SUMMARY STATEMENT

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(Privileged Communication) ***Release Date:*** **12/02/2015**

Application Number: **1 U01 HG009082-01**

Principal Investigators (Listed Alphabetically):
DING, LI PHD
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LEE, CHARLES PHD (Contact)

Applicant Organization: JACKSON LABORATORY

Review Group: **ZHG1 HGR-L (J1)**
National Human Genome Research Institute Special Emphasis Panel
Analysis Centers

Meeting Date: **11/04/2015** ***RFA/PA:*** **HG15-026**
Council: **JAN 2016** ***PCC:*** **X3AF**
Requested Start: **03/01/2016**

Project Title: **The Jackson Laboratory Center for Structural Variation Analysis**

SRG Action: **Impact Score: 36**
Next Steps: **Visit http://grants.nih.gov/grants/next_steps.htm**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **10-No live vertebrate animals involved for competing appl.**

Project Year	Direct Costs Requested	Estimated Total Cost
1	625,854	1,033,306
2	635,961	1,049,993
3	653,226	1,078,498
4	671,222	1,108,210
TOTAL	2,586,263	4,270,007

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

1U01HG009082-01 LEE, CHARLES

RESUME AND SUMMARY OF DISCUSSION: This application was submitted in response RFA-HG-15-026 "NHGRI Genome Sequencing Program Analysis Centers (U01)". The RFA invited applications that will undertake computational analyses of the data produced in the NHGRI Genome Sequencing program (GSP). Applications must describe novel, investigator-initiated analyses that will cut across the GSP projects and also undertake cross-program analyses to create common controls and standards for the GSP.

The application entitled "The Jackson Laboratory Center for Structural Variation Analysis" submitted by Drs. Charles Lee (Jackson Laboratory), Ding Li (Washington University at St. Louis) and Mark Gerstein (Yale Univ.), outlines a plan to serve as a GSP Analysis Center through identification of structural variants (SVs) and their functional impact on disease biology while also developing methods for defining metrics and common controls for future common disease studies. The application proposes to accomplish this overall goal through three aims: 1) Build an integrative pipeline for large-scale discovery of complex structural variation; 2) Develop tools to analyze the functional impact of SVs; and 3) Association of structural variants with common and rare diseases.

The Special Emphasis Panel (SEP) expressed measured enthusiasm for this application. They noted the strengths of this research team. The investigators have extensive expertise and experience in structural variation detection, computational genomics and computational tool development. The panel agreed that the proposed work had the potential for high significance since additional approaches for structural variation analysis are needed to advance the overall field.

However, the SEP had a number of concerns. First, the panel noted that the application was not well developed and did not present a cohesive plan across the three aims. Second, the panelists agreed that the proposed work was somewhat narrowly focused on structural variant analysis and common disease. On a related point, the work did not take into account the approach or samples needed for structural variant analysis in Mendelian disease studies. Third, the SEP noted that the application relied on the use of existing methods and that there was a lack of innovation in the proposed methods. Finally, the panel was concerned there was not a coordinated plan with the GSP centers as some of the proposed activities appear to be redundant with the activities in the GSP centers. Further, the application did not include a well developed common controls plan.

In summary, the SEP expressed measured enthusiasm for this application. The SEP noted the expertise of the research team and the proposal to develop additional approaches to the analysis of structural variants. The panel was concerned that the application did not present a cohesive plan which would result in the proposed outcome. Further, the panel expressed concern that this application would not address the needs of the GSP program. These concerns notably tempered the panel's enthusiasm for this application.

The comments in the CRITIQUE section were prepared by the reviewers assigned to this application and are provided without significant editing by staff. The critiques may or may not have been revised by reviewers following discussion at the review. The RESUME AND SUMMARY OF DISCUSSION documents the overall conclusions of the committee and the basis for the assigned priority score.

