deleterious role for macrophages in tumor progression. Next, using nearest neighbor analysis we examined the effect of HLA-DR and Ki67 expression on spatial distribution of CD3+ CD8+ T cells. We find that CD8+ T cells are closer to myeloid (CD68+) cells expressing HLA-DR. This is consistent with the potential of HLA-DR expressing cells to present antigens to T cells, and suggests that T cells may preferentially interact with HLA-DR expressing myeloid cells. Conversely, we find that Ki67 expression on tumor (SOX10+) cells correlates with increased distance from CD3+ CD8+ T cells relative to SOX10+ Ki67- tumor cells. This finding is consistent with the observation that more advanced tumors with higher mitotic rates have decreased T cell infiltrates, and suggests that dividing melanoma cells are less likely to interact with T cells. In addition, we performed analysis to determine whether spatial relationships defined above impact prognosis. Clinical oncology follow-up was available on 35 of the 57 patients evaluated above. We compared proximity of CD3+ CD8+ cells to both myeloid (CD68+) and tumor (SOX10+) cells in patients who recurred and those with no evidence of recurrence. We found that CD3+ CD8+ cells in patients who had recurrence were closer to CD68+ HLA-DR− cells than in patients who had no recurrence (*t*-test, $p = 0.0377$), this correlated with DSS ($p = 0.003$). Conversely, distance from CD3+ CD8+ to CD68+ HLA-DR+ in relationship to recurrence was not significant with a trend towards CD3+ CD8+ T cells being closer in nonrecurrent patients (*t*-test, $p = 0.1362$). DISCUSSION/SIGNIFICANCE OF IMPACT: Consistent with the literature, we find that densities of CD8+ T cells correlates with favorable outcomes in early stage melanoma. We also find that density of CD68+ macrophages in stroma correlates with poor outcome. If proximity is a surrogate for interaction, these data indicate that dividing, Ki67+, melanoma cells interact less with CD8+ T cells than do Ki67+ melanoma cells. Further, HLA-DR expression on CD68+ infiltrating cells likely enhances their interaction with T cells. Interestingly, on further analysis, CD3+ CD8+ cells were significantly closer to CD68+ HLA-DR− cells in patients who recurred, implying that interactions between these cell types may not be favorable. This analysis demonstrates that spatial analysis may be useful in predicting prognosis in early stage melanoma, and this is the first report of this type of analysis predicting outcomes in primary tumor specimens to our knowledge. Further staining and analysis of the complete patient cohort (n = 120) is ongoing.

2370

Understanding epicardial fat biology by imaging

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OBJECTIVES/SPECIFIC AIMS: The goal is to understand the underlying mechanism of epicaridial fat biology and its response to cardiometabolic disease by using quantitative multi-echo Dixon (mDixon) of water and lipid sequence, T2* blood-oxygen-level-dependent (BOLD) sequence of iron content, and data analysis methods to determine the quantity of brown Versus white fat. To accomplish this goal, we propose to define the histological, genetic, and metabolite state of epicardial fat and to confirm the relationship between fat phenotype and magnetic resonance (MR) characteristics. We will then investigate whether MR is more effective in identifying patients with lower cardiovascular disease risk than computed tomography (CT). METHODS/STUDY POPULATION: We will recruit 100 patients undergoing open-heart surgery and will quantify mDixon (proton density fat fraction), BOLD (T2*), and T2/T1 maps of epicardial, extrapericardial, and subcutaneous fat before their surgery. We will then (a) validate MR findings by direct depot-specific tissue analysis for lipid content, histological, and genetic markers of inflammation and brown and white fat, (b) develop plasma and fat depot specific metabolite profiling of cardiovascular disease risk and correlate with imaging characteristics. We will categorize cardiovascular risk score (Cardiovascular Health Status) of our 100 patients on quartiles. We will then build models where the categorized cardiovascular risk score are regressed on MR measures (epicardial fat fraction, T2*, and T2/T1 maps) and CT measures (epicardial fat volume and coronary calcium score). RESULTS/ANTICIPATED RESULTS: We anticipate to learn about epicardial fat biology and the role of inflammation in cardiometabolic disease. We will validate proton density fat fraction, T2* and T2 map against histology of epicardial fat for lipid content, established markers of brown and white fat and inflammation, respectively, to help us translate imaging technique to clinical practice. In respect to our second aim we anticipate that MR identifies patients at lower cardiovascular risk quartile than CT. DISCUSSION/SIGNIFICANCE OF IMPACT: Interest in epicardial fat as a visceral fat of the heart and coronary arteries is rapidly growing as the scientific based evidence indicates that the anatomic specificity is an important contributor to the cardiovascular diseases. The transformation of epicardial fat from a cardioprotective phenotype to a pro-inflammatory, atherosclerosis-promoting state triggers inflammation that is coincident with the expansion of epicardial fat volume detected by anatomic imaging. This study will impact the management of patients at risk for cardiovascular disease because it will demonstrate that quantification of epicardial fat status by MR identifies fat tissue changes validated by histology at lower cardiovascular disease risk quartile than CT.

