“ Disastrous principles of grantsmanshipwreck"

Tom Hollon, PhD

For an appointment to discuss getting funded:

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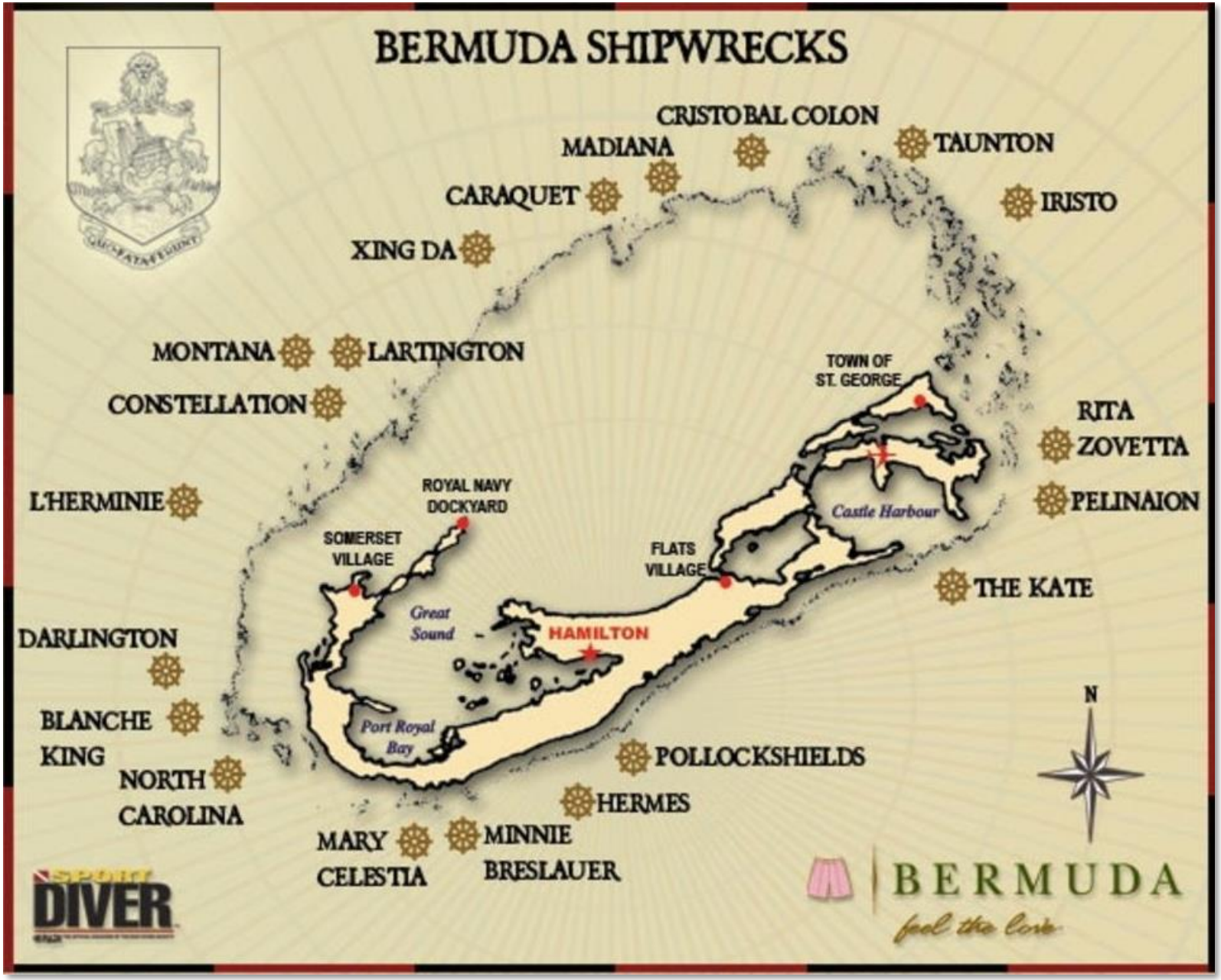
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NORTH CAROLINA
HERMES
MARY CELESTIA
MINNIE BRESLAUER
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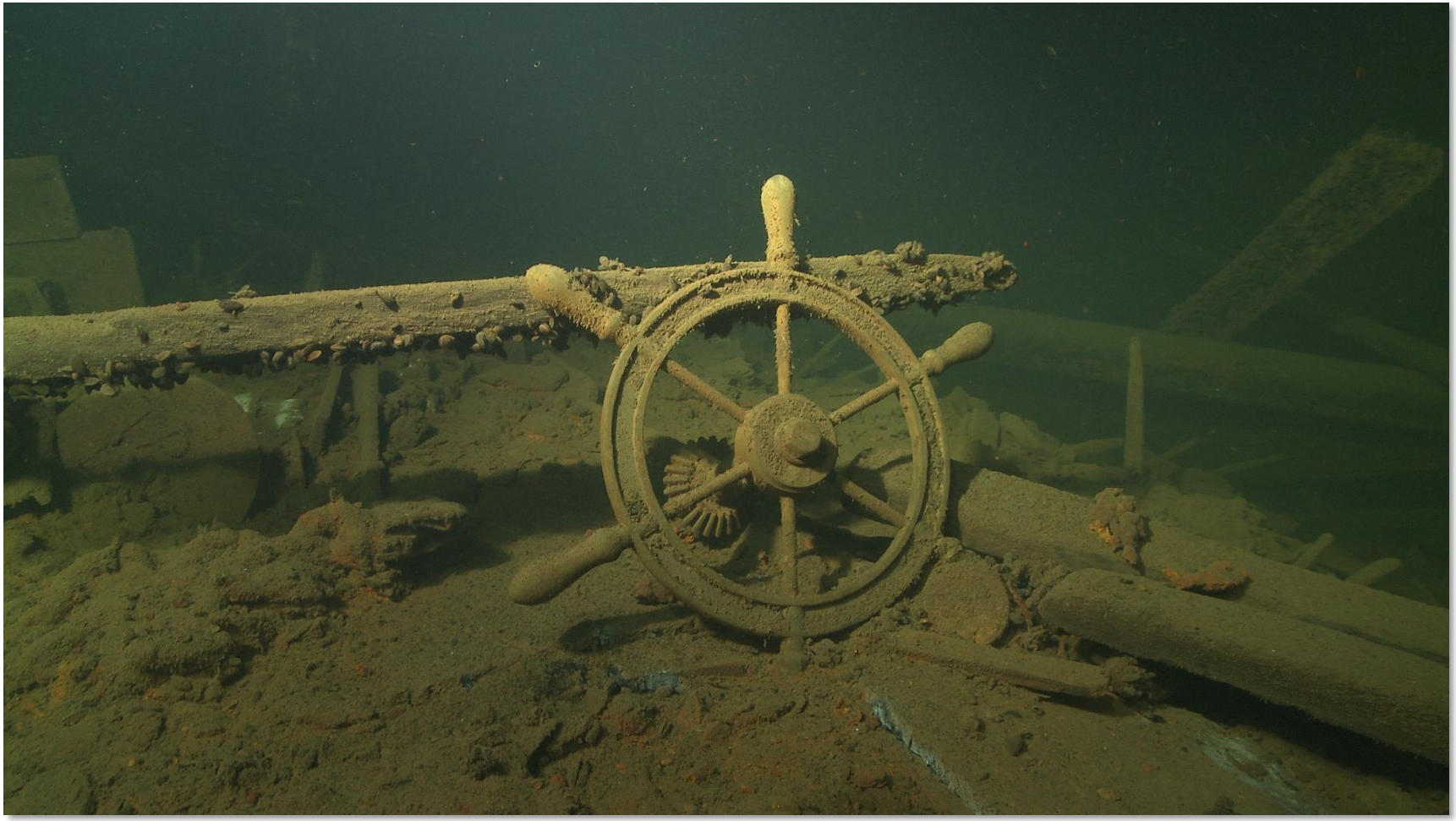
feel the love

Bermuda! Feel the love!

If I understand it, reviewers will too

Made their grant hard to review



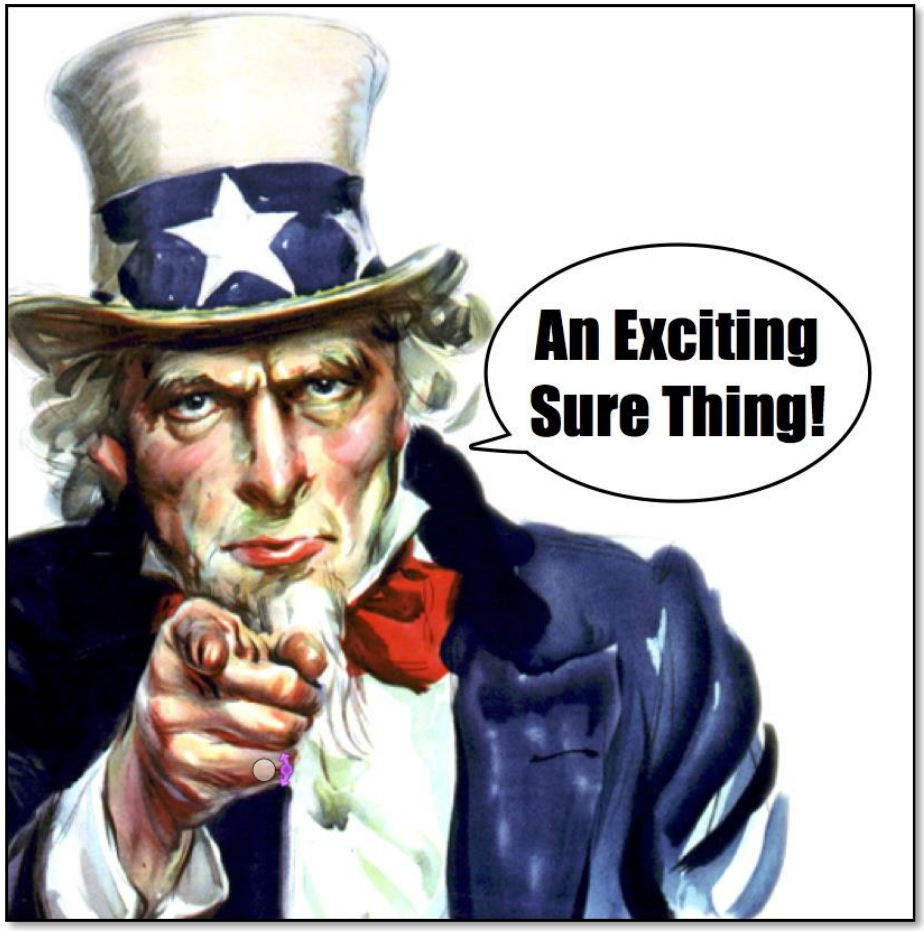
Every reviewer is alert, qualified, and fair