DESCRIPTION (provided by applicant): Structural variations (SVs), such as deletions, duplications, inversions and translocations, are among the most significant determinants of human genetic diversity to have been discovered, affecting far more bases than single-nucleotide variants in the genome. The Genome Sequencing Program (GSP) offers an exciting new opportunity to mine whole genome and

exome sequencing datasets from a large cohort of individuals for novel SV discovery, analysis and association with common and Mendelian diseases. However, SVs are inadequately covered by current computational discovery methods, making it likely that a large proportion of variants associated with human disease remain unidentified or poorly characterized. The overarching objectives of THE JACKSON LABORATORY CENTER FOR STRUCTURAL VARIATION ANALYSIS (JAX CSVA) are to i) discover SVs at high resolution and large scale, ii) functionally interpret SV origin and phenotypic effects and iii) associate specific (and rare) SVs with disease. Led by Charles Lee, Ph.D., the JAX CSVA brings together a team of pioneers with a proven record of collaboration and innovation in the field of SV discovery, genotyping and large-scale functional genome analysis. The JAX CSVA will develop and integrate novel tools for high- resolution SV discovery and, together with the primary data-producing centers of the GSP, use these to comprehensively profile all types of SVs, including complex SVs, in a large subset of the genomes being sequenced (Aim 1). To examine the functional impact of the identified SVs, we will develop cutting-edge methodologies for functional annotation of variants and characterization of associated biological processes (Aim 2), which will also enable us to prioritize subsets of SVs for association studies proposed in Aim 3. Finally, we will scale up SV detection and analysis through genotyping of all SVs detected in Aim 1 across the 200K samples of the GSP, which will provide the necessary statistical power for meaningful genotype-phenotype associations for disease-based SV association studies (Aim 3). The investigator-led component of the Center will focus on identifying SVs and their functional impact on disease biology, through which program-driven goals, i.e., methods for defining metrics and controls for future common disease studies, will be achieved. Our deliverables will be the largest library of validated SVs discovered in humans, together with an unprecedented and broadly applicable platform of pipelines for comprehensive, high-resolution and large-scale SV analysis. The JAX CSVA will leverage the extensive computational, bioinformatics and IT infrastructure at JAX and, owing to its focus on genomic analyses that cut across individual GSP projects, will be an important link among the data-producing, –analysis and coordination centers of the broader GSP.

PUBLIC HEALTH RELEVANCE The underlying mechanisms of many of humanity's most challenging diseases are linked to specific and oftentimes complex alterations to an individual's genome. THE JACKSON LABORATORY CENTER FOR STRUCTURAL VARIATION ANALYSIS (JAX CSVA) will develop computational resources and tools for discovering one type of common yet powerful genomic variation, known as structural variation, and for associating specific structural variants with disease. The analytical tools and resources we develop will be broadly applicable to studies of the genomic mechanisms of disease, towards the ultimate goal of improving human health.

CRITIQUE 1:

Significance: 2
Investigator(s): 1
Innovation: 2
Approach: 3
Environment: 1

Overall impact:
The proposed analysis center would focus on identifying, validating, wide-scale calling and analysis of structural variants (SVs), which includes deletions, duplications, inversions and translocations. The first aim would focus on a subset of ~10K NHGRI GSP samples for the discovery of SVs, including complex SVs, using tools that they've already developed as well as novel approaches. They then plan to use breakpoint assembly methods to perform *in silico* validation of the identified SVs. Aim 2 focuses on the development and application of novel approaches for functional annotation and biologic process

characterization of the identified SVs. The activities in this aim will also prioritize SVs to association analysis (including the annotations of weights for burden tests. Aim 3 will focus on calling the SVs identified in the subset of ~10K in the rest of the ~190K GSP samples and performing association analysis across relevant phenotypes. Pipelines related to SV calling, annotation, data integration and analysis will be created as part of the proposed study, and annotation of SVs will be integrated into VCF files for all GSP investigators, as well as outside researchers, to use. The proposed project would be complementary to SNP-based association analysis and has the strong potential to bring added value to all of the samples included in the GSP. The proposal has a comprehensive approach and it seems to have a high likelihood of producing data and tools that will be of wide interest to GSP investigators and the research community. Approaches developed as part of this project will be applicable to additional projects. The investigators propose an approach to select the initial discovery set of samples based on availability of multiple "omic" types of data and/or a range of measured phenotypic variables. The actual approach used should probably be discussed across the larger project to get additional input on design. The one drawback that I see is that it isn't responsive to the second overall goal stated in the RFA: i.e. that the analysis centers "will work together with the other GSP components on cross-program analyses that are directly relevant to goals defined in the Companion Funding Opportunities, which currently include defining the point at which a common disease/rare variant genome sequencing study is "comprehensive" or complete, and developing specifications for sample sets that could serve as common controls for common disease genome sequencing studies."

1. Significance:

- The proposed research has the potential for high impact by increasing the validity of structural variant calls and developing improved analytic approaches that can be used by the overall project.

