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14. ABSTRACT

Helicobacter pylori is the causative agent of gastric cancer. H. pylori infection induces DNA double-strand breaks (DSBs) in gastric epithelial cells and compromises their genomic integrity. While the genotoxicity of H. pylori promotes gastric carcinogenesis, the underlying molecular mechanisms are not fully understood. Here we show that H. pylori induces DNA DSBs in human gastric adenocarcinoma (AGS) cells through NF-κB activation. Inhibition of NF-κB in AGS cells by the expression of ΔN-IκBα, a degradation-resistant mutant of IκBα (inhibitor of NF-κB), dramatically reduces H. pylori-induced DNA DSBs. Further, type IV secretion system (T4SS)-dependent injection of H. pylori cytotoxin-associated gene A (CagA) into AGS cells promotes NF-κB activation and accumulation of a nucleic acid structure known as an R-loop, leading to DNA DSBs. Analyses of CagA mutants indicated that tyrosine phosphorylation of the EPIYA motifs is critical and a CagA mutant, AB^{T}CCC, containing three copies of the EPIYA motif was more potent than wild-type CagA (ABC) in activating NF-κB and inducing DNA DSBs, but not more carcinogenic. Our results suggest that NF-κB and R-loop-driven genomic instability caused by CagA underlies the tumorigenic effect of H. pylori. As the oncogenicity of the AB^{T}CCC strains is not significantly increased, other activities of CagA or other H. pylori virulence factors likely also play a role.

15. SUBJECT TERMS

Helicobacter pylori, gastric cancer, DNA Double-Strand Break, NF-κB

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1. INTRODUCTION:

H. pylori infection induces a massive inflammatory response that is thought to cause DNA damage and subsequent gastric cancer (GC). Despite this paradigm, molecular details of how DNA damage is induced are still poorly defined. While R-loops were originally identified in bacteria, they have since been shown to be ubiquitous in eukaryotic cells. Furthermore, they are known to play numerous physiological roles in cells, including gene regulation and Ig class switching. However, R-loops can also induce DNA damage and genome instability, which may lead to cancer. A link between *H. pylori*, R-loops and GC has not previously been investigated. Thus, the innovation in the project lies in the unique investigation of the contribution of these structures to DNA damage/GC and the exploration of the utility of targeting these structures as a novel GC therapeutic. Furthermore, if R-loops are involved in DNA damage and GC, we propose that they may also play important roles in many inflammation-based cancers.

2. KEYWORDS:

DNA damage, R-loop, *Helicobacter pylori*, Gastric Cancer

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1 (specified in proposal)	Timeline	Percent Completed
Major Task 1: Determine if R-loops are induced by *H. pylori* infection	Months	
Subtask 1: Infect AGS cells and optimize assays to monitor R-loop formation using wildtype *H. pylori*	1-2	100%
Subtask 2: Expand AGS studies to include wildtype and mutant *H. pylori* strains.	2-6	100%
Subtask 3: Monitor R-loop formation in *H. pylori*-infected Mongolian gerbils	6-12	100%
Milestone(s) Achieved: R-loop profiles in AGS and gerbil models of *H. pylori*	12	
Major Task 2: Identify R-loop sites via DRIP-seq and sites of DNA damage via ChIP-seq		
Subtask 1: Prepare DRIP-seq libraries from *H. pylori*-infected AGS cells	13-14	100%
Subtask 2: Prepare ChIP-seq libraries	15-16	100%
Subtask 3: Illumina sequencing of prepared libraries	17-18	100%
Subtask 4: Bioinformatic/data analysis	18-24	50%
Milestone(s) Achieved: Mapped sites of *H. pylori*-induced R-loop formation and DNA damage.	24	

Specific Aim 2 (specified in proposal)		
Major Task: Determine effect of blocking R-loop formation on *H. pylori*-induced DNA damage		
Subtask 1: Overexpress LV-RNase H1 and monitor R-loop formation	8-10	100%
Subtask 2: Use shRNA to knockdown CSB, XPF and XPG and monitor effects on R-loop formation	11-15	100%
Subtask 3: Data analysis and publication	16-24	50%
Milestone(s) Achieved: Knowledge whether blocking R-loop formation can inhibit DNA damage.	24	

What was accomplished under these goals?

Results

I. *H. pylori* infection induces DNA damage in gastric epithelial cells in a NF-κB-dependent manner

(a) NF-κB inhibition prevents the induction of DNA DSBs by *H. pylori*.

To investigate the role of *H. pylori* in inducing DNA DSBs in the host cells, we infected AGS cells with logarithmically growing *H. pylori* at multiplicities of infection (MOIs) of 50, 100, 150 and 200 for 8 hrs. Infected AGS cells were then harvested, and whole cell lysate was analyzed by immunoblotting for γH2Ax, a marker for DNA DSBs. The results in Fig.1 show that the γH2Ax levels in AGS cells became dramatically increased in a dose-dependent manner in response to *H. pylori* infection; this is in agreement with the notion that *H. pylori* efficiently induces DNA DSBs. Furthermore, *H. pylori*-induced DNA damage is not cell-type specific; human osteosarcoma (U2OS) cells similarly infected by *H. pylori* at a low MOI also developed DSBs as revealed by the accumulation of γH2Ax.

H. pylori infection activates NF-κB and triggers an inflammatory response in host cells. The inhibitor of NF-κB, IκBα, binds and retains NF-κB in the cytosol, thereby inhibiting the transcriptional activity of NF-κB. Stress and immune/inflammatory responses signal the phosphorylation and ubiquitin/proteasome-mediated degradation of IκBα, leading to NF-κB nuclear translocation and transcriptional activation. To test whether NF-κB is activated in *H. pylori*-infected AGS cells, we analyzed the steady-state levels of phosphorylated IκBα and IκBα by western blotting. As shown in Fig. 2A, *H. pylori* infection at MOI_{100} and MOI_{200} increased the levels of phospho-IκBα (p-IκBα) with a concomitant decrease in the steady state levels of IκBα, indicating robust NF-κB activation.

To determine whether NF-κB activation drives DNA DSBs in infected AGS cells, we blocked the activity of NF-κB by stably transducing AGS cells with a lentiviral vector, LV-ΔN-IκBα. This vector expresses ΔN-IκBα, a degradation-resistant mutant of IκBα deleted for the 36 amino-acid sequence motif in the NH_2 terminus of IκBα that mediates activation-induced IκBα phosphorylation and degradation. ΔN-IκBα constitutively retains NF-κB in the cytosol and inhibits the transcriptional activity of NF-κB. The LV-ΔN-IκBα-transduced cells were selected in puromycin

(0.5 μg/ml)-containing medium for 7 days and analyzed by immunoblotting as shown in Fig 2B, where both the endogenous wild-type IκBα and the truncated ΔN-IκBα were detected in the cell lysate (lane 2). We then infected the parental and pooled ΔN-IκBα-expressing AGS cells with *H. pylori* at MOI_{100} and MOI_{200} for 8 hrs and assessed the DNA damage responses (DDRs) by γH2Ax detection. As shown in Fig. 2C, the levels of γH2Ax were significantly lower in the ΔN-IκBα-expressing AGS cells compared to the parental cells (lanes 5 & 6 vs lanes 2 & 3), indicating that *H. pylori*-induced DNA damage in AGS cells is driven by NF-κB activation.

(b) The morphological alteration of gastric epithelial cells caused by *H. pylori* does not require NF-κB signaling.

H. pylori infection causes cell elongation in AGS cells, resulting in a "hummingbird"-like morphology. We asked whether this morphological change is a result of NF-κB signaling. To this end, we infected the parental and the ΔN-IκBα-expressing AGS cells with *H. pylori* at MOI_{100} for 8 hrs, and observed them microscopically. Both the parental and the ΔN-IκBα-expressing AGS cells exhibited the elongated phenotype as shown in Fig. 3, indicating that the morphological alteration in *H. pylori*-infected cells is not NF-κB-related.

II. *H. pylori*-induced DNA damage requires the type IV secretion system and the *H. pylori* virulence factor, CagA, but not VacA.

(a) Deletion of *PAI* or *cagA,* but not *vacA* of *H. pylori* abrogates DDR induction.

To assess the role of *H. pylori* virulence factors in inducing DSBs, we infected AGS cells with various mutant strains of *H. pylori* at MOI_{100} for 8 hrs and assessed the DNA damage response (DDR) of the infected cells. As shown in Fig. 4A, the wild-type and the Δ*vacA* strains of *H. pylori* efficiently activated NF-κB and induced DDR as revealed by the elevated levels of phosphorylated-IκBα (p-IκBα) and γH2Ax in the infected cells. In contrast, the Δ*PAI* (type IV secretion system) mutant had little effect on NF-κB activation and did not induce DNA damage. Like its Δ*PAI* counterpart, the Δ*cagA* mutant was significantly compromised in NF-κB activation and DDR induction (lane 3). However, the deficits were not as complete as in the Δ*PAI* mutant (lane 4), suggesting that the type IV secretion system likely delivered additional *H. pylori* components such as peptidoglycans and lipopolysaccharides that contributed to NF-κB activation and DDR induction. These results support the notion that CagA is the major driver of NF-κB activation and DNA DSBs induction in *H. pylori*-infected cells, while VacA plays no role.

(b) The EPIYA motifs of CagA are required for NF-κB activation and DNA damage induction.

CagA is tyrosine phosphorylated in the EPIYA (Glu-Pro-Ile-Tyr-Ala) motifs in its COOH-terminus by members of the Src family of non-receptor tyrosine kinases including c-Abl. To test the involvement of the EPIYA motifs in NF-κB activation and DNA DSB induction, we infected AGS cells with isogenic *H. pylori* strains harboring various CagA EPIYA variants. As shown in the Fig. 4B, the wild-type (EPIYA-ABC) and the EPIYA ABCCC variant (lanes 2 and 6) induced significant DNA DSBs as indicated by levels of γH2Ax induced, suggesting that tyrosine phosphorylation of CagA is essential for the induction of DSBs in the host cells. Notably, the presence of two additional EPIYA-C motifs in the EPIYA-ABCCC variant increased NF-κB activation and DNA damage, as reflected by the levels of phosphorylated IκBα and γH2Ax levels, respectively. In keeping with the critical role of the EPIYA-C motif, its deletion (EPIYA-AB, lane 4) significantly reduced NF-κB activation and DNA damage.

