thereafter ($p < 0.0001$). Etv6 expression decreases as B cells develop and is negatively correlated with Pax5 expression ($r^2 = 0.9993$; $p = 0.0167$). We next confirmed the expression patterns of ETV6 and PAX5 during B cell development in human samples. We found that ETV6 expression was higher in the early B cell fraction (CD10+, CD34+, CD19−, and CD20−) compared to the pre-B cell fraction (CD10+, CD34−, CD19+, CD20−). Conversely, we observed that PAX5 expression was higher in the preB cell fraction compared with the early B cell fraction. In Ba/F3 cells expressing ETV6 constructs, ETV6, but not ETV6 P214L overexpression significantly decreased Pax5 expression ($p \leq 0.05$). ETV6 is associated with the proximal GGAA site 72 base pairs upstream of the Pax5 TSS, but not GGAA sites further from the TSS. In addition, the transcriptional repressors SIN3A and HDAC3 were detected on the same regions of the Pax5 locus. We detected association of ETV6, SIN3A, and HDAC3 with the proximal GGAA site upon expression of WT ETV6, but not ETV6 P214L. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results provide a mechanism of interaction for ETV6 and PAX5, 2 genes often disrupted in B-cell leukemia. These findings are significant because PAX5 misregulation results in a B cell development halt, lineage infidelity, and leukemogenesis. In continuing our studies, we have generated a transgenic mouse endogenously expressing the ETV6 P214L mutation by CRISPR/Cas9 editing, and these mice appear to have a thrombocytopenic phenotype similar to that observed in patients carrying the ETV6 P214L mutation. These animals will be the focus of our continued investigation of the mechanism by which ETV6 germline mutation results in a predisposition to leukemia. Our ultimate goal is a comprehensive understanding of how this process may be targeted more efficiently in patients with both heritable and sporadic forms of leukemia involving ETV6.

2486

Sleep, biological stress, and health in a community sample of toddlers living in socioeconomically disadvantaged homes

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OBJECTIVES/SPECIFIC AIMS: The purposes of this study are to examine the relationships among sleep characteristics (duration, efficiency), stress biomarkers, and child behavior problems among toddlers living in socioeconomically disadvantaged homes and how these characteristics change over time from age of 12 months to 24 months. Aim 1: examine changes in subjective and objective sleep characteristics from 12 to 24 months of age. Aim 2: examine changes in stress biomarkers from 12 to 24 months of age. Aim 3: examine the cross sectional and longitudinal relationships between sleep characteristics and stress response. Aim 4: examine the cross sectional and longitudinal relationships between sleep characteristics and toddlers' child behavior problems. METHODS/STUDY POPULATION: In this cross-sectional study we are recruiting parents with healthy toddlers from early head start programs and a community clinic to prospectively examine the relationships among sleep characteristics, stress biomarkers, and children's health. Data on sleep characteristics will include subjective and objective measures of sleep duration and efficiency and parental interactive bedtime behaviors to assist their toddlers' sleep initiation. Multi-systemic biomarkers of stress including cortisol, CRP, IL-6, and BMI, will be measured individually. The associations between sleep characteristics and the biomarkers, considered as a latent variable of the stress response, will be explored. Health measures will include secretory IgA and parent-reported behavioral problems. Generalized linear models will be used in the data analysis. RESULTS/ANTICIPATED RESULTS: To date we have obtained objective (9 days/nights of actigraphy) measures of 33 toddlers' sleep and subjective measures of parenting interactive behaviors. Using the Parental Interactive Bedtime Behavior (PIBB) Survey and subscales [active physical comforting, encourage autonomy, settle by movement, passive physical comforting (PPC), social comforting], we are currently reporting on the associations between PIBB and toddler's sleep characteristics. The sample included 33 toddlers (mean age = 1.33 years, SD = 0.54). The toddlers' sleep duration averaged 8.22 hours (SD = 0.86). There were statistically significant moderate associations between sleep duration and parents' PPC ($r = -0.41$, $p = 0.02$). Intra-individual variability in the amount of wake after sleep onset was also significantly associated with total PIBB and PPC ($r = 0.37$, $p = 0.05$; $r = 0.52$, $p = 0.002$, respectively). Intra-individual variability in the amount of sleep fragmentation within toddlers was significantly associated with total PIBB ($r = 0.36$, $p = 0.05$). DISCUSSION/SIGNIFICANCE OF IMPACT: Although active physical comforting (eg, rocking to sleep, patting or rubbing child's back) is most commonly associated with sleep patterns in infancy and toddlerhood among samples of higher socio-economic status, findings from this study suggest a stronger association between PPC (eg, presence of the parent in the room to fall asleep) and less sleep duration and more individual variability in night wakings. The biomarker data are currently being analyzed and results will be presented within the year. Taken together, these preliminary results and pending results will inform future intervention development that may address the role of parenting behavior in promoting health sleep early in life.