2. Investigators:

- The investigative team (from 3 institutions) is highly experienced and very appropriate for the proposed work.

3. Innovation:

- There are numerous innovative aspects of the proposed study, including: expanding the types of structural variants that can be competently called; identifying structural variants in the largest sample of high coverage whole genome sequences to date; creating novel methods and tools to perform analysis of structural variants; taking a more comprehensive approach to associating structural variants with phenotypes; and utilizing weights that integrate outside information in constructing "burden" tests.

4. Approach:

- The approach describes appears to be appropriate and comprehensive for addressing structural variation in the NHGRI GSP, going from large scale discovery to establishing a pipeline for analysis and integrating structural variant information into VCF files, which can be used by investigators across the overall project. The only drawbacks that I see, with respect to the RFA that this proposal is responding to is that the proposed study would be not be contributing to the RFA goals of (1.) helping to create a common control panel for the project, as they discuss controls sets for specific tests but not in the sense of a panel that could potentially be used as a single common control panel for many outcomes (both by GSP investigators and outside researchers); and (2.) helping to determine when individual GSP projects are complete with respect to how many samples should be sequenced. This, however, is not a major concern, as I feel that the proposed study focused on issues related to structural variation will address a major issue that is currently inadequately addressed and that the proposed approach is highly appropriate for this purpose.

5. Environment:

- The investigative environment is outstanding and appropriate for the proposed research.

CRITIQUE 2:

Significance: 2
Investigator(s): 1
Innovation: 3
Approach: 2
Environment: 1

Overall Impact:
Structural variants (SV) form an under-represented class of variants that has received lesser attention in disease association studies, due primarily to the complexity of detecting and modeling them computationally. The team will make a highly significant contribution to this area while leveraging the GSP data, by developing a cloud accessible database of validated structural variants complete with methods for discovery and analysis, annotated with functional information and disease association, and validated by genotyping. The team is uniquely qualified to undertake this effort. The methods build on previous pioneering work and a strong record of developing methods for SV discovery and functional analysis, and the database and storage capabilities at Jackson Lab. The only drawback is the relatively low degree of innovation, as reflected in the presentation. Overall, this is a strong proposal that would contribute unique capabilities to the GSP in an under-represented area of variation, and therefore the impact is high.

1. Significance:
Strengths

- A database of validated SVs and methods to annotate them functionally will fill in a significant gap in the repertoire of human variation and in our knowledge of its association with disease
- The associated discovery and annotation pipelines and metrics will be shared with the consortium and will help establish standards and best practices for SV analysis

Weaknesses

- It is unclear to what extent SVs modulate common diseases

2. Investigator(s):
Strengths

- A very strong investigative team, bringing together experts and domain leaders in structural variation detection (Lee, JAX), functional annotation (Gerstein), sequencing (Weinstock), genetics and disease association experts
- The team has a long collaborative record and extensive expertise in managing very large projects

Weaknesses

- None noted

3. Innovation:
Strengths

- The project will produce new integrative models and tools for the detection of structural variants (iASV), combining read depth, paired end and split read-based tools, as well as a new integrative method (SVIM) to assess the functional impact of variants by incorporating genome annotations, feature conservation, and epigenetic and expression data from previous consortium-led projects

Weaknesses

- While it is not clear from the description how integration will be performed, it appears that most of the methods are already largely developed and will be applied here

4. Approach:

Strengths

- The team has already developed an impressive array of methods for SV detection, origin and functional characterization, and for analyzing a variety of NGS sequence data types (CHiP-seq regulatory analyses, allele-specific expression), which will provide a solid foundation for the proposed efforts
- Exquisite attention was given to experimental conditions, including sample selection, power calculations, implementation of cloud services for scalability and data protection, scaling up to intermediate and then full-size (200,000 genomes) data. The judicious project and resource planning will ensure that this large scale project can be brought to completion and will produce meaningful results.
- Aim 1 will use the vast repertoire of methods already developed by the PIs and applied in the 1000 Genomes Project to detect structural variants from WGS data at nucleotide resolution, and will use breakpoint sequence assembly to validate SVs in silico. These methods are tried and true.
- Aim 2 will combine regulatory information from NSG-powered projects (ENCODE, Epigenome Roadmap, 1000 Genomes, GTex) with genomic annotations of conserved and functional regions to assess the functional and disease impact of variants in diverse regions (coding, non-coding RNA, regulatory regions, and in the context of network and allele-specific expression. Most of these tools here have already been developed as well.
- Aim 3 will extend disease association methods (both burden tests and variant component tests) by incorporating population stratification, impact scores from functional annotation, and collapsing at gene and regulatory region levels, as needed.
- Alternate plans to use existing CCDG (Center for Common Disease genomics) and CMG (Center for Mendelian Genomics) generated data for de novo SV calling, while waiting for the GSP data, will ensure the project will meet its milestones
- Recent update: The cloud-based architecture is already in place to house the database and operations on data, a unique resource

