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TITLE: HELICOBACTER PYLORI–INDUCED DNA DOUBLE-STRAND BREAKS AND GASTRIC CANCER

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CONTRACTING ORGANIZATION: THE HENRY M. JACKSON FOUNDATION FOR THE ADVANCEMENT OF MILITARY MEDICINE, INC.

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Helicobacter pylori is a bacterial pathogen that colonizes the human gastric mucosa and contributes to the pathogenesis of gastric cancer. H. pylori infection induces DNA double-strand breaks (DSBs) in gastric epithelial cells, which compromise host cell genomic integrity. While the genotoxicity of H. pylori clearly promotes gastric carcinogenesis in infected individuals, the underlying molecular mechanisms behind this process are not fully understood. In this study, we have shown 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-I-κB, a degradation-resistant mutant of I-κBα (inhibitor of NF-κB), dramatically reduces H. pylori-induced DNA DSBs. We further showed that the H. pylori type IV secretion system and the H. pylori virulence factor, CagA, but not VacA, were required for inducing DNA DSBs in the infected AGS cells. Our results strongly support the notion that H. pylori infection triggers CagA-mediated NF-κB activation, which, in turn, promotes DNA DSBs. Current efforts focus on testing the hypothesis that NF-κB activation by CagA leads to the accumulation of R-loops, leading to DSBs.</td></tr>
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

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:** *Provide a brief list of keywords (limit to 20 words).*

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

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

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	50%
Subtask 3: Illumina sequencing of prepared libraries	17-18	Not Started
Subtask 4: Bioinformatic/data analysis	18-24	Not Started
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	25%
Subtask 3: Data analysis and publication	16-24	25%
Milestone(s) Achieved: Knowledge whether blocking R-loop formation can inhibit DNA damage.	24	

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Results

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

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

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.1A 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 developed DSBs as revealed by the accumulation of γH2Ax (Fig. 1B).

H. pylori infection activates NF-κB and triggers an inflammatory response in the host cell. The inhibitor of NF-κB, I-κBα, 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 I-κBα, 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 I-κBα and I-κBα by western blotting. As shown in Fig. 2A, *H. pylori* infection at MOI_{100} and MOI_{200} increased the levels of phospho-I-κBα (p-I-κBα) with a concomitant decrease in the steady state levels of I-κBα, 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-I-κBα. This vector expresses ΔN-I-κBα, a degradation-resistant mutant of I-κBα deleted for the 36 amino-acid sequence motif in the NH_2 terminus of IκBα that mediates activation-induced I-κBα degradation. ΔN-I-κBα constitutively retains NF-κB in the cytosol and inhibits the transcriptional activity of NF-κB. The LV-ΔN-I-κBα-transduced cells were selected in puromycin (0.5 μg/ml)-containing medium for 7 days and analyzed by immunoblotting for the expression of ΔN-I-κBα as shown in Fig 2B, where the two I-κBα bands in the lysate (lane 2) represent the endogenous wild-type I-κBα and the truncated ΔN-I-κBα. We then infected the parental and pooled ΔN-I-κBα-

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-I-κBα-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 prevented by inhibition of 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-I-κBα-expressing AGS cells with *H. pylori* at MOI_{100} for 8 hrs, and observed them microscopically. Both the parental and the ΔN-I-κBα-expressing AGS cells exhibited the elongated phenotype as shown in Fig. 3, suggesting 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 investigate the role of *H. pylori* virulence factors in inducing DSBs, we infected AGS cells with various mutant strains of *H. pylori* at MOI_{100} and MOI_{200} respectively 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* mutant strains of *H. pylori* efficiently activated NF-κB and induced DDR as revealed by the high levels of p-I-κBα, and γH2Ax and p-Kap1 in the infected cells. By contrast, the Δ*PAI* (type IV secretion system) mutant had only a marginal effect on DNA damage induction. Interestingly, while the Δ*cagA* mutant at MOI_{100} had little impact on NF-κB activation and DDR induction (lane 5), at MOI_{200} NF-κB activation and DDR induction was detected (lane 6); this suggests that *H. pylori* components other than CagA (peptidoglycan?) may also activate NF-κB and trigger DDR when a higher dose of bacteria are utilized for infection. These results indicate that the injection of *H. pylori* CagA (and perhaps peptidoglycan or other PAMP molecules) into the host cells via the type IV secretion system is directly responsible for NF-κB activation and induction of DSBs in AGS cells. The other *H. pylori* virulence factor, VacA, appears to play no detectable role.

(b) Tyrosine phosphorylation of CagA is indispensable for induction of DNA damage.

CagA is tyrosine phosphorylated within the EPIYA (Glu-Pro-Ile-Tyr-Ala) motifs by the members of Src and Abl families of kinases in the host cell. CagA exerts pleiotropic effects on various host cell signaling pathways in both phosphorylation-dependent and -independent manner. To test whether tyrosine phosphorylation of CagA by host cell kinases is required to induce DSBs, we infected AGS cells with a wild-type and a mutant strain of *H. pylori* that contained a deletion of CagA EPIYA region (ΔEPIYA) at MOI_{100} and MOI_{200}. Fig. 4B shows that the expression of EPIYA deleted CagA had minor effects on the levels of γH2Ax compared to the wild-type strain suggesting that tyrosine phosphorylation of CagA is important for NF-κB activation and the attendant DSBs induction in the host cell.