2371

Perception- and behavior-related attention systems distinguished by phase amplitude coupling and high-gamma power

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OBJECTIVES/SPECIFIC AIMS: Attention is a cognitive function that binds perception and behavior. Recent evidence suggests that attention involves phase-amplitude coupling (PAC) of neural signals. PAC occurs when the amplitude of one frequency (frequency for amplitude) is maximal at particular phases of another frequency (frequency for phase). However, some studies suggest PAC improves attention, while others maintain that PAC inhibits attention. The present study seeks to determine whether PAC promotes or inhibits neural signals that underlie attention. METHODS/STUDY POPULATION: Six adult epilepsy patients with implanted electrodes participated in a cued attention task. Subjects participated in a cued attention task where they oriented attention to one side of the screen at a time and discriminated between stimuli as fast as possible with mouse clicks. Perception-related electrodes discriminated the location and/or shape of the target. These were determined with a cluster-based permutation test. Behavior-related electrodes predicted reaction time (RT) with neural activity prior to target appearance. These were determined with correlations between PAC and RT. PAC was calculated using the modulation index (MI). RESULTS/ANTICIPATED RESULTS: We found 47 perception-related electrodes that discriminated location and/or shape of target ($p < 0.05$, FDR corrected). We found 27 behavior-related electrodes where PAC prior to the target predicted RT ($p < 0.05$ FDR corrected). There was little overlap between the perception-related and behavior-related electrodes (3%). PAC also did not discriminate left-sided and right-sided cues. In addition, behavior-related electrodes had less local neural activity and higher PAC during the period of cued attention than perception-related electrodes. DISCUSSION/SIGNIFICANCE OF IMPACT: PAC minimally facilitates perceptual aspect of visual attention. However, PAC facilitate response speed. We suggest that PAC might improve response speed by "quieting" task irrelevant neural activity. For the same reason, PAC is absent in electrodes that are actively processing meaningful streams of visual data. These findings highlight separable aspects of the human attention system and how PAC contributes to both. Future directions include determining differences in PAC for attentional disorders like ADHD and neurological neglect.

2372

Metabolite and biomarker predictors of WTC-lung injury: An integrated multiplatform pilot analysis

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OBJECTIVES/SPECIFIC AIMS: In this pilot case-control study, the metabolome was quantified in subjects with previously measured serum and clinical biomarkers. The serum metabolome was then integrated with existing serum and clinical biomarkers of WTC-exposed firefighters to identify pathways significant to loss of lung function following acute PM-exposure. This robust subset of metabolite and serum biomarkers may be clinically relevant to predicting progression to lung disease in a larger cohort. METHODS/STUDY POPULATION: Serum drawn within 6 months of 9/11 was analyzed in this pilot. Clinical measures were obtained from electronic medical records. Never-smoking, male, WTC-exposed firefighters with normal pre-9/11 lung function were segregated based on FEV1 percent predicted (FEV1 %Pred) at symptomatic presentation. Cases of WTC-LI (FEV1 %Pred < LLN, n = 15) and controls (n = 15) were identified from previous cohorts. Ultrahigh performance liquid chromatography tandem mass spectroscopy quantified the metabolomic fingerprints of a group with previously assessed (by multiplex panels; ELISA and Luminex) serum chemokines and cytokines. High-dimensional data analysis and dimension reduction techniques integrated metabolites, cytokines, chemokines, and clinical data to identify pathways of