Misunderstood what gets funded

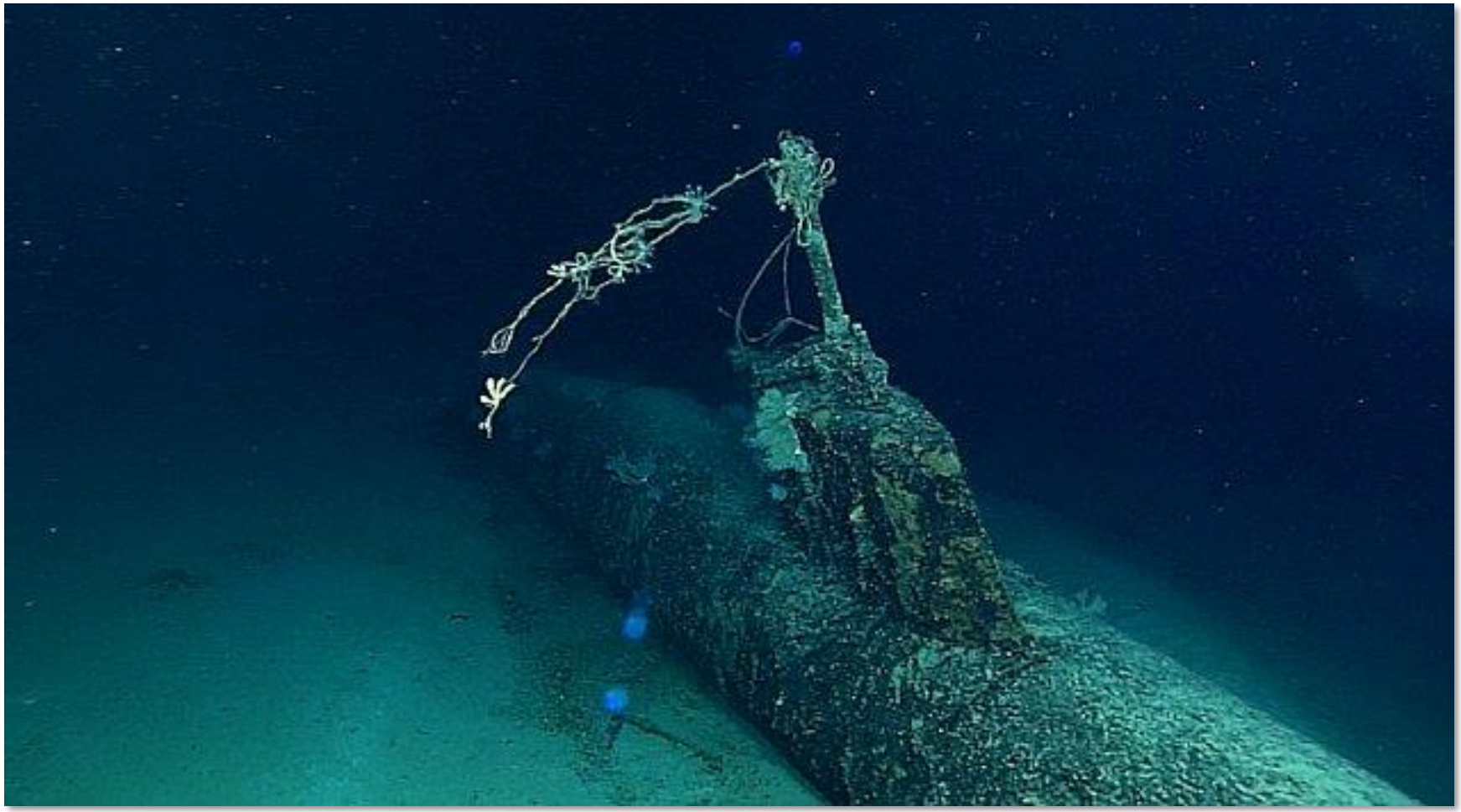
“My idea is so great it’ll sell itself!”

“Reviewers won’t notice my project is risky.”

What federal grant agencies want to buy in research projects



Foundations want the same thing



Writing grants is like writing papers

All pages are equally important

First page put reviewers to sleep

Which would you rather read at 2 am?

SPECIFIC AIMS

Approximately 40% of children with ASD exhibit clinically significant anxiety.[20] While Cognitive Behavioral Therapy (CBT) is a promising treatment for anxiety in ASD,[21] its neural-systems-level targets are unknown. We propose a randomized controlled trial of CBT vs. Psychoeducation and Supportive Therapy (PST) in 100 high-functioning 8 to 12-year-old children with ASD and moderate to severe anxiety. We will utilize fMRI to identify CBT-invoked changes in levels of activity/functional connectivity within the neural circuits involved in emotion regulation and social perception. During fMRI collected before and after a 15-week course of either CBT or PST children will: 1) regulate emotion while viewing affective images; 2) match facial expressions depicting anger and fear; and 3) perceive coherent vs. scrambled biological motion. Fifty matched typically developing (TD) children will be scanned twice, 16 weeks apart, to enable interpretation of treatment-evoked change relative to normative development. Dysfunction of the amygdala and its connectivity with the prefrontal cortex has been implicated in co-occurring ASD and anxiety.[22-24] Children with ASD also exhibit reduced activity in the ventromedial prefrontal cortex (vmPFC) and failure of amygdala down-regulation during cognitive reappraisal.[13] Plus, our pilot data reveal 1) a negative correlation between vmPFC activation and anxiety in children with ASD and 2) increase of vmPFC activity and connectivity with amygdala after CBT. Because CBT teaches emotion regulation skills, and CBT in children with anxiety disorders (without ASD) alters corticolimbic circuitry,[25] we predict that reduction of anxiety after CBT will be associated with increased prefrontal activity during emotion regulation and increased prefrontal-limbic functional connectivity. In addition, it is unknown whether the neural mechanisms of core social deficits in ASD are associated with anxiety. We will examine this relationship via the experimental manipulation of anxiety with CBT vs. PST while assaying brain activity with a biological motion perception task that robustly activated a social perception circuit including vmPFC, superior temporal sulcus (STS), fusiform gyrus (FFG) and amygdala, that is correlated with levels of social disability in children with ASD.[26] We will test whether neural markers of social impairment in ASD are associated with anxiety and its response to treatment. Lastly, we will explore amygdala-prefrontal[27] and default mode network (DMN) connectivity[28] during resting-state fMRI to corroborate task-related findings and to test the utility of large-scale neural networks analyses in developing biomarkers of treatment response in ASD. To address methodological limitations of prior clinical trials, we will test the efficacy of CBT against an active control condition.

Aim 1. To demonstrate clinical efficacy of CBT vs. PST in children with ASD and anxiety in a randomized controlled trial. *Hypothesis (H) 1a*: CBT vs. PST will lead to reduction in anxiety from baseline to endpoint on the clinician-rated Pediatric Anxiety Rating Scale. *H1b*: CBT vs. PST will lead to greater reduction in anxiety from baseline to endpoint measured by the CGI-Improvement Score assigned by the independent evaluator.

Aim 2. To examine the effects of CBT vs. PST on the neural basis of anxiety in ASD by collecting fMRI data during emotion regulation, face perception, and rest before and after treatment. *H2a*: CBT will increase ventrolateral PFC (vlPFC) and vmPFC activity, decrease amygdala reactivity, and enhance amygdala-vmPFC functional connectivity during down-regulation vs. passive viewing of affective images. *H2b*: CBT will decrease amygdala reactivity during emotional face vs. shape matching and during viewing of negative vs. neutral images. *H2c*: CBT will increase the magnitude of amygdala-vmPFC resting-state functional connectivity.

Aim 3. To explore the moderating effects of baseline fMRI indices (i.e., brain activity before treatment) of emotion regulation and social perception on response to CBT vs. PST. *H3a*: Low vlPFC and vmPFC activity and their weak functional connectivity with amygdala during down-regulation vs. passive viewing of negative affective images before treatment will be positively associated with anxiety reduction after CBT vs. PST. *H3b*: Greater activity in the amygdala during emotional face vs. shape matching and viewing of negative vs. neutral images (as a biomarker of anxiety) before treatment will be positively associated with reduction of anxiety after CBT vs. PST. *H3c*: Greater activity in the STS and FFG (as biomarkers of social function) during perception of coherent vs. scrambled motion will be positively associated with reduction of anxiety after CBT vs. PST. *H3d*: Resting-state hypo-connectivity within the DMN will be associated with poorer response to CBT vs. PST.

Aim 4. Compare activity during emotion regulation and social perception in children with ASD before and after CBT vs. PST to matched TD children scanned twice 16 weeks apart. *H4a*: Before treatment, ASD vs. TD will exhibit: 1) reduced vlPFC and vmPFC activity and connectivity with amygdala during emotion regulation; 2) decreased FFG and STS activity and increased amygdala reactivity in response to emotional faces vs. shapes; and 3) decreased activity in the social perception network during the perception of biological motion. *H4b*: After treatment, 1) in CBT vs. PST, the pre-treatment TD vs. ASD neural-systems-level differences in the emotional reactivity and regulation circuits outlined in parts 1 and 2 of *H4a* will be ameliorated, reflecting a normalization of brain function; 2) the neural circuitry of social perception will be unaffected by CBT for anxiety; 3) children with ASD who are randomized to PST will continue to show all of the aforementioned TD vs. ASD differences.

SPECIFIC AIMS

This proposal is about the role of a chromatin modifying factor in regulating uterine epithelial proliferation in response to hormonal signals. Our preliminary data strongly suggest AT-rich interactive domain-containing protein 1A (ARID1A) has a key role in implantation and decidualization, and that ARID1A expression is lost in endometriosis, a disorder characterized by overproliferation of the endometrium. This is significant for understanding both normal implantation and its dysregulation in endometriosis. Further, this proposal offers the potential to discover new therapies for infertility and endometriosis: (1) by identifying the downstream targets of ARID1A; and (2) by testing whether resveratrol, a phytoestrogen that has successfully inhibited epithelial proliferation of human cancers[85], can reverse uterine epithelial proliferation.