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

(a) . *H. pylori* infection enhances R-loop formation in 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 cause DNA damage by inducing R-loops accumulation during *H. pylori* infection. 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. The binding to the S9.6 antibody was due to RNA-DNA hybrids present in the samples and was lost upon treatment of the nucleic acid samples with RNase H, an enzyme that digests the RNA moiety in the RNA-DNA hybrid. (Fig. 5A lower panel). To further confirm that NF-κB induces R-loops accumulation in the *H. pylori* infected cells, we monitored the nuclear translocation of NF-κB subunits, p65/RelA and Rel-B, in AGS cells at various time points post-infection with wild-type *H. pylori* strain. As shown in Fig. 5B, nuclear p65/RelA and Rel-B levels gradually increased from 0 to 4.5 hrs post-infection and then subsided, showing a correlation between NF-κB activation with R-loop accumulation (Fig 5A).

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).

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

To validated the findings outlined above in an animal model of H. pylori infection and gastric tumor development, Mongolian gerbils were fed with 2x 10^9 *H. pylori* cells and sacrificed after 1, 3, 7, 21, 56 and 84 days. Nucleic acid from the lower part of the Stomach tissue was extracted and analyzed by the South-Western hybridization. As shown in Fig. 6, a significant increase in the levels of R-loops were detected after 7 and 21 days post feeding with *H. pylori*. *In situ* histological detection of *H. pylori* infection in the gastric epithelium is currently ongoing to correlate the presence of CagA in infected gerbil cells with NF-κB activation, R-loop accumulation, and DNA damage.

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 Cag A and PAI 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 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. 7A). Equimolar amounts of the two recombinant enzymes were then added 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 activated in the presence of 0.1M $CaCl_2$ for 3 min. The digested genomic DNA was extracted and analyzed by capillary gel electrophoresis. As shown in Fig. 7 B and C, GST-RHΔ-MNase digested genomic DNA from wild type *H. pylori*-infected AGS cells showed a unique banding pattern compared to the GST-MNase digested genomic DNA. Future work will focus on constructing genomic library and sequence analysis.

Future Directions

Our efforts over the past few months have firmly established the critical role of the type IV secretion system and 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, leading to gastric cancer development. Experiments are in progress to assess (a) whether *H. pylori* infection results in NF-κB-driven R-loop accumulation, and if so, (b) which genomic regions accumulate R-loops upon *H. pylori* infection.

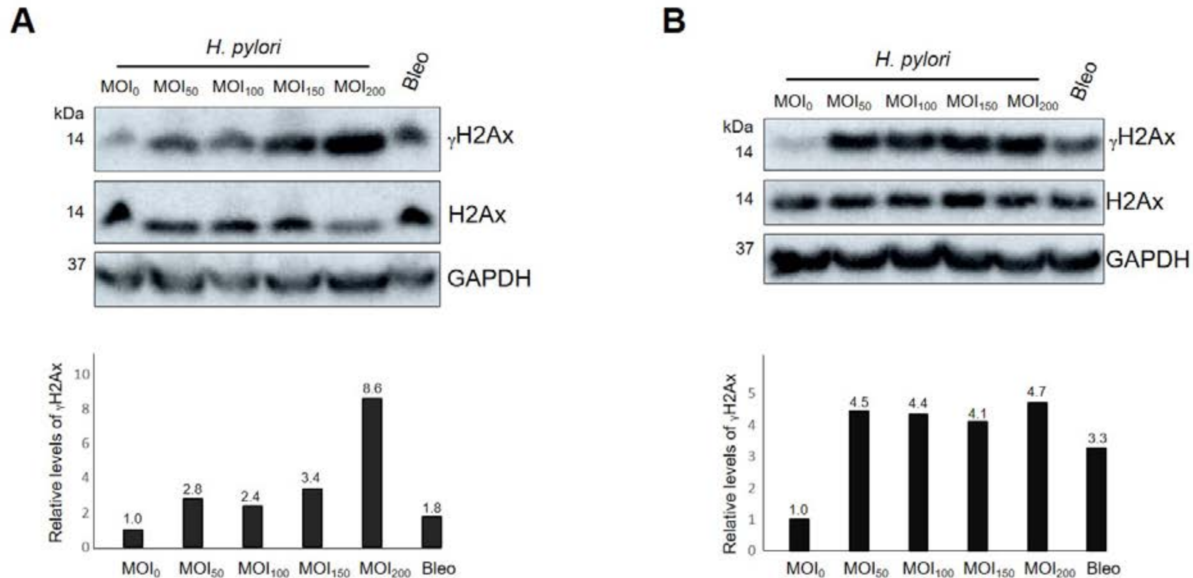

Fig. 1 *H. pylori* infection induces DNA damage in AGS and U2OS cells. (A) AGS cells were infected with *H. pylori* at various MOI (0, 50, 100, 150 and 200) for 8 hrs and DNA damage response was analyzed by immunoblotting with the γH2Ax antibody. (B) U2OS cells were infected with *H. pylori* and analyzed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