2493

GABA-A receptor binding is abnormal in sensory-motor integration brain regions in Cervical Dystonia

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OBJECTIVES/SPECIFIC AIMS: Determine whether GABA-A receptor binding is abnormal and linked to dystonia symptoms in cervical dystonia (CD). METHODS/STUDY POPULATION: There is increasing evidence that a key pathophysiological mechanism in adult-onset focal dystonia is a reduction in inhibitory control over the sensorimotor network. Results from a recent 11C-flumazenil PET imaging study suggest that abnormal inhibitory signaling in genetic and sporadic forms of dystonia may be due to reduced GABA-A binding. It remains unknown whether CD, the most common form of adult-onset focal dystonia, is associated with abnormal GABA-A binding. The goal of this research is to determine if GABA-A receptor binding is abnormal and linked to dystonia symptoms in CD. RESULTS/ANTICIPATED RESULTS: We investigated whole brain GABA-A binding in 15 CD patients (11F; 64 ± 8 y) and 15 healthy controls (10F; 64 ± 9 y) using 60-minute dynamic 11C-flumazenil PET scans. GABA-A receptor binding potential (BP) was estimated using a simplified reference tissue model. A 2-sample *t*-test was used to identify voxel-wise GABA-A BP differences between groups, and a regression analysis used to test for correlations between GABA-A BP and disease severity as measured with the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS). A conventional region of interest analysis was also conducted to quantify BP changes within the sensorimotor network using the automated anatomical labeling atlas. DISCUSSION/SIGNIFICANCE OF IMPACT: CD patients have reduced GABA-A receptor binding compared with healthy controls, with the greatest reduction seen within the sensorimotor region of the thalamus. Furthermore, reductions in GABA-A binding in brain regions associated with coupling sensory and motor information predict motor severity. These findings support that reduced GABAergic signaling within sensorimotor integration regions is a key mechanism underlying dystonic symptoms in CD and could help inform the development of better, more targeted treatment options.

2518

Development of a clinically relevant rabbit surgical model for investigation of the host response to polypropylene mesh for pelvic organ prolapse

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OBJECTIVES/SPECIFIC AIMS: Mesh properties, such as stiffness, porosity, and weight have been shown to correlate with the degree of mesh integration with vaginal tissue. Previous research in rhesus macaques implanted with polypropylene mesh differing in stiffness, porosity, and weight showed differences in vaginal deterioration following mesh implantation. These differences were correlated with a foreign body response, consisting primarily of activated, proinflammatory M1 macrophages. Previous studies have determined that the early macrophage polarization profile following biomaterial implantation is a strong indicator of overall tissue integration downstream. However, these early responses have not been previously observed in the appropriate surgical models. Prior work from our laboratory in developing a cytokine delivery system has shown that shifting the macrophage response at the host-implant interface from a pro-inflammatory M1 phenotype to an anti-inflammatory M2 phenotype in the first 14 days postimplantation resulted in enhanced integration of the mesh with the surrounding tissues. The present study develops an in vivo model clinically relevant surgical model to investigate the modulation of the host response to mesh. Utilizing a moderately-sized animal, we can feasibly implant mesh using the "gold standard" abdominal sacrocolpopexy procedure and evaluate the changes in the host immunologic response at early (14 d) and tissue remodeling outcomes at late stages (90 and 180 d) of implantation. METHODS/STUDY POPULATION: Commercially available heavyweight and lightweight mesh was used to investigate the modulation of the immune response. A custom MTI SILAR Automated Dip Coating machine is used to uniformly coat the mesh in a reproducible manner. An adapted radio frequency glow discharge method is used to create a stable negative charge on the surface of the mesh, followed by the sequential deposition of polycationic and polyanionic polymers to provide a stable, conformal, nanoscale coating. Chitosan served as the polycation, chosen because of its known antimicrobial and biocompatibility properties. Dermatan sulfate served as the polyanion, chosen for its important role in regulating extracellular matrix

