. Conclusions: In this study, parental oral health literacy was higher among urban residents compared to their rural counterparts, but the difference was not significant. Additionally, parental oral health literacy was not associated with pediatric oral health.

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192 **Abstract Title:** **Oral Health Literacy of Children's Caregivers in an Appalachian County**

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Abstract:
Purpose: The purpose of this study was to describe the oral health literacy of mothers/caregivers of kindergarten and first grade children in an Appalachian county in Kentucky. Methods: A 26 item oral health literacy questionnaire was distributed to mothers/caregivers of kindergarten and first grade children at four elementary schools in Leslie County, Kentucky. Responses were correlated with variables of insurance type and mother/caregiver's education. Results: Of 310 questionnaires administered, 178 (57%) were returned. Sixty-seven percent correctly answered the question, "When should a child have a first dental visit?" Sixty-six percent said that all children will get tooth decay at some time in their life. Eighty-three percent said they have heard of the term "baby bottle tooth decay." Seventy-five percent said they have heard of "dental sealants," but only 67% knew sealants were used to prevent decay. Fifty-eight percent reported having public insurance, while 33% had private insurance. Subjects with private insurance were more likely than those with public insurance to correctly answer the questions "Do all children get tooth decay at some time in their life?" (p=.0037), and "Have you heard of dental sealants?" (p=.0018). Subjects with education beyond high school were more likely to respond correctly to the above two questions (p=0.49; and p=.0067, respectively); and to the question "Have you heard the term baby bottle tooth decay?" than those with less than a high school education (p<.001).

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193 **Abstract Title:** **Viral Vector Gene Delivery for Management of Chronic Orofacial Pain**

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Abstract:
Objectives: We hypothesized that HSV-based vectors for gene therapy after orofacial inoculation do not spread in the central nervous system and that conditional vectors are more efficient in seeding sensory neuronal sites than defective vectors. Methods: In five separate experiments, masseter muscle (MM, n=6) or whisker pad (WP, n=23) of male Sprague-Dawley rats were injected with replication conditional (n=22) or replication defective (n=7) HSV-1-based vectors (5-10 X 107). Tissues from ipsilateral and contralateral trigeminal ganglion (TG), cerebral cortex, midbrain, pons, and brainstem were harvested ≥ 4 weeks post-vector injection. RNA from the tissues was isolated, processed, and analyzed by real-time PCR in duplicate for latency-associated transcripts LAT. Results: HSV-1 LAT was detected in all animals, and only in the ipsilateral TG, pons, or brainstem. LAT was not detected in any of the remaining tissues. The conditional vector was more effectively delivered to the TG (18/22), pons (19/22), and brainstem (7/22) than was the replication defective vector, TG (3/7), pons (4/7), and brainstem (0/7). Masseteric injections resulted in more efficient delivery of HSV-1 to the TG (6/6) than WP injections (13/16) based on LAT detection, irregardless of the vector backbone. Conclusion: This appears to be the first study demonstrating that defective HSV-1-based vectors delivered intradermally and intramuscularly migrate to, but not beyond, the TG, pons. Second order synaptic transmission does not appear to occur. These preliminary findings suggest that potentially therapeutic genes can be delivered within this viral vector to nervous system tissues that innervate orofacial regions.

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194 **Abstract Title:** **Use of an Electronic Nutritional Assessment in Pediatric Dentistry: A Pilot Study**

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Abstract:
Purpose: To assess the effectiveness of an electronic nutritional assessment in Pediatric Dentistry. Methods: Children and their parents who presented to the University of Kentucky Pediatric Dental Clinic for their routine dental appointments were asked to complete the electronic assessment. Then, participants received a brief session of nutritional counseling by a member of the research staff. Additionally, an oral health screening was completed on all participants. Bivariate analysis and unadjusted odds ratio were calculated. Results: Children ranged from 2-17 years old. Eighty four children and their parents agreed to participate and completed the electronic assessment. Of these children, 42% (35) had untreated dental caries and 27% (23) had a BMI that placed them in the overweight or obese categories. The assessment revealed that the daily consumption of three or more drinks with a high sugar content, as well as, low socioeconomic status were linked to children with untreated decay. Children who reported drinking 3 or more drinks with a high sugar content, per day, were 2.15 times (95%CI: 0.83- 5.59, P<.07) more likely to have untreated decay, than children who reported drinking 1 to 2 drinks per day. The odds of having untreated dental caries in children who received food stamps or WIC were 4.50 (95%CI: 1.58-12.8, P< .005) and 3.41 (95%CI: 1.24-9.43, P< .018) times those who did not receive food stamps and WIC, respectively. Conclusions: In this population, low socioeconomic status and consuming a high number of sugary drinks were both risk factors for having untreated dental caries.

