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Oral Presentation Abstracts

Abstract Title:	**Combined genetic polymorphisms and environmental factors in the etiology of a chronic TMD murine model**
Author(s):	K. N Westlund, Dept of Physiology, University of Kentucky S. L McIlwrath, Dept of Physiology, University of Kentucky H. S Oz, Dept of Physiology, University of Kentucky

Abstract: Temporomandibular joint (TMJ) Inflammation and hypersensitivity were induced in mice lacking both TNFalpha receptors (TNFR1/R2 KO). After recovery from the initial priming TMJ insult with complete Freund's adjuvant, a minor inflammatory "double-hit" initiated a recrudescence of inflammation and hypersensitivity two weeks later persisting at least 18 weeks. While mechanical thresholds and heat response latencies in WT mice returned to baseline after both insults, the TNFR1/R2 KO developed chronic mechanical and heat hypersensitivity persisting 18 weeks. Both mechanical and heat hypersensitivity were attenuated by NMDA receptor antagonist MK801, P2X7 inhibitor A438079, reactive oxygen species scavenger phenyl-N-t-butyl nitrone (PBN), and human TNFalpha neutralizing antibody Etanercept. At weeks 2 and 18, the TNFR1/R2 KO and WT mice had very different cytokine profiles. At 2 weeks when the initial inflammation induced hypersensitivity had resolved, serum levels of TNFalpha, RANTES, and MIG were twice as high in TNFR1/R2 KO while CXCL11 was decreased compared to WT mice. After 18 weeks when hypersensitivity is chronic, G-CSF, IFNg and TNFa serum levels in TNFR1/R2 KO significantly increased relative to WT. IL-16 and CXCL9 decreased compared to WT. Conclusions: The "double hit" inflammatory model demonstrated TMD can be "re-ignited" by minor GI insult to become a chronic condition in mice with dysregulated TNFalpha. Increased TNFalpha was accompanied by unique profile of inflammatory mediators.

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Abstract Title:	**Catechol-O-methyltransferase Inhibition Alters Pain and Anxiety-Related Volitional Behaviors Through Activation of β-adrenergic Receptors in the Rat.**
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Abstract: Reduced catechol-O-methyltransferase (COMT) activity resulting from genetic variation or pharmacological depletion results in enhanced pain perception in humans and nociceptive behaviors in animals. Using phasic mechanical and thermal reflex tests (e.g. von Frey, Hargreaves), recent studies show that acute COMT-dependent pain in rats is mediated by β-adrenergic receptors (βARs). In order to more closely mimic the characteristics of human chronic pain conditions associated with prolonged reductions in COMT, the present study sought to determine volitional pain-related and anxiety-like behavioral responses following sustained as well as acute COMT inhibition using an operant 10-45°C thermal place preference task and a light/dark preference test. In addition, we sought to evaluate the effects of sustained COMT inhibition on generalized body pain by measuring tactile sensory thresholds of the abdominal region. Results demonstrated that acute and sustained administration of the COMT inhibitor OR486 increased pain behavior in response to thermal heat. Further, sustained administration of OR486 increased anxiety behavior in response to bright light, as well as abdominal mechanosensation. Finally, all pain-related behaviors were blocked by the non-selective βAR antagonist propranolol. Collectively, these findings provide the first evidence that stimulation of βARs following acute or chronic COMT inhibition drives cognitive-affective behaviors associated with heightened pain that affects multiple body sites.

