that if a family was known for having any problem, gossips would start and spread quickly wherever they go, and pretty soon, the family would be looked down by the entire community. "I think for Vietnamese people, they don't help those that are in need. They know of your situation and laugh about it, see less of you, and distant themselves from you" (Older women FGD). Culture and mental illness stigma, much of the described stigma and discrimination expressed, and consequently the reluctance to seek help, was attributed to the lack of awareness of mental health and of mental health disorders. Many study participants across groups also emphasized a belief that Vietnamese Americans were often known for their perseverance and resilience, overcoming wars and natural disasters on their own. Mental disorders were reportedly seen as conditions that individuals and families needed to overcome on their own, rather than asking for help from outsiders. This aspect of Vietnamese culture is intertwined with the need to protect one's family's reputation, being passed on from one generation to the next, reinforcing the beliefs that help for mental disorders should come from within oneself and one's family only. Consequently persons with mental health problems would be "Keeping it to themselves. Holding it in and believing in the power of their friends" (Middle-aged FGD) instead of seeking help. Another dimension of culture that was apparent from FGDs (as well as KIIs) was the mistrust in Western medicine. Not understanding how counseling or medicines work made one worry about approaching service providers or staying in treatment. The habit of Vietnamese people to only go see a doctor if they are sick with physical symptoms was also a hindrance to acknowledging mental illness and seeking care for it. Challenges, including the lack of vocabulary to express mental illness and symptoms, in the Vietnamese language, exaggerated the problem, even among those who had some understanding of mental disorders. It was said in the young men FGD that: "when you classify depression as an illness, no one wants to be sick,… if you call it an illness, no one wants to have that sort of illness, and it's not an illness that you can physically see…" (Young men FGD). Another young man summarized so well the influence of culture on mental illness stigma: "Us Southeast Asian, like, from my parents specifically has Vietnam War refugees. I think the reason why they don't talk about it is because it's a barrier that they have to overcome themselves, right? As refugees, as people who have been through the war… [omitted]They don't want to believe that they need help, and so the trauma that they carry when they give birth to us is carried on us as well. But due to the language barrier and also the, like, they say with the whole health care, in Vietnam I know that they don't really believe in Western and Eurocentric medicine. So, from their understanding of how, like from their experience with colonization or French people, and how medicine works, they don't believe in it" (Young men FGD). One characteristic of the Vietnamese culture that was also often mentioned by our FGD participants (as well as KIIs) was the lack of sharing and openness between generations, even within a family. Grandparents, parents, and children do not usually share and discuss each other's problems. Parents and grandparents do not talk about problems because they need to appear strong and good in front of their children; children do not talk about problems because they are supposed to do well in all aspects, particularly in school. The competitiveness of Vietnamese and high expectations of younger generations again come into play here and create a vicious cycle. Young people are expected to do well in school, which put pressure on them and may result in mental health problems, yet, they cannot talk about it with their parents because they are not supposed to feel bad about school, and sharing is not encouraged. The Asian model minority myth and the expectations of parents that their children would do well in school and become doctors and lawyers were cited by many as a cause of mental health problems among young people. "Our parents are refugees, they had nothing and our parents want us to achieve this American Dream…. [omitted] It set expectations and images for us…. It was expected for all the Asians to be in the top 10, and for, like a little quick minute I thought I wasn't going to make it, I was crying" (Yong men FGD). As a result, the mental health problems get worse. "If you're feeling bad about something, you don't feel like you can talk about it with anyone else, especially your family, because it is not something that is encouraged to be talked about anyway, so if you are feeling poorly and you don't feel like you could talk to anybody, I think that just perpetuates the bad feelings" (Middle-aged women FGD). Acculturation and mental illness stigma Acculturation, the degree of assimilation to the host society, has changed some of the understanding of mental illness and stigmatizing attitudes. Differences across generations expressed in different FGDs indicated differences in perceptions towards mental illness that could be attributed to acculturation. For example, the young generation understood that mental illness was a health problem that was prevalent but less recognized in the Vietnamese community, whereas a prominent theme among the older participants was that mental