III. *H. pylori*-infection increases R-loop formation in AGS cells and Mongolian gerbils.

(a) . *H. pylori* infection enhances R-loop formation in a CagA- and PAI-dependent manner in AGS cells

An R-loop is a three-stranded nucleic acid structure consisting of a RNA-DNA hybrid and a displaced single-stranded DNA loop. R-loop formation mediates immunoglobulin isotype switching, CRISPR-mediated DNA excision, transcription-coupled nucleotide excision repair (TC-NER), and chromatin structure and transcription. Transcriptional de-repression and RNA splicing/elongation/

processing/export deficiencies are known to cause R-loop accumulation, leading to DNA damage and genomic instability (GI). Furthermore, R-loop processing by the TC-NER endonucleases, Xeroderma pigmentosum F (XPF) and XPG, has been shown to induce DNA DSBs and GI. To investigate the mechanism underlying NF-κB mediated DSBs in *H. pylori*-infected cells, we ask whether NF-κB may drive R-loop accumulation during *H. pylori* infection, leading to DNA DSBs. To address this question, we infected AGS cells with wild-type *H. pylori* at MOI_{200} and extracted total host cell nucleic acid at various times after infection. One microgram of nucleic acid from each sample was transferred onto the nylon membrane and analyzed by South-Western hybridization using the S9.6 antibody that specifically binds RNA-DNA hybrids. As shown in the Fig 5A, *H. pylori* infection induced an increase in the levels of R-loops in AGS cells at 3 and 4.5 hrs post-infection, but the R-loop signal did not persist and subsided at 6 hrs post-infection. S9.6 antibody binding reflected R-loop formation, and disappeared when the nucleic acid samples were treated with RNase H, an enzyme that digests the RNA moiety in the RNA-DNA hybrid. (Fig. 5A lower panel). As expected, the time course of R-loop accumulation in *H. pylori* infected AGS cells correlated with the kinetics of the nuclear translocation of NF-κB subunits, p65/RelA and Rel-B, steadily increasing between 0 to 4.5 hrs post-infection, and then subsided afterwards (Fig 5B).

We next assessed whether Cag A and Type IV secretion system (encoded by the pathogenicity island, PAI) of *H. pylori* are required for R-loop accumulation. We infected AGS cells with wild-type, *ΔcagA* and *ΔPAI* mutant strains of *H. pylori* at MOI_{200} for 4.5 hrs and analyzed R-loops by South-Western hybridization. As expected, a significant reduction in R-loops (40%) is observed in AGS cells infected with *ΔcagA* and *ΔPAI* mutant *H. pylori* strains compared to wild-type strain (Fig. 5C). To test whether the *H. pylori* induced R-loop accumulation is dependent on NF-κB activation, we infected cells of wild-type AGS and an AGS clone that stably expressed ΔN-IκBα with the wild-type *H. pylori* strain at MOI_{200} for 4.5 hrs and analyzed R-loop accumulation by South-Western hybridization. The result in Fig.6A shows that a significant reduction in R-loop accumulation occurred in ΔN-IκBα-expressing AGS cell line compared to the wild-type control, suggesting that *H. pylori* induced R-loop formation is dependent on NF-κB activation. IN agreement with these results, the immunoblots of the whole cell lysates (Fig. 6B) show that the R-loops formation correlates with the DNA DSBs as indicated by elevated level of γH2Ax levels in AGS wild-type cells compared to the ΔN-IκBα cells (lane 2 and 4).

We also assessed the *H. pylori* induced NF-κB activation, R-loops accumulation, DNA DSBs in AGS cell by Immunofluorescence. AGS cells were infected by *H. pylori* wild-type, EPIYA (ABCCC) variant and *ΔPAI* mutant strain at MOI_{200} for 4.5 hrs and fixed using paraformaldehyde or methanol. The fixed cells were immunostained with S9.6 antibody, γH2Ax antibody, and p65/RelA antibody to detect R-loops, DNA DSBs and nuclear NF-κB, respectively. As shown in Fig. 7 A, B & C, *H. pylori* wild-type and EPIYA ABCCC variant activated NF-κB (nuclear localization of p65) and increased the levels of R-loops and γH2Ax in AGS cells whereas *ΔPAI* mutant strain did not.

(b) . *H. pylori* infection increases R-loop formation in Mongolian gerbils.

To validate the findings outlined above in an animal model of *H. pylori* infection and gastric cancer development, Mongolian gerbils were fed with $2x10^9$ *H. pylori* cells and sacrificed 7 and 21 days after. Nucleic acid from the lower part of the stomach tissue was extracted and analyzed by the South-Western hybridization. As shown in Fig. 8A, a significant increase in the levels of R-loops were detected in the stomach tissue of the *H. pylori*-fed Mongolian gerbils.

IV. Genome wide analysis of R-loops accumulation in *H. pylori* infected AGS cells.

Having shown that *H. pylori* infection enhances R-loops accumulation in AGS cells in a CagA- dependent manner, we next aimed to map R-loops in the genome of the AGS cells infected with either wild-type or *ΔPAI H. pylori* strains. To that end, we utilized a recently developed R-loop

mapping technique known as MapR to release DNA-RNA hybrids (R-loops) from the nuclei of infected AGS cells. We initially expressed micrococcal nuclease (GST-MNase) and a catalytically dead mutant of RNase H fused to micrococcal nuclease (GST-RHΔ-MNase) in *E. coli* and purified them using glutathione S-transferase (GST)-agarose beads (Fig. 9A). Equimolar amounts of the two recombinant enzymes were then added respectively to the digitonin-permeabilized AGS cells that were immobilized on concanavalin A-coated beads. The recombinant enzymes were incubated with AGS cells overnight at 4°C and the micrococcal nuclease activated in the presence of 0.1M $CaCl_2$ for 2 min. The digested genomic DNA was extracted and analyzed by 2% agarose and capillary gel electrophoresis. As shown in Fig. 9B and C, GST-RHΔ-MNase-digested genomic DNA from wild-type *H. pylori*-infected AGS cells showed a unique banding pattern compared to those of similarly treated genomic DNAs of uninfected and *ΔPAI* mutant strain infected AGS cells, likely reflecting R-loops induction in wild-type *H. pylori*-infected AGS cells. We then enriched the 400 bp fragments from the GST-RHΔ-MNase-digested genomic DNA and used them to construct R-loop libraries for NextGen sequencing using the Illumina platform. Future work will focus on comparative bioinformatic analyses of the sequence data of these R-loop libraries.

Future Directions

Our results firmly established the role of CagA-mediated NF-κB activation as the principal driver of DNA damage and genomic instability caused by *H. pylori* infection. Current efforts focus on testing the hypothesis that NF-κB activation promotes excess RNA polymerase II (RNAPII) transcription of NF-κB-regulated genes, causing RNAPII stalling and co-transcriptional R-loop accumulation. This, in turn, induces TC-NER-mediated R-loop excision and DSBs, promoting gastric cancer development. Bioinformatics analysis of the R-loops sequence data is in progress to identify (a) which genomic regions accumulate R-loops upon *H. pylori* infection, (b) whether the sites of R-loop accumulation correlate with the sites of DNA DSBs, and (c) how these DNA DSBs impact gastric cancer development. We expect at least one substantial manuscript that will detail all of our results.

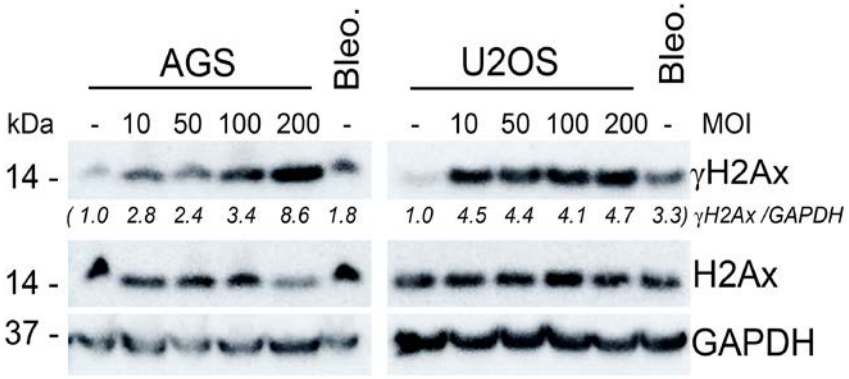

Fig. 1 *H. pylori* infection induces DNA damage in AGS and U2OS cells. AGS and U2OS cells were infected with *H. pylori* at various MOI (0, 50, 100, 150 and 200) for 8 hrs and DNA damage response was analyzed by immunoblotting with the γH2Ax antibody. Bleomycin-treated AGS and U2OS cells were used as positive controls for DNA DSBs. Bands were quantified and normalized to the loading control.