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195 **Abstract Title:** **Sampling Techniques in Analysis of Salivary Biomarkers of Myocardial Injury**

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Abstract:
Objective: To evaluate three methods of oral fluid sample collection for assessing oral fluid biomarker levels compared with those in serum following alcohol septal ablation (ASA) - a model of acute myocardial injury. Methods: Unstimulated whole saliva (UWS), buccal gingival swabs (BGS), and sublingual swabs (SLS) and serum samples were collected from individuals undergoing ASA (n=19) and non-AMI control subjects (n=99) at t=0, 4, 8, 16, 24, and 48 hours post-ablation. BGS and SLS were collected using Aware® Messenger Swabs. Non-AMI control subjects provided a UWS, BGS, SLS, and serum sample. BGS was collected from the attached facial gingiva of both arches after 30 sec absorption per arch. SLS was obtained following placement of the swab under ventral tongue adjacent to the lingual salivary duct for 2 minutes. Luminex® immunoassays were performed in duplicate to quantify thirteen relevant biomarkers of cardiovascular disease. Results: Levels of cardiac enzymes troponin I (TnI) and creatinine kinase (CK-MB) showed significant elevations in SLS samples at 16-48 hr and 4-48 hr, respectively compared with baseline ($p<0.05$). Interleukin (IL)-6 and C-reactive protein (CRP) showed elevations at 8-48 hr in BGS samples ($p<0.05$). IL-1β, sICAM-1, and adiponectin were significantly decreased from 8-48 hr in BGS samples ($p<0.05$). Only brain natriuretic peptide (BNP) showed marked increases in both SLS and BGS samples, each being elevated at 16-48 hr ($p<0.05$). Biomarkers showing significance in BGS and SLS were consistent with the kinetic profiles in UWS and serum, but in lower concentration. Conclusion: Biomarkers of ablation (TnI, CK-MB, CRP, IL-6) appear within 8 to 16 hours of myocardial injury and are more effectively identified in UWS > SLS > BGS. These data suggest that the location and method of oral fluid sampling is important for accurate biomarker detection.

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196 **Abstract Title:** **Prevalence and Severity of Periodontal Disease in Crohn's Disease Patients**

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Abstract:
The focus of this study is to establish the incidence and prevalence of periodontial disease in Crohn's disease. In addition it may provide a rationale for new treatment modalities for periodontitis based upon periodontal status of individuals undergoing extensive biologic therapies for Crohn's or other IBD. This proposal investigates the association between periodontal disease and Crohn's disease using the general hypothesis that "patients with Crohn's Disease will have an increased prevalence of periodontal disease. Crohn's disease is a chronic disease of inflammatory dysregulation due to interaction with gut microflora. It has been shown that both the innate and adaptive branches of the immune system are involved. Within the cohort of patients with Inflammatory Bowel Disease (IBD) there are significant numbers with oral inflammatory disease, of which prevalence has not been adequately established. Periodontal diseases manifest as chronic inflammatory lesions of the supporting structures of the dentition. For the purpose of this study periodontal disease will be defined as it is by the American Academy of Periodontology. There are limited previous studies. Some authors have found observable differences in probing depths amongst Crohn's disease patients (Flemming et al.). A study by Brito in 2008 looked at periodontitis and DMFT scores in patients with Ulcerative Colitis and Crohn's disease and compared them to healthy controls. They found an increase in periodontitis and DMFT in IBD patients as compared to their controls. The results of this study show similar results but a focus on patient medications and periodontal inflammation as measured by bleeding.