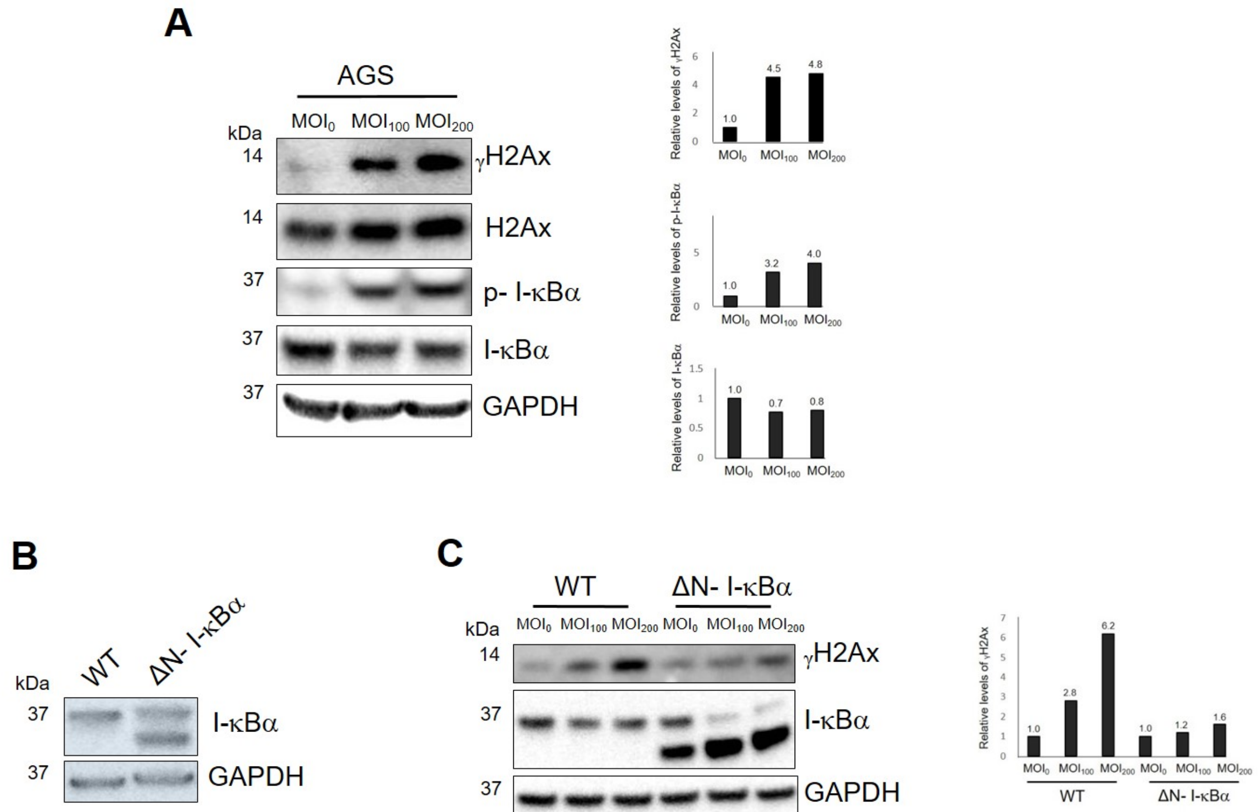

Fig. 2 ***H. pylori*** **infection activates NF-κB in AGS cells:** (A) AGS cells were infected with *H. pylori* at MOI_{100} and MOI_{200} for 8 hrs and whole cell lysate was immunoblotted with the indicated antibodies. (B) AGS cells were transduced with LV-ΔN-I-κBα. Transduced cells were selected in puromycin for 7 days and verified for ΔN-I-κBα expression by immunoblotting. (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.

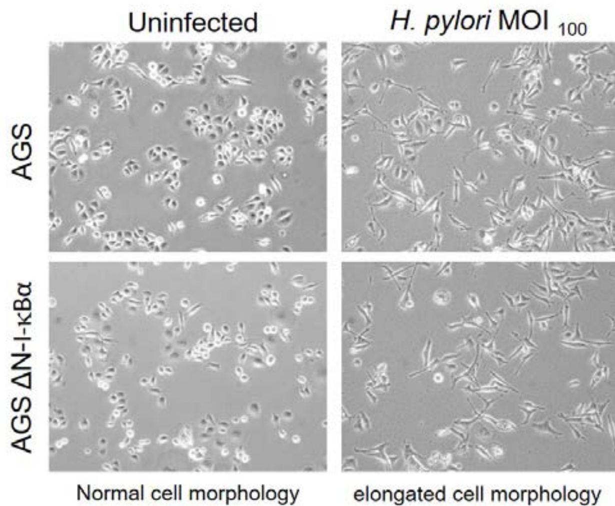

Fig. 3 ***H. pylori*****-induced morphological changes in AGS cells are independent of NF-κB signaling**. AGS WT and ΔN-I-κBα-expressing cells were infected with *H. pylori* at MOI_{100} and photographed 8hrs post-infection.