WTC-LI on curated data. Random Forest (RF) out-of-bag estimated success rates were used to measure classification utility of the refined biomarker profile. Principal components analysis (PCA) was used to visualize class separation produced by the refined profile. RESULTS/ANTICIPATED RESULTS: Of the 765 metabolites detected, 580 metabolites were quantified in more than 80% of subjects/group with relative standard deviation $\geq$15%. Relevant chemokines, cytokines, and clinical biomarkers were included based on previously established clinical importance. Initial PCA explained 34.7% of the variance in the first 3 components. RF was used to identify the top 5% of biomarkers important to class separation. RF of the refined biomarker profile correctly classified cases and controls with a 96.7% estimated success rate. A PCA of the refined metabolic profile now explained 46.2% of the variance in components 1–3, demonstrating improved class separation. Differentiators between cases of WTC-LI and controls included elevated sphingolipids in cases of WTC-LI. The metabolic-inflammatory serum biomarkers MDC, Apo AI, GM-CSF, and heart rate play an important role in class separation. Phospholipids and lysolipids also appeared to differentiate cases of WTC-LI from controls. Specifically, several glycero-phosphatidylcholines (GPC) were elevated in cases of WTC-LI. DISCUSSION/SIGNIFICANCE OF IMPACT: High-dimensional data analysis on the metabolic fingerprints, serum, and clinical biomarker data of a subset of WTC-exposed 9/11 rescue workers has identified pathways associated with the loss of lung function. Sphingolipids, known to function as inflammatory signaling mediators, are thought to play important roles in lung function under both physiological and pathological conditions. Changes in sphingolipid metabolism have been linked to several pulmonary disorders, including asthma, COPD, and acute lung injury. Interestingly, a relation between sphingolipid metabolism and the metabolic-inflammatory pathway is suggested by similarities observed in PCA. Findings of elevated GPCs are similar to COPD literature. Higher levels of GPCs could correspond to elevated levels of lysophosphatidic acid (LPA), a ligand of RAGE. RAGE is a known proinflammatory mediator; LPA species have well-described roles as lipid signaling molecules, function as synthetic intermediates in other metabolic pathways, and were found to be predictive of WTC-LI. Since metabolites are more proximal markers of disease processes, metabolites could capture the complexity of past exposures and, therefore, may better inform treatment. These pathways warrant further investigation into their mechanisms and therapeutic importance.

2383

Electrical stimulation to the subcallosal cingulate and amygdala drive shifts in affective bias across patient populations

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OBJECTIVES/SPECIFIC AIMS: Deep brain stimulation is currently being evaluated as an experimental therapy for various psychiatric disorders, as well as being investigated as a method for mapping emotional brain functions. This growing area of research requires sensitive measures to quantify effects of stimulation on emotional processing. The current study examined the effects of acute stimulation to 2 limbic regions—the subcallosal cingulate (SCC) and the amygdala—on bias in the perception and evaluation of emotional facial expressions. We hypothesized that transient electrical stimulation to the limbic system would produce acute reductions in negative bias, consistent with its antidepressant effects in patients with severe depression. METHODS/STUDY POPULATION: The current study uses a novel affective bias task, developed to rapidly and covertly quantify emotional state. Over 4–6 minutes, patients rate the intensity and valence of static images of emotional facial expressions. We examined effects of electrical brain stimulation in 2 groups: patients with treatment-refractory depression undergoing SCC DBS therapy, and epilepsy patients undergoing amygdala stimulation via stereo-EEG electrodes during inpatient intracranial monitoring. DBS patients completed the task under stimulation and sham conditions during monthly visits over the first 6 months of therapy, as well as daily during a 1 week, blinded period of DBS discontinuation at the 6-month time point. Epilepsy patients completed the task under stimulation and sham conditions at a single visit. Mixed linear models and paired-samples *t*-test were used to investigate effects of stimulation as well as depression scale scores on affective bias ratings. RESULTS/ANTICIPATED RESULTS: Four SCC DBS patients showed significant effects of stimulation ($p < 0.0001$) and depressive state ($p < 0.0001$) on affective bias scores across 6 months of chronic DBS therapy, where emotional faces were perceived as less sad with stimulation ON, as well as during visits in which patients were nondepressed (typically later in the treatment course). Furthermore, 2 DBS patients showed rapid negative shifts in bias following acute blinded discontinuation of chronic stimulation, an effect which persisted over the 1-week period of discontinuation ($t_{29} = -2.58$, $p = 0.015$), in the absence of any self-reported change in mood. Likewise, 6 epilepsy patients showed significant positive shifts in affective bias with acute amygdala stimulation ($t_5 = -4.75$, $p = 0.005$). Current analyses are investigating electrophysiological, autonomic and facial motor correlates to affective bias in these patients. DISCUSSION/SIGNIFICANCE OF IMPACT: Affective bias has revealed rapid, significant changes with stimulation at 2 limbic targets—one a white matter hub and one a nuclear subcortical structure—suggesting the task's utility as an emotional outcome measure in brain stimulation studies. These stimulation-sensitive measures may provide a new metric to track treatment response to deep brain stimulation therapy for affective disorders. Future studies will determine whether affective bias can predict neuropsychiatric complications in patients undergoing stimulation mapping of brain circuitry ahead of resection surgery for epilepsy.

2392

Detecting cardiometabolic disease through breath analysis: A metabolomic approach

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OBJECTIVES/SPECIFIC AIMS: The purpose of this study is to use the baboon as a novel animal model for breath research and to identify and characterize baboon breath metabolites that reflect cardiometabolic function to inform us in the development of a noninvasive, cost-effective, and repeatable point-of-care diagnostic breath test. METHODS/STUDY POPULATION: Blood and urine was collected from control and IUGR at the approximate age of 3.5 years. Both groups were then placed on a high fat, high sugar, high salt diet for 7 weeks, after which blood, urine, and breath were collected. The breath samples were then subjected to comprehensive, 2-dimensional gas chromatography coupled with time-of-flight mass spectrometry. Using ChromaTOF software, breath VOCs were identified with at least an 80% spectral match against the National Institute of Standards and Technology (NIST) chemical reference library. The raw data were then statistically analyzed using MetaboAnalyst. We then interrogated multiple online databases to characterize and identify the role of VOCs that were present in both control and IUGR groups. RESULTS/ANTICIPATED RESULTS: Preliminary analyses of the breath VOCs indicate differences in expression between sexes and in control Versus IUGR groups. These results indicate unique "breath signatures." Further analysis of the breath VOCs reveals the presence of metabolites that are involved in β-oxidation and oxidative stress pathways. DISCUSSION/SIGNIFICANCE OF IMPACT: This breath study, a first of its kind, will develop the baboon as a superior animal model for breath biomarker research. Our observed unique "breath signatures" indicate changes in lipid metabolism and oxidative stress pathways, which we hypothesize are the early metabolic changes at the cellular level that are not yet reflected in clinical lab measures. Future directions include analyzing breath VOCs that did not meet 80% spectral match, validation using SPME technology and commercial standards, and initiating a human pilot study in clinically obese, at-risk children in collaboration with physicians at the Children's Hospital of San Antonio to develop a noninvasive, cost-effective, rapid, and repeatable point-of-care diagnostic breath test.

2399

Protective immunity to live vaccines among children with solid tumors

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OBJECTIVES/SPECIFIC AIMS: Determine whether children with solid tumors maintain intact protective immunity to live vaccines during cancer therapy and after completing cancer therapy (postTx). METHODS/STUDY POPULATION: We will perform a prospective cohort study of children with solid tumors (Hodgkin lymphoma, brain, Wilms, and germ cell tumors) followed at the Puerto Rico's University Pediatric Hospital. Protective immunity will be measured with antibody titers against live vaccines (Measles, Mumps, Rubella, and Varicella) at diagnosis, during cancer therapy, upon completion and