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Orexin/hypocretin receptor 2 (HCRTR2) in alcohol dependence diagnosis and severity: An exploratory investigation in the role of HCRTR2 rs2653349 polymorphism

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OBJECTIVES/SPECIFIC AIMS: The preliminary analysis sought to retrospectively characterize the role of hypocretin receptor 2 (HCRTR2) in the development and prognosis of AD along with associated behavioral measures including smoking, self-reported drinking history, and neuroticism. Given the results in this study along with the paucity of information regarding the functional significance of rs2653349, we intend to comprehensively characterize HCRTR2 using haplotype analyses. We will then identify relationships between our haplotype analysis and IV alcohol self-administration using the Computer-Assisted Infusion System, and phenotypes identified in a sleep study. Furthermore, we aim at identifying functional loci in the hypocretin/orexin system by investigating differential allele expression in the orexin receptors in hippocampus tissue obtained from postmortem human brains. METHODS/STUDY POPULATION: This study examined 1569 European American and African American individuals between 18 and 65 years old, 922 of whom with a current diagnosis of AD. Participants were genotyped for HCRTR2 rs2653349 and ancestry was determined via a genome-wide panel of ancestry informative markers. AD was diagnosed using the Structured Clinical Interviews for DSM-IV (SCID-IV) for psychiatric disorders and recent alcohol use was assessed by 90-day Timeline Follow-back (TLFB) interviews. Smoking was assessed using the Fagerström Test for Nicotine Dependence and neuroticism was measured using the NEO Personality Inventory. RESULTS/ANTICIPATED RESULTS: In European Americans, a significant difference was found in current AD diagnosis between AX carriers and GG carriers ($z = -2.390$, $p = 0.017$). This relationship remained significant in a logistic regression model controlled for age and gender ($R^2 = 0.269$, $p = 0.015$). TLFB drinking measures were compared based on the median values to correct for the ceiling effect resulting from the assessment covering the past 90 days. Total drinks ($U = 8.280$, $p = 0.004$), number of drinking days ($U = 6.983$, $p = 0.008$), and average drinks per days ($U = 7.221$, $p = 0.007$) were all noted to significantly differ between the two allele groups among Caucasians. The associations between rs2653349 and total drinks ($R^2 = 0.115$, $p = 0.023$) and heavy drinking days ($R^2 = 0.190$, $p = 0.015$) remained significant in linear regressions controlled for age and gender. Furthermore, Caucasian AX carriers had a higher median number of drinking days relative to GG homozygotes among current AD positive subjects ($U = 6.937$, $p = 0.012$) and a lower median number of drinking days among current AD negative subjects ($U = 4.430$, $p = 0.035$). Among Caucasian AD negative subjects, there was a significantly greater frequency of smokers ($\chi^2 = 3.550$, $p = 0.046$). In African American participants, there were no significant differences in AD diagnosis and in measures of AD severity by genotype. African American males diagnosed with current AD had higher rates of smoking in the AX group ($\chi^2 = 4.969$, $p = 0.017$). No significant associations were found between rs2653349 and neuroticism in any of the cohorts analyzed in this sample. DISCUSSION/SIGNIFICANCE OF IMPACT: The results suggest that, among Caucasians, AX carriers have an increased risk to develop AD independently of their age and gender. In addition, among individuals with a diagnosis of AD, AX carriers reported a greater number of drinking days, as measured by the TLFB, suggesting that this polymorphism also exerts an effect on the severity of the disease. This effect on increased alcohol consumption was absent in Caucasian AX carriers without current AD diagnosis. In future analysis, we will explore how different genetic profiles in HCRTR2, and also HCRTR1, may alter the orexin signaling pathway and how such alterations may predispose patients to develop AD and exacerbate AD once it develops.