Supported by: Support through the University of Kentucky College of Dentistry

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197 **Abstract Title:** **Programatic Assessment of Implant Success and Survival in a Graduate Training Program**

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Abstract:
Background: Implant therapy has become a common therapeutic modality in dentistry today and is considered to be a standard of care in patient's seeking replacement of missing teeth1. More and more patients today are aware of the restorative options available to them before receiving treatment, thus it is crucial for implant education to continue be a standard within professional training program's curriculum. Our objective was to assess the effectiveness of a University-based implant training program in relation to implant survival and success. Methods: We conducted a survey of 415 patients treated between the years 2004-2006 at the University of Kentucky College of Dentistry in accordance with the University of Kentucky's Implant Quality Assurance Protocol (IQAP). Information was collected on these de-identified patients regarding implant site, number of fixtures, service placed implant, specific medical questions regarding Diabetes, smoking, history of periodontal disease, bisphosphonate use, pain, mobility, and survival and satisfaction of the implant(s). Survival was defined, as the implant remains in-situ after placement. Success was defined in the present study as patient reported satisfaction with the function, appearance and surgical experience. Loss was defined as the implant not being in the mouth for any reason. Finally, failure was defined as a patient reporting being not satisfied with any component of the implant itself, or the surgical experience. Results: A total of 962 implants were placed. The satisfaction and survival of implants placed was very high with corroborates with other long-term outcome studies. Overall, on a patient level, the survival and success was calculated as 97.8% and 90.1% respectfully. On an implant level, survival and success was reported as 97.5% and 89.5%. Overall, only 77 implants (8.20%) were considered failures and only 27 (2.81%) were lost. The results of this study showed that none of the following variables contributed to failure of implants: gender, age, smoking status, diabetes, osteoporosis or bisphosphonate use. Conclusions: Patient reported outcomes of implants placed in a training program reflect a high level of survival and success. The results of this study showed very low failure and loss rates.

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198 **Abstract Title:** **Differential Phagocytosis of Commensal and Pathogenic Oral Bacteria by Immature Dendritic Cells.**

Author(s):
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C. B. Huang, Center for Oral Health Research, College of Dentistry, U of Kentucky

Abstract:
It is clear that the host immune system has to respond to both commensal and pathogenic bacteria; however, it would be expected that these bacteria interact differently with the immune system. Minimal information is available delineating characteristics of the immune system responding differently to commensal and pathogenic mucosal bacteria. This study examined how immature dendritic cells (iDCs) interact with commensal and pathogenic oral bacteria. Methods: THP-1 cells were grown for 7 days and treated with GM-CSF and IL-4 to drive them into iDCs. Afterwards, the iDCs were challenged with single and various combinations of labeled commensal and pathogenic oral bacteria (P. gingivalis, F. nucleatum) and commensal oral bacteria (S. gordonii) for an hour. Cells were washed with 1XPBS and fixed with 0.01% paraformaldehyde. FACS analysis was performed to determine total uptake and phagocytosis of bacteria by the iDC. Results: 100% of the iDC showed uptake of Pg and Fn at a concentration of 3X106/ml of bacteria; however ingestion/phagocytosis was up to 90% for Pg and only 40% for Fn. Sg was absorbed by iDC at a lower rate than both Pg and Fn, and the ingestion of Sg was substantially lower. Meanwhile if iDC were exposed to Fn and Pg (1:1 ratio) sumultaneously, Fn demonstrated the ability to compete with Pg by lowering the ingestion by the iDC. This effect showed a dose response with even more substantial interference at a Pg:Fn ratio of 1:10. Commensal Sg mixed with Pg did not demonstrate the ability to lower ingestion of Pg by iDC at both a 1:1 and 1:10 ratio Conclusions: Variations in ingestion of Pg, Fn and Sg by immature dendritic cells were demonstrated. Fn showed a competitive ability with the oral pathogen, Pg, while the commensal, Sg, did not influence the uptake of Pg by the iDCs. These data suggest that the 3 bacteria are identified by different pattern recognition receptors with varying effectiveness in triggering ingestions for antigen presentation. Additionally, it appeared that Fn and Pg may compete for similar receptor(s), potentially TLR4.