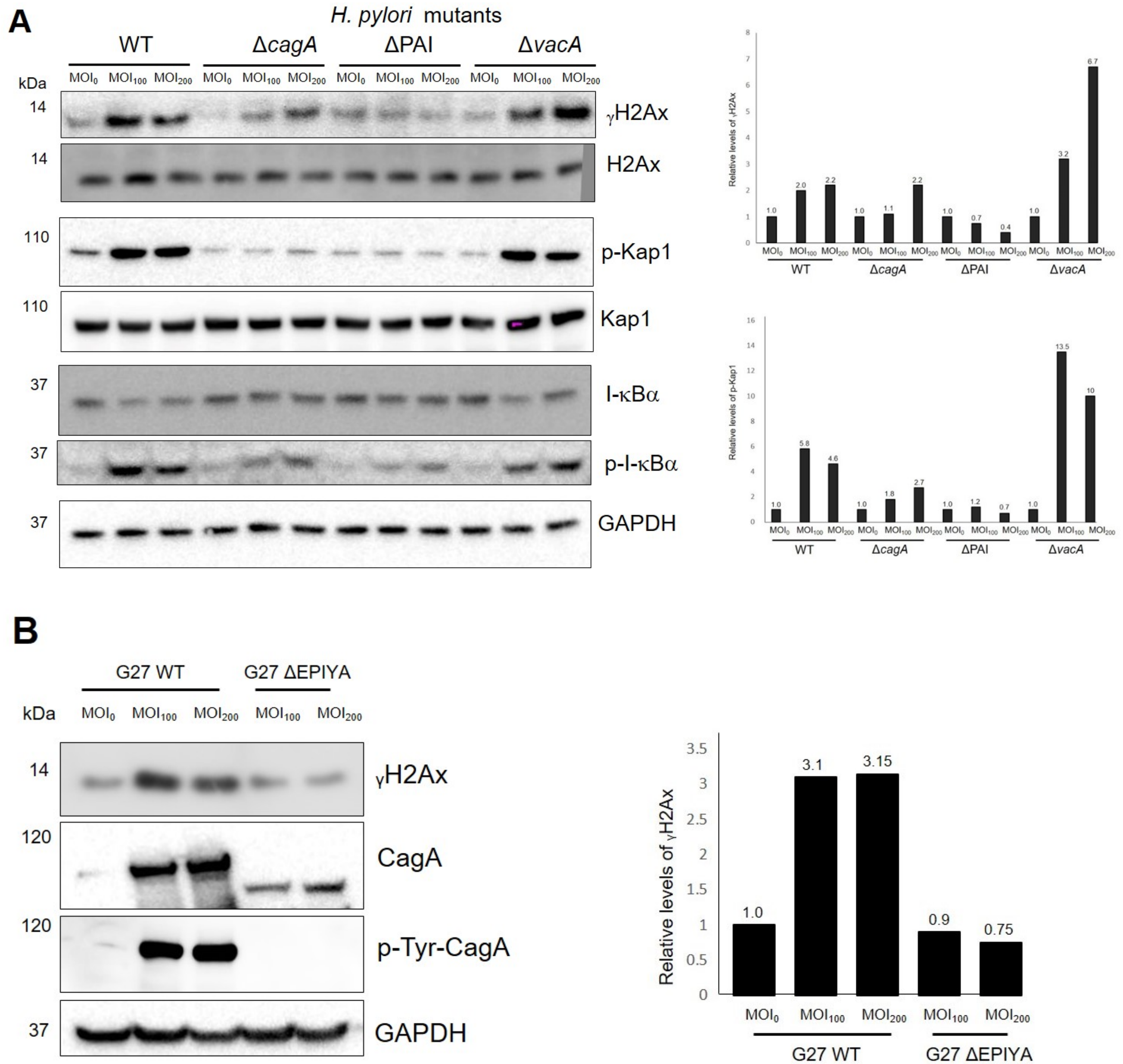

***Fig. 4 H. pylori* type IV secretion system and virulence factor CagA are required for the induction of DNA DSBs in AGS cells** (A) AGS cells were infected with Δ*cagA*, ΔPAI, and Δ*vacA* mutant strains of *H. pylori* at MOI_{100} and MOI_{200} for 8 hrs, and whole cell lysate was analyzed by immunoblotting with the indicated antibodies. (B) AGS cells were infected with wild-type and EPIYA deleted CagA mutant strains of *H. pylori* at MOI_{100} and MOI_{200} for 8 hrs and whole cell lysate was analyzed by immunoblotting. All bands were quantified using ImageJ software and normalized to the loading control.