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Abstract Title:	Facial Shape Analysis of 13 Brazilian Families with Class III Malocclusion
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Abstract: Objective: Phenotypic heterogeneity among Class-III malocclusion cases makes this malocclusion challenging to study its etiologies and to treat. Our purpose was to perform preliminary cluster-analysis of cephalometric data from 29 Class-III patients from 12 Brazilian-families. Skeletal features both in and among Class-III subjects from different families have not previously been compared. Our goal was to sub-classify Class-III skeletal features, and determine whether specific features ran within the families. Methods: Class-III diagnosis and informed consent were obtained for a study initiated at the Universidade De-Brasilia, Brazil. Initial-treatment-cephalographs were digitized, values determined for 11 variables. A cluster-analysis of the data performed using JMP-statistical software. Results: Five unique Class III sub-types were identified; Cluster-1 (CL1)(n=12) Small upper-face height, small maxilla, small mandible, average mandibular-plane angle, CL2(n=5) , Small upper-face height, large mandibular-plane angle; CL3, (n=9) Small upper-face height, protrusive-maxilla, protrusive-mandible, small mandibular-plane angle; CL4(n=2), Large mandible, small mandibular-plane angle , severest Class-III based on Wits; and CL5(n=1) Large upper-face height; large maxilla, large mandible , small mandibular-plane angle, least Class-III according to Wits. A single facial-cluster did not consistently run within a family with more than one member. Conclusions: All related family members did not segregate to same cluster. Family members were not segregated by age or sex into different clusters. While it is a tenet of genetics-analysis to study phenotypes with the same genetic etiology, the variability of phenotypic expression, as well as incomplete penetrance observed in other studies indicate the genetic studies of Class-III should be on a linkage within a family-basis more than genetic-association basis.

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Abstract Title:	**Bisphosphonates and Skeletal Metastasis: Resorption Agonists Reduce Zoledronic acid's Effectiveness in Bone-Tumour Microenvironment by Modulating PTP Expression in Osteoclasts**
Author(s):	S. N. Pandruvada, College of Dentistry, U of Kentucky J. L. Ebersole, Center for Oral Health Research, U of Kentucky S. S. Huja, College of Dentistry, U of Kentucky

Abstract: Zoledronic acid (ZOL) is a bisphosphonate (BP) currently in use to treat bone loss and skeletal complications of several neoplasms. However, the effectiveness of ZOL in tumor microenvironment is drastically reduced when compared to its efficacy in the treating post-menopausal osteoporosis. Bone-tumor microenvironment is a complex niche due to the presence of numerous bone resorption agonists (RA) secreted by tumor cells. Our overall objective was to investigate the mechanisms of resistance developed by osteoclasts (OC) to escape the detrimental effects of ZOL in tumour microenvironment simulated using an in vitro model. OC prepared from human peripheral blood mononuclear cells were differentiated in the presence of ZOL and conditioned media from oral squamous cell carcinoma cell line OSCC12 (OSCC-CM) as a source of RA. Quantification of TRAcP positive OC revealed a 33% reduction in OC number following ZOL (10nM) treatment, but this inhibition by ZOL was reduced to ½ when cultured along with OSCC-CM (ZOL+OSSCC-CM). QPCR analyses done following a 48h treatment with ZOL revealed that non-receptor type protein tyrosine phosphatase 13 (PTPN13) gene expression levels were reduced by 6-fold. In contrast, there was a 3-fold increase of PTPN13 levels following ZOL+OSCC-CM treatment when compared to ZOL alone. In vitro inhibition of PTPs using Orthovanadate (5μM) for 48h made OC susceptible to ZOL-induced effects. Based on these results, we propose that sustained PTP elevation in OC is responsible for reduced efficacy of ZOL and simultaneous inhibition of PTPs would help overcome the development of resistance and improve the outcomes of BP treatment in tumour microenvironments.

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Abstract Title:	**S. gordonii-induced Oral Epithelial Chemokine Transcription and Translation are disconnected**
Author(s):	J.L. Ebersole, Center for Oral Health Research, U of Kentucky S. Kirakodu, Center for Oral Health Research, U of Kentucky Y. Alimova, Center for Oral Health Research, U of Kentucky O.A. Gonzalez, Center for Oral Health Research, U of Kentucky