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199 **Abstract Title:** **The Effect of Omega-3 Polyunsaturated Fatty Acids (n-3 PUFA) on Oral Biofilms**

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Abstract:
Introduction: Fish oils have recently been shown to effectively inhibit the growth of many planktonic oral bacteria, such as Streptococcus mutans. However, little is known about the effect of n-3 PUFA on the oral bacterial biofilms, which is the principal form of oral bacteria in situ. This study tested the ability of n-3 PUFAs [α-linolenic acid (ALA), eciosapentanoic acid (EPA) and docasapentanoic acid (DHA)] to alter the formation of S. mutans in vitro biofilms. Methods: ALA, EPA and DHA were used to treat monospecies biofilms grown in 96-well or 48-well plates for 2 days. The biofilms were analyzed after the n-3 PUFA treatments, using microplating, MTT metabolic marker, and crystal violet assays. Results: Low concentrations (25μg/ml) of fatty acid treatment showed significant prevention and inhibition of mono-species biofilm growth, as well as killing the biofilm bacteria, in vitro. Conclusion: The study demonstrated that n-3 PUFAs have a bactericidal effect on S mutans biofilm, yielding both biofilm-static and biofilm-cidal outcomes. These findings suggest that n-3 PUFA may be used as preventive and/or therapeutic compounds for dental caries by preventing this pathogenic species biofilms.

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200 **Abstract Title:** **Exploring the Prescribing Practices of Dentists in Kentucky**

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Abstract:
It is established that “pill mills” and unethical practitioners are at the core of prescription drug misuse and abuse. Corruption and malpractice exist in any field of medicine, including dentistry, and can only be resolved when discovered. However, the other contributing factor lies in well-intentioned health care professionals prescribing these pain medications legally and, for the most part, appropriately. The Department of Justice reports that “young people rarely obtain prescription drugs using methods commonly associated with pharmaceutical diversion such as pharmacy theft, prescription fraud, or doctor shopping—visiting numerous doctors to obtain multiple prescriptions. Instead, adolescents typically obtain prescription drugs from peers, friends, or family members”. This study is an attempt to track the prescribing practicing of dentists in Kentucky and evaluate the dental community’s contribution to the availability of addictive substances. The study population consists of a sample of currently practicing dentists (D.M.D or D.D.S) who were surveyed about their prescribing practices. The data is currently being entered and analyzed. In addition, a de-identified data set from the Kentucky All State Prescribing Electronic Record (KASPER) system has been requested and will be analyzed separately.

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201 **Abstract Title:** **Genetic Association Analysis of CYP19A1 in Pubertal Sagittal Facial Growth**

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Abstract:
Objective: A more accurate facial growth prediction would take into account the genetics of each individual patient. The CYP19A1 gene encodes the aromatase enzyme which catalyzes estrogen biosynthesis via the conversion of androgens. The androgen/estrogen ratio affects the difference in development of male and female facial characteristics. Genetic variation in CYP19A1 has been associated with different average sagittal facial growth in Chinese males. Our null hypothesis (HO) was that the single nucleotide polymorphisms (SNPs) rs2470144, rs2445761 and rs730154, located within in the CYP19A1 gene, are not associated with variation in average sagittal jaw growth during puberty in Caucasians. Method: Genotypes for rs2470144, rs2445761, and rs730154 were determined by Taqman®-based methodology in 141 subjects who began orthodontic treatment at cervical stages (CS) 2 or 3 and progressed to CS4 or CS5. Pre- and post-treatment lateral cephalometric radiographs were measured with Dolphin software. Pre-orthodontic measurements and annualized average changes of maxillary and mandibular sagittal lengths were compared among genotypes for each SNP by ANOVA ($p \leq 0.05$). Results: Based on linear regression modeling assuming a co-dominant mode of inheritance (MOI), the heterozygous females (TC) at rs2470144 are associated with increased annualized maxillary growth compared to females with the homozygous major allele (TT) ($p=0.03718$). Similarly, the heterozygous females (TC) at rs2445761 are associated with increased maxillary growth compares to females with the homozygous major allele (TT) with a co-dominant MOI ($p=0.05086$). Statistically significant differences were not observed in the males. Conclusion: We rejected the null hypothesis for females and accepted the null hypothesis for males.