A

H. pylori MOI$_{200}$

0 3.0 4.5 6.0 hr post-infection

R-DH

(1.0 1.2 1.8 1.1)

NA

RNase H

R-DH

(unquantified)

NA

B

0 3.0 4.5 6.0 8.0 hrs

C N C N C N C N C N

70 Rel-B

70 p65

37 GAPDH (Cytosolic marker)

70 Lamin (Nuclear marker)

C

H. pylori strains MOI$_{200}$ for 4.5 hrs

WT *cagAΔ* *PAIΔ*

R-DH

(1.0 0.63 0.61)

NA

Fig. 5 H. pylori-infection modestly increases R-loop formation in AGS cells (A) AGS cells were infected with wild-type strain of *H. pylori* at MOI$_{200}$ for indicated time-points and total cell nucleic acid was analyzed by South-Western blotting with the S9.6 antibody. (B) AGS cells were infected with wild-type *H. pylori* strain for various time-points and sub-cellular factions were analyzed by immunoblotting with indicated antibodies. (C) AGS cells were infected with wild-type, *ΔcagA* and *ΔPAI* mutant *H. pylori* strains at MOI$_{200}$ for 4.5 hrs and total nucleic acid was analyzed by South-Western blotting with the S9.6 antibody.

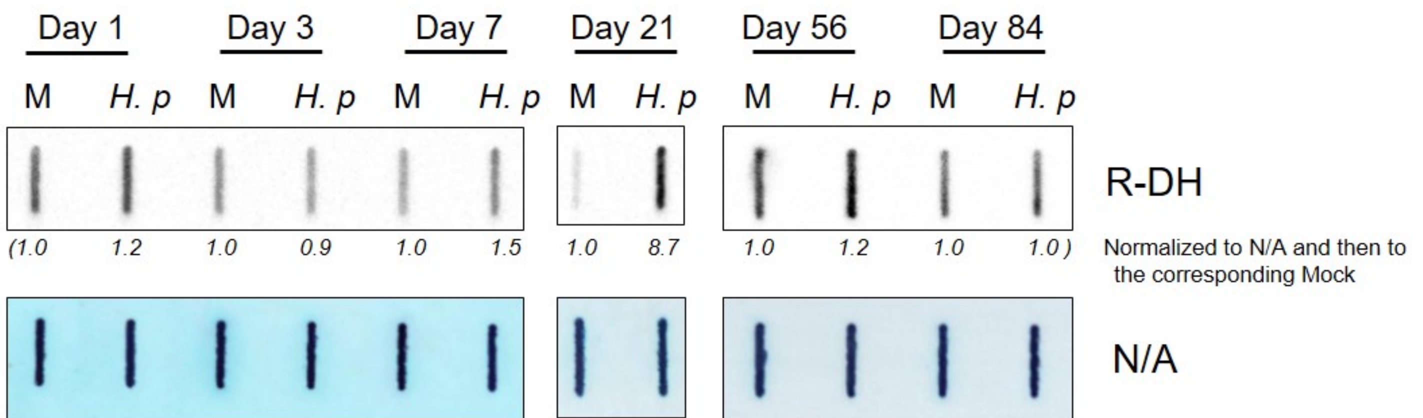

Fig. 6 R-loop formation increases in Mongolian gerbils **after** ***7 and 21 days of H. pylori infection***. Mongolian gerbils were fed with 2x10^9 *H. pylori* cells and sacrificed at indicated time points. Nucleic acids were extracted from the lower part of the Stomach tissue and analyzed as in (Fig. 5A).