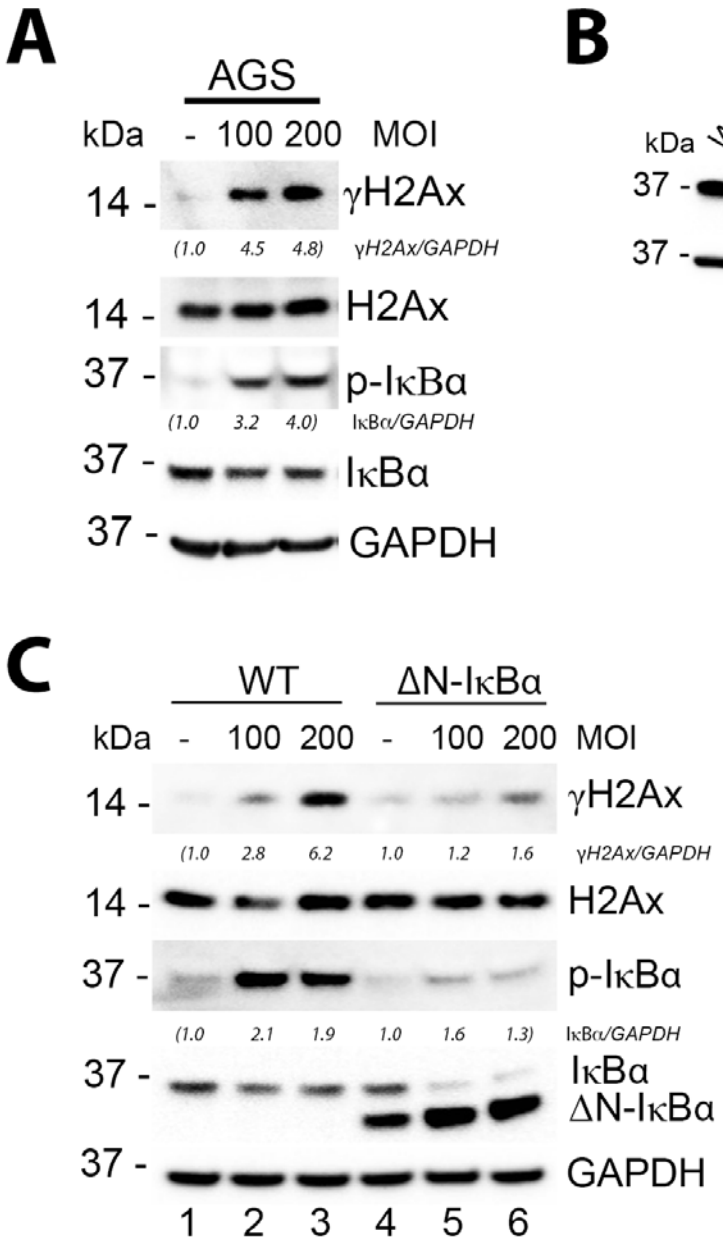

Fig. 2 *H. pylori* infection activates NF-κB in AGS cells: (A) AGS cells were infected with *H. pylori* at MOI_{100} and MOI_{200} for 8 hrs and whole cell lysate was immunoblotted with the indicated antibodies. (C) AGS parental cells and pooled ΔN-I-κBα-expressing cells were infected with *H. pylori* at MOI_{100} and MOI_{200} for 8 hrs, and whole cell lysates were analyzed by immunoblotting with the indicated antibodies. All bands were quantified using ImageJ software and normalized to the loading control.

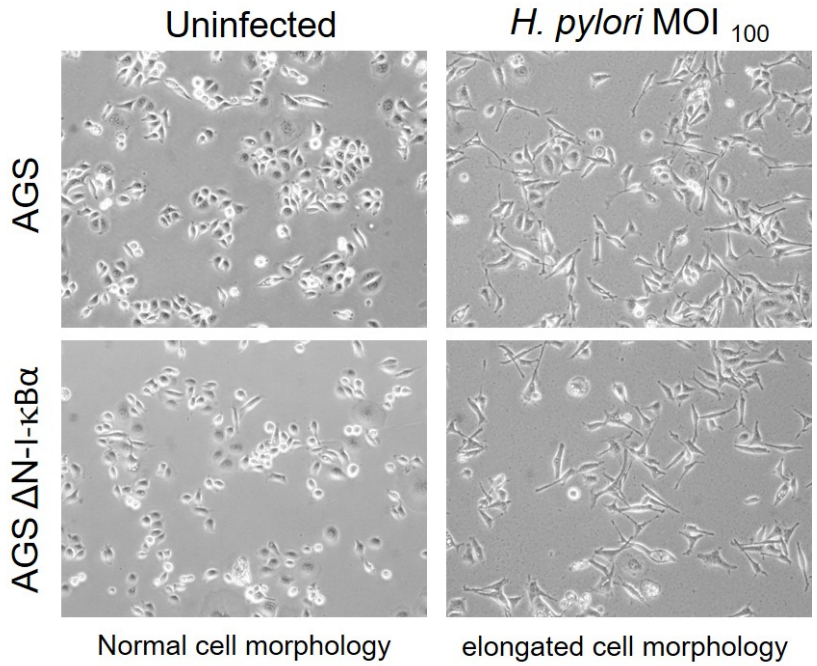

Fig. 3 *H. pylori*-induced morphological changes in AGS cells are independent of NF-κB signaling. AGS WT and ΔN-IκBα-expressing cells were infected with *H. pylori* at MOI_{100} and photographed 8 hrs post-infection.

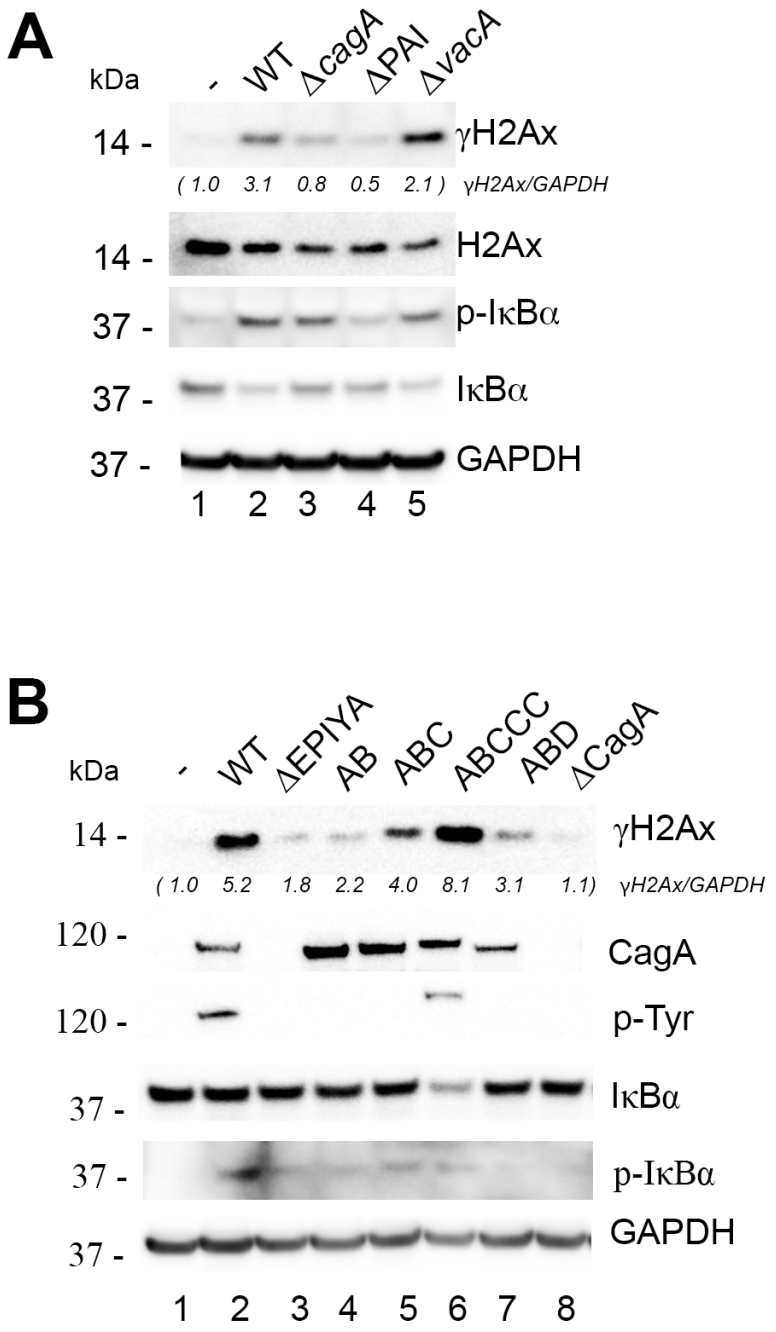

***Fig. 4 H. pylori* type IV secretion system and virulence factor CagA, but not VacA, are required for the induction of DNA DSBs in AGS cells** (A) AGS cells were infected with Δ*cagA*, ΔPAI, and Δ*vacA* mutants of *H. pylori* at MOI_{100} for 8 hrs, and whole cell lysate was analyzed by immunoblotting with the indicated antibodies. . (B) AGS cells were infected with wild-type and CagA mutant strains of *H. pylori* at MOI_{100} and whole cell lysate was analyzed by immunoblotting. All bands were quantified using ImageJ software and normalized to the loading control. (C) AGS cells were infected with isogenic wild-type and various EPIYA allele strains of *H. pylori* at MOI_{100} for 8 hrs and whole cell lysate was analyzed by immunoblotting.

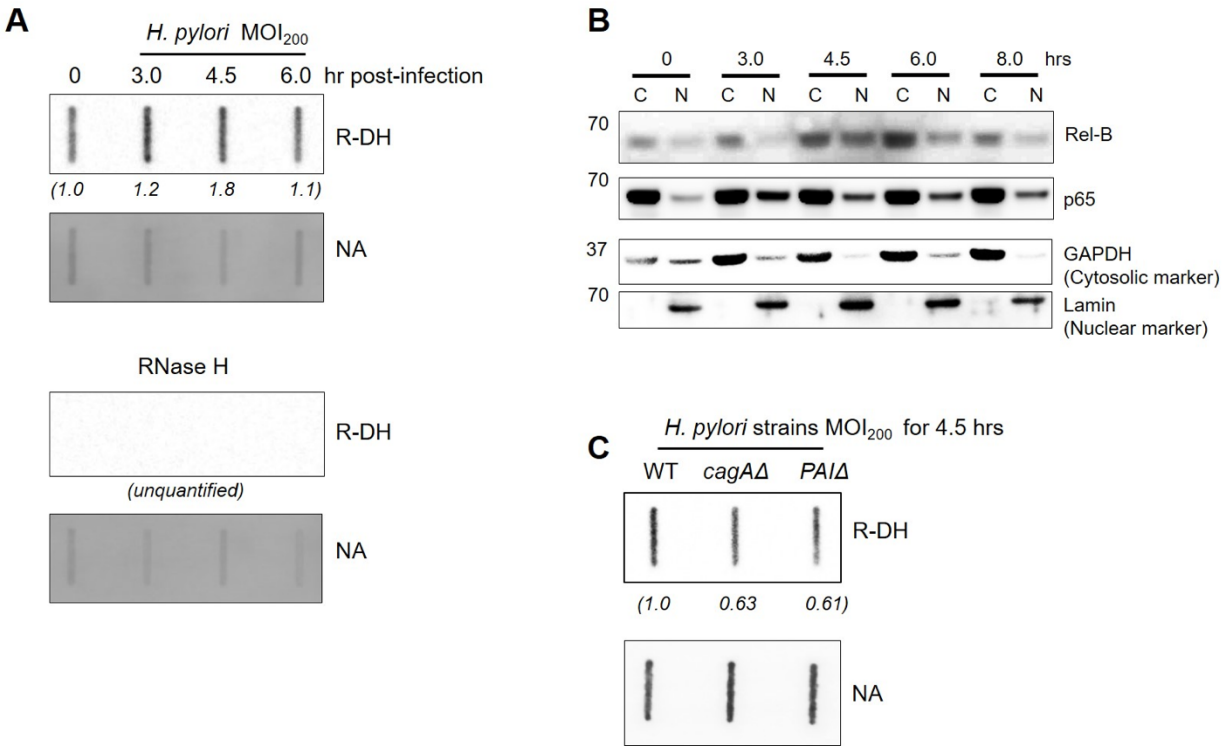

Fig. 5 H. pylori-infection increases R-loop formation in AGS cells (A) AGS cells were infected with wild-type *H. pylori* at MOI_{200} for the indicated times. Total cell nucleic acids were extracted from infected- and uninfected-AGS cells and analyzed by South-Western blotting with the S9.6 antibody. (B) AGS cells were infected with wild-type *H. pylori* for the indicated times and sub-cellular factions were prepared and analyzed by immunoblotting with the indicated antibodies. (C) AGS cells were infected with wild-type *H. pylori*, and *ΔcagA* and *ΔPAI* mutants at MOI_{200} for 4.5 hrs and total nucleic acids were analyzed as in (A).