illness was a temporary condition due to psychological stress, that it was a condition that only Caucasians had. Some of the components of public stigma related to mental illness seemed to vary between generations, for example the youngest participants were less likely to put a label on a person with mental health problems, or to stereotype them, compared to the oldest and middle-aged participants. This was attributed to their education, exposure to the media and information, and to them "being more Americanized." However, there was no evidence that acculturation played an important role in changing the other components of public stigma, including stereotyping, separating, and status loss and discrimination. For example, the need to protect the family reputation was so important that our young participants shared: "If you damage their image, they will disown you before you damage that image" (Young men FGD). Young people, more likely to recognize mental health problems, were also more likely to share within the family and to seek help, but no more likely than their older counterparts to share outside of the family—"maybe you would go to counseling or go to therapy, but you wouldn't tell people you're doing that" (Young women FGD). The youngest participants in our study were facing a dilemma, in which they recognized mental health problems and the need for care, yet were still reluctant to seek care or talk about it publicly because of fears of damaging the family reputation and not living up to the parents' expectations. Many young participants reported that it actually made it very difficult for them to navigate mental health issues between the 2 cultures, despite the awareness of the resources available. "I think it actually makes it harder. Only because you know to your parents and the culture, and your own people, it's taboo, and it's something that you don't talk about. Just knowing that you have the resources to go seek it… You want advice from your family also, but you can't connect the appointment to your family because you're afraid to express that to your parents, you know? So I think that plays a big part, and knowing that you are up and coming, but you don't want to do something to disappoint your family because they are so traditional" (Young men FGD). Some participants felt more comfortable talking about mental health problems, like depression, if it was their friend who experienced it and confided in them, but they would not necessarily felt open if it was their problem. Subtle cultural differences like this are likely overlooked by Western service providers. One older participant summarized it well "They [the young generation] are more Americanized. They are more open to other things [but] I think that mental health is still a barrier." DISCUSSION/SIGNIFICANCE OF IMPACT: This study investigated how different components of public stigma related to mental illness manifest among Vietnamese Americans, a major ethnic group in the United States, and how acculturation may influence such stigma. The findings highlighted important components of public stigma, including labeling and status loss, but did not provide strong evidence of the other components within our study population. Strong cultural beliefs underlined the understanding of mental health and mental illness in general, and how people viewed people with mental illness. Several findings have been highlighted in previous studies with Asian immigrants elsewhere; for example, a study from the perspectives of health care providers in Canada found that the unfamiliarity with Western biomedicine and spiritual beliefs and practices of immigrant women interacted with social stigma in preventing immigrants from accessing care (O'Mahony and Donnelly, 2007). Fancher *et al.* (2010) reported similar findings regarding stigma, traditional beliefs about medicine, and culture among Vietnamese Americans. Acculturation played a role in changing stigmatizing attitudes as evidenced in intergenerational differences. However, being more Americanized did not equate to being more open, having less stigmatizing attitudes, or being more willing to seek care for mental health issues. Consistent with previous studies (Pedersen and Paves, 2014), we still found some level of stigma among young people aged 18–35, although some components were lessened with an increased level of acculturation. There was also a conflict among the younger generation, in which the need for mental health care was recognized but accessing care was no easier for them than for their parent and grandparent generations. The study's findings are useful to adapt existing instruments to measure stigma to this population. The findings also have important program implications. One, they can be directly translated into basic supports for local primary and behavioral health care providers. Two, they can also be used to guide and inform the development and evaluation of an intervention and an additional study to validate the findings in other immigrant ethnic groups in the United States. Finally, based on results of the study, we can develop a conceptual framework that describes pathways through which social, cultural, and ecological factors can influence stigma and the ways in which stigma acts as a barrier to accessing mental health care among Vietnamese Americans. The guiding framework then can be validated and applied in future programs aimed to improve mental health care utilization among ethnic minorities.