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202	**Abstract Title:**	**Unraveling A Genetic Association Between Hypodontia and Epithelial Ovarian Cancer (EOC)**

Author(s): A.N. Vu, Division of Orthodontics, U of Kentucky; K.C. Kirk, U of Kentucky; M. Gilbey, College of Dentistry, U of Kentucky; G. Falcão-Alencar, Department of Oral Health Practice, U of Kentucky; M. Sakamoto, Division of Orthodontics, U of Kentucky; J.K. Hartsfield, Division of Orthodontics, U of Kentucky; L.A. Morford, Department of Oral Health Practice, U of Kentucky

Abstract:
Objectives: Women diagnosed with EOC are 8.1 times more likely to have hypodontia than women without EOC. The genetic link for this remains unknown. This study investigates whether single nucleotide polymorphisms (SNPs) located within/near the RUNX2 and FGFR2 genes are associated with hypodontia. If a genetic association is identified, the SNPs will be tested for a dual association of hypodontia with a cancer family history. Methods: Female and male orthodontic patients are being recruited into two groups: hypodontia patients and controls. DNA is being collected from saliva along with a three-generation family health history. SNPs are being genotyped with Taqman® methodology. Chi-square analysis will access Hardy-Weinberg equilibrium (HWE) in the controls and test for association with hypodontia (significance at $p<0.05$). Results: Hypodontia was identified more often in women than men; with maxillary lateral incisors being the most common teeth affected followed by mandibular 2nd premolars. DNA has been genotyped in 26 hypodontia patients and 37 controls and is still ongoing. All genotypes tested have maintained HWE within the control population. Thus far, FGFR2 (rs2981578) was analyzed in 25 hypodontia-patients and 34 controls ($X2=2.259$; $p=0.323$) and RUNX2 (rs1406846) analyzed in 26 hypodontia-patients and 37 controls ($X2=2.813$; $p=0.2449$). Similar subject numbers have been analyzed for RUNX2-related SNPs, rs4714854 and rs1928533, with $p=0.4628$ and 0.7528, respectively. Conclusion: Hypodontic teeth reported in this study are consistent with those previously reported missing in women diagnosed with EOC plus hypodontia. The influence these variations have on hypodontia will be revealed when recruitment and genotyping is complete.

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203	**Abstract Title:**	**White Spot Lesions: Etiology, Prevention and Treatment**

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Abstract:
The development of decalcifications, also known as white spot lesions, during orthodontic treatment is a significant problem. Previous research demonstrates up to 97% of orthodontic patients develop these lesions during treatment, with up to 50% of patients developing them during the first month. On average, the lesions affect 1/3 of all buccal tooth surfaces, but are more prevalent in males than females. Their development is directly correlated with the level of hygiene practiced by the patient. Their opaque clinical appearance differs from fluorosis, hypomaturation in amelogenesis imperfecta, and other classes of decalcifications. Maintaining excellent oral hygiene is the most effective method to prevent the lesions. Additionally, research suggests using RMGI instead of composite to bond the brackets, applying periodic fluoride varnishes or using a sealant such at Pro-Seal at the initiation of treatment, and recommending the use of MI Paste may also reduce the incidences of the lesions. If the patient begins developing lesions while in orthodontic appliances, oral hygiene instruction should be given, MI Paste should be recommended, and a frank discussion of the possibility of early debonding should be undertaken. When lesions are identified, fluoride should not be used as it arrests the lesions and does not allow for remineralization. Once appliances are removed, an average of 33% of lesions will remineralize naturally within 6 weeks and up to 50% within 12 weeks. It is recommended to allow 6 months for natural remineralization before pursuing more aggressive treatment (such as microabrasion) or initiating fluoride therapy.

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204	**Abstract Title:**	**Association Analysis of Genetic-Variations in Genes Establishing Dental-Fields and Palatally-Displaced-Canines**

Author(s): N. Williams, Hereditary Genomics Laboratory, College of Dentistry, U of Kentucky
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J.K. Hartsfield, Oral Health Science, College of Dentistry, U of Kentucky
L.A. Morford, Oral Health Practice, College of Dentistry, U of Kentucky