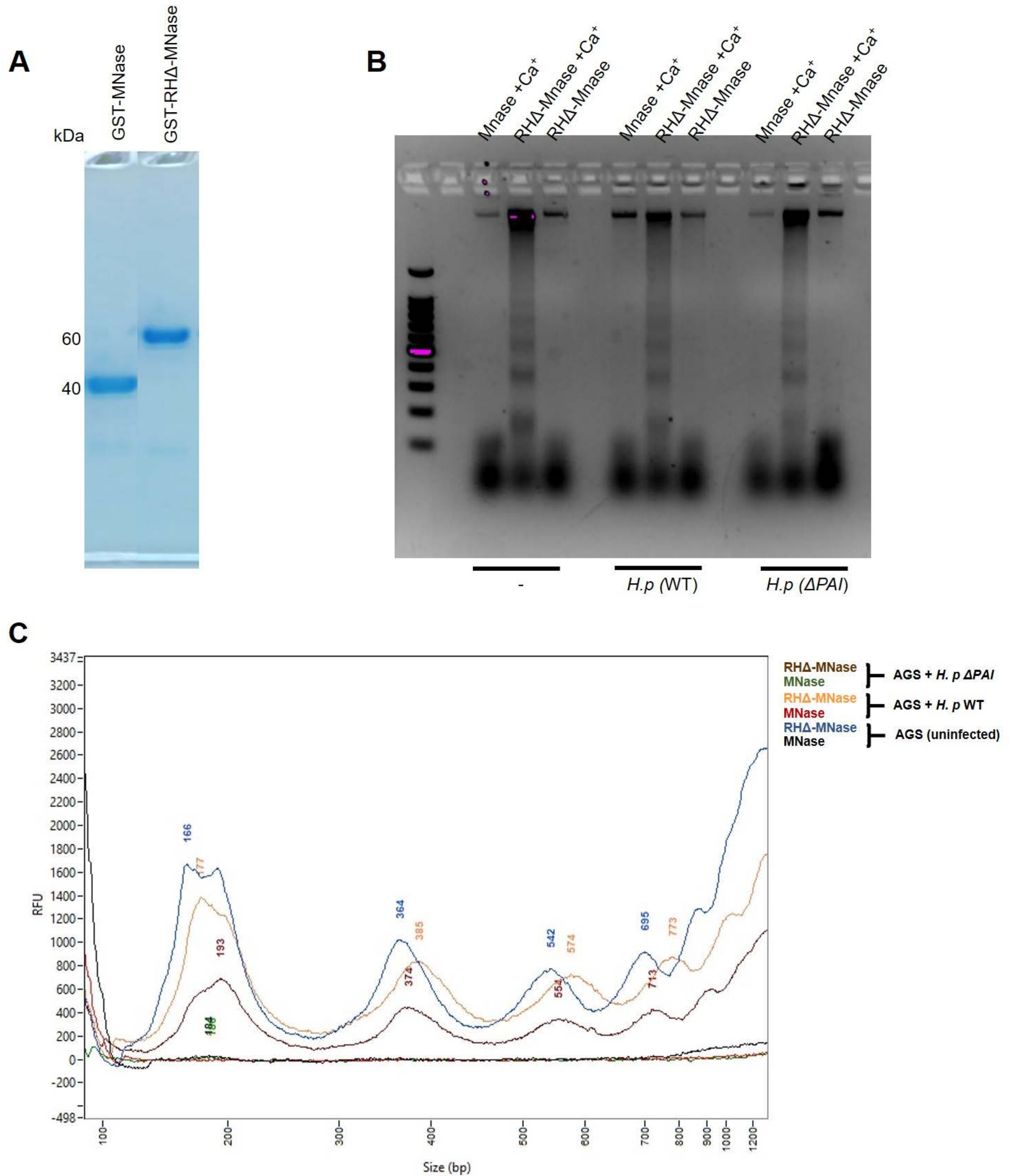

Fig. 7 Mapping R-loops in the genome of H. pylori infected AGS cells. (A) Purification of recombinant enzymes GST-MNase and GST-RHΔ-MNase from *E.coli.* (B & C) 2% Agarose Gel Electrophoresis and Capillary Gel Electrophoresis analysis of Micrococcal Nuclease digested genomic DNA of AGS cells.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the next reporting period all of the remaining tasks under Aim 1, Major Task 2 and Aim 2 will be completed as described in the original proposal.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Our current data clearly indicate that *H. pylori* infection induces the formation of DNA damage and R-loops. 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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report at this stage.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

We've experienced several delays in execution of the items we expected to complete during year one and two. The delays in year one were related to delays in hiring of personnel and time needed to construct additional bacterial strains. The delays in year two were due to COVID-19; our laboratories were completely closed for months and then operated at reduced capacity for an extended time. We were grateful for the cost-extension that was provided as a way to move the project forward. Given the cost extension the original timing delays are not expected to result in any significant changes to the project or its direction.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Originally we experienced a short delay in recruiting suitable personnel to the project, which has delayed the execution of some of the tasks. These individuals were recruited and work was back on track until COVID-19. Now that we are reopened, we have resumed work and will continue with execution of the experiments as described in the original proposal. The only significant change will be to the timing of completion of the studies.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

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 were 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
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee

(or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*

- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

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 interpretatioin 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: 8.41
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: Garima Bansal
Project Role: Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 7.73
Contribution to Project: Dr. Bansal is a new Postdoctoral Fellow that works on the project within Dr. Merrell's laboratory. She worked 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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

D. Scott Merrell, Ph.D.
Other Research Support
Current Support:
Supporting Agency: Uniformed Services University of The Health Sciences/ MIDRP
Address and POC of Funding Agency: 4301 Jones Bridge Rd, Bethesda, MD 20814, Rita Holliday
Period of performance: 08/01/2021– 09/30/2023
Title: A Novel Antimicrobial to Prevent and Treat Staphylococcus Aureus-Associated Infections
Brief description of goals: This Study Will: 1)Identify the C. Pseudodiphtheriticum Genes responsible for the secreted Bactericidal Factor(S), 2) Purify And Identify the secreted factor(s) using Chromatographic Purification and Mass Spectrometry, and 3) Test the efficacy of the Identified Factor(S) as a S. Aureus Therapeutic using a Colonization and Abscess Model. **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: 06/21/2021– 05/31/2023
Title: Contribution of Helicobacter pylori HomA and HomB to colonization and disease
Brief description of goals: The goal of this project is to investigate the biological function and role of the HomA and HomB outer membrane proteins in bacterial adaptation, adherence to host cells, colonization and development of gastric cancer.
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: Uniformed Services University of The Health Sciences/ MIDRP
Address and POC of Funding Agency: 4301 Jones Bridge Rd, Bethesda, MD 20814, Rita Holliday
Period of performance: 02/01/2021– 09/30/2022
Title: Directed Approach for the Human Monoclonal Antibody Platform (HMAP) Against Surface Targets Required Virulence in Gram-negative Bacteria
Brief description of goals: The goal of this project is to develop, and test monoclonal antibodies directed to specific targets on two pathogens, Acinetobacter baumannii and Klebsiella pneumoniae.
Time Commitment/Effort: 0.6 calendar months (5% effort)
List of specific aims:
- Aim 1: Clone up to 10 gene targets from *Acinetobacter baumannii* and Klebsiella pneumoniae into the pGEX-6P-1 vector. Resulting constructs will be moved into E. coli BL21 plysS and then recovered for sequencing verification.
- Aim 2: Use the constructs described in Major Task 1 to express small concentrations of protein that correlate with each of the cloned genes. Said proteins will then be purified via the tag provided in the pGEX-6P-1 vector. Proteins will then be transferred to WRAIR.
- Aim 3: Use antibodies generated at WRAIR to treat infected Galleria to determine whether the generated antibodies can increase survival of these caterpillars.