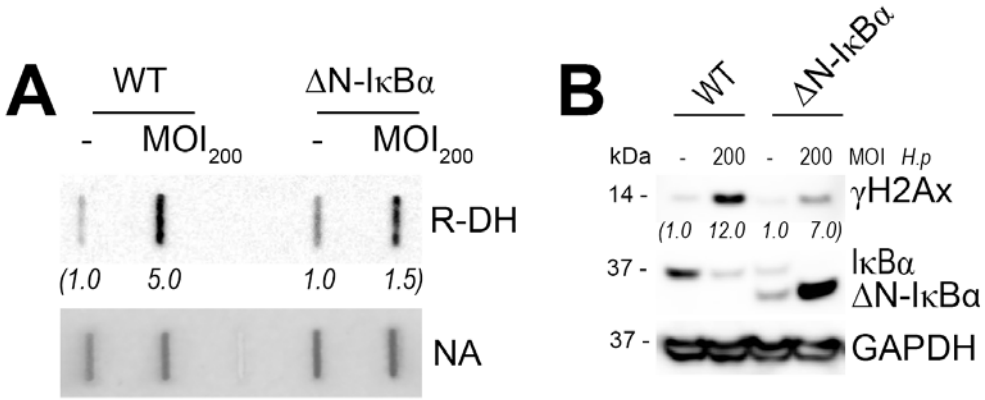

Fig.6 H. pylori-induced R-loop formation depends on NF-κB signaling. (A) Wild-type and a ΔN-IκBα-expressing AGS cell clone (clone #9) were infected with wild-type *H. pylori* at MOI_{200} for 4.5 hrs and total nucleic acids were extracted and analyzed as in Fig. 5A. (B) Whole cell lysates were analyzed by immunoblotting with the indicated antibodies.

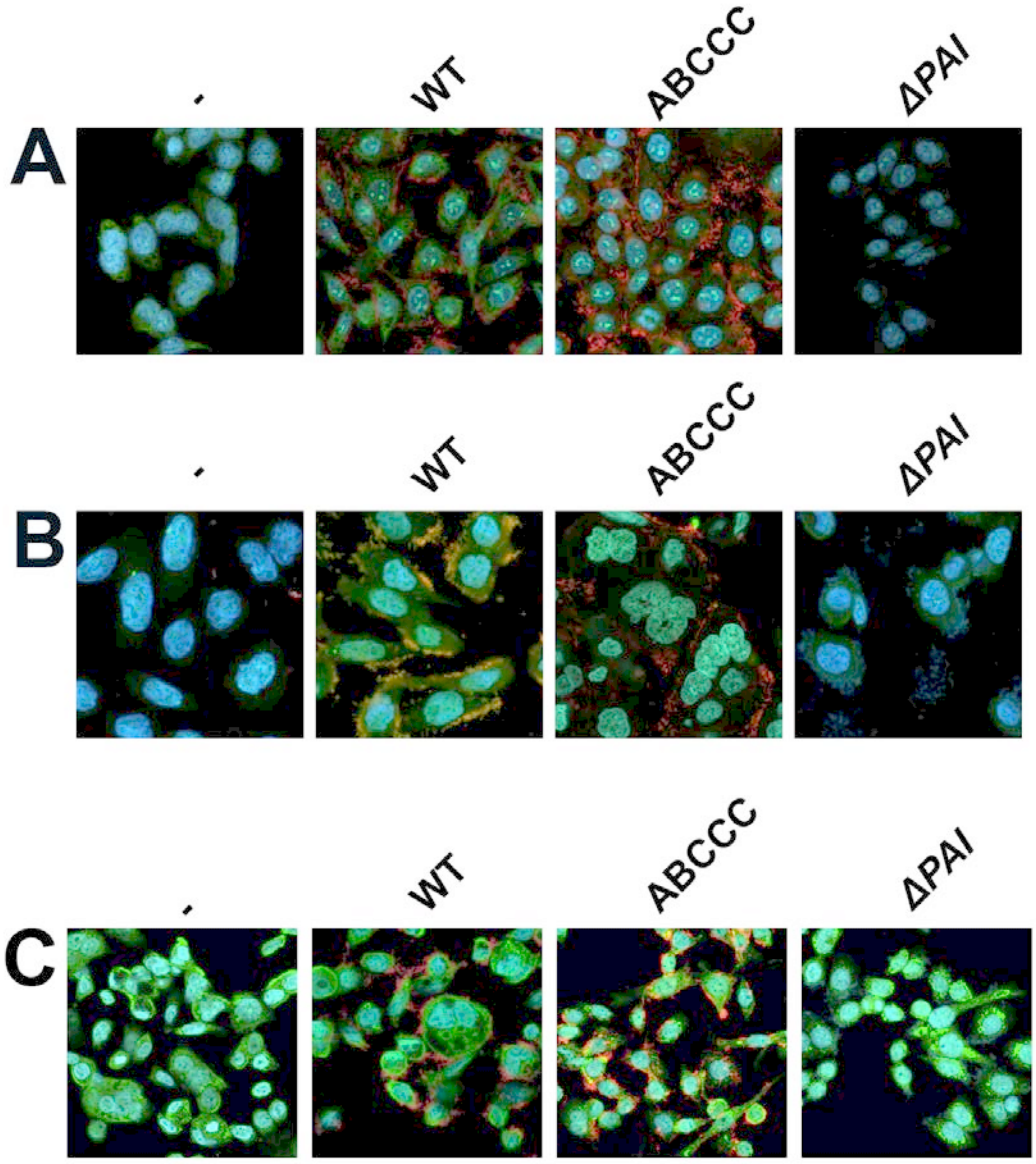

Fig.7: Immunofluorescence detection of R-loops, DNA DSBs and NF-κB activation in H. pylori-infected AGS cells. AGS cells were mock infected (left panels) or infected by *H. pylori* strains expressing wild-type CagA (WT), EPIYA ABCCC CagA (ABCCC), or an isogenic *H. pylori* strain deleted for the type IV secretion system (*ΔPAI*). The fluorescence signals for CagA (red) and DAPI (nuclei, blue) are the same for all rows. The green fluorescence signals in rows ***(***A), (B) and (C) represent R-loops, γH2Ax, and p65/RelA, respectively.

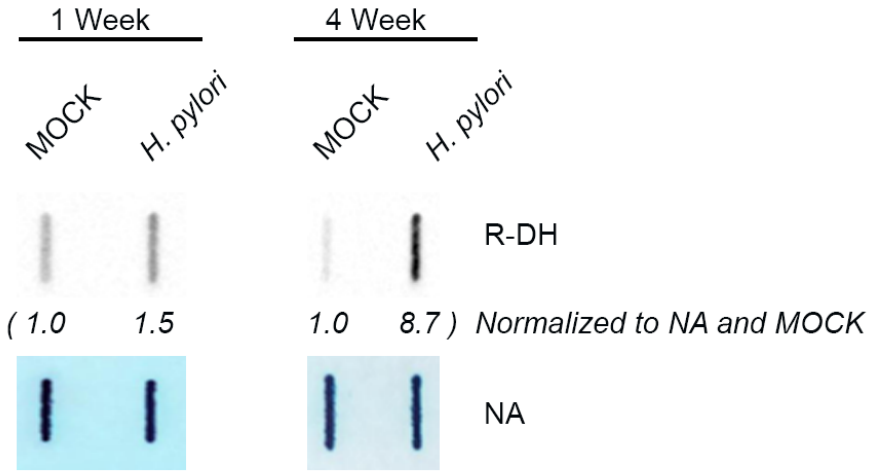

Fig. 8 H. pylori infection increases R-loop formation in gastric tissues of Mongolian gerbils. Mongolian gerbils were fed with $2x10^9$ *H. pylori* cells and sacrificed at the indicated times post-infection. Total nucleic acids were extracted from the tissues collected from the lower part of the stomachs and analyzed as in Fig. 5A.