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Soluble adenylyl cyclase (sAC) regulates melanogenesis and melanocyte response to UVB

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OBJECTIVES/SPECIFIC AIMS: Our objective is to study the role of soluble adenylyl cyclase in the melanocyte regulation of pigment in response to ultraviolet radiation. Melanocytes are specialized cells that produce melanin in organelles called melanosomes, and melanin determines the pigmentation of hair and skin. cAMP is a master regulator of pigmentation and transmembrane class of adenylyl cyclases are essential for expression of important enzymes involved in melanogenesis. However, pigmentation is also controlled by melanosomal pH, which regulates melanogenesis, tyrosinase activity, and melanosome maturation. The relationship between melanosomal pH and cAMP has been elusive. Soluble adenylyl cyclase is a noncanonical source of cAMP that is not responsive to G proteins but rather functions as a pH sensor. We recently demonstrated that loss of soluble adenylyl cyclase (sAC) activity leads to increased melanosomal pH as well as increased pigmentation in cells and hair. We expanded our research to investigate the role of sAC in the intrinsic response of melanocytes to ultraviolet radiation. METHODS/STUDY POPULATION: We utilized sACfl/fl (wild type) and sACKO mouse melanocytes and compared their change in pigmentation in response to ultraviolet radiation. Melanin was used as a measure of pigmentation. We irradiated these cells at differing doses of UVB (0, 1, 2, or 3 mJ/cm^2) daily for 3 days. After UVB treatment, cells were observed and the surviving cell numbers were determined. Cells were then analyzed for melanin content using spectroscopy. RESULTS/ANTICIPATED RESULTS: We found that while both sACfl/fl and sACKO cells had increased melanin content in response to UVB, the melanin content of sACKO cells increased more compared with sACfl/fl cells ($p = 0.001$ at daily dose of 3 mJ/cm^2). In addition, sACKO cells required less UVB dose to induce a response. We also observed that sACKO cells show increased cell death compared with sACfl/fl cells. DISCUSSION/SIGNIFICANCE OF IMPACT: Although both sACfl/fl and sACKO cells can induce melanin production in response to UV, our results suggest that sACKO cells are more sensitive. We believe that this increased response in sACKO cells is due to increased melanosomal pH. In addition, sACKO cells show increased cell death, suggesting that sAC is important in the damage response secondary to UV exposure. UV plays a wide range of roles in skin biology such as contributing to cancer risk and pigmentation. Since pigmentation is essential for the protection of the skin from UV insult, further investigation of possible mechanisms in which sAC can influence pigmentation in response to UV is warranted.

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Doxorubicin exposure in vitro stimulates ROS production and directly suppresses cardiac fibroblast proliferation

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OBJECTIVES/SPECIFIC AIMS: Our research strives to understand the pathophysiology of doxorubicin cardiotoxicity, focusing on the understudied non-myocyte cardiac cells. Our understanding will enable researchers to develop protective or alternative therapies for cancer patients and treatments for cancer survivors. METHODS/STUDY POPULATION: Early studies have been carried out in isolated primary cardiac fibroblasts. Cells were treated with varying doses of doxorubicin. Cell viability, proliferation, and reactive oxygen species generation have all been studied. Future studies will focus on mitochondrial assessment in treated cells and confirmation of findings in animal models. Potential therapies discovered in these studies will also be conducted in animal models. RESULTS/ANTICIPATED RESULTS: Our results show a direct effect of doxorubicin on cardiac fibroblasts in vitro. Treated cells show a decreased rate of proliferation and increased production of reactive oxygen species. Similarly to cardiomyocytes, we hypothesize that reactive oxygen species damage the mitochondria of cardiac fibroblasts thereby altering their function and playing a role in doxorubicin cardiotoxicity. DISCUSSION/SIGNIFICANCE OF IMPACT: Current therapies have not been able to adequately protect patients from the cardiotoxicity of doxorubicin and other anthracyclines. A complete understanding of how doxorubicin damages cardiac tissue will only be possible by studying all cell types of the heart. With a better understanding, alternative therapies can be developed to prevent or treat doxorubicin cardiotoxicity without sacrificing the efficacy of doxorubicin in treating cancer.

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Receptor for advanced glycation end-products: Mitigating the persistent effects of particulate matter induced airway injury

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OBJECTIVES/SPECIFIC AIMS: Obstructive lung disease following particulate matter (PM) exposure is a major health concern. Coexisting metabolic

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