The 1st Review Committee of Experimental Animals (A)
Evaluation and Suggestions

(January 22, 2019)

Division/Team Name: Next Generation Human Disease Model Team
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1. Achievements and plans for the Team

（1） Have the current achievements reached the standards of those made by the major international bioresource centers?

- At the former post, the PI built a solid track record in the research on the control of gene expression. Achievements have reached the standards of research projects in international bioresource centers.
- As this Team has only just been launched, evaluation of performance as a BRC project will depend upon the achievements of its future activities.
- The PI is viewed as having enough capability to produce satisfactory research results tied to the Next Generation Human Disease Model Team. The PI is further assessed as having sufficient ability to advance the bioresource center's projects, judging from following achievements in the previous position: 1) developmental genetics, 2) the study of gene regulation by non-coding regions, 3) the functional analysis of regulatory variants at the *Shh* locus, 4) genomics analysis, and 5) proposal of the threshold model of inactivation of multiple enhancers for multifactorial diseases.

（2） Have sufficient achievements been made for contributing to society and to the research community within Japan and overseas?

- The PI’s notion that study of function of non-coding variants is necessary for comprehensive understanding of the cause of human disease is important for the disease research community. Aiming to achieve the precision medicine and its optimization, the Team has already begun joint research with disease genome analysis researchers and clinicians. The Team has begun generating human disease models by introducing point mutations using the CRISPR/Cas9 genome editing.
- This is a newly established team. However, going forward, the committee hope that the Team will produce results.

（3） Are current activities and plans based on the results of the 3rd Mid- to Long-Term Plan or the achievements in the previous position? Are they in line with the BRC's 4th Mid- to Long-Term Plan (7 years from 2018 to 2024)? Are they appropriate and do they contribute to the development of the center?

- As the BRC's 4th Mid- to Long-Term Plan sets out a policy for the active development of human disease models based on the development of genome editing techniques, the action plan of the Team is in accordance with the plan of the center.
- The following endeavors are also commendable: 1) the Team's focus on explaining the function of non-coding variants observed in human disease, 2) the generation of knock-in alleles using genome editing, and 3) detailed planning for functional analyses of regulatory elements.
- The Team made the following detailed plans "toward the realization of personalized and precision medicine": 1) functional validation of Mendelian genetic diseases, 2) functional analysis of regulatory variants, and 3) the development of complex mouse models necessary to elucidate the mechanisms of multifactor disease onset. These are important to the center's projects, and require efforts in collaboration with the International Mouse Phenotype Consortium (IMPC) and public databases. These efforts allow phenotype analysis of mice from the fetal stage to old age, and are expected to contribute to the center's future development.
- A research group in Johns Hopkins University has also been working intensely on enhancer-variant analysis of human *RET* in Hirschsprung's disease. Thus, considering this competition, it is necessary to demonstrate the superiority and significance of the Team's endeavors.
- Introduction and development of experimental animal models which reflect the diversity of human populations is useful to elucidate pathogenesis. Nevertheless, more careful planning, taking into account their usability as wide-ranging resources, is necessary. For example, complete replication of multifactorial inheritance, similar to that in humans, may then require complex breeding to obtain mice with a phenotype. These disease model mice may be difficult to use in the development of treatment. Disease model mice should be developed not only to elucidate etiology, but also to develop treatments of the diseases.

（4） What are resources to be developed and research/ technological development to be undertaken in addition to those currently planned in the initial 4th Mid- to Long-term Plan?

- The Team is planning to generate model mice for intractable diseases designated by the Ministry of Health, Labor, and Welfare in Japan. This is viewed as a good plan, considering social needs, the number of predicted users of the mice, etc. On the other hand, there is a plan adopting JF1 mice as a reference strain. Although the importance of this particular plan can be fully understood, the basis for estimating the extent of its use is not explained sufficiently. If not used, there is a high probability that evaluation will be low. Therefore, a preliminary study prior to conducting full-scale research using JF1 may be necessary. In addition, because making of multifactorial

disease models are laborious, it is necessary to limit the number of diseases to be investigated.

- In order to achieve precision medicine for human disease, the Team is planning to use JF1, which is derived from *Mus musculus molossinus* subspecies originated in Japan and East Asia, as an experimental animal to reflect the diversity of human populations. This can be evaluated as an appropriate plan, as the center maintains dozens of *molossinus*-derived strains as well as the know-how of reproductive engineering for this strain.
- Attempts to identify modifier factors by analyzing gene expression networks using JF1 strain in addition to B6 are of great academic interest. However, it is very difficult to consider that two subspecies can mimic the diversity of human populations. Thus, this must be presented in a way that does not cause misunderstanding.
- Instead of making JF1 mutants, perhaps the Team should examine the phenotype in F1 hybrid mice between JF1 and B6 mutant mice. It seems a priority to show JF1's utility by introducing known disease mutations into JF1 background, and comparing of phenotype data of the JF1 mutants with those of B6 mice possessing the same mutation.
- For the development of next-generation human disease models, projects can be set up using a comprehensive perspective which spans various levels. The use of different technologies, such as chromosomal engineering, is also recommended.
- Even with the genome editing technique, the generation of knock-in mice with long insert fragments is complicated. To overcome this problem, development of technologies to lower this hurdle may be necessary.