2322

Modulation of autophagy in intestinal health and inflammation

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OBJECTIVES/SPECIFIC AIMS: Modulation of autophagy has the potential to treat inflammatory bowel disease (IBD). IBD is characterized by dysregulated inflammatory pathways and a defective intestinal epithelial barrier. We sought to better understand how autophagy can be utilized to regulate both inflammation and the intestinal barrier. METHODS/STUDY POPULATION: We examined mice with an autophagy defect in only macrophages in an animal

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model of IBD. To understand the phenotype, we utilized macrophages to investigate the mechanism behind autophagy and proinflammatory cytokine secretion. In addition, we analyzed the development of colonoids in a co-culture system with macrophages with or without a functional autophagy pathway. Lastly, pharmacological modulation of autophagy to control inflammation was assessed. RESULTS/ANTICIPATED RESULTS: Mice with autophagy-deficient macrophages were highly susceptible to intestinal barrier disruption. Susceptibility was due to enhanced proinflammatory cytokine secretion and intestinal permeability. Furthermore, proinflammatory macrophages (due to an autophagy defect) co-cultured with colonoids, significantly decreased the number of mucus producing goblet cells. Finally, pharmacologically modulation of autophagy reduced the secretion of proinflammatory cytokine by macrophages and reduced intestinal permeability. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results strongly suggest autophagy modulation can dampen inflammation and enhance the intestinal epithelial barrier.

2466

MicroRNA-451: A potential key player in the development of diabetic nephropathy in an insulin resistant mouse model

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OBJECTIVES/SPECIFIC AIMS: MicroRNAs (miRNA) affect transcription of a number of genes involved in the development and progression of diabetic nephropathy (DN), and have become attractive therapeutic targets and biomarkers. Elevated renal gluconeogenesis, fibrosis, and albuminuria are early markers of incipient DN. Recent studies report that renal miRNA-451 may protect against DN and reduce renal gluconeogenesis in rodent models. MiRNA-451 is thought to act by targeting select factors resulting from disrupted insulin and growth factor signaling and the mechanistic-target of rapamycin (mTOR) in early DN. This study aimed to elucidate the role of miRNA-451 in the development and progression of DN. METHODS/STUDY POPULATION: To further elucidate the role of miRNA-451 in DN, we placed male insulin-resistant, TALLYHO/Jng mice on a high-fat diet (60% kCal). The mice were divided into 2 treatment groups and received 8 consecutive weekly intraperitoneal injections of locked nucleic acid (LNA) miR-451-inhibitor or LNA-scrambled compound (2 mg/kg·bw; n = 8/treatment). Mice were euthanized after 12 weeks (4 weeks sans injections) and kidneys, liver, pancreas and abdominal adipose tissue were harvested for analysis. RESULTS/ANTICIPATED RESULTS: Renal homogenate expression of miRNA-451 was drastically reduced in inhibitor-treated mice (~6-fold) in comparison with scramble-treated mice. Western blotting of cortex homogenates for indicators of fibrosis and targets of miRNA-451 revealed a significant reduction in collagen IV (marker of epithelial integrity) in inhibitor-treated mice. In addition, metalloproteinase type 9 (MMP9, a known type IV collagenase), YWHAZ (a scaffolding protein and known target of miR-451), mTOR, and fructose bisphosphatase (FBP1, a rate-limiting gene in gluconeogenesis) were significantly increased in this group in comparison to scramble-treated mice. However, no differences were found in protein levels for glucose-6-phosphatase (G-6-Pase) or phosphoenolpyruvate (PEPCK), 2 additional gluconeogenic rate-limiting genes. MiRNA-451 antagonist did not significantly affect final body weight or blood glucose; however, mean blood sodium concentrations were slightly, but significantly higher (2%) in the LNA-inhibitor treated group (when compared with the scramble-treated group). No differences in blood potassium or chloride were found. Anion gap was 90% higher in the LNA-inhibitor treated group when compared with scramble-treated mice. No differences in urinary albumin to creatinine ratio were found between the two treatment groups. However, Masson Trichrome scoring revealed a 59% increase in fibrosis in inhibitor-treated mice. DISCUSSION/SIGNIFICANCE OF IMPACT: Collectively, these findings support a potentially protective role of miRNA-451 in attenuating signaling via mTOR that may alter both renal gluconeogenic potential (contributing to the diabetic phenotype) and activation and progression of renal fibrosis. Therapies to enhance miRNA-451 signaling may be beneficial to reduce renal pathology associated with DN.

