Instant Grant Improvement

Grantsmanship tricks you can apply this very day

Tom Hollon, PhD

I help people win grants

For 1-hour appointments on campus

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Agenda

- Some truths about writing and reviewing grants and what that implies about winning
- Advanced grantsmanship tips you can use immediately in most types of grants

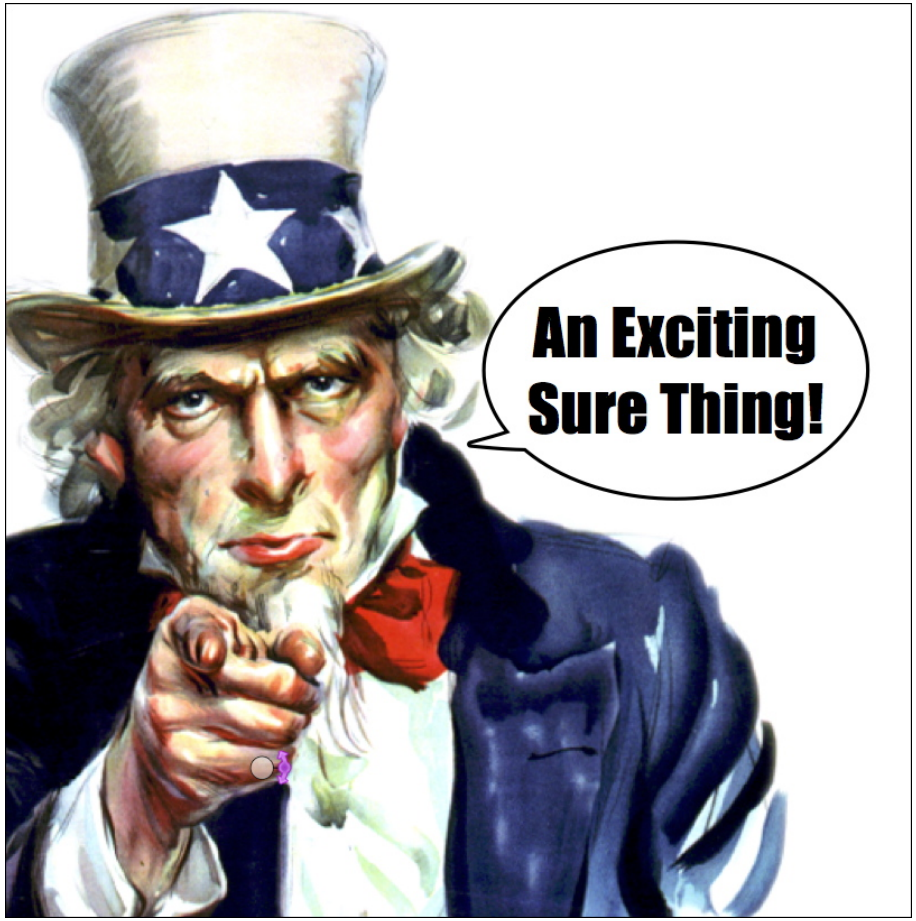
Deep truths about grants

1. Most grants don't do the people who wrote them justice
2. Writing a grant is not like writing a journal article
3. There is no guarantee your reviewers will be alert, qualified, or fair
4. If your grant is easy to review, reviewers will be more likely to give you high scores

Deep truths about grants

5. Sometimes losing has to do with the grant agency, not you. It's not your fault you lost.
6. Grantsmanship will help you win sooner and more often
7. A grant writing trick for one kind of grant (e.g., NIH) can be used in many others (NSF, DoED, foundation grants, etc.)

Deep truth #8: What federal grant agencies want to buy in research projects



Foundations want the same thing

A tale of 4 MSU reviewers: the good, the bad, the sleepy, and the angry

… to explain that grant review is not all bloodless and mechanical -- so you must write for reviewers as they really are, and not as you wish they were.

So this should be your grant writing strategy

To describe a project so interesting, and to make it so easy to review, that it casts a spell and makes them…

- ✓ forget they're tired
- ✓ forget their bad mood
- ✓ forget resenting spending time on your grant

This is a good strategy because…

"A happy reviewer tends to be a positive reviewer."

– Liane Reif-Lehrer "Confessions of an NIH Proposal Reviewer"

Dead-on-Arrival on page 1

More than a few reviewers decide a grant will lose based just on the first page. And as soon as they believe you've lost, they have far less motivation to read with care. Page 1 must make them think your project may be a winner.

Which would you rather read at 10 pm?

B. Significance

This proposal is devoted to addressing the pressing issue of discovering those individually weak, but collectively significant, genetic associations and interactions through genome-wide genetic data. It will apply the detected genetic variants to personalized medicine for more effective smoking cessation interventions, though the developed theory and methods will be more generally applicable.

B.1. Motivating Applications: Genetics for Smoking Cessation and Personalized Medicine

As indicated in [15], *"Smoking is the greatest modifiable contributor to premature death in the United States and the world [91, 135, 22], and cigarette use will kill one in two long-term smokers [21]. Each year over 400,000 people in the United States die of smoking related illnesses [20], and because of increasing cigarette use in developing nations, it is predicted that the worldwide death from smoking will increase from the current 5.4 million persons per year to more than 8 million persons per year by 2030 [135]. The economic burden of smoking is correspondingly high. In the United States alone, annual costs are estimated at $96 billion in direct medical expenses and $97 billion in lost productivity [20]. These important public health issues and associated economic costs motivate studies to identify and understand biological pathways that drive smoking behaviors so that more effective prevention and cessation treatments can be developed."*

Multiple genetic variants have been established to be associated with nicotine dependence or abuse and success of smoking cessation or withdrawal, such as those in the nicotine receptor gene cluster CHRNA5-CHRNAS3-CHRNB4, GABBR2, 5HTT, CYP2A6, DAT1, DRD2 and BDNF [72]. In particular, three common haplotypes defined by SNPs rs16969968 and rs680244 in CHRNAS5-CHRNAS3-CHRNB4 region predicted abstinence at the end of treatment in individuals receiving placebo treatment, but not among individuals receiving active medication [28]. These results suggest that personalized *smoking cessation intervention based upon genotype could meaningfully increase the efficiency of such treatment.*

On the other hand, existing studies are largely based on a few candidate genes, not fully taking advantage of genome-wide association studies (GWASs) and sequencing studies. The major obstacle lies in the difficulty in identifying those SNPs that are individually weakly, but collectively strongly, associated with a complex trait like smoking cessation; see §B.2 for more discussions. At the same time, it is fully recognized using a simple genotype risk score (GRS) by summing up the total number of risk alleles in the confirmed loci is an effective approach [36, 88], though it is necessarily conservative [10] in that i) it omits many associated SNPs yet to be identified and ii) it incorrectly and over-simplifyingly assumes a common effect size across all risk loci. We will propose new methods that fully address these issues.

B.2. Genome-Wide Association Studies: Opportunities and Challenges

Genetic association studies aim to map disease genes by comparing frequencies of genetic variants among affected and unaffected individuals. The idea of genome-wide association studies (GWASs) was proposed to systematically survey common genetic variants, such as single nucleoside polymorphisms (SNPs), and to test the variants for association with common and complex diseases [31, 68, 104]. The first wave of GWASs have reported the localization of many SNPs associated with a wide range of common diseases and clinical outcomes, including nicotine dependence, lung function and smoking quitting targeted in this proposal.

The importance of statistical analysis for GWASs is being recognized. As pointed out in [1]: *"Recognizing causal loci amid a genome's worth of random fluctuation required advances in statistical design, analysis and interpretation". Here we highlight the necessity and urgency of developing and applying powerful statistical methods, the overarching goal of this proposal.* Our proposed de novo discovery of gene pathways containing multiple SNPs with individually weak, but collectively significant, associations with an outcome is best motivated from two lessons learned from the first wave of GWASs [1]. First, effect sizes for the identified common variants are typically small to modest with the estimated relative risks at a mode of only 1.2 [43]. Given small effects sizes and that for most GWASs the feasible sample size is about a few thousand individuals, the power of the current standard approaches, as single SNP-based testing as used by most GWASs, will be necessarily low to detect weak associations without fully accounting for genetic heterogeneity. Second, in spite of the success of GWASs, for most common diseases the proportion of the overall phenotypic variance explained by discovered disease-susceptibility loci remains very low [85]. Hence, most likely only a small fraction of causal loci have been identified. Because most GWASs apply only the univariate single SNP analysis, which is low powered in most situations with multi-locus and weak associations, the development and application of more powerful multi-locus methods will be necessary for discovery of more disease loci. Although recent sequencing studies have expanded GWAS to include rare variants, the same issue remains with extreme genetic heterogeneity. *The issue becomes even worse in detecting gene by environment (e.g. treatment) interactions, which is the driving force behind personalized medicine.* Therefore, our proposed research addresses the aforementioned urgent issues in GWASs and sequencing studies. Our proposed methods for de novo discovery of gene pathways (i.e. a set of the genes) containing genetic variants with weak individual effects, but strong effects in aggregation, are powerful and useful *multi-locus and global* approaches, aiming to detect globally many weak gene by environment interactions, deviating from the current standard approaches that are local (e.g. single SNP- or region-based). The new methods can

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.

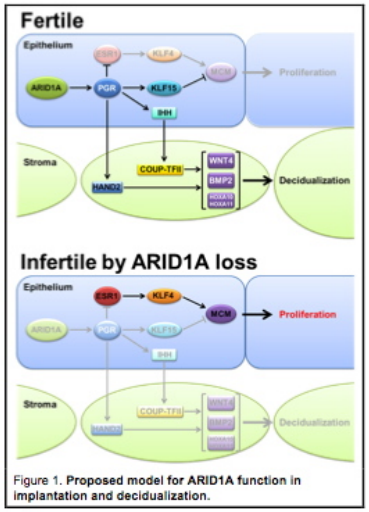


Figure 1. **Proposed model for ARID1A function in implantation and decidualization.**

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.

7 ways to improve your grant's critical first page (that most of your competitors won't use)

1. **Tell reviewers what your project is about in the first sentence. For example:**

   This project is about [fill in].

   This goal of this proposal is to [fill in].

   Many of your competitors won't tell reviewers what their grant is about until the second or third paragraph. They're writing as if grants are journal articles.

Example

-- Jae-Wook Jeong of MSU

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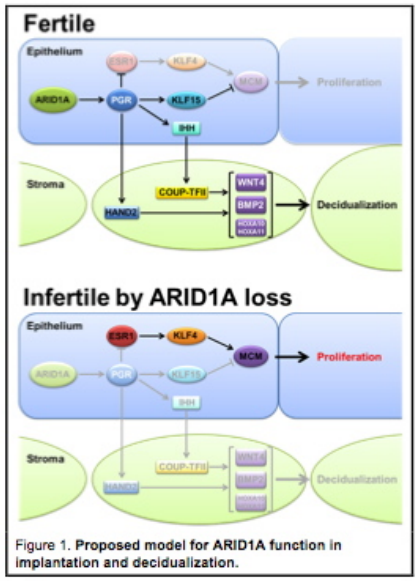

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2. **Include a graphical abstract to help reviewers understand what your project is about. And use its legend to help them see what makes your project special or guaranteed to work.**

A graphical abstract doesn't have to take much room. Note sentences in bold for easier scanning, brevity of the Aims, and Overall Impact at bottom.

-- Jim Pestka of MSU

SPECIFIC AIMS

i) Can dietary lipids block environmental triggers of autoimmune disease? Autoimmune disease (AD), a mosaic of over 80 chronic debilitating illnesses afflicting some 25 million Americans, results from loss of immunological tolerance to the body's own tissues. Heredity (genome) is a primary predisposing factor for AD. However, cumulative exposure to environmental factors (exposome) such as toxic stressors and diet also critically impacts latency and severity of AD in genetically prone individuals. Our overall goal is to understand how triggering prototypical AD lupus by a toxic stressor can be prevented by dietary modulation of cellular lipids (lipidome) (Fig. 1).