ARID1A belongs to the SWI/SNF family and is required to activate transcription of genes normally repressed by chromatin[4,5]. ARID1A loss is uniquely associated with endometriosis-associated ovarian carcinomas[6-8]. However, how ARID1A works in the female reproductive track in both health and disease is unclear.

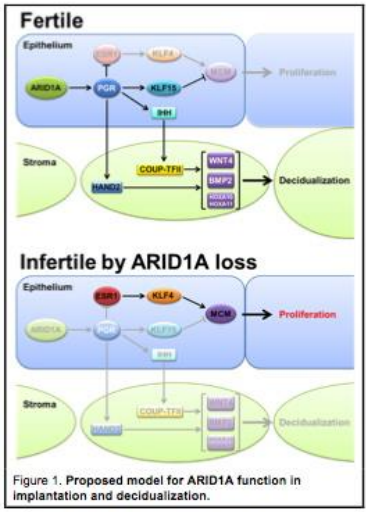


Figure 1. **Proposed model for ARID1A function in implantation and decidualization.**

Our experiments will comprehensively test the interactions between ARID1A and progesterone receptor (PGR), identify the gene targets of ARID1A, and test the ability of resveratrol to reverse uterine epithelial proliferation caused by ARID1A loss. There is strong innovation in the novelty of our hypotheses and our cutting-edge technical approaches. In particular, our experiments will employ the first low cost animal model that closely resembles human endometriosis.

Aim 1. Determine the role of ARID1A in suppressing epithelial cell proliferation for uterine receptivity.

A. Determine if ARID1A negatively regulates E2-induced epithelial cell proliferation through PGR interactions
B. Characterize transcriptional regulation of P4 target genes by ARID1A
C. Evaluate ARID1A loss in tissues of infertile women with endometriosis compared to controls

Aim 2. Determine the importance of ARID1A loss in decidualization, infertility and endometriosis.

A. Determine whether ARID1A loss causes a decidualization defect in conditional *Arid1a* KO ($Pgr^{cre/+}$ $Arid1a^{f/f}$; $Arid1a^{d/d}$) mice and human endometrial stromal cells
B. Determine if ARID1A loss causes P4 resistance in endometriosis using a mouse model that realistically resembles human endometriosis
C. Determine if ARID1A loss causes endometriosis-related infertility using a mouse model

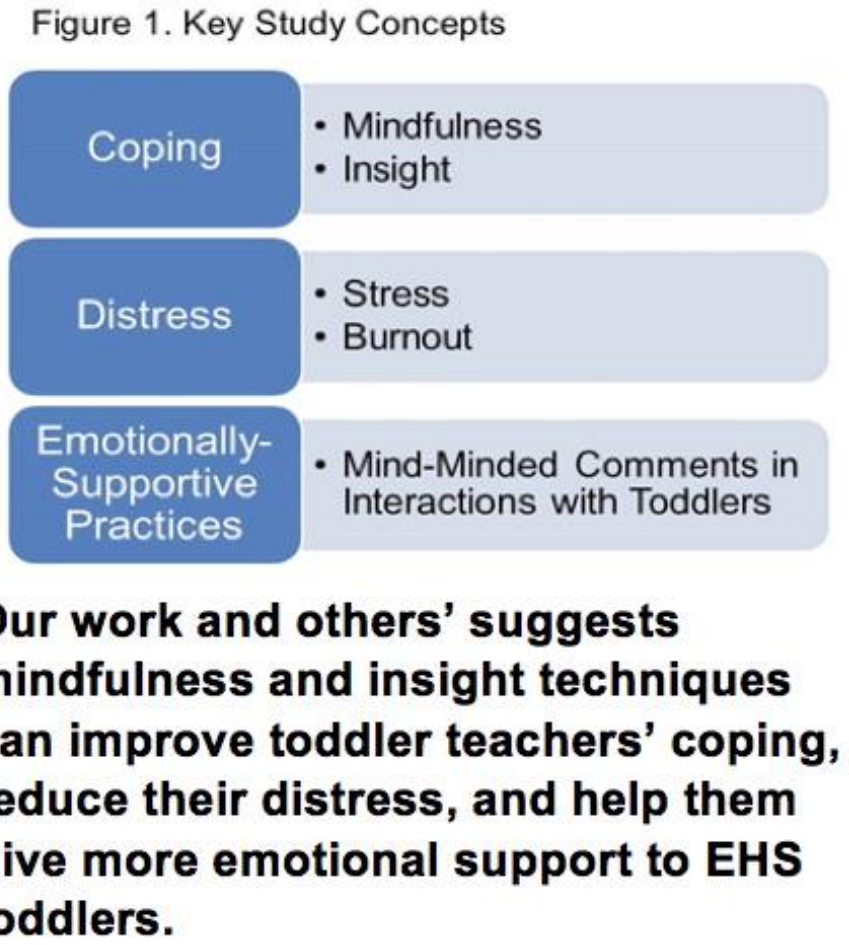
Aim 3. Evaluate the ability of resveratrol to restore uterine function in cases of infertility and endometriosis due to ARID1A loss.

A. Determine if resveratrol overcomes aberrant epithelial proliferation and implantation defects in $Arid1a^{d/d}$ mice
B. Determine effect of resveratrol on establishment of endometriotic lesions and infertility
C. Determine effect of resveratrol in a Xenograft model using human endometrial tissue

OVERALL IMPACT: We will clarify how ARID1A mediates P4 inhibition of E2 signaling in the uterus, and test using mice and human tissues whether a phytoestrogen, resveratrol, can help treat infertility and endometriosis. Our experiments will employ the first low cost animal model that closely resembles human endometriosis.

Graphical abstracts aren’t needed

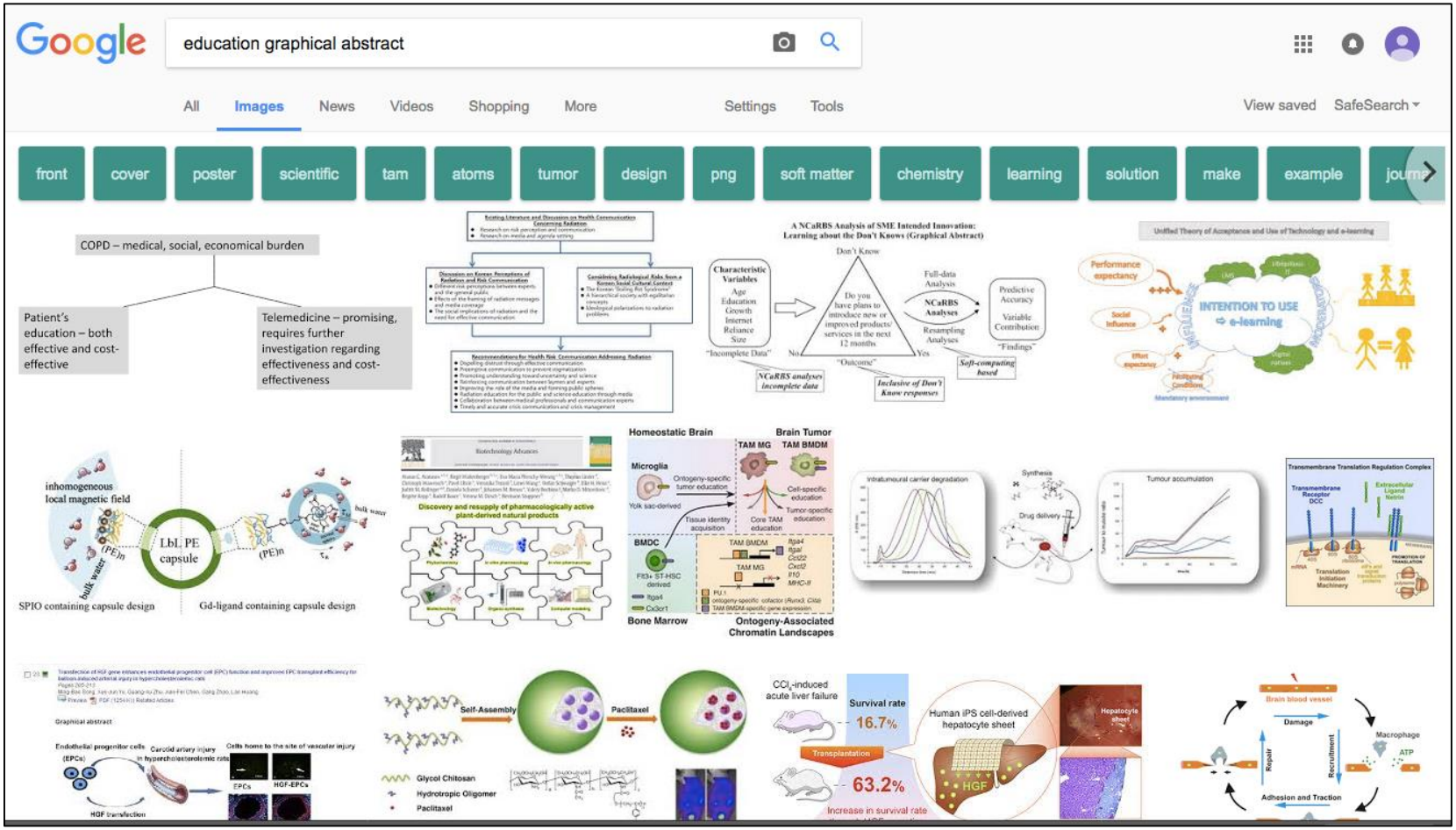
Graphical abstracts don’t have to be fancy



-- Holly Brophy-Herb of MSU

To find graphical abstract ideas:

Google: “graphical abstract” + your keyword



Grants don’t need elevator pitches

Nancy Roman's pitch to get Congress to fund the Hubble Space Telescope

NIH grant elevator pitch

"**OVERALL IMPACT**: We will clarify how ARID1A mediates P4 inhibition of E2 signaling in the uterus, and test using mice and human tissues whether a phytoestrogen, resveratrol, can help treat infertility and endometriosis. Our experiments will employ the first low cost animal model that closely resembles human endometriosis." (48 words)

-- Jae-Wook Jeong of MSU

NIH grant elevator pitch

Overall Impact: Revealing mechanisms by which DHA blocks $cSIO_2$-triggered lupus will bring novel insights into the disease's initiation and how manipulating the lipidome through diet can prevent it.

(29 words)

-- Jim Pestka of MSU

Didn’t repeat key selling points

Repeating what makes your project special is ok. Red: a novel therapy; Blue: new gene targets; Yellow: an innovative disease model.