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components and enhancing the activity of cytokines. Interleukin-4 (IL-4) is incorporated into the coating to be released in a controlled manner upon implantation. In vitro controlled release profiles were assessed to demonstrate efficient and local release of IL-4. Utilizing a New Zealand white rabbit surgical model, we implant mesh using the "gold standard" abdominal sacrocolpopexy procedure and evaluate the changes in the host immunologic response at early (14 d) and tissue remodeling outcomes at late stages (90 and 180 d) of implantation. The mesh-tissue complex was removed from each rabbit and processed for histological staining as well as immunolabeling of immune cells, such as macrophages. Determination of matrix metalloproteinases and fibrotic capsule formation also helps characterize the overall inflammatory response associated with each implant. RESULTS/ANTICIPATED RESULTS: We have developed a clinically relevant rabbit surgical model to implant different conditions of surgical mesh into 2 different sites, including the vagina and the abdomen. The results of this study show that implants into vaginal tissues elicited an increased host inflammatory response at 14 days as compared with those in the abdominal wall. However, at chronic time points the inflammatory response in the vagina was reduced as compared to that in the abdominal cavity. The present study also demonstrates the scale-up of a previous methodology for nano-scale coating. We present a nanometer thickness, tunable, and uniform coating capable of releasing bioactive IL-4. In vitro assays confirm the bioactivity and the controlled local release allowing for shifts in the immune response to promote implant integration. Improved remodeling has been observed to correlate with a shift in the early host response from an M1 to an M2 phenotype, however, there is limited information on the exact mechanism. Our strategy to achieve enhanced tissue remodeling demonstrate outcomes such as minimal changes to the structural properties of the mesh and a controlled release profile to sufficiently polarize macrophages around the mesh to a pro-remodeling state. DISCUSSION/SIGNIFICANCE OF IMPACT: Pelvic organ prolapse is a condition where the pelvic floor muscles weaken over time resulting in the downward shift of the pelvic organs into the vaginal canal. Moreover, factors such as obesity, age, and vaginal birth increase the susceptibility of being diagnosed with pelvic organ prolapse. Direct costs of reconstructive procedures exceed $1 billion each year in the United States. Synthetic mesh has been used to repair abdominal hernias for over half a century. Biomedical companies, through 510k and the 1976 Medical Device Amendments Act, were able to resell their hernia repair mesh as a treatment for pelvic organ prolapse. However, women who have had vaginal mesh implants have reported an increasing number of complications including chronic pain and mesh erosion/exposure at rates as high as 10%–20%. In fact, in 2008 and 2011, the US Food and Drug Administration issued warnings to doctors and patients about the mesh. In January 2016, the FDA officially had to reclassify surgical mesh for transvaginal repair of pelvic organ prolapse from a class II, moderate risk device, to a class III, high-risk device. Presently, data for the use of synthetic mesh has largely derived from abdominal hernia repair, instead of vaginal repair of prolapse. In the rodent model, the vagina is too small to implant mesh in an analogous manner to human implantation. Instead, implantations are done in the abdomen, a different tissue composition and host response profile than the vagina. Primate models of pelvic organ prolapse have been utilized, but are associated with high costs and investigation of acute immune responses are not considered ethical due to the short time of survival. Thus, our presented work will not only show the development of an improved material for implantation, but also the development of an in vivo model clinically relevant to understanding the early host response to mesh.