Abstract: Objective: The role of commensal bacterial species in maintaining oral health, or contributing to an inflammatory disease process remain unclear. Activation of oral epithelial cell (OEC) innate responses (e.g., chemokine production) by oral commensal bacteria needs to be tightly and specifically regulated to avoid inflammatory disease. We sought to determine the OEC chemokine response profiles induced by the oral commensal S. gordonii (Sg), and the Sg effect on the expression of specific miRNAs with potential for regulating chemokine production. Methods: Human oral epithelial cells were challenged with Sg or F. nucleatum (Fn) as positive control. Abundance of transcripts for 25 chemokines was analyzed using NanoString technology. Time and dose-responses for mRNA and protein levels of representative chemokines (i.e., IL-8, CCL20 and CXCL10) were determined by qRT-PCR and ELISA. The ability of Sg to modulate expression of miRNAs was evaluated using the GeneChip miRNA 4.0 Array (Affymetrix) and miRTarBase. Results: Higher number and levels of chemokines were transcriptionally activated by Sg (17/25) compared with Fn (10/25). Transcriptional activation of IL-8, CCL20 and CXCL10 by both bacteria occurred early (4h); however mRNA levels increased or remained elevated until 24h in cells exposed to Sg but not Fn. Lower Sg concentrations induced higher CXCL10 mRNA levels. IL-8 protein levels were more rapidly and efficiently increased by Fn, and constitutive CCL20 and CXCL10 protein expression was inhibited by Sg and increased by Fn. miR-663a, miR-4516, miR-492 and miR-193a-5p were identified as candidates for regulating Sg-induced chemokine transcription and translation. Conclusion: Chemokine transcription and translation activated by the oral commensal Sg in OECs is disconnected and could involve regulation through specific miRNAs. A better understanding of the OEC tolerogenic mechanisms to oral commensal bacteria and how disruption of those mechanisms can lead to disease could help to develop strategies to prevent oral chronic inflammation.

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Abstract Title:	**Apoptotic pathways during initiation, progression, and resolution of periodontitis across the lifespan**
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Abstract: Growing evidence indicates that apoptosis regulates the inflammatory response through the generation of anti-inflammatory signals for phagocytes and removal of inflammatory cells from the tissues. Although variations in apoptotic events during periodontitis have been previously shown, the role of apoptosis in the pathogenesis of periodontal disease remains unclear. Objective: To develop ontologic analysis of apoptotic genes/pathways during initiation, progression, and resolution of periodontitis. Methods: A cross-sectional study of healthy tissues from 4 age groups of rhesus monkeys (M. mulatta; young, adolescent, adult, aged) (n=23) and periodontitis (adult, aged; n=11) animals examined naturally existing apoptotic gene profiles. The ligature-induced periodontitis model was used in animals from the same age groups (n=45). Gingival tissues samples were taken at baseline pre-ligatures, at 2 weeks and 1 month (Initiation), and at 3 months (Progression) post-ligation. Ligatures were removed and samples taken 2 months later (Resolution). Total RNA was isolated from tissues and the Rhesus Gene 1.0 ST (Affymetrix) used for expression analysis of 87 apoptotic genes. Gene expression profiles were mapped to the KEGG database for ontology comparative analysis of different time points. Results: Cross-sectional results demonstrated unique profiles of pro- and anti-apoptotic genes in periodontitis versus healthy tissues, and mutually exclusive gene changes in the adult compared to the aged animals. Furthermore, a set of apoptotic genes was significantly correlated with aging in healthy tissues, with specific genes increasing or decreasing across the age range. Initiation of periodontitis demonstrated significant clinical differences across the age groups, with younger animals exhibiting lower levels of destructive disease. The gingival gene expression studies showed a greater balance in alterations in pro- and anti-apoptotic genes in the young versus aged animals. Interesting gene targets during disease progressing showing these differences included members of the PI3-kinase family, a group of IL-1 genes, and PRKA members. With resolution, calcineurin regulatory genes and IKKA, an inhibitor of NF□B differentiated the age groups. Conclusion: Intrinsic and extrinsic apoptotic genes/pathways are engaged with the initiation of periodontitis, while variation in the expression of specific subsets of apoptotic genes is associated with both progression and resolution of disease. Persistently altered balances in expression of apoptotic molecules during the course of disease in aged individuals could suggest an age-related impaired apoptotic response in periodontitis.

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