2. SWOT analysis

（1） Are the results of the presented SWOT analysis valid?

- It is a reasonable analysis. A weakness is a lack of manpower at this point.
- Based on achievements in the former work, and considering the Team's mission as set out in the 4th Mid- to Long-Term Plan, the results of the SWOT analysis are adequate.

（2） Are the countermeasures for the results of the SWOT analysis appropriate?

- The Team is currently recruiting new members and hopes to hire good ones. Along with the hiring of new staff (which facilitates the progress of joint research), it is recommended that collaborative research should be actively pursued within RIKEN to reduce the Team's burden.
- Balance is needed between resource development vs. research on technology development.
- It is necessary to strengthen mathematical and statistical modelling power to utilize big data in order to develop new disease models. The center ought to provide sufficient support in this regard.

3. International collaboration

（1） Is the international exchange being actively addressed, and is the Team functioning as a hub of international science and technology?

- The PI has been actively participating in international collaboration such as the IMPC and the Asian Mouse Mutagenesis Resource Association (AMMRA).
- The Team has only just been founded, and the Team looks forward to future activities. Establishing linkages to international researchers in disease genome analysis is particularly important in empathizing this Team's presence.
- The Team can be commended for a number of initiatives, including: 1) participating in the IMPC for KO mouse generation, 2) cooperating in international phenotype analysis (as well as making efforts to share and internationally standardize methods of phenotype analysis), 3) making efforts to collect disease model information while engaging discussions among model animal researchers, 4) participating in AMMRA, and 5) making efforts to generate model animals with Japanese-specific variants.

4. PI assessment

（1） Is the PI fulfilling the role in line with the BRC mission?

- The PI has only just been assigned. However, he understands the BRC’s mission well, and is setting forth plans. Considering detailed needs for the disease models, trajectory corrections of the plans may be required.
- Since the development of model mice reflects the needs of clinical researchers, it is commendable that the Team has made active efforts to communicate with clinical researchers and gather information. Moreover, the Team is striving to achieve personalized medicine and precision medicine by recapturing patients’ genotypes into mouse models. Lastly, they are attempting to use mouse models to evaluate diseases for which a definitive diagnosis cannot be made.
- Based on social needs, the Team selected Alzheimer's disease, frontotemporal lobar degeneration, Hirschsprung's disease, and branchio-oto-renal syndrome as target diseases.
- The PI's strength in cis-regulatory elements is also important. However, in order to achieve maximum results with limited funds, they should first focus on development of novel resources.
- Considering the time needed for manpower development and team establishment, it is necessary to emphasize efficiency by clarifying the priority levels of their many plans.
- Collaboration with researchers in the field of disease genome analysis is essential. First, it is important to strengthen collaboration by producing results. To this end, it is initially necessary to focus on introducing powerful disease-inducing point mutations. Doing so will help establish a system to advance efforts toward challenging matters, such as multifactorial diseases, from a

long-term perspective.

（2） Do the PI's achievements in research and development (R&D) satisfy international standards in light of the following three aspects? (i) Results output and impact, (ii) Contribution to specific missions of each laboratory regarding research support and collaborative exchange programs within RIKEN, (iii) Pioneering new fields of research, acquisition, and commercialization of intellectual property rights, social education for science, the fusion of different fields, and social contribution

- The PI satisfies the three perspectives (i), (ii), and (iii) simultaneously. The plan will also satisfy international standards if it is implemented.
- Because the PI has only just been appointed, evaluation of these items should be done based on future activities and results.
- The Team is collaborating with clinical experts on issues, and actively promoting such cooperation since it is essential for generation of human disease model animals. The Team is striving for collection and sharing of information regarding genomic medicine. Moreover, in order to open up new fields of research, the Team is working on the development and maintenance of new reference mouse strains.
- Why not begin a genome editing support service using JF1?

（3） Is the PI appropriately tackling the management and operation of the Team? In addition, does the PI make efforts for training and development of young talent?

- As the PI has just been appointed, selection of staff for the generation of mouse models and phenotype analysis are now in process. The committee hope for the appropriate personnel to be brought onboard.
- Establishment of a phenotyping platform to evaluate phenotype is important in disease model development. On this point, it can be appropriate that the Team is to strengthen collaborations with external clinical experts.

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