2058

miRNA manipulation to improve CFTR correction in cystic fibrosis

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OBJECTIVES/SPECIFIC AIMS: CFTR is the mutant protein that causes cystic fibrosis (CF), a fatal respiratory diseases affecting 1 in 3500 children. CFTR modulators are small molecules that directly address mutant CFTR function. Improving correction of the F508del CFTR mutation (affecting 90% of CF patients) is one of the most pressing unmet needs in CF. Currently available F508del therapeutics only marginally improve CF, In vitro, we have identified a miRNA that impairs utility of CFTR directed therapies. miR-145 is upregulated by TGF-β (a genetic modifier of CF lung disease) with a direct binding site on the 3'-untranslated region of CFTR mRNA. Binding of miR-145 to CFTR destabilizes mRNA transcript and impedes protein translation. Overexpression of miR-145 abolishes benefit of F508del CFTR correction. Antagonists to miR-145 block TGF-β suppression of CFTR function and augment response to CFTR correction. This project evaluate in vivo impact of TGF-beta and miRNA manipulation on CFTR functional readouts including nasal potential difference (NPD) and short circuit current (Isc) across tracheal explants in addition to standard biochemical measures. METHODS/STUDY POPULATION: Wild-type Sprague-Dawley rats were inoculated with an adenoviral vector containing bioactive TGF-beta or sham at 1×10^9 pfu/animal placed in the left nares. Seven days post-inoculation, functional, and biochemical measures were conducted. NPD was measured with a microelectrode placed in the left nare and grounded the tail. The nare was sequentially perfused with standard Ringer's solution, amiloride (to block the ENaC sodium channel), low chloride Ringer's (to stimulate chloride efflux), forskolin (to open the CFTR channel) and CFTRinh-172 (to block the CFTR channel. Tracheal explants were harvested, microdissected, and placed on modified Ussing chambers. RESULTS/ANTICIPATED RESULTS: We have inoculated WT rats with bioactive TGF-β Versus sham delivered by intranasal inoculation of an adenoviral vector. Functional readout of CFTR function is by Isc across tracheal epithelia and NPD. Lung homogenates are analyzed for TGF-β signaling, miRNA expression, and CFTR transcripts. Both tracheal explants and NPD indicate TGF-β stimulation diminishes CFTR function in vivo. In tracheal explants, TGF-β exposure diminishes CFTR response to forskolin-stimulation by 75%. Loss of current after CFTR inhibition (CFTRinh-172) is halved. By nasal PD, TGF-β inoculation similarly halves the bioelectric response to low chloride and forskolin stimulation. Evaluation by qPCR reveals a strong increase in TGF-β signaling demarcated by PAI-1, prompting a reduction in CFTR mRNA. miR-145 is expressed highly in rat pulmonary tissue, but no change in overall miR-145 levels was detected between TGF-β and sham exposed rats. This finding reflects what we have observed in human lungs, with a localized increased miR-145 expression in CF epithelia, but similarly high levels of miR-145 in both CF and non-CF whole lung homogenates. Although expressed at lower levels than miR-145, we did find increased expression in TGF-β relevant miR-101, miR-494, and miR-144 that have a predicted binding site on rat 3'-UTR in TGF-β exposed Versus sham lungs. DISCUSSION/SIGNIFICANCE OF IMPACT: Our data indicate the relevance of TGF-β stimulation to suppress CFTR synthesis and function in vivo. Future work will evaluate whether these additional miRNA with CFTR binding sites may mediate TGF-β suppression of CFTR in the rat model, and the utility of miRNA manipulation to augment F508del CFTR correction.

2301

Mucoidal pseudomonas aeruginosa infection is associated with regional inflammation in the cystic fibrosis lung

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OBJECTIVES/SPECIFIC AIMS: Cystic fibrosis (CF) is a life-shortening genetic disease that affects approximately 30,000 patients in the United States. CF patients suffer from chronic pulmonary infections that are associated with hyperinflammation and irreversible damage to the lower airways. As CF patients age, Pseudomonas aeruginosa (P.a.) is the predominant pathogen that infects the respiratory tract. The P.a. strains initially infecting the CF lung have a nonmucoid colony morphology, whereas, once chronic infection is established, these bacteria mutate leading to the emergence of mucoid P.a. variants with heightened resistance to both antibiotics and host immunity. Both nonmucoid and mucoid P.a. variants are often co-isolated on microbiological cultures of sputum collected from CF patients. However, the CF lung is known to exhibit heterogeneity in inflammation and infecting microbes across different lung regions that cannot be studied using routine sputum collection alone. Here, using a standardized bronchoscopic protocol, bronchoalveolar lavage (BAL) fluid was prospectively collected from each lobe of a CF cohort undergoing clinically indicated surgical procedures. We sought

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