Abstract:
Objective: Palatal-displaced-canines (PDCs) are maxillary canines which have been developmentally displaced to the palate, frequently resulting in impaction. PDCs often occur concurrently with several other tooth anomalies, including small or pegged-shaped lateral incisors, agenesis of lateral incisors, and agenesis of second and/or third molars. The concurrent lack of tooth development with PDCs may be indicative of alterations in dental field patterning. This study investigated the hypothesis that variations within genes that establish dental-fields, such as BMP4-(rs17563) and FGF8-(rs1348870), will be associated with the formation of PDCs. Method: DNA was isolated from buccal swabs of 130 Caucasian subjects undergoing orthodontic treatment (35 subjects with unilateral/bilateral PDCs and 95 controls). All TaqMan® genotyping was completed on the Roche LightCycler480®. PDC-phenotypes were determined by treatment records and radiographs. Hardy-Weinberg-Equilibrium (HWE) and Chi-Square analysis for association were performed assuming a co-dominant mode of inheritance with significance at ($p<0.05$). Result: All genotypes tested in this study maintained HWE within the control population. BMP-4 (rs17563) was analyzed in 35 subjects with PDCs (14GG, 17GA, 4AA) and 95 controls (30GG, 45GA, 20AA) and is being analyzed with expanded subject numbers ($X2 = 1.872$; $p=0.4011$). FGF8 (rs1348870) was analyzed in 31 subjects with PDCs (7GG, 12GA, 12AA), and 51 controls (7GG, 23GA, 21AA) and is also undergoing additional subject analyses ($X2 =1.099$; $p=0.57724$). Conclusion: Preliminary findings suggest that the genetic variations in dental-field-defining-genes warrant further investigation. We are expanding the number of subjects with PDCs and controls in our study, in addition to testing additional SNP-markers in dental-field-defining-genes.

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205	**Abstract Title:**	**Genetic Association Study of P2RX7, CASP1, and IL1-B Single Nucleotide Polymorphisms (SNPs) and External Apical Root Resorption (EARR) During Orthodontia**

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Abstract:
Objective: Genetic variation in the IL-1ß gene is associated with increased external-apical-root-resorption (EARR) in orthodontic patients. Caspase1 and P2RX7 are upstream regulators of mature IL-1ß protein activation. This study investigated the potential association of SNPs within or near the P2RX7 (rs1718119 and rs208294), CASP1 (rs530537 and rs580253), and IL-1ß (rs1143634) genes with EARR of the maxillary incisors in orthodontic patients. Methods: DNA was isolated from buccal swabs of up to 424 Caucasian patients. SNPs were analyzed by Taqman® genotyping. Pre and post-treatment radiographs were visually analyzed for EARR of the maxillary-incisors by 3 reviewers. Subjects were considered “affected” when 2-of-3 reviewers agreed that EARR was present. Both Hardy-Weinberg Equilibrium (HWE) and Chi-square analysis for association were performed under a co-dominate mode of inheritance, with significance set at ($p<0.05$). Results: All five analyzed SNPs maintained HWE within the control population. Seventy one subjects with EARR (39GG/24GA/8AA) and 300 control subjects (183GG/108GA/9AA) were analyzed for the IL-1ß SNP, rs1143634. This SNP was the only genetic marker tested with a significant association with EARR ($X2=8.996$, $p= 0.01113$). The p-values for the other SNPs tested ranged from $p=0.3725$ (for Caspase1 rs580253) to $p=0.75729$ (for P2RX7 rs1718119). Conclusions: This study further confirms that IL-1 ß plays a critical role inflammatory resolution during orthodontic treatment (versus the RANK-RANKL-OPG side).

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206	**Abstract Title:**	**Patients with Myofascial Pain Who Smoke Report Significantly More Sleep Disturbances than Those Who Don't Smoke**

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Abstract:
Cigarette smoking has been associated with sleep disturbances in the pain population. We hypothesize that patients with masticatory myofascial pain who smoke will present with more sleep disturbances than those who don't. Patients referred to the Orofacial Pain Center of the University of Kentucky, who completed a pain questionnaire and a battery of psychometric tests, and were clinically diagnosed with myofascial pain according to the RDC/TMD were included in the study. The sample consisted of 996 patients (87.1% female). Two-hundred-eighty-seven (28.8%) endorsed to be smokers. Smokers reported significantly more pain on several pain measures as demonstrated by independent samples t-tests. Except for the use of sleep medications, smokers reported significantly more issues with sleep. Although the total sleep score was elevated in both smokers and non-smokers, the score was significantly higher in smokers. Finally, smokers also reported more depression and anxiety (all p's < 0.0001). Multiple regression models indicated that smoking predicts quality of sleep (β=.23, p<0.0001, r2= .051). However, after controlling for the co-variables anxiety, depression and pain severity, the relationship between smoking and sleep quality was attenuated (β=.131, p<0.0001 for smoking, β=.011, p=.55 for anxiety, β=.166, p<0.0001for depression, and β=.350, p<0.0001 for pain severity). The overall model predicted significantly more variability (r2= .207, Δ r2=.156). In conclusion, cigarette smoking seems to be a good predictor of sleep quality, although after including the co-variables this relationship was diminished. Anxiety does not appear to predict sleep quality above smoking; conversely pain severity appears to be the best predictor.