-- Jae-Wook Jeong of MSU

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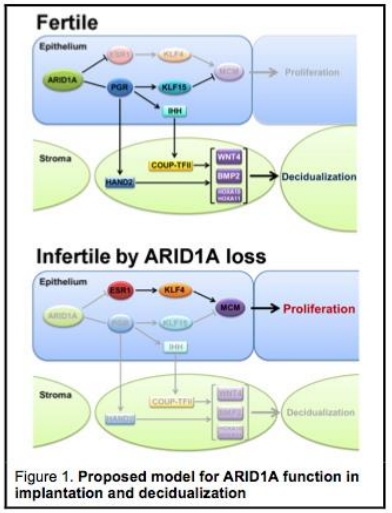

Figure 1. **Proposed model for ARID1A function in implantation and decidualization**

AT-rich interactive domain-containing protein 1A (ARID1A) belongs to the SWI/SNF family and is required to activate transcription of genes normally repressed by chromatin[12,13]. ARID1A loss is uniquely associated with endometriosis-associated ovarian carcinomas[14-16]. However, how ARID1A works in the female reproductive track in both health and disease is unclear.

Our experiments will comprehensively test the interactions between ARID1A and the estrogen receptor and progesterone receptor, identify the gene targets of ARID1A, and test the ability of resveratrol to reverse uterine epithelial proliferation caused by ARID1A loss. There is strong innovation in the novelty of our hypotheses and our cutting-edge technical approaches. In particular, our experiments will employ the first low cost animal model that closely resembles human endometriosis.

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Stopped selling before the last page

Didn’t anticipate reviewers’ objections

Didn’t justify key decisions

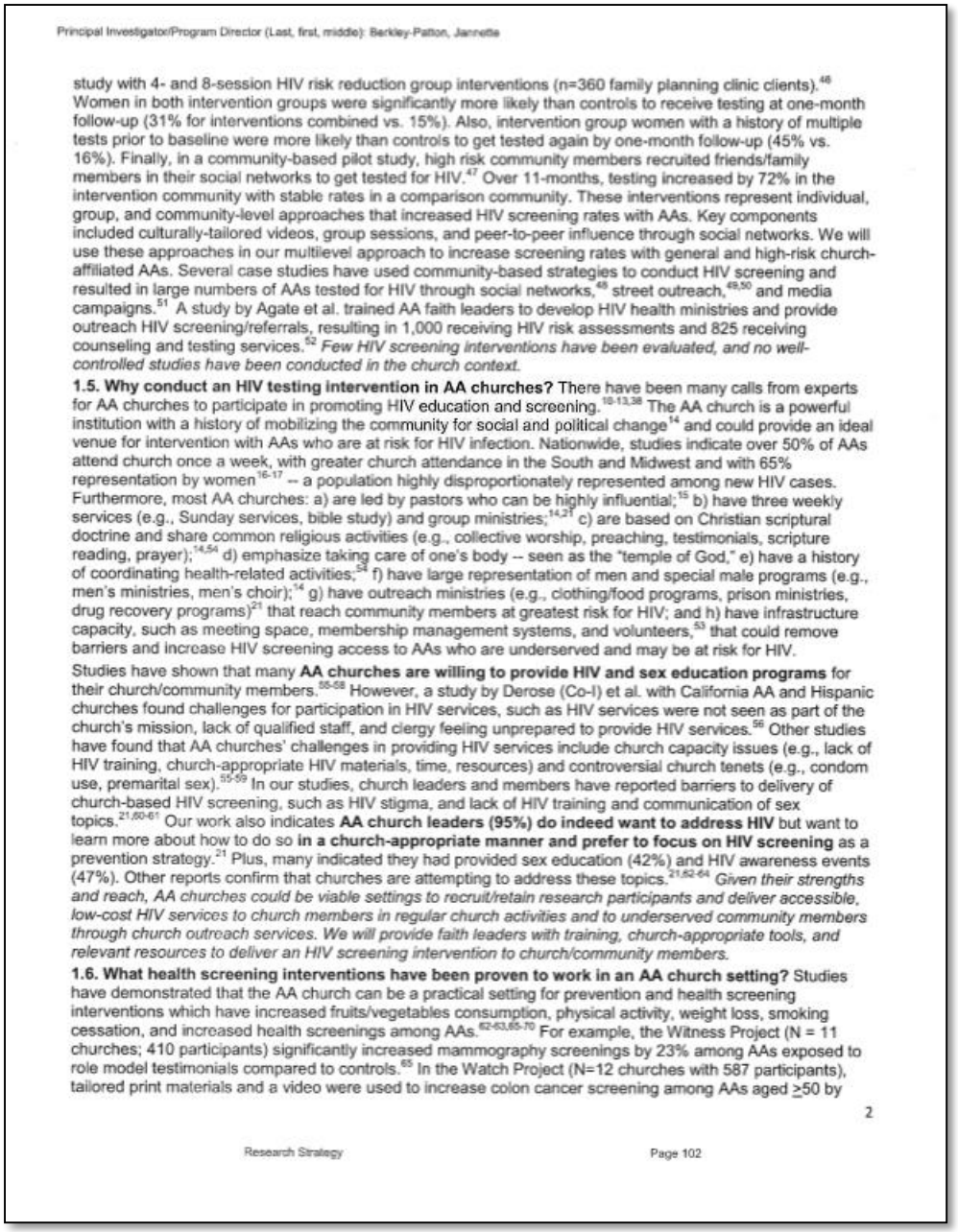
Some justifications take only a sentence:

"The justification for this approach is that insulin [55-59] and IGF-I [60, 61] have both been shown to be stimulators of trophoblast amino acid and/or glucose transport."

-- Thomas Jansson

Why & What paragraphs can help reviewers accept how you decide to run your project

-- Jannette Berkley-Patton

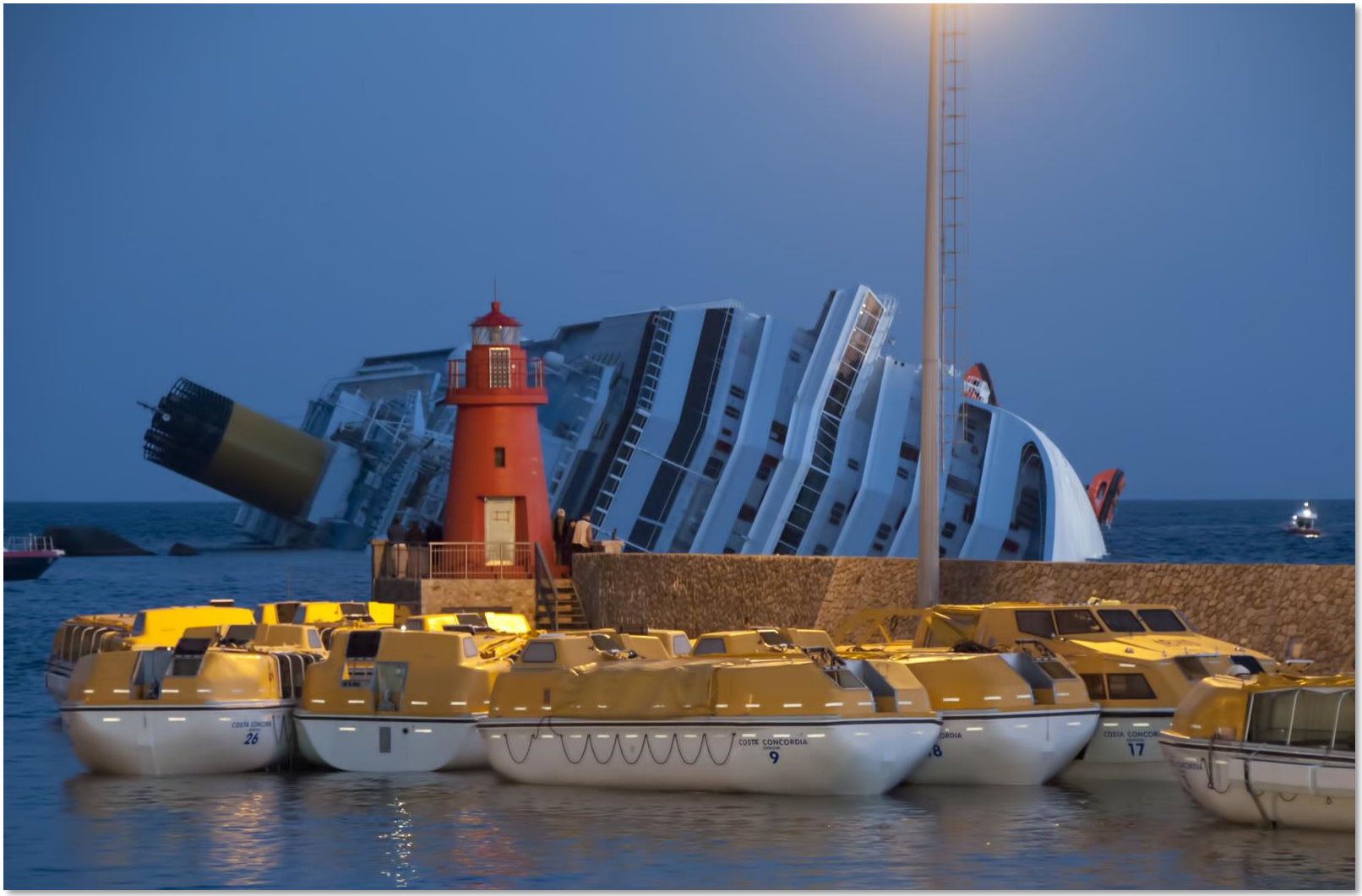


study with 4- and 8-session HIV risk reduction group interventions (n=360 family planning clinic clients).[46] Women in both intervention groups were significantly more likely than controls to receive testing at one-month follow-up (31% for interventions combined vs. 15%). Also, intervention group women with a history of multiple tests prior to baseline were more likely than controls to get tested again by one-month follow-up (45% vs. 16%). Finally, in a community-based pilot study, high risk community members recruited friends/family members in their social networks to get tested for HIV.[47] Over 11-months, testing increased by 72% in the intervention community with stable rates in a comparison community. These interventions represent individual, group, and community-level approaches that increased HIV screening rates with AAs. Key components included culturally-tailored videos, group sessions, and peer-to-peer influence through social networks. We will use these approaches in our multilevel approach to increase screening rates with general and high-risk church-affiliated AAs. Several case studies have used community-based strategies to conduct HIV screening and resulted in large numbers of AAs tested for HIV through social networks,[48] street outreach,[49,50] and media campaigns.[51] A study by Agate et al. trained AA faith leaders to develop HIV health ministries and provide outreach HIV screening/referrals, resulting in 1,000 receiving HIV risk assessments and 825 receiving counseling and testing services.[52] *Few HIV screening interventions have been evaluated, and no well-controlled studies have been conducted in the church context.*

1.5. Why conduct an HIV testing intervention in AA churches? There have been many calls from experts for AA churches to participate in promoting HIV education and screening.[10-13,38] The AA church is a powerful institution with a history of mobilizing the community for social and political change[14] and could provide an ideal venue for intervention with AAs who are at risk for HIV infection. Nationwide, studies indicate over 50% of AAs attend church once a week, with greater church attendance in the South and Midwest and with 65% representation by women[16-17] -- a population highly disproportionately represented among new HIV cases. Furthermore, most AA churches: a) are led by pastors who can be highly influential;[15] b) have three weekly services (e.g., Sunday services, bible study) and group ministries;[14,21] c) are based on Christian scriptural doctrine and share common religious activities (e.g., collective worship, preaching, testimonials, scripture reading, prayer);[14,54] d) emphasize taking care of one's body -- seen as the "temple of God," e) have a history of coordinating health-related activities;[54] f) have large representation of men and special male programs (e.g., men's ministries, men's choir);[14] g) have outreach ministries (e.g., clothing/food programs, prison ministries, drug recovery programs)[21] that reach community members at greatest risk for HIV; and h) have infrastructure capacity, such as meeting space, membership management systems, and volunteers,[53] that could remove barriers and increase HIV screening access to AAs who are underserved and may be at risk for HIV.

Studies have shown that many **AA churches are willing to provide HIV and sex education programs** for their church/community members.[55-58] However, a study by Derose (Co-I) et al. with California AA and Hispanic churches found challenges for participation in HIV services, such as HIV services were not seen as part of the church's mission, lack of qualified staff, and clergy feeling unprepared to provide HIV services.[56] Other studies have found that AA churches' challenges in providing HIV services include church capacity issues (e.g., lack of HIV training, church-appropriate HIV materials, time, resources) and controversial church tenets (e.g., condom use, premarital sex).[55-59] In our studies, church leaders and members have reported barriers to delivery of church-based HIV screening, such as HIV stigma, and lack of HIV training and communication of sex topics.[21,60-61] Our work also indicates **AA church leaders (95%) do indeed want to address HIV** but want to learn more about how to do so **in a church-appropriate manner and prefer to focus on HIV screening** as a prevention strategy.[21] Plus, many indicated they had provided sex education (42%) and HIV awareness events (47%). Other reports confirm that churches are attempting to address these topics.[21,62-64] *Given their strengths and reach, AA churches could be viable settings to recruit/retain research participants and deliver accessible, low-cost HIV services to church members in regular church activities and to underserved community members through church outreach services. We will provide faith leaders with training, church-appropriate tools, and relevant resources to deliver an HIV screening intervention to church/community members.*

1.6. What health screening interventions have been proven to work in an AA church setting? Studies have demonstrated that the AA church can be a practical setting for prevention and health screening interventions which have increased fruits/vegetables consumption, physical activity, weight loss, smoking cessation, and increased health screenings among AAs.[62-63,65-70] For example, the Witness Project (N = 11 churches; 410 participants) significantly increased mammography screenings by 23% among AAs exposed to role model testimonials compared to controls.[65] In the Watch Project (N=12 churches with 587 participants), tailored print materials and a video were used to increase colon cancer screening among AAs aged ≥50 by



Reviewers never skim instead of read

The first sentences in bold in Jose Luchsinger's paragraphs summarize why his project is important and allow reviewers to skim

3. RESEARCH STRATEGY.

3.1. Significance.

3.1.1. Dementia is an increasing problem in our aging societies and is more prevalent in Hispanics. Dementia is a syndrome characterized by impairment of memory and other cognitive abilities as well as behavior, severe enough to impair the ability to live independently.[6] The most common cause of *late onset dementia* is Alzheimer's disease,[7,8] comprising approximately 70% of cases, but vascular and mixed dementias are also common, comprising up to 25% of cases.[9] Dementia prevalence increases after the age of 70 years[10] and may reach 50% in persons 85 years and older.[11] In 2011 the Alzheimer's Association estimated that 5.4 million people (1 in 8 elders), have Alzheimer's dementia (AD) cared for by 14.9 million unpaid caregivers, resulting in $183 billion in annual costs.[6] Despite increased understanding of dementia, no preventive or curative measure exists,[12] and trials of new agents are discouraging.[13] Consequently, the numbers of caregivers burdened by dementia is increasing. Hispanics, the fastest growing ethnic group in the United States,[14] is also the group with the fastest growing number of dementia cases.[15] Dementia prevalence in Hispanics is several times higher than in Non-Hispanic Whites (NHW) nationally (27.9% vs. 10.9% in persons aged 75-84 years; 62.9% vs. 30.2% in persons 85 years and older)[8] and in New York City.[16,17] *NHiRP focuses on Hispanics because they suffer disproportionately from dementia and its related caregiving burdens.*

3.1.2. Caregivers of persons with dementia suffer from depression, potentially exacerbated in Hispanics by unique caregiving experiences and stressors. The care of persons with dementia is challenging[18-20]. They require intense supervision and care, risking caregivers' psychological, physical,[21-23] and financial health.[8] Caregiver stress leads to nursing home placement (NHP) for the person with dementia,[23,24] but caregivers report emotional and physical stress even after NHP.[25,26] Most caregivers report no guilt after NHP,[8] but this is less common in Hispanics,[3,8] who delegate less care of affected relatives.[3] Hispanic caregivers are more depressed than other racial/ethnic groups,[27] *but the interplay of risk and protective factors is poorly understood*, due to limitations of the literature. A 2011 nationwide *telephone survey* conducted by the Alzheimer's Association highlights Hispanic caregivers' unique characteristics. They are younger than NHW and Non-Hispanic Black (NHB)[8]; less likely to be married than NHW; more likely to have children or grandchildren under age 18 in their household than NHW and NHB; more likely to be a primary caregiver than NHW and Asian-Americans (AA); more likely to make <$50,000 annually than NHW and AA; and more likely to need help balancing work and family and finding personal time than NHW[8]. Small studies show Hispanics experience more strain and less social support than other racial/ethnic groups,[28,29] despite extensive social networks,[30] and less acculturated Hispanic caregivers experience more depression.[31] Yet, mixed results from small studies have not clarified the association between social support and caregiver depression in Hispanics.[29,30] The role of coping in attenuating caregiver depression in Hispanics remains poorly elucidated;[32] effortful coping[33] has never been investigated in Hispanic caregivers. Knowing whether and how these key characteristics operate together can inform interventions. *NHiRP will collect comprehensive data through in-person interviews that shall foster understanding of the interplay of key socio-demographic characteristics of Hispanic caregivers, caregiver burden, stress, and depressive symptoms in one of the largest studies to date.*

3.1.3. There is limited information on caregiver interventions for Hispanics. Caregiver interventions can be classified broadly as counseling or as psycho-educational. Some interventions combine both, such as the NYUCI,[5] and the Resources for Enhancing Alzheimer's Caregiver Health (REACH).[34,35] These interventions decrease caregiver burden and depressive symptoms by increasing self-efficacy and teaching coping mechanisms. REACH has demonstrated efficacy among Hispanics in South Florida,[34] but there is no comparable data for the NYUCI, which is the reason for conducting NOCIP. Psycho-educational interventions are also effective tools[36-39] that provide knowledge and skills that improve self-efficacy. However, there is limited evidence that psycho-educational tools are effective in Hispanics.[40] Psycho-educational tools lend themselves particularly well to technology-based interventions, but studies of their efficacy in Hispanics are needed. Given the cost of in-person interventions, technology-based interventions are increasingly being designed and tested. *NHiRP will study the long-term effectiveness of the NYUCI in Hispanics and will develop and test a novel technology-based intervention that supports education and health management.*

3.1.4. Technology-based family caregiver interventions offer encouraging results, but knowledge gaps exist regarding web-based health information management systems by caregivers. Technology-based caregiver interventions can improve decision confidence, reduce emotional strain, improve spousal relationship conflict, decrease activity restriction, increase self-efficacy, and decrease burden.[41-43] The most promising interventions are based on computer networks, interactive telephonic and video platforms, and the internet to provide direct caregiver support. The earliest computer-based intervention used computer networks to provide

Doug Postels of MSU used Luchsinger's technique in his R01 clinical trial grant worth $9 million

Significance

In Africa, malaria kills over 500,000 people annually[1]. Many die of cerebral malaria (CM), defined as an otherwise unexplained coma in someone with *Plasmodium* parasitemia. Ninety-five percent of the burden of African CM mortality falls onto children. We have been working to understand the underlying mechanisms of mortality in pediatric CM, and recently identified a strong association between increased brain volume and death in children with retinopathy-positive CM, publishing our study in the *New England Journal of Medicine*[2]. The high strength of the association, its biological plausibility, and analogy with other disease processes suggests that brain swelling is not an epiphenomenon, but a mediator on the causal pathway between malaria infection and death. In this application we propose a clinical trial of 2 interventions targeting this key pathophysiological step. We hypothesize that one or both of our proposed interventions will decrease mortality without concurrently raising rates of neurological morbidity in survivors. Our overarching goal is to decrease rates of death *and* neurological disability from this devastating infectious disease.

Numerous clinical trials have been performed in cerebral malaria, none of which has shown a therapeutic benefit of interventions compared to a control population. Several of these trials had limited study power by including children at low risk of mortality[3]. Our proposed clinical trial improves on these previous designs by using newfound knowledge about the importance of increased brain volume, a key step on the pathway to death in children with CM. This knowledge allows us to enrich our study population to children at very high risk of mortality, those with highly increased brain volumes. While enrollment in the clinical trial includes children who fulfill the broad diagnostic criteria for CM, our primary analysis will be for children with retinopathy-positive CM, where the association between increased brain volume and mortality is clear.