Weaknesses

- The integrative methods are not sufficiently described, so it is hard to assess their capabilities and novelty

5. Environment:

Strengths

- The database and structural variant pipelines will be stored and operated from the computing facilities at the new Jackson Lab Genomic Medicine Center, which houses 1,700 cores and 1.4 PB of storage. These facilities are more than adequate for the computational tasks. Co-PIs' computing and research environments at WUST and Yale are also well matched to the project's needs.
- The cloud-based environment is a unique resource that JAX-GM brings to the GSP project and the larger community

Weaknesses

- None noted

Resource Sharing Plans:

Acceptable

Budget and Period of Support:
Recommend as Requested

CRITIQUE 3:

Significance: 6
Investigator(s): 1
Innovation: 6
Approach: 6
Environment: 3

Overall Impact:
Drs. Lee, Gerstein, and Ding propose to discover SVs with a multi-method analysis pipeline in 10K genomes, integrate genomic annotations of genes and regulatory effects into an SV impact score, and genotype the discovered and scored SVs across 200K samples to discover associations between SVs and disease risk. In a recent update, they suggest that discovery in more than 10K samples may be feasible. The investigators have a substantial track record in each main area of the proposal. However, there are major weaknesses that limit the overall impact of the proposed work. First, there are likely to be considerable redundancies in time, effort, and compute costs with SV identification efforts from each internal CMG or CCDG study, and it's unclear how much marginal value would be generated here beyond those individual CMG/CCDG efforts. Second, a focus on genotyping SVs >0.1% MAF means that many very rare variants will be missed, including singletons in the large majority of samples not subject to the discovery step. These data are essential, particularly for Mendelian disease analyses. This concern is attenuated but not eliminated with the recent update suggesting more comprehensive (i.e., in more than 10K samples) SV discovery may be feasible. Third, the lack of SV calling in WES data leaves a lot of current and planned CMG/CCDG data and potential discovery opportunities left untapped. Fourth, minimal conceptual innovations in SV discovery are proposed in an area where such innovations are desperately needed. Heuristic integration of SV calls from a diverse set of methods, as proposed here, is the standard approach but one that is sub-optimal in many ways and would ideally be replaced by fewer, more elegant and innovative methods in the future. Finally, there is minimal cross-site work proposed and thus the benefit of the somewhat complicated structure -- 3-PIs separated across 3 physically distant sites is unclear.

1. Significance:
Strengths
- Discovery of SVs is difficult and yet important to overall success of any sequence-based disease genetic study
- Plan to integrate SV callers into a standard pipeline may improve success rates of individual studies, particularly for smaller sample groups

Weaknesses
- Given a focus on common SV discovery and genotyping, proposal will have less impact on Mendelian disease studies, who will need complete SV discovery in each genome to discover all rare variants, including (especially) singletons
- Except for citation to previous methods, no mention of analyzing WES data; there are already 100s of thousands of exomes (e.g., ExAC), more continually being generated, and many planned, thus, more and better SV calls from WES data could provide both an immediate and ongoing resource of great value
- Individual CMGs and CCDGs have or will almost certainly implement SV pipelines, leading to redundancies of time/effort and compute costs; especially for large CCDG efforts that will involve uniform processing of tens of thousands of samples, it is unclear how much additional benefit this proposal will add to what will already be produced

2. Investigator(s):
Strengths
- Investigators have outstanding records in SV discovery, disease analyses, and large project management and coordination