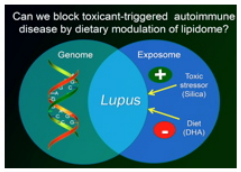


Fig. 1: Environmental factors interact with genetic predisposition to influence development of AD. Our R21 data show silica induces inflammation and autoimmunity in lupus-prone NZBWF1 mice (+) whereas dietary DHA counteracts these effects (-).

ii) Dramatic evidence a fatty-acid dietary supplement blocks toxicant triggering of lupus. Our goal is based on our NIEHS R21 research revealing that airway exposure to crystalline silica ($cSiO_2$) triggers early loss of self-tolerance that accelerates onset of systemic AD and exacerbated glomerulonephritis in female NZBWF1 mice, a widely used model for lupus. The forefront for this AD triggering is the lung, where $cSiO_2$ elicits marked cytokine secretion and leukocyte infiltration, including T and B cells. The latter structurally organize into ectopic lymphoid tissue (ELT) to orchestrate production of destructive autoantibodies, and potentially direct autoreactive lymphocytes to distal tissues. Amazingly, supplementing NZBWF1 mouse diets with the ω-3 polyunsaturated fatty acid (PUFA) docosahexaenoic acid (DHA), a well-known dietary supplement extracted from cold-water fish, dose-dependently prevents $cSiO_2$-triggered pulmonary inflammation and ELT development, systemic autoimmunity and nephritis. This extraordinary finding is highly relevant to human health: lupus strongly associates with both airway exposure to $cSiO_2$ and insufficient dietary ω-3; and preclinical and clinical evidence show ω-3s and their metabolites can reduce inflammation and mitigate AD.

iii) How does DHA prevent silica-triggered lupus? Discovering $cSiO_2$-triggered lupus is preventable in NZBWF1 mice by diet opens a wondrous possibility: What if lupus and other AD could be prevented in humans the same way? Our current perspective is so far limited to analyses conducted after glomerulonephritis onset, 3 months after $cSiO_2$ exposure. In this proposal, we will unravel the early molecular targets and mechanisms by which dietary modulation of the lipidome prevents $cSiO_2$-triggered lupus. Our prior work indicates phagocytosis of $cSiO_2$ by alveolar macrophages (AMϕ) drives a vicious cycle: inflammasome activation → cytokine release / cell death → release of free silica particles → phagocytosis. This depletes AMϕ, resulting in self-antigen accumulation and unresolved inflammation that together initiate loss of tolerance and production of autoantibodies. Recent work reveals ω-3s, notably DHA and its potent metabolites (a.k.a. specialized pro-resolving mediators [SPMs]), potentially stop this vicious cycle by altering the lipid composition of AMϕ cell membranes and enhancing phagocytosis, or by activating anti-inflammatory pathways via G protein-coupled receptors. Our core hypothesis is that *dietary DHA consumption at physiologically relevant concentrations mitigates* $cSiO_2$*-triggered lupus by suppressing inflammasome activation and cell death in AMϕ, thus preventing self-antigen overload in the lung* (Fig. 2). We will test this hypothesis in 3 Specific Aims:

Aim 1: Test the hypothesis that dietary DHA suppresses *in vivo* $cSiO_2$-triggered inflammasome activation, inflammation, cell death and self-antigen release in the lungs of lupus-prone NZBWF1 mice.

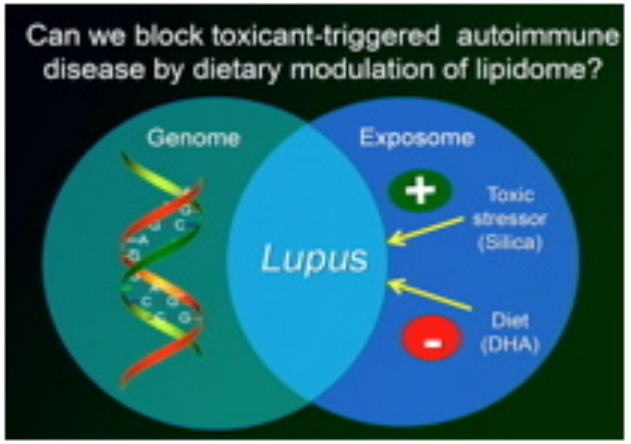
Aim 2: Test the hypothesis that DHA consumption by NZBWF1 mice alters *ex vivo* AMϕ function by: a) reducing $cSiO_2$-triggered inflammasome activation, apoptosis and self-antigen release; b) enhancing removal of apoptotic cells by efferocytosis; and c) increasing DHA-derived SPMs after $cSiO_2$ treatment.

Aim 3. Test the hypothesis that *in vitro* exposure of naïve mouse AMϕ and human blood monocyte-derived Mϕ to DHA and DHA-derived SPMs will: a) suppress $cSiO_2$-induced inflammasome activation, apoptosis and self-antigen release; and b) enhance removal of apoptotic cells by efferocytosis.

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.

Using a Venn diagram to highlight the grant's main question

The legend ties the question to supporting preliminary data.



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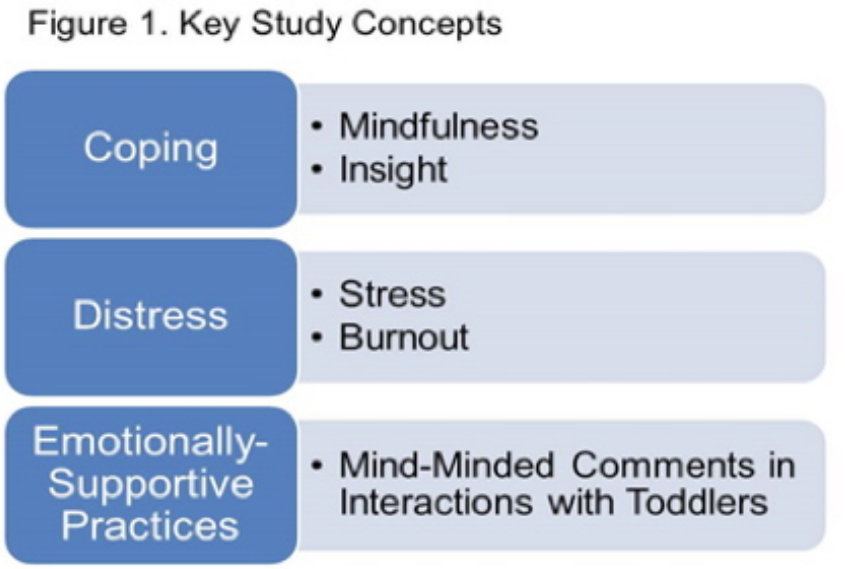
-- Jim Pestka of MSU

Use the legend to make your project easier to understand. Or say what makes it special or guaranteed to work.



Our work and others' suggests mindfulness and insight techniques can improve toddler teachers' coping, reduce their distress, and help them give more emotional support to EHS toddlers.

-- Holly Brophy-Herb of MSU

Graphical abstract & lengthy legend make central hypothesis easier to understand

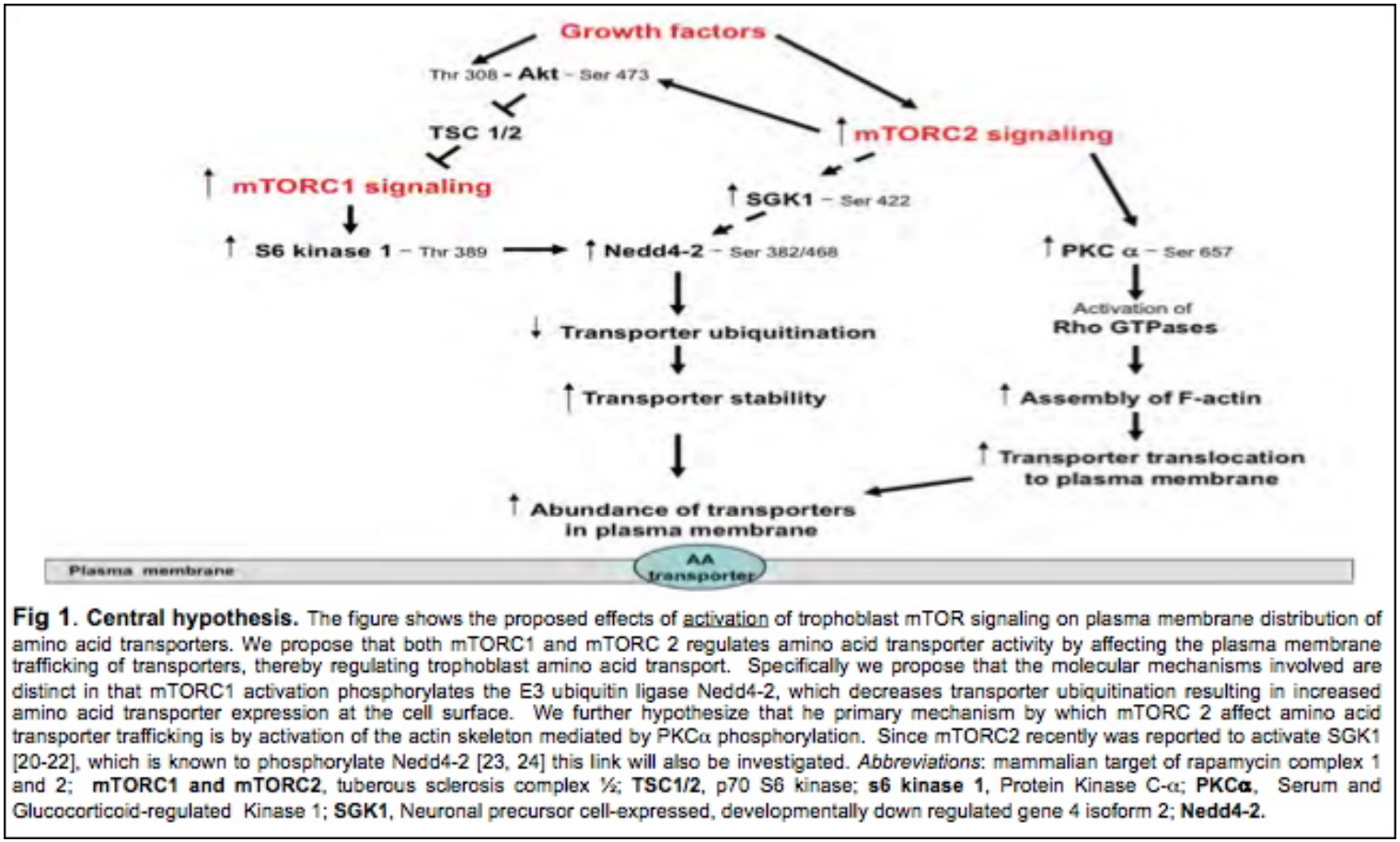


Fig 1. Central hypothesis. The figure shows the proposed effects of activation of trophoblast mTOR signaling on plasma membrane distribution of amino acid transporters. We propose that both mTORC1 and mTORC 2 regulates amino acid transporter activity by affecting the plasma membrane trafficking of transporters, thereby regulating trophoblast amino acid transport. Specifically we propose that the molecular mechanisms involved are distinct in that mTORC1 activation phosphorylates the E3 ubiquitin ligase Nedd4-2, which decreases transporter ubiquitination resulting in increased amino acid transporter expression at the cell surface. We further hypothesize that he primary mechanism by which mTORC 2 affect amino acid transporter trafficking is by activation of the actin skeleton mediated by PKCα phosphorylation. Since mTORC2 recently was reported to activate SGK1 [20-22], which is known to phosphorylate Nedd4-2 [23, 24] this link will also be investigated. *Abbreviations*: mammalian target of rapamycin complex 1 and 2; **mTORC1 and mTORC2**, tuberous sclerosis complex ½; **TSC1/2**, p70 S6 kinase; **s6 kinase 1**, Protein Kinase C-α; **PKCα**, Serum and Glucocorticoid-regulated Kinase 1; **SGK1**, Neuronal precursor cell-expressed, developmentally down regulated gene 4 isoform 2; **Nedd4-2.**

-- Thomas Jansson

Is it too big for page 1? Use page 2.