We propose a clinical trial of intravenous hypertonic saline or early mechanical ventilation in children with CM at high risk of death. We will compare mortality rates in children enrolled in these two treatment arms to rates in control children treated with the current standard of care—elevation of the head of the bed by 30 degrees and intravenous antimalarials. We chose the two interventions for different reasons. Hypertonic saline is commonly used in high income countries to decrease brain swelling in those with increased intracranial pressure of multiple etiologies, while early mechanical ventilation alone may be helpful in children with CM due to the quick reversibility of coma and the rapid improvement in multiple biomarkers in children who survive this illness. Hypertonic saline targets increased brain volume, while mechanical ventilation supports life while the natural history of the disease unfolds.

In high income countries, adjunctive hypertonic saline administration is the standard of care for initial treatment of many causes of diffuse brain swelling. A large body of medical literature supports bolus or continuous hypertonic saline infusion therapy for patients with brain abnormalities accompanied by neuroradiological or clinical signs of increased intracranial pressure. The intervention is well tolerated, easy to administer, and requires little technology to implement.

We believe that supportive mechanical ventilation alone may decrease death rates in children with CM due to the rapid reversibility of the massive brain swelling in survivors. Additionally, clinical characteristics and biomarkers of cerebral dysfunction seen in children with CM demonstrate that this may be a uniquely rapidly reversible illness. Clinically, children with CM who recover often do so extremely quickly—a child's Blantyre coma score of 1 may improve to a normal score of 5 within 24 hours. Electroencephalography (EEG) studies in children with CM demonstrate that patterns typically considered as precursors to death in other disease processes (e.g. burst suppression) may be followed in 24 hours by complete recovery (Dr. Gretchen Birbeck, personal communication). Diffusion weighted imaging (DWI) sequences on brain MRI of children with CM may demonstrate multifocal areas of restricted diffusion, which is usually assumed to represent an area of cerebral infarction --- but in CM, these can resolve completely followed within 24 hours (Dr. Sam Kampondeni, personal communication) followed within 24 hours by complete resolution. Admission retinal angiography in children with retinopathy positive CM can show complete vessel occlusion which clears within 24 hours. Taken together, these rapid clinical and biomarker improvements suggest that supportive mechanical ventilation alone may be sufficient to increase survival, allowing the brain to heal itself while the body's vital functions are supported.

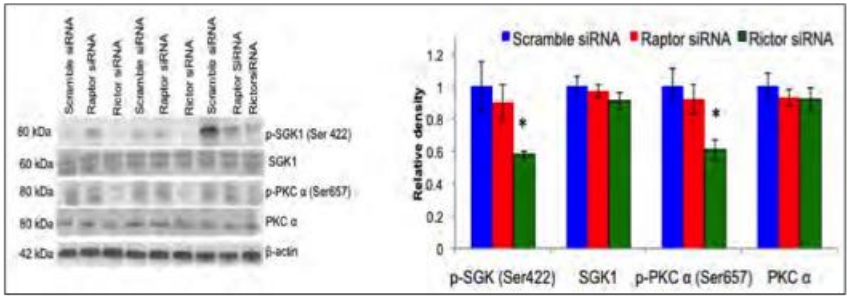
Interventions that decrease mortality may be accompanied by an increase in rates of neurological morbidity in CM survivors. Though mortality is our primary endpoint, we will closely document, follow, analyze, and compare the rates of neurological morbidity in survivors in both intervention arms and the control

Didn’t tell reviewers what to think about the tables and figures

Write your legends to get your point across and let reviewers skim at the same time

--Thomas Jansson

Which legend is safest for writing to the reviewer reading in bed?



A

Fig 12. Cultured trophoblast cells were transfected with siRNA at 18 hours of culture and harvested at 90 hours. Protein expression of total and phosphorylated PKCα and SGK1 was determined in whole cell lysates using Western blot. Mean ± SEM, n = 3-6 placentas. ANOVA, *p<0.

B

Fig 12. Cultured trophoblast cells were transfected with siRNA at 18 hours of culture and harvested at 90 hours. Protein expression of total and phosphorylated PKCα and SGK1 was determined in whole cell lysates using Western blot. Mean ± SEM, n = 3-6 placentas. ANOVA, *p<0. The data shows that silencing rictor, but not raptor, inhibits PKCα and SGK1 phosphorylation.

C

Fig 12. Silencing of rictor, but not raptor, inhibits PKCα and SGK1 phosphorylation. Cultured trophoblast cells were transfected with siRNA at 18 hours of culture and harvested at 90 hours. Protein expression of total and phosphorylated PKCα and SGK1 was determined in whole cell lysates using Western blot. Mean ± SEM, n = 3-6 placentas. ANOVA, *p<0.

Answer: The one at bottom is best because it begins in bold text with the conclusion the author, Thomas Jansson, wants the reviewer to take away. If the reviewer is tired and tempted to skim the rest of the text, the amount of harm is minimized because at least the reviewer has read the conclusion. The legend in the middle isn't as good because it forces the reviewer to read until the end for the conclusion, which isn't set off by bold letters to make it more noticable. The legend at the top is the worst, because it has no conclusion at all: it's left up to the reviewer to decide what the data means — a dangerous idea. Legend C is the one Jansson actually included in his winning R01 grant. Jansson is a master grant writer, winning 3 R01s in 5 years. Notice that Jansson used 10-point font for his conclusion and 9-point for the rest, saving a little space.

This technique makes conclusions from many studies easier to grasp quickly

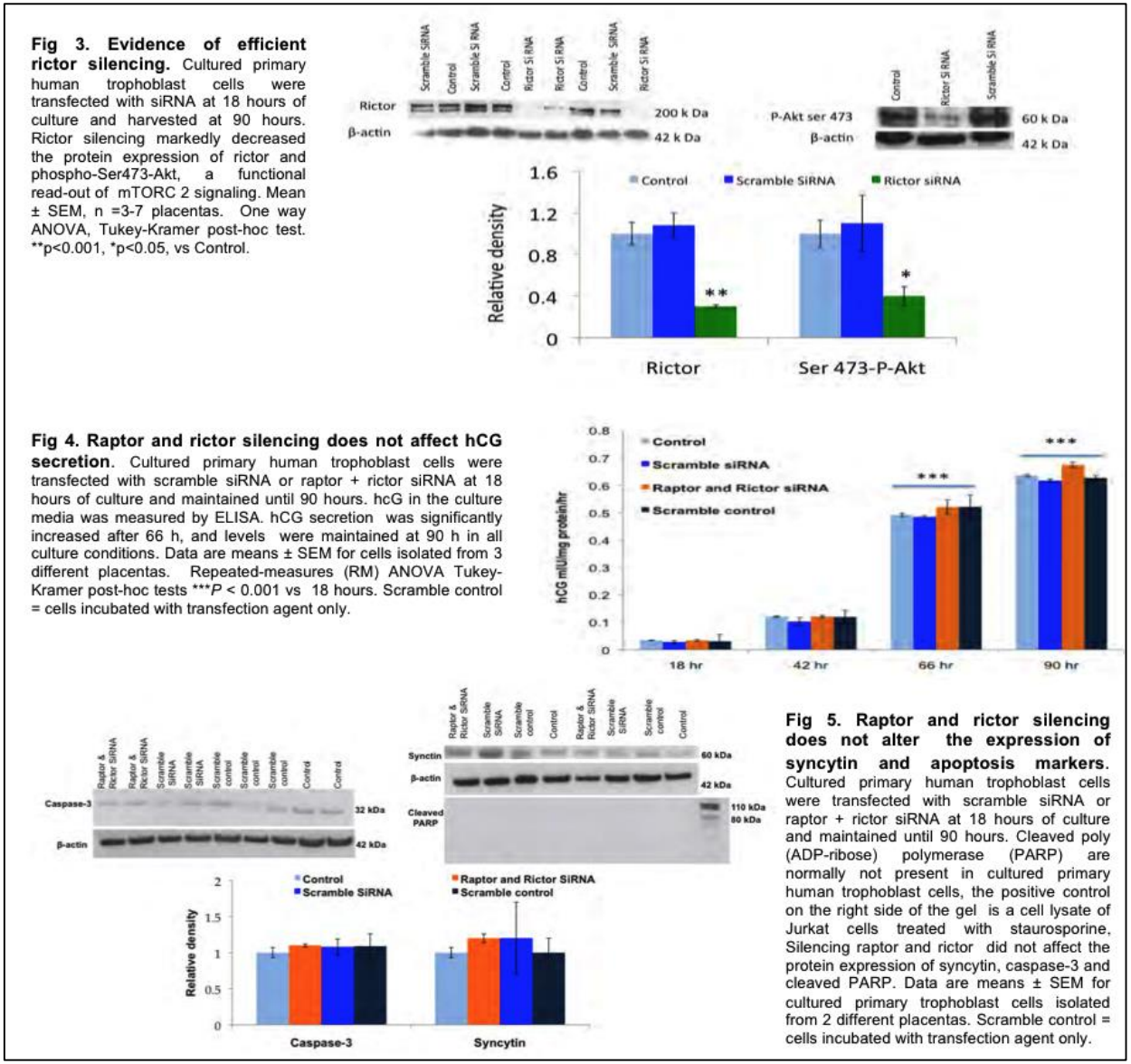


Fig 3. Evidence of efficient rictor silencing. Cultured primary human trophoblast cells were transfected with siRNA at 18 hours of culture and harvested at 90 hours. Rictor silencing markedly decreased the protein expression of rictor and phospho-Ser473-Akt, a functional read-out of mTORC 2 signaling. Mean ± SEM, n =3-7 placentas. One way ANOVA, Tukey-Kramer post-hoc test. **p<0.001, *p<0.05, vs Control.

Fig 4. Raptor and rictor silencing does not affect hCG secretion. Cultured primary human trophoblast cells were transfected with scramble siRNA or raptor + rictor siRNA at 18 hours of culture and maintained until 90 hours. hcG in the culture media was measured by ELISA. hCG secretion was significantly increased after 66 h, and levels were maintained at 90 h in all culture conditions. Data are means ± SEM for cells isolated from 3 different placentas. Repeated-measures (RM) ANOVA Tukey-Kramer post-hoc tests ***$P < 0.001$ vs 18 hours. Scramble control = cells incubated with transfection agent only.

Fig 5. Raptor and rictor silencing does not alter the expression of syncytin and apoptosis markers. Cultured primary human trophoblast cells were transfected with scramble siRNA or raptor + rictor siRNA at 18 hours of culture and maintained until 90 hours. Cleaved poly (ADP-ribose) polymerase (PARP) are normally not present in cultured primary human trophoblast cells, the positive control on the right side of the gel is a cell lysate of Jurkat cells treated with staurosporine, Silencing raptor and rictor did not affect the protein expression of syncytin, caspase-3 and cleaved PARP. Data are means ± SEM for cultured primary trophoblast cells isolated from 2 different placentas. Scramble control = cells incubated with transfection agent only.