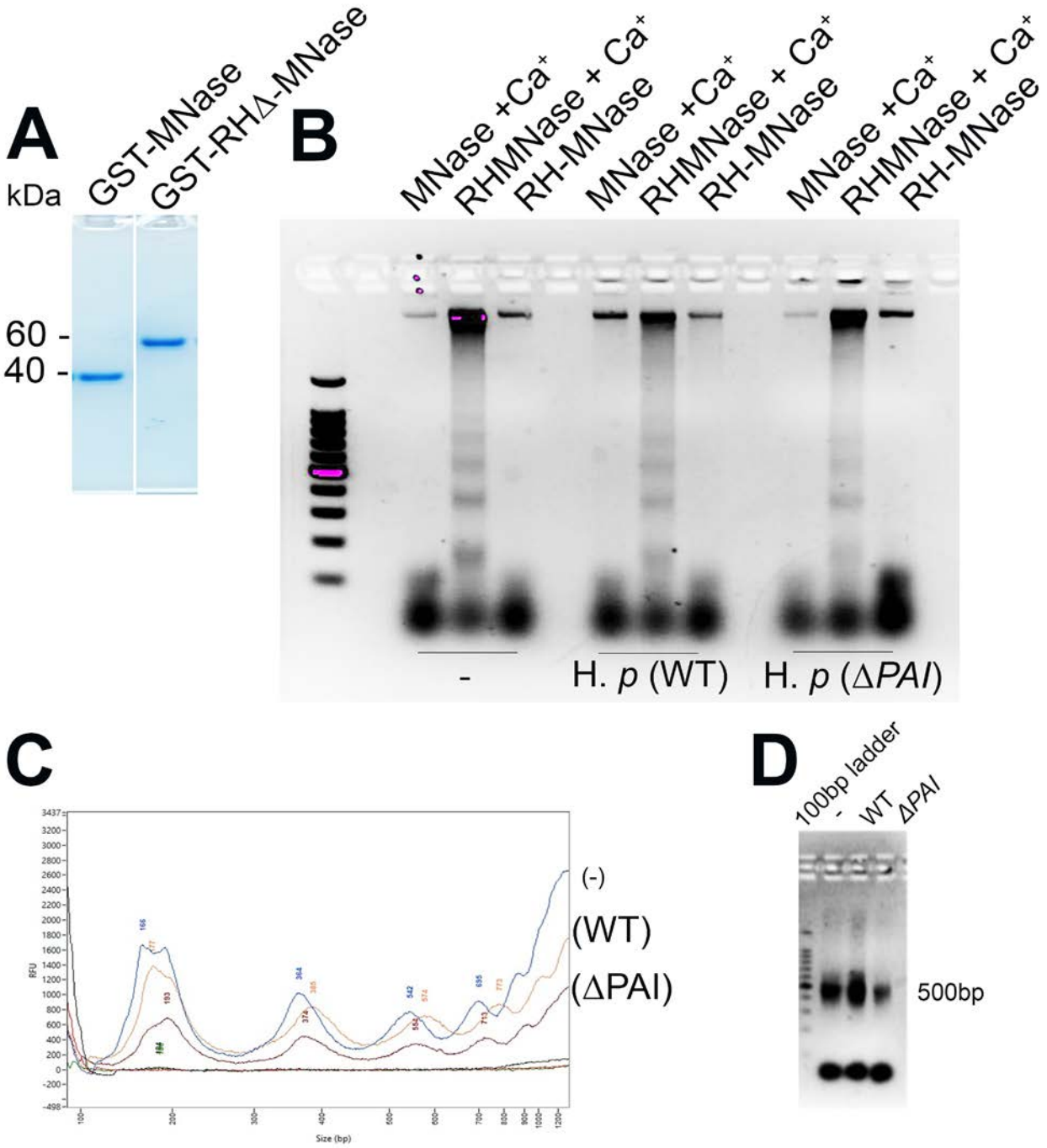

Fig. 9 Mapping R-loops in the genome of H. pylori-infected AGS cells. (A) Purification of recombinant enzymes GST-MNase and GST-RHΔ-MNase from *E.coli.* Agarose gel (2%) electrophoresis (B) and capillary gel electrophoresis (C) of micrococcal nuclease digested genomic DNAs of uninfected (-), wild-type *H. pylori-*

and ΔPAI mutant-infected AGS cells. (D) Construction of DNA libraries of putative “R-loop”-derived DNA fragments (≅400 bp) from the AGS cells in (B&C).

What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report at this time, but one substantial manuscript is expected in the coming months.

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to Report.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Our current data clearly indicate that *H. pylori* infection induces the formation of DNA damage. This knowledge may have significant future implications for strategies seeking to prevent *H. pylori*-induced DNA damage and subsequent development of gastric cancer.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

We experienced several delays in execution of the items we expected to complete during the project. The delays in year one were related to delays in hiring of personnel and time needed to construct additional bacterial strains. The delays in subsequent years have been due to COVID-19; our laboratories were completely closed for months and operated at reduced capacity for a significant amount of time. Overall, these delays resulted in us pouring greater effort into some of the tasks (those marked above as 100% completed) and deprioritization of some of the others (those marked above as <100% completed). Despite these minor changes, the overall goals of the project have still been accomplished.

Actual or anticipated problems or delays and actions or plans to resolve them

Originally we experienced a short delay in recruiting suitable personnel to the project, which delayed the execution of some of the tasks. These individuals were recruited and work was back on track until COVID-19. Overall, these delays resulted in us pouring greater effort into some of the tasks (those marked above as 100% completed) and deprioritization of some of the others (those marked above as <100% completed). Despite these minor changes, the overall goals of the project have still been accomplished.

Changes that had a significant impact on expenditures

Personnel and supply expenditures have been lower than expected in year one because of the delays in recruiting suitable personnel to execute the project. Supply expenditures in year two are also lower due to the closure due to COVID-19.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**
  Journal publications.

Nothing to report at this time.

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Nothing to report

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: D. Scott Merrell
Project Role: Principal Investigator
Research Identifier (e.g. ORCID ID): 0000-0001-7095-5177
Nearest person month worked: 1.2
Contribution to Project: Dr. Merrell is an expert in H. pylori and has contributed expertise to the design and execution of the infection experiments. He has helped with data interpretation and experimental design of the upcoming experiments.
Funding Support: Salary support covered as DoD employee

Name: Chou-Zen Giam
Project Role: Co-Principal Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.2
Contribution to Project: Dr. Giam is an expert in infection-associated cancers. He provided resources such as reagents, antibodies and proteins to support this study. Dr. Giam assisted with data interpretation and provided extensive knowledge in this area of research.
Funding Support: Salary support covered as DoD employee

Name: Nagesh Pasupala
Project Role: Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 11.2
Contribution to Project: Dr. Pasupala is a Postdoctoral Fellow that works on the project within Dr. Giam's laboratory. He has generated the majority of the *in vitro* data that is presented in the progress report.
Funding Support: This award

Name: Faith Blum
Project Role: Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2.4
Contribution to Project: Dr. Blum is a Postdoctoral Fellow that works on the project within Dr. Merrell's laboratory. She assisted with growth and maintenance of *H. pylori* cultures, and helped with the execution of the in vivo studies.
Funding Support: This award and National Organization for Rare Disorders.

Name: Garima Bansal
Project Role: Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 12
Contribution to Project: Dr. Bansal is a new Postdoctoral Fellow that works on the project within Dr. Merrell's laboratory. She worked with Dr. Blum on the growth and maintenance of *H. pylori* cultures and helped with the execution of the in vivo studies.
Funding Support: This Award

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

D. Scott Merrell, Ph.D.
Other Research Support
Current Support:
Supporting Agency: Military Infectious Diseases Research Program (MIDRP)/DoD
Address and POC of Funding Agency: MIDRP, Fort Detrick, MD
Period of performance: 8/1/2021 – 9/30/2023
Title: A Novel Antimicrobial to Prevent and Treat Staphylococcus aureus-Associated Infections
Brief description of goals: This project seeks to identify novel antimicrobials that work against. *S. aureus.*
Time Commitment/Effort: 1.2 calendar months (10% effort)
List of specific aims:

- Aim 1: Identify the *C. pseudodiphtheriticum* genes responsible for the secreted bactericidal factor(s).
- Aim 2: Purify and identify the secreted factor(s) using chromatographic purification and mass spectrometry.
- Aim 3: Test the efficacy of the identified factor(s) as a *S.aureus* therapeutic using a Cotton Rat Model of nasal colonization and a Balb/c thigh abscess model.

Supporting Agency: National Institute of Health, NIAID
Address and POC of Funding Agency: Melody Mills, NIH, 5601 Fishers Lane, Rockville, Maryland 20892
Period of performance: 6/21/2021 – 5/31/2023
Title: Contribution of Helicobacter pylori HomA and HomB to Colonization and Disease
Brief description of goals: The goal of this project is to define the role of HomA and HomB in the ability of H. pylori to colonize and cause disease.
Time Commitment/Effort: 1.2 calendar months (10% effort)
List of specific aims:

- Aim 1: Characterize hom expression in response to environmental stress and determine whether genomic location of homA and homB affects expression and function of the Hom proteins.
- Aim 2: Characterize the role of HomA and HomB in colonization and in development of gastric cancer in the gerbil model of H. pylori infection.

Supporting Agency: Military Infectious Diseases Research Program (MIDRP)/DoD
Address and POC of Funding Agency: MIDRP, Fort Detrick, MD
Period of performance: 9/1/2022 – 8/31/2024
Title: Copper-Laden Hydrogels and Nanocrystalline Cellulose Films as Novel Wound Infection Therapeutics
Brief description of goals: This project seeks to develop and characterize novel copper-based antimicrobials.
Time Commitment/Effort: 1.2 calendar months (10% effort)
List of specific aims:

- Aim 1: Assess the ability of copper to kill *S. aureus* and *P. aeruginosa* on liquid and solid surfaces and determine if copper supplementation can increase antibiotic efficacy against *A. baumannii, S. aureus* and *P. aeruginosa*.
- Aim 2: Aynthesize and characterize impregnated hydrogels and nanofibrillated cellulose films containing copper solutions and nanoparticles and assess their *in vitro* efficacy against *A. baumannii, S. aureus* and *P. aeruginosa*.
- Aim 3: Test the *in vivo* efficacy of the copper-based materials using waxworm and mouse dorsal wound infection models.

Supporting Agency: DoD/Uniformed Services University of the Health Sciences
Address and POC of Funding Agency: Gale Morgan, USUHS, Office of Research, 4301 Jones Bridge Road, Bethesda, MD 20814
Period of performance: 9/29/2022 - 9/3/2025
Title: Targeting Helicobacter pylori Biofilms to Prevent Gastric Cancer
Brief description of goals: The project seeks to characterize *H. pylori* biofilm formation and identify antimicrobials that target biofilm bacteria.
Time Commitment/Effort: 1.2 calendar months (10% effort)
List of specific aims:

- Aim 1: Identify and characterize genes involved in *H. pylori* biofilm formation.
- Aim 2. Identify and characterize compounds that kill or disrupt *H. pylori* biofilms.