Contact PD/PI: Noble, William, S

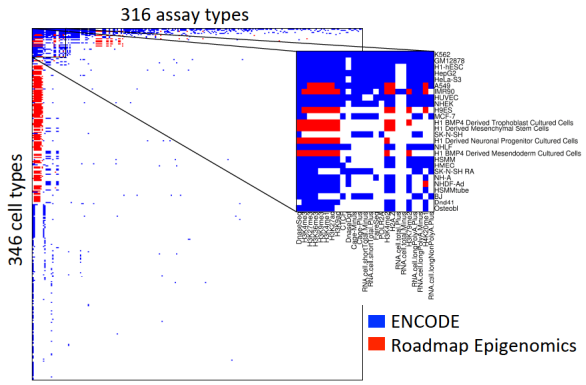


Figure 1: **Pattern of available data.** The matrix summarizes the epigenomic experiments that have been carried out by Roadmap Epigenomics (red) and ENCODE (blue). In both figures, rows correspond to cell types and columns correspond to assay types. A filled cell indicates that a particular assay has been performed on a particular cell type. Rows and columns are sorted by number of available experiments. The inset shows details for the first 25 cell types and 25 assay types.

1 Research strategy

1.1 Significance

A huge proportion of current scientific progress in genomics is driven by technological advances associated with high throughput sequencing. The primary significance of the proposed work will be the development and application of machine learning tools that leverage these big, heterogeneous genomic data sets.

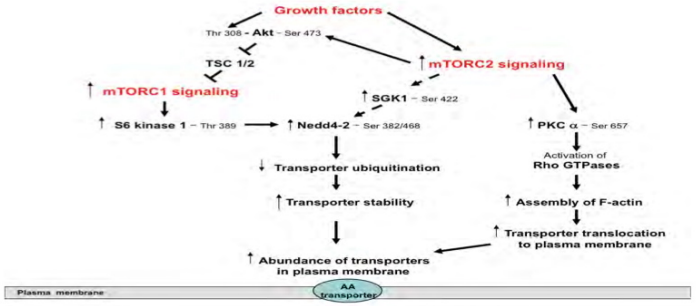
Our first aim combines Roadmap Epigenomics data with ENCODE data (Figure 1) to produce *virtual experiments*. For example, a researcher interested in studying breast cancer might be excited to find that Roadmap Epigenomics and ENCODE have, together, performed 33 assays in the MCF-7 cell line, but disappointed to find that many important histone modification ChIP-seq experiments have not yet been carried out in MCF-7. Rather than doing these experiments or waiting until someone else does them, our project will provide this researcher with a computationally estimated version of the missing experiments. Although not as accurate as the real experiment, our preliminary results suggest that this type of virtual experiment can be a useful way to generate hypotheses for further validation and can provide a way to prioritize future experiments.

Our second and third aims develop and apply methods to summarize big, heterogeneous functional genomics data sets into a format easily interpretable by humans. Methods such as Segway [1], which we developed as part of the second phase of the ENCODE project, coalesce a large collection of high throughput sequencing assays into a *genome annotation*—a simultaneous partitioning and labeling of the genome, where the labels correspond to different types of biochemical activities: promoters, enhancers, insulators, various portions of gene bodies, repressed regions, etc.

In this project, we will significantly improve upon this general class of methods in two ways. First, we propose a graph-based regularization (GBR) method that allows us to analyze, in a principled fashion, data derived from multiple cell types, producing cell type-specific annotations whose labels share a common semantics. As outlined below, this method improves significantly upon existing, simple methods for analyzing such data sets. Having a unified label semantics is doubly impactful: (1) it leads to easier interpretation of annotations of various cell types, and (2) it eliminates the need for a human to re-interpret the labels after each new cell type is annotated.

-- *William Noble*

Principal Investigator/Program Director (Last, first, middle): Jansson, Thomas



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3. RESEARCH STRATEGY

a. Significance. This proposal is **significant** because:

1. The activity of key placental amino acid transporters is decreased in IUGR [3-5, 8] and up regulated in fetal overgrowth [9], suggesting that altered placental nutrient transporter activity contributes to abnormal fetal growth [10-12]. Since this work focuses on mechanisms by which placental amino acid transport is regulated, it addresses questions critical to the understanding of how important pregnancy complications develop.

2. Placental mTOR signaling activity is decreased in IUGR [1, 2] and preliminary data show an activation of placental mTOR signaling in fetal overgrowth [25]. Our preliminary data demonstrates that mTORC 1 and mTORC2 signaling has a profound impact on trophoblast amino acid transporter activity, suggesting that we have identified important mechanisms for the regulation of placental amino acid transport and fetal growth.

3. Functional data (nutrient transport activity) will be obtained in primary human trophoblast cells, using growth factors in physiological concentrations, which contributes to the physiological relevance of the proposed studies. Furthermore, the systematic utilization of gene silencing approaches in cultured human primary trophoblast cells will allow us to obtain specific mechanistic information on mTORC 1 and mTORC2 signaling pathways in the human placenta, contributing to the significance of the work.

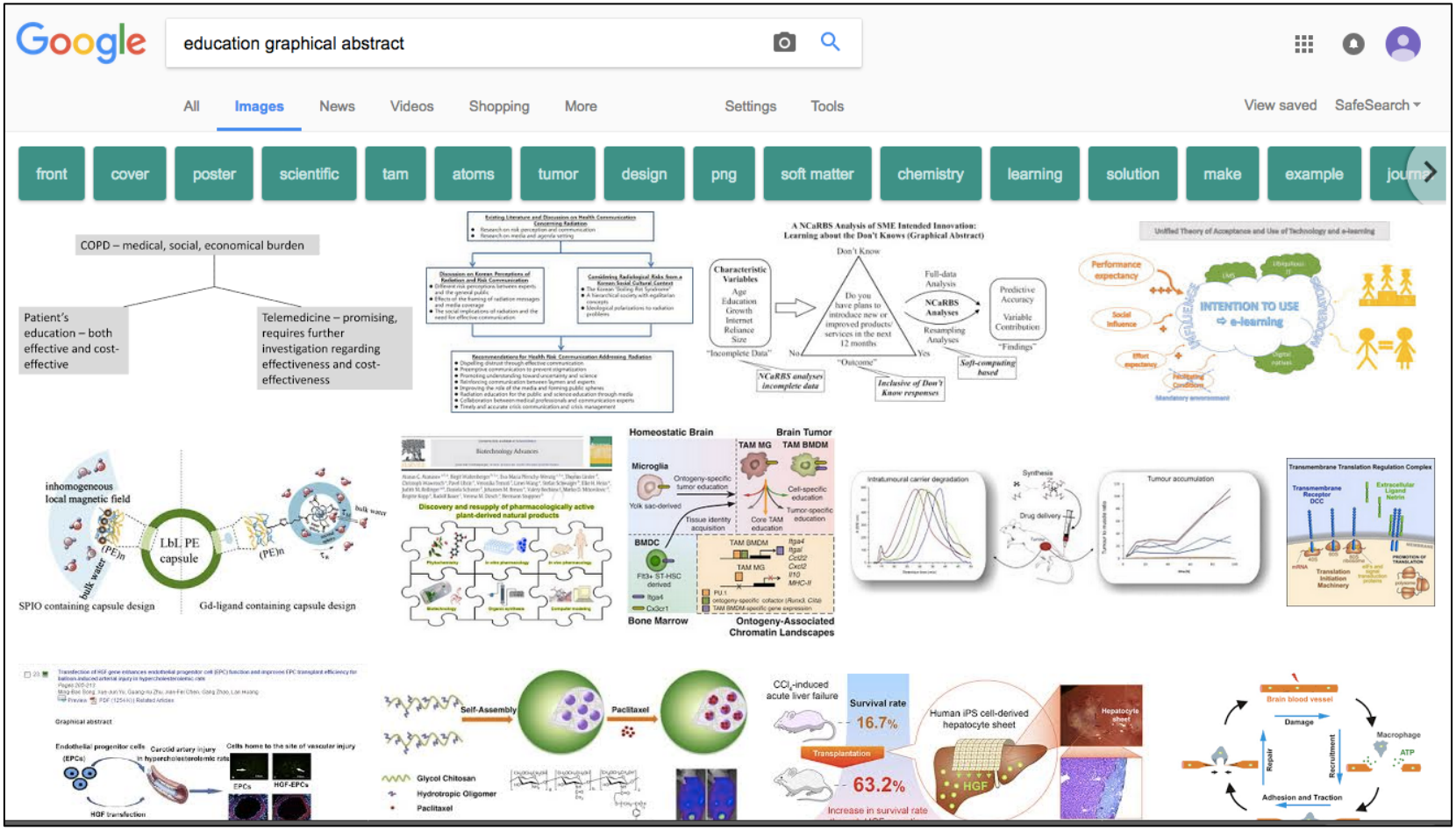
4. In addition to playing a role in abnormal fetal growth, regulation of nutrient transporters has been implicated in many other diseases, including cancer [26]. However, the authors of several recent reviews have highlighted the existence of a major gap in knowledge with respect to the mechanisms regulating nutrient transporters. For example, Edinger concludes '*Despite the clear implications for human disease, there are large gaps in our knowledge of how nutrient transporter expression is regulated*' [27] and '*.. virtually nothing is known about how nutrient transporter internalization and trafficking is regulated in mammalian cells*' [28]. Thus, the proposed research is significant because it addresses a major gap in knowledge and mechanisms shown to regulate amino acid transporters in human primary trophoblast cells are likely to be relevant for other human cells.

b. Innovation. The proposed research is **innovative** because

1. A number of the molecular links that we propose in Fig 1 have not been clearly demonstrated previously, **in any mammalian tissue**, and are therefore novel. These include the regulation of amino acid transporter by altered f-actin assembly and mTORC1 regulation of Nedd4-2.

-- *Thomas Jansson*

Graphical abstract ideas aren't hard to find



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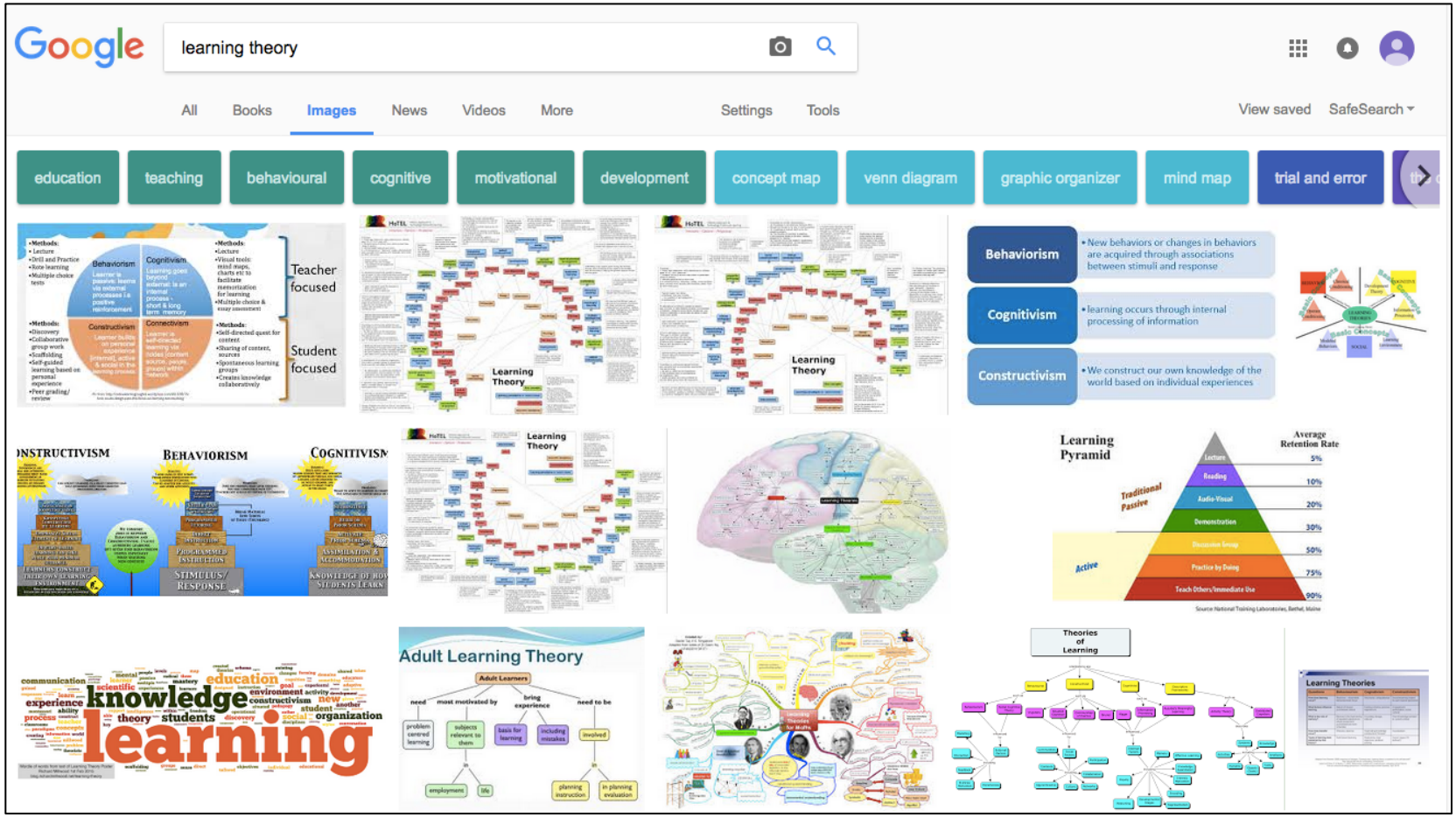

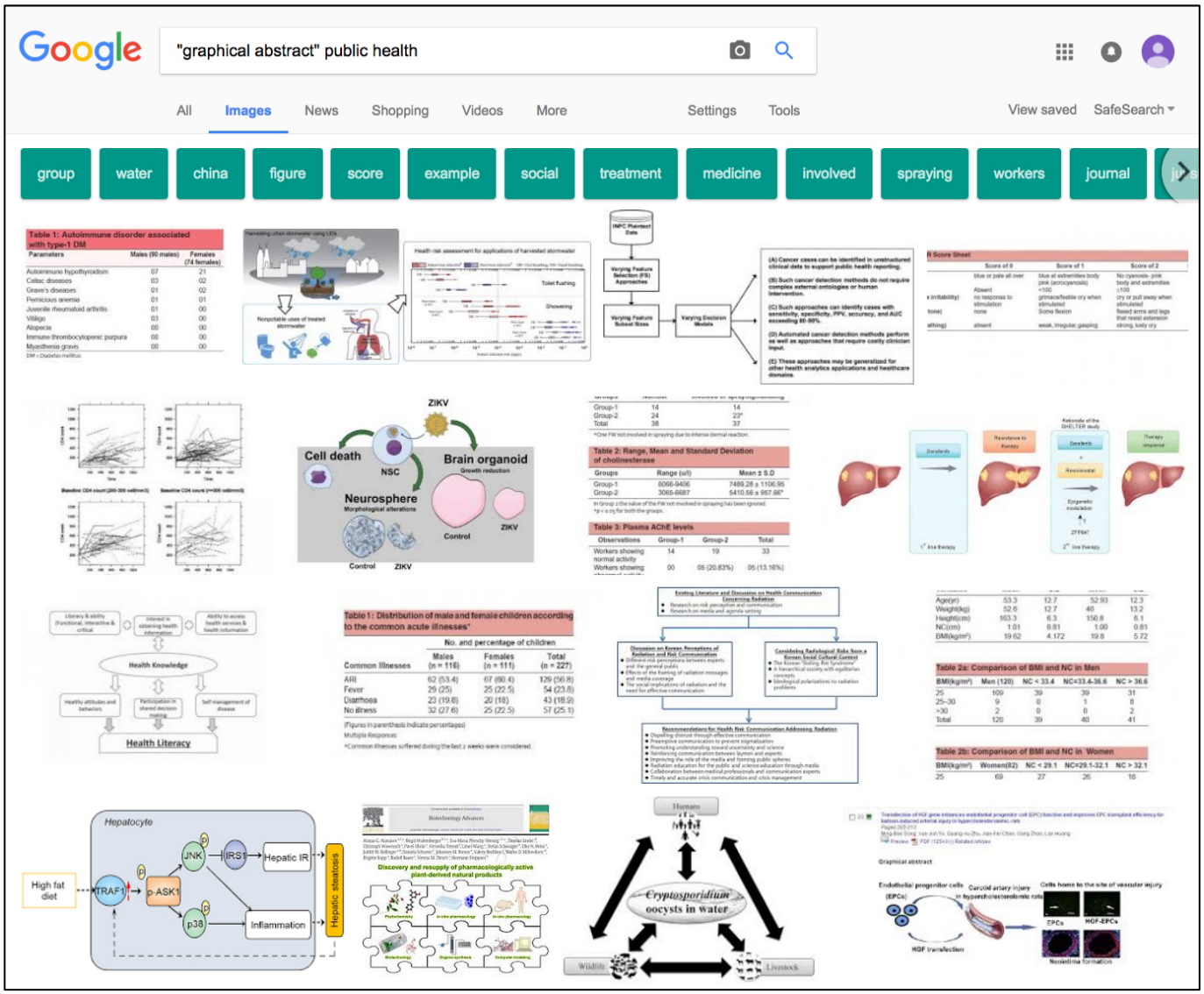
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Tools
View saved
SafeSearch
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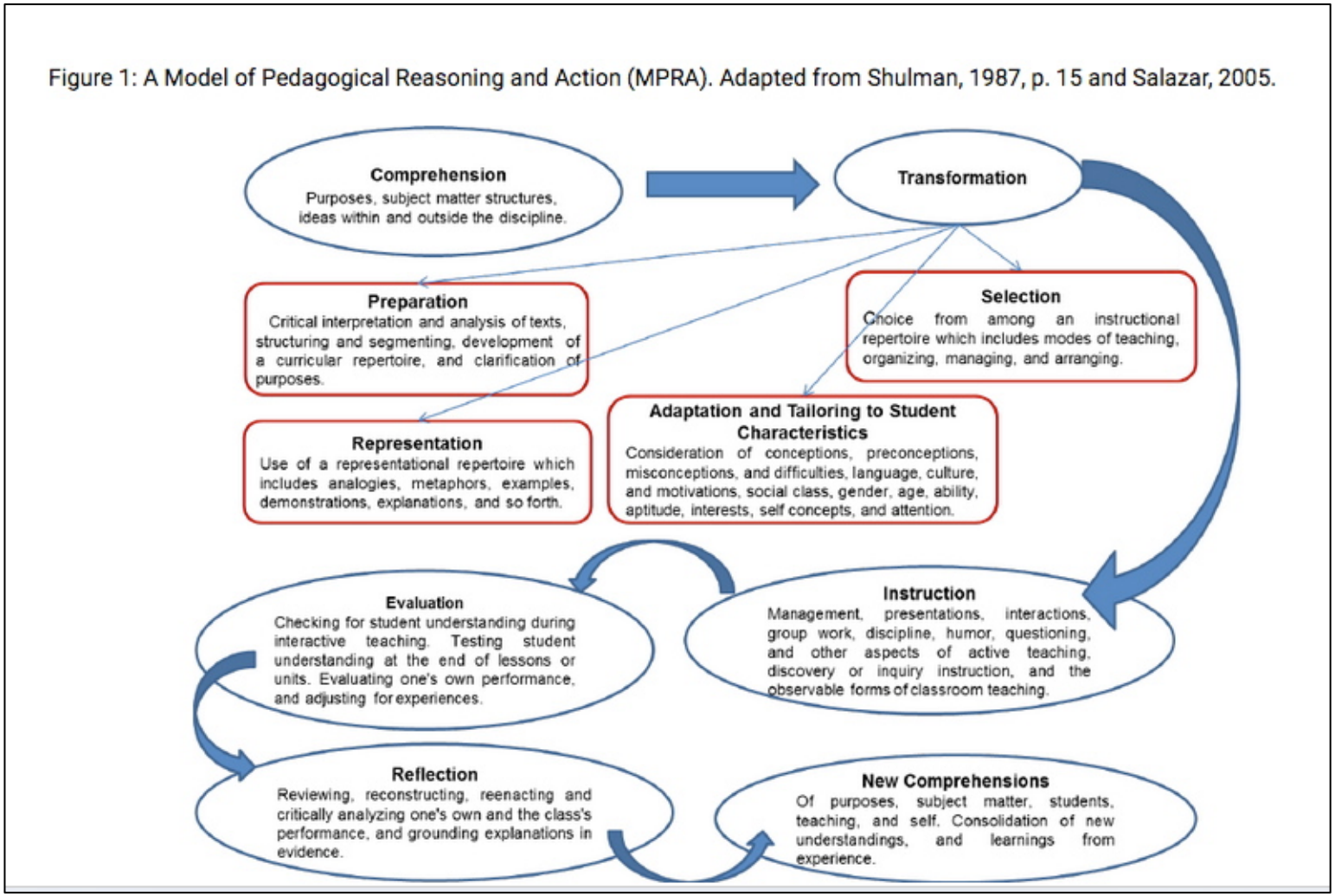
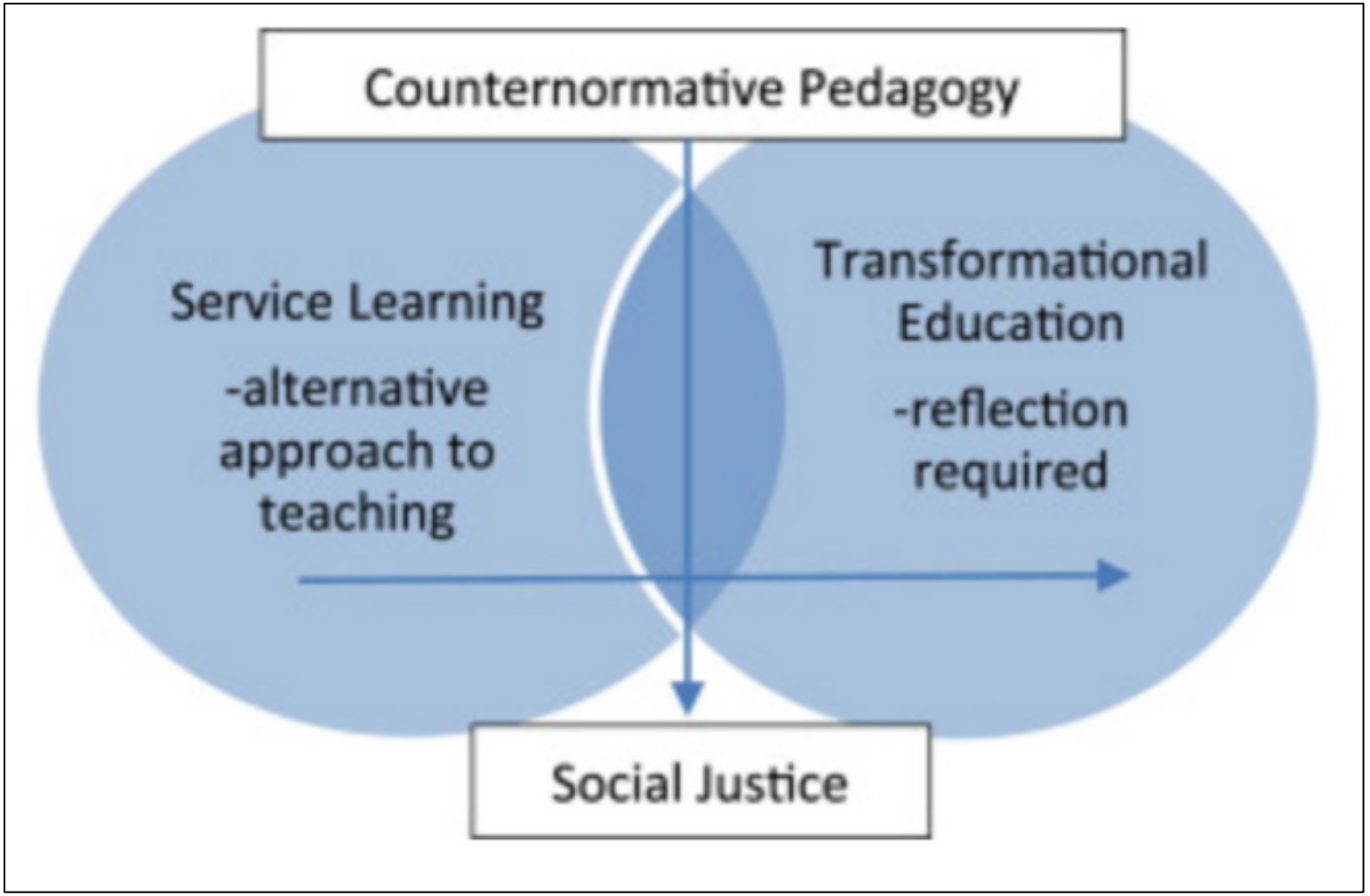
social
treatment
medicine
involved
spraying
workers
journal
Table 1: Autoimmune disorder associated with type-1 DM
Parameters
Males (90 males)
Females (74 females)
Autoimmune hypothyroidism
Celiac diseases
Grave's diseases
Pernicious anemia
Juvenile rheumatoid arthritis
Vitiligo
Alopecia
Immune thrombocytopenic purpura
Myasthenia gravis
Harvesting urban stormwater using LIDs
Non-potable uses of treated stormwater
Health risk assessment for applications of harvested stormwater
Toilet flushing
Showering
INPC Plaintext Data
Varying Feature Selection (FS) Approaches
Varying Feature Subset Sizes
Varying Decision Models
(A) Cancer cases can be identified in unstructured clinical data to support public health reporting.
(B) Such cancer detection methods do not require complex external ontologies or human intervention.
(C) Such approaches can identify cases with sensitivity, specificity, PPV, accuracy, and AUC exceeding 80-90%.
(D) Automated cancer detection methods perform as well as approaches that require costly clinician input.
(E) These approaches may be generalized for other health analytics applications and healthcare domains.
Score of 0
Score of 1
Score of 2
ZIKV
Cell death
NSC
Brain organoid
Growth reduction
Neurosphere
Morphological alterations
Control
ZIKV
Control
ZIKV
Table 2: Range, Mean and Standard Deviation of cholinesterase
Groups
Range (u/l)
Mean ± S.D
Group-1
6066-9406
7489.28 ± 1106.95
Group-2
3065-6687
5410.66 ± 957.66*
Table 3: Plasma AChE levels
Observations
Group-1
Group-2
Total
Workers showing normal activity
Workers showing
Literacy & ability (Functional, interactive & critical)
Interest in obtaining health information
Ability to access health services & health information
Health Knowledge
Healthy attitudes and behaviors
Participation in shared decision making
Self management of disease
Health Literacy
Table 1: Distribution of male and female children according to the common acute illnesses*
No. and percentage of children
Common Illnesses
Males (n = 116)
Females (n = 111)
Total (n = 227)
ARI
62 (53.4)
67 (60.4)
129 (56.8)
Fever
29 (25)
25 (22.5)
54 (23.8)
Diarrhoea
23 (19.8)
20 (18)
43 (18.9)
No illness
32 (27.6)
25 (22.5)
57 (25.1)
(Figures in parenthesis indicate percentages)
Multiple Responses
*Common illnesses suffered during the last 2 weeks were considered.
Existing Literature and Discussion on Health Communication Concerning Radiation
Research on risk perception and communication
Research on media and agenda setting
Discussion on Korean Perceptions of Radiation and Risk Communication
Considering Radiological Risks from a Korean Social Cultural Context
Recommendations for Health Risk Communication Addressing Radiation
Age(yr)
Weight(kg)
Height(cm)
NC(cm)
BMI(kg/m²)
Table 2a: Comparison of BMI and NC in Men
BMI(kg/m²)
Men (120)
NC < 33.4
NC=33.4-36.6
NC > 36.6
Table 2b: Comparison of BMI and NC in Women
BMI(kg/m²)
Women(82)
NC < 29.1
NC=29.1-32.1
NC > 32.1
Hepatocyte
High fat diet
TRAF1
p-ASK1
JNK
IRS1
Hepatic IR
p38
Inflammation
Hepatic steatosis
Biotechnology Advances
Discovery and resupply of pharmacologically active plant-derived natural products
Humans
Cryptosporidium oocysts in water
Wildlife
Livestock
Graphical abstract
Endothelial progenitor cells (EPCs)
Carotid artery injury in hypercholesterolemic rats
Cells home to the site of vascular injury
EPCs
HGF-EPCs
HGF transfection
Neointima formation

Education paper's graphical abstract



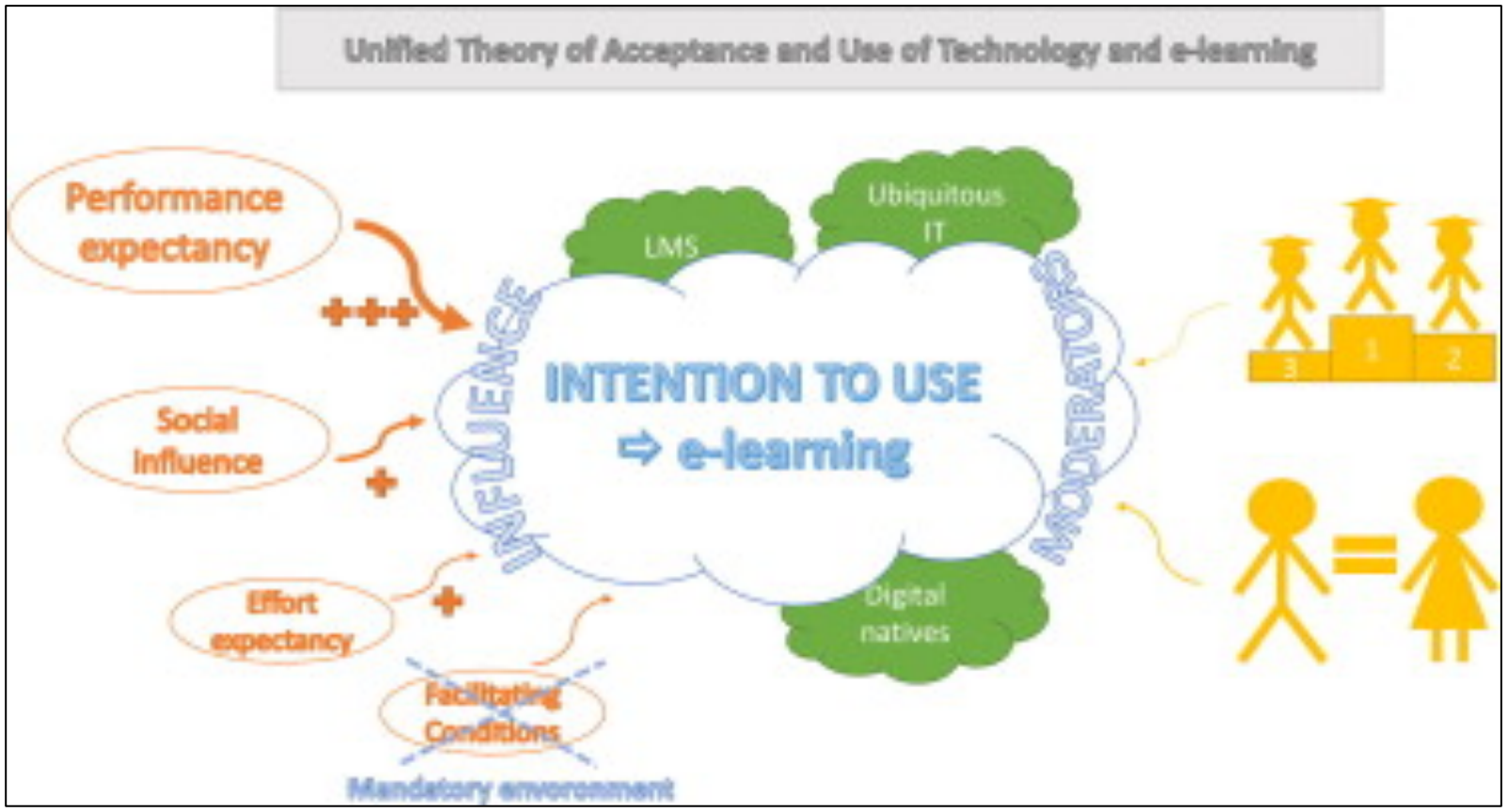
from researchgate.com

Education paper's graphical abstract



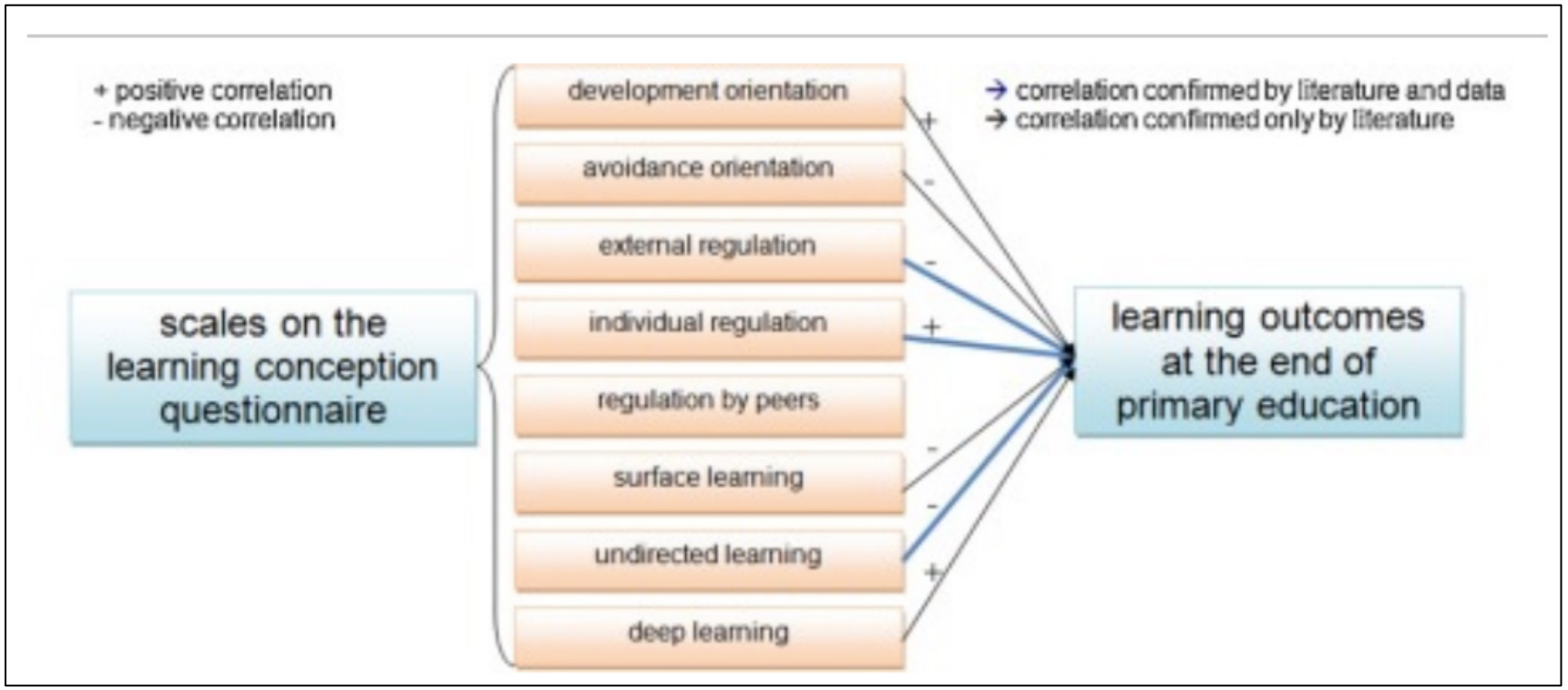
from sciencedirect.com

Education paper's graphical abstract



Computers in Human Behavior

Education paper's graphical abstract



International J. of Educational Research

3. **Before the first page ends, tell reviewers in 50 words or less the impact your project's success will have on your field.**

   This statement summarizes what you will give the funders in return for their money.