-- Thomas Jansson

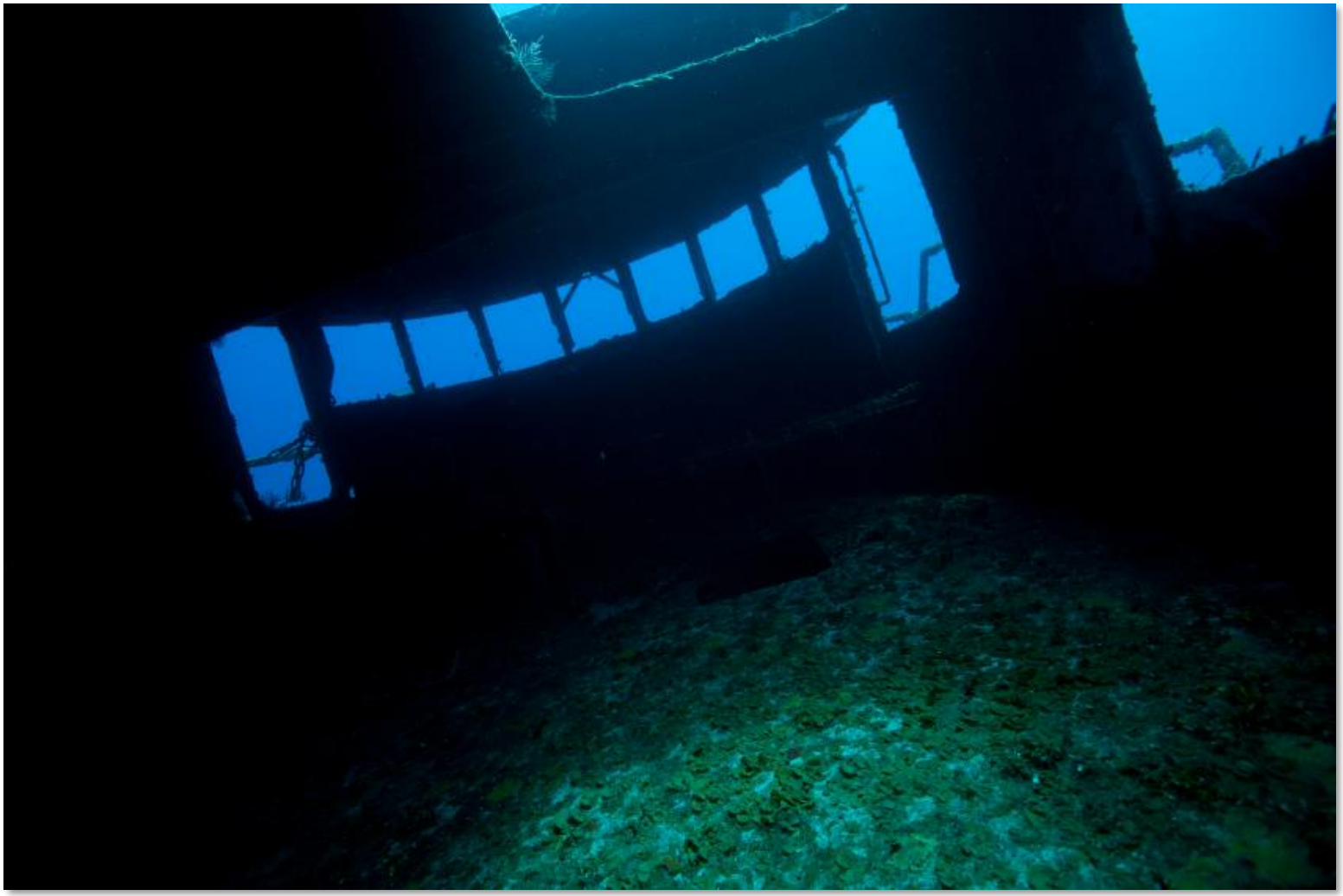
Promising the impossible – the 2-grants-in-1 gambit

Not all collaborators collaborate

Waited too long to start writing

2013's natural experiment in NIH grant writing

Gave up too soon



In the dark about funding sources (and didn’t use FDOP)

Worked on the grant alone

No peer review before submission

An all-day grant hotseat is one way to get colleagues to review your grant

Yanni Sun, Kevin Liu, Greg Bonito and me, all-day NSF grant hotseat

Don’t be outside looking in. Let MSU help you win.

I help people
win grants
(and not just from NIH)

For 1-hour appointments on campus

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If you're interested in NSF grants, MSU's NSF grant consultant is

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Acknowledgements

Steve Hsu
Corey Washington

END

Bonus tip

If you anticipate reviewers will have an immediate objection about your project once they understand what it is, begin on the first page to explain why your project is consistent with their organization's mission. On the following pages keep reminding them. If you have evidence to back you up, don't wait long to show it.

Example: Gita Coaker knew she had a problem

Coaker is a botanist, and NIH spends hardly anything on botany research. Unless she convinced reviewers the immune system proteins she studied in a plant were conserved during evolution all the way to humans, she very likely would not win NIH funding and would have to apply to NSF for a whole lot less money.

Coaker starts selling NIH on plant research beginning in the abstract

Project Summary

Multiple key components of the innate immune system are conserved across eukaryotes. In plants, the innate immune system serves as a barrier to inhibit both pathogen entry and multiplication. Despite the importance of the innate immune system, scientists still have a limited understanding of how plant immune complexes are assembled and regulated in response to pathogen perception. A key regulator of the plant immune system is the *Arabidopsis* gene RIN4. RIN4 is conserved among all land plants and acts to regulate immune perception of the bacterial pathogen *Pseudomonas syringae* pv. *tomato* in *Arabidopsis*. Preliminary data within this proposal demonstrate the purification of RIN4 protein complexes in the absence and presence of pathogen stimulus. Fifteen novel proteins were identified by mass spectrometry and multiple proteins were subsequently shown to interact with RIN4 by yeast two-hybrid. *Arabidopsis* knockout or overexpression lines for three of these RIN4 associated proteins display altered defense responses to *P. syringae* pv. *tomato*, suggesting that they are important components of the plant immune response. One RIN4 associated protein is the H^+-ATPase AHA1. Experiments indicate that RIN4 can directly regulate AHA1 enzymatic activity. RIN4 can work in concert with AHA1 to regulate leaf stomatal opening during the innate immune responses, thus blocking the entry of bacterial pathogens into the leaf interior. The central hypothesis of the proposed research is that RIN4 complex constituents will be key components controlling innate immune signaling. Several proposed experiments seek to understand RIN4 protein complex assembly and RIN4-mediated cellular signaling cascades using the *P. syringae-Arabidopsis* pathosystem. This pathosystem is an excellent model system to study eukaryotic innate immune signaling because of the extensive genetic resources available, the fast generation time of *Arabidopsis*, and the similarities between innate immune systems in plants and other eukaryotes.

The specific aims of this research proposal are the following:
1) Elucidate the mechanism RIN4 uses to regulate plasma membrane H^+-ATPase activity; 2) Investigate the spatial and temporal components of the RIN4 protein network; 3) Functionally characterize *Arabidopsis* RIN4 associated proteins.

She continued selling NIH on her work in her grant's first page.

A. SPECIFIC AIMS

Plants are constantly exposed to infectious microorganisms. However, the development of disease is the exception rather than the rule due to the evolution of highly coordinated passive and active defense systems in higher plants. There are two active branches of the plant immune system [3,5]. One branch consists of extracellular receptors recognizing conserved microbial features and functions to inhibit initial pathogen colonization. The second branch consists of intracellular receptors that recognize the presence of pathogen proteins present inside plant cells during infection. Despite the importance of the innate immune system, scientists still have a limited understanding of the composition and regulation of immune complexes in plants. The PI's laboratory investigates how the plant immune system recognizes bacterial pathogens. To date there is only one gene that can regulate both branches of the plant immune system: *RIN4* [6,7,8,9]. *RIN4* is highly conserved among all land plants, yet the mechanisms it uses to regulate defense signaling are largely unknown. RIN4 is a central player in regulating innate immunity at the membrane and its presence in more than one protein complex indicates that RIN4 is an important signaling molecule to study the regulation and activation of protein complexes controlling plant immunity. Our *long term goal* is to elucidate the signaling overlap between both branches of the plant innate immune system. This proposal seeks to understand how plant immune signaling unfolds by investigating RIN4-mediated cellular signaling cascades. *The central hypothesis of the proposed experiments is that RIN4 protein complex constituents will be key components controlling innate immune signaling.* A mechanistic understanding of how plant immune signaling unfolds will lead to innovative strategies to control and prevent plant disease. Furthermore, fundamental insight into how protein complexes are assembled, activated, and regulated at the membrane can also be applied to other eukaryotic systems, including human disease.

Using immunoaffinity chromatography, we have purified RIN4 associated proteins (RAPs) from the plant *Arabidopsis*. Purified proteins were identified by mass spectrometry, enabling the detection of RAPs in the absence and presence of pathogen stimulus. RIN4 associates with a different set of proteins during immune signaling, indicating that it may function as an adapter, transferring the signal of pathogen perception to intracellular signaling pathways. One RAP is a plasma membrane H^+-ATPase (AHA) that is expressed in guard cells, which make up stomatal pores. Our results indicate that RIN4 functions in concert with AHA to regulate leaf stomata during the innate immune response, thus blocking the entry of bacterial pathogens into the leaf interior. The discovery that RIN4 is a molecular link between immune signaling and stomatal movement provides an explanation for how this important defense regulator can act to control immunity at the level of pathogen invasion. Recently, we have also shown that two additional RAPs play a role in plant innate immunity.

The Specific Aims of this application are:

1) Elucidate the mechanism RIN4 uses to regulate plasma membrane H^+-ATPase enzymatic activity. RIN4 is posttranslationally modified during pathogenesis and we are unable to detect an interaction between modified RIN4 and AHA1. We will test the hypothesis that RIN4's phosphorylation status controls its interaction with AHA1, leading to the regulation of stomatal apertures during innate immune defenses.

2) Investigate the spatial and temporal components of the RIN4 protein network. We hypothesize that RIN4 is an adapter protein for multiple protein complexes and exists in distinct pools within plant cells. We will generate a RIN4 interactome map by conducting targeted yeast-two hybrid with RIN4 and RAPs as well as RAPs with one another. We will also analyze complex assembly *in planta* in different sub-cellular regions and tissues using a combination of Blue-Native PAGE and bimolecular fluorescence.