Weaknesses
- None noted

3. Innovation:
Strengths
- Groups have historical records of novel methods development that is relevant

Weaknesses
- No major conceptual approaches or methods are proposed for SV identification, with an emphasis on integrating calls from a mix of pre-existing software, essentially replicating at larger scale 1000G work from Drs. Lee and Gerstein
- No mention or inclusion of new software or hardware solutions to accelerate compute-intensive genomic analysis steps and thereby remove bottlenecks that prevent complete SV analysis across all samples
- Claimed innovation strengths are not well connected across aims; for example, nucleotide level resolution of SVs is emphasized in Aim 1, but no mention is made of special annotation approaches or methods to leverage such precision in Aim 2, and Aim 3 will explicitly bin events using simple overlap criteria (e.g., 80% reciprocal overlap or breakpoints +/- 1kb), rendering precision in breakpoints largely irrelevant

4. Approach:
Strengths
- Effort to uniformly call SVs across a large cohort
- Integration of SVs with regulatory element and transcript data

Weaknesses
- No exome analysis proposed
- Unclear that compute demands, especially in light of dramatically accelerating compute resources specifically for genome compute-intensive steps (e.g., alignment and variant calling on DRAGEN hardware), are truly insurmountable for SV discovery in all samples.
- Lack of de novo SV discovery in vast majority of samples, and focus on variants seen in at least 3 individual carriers, will lead to missed discovery opportunities to evaluate very rare SVs, which are in turn likely to exhibit distinct biological properties and are essential to Mendelian disease studies.
- Special considerations, caveats, or advantages to studying complex and/or multi-allelic events at a given site are not considered in either of Aims 2 or 3.
- Difficulties in accurately estimating impact of diverse SV types and sizes (spanning several orders of magnitude) seem under-estimated.

5. Environment:
Strengths
- All 3 sites are individually well-equipped for genome-scale data analyses

Weaknesses
- Physically distant 3-site structure with minimal cross-site plans

Inclusion of Women, Minorities and Children:
G4A - Gender Unknown, Acceptable

M4A - Minority Representation Unknown, Acceptable
C4A - Children Representation Unknown, Acceptable

Resource Sharing Plans:
Acceptable

Budget and Period of Support:
Recommend as Requested

CRITIQUE 4:

Significance: 2
Investigator(s): 2
Innovation: 3
Approach: 3
Environment: 2

Overall Impact:
This proposal is narrowly focused on the detection of structural variation. The proponents at Jackson Labs are ideally suited to develop and maintain this pipeline. This would be a valuable pipeline to have for computational genomics elsewhere particularly if programmatic interoperability is well supported. As far as this reviewer can tell from the proposal itself, the original approach did not contemplate its use as a SaaS as this would be desirable in an API ecosystem/economy. However, their approach has changed since the original submission, as documented by the additional update material. This changes the flavor of the proposal and increased the enthusiasm of this reviewer.

1. Significance:
Strengths
- The ability to engage a structural variant pipeline from any genomic computing infrastructure is a very desirable feature in for clinical genomics applications

Weaknesses
- The realization of this strength depends critically on the convenience of the engagement, i.e. on the degree of programmatic interoperability. The updated material suggests this has since been realized by the proponents and it is suggested that, if selected for funding, the proponents submit and amended proposal.

2. Investigator(s):
Strengths
- This is an outstanding team as regards the core pursuit of establishing a structural variation pipeline.

Weaknesses
- The lesser focus put on interoperability is reflected by the team composition. This may require an amended research plan and resource allocation.

3. Innovation:
Strengths
- The orchestration of resources at Jackson Labs and at the Gernstein lab at Yale is innovative.

Weaknesses
- The strong innovative component in structure variation workflows was improved by an equally innovative approach to its program, which was supplied in the update. See related comments in the other sections.

4. Approach:
Strengths

- Solid track record and resources developed by the proponents offers ample assurances regarding the effectiveness and quality of the proposed structural variation pipeline. Reliance on cloud computing to deliver scalable services is also a particular strength of the proposal.

Weaknesses

- The narrow focus of the computational constructs on structural annotation missed a complementary effort in interoperability in the original proposal. This problem was addressed in principle by the updated material but this needs to be reflected in the research plan and resource allocation.

5. Environment:
Strengths

- Outstandingly suited for pursuing the structure variation pipeline goal

Weaknesses

- None noted

Additional Comments to Applicant (Optional):
THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

MULTIPLE PI PLAN: Adequate

RESOURCE SHARING PLAN: Adequate

HUMAN SUBJECTS: Adequate

LMP

MEETING ROSTER

National Human Genome Research Institute Special Emphasis Panel
NATIONAL HUMAN GENOME RESEARCH INSTITUTE
Analysis Centers
ZHG1 HGR-L (J1) 1
November 04, 2015

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Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.