EXAMPLE:

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

EXAMPLE:

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

4. **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

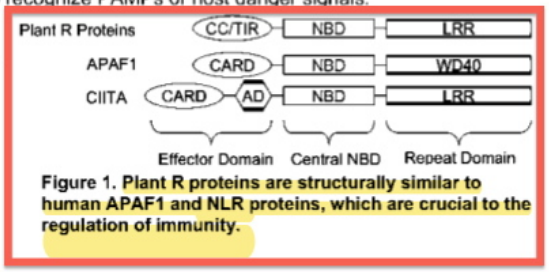


Figure 1. Plant R proteins are structurally similar to human APAF1 and NLR proteins, which are crucial to the regulation of immunity.

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.

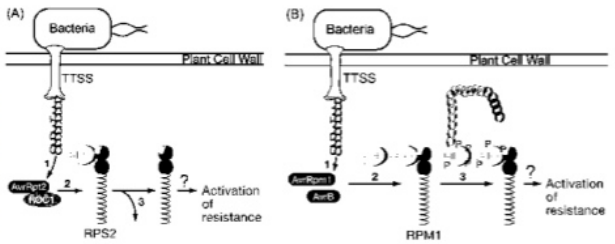


Figure 2. RIN4 negatively regulates both RPS2 (A) and RPM1 (B) specified disease resistance. Adapted from [3].

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.

Example of stealing from Gita's playbook: On his 3rd and final try to win his R01, Adam Ratner knew he had a problem

INTRODUCTION TO RESUBMISSION APPLICATION

This is the second (A2) resubmission of application R01 HD061371-01, *"Gardnerella vaginalis:* toxin production and pathogenesis," which was reviewed in February 2009 and then in June 2009 at the HIBP study section. The initial submission received a 35.5 percentile (priority score 203) and the A1 resubmission a 15 percentile (impact priority score 28.) Under FY10 paylines, it was not funded by NICHD or NIAID . As a new investigator, I am grateful for the opportunity to present this revised application. I have made every effort to address the critique thoroughly, and I believe that the proposed studies have emerged considerably stronger and more focused on relevant aspects of pathogenesis. This resubmission has undergone very substantial revision, both in response to the reviewers' comments and in order to meet the new page limit guidelines for R01 applications. For that reason, changes are not marked in the text.

My impression from the summary statement for the A1 application was that the reviewers found the subject matter of interest, and there was substantial enthusiasm for studies of pathogenic mechanisms of G. *vagina/is* focused on the new human-specific cytolysin (vaginolysin, VLY) and its receptor, human CD59. The summary of discussion described the strengths of the application as *"the expertise and productivity of the investigator in the field, the supportive preliminary data ensuring feasibility, the innovative approach, the adequate response to previous critiques, and the significance of this understated female problem."* The weaknesses identified were *"the ambitious nature of the project, the lack of* a *transgenic model, and the relevance to humans."* However, the proposed research was felt to be *"potentially very important with* a *high probability of it being successful."*

The major concern of the reviewers surrounded the hCD59-transgenic murine lines for the in vivo studies in Aim 2. I have approached this problem in three ways. First, I provide data demonstrating that we have generated C57BLI6 lines with hCD59 under the control of the ubiquitously expressed EF-1a promoter. Second, we have initiated a collaboration with XXX [redacted] is an expert in the biology Of CD59 and other complement regulatory molecules, and he has created and characterized several hCD59-transgenic murine lines. He has generously agreed to share his expertise as well as these mice with us for our studies. Third, we provide new data in this resubmission that even if the hCD59-transgenic colonization studies are unsuccessful we will still be able to study the role of the toxin/host interaction in the initiation and maintenance of G. *vaginalis* colonization. We have developed a new model of murine G. *vaginalis* vaginal colonization using coinoculation of a non-species-specific cholesterol-dependent cytolysin (PLY), which unlike VLY does not require hCD59 for its activity. Codelivery of this toxin with G. *vaginalis* enhances colonization, allowing high-level murine colonization with G. *vaginalis* for the first time. This both lends credence to our hypothesis that toxin function is crucial to establishment of colonization and provides a novel platform with which we can test various knockout strains and potential inhibitors of colonization in case there are unforeseen technical issues with the hCD59 transgenic approach.

We have addressed the other concerns of the reviewers as well. We provide additional evidence of our ability to manipulate G. *vaginalis,* including the construction of a GFP-expressing strain and streptomycin-resistant mutants useful in vivo. We include plans for correcting for growth defects in mutant strains, testing biofilm mutants both for changes in VLY production and in the in vivo models, addressing LPS contamination more rigorously, and preparing targeted mutations of the most interesting genetic loci as suggested in Critique 1. We also describe a more detailed approach to our analysis plan for the bleb studies and to ensuring that candidate inhibitors do not sensitize human cells to complement attack, as suggested in Critique 2. I believe that this research plan will help us to understand the pathogenesis of bacterial vaginosis and will lead to new therapeutic strategies for this important and difficult to treat disease. Thank you for your consideration.

Ratner's paragraph repeats over and over: This is a low-risk experiment

The major concern of the reviewers surrounded the hCD59-transgenic murine lines for the in vivo studies in Aim 2. I have approached this problem in three ways. First, I provide data demonstrating that we have generated C57BLl6 lines with hCD59 under the control of the ubiquitously expressed EF-1a promoter. Second, we have initiated a collaboration with XXX [redacted] is an expert in the biology Of CD59 and other complement regulatory molecules, and he has created and characterized several hCD59-transgenic murine lines. He has generously agreed to share his expertise as well as these mice with us for our studies. Third, we provide new data in this resubmission that even if the hCD59-transgenic colonization studies are unsuccessful we will still be able to study the role of the toxin/host interaction in the initiation and maintenance of G. *vaginalis* colonization. We have developed a new model of murine G. *vaginalis* vaginal colonization using coinoculation of a non-species-specific cholesterol-dependent cytolysin (PLY), which unlike VLY does not require hCD59 for its activity. Codelivery of this toxin with G. *vaginalis* enhances colonization, allowing high-level murine colonization with G. *vaginalis* for the first time. This both lends credence to our hypothesis that toxin function is crucial to establishment of colonization and provides a novel platform with which we can test various knockout strains and potential inhibitors of colonization in case there are unforeseen technical issues with the hCD59 transgenic approach.

5. **Anything that can distract reviewers from getting excited about your grant by the bottom of the first page should be removed. Put it on some other page.**

   If reviewers aren't excited about your grant by the bottom of the first page you'll probably lose.

Example. In editing Jae-Wook Jeong's first page, I moved details unnecessary to getting reviewers excited about his work (yellow) to another page.

SPECIFIC AIMS

According to the CDC, 6% of married women between the ages of 15-44 are infertile, and 12.1% of women display difficulties getting pregnant or carrying a pregnancy to term[1]. The loss of pregnancy before 20 weeks is called early pregnancy loss or miscarriage. This occurs in about 15% of known pregnancies. **Implantation defects contribute to over 75% of failed pregnancies**, suggesting a need to focus research efforts towards understanding the complex mechanisms necessary for uterine receptivity and successful implantation[2]. The uterus is an endocrine organ, responsive to the presence of the ovarian steroid hormones, estrogen (E2) and progesterone (P4), which activate transcription of target genes through the binding of their cognate receptors, the estrogen receptors (ESRs) and the progesterone receptor (PGR)[3]. P4 signaling through the PGR is responsible for activating a complex paracrine signaling network that is imperative in pregnancy. PGR is also found to be dysregulated in many malignancies such as endometriosis[4], endometrial carcinoma[5,6], and breast cancer[7]. However, the cause of PGR dysregulation is not well known. AT-rich interactive domain-containing protein 1A (ARID1A) is a member of the SWI/SNF family and is required for transcriptional activation of genes normally repressed by chromatin. ARID1A loss is a frequent event in endometrial cancers and endometriosis-associated ovarian carcinomas. However, the pathophysiological effect of ARID1A loss remains quite poor and the function of ARID1A in the female reproductive track has remained elusive. In our Preliminary studies, **ARID1A loss is implicated in infertile women with endometriosis.** Furthermore, uterine-specific *Arid1a* knock-out mice (*PGR*$^{cre/+}$*Arid1a*$^{f/f}$; *Arid1a*$^{f/f}$) were sterile due to a defect of implantation and decidualization. E2 signaling and epithelial proliferation were significantly increased at the peri-implantation in mutant mice. Epithelial PGR and its target genes were downregulated in mutant mice. Bases on these findings, **we hypothesize that ARID1A plays an important role in implantation and decidualization by suppressing ESR1 activities and enhancing PGR activities (Fig. 1)**. The goal of this proposal will be accomplished by achieving the following specific aims.

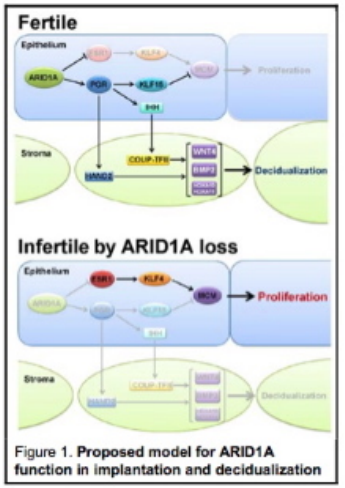


Figure 1. **Proposed model for ARID1A function in implantation and decidualization**

Specific Aim 1. To determine the role of ARID1A on epithelial cell proliferation and implantation. We will determine if ARID1A negatively regulates E2-induced epithelial cell proliferation through ESR1 and PGR interactions. Transcriptional regulation of *ARID1A* target genes will be investigated in mouse models as well as epithelial cell lines by using promoter assay and CRISPR-Cas9 gene editing.

Specific Aim 2. To examine the importance of ARID1A loss in decidualization, infertility and endometriosis. The effect of ARID1A loss on decidualization will be investigated in uterine specific *Arid1a* knock-out mice and human endometrial stromal cells. The effect of ARID1A loss on P4 resistance and endometriosis development will be examined in mouse models with a fluorescence reporter. We will utilize a large endometrial tissue repository to quantify ARID1A and associated down-stream targets to correlate findings with the stage of endometriosis and the degree of infertility.

Specific Aim 3. To evaluate the ability of resveratrol to restore P4 resistance and uterine functioning in infertility and endometriosis by ARID1A loss. We will determine the therapeutic potential of resveratrol to recover aberrant epithelial proliferation and decidualization defects in A*RID1A* loss. The effect of resveratrol on human endometriotic xenografts established in an immunocompromised mouse model will provide a novel therapeutic approach to inhibit the pathologic processes associated with the disease.