3) Functionally characterize *Arabidopsis* RAPs. We have reproducibly identified 15 novel RAPs. Several of these RAPs are differentially regulated during infection and some have been implicated in immune signaling in either plants or vertebrates. Available RAP knockout lines will be analyzed for altered disease phenotypes. Two informative RAPs that can interact with RIN4 and are involved in defense signaling will be characterized in-depth using a combination of genetics, cell biology, and biochemistry.

Then she continued to sell on the next page and added evidence (Fig. 1).

B. BACKGROUND AND SIGNIFICANCE

1. Plant Defense Mechanisms

1.1 PAMP-Triggered Immunity (PTI)

In order to successfully avoid infection, plants have evolved a series of defense mechanisms that work in concert to limit pathogen invasion and multiplication [3,5]. Unlike vertebrates, plants lack an adaptive immune system and rely on their innate immune system to recognize and restrict pathogenic microbes. Conceptually, there are two primary branches of plant innate immunity. One branch employs extracellular receptors to recognize Pathogen Associated Molecular Patterns (PAMPs), resulting in PAMP-triggered immunity (PTI). PAMPs are conserved microbial features, such as bacterial flagellin or fungal chitin, which fulfill a function crucial to the lifestyle of the organism. The activation of PTI leads to the induction of mitogen-activated protein kinase signaling, transcriptional reprogramming, production of reactive oxygen species, and callose deposition, which serves as a physical barrier at infection sites (reviewed in [10,11]). The second branch uses intracellular plant resistance (R) proteins to recognize pathogen effectors delivered inside host cells during infection, resulting in effector-triggered immunity (ETI). Although both branches result in disease resistance, ETI activation induces in a faster and stronger response, cumulating in programmed cell death surrounding the infection site. Despite the importance of plant innate immunity, how pathogen perception activates immune responses and signaling overlap between PTI and ETI remain elusive.

In order to colonize plants, virulent microorganisms need to overcome PTI. It is likely that all pathogenic microbes possess effectors that interfere with PAMP perception, but the best-characterized ones come from Gram-negative bacteria. Gram-negative bacteria use the Type Three Secretion System (TTSS) to translocate effectors into human, animal and plant cells. On average, individual plant pathogenic bacteria possess an arsenal of 20-30 effector proteins. Collectively, these proteins are required for pathogenicity and manipulate host cells to optimize their environment and subvert the host defense response [12]. Effectors play dual roles as pathogen virulence and avirulence factors; in the absence of plant R proteins, effectors enhance pathogenicity but in the presence of a corresponding R protein, defense signaling is activated. The enzymatic activities and targets of effectors are largely unknown, but emerging evidence indicates that effectors act as eukaryotic enzymes to suppress host immune responses [13,14,15,16,17].

1.2 Effector-Triggered Immunity (ETI)

Despite the wide range of pathogens recognized, the majority of R genes can be grouped into one large family of NLR (nucleotide-binding domain, leucine-rich repeat containing) immune receptors [18] [19] (Fig 1). Plant R proteins can be subdivided into two classes: proteins that contain a Toll/Interleukin-1 receptor-like region and those that contain a coiled-coil region near their N-termini. The distinct N-terminal domains of plant NLR proteins influence the requirement for downstream signaling components. NLRs have also been described as integral members of innate immunity and mediators of inflammatory diseases in vertebrates [20]. Although it is thought that the function of NLR proteins have evolved independently through convergent evolution, investigation of plant NLRs have provided important insights into the function of vertebrate NLRs [21]. For example, several NLRs (including plant R proteins) interact with the HSP90 chaperone and the ubiquitin ligase-associated protein SGT1 [22,23,24,25]. In plants, NLR proteins have evolved to recognize pathogen effectors, while in vertebrates, NLR proteins have evolved to recognize PAMPs or host danger signals.

Despite the prevalence of R genes throughout the plant kingdom, an in-depth comprehension of how they are activated and initiate defense signaling is lacking. The simplest explanation for ETI is the receptor-ligand model, where R proteins directly recognize the ligands specified by pathogen effector proteins. Although many R genes and their corresponding bacterial effectors have been cloned, direct binding between them

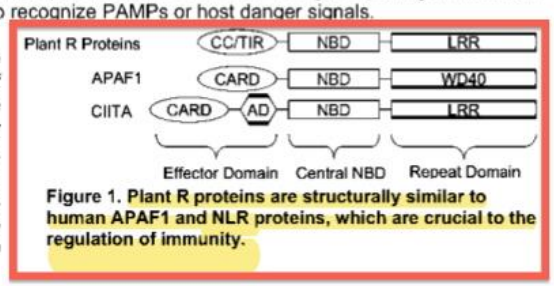

Figure 1. Plant R proteins are structurally similar to human APAF1 and NLR proteins, which are crucial to the regulation of immunity.

More selling from Coaker on the next page

has rarely been demonstrated [26]. Rather than directly detecting bacterial effectors, plant R proteins can detect effectors indirectly, by monitoring for effector-mediated perturbations of host proteins [3].

In the last three years, a new paradigm for R protein signaling has emerged. At least four plant R proteins have been shown to dynamically re-localize to the nucleus upon pathogen perception (reviewed in [27]). Furthermore, nuclear localization was shown to be critical for R protein function, as these R proteins were rendered nonfunctional after the addition of a nuclear export signal. The importance of a chloroplast protein, NRIP1, has also been demonstrated to be required for the recognition of the tobacco mosaic virus effector by the plant R protein N [28]. During ETI, NRIP1 shuttles to both the cytoplasm and nucleus [28]. Taken together, these results highlight the importance of sub-cellular trafficking of immune receptors and key signaling proteins in the plant innate immune response. In this proposal, we will investigate the protein dynamics of RIN4 and key RAPs at the plant plasma membrane, where many plant immune receptors are localized, during innate immune signaling.

2. RIN4 Negatively Regulates both PAMP and Effector-Triggered Immunity.

Pseudomonas syringae pv. *tomato* (*Pst*) is the causal agent of bacterial speck disease on tomato and *Arabidopsis*. For the last 20 years, both *Pseudomonas syringe* and *Arabidopsis* genetics have been intensely studied. This research has resulted in the generation of the *Pseudomonas* genome database and extensive genetic collections of *Arabidopsis* mutant lines. In the same way that *Saccharomyces cerevisiae*, mouse, and *Drosophila* contribute as model systems to the comprehension of human disease, *Arabidopsis* has revealed advances in the understanding of plant innate immune function, which will ultimately result in superior agricultural disease control strategies.

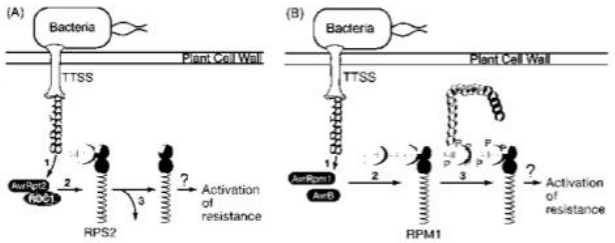

Figure 2. RIN4 negatively regulates both RPS2 (A) and RPM1 (B) specified disease resistance. Adapted from [3].

To date, RIN4 is the only known protein that can regulate both branches of the plant immune system. *RIN4* overexpression lines exhibit decreased callose deposition after PAMP treatment as well as enhanced growth of virulent and type III secretion-deficient *Pst*, indicating a reduction in PTI [6]. *rin4* knockout lines exhibit increased callose deposition after PAMP treatment and decreased *Pst* growth, consistent with enhanced PTI signaling [6]. These data indicate that RIN4 is a negative regulator of PTI. In addition, two R proteins, RPM1 and RPS2, monitor RIN4 (Fig 2). In the absence of pathogen perception, RIN4 acts as a negative regulator of RPM1 and RPS2. When the *P. syringae* effectors AvrRpm1 or AvrB are delivered to the plant cell RIN4 is hyper-phosphorylated, which in turn leads to the activation of RPM1-mediated resistance [8] (Fig 2B). Another *P. syringae* effector, AvrRpt2, is a protease that directly targets RIN4, leading to the activation of RPS2-mediated resistance [7,9,13,29] (Fig 2A). Investigation of the *Arabidopsis- P. syringae* interaction has identified RIN4 is a point of convergence for the regulation of both PTI and ETI. However, a detailed mechanistic understanding of how this is achieved remains elusive.

3. Guard Cells Actively Signal to Prevent Pathogen Entry

Many pathogenic bacteria can proliferate as epiphytes on the plant leaf surface, but in order to infect a plant they must colonize host tissues. Bacterial pathogens gain entry inside plant leaves through wounds or natural openings like stomata. A pair of guard cells surrounds stomatal pores. Guard cell turgor controls opening and closure of the aperture, permitting gas exchange between plants and the atmosphere. Guard cells respond to

Coaker sold her work as relevant to NIH's mission on the page after that

4. Rationale

In this proposal, we seek to investigate how plant immune signaling unfolds by studying RIN4-mediated cellular signaling cascades. RIN4 is a key plant protein involved in both branches of the plant innate immune system and homologs can be detected across land plants, highlighting its importance during immune signaling. Despite the importance of RIN4, an understanding of how this protein mediates both PTI and ETI signaling remains elusive. We have recently isolated the RIN4 protein complex in the presence and absence of pathogen stimulus and have identified 15 novel proteins, three of which exhibit strong disease related-phenotypes. We will investigate how the RIN4 complex is assembled in different subcellular compartments and tissues as well as the importance of individual complex constituents. The completion of the proposed research will have broad-reaching impact not only for our understanding of immune signaling cascades in plants, but will further our knowledge of the mechanisms governing protein dynamics. Several members of the RIN4 complex are widely conserved among eukaryotes. A greater understanding of how plant immune complexes assemble and signal in response to pathogen perception will provide fundamental knowledge that can be used to understand complex formation and cellular signaling in eukaryotes. Because there are significant similarities between the innate immune system in plants and mammals, our research discoveries will facilitate a general understanding of immune signaling and will be relevant to NIH's mission.

8

How many times Gita Coaker mentions conservation of her system during evolution — *which reviewers must accept for her to win* — in her Abstract and first 4 pages.

Stealing a page from Gita's playbook

If there's a point you absolutely must convince reviewers about or not be funded, do what Gita Coaker did: Say it multiple times, say it early, and add evidence to back it up.

END