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 – 04/30/2022
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 – 08/14/22
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.

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 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:
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.

Prior Support/Completed Funding:
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 of the project: Evolution of Homologous recombination mechanisms
Funding agency: NIH
Goals of the project: to unravel the mechanisms that affect recombination efficiency and distribution in mammals, the factors that may interfere with recombination progression, and the mechanisms involved in mammalian speciation.
Specific aims/tasks:
1. We will employ our ChIP/seq-based approach25 to build high-resolution genome-wide maps of DSB hotspots in two mammals that lack Prdm9: the dog and the short- tailed opossum. We will compare these maps to the maps we recently generated for mice7 and

human10 to determine the common features of DSB hotspots and their distributions in animals that have and that lack PRDM9, as well as their differences.
2. We will generate two mouse models to determine the biological function of the KRAB domain of PRDM9. These will include (a) a line expressing the truncated version of PRDM9 restricted to the N-terminal part of PRDM9 without the DNA binding domain (the form that is found in opossums) and (b) the line expressing the full length PRDM9 with a mutant KRAB domain. We will evaluate both models with respect to distribution of PRDM9-dependent H3K4me3 marks, the ability to initiate homologous recombination, the distribution of DSB hotspots, and the ability to complete recombination

Start and end date: 03/17/2016 – 02/28/2021
Level (%) of effort in the project: 50
Project Direct Costs:
Point of contact at the funding agency: Grants Management Specialist: Romeo Tengey;
Email: tengeyr@mail.nih.gov Phone: (301) 594-5144

Title of the project: Targeted recombination to pinpoint responsible regions within large susceptibility loci in mice
Funding agency: NIH/NIGMS
Goals of the project: The goal of this project is to develop the approach to artificially induce recombination at defined locations within the susceptibility locus (targeted recombination).
Specific aims/tasks:

1. To generate the mouse strain (*Mei-Cas9*) that expresses the *Cas9* gene specifically in meiosis, where recombination takes place. The double stranded DNA breaks generated by Cas9 are expected to be repaired by the homologous recombination mechanism, because non-homologous end joining DNA repair pathway is not functional in meiosis.
2. To generate the mouse strains expressing (i) one or (ii) three guide RNAs to target Cas9 to four specific sites within a specific locus.
3. To evaluate the efficiency of targeted recombination in the mice expressing both *Cas9* and guide RNAs.

Start and end date: 9/1/19 – 8/31/21
Level (%) of effort in the project: 25
Project Direct Costs:
Point of contact at the funding agency: Paul J Sammak

<u>Pending Support</u>
Title of the project: Mechanisms of homologous recombination
Funding agency: NSF
Goals of the project: To evaluate recombination landscape in species that lost *Prdm9.*
Specific aims/tasks:

1. To determine whether recombination outside hotspots is common in species that lost *Prdm9*
2. To confirm the lack of recombination hotspots in opossums
3. To determine the mechanism for lack of hotspots in opossums

Start and end date: 3/1/20 – 2/28/24
Level (%) of effort in the project: 25
Project Direct Costs
Point of contact at the funding agency: TBD

<u>Prior Support/Completed Funding:</u>
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, (914) 997-4488

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 Support:
5 U01 AG057659 (Pericak-Vance) 9/30/2017-8/31/22 0.60 calendar
National Institute of Aging
Whole Genome Sequencing in Ethnically Diverse Cohorts for the ADSP Follow-Up Study (FUS)

- Goal 1. Increase the ethnic diversity of the Alzheimer's disease Sequencing Project (ADSP) by assembling samples from existing cohorts with African-American and Hispanic LOAD cases and controls as well as expand our Non-Hispanic White dataset using autopsy cases and controls from the Alzheimer's disease Centers (ADC). Adjudicate and harmonize all data to consistently determine AD Diagnosis. Cohorts include MHAS, REGARDS, NOMAS, the Puerto Rican 1066 Study, PRADI, REAAADI, EFIGA, MESA and ADC.
- Goal 2. Collaborate with the National Cell Repository for Alzheimer's disease (NCRAD) in assembling DNA on these existing cohorts. This will serve as a central resource for the Alzheimer's disease research community. To accomplish this we will collect, perform quality control checks, store and allocate DNA for genome-wide SNP array genotyping using the Illumina Global Screening Array (GSA) to the HIHG and for WGS of all individuals to the Uniformed Services University of the Health Sciences (USUHS).
- Goal 3. Generate genome-wide SNP array data and whole genome sequencing data for all collected samples using established resources. Goal 4. Collaborate with the NIA Genetics of Alzheimer's Disease Data Storage Site (NIAGADS) and the Genome Center for Alzheimer's disease (GCAD) in processing, quality control, storage, and make the final datasets available for qualified access. This includes working with GCAD in genotype calling, quality control, and harmonization of all array and sequencing data and collaborating with NIAGADS in the storage of phenotype, genotype and sequence data and in the transfer of the final datasets.

