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TITLE:	Genome-Wide Association Study in Acquired Aplastic Anemia
PRINCIPAL INVESTIGATOR:	Dr. Neal Young
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14. ABSTRACT

Aplastic Anemia (AA) is a previously universally fatal disease characterized by a hypocellular bone marrow and pancytopenia. AA incidence is skewed across the globe, being 2-3 fold higher in Asia than in Europe. In a variety of studies, chemical exposures and infections in different geographic areas have been described as risk factors for AA, but inconsistently, based on small sample sizes, and at prevalence too low to account for large geographic differences. Genome wide association study GWAS is a powerful research method that compiles genetic variants from many individuals to investigate the association of common genetic markers with a disease phenotype. It has led to the discovery of important new genes and associations in a number of medical conditions.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

DNA samples of AA patients from United States/Europe (n=840) and Asia (n=900) will be genotyped on the GSA v3 Illumina chip in the National Institutes of Health, USA. The new data scan will undergo meta-analysis with existing data from American (n=802), UK (n=665), and Korean populations (n=131) to identify major differences in allele frequencies by ancestry. Experimental follow up will be performed on potentially identified loci in order to investigate novel biological mechanisms.
This is a pioneering international multi-ethnical GWAS study that aims to investigate still unexplained higher AA incidence in those of Asian ancestry. As the largest and most ethnically diverse GWAS in AA we aim to have sufficient power to reveal new traits that account for the heterogeneity and give insight into the genomic landscape of AA
Immune AA is a complex disease in which the pathophysiology is still not completely understood. By unraveling the genetics of AA through the unbiased approach of GWAS, we expect to identify novel mechanisms that underlie AA and potentially reveal new targets for therapies.

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

Aplastic Anemia, Hematology

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: Genome Wide Association Study in Aplastic Anemia
Specific Aim 2: Discovery of novel common variants associated with increased AA risk and etiology in different populations.
Specific Aim 3: Meta-analysis to increase statistical power to detect common variants
Specific Aim 4: Post-GWAS experiments to validate identified risk loci with translational potential

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.

Aim 1: The project protocol has been written and reviewed by the Institutional Review Board of the National Institutes of Health as well as the HPRO. It has been determined non-human subject research. Additionally, a proforma has been devised in order to allow for collection of clinical information to send with de-identified samples.

The project was also presented and passed at the local clinical genetics branch for scientific validity approval.

An internal funding CAN for $95,000 has been established for to allow us to pay for shipping and sequencing costs internal to NIH.

All NIH samples (n=600) have been prepared for the GWAS. DNA extraction and quality control has been performed and samples are now stored and awaiting sequencing. All samples have been listed on a spreadsheet, de-identified, and associated with relevant clinical information per a standard proforma.

A Research Cooperative Agreement has been prepared by the NIH Office of Technology Transfer. So far this been signed by the following collaborating institutions: Kanazawa University (Japan), Nagoya University (Japan), Chonnam University (Korea), and National Institute of Hematology – Blood Transfusion (Vietnam). Other collaborators we are still awaiting their institutional ethical approval to allow them to sign. Multiple correspondences have occurred between NIH and the collaborating institutions in order to trouble shoot delays in local ethical approval. Locally, collaborators are preparing samples via de-identification and association with clinical information per proforma. One test sample has successfully been sent from Nagoya University and others are pending from signatories shortly.

Given the delays in sample ethical approval in some institutions, the NIH office of tech transfer has elected to honor each signature as an independent agreement between institutions so we will not need to wait for all signatories for the agreement to be valid – this will allow us to imminently start shipping samples from those collaborators who have completed the agreement.

We hope to complete aim 1 in early/mid 2022 – this will involve transfer of all de-identified DNA to the National Cancer Institute to be run on the GWAS chip. Aims 2-4 will depend on the successful completion of aim 1.

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

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

Our main goal will be to start shipping samples – this cannot occur until the agreement is fully executed by all parties, and as a result shipping has been delayed. Institutions outstanding expect their ethical approval by winter and we are aiming to ship samples this winter to allow for GWAS sequencing this spring.

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).

Nothing to report

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

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:*

Nothing to report

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.

We have encountered a significant delay in sample shipment because each collaborator must get independent ethical approval from their institutions to allow for sample shipment and this has taken a significant period for many institutions. Many of these delays are due to COVID as many of our collaborators were unable to prioritize getting ethical approval for this project due to onerous clinical duties resource issues. Additionally, all of our collaborators are international and there is a great deal of variability in getting ethical approval in each country. Due to the nature of our research agreement, samples initially could not be shipped until the agreement was fully executed by all parties. After meeting with out tech transfer office 1 month ago, we have decided to execute the agreements individually between us and each collaborating institution to allow for sample shipment and other institutions have a timeline in place for approval in early 2022.

However, the GWAS sequencing cannot be performed until all samples are collected as they must be batched together to avoid assay variability. Therefore, a delay has occurred in the SOW which originally predicted genotyping would start between 6-8 months and now likely to occur in early spring 2022 (delay of 6+ months). Due to shifting the completion of aim 1 to Spring 2022, aims 2-4 will also be shifted as they depend on the results of aim 1. Due to these shifts in time it is possible we will need a NCE.

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.

Nothing to report

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: *Emma Groarke*
Project Role: *Co-Investigator*
Researcher Identifier (e.g. ORCID ID): *0000-0002-4648-5926*
Nearest person month worked: *6*

Contribution to Project: *Dr. Groarke has authored the study protocol, obtained approval from the NIH IRB and HPRO, obtained clinical data for 600 NIH samples, coordinated with NCI regarding study design and sample specifications, acted as a coordinator with all international collaborators to allow for local ethical approval and signing of research agreements, and liaised with Geneva regarding all required documentation.*

Name: *Fernanda Gutierrez-Rodrigues*
Project Role: *Co-Investigator*
Researcher Identifier (e.g. ORCID ID): *0000-0003-3116-4588*
Nearest person month worked: *6*

Contribution to Project: *Dr. Gutierrez-Rodrigues has coordinated the processing of samples within the lab including sample selection, sorting, and DNA extraction of all 600 samples .*

Name: *Neal S. Young*
Project Role: *PI*

Contribution to Project: *Dr. Young has provided oversight for the project, was involved in the study design and editing of the protocol, and his lab and staff have carried out processing of samples.*
.
Name: *Sharon Savage*
Project Role: *Collaborator*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *0.5*

Contribution to Project: *Dr. Savage has been coordinating things in regards to the technical aspects of sample preparation for the GWAS as well as organizing local genomics committee within the National Cancer Institute authorization of the project to go ahead.*

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.

One of the collaborators (Chinese Academy of Medical Sciences) sought ethical approval for sample transfer and were denied and therefore have had to cease being collaborators. Given the numbers of other samples from Asia, after discussion we do not feel this should greatly impact the eventual study results.

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.*

Kanazawa University, Japan – collaborators who are providing samples
Chonnam National University, South Korea - collaborators who are providing samples + controls
King's College London, UK - collaborators who are providing samples
Hospital Saint-Louis, France - collaborators who are providing samples
Universita Degli Studi Di Napoli Federico II, Italy - collaborators who are providing samples
RIKEN, Japan - collaborators who are providing control data
National Institute of Haematology – Blood Transfusion, Vietnam - collaborators who are providing samples
Nagoya University, Japan - collaborators who are providing samples
St. James's Hospital, Dublin, Ireland - collaborators who are providing samples

8. SPECIAL REPORTING REQUIREMENTS

Award Chart- See Attached

9. APPENDICES: *N/A*