Prior Support/Completed Funding:
Supporting Agency: Military Infectious Diseases Research Program (MIDRP)/DoD
Address and POC of Funding Agency: MIDRP, Fort Detrick, MD
Period of performance: 12/10/2018 – 12/09/2020
Title: Identification of bacterial biomarkers associated with biofilm and host innate immune response during wound infection
Brief description of goals: This project seeks to understand the role of biofilm formation in wound healing and innate immune responses.
Time Commitment/Effort: .48 calendar months (4% effort)
List of specific aims:

- Aim 1: Identification and characterization of bacterial isolates from wound by using BD-Phoenix Automated Microbiology system.
- Aim 2: Investigate the biofilm formation in the clinical isolates of wound infection in vitro by using 96 wells peg lids biofilm assay.
- Aim 3: Investigate the role of biofilm bacteria in modulating innate immune response

Supporting Agency: National Institute of Health, NIAID
Address and POC of Funding Agency: Melody Mills, NIH, 5601 Fishers Lane, Rockville, Maryland 20892
Period of performance: 05/23/18 – 04/30/2021
Title: A Novel Helicobacter Pylori Strain to Study Gastric Cancer Development
Brief description of goals: The goal of this project is to characterize a new cancer-causing strain of H. pylori
Time Commitment/Effort: 1.2 calendar months (10% effort)
List of specific aims:

- Aim 1: To characterize USU101 infection and disease pathology in the Mongolian gerbil model.
- Aim 2: To characterize effects on host cell singling pathways following infection with USU101

Supporting Agency: USAMRAA/CDMRP
Address and POC of Funding Agency: Darrell Beaver, 820 Chandler Street, Fort Detrick, MD 21702-50147
Period of performance: 08/15/18 – 08/14/21
Title: Helicobacter pylori-Induced DNA Double Strand Breaks and Gastric
Brief description of goals: This project seeks to understand how H. pylori-induced DNA damage contributes to cancer formation.
Time Commitment/Effort: 1.2 calendar months (10% effort)
List of specific aims:

- Aim 1: To determine if R-loops are induced by H. pylori infection and where these structures form on the host genome.
- Aim 2: To determine if blocking R-loop accumulation will decrease DNA damage induced by H. pylori.

Supporting Agency: National Organization for Rare Disorders (NORD)
Name and Address of Funding Agency: 1779 Massachusetts Ave NW, Washington, DC 20036
Period of performance: 01/01/18 – 12/31/20
Title: "Role of Bacteria in the Development and Progression of Pseudomyxoma peritonei"
Brief description of goals: The R21 seeks to understand the role of the Hom proteins in pathogenesis of H. pylori infection.
Time Commitment/Effort: 1.2 calendar months (10% effort)
List of specific aims:

- Aim 1: To determine whether the bacteria present in PMP patients undergoing standard treatment are different from the bacteria present in patients treated with antibiotics.
- Aim 2. To determine whether the bacteria present in patients are correlated to PMP form (DPAM or PMCA), disease severity, and disease recurrence and/or outcomes.

Supporting Agency: Military Infectious Diseases Research Program (MIDRP)/DoD
Name and Address of Funding Agency: MIDRP, Fort Detrick, MD
Period of performance: 07/01/2015-06/30/2020
Title: "Natural History of Staphylococcus aureus Colonization, Infection, and Immune Response in Military Trainees"
Brief description of goals: The longitudinal study investigates microbiome changes associated with wounds SSTI development.
Time Commitment/Effort: 1.2 calendar months (10% effort)
List of specific aims: Dr. Merrell's component is as follows:

- Specific Aim 3. Determine the relative abundance and distribution of bacterial species colonizing the bodies of military trainees.

Period: December 1, 2006 – November 30, 2013
"Regulatory Networks of Helicobacter pylori" 1 R01 AI065529 Principal Investigator: D. Scott Merrell, Ph.D.
Agency: NIH
Total Direct Costs:

Period: July 1, 2011 – February 28, 2014
"Differential Interaction of the Highly Polymorphic CagA Toxin from Helicobacter pylori with Host Cell Targets".
Principal Investigator: Myron Levine, Ph.D.
Component Project Principal Investigator: D. Scott Merrell, Ph.D. Agency: NIH Program Project No. 2 U54 AI57168-06
Total Direct Costs:

Period: March 9, 2014 – March 8, 2015
"Activity of Solithromycin Against Helicobacter pylori" (CRADA) Principal Investigator: D. Scott Merrell, Ph.D.
Agency: Cempra
Total Direct Costs:
Period: August 1, 2009 – June 30, 2013 No-cost extension until June 30, 2015 "Bacterial and Chemical Carcinogens in Gastric Oncogenesis" R01 CA082312
Principal Investigator: D. Scott Merrell, Ph.D. PI status assumed after the untimely death of Dr. Andre Dubois Agency: NIH
Total Direct Costs:

Period: August 15, 2013 – July 31, 2014 No-cost extension until July 31, 2015 "Regulatory Networks of Helicobacter pylori" 1 R56 AI065529
Principal Investigator: D. Scott Merrell, Ph.D. Agency: NIH
Total Direct Costs:

Period: July1, 2012 – June 30, 2014 No cost extension until June 30, 2015

"Skin and Soft-Tissue Infection in the MRSA Era: Etiology and Humoral Immunity" Principal Investigator: Eric Hall, Ph.D.
Role on Project for D. Scott Merrell, Assistant Principal Investigator Agency: MIDRP-DOD
Total Direct Costs: in year two of the project 07/01/13 – 06/30/14
Supporting Agency: DoD/Uniformed Services University of the Health Sciences
Address and POC of Funding Agency: Jeannienne Paschall, USUHS, Office of Research, 4301 Jones Bridge Road, Bethesda, MD 20814
Period of performance: 07/31/12-12/31/17 (NCE through 2017)
Title: Skin and Soft Tissue Infection in Soldiers, Epidemiology, Treatment and Prevention Brief description of goals: The project investigates microbiome changes associated with SSTI.
Time Commitment/Effort: 1.8 calendar months (15% effort)
List of specific aims:

- Aim 1: Determine the relative abundance and distribution of bacterial species within the external nares and wounds of individuals that develop purulent SSTI.
- Aim 2: Determine the relative abundance and distribution of bacterial species found within nonpurulent SSTI (cellulitis).
- Aim 3: Examine the expression of known virulence genes in the major microbial species identified in the SSTI samples and correlate expression to disease severity.

Supporting Agency: National Institute of Health, NIAID
Address and POC of Funding Agency: Laura Eisenman, NIH, 5601 Fishers Lane, Room 3D10, Rockville, Maryland 20892
Title: Helicobacter pylori CagA toxin polymorphism
Period of performance: 04/01/2016-03/31/2018
Brief description of goals: This R21 investigates the role of CagA toxin polymorphism in H. pylori virulence. **Role:** Principal Investigator
Time Commitment/Effort: 2.4 calendar months (20% effort)
List of specific aims:

- Aim 1: Characterize the role of CagA polymorphism in development of gastric cancer in a Mongolian gerbil model of H. pylori infection.
- Aim 2: Characterize the role of CagA polymorphism on host cell signaling pathways known to be associated with gastric cancer development.

Funding Agency: Naval Medical Research Center / DoD
Address and POC of Funding Agency: Chaselynn Watters, U.S. Army Forest Glen Annex, Silver Spring, Maryland
Period of performance: 08/01/2015-08/31/2019
Title: Wound Infections: Novel Therapeutics, Diagnostics, and Dressings
Brief description of goals: The project investigates microbiome changes associated with wounds and treatment of wounds with phage therapy.
Time Commitment/Effort: 1.2 calendar months (10% effort)
List of specific aims:

- Task 3. Beginning with the WID 5-member phage cocktail that effectively treats A. baumannii infected wounds in mice, WID and USU collaborators will determine the extent to which this phage therapeutic disrupts normal host microbiota.

- Task 4. NMRC WID and USU collaborators will extend microbiota analysis to the additional cocktails to the ESKAPE pathogens that show efficacy in animal models.
- Task 5. NMRC WID will test PDT and any appropriate emergent antibacterial modalities in the appropriate animal models. If efficacious, these studies will then be extended to include microbiota studies.

Overlap:
None

Chou-Zen Giam, Ph.D.
Other Research Support
Current Support:
1R21AI173635-01 **11/14/2022 – 10/31/2023**
PI: Chou-Zen Giam, PhD (20% effort)
Title: HTLV-1 Replication/Reactivation-Induced DNA Damage: Mechanisms and Pathogenesis
Agency: NIH/NIAID **POC:** Eun-Chung Park
Goals: The goal of this proposal is to study the contribution of DNA damage to the HTLV-1 lifecycle and associated disease.
Specific Aims:
- Aim 1: To determine how Tax/NF-kB-induced R-loops impact host cell genome and cell fate.
- Aim 2: To elucidate how ATL cells accommodate chronic NF-kB activation

Prior Support/Completed Funding:
1R21CA216660-01A1 **12/27/17-11/30/21**
PI: Chou-Zen Giam, PhD (30% effort)
Title: "Clonal Expansion of HTLV-1-Infected Cells and Adult T Cell Leukemia"
Agency: NIH/NCI **POC:** Betsy Read-Connole, PhD
Goals: The goal of this proposal is to elucidate the mechanisms underlying the clonal expansion of HTLV-1-infected T cells so as to facilitate the development of ATL treatment.
Specific Aims:
- Aim 1 To determine whether ATL-specific activating (ATLA) mutations facilitate clonal expansion and transformation of HTLV-1-infected T cells.
- Aim 2 To determine the role of Foxp3 in the proliferative expansion of HTLV-1-infected T cells.

W81XWH1810325
PI: Chou-Zen Giam, PhD (10% effort) **08/15/18-08/14/21**
Title: "Helicobacter pylori-Induced DNA Double Strand Breaks and Gastric Cancer"
Agency: DoD/CDMRP **POC:** TBD
Goals: This project seeks to understand how H. pylori-induced DNA damage contributes to cancer formation.
Specific Aims:
- Aim 1: To determine if R-loops are induced by H. pylori infection and where these structures form on the host genome.

- Aim 2: To determine if blocking R-loop accumulation will decrease DNA damage induced by H. pylori.