2519

A quantitative disintegration method to evaluate polymeric films

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OBJECTIVES/SPECIFIC AIMS: To establish an in vitro quantitative method for the evaluation of polymeric film disintegration that can be applied to predict in vivo behavior. METHODS/STUDY POPULATION: Two clinically advanced vaginal microbicide film products containing tenofovir and dapivirine were used as model films throughout this work. Films were made using the solvent cast manufacturing method in which polymers, excipients, plasticizer, and APIs were either dissolved or dispersed in water, mixed, and cast on a heated substrate. The novel, quantitative method was developed using a TA.XT Plus Texture Analyzer® (Texture Technologies) in combination with a TA-108S5 fixture and the TA-8A: 1/8″ diameter rounded end ball probe. Exponent® was used as the data analysis software. In this method, the film was placed and secured in the fixture, the probe applied a constant force to the film product, and a biologically relevant amount of fluid was applied to the film. The probe was able to penetrate the film upon disintegration resulting in an applied force of zero at that point. A curve of force Versus time was plotted, and disintegration time was defined as the time between fluid addition until the probe force reached zero. Test parameters were optimized in order to reduce error. Visual observation of film disintegration was conducted in the in vivo macaque model using films that included a water-soluble blue dye for film visualization. Colpophotography was also used to confirm film disintegration. In vitro results were compared with in vivo findings. RESULTS/ANTICIPATED RESULTS: The Texture Analyzer disintegration method developed provided quantitative disintegration times and did not rely on user defined endpoints which is common in many visual disintegration tests. The disintegration method was able to distinguish differences between the 2 clinical film products and produced reproducible disintegration times for the tenofovir and dapivirine films. The tenofovir film had a shorter disintegration time (41.28 ± 2.85 s) compared with that of the dapivirine film ($88.3\ 6 \pm 9.82$ s). This method was also able to distinguish changes made to these 2 clinical film products in terms of volume and formulation alterations. In vitro and in vivo disintegration times differed by orders of magnitude, with in vitro time being measured in seconds and in vivo time being measured in days, for a variety of factors, mainly the application of constant force to the film product. Regardless of these differences, the rank order of film disintegration remained constant for in vitro and in vivo disintegration and an In Vitro In Vivo Correlation (IVIVC) trend could be seen. DISCUSSION/SIGNIFICANCE OF IMPACT: Standardization of preclinical in vitro assessments which minimize user bias are crucial to the field of pharmaceutical film development. As this field continues to develop and more products advance for pharmaceutical application, this method has the potential to become a standard assessment of film functionality. This study represents a first step in the process of developing an IVIVC. More films will need to be tested using both in vitro and visual methods in order to establish and accurate factor to predict in vivo behavior.

2529

Clinical determinants of clopidogrel responsiveness in a heterogeneous cohort of Caribbean Hispanics

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OBJECTIVES/SPECIFIC AIMS: To determine the association between clinical characteristics and platelet reactivity in Hispanic patients on clopidogrel therapy. METHODS/STUDY POPULATION: A cross-sectional pilot study was performed in 58 Puerto Rican patients diagnosed with any type of vascular disease and actively receiving a maintenance dose of clopidogrel for at least 7 days. The study population was divided into 2 groups: Group I with non-high on-treatment platelet reactivity (TPR); Group II with high TPR. To determine the platelet function, P2Y12 reaction units (PRU) were obtained by VerifyNow® P2Y12 assay (Accumetrics, USA). RESULTS/ANTICIPATED RESULTS: We studied a heterogeneous cohort of patients with coronary artery disease (57%), peripheral artery disease (30%), carotid artery stenosis (7%), cerebral artery aneurysm (3%), and stroke (3%) on clopidogrel therapy for secondary prevention of thromboembolic events. The mean TPR was 205 ± 49 PRU (range: 61–304), with a prevalence of 28% patients with high TPR (PRU $\geq$ 230). No significant clinical differences were found between the non-high TPR and high-TPR groups ($p > 0.05$). However, multivariable logistic regression analysis showed that both diabetes mellitus (OR = 7.5; CI: 1.01–51.9) and proton-pump inhibitors (OR = 13.6; CI: 1.3–142.0) were independently correlated with high TPR ($p < 0.05$) after adjusting for other clinical variables. DISCUSSION/SIGNIFICANCE OF IMPACT: These results provide new insight into the importance of clinical characteristics on platelet reactivity in this Caribbean population. Further studies are warranted to determine whether important clopidogrel pharmacogenes are related with platelet function in Hispanics, as well as the role of TPR in guiding antiplatelet therapy and predicting future adverse cardiovascular events in this population.

OUTCOMES RESEARCH/HEALTH SERVICES RESEARCH/COMPARATIVE EFFECTIVENESS

2025

Institutional and community involvement establishing ARresearch.org and innovative recruitment results in diverse registrants

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OBJECTIVES/SPECIFIC AIMS: To establish a state-wide research registry of diverse participants. METHODS/STUDY POPULATION: We garnered broad institutional and community support by involving TRI's Community Engagement team, its Community Advisory Board (CAB), and 3 UAMS patient CABs in

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