1 R01 AG061837 (Lee) 9/1/2018-8/31/23 0.60 calendar
National Institute of Aging
Identification of protective factors for cognitive resilience in adults with Down Syndrome: A multi-omic study We will:

1. Characterize older, non-demented vs. the remaining cohort, particularly younger, demented adults with DS to generate a metabolomic profile of successful cognitive aging;
2. Identify proteins that distinguish older non-demented adults with DS from the remaining cohort, particularly younger, demented adults to generate proteomic profiles of successful cognitive aging;
3. Identify genetic variants or genes that are associated with protective metabolomic and proteomic profiles identified from Aims 1 and 2, and then perform data-driven multi-omic analysis; (4) Using the protective factors identified from Aims 1-3, we will examine the

associations between protective biomarker profiles and neuropsychological and functional performance scores. Once protective factors identified, we will confirm our findings using independent, external DS and non-DS cohorts.

1 R01 AG058918 (Lee) 8/15/18-3/31/23 0.60 calendar
National Institute of Aging
Protective genetic factors of Alzheimer disease in PSEN1 Mutation Carriers in Puerto Rico. We will:

1. conduct a WGS study to identify novel variants;
2. characterize the relations between novel modifier variants and AAO or memory through examination of transcriptomic and external genomic data of EOAD and LOAD; and
3. investigate the mechanisms of action of modifiers of PSEN1 using human fibroblast and iPSC-derived neuronal cultures. If successful, it may be possible to identify a potential therapeutic target(s) that might delay or prevent AD or maintain healthy cognition.

AC16OM07 (Beachkofsky) 2/1/17-6/30/20 0.60 calendar
Air Force Medical Support Agency
Comprehensive Analysis of Severe Cutaneous Adverse Drug Reactions
The project goal is to identify candidate genetic biomarkers of cutaneous drug reactions. The aim is to perform comprehensive genomic analysis of whole genome sequencing and epigenetic analysis to associate with adverse drug reactions from potential SJS/TEN phenotypes

DM160477 (Browning) 9/1/2017-9/30/21 0.60 calendar
Congressional Medically Directed Research Program
Genomics of Early Lung Cancer Among Military Personnel (GELCAMP)
The project goal is to develop clinical protocol, establish iMedidata RAVE and operational & administrative workflow. We are testing for genomic biomarkers for the detection of early lung cancer using whole genome sequencing of DNA specimens from blood, endobronchial brushings and lung tumor specimens (for those with lung cancer). The DECAMP cohort is the only cohort that specifically enrolls military personnel and offers the potential to identify mutations that might be linked to military specific exposures.

1 R01 AG060747 (Grecius) 9/15/18-5/31/23 0.60 calendar
National Institute of Aging
The Stanford Extreme Phenotypes in Alzheimer's Disease (StEP AD) Cohort

- In Aim 1 the research team will look for rare genetic variants seen more often in protected APOE4 carriers than in AD patients.
- In Aim 2 the team will look for rare genetic variants seen in APOE4-negative EOAD patients but not in healthy older controls. Most of the StEP AD cohort will undergo "deep phenotyping" to include structural and molecular brain imaging, spinal fluid analysis, immunophenotyping, and culturing of participant-specific neurons.
- In Aim 3, the deep phenotyping data will be used to begin to understand the molecular effects of the rare protective or causal genetic variants identified in Aims 1 and 2.

1 UG3 NS103870 (Rademakers) 09/25/2017-08/31/2022. 0.60 calendar

National Institutes on Aging
Whole Genome Sequencing Consortium on Frontotemporal Dementia with Underlying TDP-43 Pathology.
The goal of this UG3/UH3 is to bridge a knowledge gap between the establishment of an FTLD-TDP sequencing consortium and the identification and functional validation of novel FTLD disease genes. The overarching hypotheses is that new FTLD-TDP candidate genes will lead to disruption of neuronal homeostasis through effects on proteostasis and/or RNA metabolism. This study will be the first of its kind and is now possible through the availability of whole genome sequencing (WGS) technologies and unparalleled cohorts of well-characterized patients.

Pending Support:
National Institute of Aging (Vardarajan) 4/1/19-3/31/24 0.60 calendar
Genetic Etiology of late-onset Alzheimer's disease in multi-ethnic families
The major goal of this proposal is to analyze sequencing data from ~1000 families of non-Hispanic white, Hispanic and African American ancestry to identify variants and genes that confer risk of late-onset Alzheimer's Disease (LOAD).

Overlap:
The laboratory tasks of genomic profiling in 5 U01 AG057659, 1 R01 AG058918, 1 R01 AG060747 and the current application do not have overlapping performance. However, the development and validation the genomics core workflows from 5 U01 AG057659 are foundational for the proposed work in this application.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*