R073248116
PI: Chou-Zen Giam (5% effort) **10/01/13-02/28/2018 (no cost extension)**
Title: "Genes Involved in Regulating IKK/NF-κB and Senescence" 0.6 calendar months
Agency: USU **POC:** Toya Randolph, PhD
Goals: The goal of this project is to identify hitherto unknown genes involved in IKK/NF-κB regulation, genes or pathways that pre-dispose pre-malignant cells to chronic IKK-NF-κB activation, and genes involved in regulating DNA damage response and apoptosis.
Specific Aims:
- Aim 1: To use insertional mutagenesis to identify genes involved in NF-κB regulation and senescence. HeLa cell lines resistant to Tax-induced senescence will be isolated, characterized, and the sites of retroviral insertion mapped and sequenced.
- Aim 2: To adapt the strategy in Aim 1 to isolate and characterize cellular genes involved in facilitating the deregulation of G1 cyclin-dependent kinases.

MIC-73-4238 **10/1/16-9/30/17**
PI: Chou-Zen Giam, PhD (10% effort)
Title: "Genomic Instability and Adult T-cell Leukemia"
Agency: USU **POC:** Toya Randolph, PhD
Goals: In this study, we seek to elucidate how Tax activates Rnf8, determine whether and how aberrant Rnf8 activation by Tax impacts genomic instability, and assess the role of Rnf8 deficiency in ATL development and treatment.
The proposed specific aims are as follows:
1. To elucidate how Tax activates Rnf8;
2. To determine the biological impact of Rnf8 dysregulation by Tax; and
3. To investigate the causes of Rnf8 deficiency in ATL cells and explore treatment implications of Rnf8 deficiency.

1 RO1 CA115884 (Giam) **07/01/05-02/28/16**
Agency: NIH/NCI **POC:** Betsy Read-Connole, PhD
"HTLV-1 Tax Activates the Anaphase Promoting Complex"
Goals: The goals of this project are;
- To investigate the mechanism by which Tax activates APCCdc20,
- To investigate the biological characteristics of and biochemical basis for the senescence-like state induced by Tax, and
- To investigate the "suppressor" mechanism(s) in HTLV-I transformed cells that allow them to escape the rapid senescence induced by Tax.

5 R01 CA140963 (Giam) **07/01/05-01/31/16**
Agency: NIH/NCI **POC:** Betsy Read-Connole, PhD
"Cell Cycle Regulation and Adult T-Cell Leukemia"

Goals: The goals of this application are

- To delineate the pathway leading from persistent NF-κB activation by Tax to senescence;
- To investigate the cause and biological effects of HTLV- and Tax-induced chromosome instability; and
- To elucidate the mechanisms by which cells become adapted to (transformed by) Tax and HTLV-1.

3 R01 CA140963-02S1 (Supplement) **06/07/11-09/30/14**
Agency: NIH/NCI **POC:** Betsy Read-Connole, PhD
"Cell Cycle Regulation and Adult T-Cell Leukemia" (Supplement)
Goals: The goal of this application is to support the research training of Ms. Adeola Obajemu in areas of viral oncogenesis and genomics.

Overlap:
None

Dr. Galina Petukhova
Other Research Support
Current Support:
TITLE: Evolution of Homologous Recombination Mechanisms
GRANT#: U01GM114173/R01GM114173
PERIOD: 3/17/16 - 3/31/25
EFFORT: 3.6 calendar months
TOTAL COSTS:
The goal of this grant is to elucidate the mechanisms underlying formation of recombination hotspots in mammals that lost the Prdm9 gene. We will map recombination hotspots in three species that lost canonical Prdm9 and examine three specific mechanisms that may define recombination landscape in such species.

Prior Support/Completed Funding:
TITLE: Targeted recombination to pinpoint responsible regions within large susceptibility loci in mice
GRANT#: R21GM135494
PERIOD: 9/20/19 - 8/31/22
EFFORT: 3 calendar months
TOTAL COSTS:
The goal of this project is to develop the approach to artificially induce recombination at defined locations within the susceptibility locus (targeted recombination).

Title of the project: Molecular Mechanisms of Genetic Recombination in Mammals
Funding agency: NIH
Goals of the project: In this study we will take a genome-wide approach to define the mechanisms of crossing-overs (CO) placement in the mouse
Specific aims/tasks:

1. Using chromatin immunoprecipitation followed by direct high-throughput sequencing we

will map the hotspots of meiotic DSBs in the mouse and identify the particular features associated with the hotspot regions. This information is necessary to delineate the mechanism behind hotspot formation.

2. We will map the hotspots of meiotic COs using a similar approach, and carry out a comparison between the hotspots of COs and the hotspots of DSBs. This will allow us to establish whether the sites of DSBs in mammals always coincide with recombination (CO) hot spots, and whether a subset of the DSBs is destined to become COs. Such information will advance our understanding of the pathways leading to CO formation in mammals and the mechanisms involved in the CO/NCO designation.

Start and end date: 5/1/09 – 4/30/17
Level (%) of effort in the project: 50
Project Direct Costs:
Point of contact at the funding agency: Grants Management Specialist: Richard Brundage Email: brundagerc@mail.nih.gov Phone: (301) 594-1805

Title of the project: The Role of Faulty Genetic Recombination in Infertility, Aneuploidy and Birth Defects
Funding agency: March of Dimes Foundation
Goals of the project: We propose to map the preferred sites of homologous recombination (recombination hotspots) in mouse females genome-wide.
Specific aims/tasks:

1. Generation of the recombination hotspot map in female mice
2. Development of medium-throughput approach for mapping female recombination hotspots in the mouse
3. Determination of underlying causes of perinatal and embryonic lethality inprogeny of female mice with reduced recombination activity
4. Study the effect of environmental and genetic factors on the distribution of recombination hotspots in females.

Start and end date: 6/1/13 – 11/30/16
Level (%) of effort in the project: 25
Project Direct Costs:
Point of contact at the funding agency March of Dimes Research and Global Programs, 1275 Mamaroneck Avenue, White Plains, NY 10605,

Title of the project: Mechanisms controlling common DNA fragile sites in human
Funding agency: Uniformed Services University of the Health Sciences (Exploratory Grant)
Goals of the project: In this study we will take a genome-wide approach to address several aspects of DNA fragile site biology in human:
Specific aims/tasks:

1. We will use a novel DSB mapping technique to map DNA fragile sites in human and to determine their characteristic features.
2. We will test the hypothesis that the susceptibility of different DNA fragile sites to replication stress depends on the nature of the damaging agent.
3. We will test the hypothesis that there is a substantial variability in susceptibility of the particular fragile sites to damage between individuals.

Start and end date: 10/1/12 – 9/30/15

Level (%) of effort in the project: 20
Project Direct Costs:
Point of contact at the funding agency: Toya V. Randolph, PhD, MSPH, Assistant Vice President for Research Administration

Dr. C.L Dalgard
Other Research Support
CURRENT AWARDS:

*Title: Whole Genome Sequencing in Ethnically Diverse Cohorts for the ADSP Follow up Study (FUS)- FUS 2.0

*Major Goals: The Alzheimer's Disease Sequencing Project uses a large-scale sequencing effort to increase knowledge about the genetic variation that influences Alzheimer's Disease.
This is a competitive revision application of a funded cooperative agreement that's designed to generate high quality sequence data in this follow up sample.
*Status of Support: Active
Project Number: 5U01AG057659
Name of PD/PI: Margaret Pericak-Vance
*Source of Support: NIH
*Primary Place of Performance: USUHS
Project/Proposal Start and End Date: 09/01/2019 – 03/31/2023
* Total Award Amount:

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2019	0.6
2. 2020	0.6
3. 2021	0.6
4. 2022	0.6
5. 2023	0.6

*Title: Genomic Characterization of Alzheimer Disease Risk in Admixed Populations with Native American and Southern European Genetic Ancestry
*Major Goals: This project will study the genomics of AD in Hispanic populations with Amerindian and southern European ancestry. We will use whole-genome sequencing in these populations together with information about ancestry and ethnicity to identify genetic variation influencing AD and to generalize existing AD genetic discoveries to this underrepresented groups.
*Status of Support: Active
Project Number: R01AG070864
Name of PD/PI: Margaret Pericak-Vance
*Source of Support: NIH
*Primary Place of Performance: USUHS
* Project/Proposal Start and End Date: 04/01/2021 – 01/31/2026

* Total Award Amount:

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2021	0.6
2. 2022	0.6
3. 2023	0.6
4. 2024	0.6
5. 2025	0.6
6. 2026	0.6

*Title: Sequencing of the NIH NeuroBioBank for Centralization of Genomic Data of the Human Brain
*Major Goals: The purpose of this project is to provide genetic data analysis for precision and genomic medicine research and applications with The National Institutes of Health and non Federal collaborators.
*Status of Support: Active
Project Number: HU00012220044
Name of PD/PI: Clifton Dalgard
*Source of Support: NIH
*Primary Place of Performance: USUHS
Project/Proposal Start and End Date: 8/1/2022-7/31/2023
* Total Award Amount:

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	0.6
2. 2023	0.6

*Title: Collaborative Health Initiative Research Program
*Major Goals: This program will characterize genomic determinants for cardiovascular, pulmonary and sleep diseases in US service members from minority ethnic backgrounds. Whole genome profiling at population scale will be performed using genomic core resources established by the program.
*Status of Support: Active
Project Number: NHBLI HU001-14-2-0041
Name of PD/PI: Clifton Dalgard
*Source of Support: DoD
*Primary Place of Performance: USUHS
*Project/Proposal Start and End Date: 09/01/2014 – 03/31/2023
* Total Award Amount:

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2014	6
2. 2015	6
3. 2016	6
4. 2017	6
5. 2018	6
6. 2019	0.6
7. 2020	0.6
8. 2021	0.6
9. 2022	0.6

*Title: Genetics of Frontotemporal Degeneration in Diverse Populations ("Project")
*Major Goals: To identify and characterize genetic factors and networks contributing to FTD in diverse populations. Identification of disease contributing factors in diverse groups will not only help further elucidate underlying biological mechanisms of dementia but will also improve diagnosis and treatment in these groups reducing future health disparities.
*Status of Support: Active
Project Number: W81XWH2110437
Name of PD/PI: Karen Nuytemans
*Source of Support: DoD
*Primary Place of Performance: USUHS
*Project/Proposal Start and End Date: 07/01/2021 – 06/30/2025
* Total Award Amount:
* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2021	0.6
2. 2022	0.6
3. 2023	0.6
4. 2024	0.6
5. 2025	0.6