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207	**Abstract Title:**	**Genetic Association Analysis of Growth Hormone Receptor (GHR) in Pubertal Sagittal Facial Growth**

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B. Sloan, College of Dentistry, U of Kentucky; J.K. Hartsfield Jr., College of Dentistry, Div of Orthodontics, U of Kentucky

Abstract:
Background: Understanding how facial dimensions change during the pubertal growth spurt (PGS) is essential for effective orthodontic treatment planning. A main determinate of growth during the PGS appears to be associated with Growth Hormone/Growth Hormone Receptor (GHR) signaling. The aim of this study was to analyze three Single Nucleotide Polymorphisms (SNPs) in the GHR for their impact on maxillary (Co-ANS) and mandibular growth (Co-POG). Methods: Caucasian subjects are being chosen for the study based on cervical vertebral maturation stage from a database of ~1,480 patients. For each subject, pre and post treatment lateral cephalometric radiographs are being analyzed using Dolphin Cephalometric software. The corresponding DNA sample is being genotyped utilizing Taqman® Methodology for SNPs rs6180, rs4130114 and rs2972408 within or near the GHR gene. Data analysis with the ANOVA test is being used (significance at p<0.05). Results and Conclusions: SNPs in the GHR gene have previously been associated with variations in adult mandibular ramus height in Asian samples. Since the previous SNPs analyzed are uncommon in Caucasians, SNPs were identified in Caucasians to investigate an GHR-association with growth in sagittal length of the jaws during orthodontic treatment for an average of two years during the PGS. There is a clear effect on average growth change and genotype for the mandible (and less for the maxilla) with males and females at rs6180, however the statistical significance is borderline with the small sample size. The sample size will be increased and additional cephalometric measurements such as ramus height (Co-Go) will be analyzed.

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208 **Abstract Title:** **Effect of Treatment of Gingivitis on Select Salivary Biomarkers Levels**

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Abstract:
Purpose: The purpose of this study is to determine the level of select inflammatory and connective tissue destructive biomarkers, interleukin-1β (IL-1β), interleukin-6 (IL-6), matrix metalloproteinase-1 (MMP-1), matrix metalloproteinase-8 (MMP-8), macrophage inflammatory protein-1-alpha (MIP-1α), and prostaglandin E2 (PGE2) in whole saliva of persons diagnosed with gingivitis before and after treatment. Specific Aims: 1) To identify salivary biomarkers associated with gingivitis. 2) To determine if levels of salivary biomarkers of gingivitis change after standard treatment of gingivitis. 3) To identify salivary biomarkers associated with response to treatment. Research Hypothesis: Select inflammatory and connective tissue destructive biomarkers are present in saliva in greater quantities in gingivitis subjects prior to receiving dental prophylaxis treatment compared to post-treatment. Background & Significance: The gingival tissue is consistently subjected to bacterial assult. Gingivitis is a reversible inflammation of gingival tissue without the loss of attachment. It manifests as redness, edema, and bleeding on external stimulus. It has not been determined the amount or severity of gingival inflammation that must be present in an individual to be considered a gingivitis case. It has been shown that the time necessary for the development of clinically detectable gingivitis ranges between 10-21 days after the cessation of oral home care. Gingivitis or Gingival inflammation is a reversible entity and resolves in about 1 week after the reinstitution of oral hygiene procedures.9 However, gingivitis may ultimately progress in a subset of individuals to periodontitis if left untreated. The purpose of the present study is to assess the level of select biomarkers in gingivitis subjects before and after treatment.

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