Progress in our understanding of the etiology and pathophysiology of infertility and endometriosis and potential therapeutic interventions by targeted pharmacological agents has been hampered due, in part, to the lack of defined molecular mechanisms and animal models. ***If successful, the proposed Specific Aims will motivate the translation of animal models for biomarker identification as well as the development of new therapeutic approaches for infertility and endometriosis.***

Moving some info elsewhere made room to repeat key points. Red: a novel therapy; Blue: new gene targets; Yellow: an innovative disease model

SPECIFIC AIMS

This proposal is about the role of the chromatin modifying factor ARID1A in regulating uterine epithelial proliferation in response to hormonal signals. Our preliminary data strongly suggest 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 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.

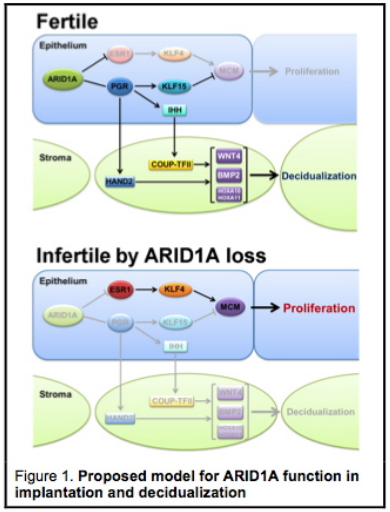


Figure 1. **Proposed model for ARID1A function in implantation and decidualization**

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.

Aim 1. Determine the role of ARID1A on epithelial cell proliferation and implantation.

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 KO *Arid1a*$^{d/d}$ mice and human endometrial stromal cells
B. Determine if ARID1A causes P4 resistance in endometriosis using a mouse model that realistically resembles human endometriosis

Aim 3. Evaluate the ability of resveratrol to restore P4 resistance and uterine functioning in cases of infertility and endometriosis due to ARID1A loss.

A. Determine if resveratrol overcomes aberrant epithelial proliferation and implantation defects in conditional KO *Arid1a*$^{d/d}$ mice
B. Determine effect of resveratrol on establishment of endometriotic lesions
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.

6. Use simple English as much as possible. Avoid really long sentences. Minimize jargon. Use the active voice.

Reviewers in pajamas will love you.

7. Do not misoverabbbreviate*!

Using lots of abbreviations in your grant that reviewers don't know the meaning of won't help you win.

** New word inspired by Pres. G.W. Bush*

Some grant tips for later pages

Write a figure legend so it can be scanned: Do this by beginning the legend with your conclusion about what the figure means in bold.

Begin the legend with the conclusion in bold

-- Mengxi Jiang

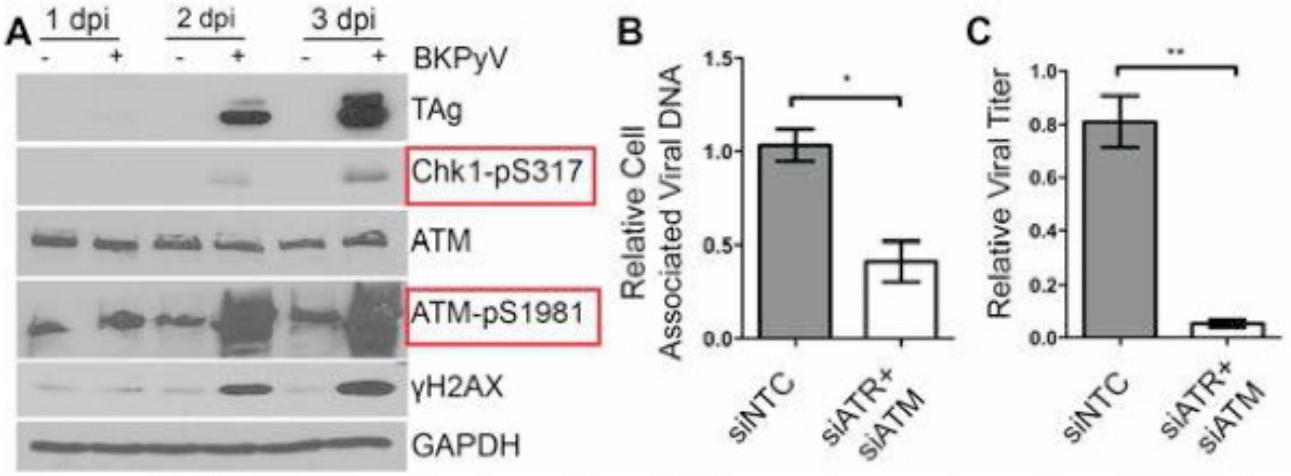


Figure 3. BKPyV activates the DDR and requires the DDR for productive infection. (A) RPTE cells were infected with BKPyV at an MOI of 5 infectious units (IU)/cell. Whole cell lysates were harvested at 1-3 days post infection (dpi) and immunoblotted for ATR and ATM activation markers. (B+C) RPTE cells were transfected with indicated siRNAs followed by BKPyV infection at an MOI of 0.5 IU/cell. Cell-associated viral DNA (B) and virus titer (C) were determined at 2 dpi by a real-time PCR assay and a fluorescence IU assay, respectively. Data were normalized to no siRNA control. *, $p< 0.05$; **, $p<0.01$.

This technique makes conclusions from many studies easier to grasp quickly

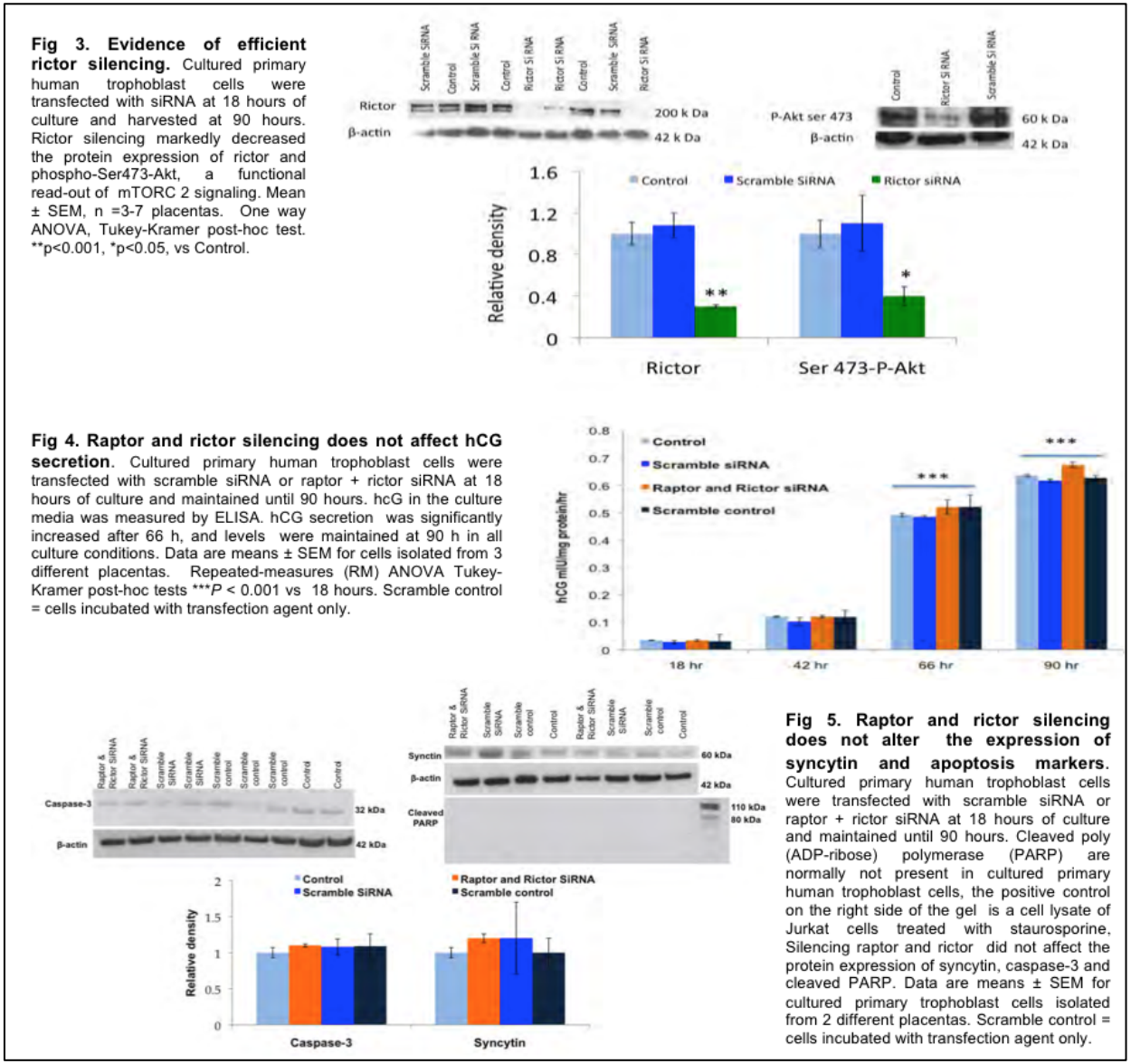


Fig 3. Evidence of efficient rictor silencing. Cultured primary human trophoblast cells were transfected with siRNA at 18 hours of culture and harvested at 90 hours. Rictor silencing markedly decreased the protein expression of rictor and phospho-Ser473-Akt, a functional read-out of mTORC 2 signaling. 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

A Jansson-style legend not only makes understanding the data easier – it also makes it easier for a reviewer to defend your grant during a review meeting.

Reviewers love lists and summaries because they make reviewing easier

"A happy reviewer is a positive reviewer."

-- Liane Reif-Lehrer

Thomas Jansson used a list to explain why his proposal was important

a. Significance. This proposal is **significant** because:

1. The activity of key placental amino acid transporters is decreased in IUGR [3-5, 8] and up regulated in fetal overgrowth [9], suggesting that altered placental nutrient transporter activity contributes to abnormal fetal growth [10-12]. Since this work focuses on mechanisms by which placental amino acid transport is regulated, it addresses questions critical to the understanding of how important pregnancy complications develop.

2. Placental mTOR signaling activity is decreased in IUGR [1, 2] and preliminary data show an activation of placental mTOR signaling in fetal overgrowth [25]. Our preliminary data demonstrates that mTORC 1 and mTORC2 signaling has a profound impact on trophoblast amino acid transporter activity, suggesting that we have identified important mechanisms for the regulation of placental amino acid transport and fetal growth.

3. Functional data (nutrient transport activity) will be obtained in primary human trophoblast cells, using growth factors in physiological concentrations, which contributes to the physiological relevance of the proposed studies. Furthermore, the systematic utilization of gene silencing approaches in cultured human primary trophoblast cells will allow us to obtain specific mechanistic information on mTORC 1 and mTORC2 signaling pathways in the human placenta, contributing to the significance of the work.

4. In addition to playing a role in abnormal fetal growth, regulation of nutrient transporters has been implicated in many other diseases, including cancer [26]. However, the authors of several recent reviews have highlighted the existence of a major gap in knowledge with respect to the mechanisms regulating nutrient transporters. For example, Edinger concludes *'Despite the clear implications for human disease, there are large gaps in our knowledge of how nutrient transporter expression is regulated'* [27] and '*.. virtually nothing is known about how nutrient transporter internalization and trafficking is regulated in mammalian cells*' [28]. Thus, the proposed research is significant because it addresses a major gap in knowledge and mechanisms shown to regulate amino acid transporters in human primary trophoblast cells are likely to be relevant for other human cells.

John Gabrieli didn't trust reviewers to know what his preliminary experiments meant. So he summarized it for them inside text boxes they couldn't overlook.

Preliminary Study 3. *(Gabrieli lab)* Here we show how fMRI and DTI measures may predict long-term reading outcomes in dyslexia better than conventional behavioral tests, and how multivariate voxel pattern analysis (MVPA) may be a useful tool for enhancing single-subject diagnostic prediction. Children ages 10-16 with dyslexia (N = 25) were initially evaluated with fMRI and DTI scanning, and with 17 widely-used reading and language measures. The children's progress in reading was measured 2.5 years later. We examined gains in single-word reading accuracy and passage comprehension as outcome measures. We related initial behavioral and brain measures to reading gains across the following 2.5 years either as continuous measures on reading scores or by dividing dyslexic readers into a compensated group who improved across years (final reading scores within 1.5 SD of standardized norms) and a struggling group (final reading scores remaining >1.5 SD below the standardized norms).

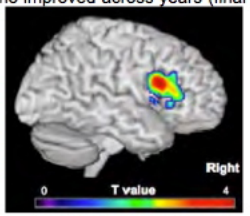


Figure 6. Right frontal brain area identified in study 3.

Results: None of the 17 behavioral measures, alone or in combination, predicted future reading gains in children. Both fMRI and DTI measures from right frontal cortex significantly predicted future reading gains. Strikingly, the best correlation between both fMRI and DTI occurred in right prefrontal cortex, a region not typically engaged in reading. This suggests that older dyslexic children compensate to the extent they use an atypical brain pathway rather than the typical left-hemisphere pathway (this is consistent however with another study on compensated versus struggling children (104). Critically, these brain correlations with future reading levels exceeded all the behavioral measures, which included of the most widely used reading measures. We then used multi-voxel pattern analysis (MVPA) to examine how accurately we could predict for each of the 25 dyslexic children whether they would belong to the compensated or struggling group. The MVPA classification accuracy was 92%, which far exceeded the accuracies of the univariate fMRI and DTI analyses or the behavioral analyses. The limitation of the MVPA approach is that it identifies brain differences that are difficult to understand at present (in Fig.7, one critical region is in the right prefrontal cortex, but others occur in multiple regions). From a practical perspective, this form of analysis may be optimal for predicting later reading performance. From a scientific perspective, areas that appear important in this analysis become targets for further analysis of what those regions contribute to reading and risk for reading. Note that brain measures did not predict reading gains for typical readers who made expected reading gains in 2.5 years.

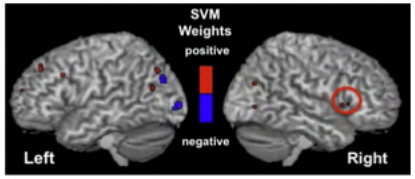


Figure 7. Regions identified using MVPA.

•• ***Preliminary Study 3 shows that brain measures can be more predictive of reading ability in future years than behavioral testing, and that MVPA is a promising method for maximizing accuracy in predicting reading ability years into the future for poor readers.***

Preliminary Study 4. *(Gabrieli lab)* Here we show our ability to use event-related potentials (ERPs) to study auditory processes in children. The mismatch negativity (MMN) is observed when a participant is presented with a continuous series of sounds of an identical stimulus intermixed with an occasional deviant stimulus. The MMN is thought to reflect an automatic, pre-attentive process and therefore does not require that the participant be paying attention to the stimulus. We have been recording MMNs from children 7-11 years old that are normal reading or have dyslexia. Following Maurer's (105) methodology we are using speech sounds as the standard (ba) and deviant (da).

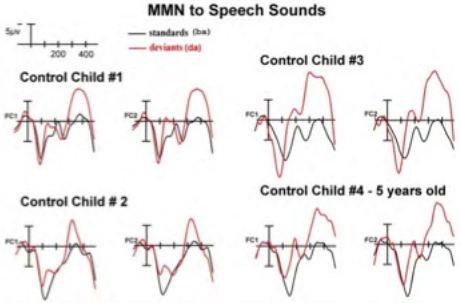


Figure 8. ERP mismatch negativity waveforms from three typical children (ages 7-11) and one 5-year-old child.

Each speech sound is presented for approximately 200 ms with a 200 ms inter-

Gabrieli's summaries-in-a-box tell reviewers what his preliminary data means for project feasibility

•• *Preliminary Study 1 documents our ability to perform an auditory phonological fMRI task with kindergartners that yields findings highly relevant to reading disability.*

•• *Preliminary Study 2 documents our ability to perform an auditory phonological fMRI task with young kindergartners, our ability to see both functional and structural brain differences in children at risk for dyslexia, and our ability to recruit and characterize large numbers of children in this age range.*

•• *Preliminary Study 3 shows that brain measures can be more predictive of reading ability in future years than behavioral testing, and that MVPA is a promising method for maximizing accuracy in predicting reading ability years into the future for poor readers.*

•• *Preliminary Study 4 shows that we can perform ERP MMN studies with children.*

To make your grant easy for lazy reviewers to scan without missing why your work is important

1. Put the first sentence in each paragraph in **bold**.

2. Write these first sentences so when a reviewer scans them they'll summarize what you want the reviewer to know.

Read just the first sentences in bold in Jose Luchsinger's paragraphs. You'll see they summarize why his project is important.

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

How to write background info

When you write background information that reviewers need to understand your project, weave together background about the problem and what you intend to do about the problem.

Do this using words like “we”, “us”, “this project”, “preliminary data”, “understanding”, “this work”, etc.

Weaving background and solution

Colin Parrish

A1) Importance: understanding fundamental aspects of virus infection processes controlled by receptor or antibody binding, and the controls of viral host ranges. The parvoviruses include many different human and animal pathogens, including the long-known B19 virus which causes the childhood fifth disease and more severe diseases of adults, as well as the recently identified human bocavirus and Parv4. The adeno-associated viruses (AAVs) are parvoviruses that are not associated with disease, but are being developed as human gene therapy vectors and the same issues of receptor and antibody recognition are important for vector optimization. The viruses we are studying in this model are the canine parvovirus (CPV) and its close relative feline panleukopenia virus (FPV), which bind to the host transferrin receptor type-1 (TfR) to infect cells (53). The parvoviruses have a 25 nm diameter T=1 capsid that is assembled from 60 copies of two or three versions of a single capsid protein, and the single stranded DNA of the virus is packaged into the pre-formed capsid by the action of the larger non-structural protein (NS1). Although those capsids are remarkably robust and survive in the environment, structural variation results in viruses with different properties, and those also show structural changes during the process of cell entry and nuclear trafficking. The simple and well defined structures of the parvovirus capsids, the known properties of the TfR, and the well characterized antibodies available for these studies allow us to examine several processes of viral infection. Variant viruses with extended host ranges can cause new outbreaks or epidemics of infectious disease. The viruses that we are examining include the comparison of such a system, where one variant arose as a pandemic pathogen in a new host through the acquisition of mutations in the capsid protein that altered its structure to change host-specific receptor binding, and also to change its antigenic structure.

A2) Critical barriers: to antiviral therapy and vaccination success. Animal viruses are complex biological machines that engage host cell receptors and undergo a series of varying structural and functional changes to allow cell penetration and release of the genome for replication. Those infection processes are key to the success of any virus, and are targets of various anti-viral drugs. A better understanding of the details of the general processes involved will likely allow the development of more effective and broadly acting antiviral drugs. Although antibodies are critical components of immune responses of all vertebrates, in many cases they are poorly effective so that viruses maintain persistent infections or vaccines do not work well. Understanding the underlying rules that determine how antibodies bind to viruses and block the processes of cell infection will reveal how effective antibody responses might be elicited against different viruses.

A3) Improvement of scientific knowledge: understanding fundamental viral mechanisms and clarifying textbook knowledge of virus structures and functions. This project addresses several mechanisms important for all viruses of humans and other animals. In general terms those include understanding viral recognition of cell receptors, how changes in receptor binding sites lead to alterations of binding and host range, capsid and receptor structures and the interactions that control uptake and trafficking within cells. During each of these steps the viral proteins must assume the correct conformations, bind receptors with the correct contacts, and in the process undergo a variety of structural transitions to release internal peptides, protein domains, and the viral DNA. Here we will investigate the roles of flexible and variable structures in the parvovirus capsid and show how receptor and antibody binding control cell infection.

Weaving background and solution

a. Significance. This proposal is **significant** because:

1. The activity of key placental amino acid transporters is decreased in IUGR [3-5, 8] and up regulated in fetal overgrowth [9], suggesting that altered placental nutrient transporter activity contributes to abnormal fetal growth [10-12]. Since this work focuses on mechanisms by which placental amino acid transport is regulated, it addresses questions critical to the understanding of how important pregnancy complications develop.

2. Placental mTOR signaling activity is decreased in IUGR [1, 2] and preliminary data show an activation of placental mTOR signaling in fetal overgrowth [25]. Our preliminary data demonstrates that mTORC 1 and mTORC2 signaling has a profound impact on trophoblast amino acid transporter activity, suggesting that we have identified important mechanisms for the regulation of placental amino acid transport and fetal growth.

3. Functional data (nutrient transport activity) will be obtained in primary human trophoblast cells, using growth factors in physiological concentrations, which contributes to the physiological relevance of the proposed studies. Furthermore, the systematic utilization of gene silencing approaches in cultured human primary trophoblast cells will allow us to obtain specific mechanistic information on mTORC 1 and mTORC2 signaling pathways in the human placenta, contributing to the significance of the work.

4. In addition to playing a role in abnormal fetal growth, regulation of nutrient transporters has been implicated in many other diseases, including cancer [26]. However, the authors of several recent reviews have highlighted the existence of a major gap in knowledge with respect to the mechanisms regulating nutrient transporters. For example, Edinger concludes '*Despite the clear implications for human disease, there are large gaps in our knowledge of how nutrient transporter expression is regulated*' [27] and '*.. virtually nothing is known about how nutrient transporter internalization and trafficking is regulated in mammalian cells*' [28]. Thus, the proposed research is significant because it addresses a major gap in knowledge and mechanisms shown to regulate amino acid transporters in human primary trophoblast cells are likely to be relevant for other human cells.

-- Thomas Jansson

By the way, too much background info can kill your grant

The more background you have, the less space you'll have to explain your strategy and careful planning to guarantee your study will work. Reviewers will complain your plans of execution lack detail and kill your grant.

Anticipate reviewers' objections by justifying any unusual decision you made about how to run your studies

If reviewers don't understand why you decided upon approach A when 9 out of 10 in your field choose B, you've given them all the ammunition they need to say you don't know what you're doing.

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

A *Why* paragraph is a great way to justify a decision that needs a lengthy explanation

Why a subunit strategy?
The Global Enteric Multicenter Study (GEMS) funded by the Bill & Melinda Gates Foundation (BMGF) showed that ETEC and *Shigella* are the two principle bacterial diarrheal pathogens in young children. While other approaches are being investigated, including heterologous expression of ETEC antigens in attenuated strains of *Shigella*, and whole vaccine mixtures of ETEC expressing multiple CF antigens, a subunit strategy is more amenable to testing in an iterative polyvalent approach that could also incorporate Shigella subunit antigens, which already show excellent promise, in a vaccine that covers multiple pathogens (similar to current acellular pertussis vaccines that combine tetanus and Diphtheria toxoids). Our decision is informed by practicality and discussions with PATH and BMGF, critical partners in any downstream development efforts (see attached letter from PATH Enteric Vaccine Initiative). Nevertheless, this strategy does not preclude concurrent development of alternative strategies including co-expression of novel antigens in Shigella vaccine vectors in clinical trials.

-- James Fleckenstein

At the end of your grant, say thank you

Your last sentence should be, “Thank you for your consideration.” None of your competitors will do this, so you will definitely be remembered. And if reviewers have to turn you down now, they’ll be encouraged to give you better advice on on winning next time than they might give you otherwise.

Benefits from saying, “Thank you.”

1. Reviewers will be less likely to forget what your grant is about.
2. They may give you better advice in your Summary Statement.
3. They may even bump up your scores a bit.

Acknowledgements

Steve Hsu
Corey Washington