*Title: Inclusion of sub-group of ASPREE samples into the ADSP– FUSJoey
*Major Goals: ASPREE recently joined the Alzheimer Disease Sequencing Project (ADSP). This supplement requests funds to process these 2,796 samples through GCAD.Specific aim 1 is adjudicate and harmonize clinical data from the two ASPREE datasets to generate high quality inferentially equivalent phenotypes and endophenotypes; Aim 2 is to conduct wholegenome sequencing of 424 high-risk, unaffected samples with longitudinal data ; Aim 3 is to perform

biomarker studies on two separate plasma samples (at enrollment and at 3 year followup) on all 926 high-risk ASPREE samples; aim 4 is to collaborate with NIAGADS, GCAD and the Penn Neurodegeneration Genomics Center (PNGC and HIHG CGESG QC Teams in processing, storage, and delivery of final datasets to NIAGADS for public data release and aim 5 is to harmonize clinical data from newly acquired and existing FUS datasets to generate high quality inferentially equivalent phenotypes and endophenotypes.
Project Number: 3U01AG066767-02S1
Name of PD/PI: Jeffrey Vance
*Source of Support: NIH
*Primary Place of Performance: USUHS
Project/Proposal Start and End Date: 09/30/2021-06/30/2023

* Total Award Amount:

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2021	0.6
2. 2022	0.6
3. 2023	0.6

*Title: Identifying Variants that Increase Risk for Alzheimer's Disease
*Major Goals: The goal of this study is to identify new genes and variants that increase risk for AD, as well as protective factors
Project Number: 2RF1AG044546
Name of PD/PI: Carlos Cruchaga
*Source of Support: NIH
*Primary Place of Performance: USUHS
Project/Proposal Start and End Date: 3/01/2022 – 2/28/2023

* Total Award Amount:

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	0.6
2. 2023	0.6

*Title: The Stanford Extreme Phenotypes in Alzheimer's Disease (StEP AD) Cohort
*Major Goals: The overall goals of the Stanford Extreme Phenotypes in AD (StEP AD) project will be to identify and characterize novel genetic variants that promote resilience to AD pathology in the presence of the APOE4 allele or that drive pathogenesis in the absence of the APOE4 allele.
Project Number: 1R01AG060747
Name of PD/PI: Michael Grecius
*Source of Support: NIH
*Primary Place of Performance: USUHS
Project/Proposal Start and End Date: 09/15/2018 – 05/31/2023

* Total Award Amount:

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2018	0.6
2. 2019	0.6
3. 2020	0.6
4. 2021	0.6
5. 2022	0.6
6. 2023	0.6

*Title: Covid POTS
*Major Goals: The public purpose of this award is to improve basic and applied knowledge about long haul COVID-19 disease and its associated nervous system consequences. The funding is to develop and implement cellular and molecular characterization of long haul COVID-19 disease participants within a clinical trial for Ivabradine responsiveness for reduction of tachycardia. Furthermore, this award will generate a data repository for long haul COVID-19 clinical, translational and molecular data for utilization by the research community. The clinical study infrastructure and capabilities will benefit the public by reporting results and sharing data that will contribute to generalized knowledge about long haul COVID-19 disease consequences, as well as identify and innovate treatment paradigms and therapeutic targets for improving recovery and enhancing health for those affected by long haul COVID-10 disease.
Project Number: HU00012120087
Name of PD/PI: Mark Haigney
*Source of Support: DHA
*Primary Place of Performance: USUHS
Project/Proposal Start and End Date: 9/1/2021-8/31/2025

* Total Award Amount:

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2021	0.6
2. 2022	0.6
3. 2023	0.6
4. 2024	0.6
5. 2025	0.6

*Title: The American Genome Center
*Major Goals: The purpose of this Cooperative Research is to provide genetic and genomic data generation and analysis for precision health and genomic medicine research and applications. These collaborations have the objective of discovering and evaluating genetic influences and factors associated with common complex human phenotypes, disease and refine therapeutic strategies. This

award is being made with continuing goals of improving clinical utility with certification of laboratories for clinical testing and understanding of genetic and molecular factors for cancer predisposition, cancer progression and treatment response, cardiovascular disease, mental illness, dementia and infectious diseases in consideration of ancestrally diverse populations relevant to those enriched by military servicemembers and the general population.
*Status of Support: Active
Project Number: HU0001-18-2-0038 (TAGC 3) / HU0001-222-0014 (TAGC 4)
Name of PD/PI: Clifton Dalgard
*Source of Support: DoD
*Primary Place of Performance: USUHS
Project/Proposal Start and End Date: 6/01/2021 - 05/31/2023 (TAGC 3)/ 09/01/2022-08/31/2023 (TAGC 4)

* Total Award Amount:

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2021	4.56
2. 2022	4.56
3. 2023	4.56

*Title: Whole Genome Sequencing in Ethnically Diverse Cohorts for the ADSP Follow-Up Study (FUS)
*Major Goals: The specific hypothesis is that genetic variation in ethnically underrepresented populations influence and modify Alzheimer's Disease risk and progression.
*Status of Support: Active
Project Number: 5U01AG057659
Name of PD/PI: Margaret Pericak-Vance
*Source of Support: NIH
*Primary Place of Performance: USUHS
Project/Proposal Start and End Date: 09/30/2018 – 08/31/2023

* Total Award Amount:

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2018	0.6
2. 2019	0.6
3. 2020	0.6
4. 2021	0.6
5. 2022	0.6
6. 2023	0.6

Title: Sudden Death Genomics

*Major Goals: This collaborative research will develop and implement genomic evaluation tools for clinically adjudicated selected individuals with unknown etiology for sudden death or common human disease that results in early mortality.
*Status of Support: non-active
Project Number: HU00012120102
Name of PD/PI: Clifton Dalgard
*Source of Support: NIH
*Primary Place of Performance: USUHS
Project/Proposal Start and End Date: 09/07/2021 – 09/26/2024

* Total Award Amount:
* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2021	0.6
2. 2022	0.6
3. 2023	0.6

*Title: Additional Sequencing Cohorts for the Alzheimer's Disease Sequencing Project- FUS Mini Me
*Major Goals: The focus and goal of this project is identifying genetic risk and protective factors for Alzheimer's Disease in an effort to identify new pathways for prevention and new targets for drugs development.
Project Number: 1U01AG062943
Name of PD/PI: Margaret Pericak-Vance
*Source of Support: NIH
*Primary Place of Performance: USUHS
Project/Proposal Start and End Date: 09/01/2019 – 08/31/2023

* Total Award Amount:
* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2019	0.6
2. 2020	0.6
3. 2021	0.6
4. 2022	0.6
5. 2023	0.6

NON-ACTIVE AWARDS:

*Title: Phenotype and Genotype of Autosomal Dominant Alzheimer's Disease in Jalisco, Mexico
*Major Goals: In this project we propose to facilitate research in ADAD in Jaliscostate by providing the technology for research and clinical genetics as well as funding and training to develop a cohort

that will prospectively be followed using standardized clinical and cognitive measures, enabling additional research studies.
Project Number: R01AG069013
Name of PD/PI: John Ringman/Figuera
*Source of Support: NIH
*Primary Place of Performance: USUHS
Project/Proposal Start and End Date: 06/01/2022 – 05/31/2025

* Total Award Amount:

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	0.6
2. 2023	0.6
3. 2024	0.6
4. 2025	0.6

*Title: Alzheimer's Disease Genetics Consortium

*Major Goals: The goal of this study is to completely resolve Alzheimer's disease (AD) genetics by identifying both common and rare variants that cause, alter risk and protect against (AD).
*Status of Support: Active
Project Number: 5U01AG032984
Name of PD/PI: Gerard Schellenberg
*Source of Support: NIH
*Primary Place of Performance: USUHS
Project/Proposal Start and End Date: 04/15/2020 – 03/31/2025

* Total Award Amount:

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2020	0.6
2. 2021	0.6
3. 2022	0.6
4. 2023	0.6
5. 2024	0.6
6. 2025	0.6

*Title: APOE in the Predisposition to, Protection from and Prevention of Alzheimer's Disease
*Major Goals: We propose to establish, use, and extensively share two important resources to advance the study of cognitively unimpaired (CU) persons at six levels of genetic risk for Alzheimer's disease (AD) due to their apolipoprotein E (APOE) genotype, including understudied APOE2 and APOE4 homozygotes (HMs).
Project Number: 1R01AG069453-01

Name of PD/PI: Eric Reiman
*Source of Support: NIH
*Primary Place of Performance: USUHS
Project/Proposal Start and End Date: 4/1/2022 – 3/31/2025
* Total Award Amount:
* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	0.6
2. 2023	0.6
3. 2024	0.6
4. 2025	0.6

*Title: Additional Sequencing for the Alzheimer's Disease Sequencing Project (ADSP)" – FUSaRoo
*Major Goals: Overall goal is to increase the ethnic diversity of the Alzheimer's Disease Sequencing Project.
*Status of Support: Year 3 MOD pending
Project Number: 1U01AG066767
Name of PD/PI: Jeffrey Vance
*Source of Support: NIH
*Primary Place of Performance: USUHS
Project/Proposal Start and End Date: 07/01/2020 – 06/30/2024
* Total Award Amount:
* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2020	0.6
2. 2021	0.6
3. 2022	0.6
4. 2023	0.6
5. 2024	0.6

PENDING SUPPORT:

*Title: Additional Sequencing for the Alzheimer's Disease Sequencing Project (ADSP) the Follow-Up Study (FUS), The Diverse Population Initiative- FUSing Around Again
*Status of Support: Pending
Name of PD/PI: Pericak-Vance
*Source of Support: UM/NIH
*Primary Place of Performance: USUHS

Project/Proposal Start and End Date: 9/1/2022-8/31/2027

* Total Award Amount:

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	0.6
2. 2023	0.6
3. 2024	0.6
4. 2025	0.6
5. 2026	0.6
6. 2027	0.6

*Title: The Countermeasures for Complications of Pandemic Illness (CCOP)
*Status of Support: Pending
Project Number: N/A
Name of PD/PI: John Dumler
*Source of Support: USU
*Primary Place of Performance: USUHS
Project/Proposal Start and End Date: 10/1/2022-9/30/2024

* Total Award Amount:

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	0.6
2. 2023	0.6
3. 2024	0.6

***Overlap** (summarized for each individual):

No overlap.

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: QUAD CHARTS:

9. APPENDICES: