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Reproducibility and Research Integrity

Admitting its shortcomings is the foundation of reliable research

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AMED “International Journals Project”

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Introduction

Science and technology have made remarkable advances as the result of the cumulative effort of many researchers. Many erroneous hypotheses have been proposed and, at times, widely accepted. These errors were due mainly to inappropriate methods, insufficient data, or mistaken data interpretations. The faulty hypotheses were subsequently corrected either by their original proponents or by other scientists. In the long run, research in science and technology progresses through such self-correction.

Unfortunately, but inevitably, substantial resources are often required for the self-correction process, and this is increasingly so at present. The rate of progress today is markedly faster. This, in turn, has prompted the swift growth of investment in research. Over the last half century, for example, the budget of the U.S. National Institutes of Health normalized for GDP doubled,[1] while the investment in research by private industry likewise increased substantially, with the latter being more than double the former in biomedical research. In concert with this trend, research institutions have taken their own measures to promote research by placing greater emphasis on the publication record of researchers in recruitment and promotion. This, in turn, has prompted researchers to seek more grants that enable them to publish more papers in more widely recognized journals.

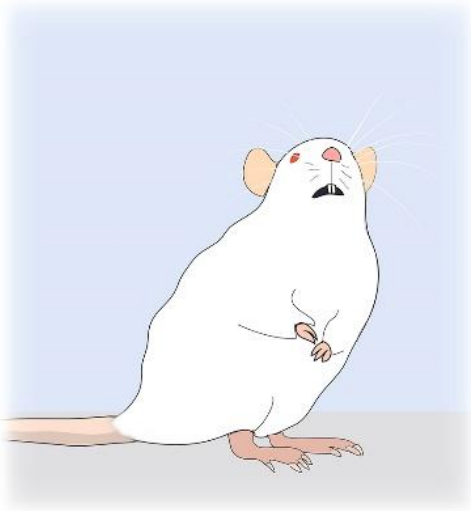
As great amounts of resources are invested in rapidly expanding research, substantial portions are devoted to the process of testing published hypotheses and correcting those found to be incorrect. This is costly but unavoidable, as throughout the history of science most published hypotheses have ultimately proven to be incorrect. Most incorrect hypotheses were the result of honest errors by investigators. However it is unforgivable if the erroneous hypothesis was the result of research misconduct rather than honest error. Recently, another serious issue has also come to the attention of public stakeholders and members of the research community as a significant factor in wasting resources, namely the publication of irreproducible research.

After reviewing this module, the reader should be able to

- ✓ Explain the importance of reproducibility and objectivity in biomedical research.
- ✓ Explain the reasons why biomedical research tends to suffer from low reproducibility depending on research contents.
- ✓ Discuss several aspects of researchers' attitude that hinder the reproducibility of research.
- ✓ State what researchers can do to improve the reproducibility of their research.
- ✓ Explain the factors leading to disputes on authorship among researchers in collaborative research.

Poorly Reproducible Research Wastes Resources

Irreproducible nature of biomedical research has been widely recognized as a serious problem. Clinical trials are experiments through which both safety and efficacy need to be validated before new drugs are placed on the market. Each trial involves studies on a large number of human subjects, requiring a major financial investment. For the development of one new drug, the total cost is said to be between $160 million and $2 billion.[3] It has become a serious issue from the fact that the rate of successfully marketing the test drug through trials, i.e., the success rate of experiments showing the safety and the efficacy of drugs being tested, is average 10 %, according to (Hay et al. 2014).[2] The latter phenomenon has triggered serious scrutiny of preclinical animal studies on which clinical trials are based. Critical reviews of these animal studies conducted using various approaches have all shown that published animal study data in cancer research are poorly reproducible.[4] It has been reported that, in the U.S.A. alone, 28 billion dollars are invested in non-reproducible research annually, an amount almost equal to the budget of the National Institutes of Health (NIH) in U.S.A.[5]



As humans and mice or rats are distinctively different species, it is natural that their responses to a given drug is found to be different. However, what is troublesome is that reported results from mouse/rat studies, in and of themselves, are poorly reproducible. Although similar problems have been reported outside of biomedical science,[6] this degree of irreproducibility is much more serious than those of studies in physics or chemistry.[4]

Defining "Reproducibility"

It is important to distinguish "reproducibility" from other related but distinct terms. In this module, the definitions of these terms are follows:

- Replicability: Previous results can be duplicated when the same person or team of persons use identical methods and materials.
- Reproducibility: Previous results can *largely* be duplicated when another person or team of persons use comparable methods and materials. Replicability is thus a prerequisite for reproducibility, and verifying a study's replicability improves its reproducibility.
- Generalizability: Results obtained under a specific condition, circumstance or system can *largely* be duplicated in different, broader conditions/systems. Reproducibility is a prerequisite for generalizability, and verification of a study's reproducibility improves its generalizability
- Translationability: Results obtained with animal model(s) can be applied to humans. Generalizability is thus a prerequisite for translationability, and verification of a study's generalizability improves its translationability.

One should recognize the presence of a sizable spectrum existing within "reproducibility." What one considers to be the same reagents, for example, may have been synthesized at

different times or in different factories, which may lead to results which differ from earlier studies. This is particularly common when biological reagents are used, as they usually contain undefined substances. Or, the same strain of mice or rats may respond differently to the identical drug, depending on the vendors from whom the animals were acquired; this can be expected, as the study subjects were reared in different environments. Likewise, “generalizability” means the ability to apply variably. For example, a given experimental observation may be generalized only for elderly persons, or on the contrary, may be applicable regardless of age.

Biological Causes Prevent Perfect Reproducibility in Biomedical Research

It is well known in the field of biology that the same person, using the same methods and materials, may not be able to obtain the same results as he or she previously obtained. Such lack of replicability can occur for many reasons, seasonal variations, time of the day, temperature and humidity, or how gently the animals were handled. Many other unknown internal and external environmental parameters could also potentially influence the response of animals under study. In biomedical studies, antibodies are used for assessing a variety of phenotypes. Those antibodies, too, are synthesized from animals, adding another possible cause of variability in animal studies.

Statistical Analysis as a Method of Estimating Reproducibility

Biostatistics is the method used to quantitatively determine the validity of an estimation. Owing to the establishment of biostatistical analyses as a credible scientific method, biological research has now been accepted as a respected part of the scientific field.

The “P value” (or “confidence interval”) documented in a publication indicates (within the framework of the model being used) the degree of probability that the null hypothesis (the absence of an effect) would be rejected in error. By expressing the uncertainty in this fashion, biomedical research is following best practice in all experimental and scientific fields. Of note, statistical analysis is as such that it cannot recognize a system error outside the model employed, so that even the best statistical analysis can only give an optimistic estimate at best. Researchers, therefore, should not treat statistical analysis as a “black box”, but instead need to understand the fundamental principle of statistical analysis.

Causes of Irreproducibility Attributed to Researchers

Examples of irreproducible results due to researchers’ insufficient knowledge or lack of integrity include the following:

- Lack of the right knowledge/skills to appropriately determine the sample size prior to the onset of a study, data collection or statistical analysis.
- Inability to take appropriate measures in planning to prevent foreseeable bias or to identify potential bias in analyzing results epidemiologically.
- Lack of adherence to data collection and analysis protocols set forth prior to the onset of a study.
- Repeating studies until results supporting the hypothesis are obtained, while suppressing the unsatisfactory results; or, after collecting samples, choosing a mode of statistical analysis that inappropriately gives a result consistent with the hypothesis.

- Measuring multiple parameters to look for one(s) that show very low P value, e.g., <0.05, and results with low P value as though they were result of a test of a well defined hypothesis. This is sometimes called “P-hacking” or a “fishing expedition.”
- Hypothesizing after results are known, (i.e., so-called HARK). Often practiced in combination with P-hacking, this is when a researcher dishonestly claims that a hypothesis set forth prior to the study was proven valid. Results from an exploratory study should not be presented as though they were the results of a hypothesis-driven study. Data obtained from an exploratory study, but published as if a result of a hypothesis-driven study, are highly likely to be non-reproducible.
- Failing to disclose negative data. As negative data are likely not to be accepted for publication in high-impact journals, researchers are tempted not to submit them. They are also tempted not to publish data that speak against the hypothesis they have been promoting.
- Reporting data resulting from specific conditions as ones collected in a more general condition, e.g., publishing data received only from male subjects without documenting the animals’ gender in the “methods” section.
- Failing to disclose information necessary for other researchers to verify results.

Researchers who commit improper acts of the type listed above are often under professional pressure to publish high impact papers, to get better jobs, promotions and research grants. Knowingly or not, they are tempted to:

- ✓ Collect data in search of evidence supporting their hypothesis, while rejecting evidence against it
- ✓ Place too much weight on “unexpected findings” while downplaying “expected findings
- ✓ Make up ad hoc explanations for any and every observation

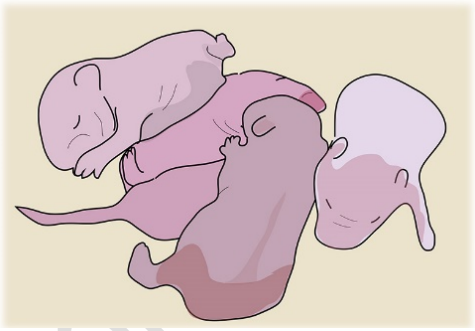
For Better Reproducibility

The scientific value of a set of research results is recognized only through confirmation of the results by others. It is therefore important for researchers to acquire the knowledge and

skills necessary for their work to be reproducible.

Despite inherently imperfect reproducibility, biomedical research can still be reliable. It is so by employing appropriate statistical analysis along with unrestricted disclosure of the data and their analysis, thereby accurately estimating the degree of reproducibility. Vigorous disclosure of conflict of interest, another potential cause of researchers' bias, is also an important corner stone for research reliability.

Biological Factors Causing Variability among Study Results



It is important to recognize that, in conducting research, a number of variables can affect the observed phenomena. In animal research the variables inherent to animals will add to the complexity. These include:

Animal Subjects:

a. Being of the same species but a different strain or genetic background, gender, age, or environment, e.g., breeder or farm.

b. Factors with regard to the environment in which the experiments are conducted: (breeding) season, temperature, humidity, brightness, time of the day, food, water, pre vs. post prandial time, noise, and smells.

Personnel Conducting the Research:

c. The level of experience and the maturity of animal farm personnel and those conducting experiments in handling animals.

Foods, Reagents, and/or Chemicals

d. Manufacturer
e. Lot number
f. Method and duration of storage
g. Method/Route of administration

There are a number of variables that can affect the results of not only *in vivo* live animal studies, but also *in vitro* cultured cell studies.

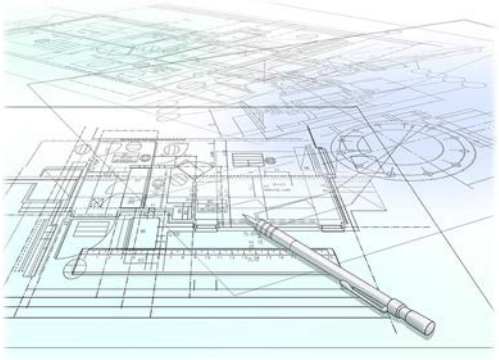
By making the methods and materials used as close as possible to those of the study being replicated, one can reasonably expect the new data to be similar to those of the earlier study. In some studies, one may elect to conduct studies at varying experimental settings, to be able to determine whether the phenomena being observed are generalizable. For a study to be reproducible, it is critical to document and disclose the details of the experimental conditions employed. In addition, if you obtained negative results under different conditions before, during or after the publication of positive results, you still need to publish those data, and make yourself available to provide detailed information about those negative results.

An animal's health is largely determined by how they were raised and cared for. It is therefore important to closely monitor and document their health. The use of a checklist prevents overlooking signs of illness. Researchers conducting experiments and animal farm staff need to communicate closely, the former explaining the purpose and the experimental method while the latter provides information regarding the animals' conditions. Veterinarians and other animal farm personnel often provide researchers with

timely and valuable information.

It is most important, at both the animal farm and in the laboratory, to handle animals gently to minimize the stress they experience. This is critically vital in order to obtain truly meaningful data.

Planning and Executing Research



A typical biomedical research aims to estimate a phenomenon occurring in a population by observing a few extracted from it. Statistics is an analytical method that quantitatively assesses the validity of this estimate. Owing to the recent major advancement in biostatistics, the degree of reproducibility can be presented in an objective manner. As a result, some human research data are now published in highly respected science journals, such as *Nature*. If one estimates the probability using an inappropriate statistical analysis, it is hardly surprising for other researchers unable to reproduce the results. It is therefore essential for researchers to use statistical methods correctly.

Methods for minimizing the chances of incorrect application of statistics include:

a. Invite researchers who carry a theory different from your own.
b. At the outset of research, propose two hypotheses that are mutually unacceptable.
c. Determining, prior to data collection, the sample sizes that will give sufficient chance to detect a signal, given the variance of individual observables.
d. Taking a training course for biostatistics and/or reading works on statistics in general and applications of statistics to bioscience in particular.
e. Selecting the method of statistical analysis prior to collecting data, and consulting a specialist when uncertainties remain.
f. Establishing the method of handling “anomalous” data prior to collecting data.

And in executing the planned experiments,

g. Randomizing sample collections
h. Using “blind” methods for data analysis.
i. Adhering to the protocol for statistical analysis established at the study’s onset.

In summary, researchers must:

1) Determine, prior to the start of the experiments:
   - the critical parameters for testing their hypothesis.
   - the methods of data collection and analyses, including the statistical methods.
   - the sample size(s) based on the acceptable signal-to-noise ratio.
2) Adhere, during experimentation, to the above protocols, established prior to the onset of the experiments.

In principle, researchers should not repeat experiments or use unplanned statistical

analyses, whether or not the planned statistical analysis on the collected data show the desired pattern. However, in the event they do so, this must be clearly stated and the reasons explained in their reports and papers on the experiments. Failure to make such a statements constitutes research misconduct.

Publishing Research

The readers of scientific publications are entitled to assume that the authors have complied with guidelines established by the journal. Non-compliant articles may cause misunderstanding by the readers, and might lead them to waste resources by conducting attempts to replicate the fraudulent results. Papers should therefore be retracted once FFP or other significant non-compliance has been identified; the authors of retracted research may be subject to disciplinary action. One example is the now well-known scandal involving the antihypertension drug Valsartan manufactured by Company N. Articles on this drug from multicenter studies were retracted due to deviation from the journal's guidelines for conflict of interest. It is noteworthy that Japan did not have governmental or institutional guidelines for conflict of interest at that time.

Authors need to be fully familiar with the "Instructions/Information for Authors" of the journal to which they are submitting their manuscript. These instructions/information establish rules about authorship, copyright etc., together with the following "must-include" items. It is critical for the integrity of published articles that the following items be accurately and appropriately reported.

A. Statistical Analysis
B. Processing Images and Figures
C. Conflict of Interest (COI)
D. Disclosure · Sharing · Offering

A. Statistical Analysis

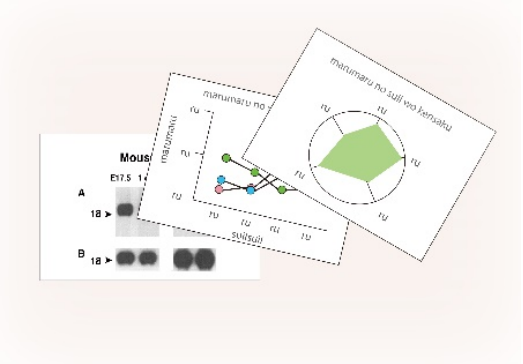
In order to assure reproducibility, it is critically important to clearly document the methods and the results of statistical analysis, including how adjustments were made when the sample size was substantially smaller than the original protocol, or data points did not show normal distribution, or multiple endpoints were assessed for testing hypotheses. In addition, some journals require authors to express the variability of data in a specific fashion.[7,8] Or, often, journals refer authors to common guidelines which are specifically designed for human subject vs. non-human subject studies; or qualitative vs. quantitative data analyses.[9] Compliance is essential to achieve transparency, and hence reproducibility of

published results.

An interesting article entitled, "Chocolate Consumption, Cognitive Function, and Nobel Laureates" has recently been published in New England Journal of Medicine[30]. The article quoted data comparing chocolate consumption among different countries. When the amount of chocolate consumption was related to the number of Nobel Laureates, Peason correlation coefficient was found to be 0.791 with P value of less than 0.0001, i.e., a significant relationship was present between the two variables.

It is obviously silly to conclude, on basis of this finding, that the more you eat chocolate, the smarter you become. This is one of the typical biases known in epidemiology as "ecological fallacy", i.e., a form of erroneous interpretation of statistical data in which the characteristics of a group is extrapolated into the characteristics of individuals who belong to the group. Switzerland, Sweden, Austria, Denmark, Norway do, indeed, have relatively large number of Novel Laureates and large amount of chocolate consumption; however, this cannot be taken as an evidence indicating that eating chocolate increases the probability of winning Novel Prize. Surprisingly, however, this form of error is not rare.

B. Processing Images and Figures



In biomedical research data are presented in various forms of photographic images in both oral and written presentations. Some authors are tempted to commit misconduct or misrepresentation in presenting images. For example, the best image may be described falsely as "representative." Data presented in this misleading way are not reproducible, and hence lack integrity.

In recent biomedical research, photographic images are captured and stored in digital format. As a result, original images can be easily manipulated. In fact, many of the misconduct cases that surfaced in recent years involved image manipulation. In some cases item that did not exist in the original was added, or changes were made in the brightness or color of a selected portion of the original. In other cases multiple different images were presented as a single image by combining them. All of these practices are explicitly prohibited by journals' rules and government regulations today, except that brightness or tone of color may be altered non-selectively throughout a given image if this is explicitly stated and if the authors make the original available upon request or include it in an electronic supplement to the paper.[10,11,12]

Graphs are another tool to help readers (or the audience at oral or poster presentations) to grasp the essence of data. In the past, graphs were drawn manually, often by the researchers themselves. Today, most journals require authors to submit graphs that drawn by computer software so that errors can be minimized. Improperly manipulating software to produce false or misleading graphs is research misconduct. Misconduct can also occur in inputting raw data into software and in handling the software.

C. Conflict of Interest (COI)

Conflict of Interest (COI) per se is not considered unethical, but the failure to properly disclose the existence of COI may in many cases constitute research misconduct. A typical example of COI in research is the presentation of data relating to merchandise from a company with which the presenter has a monetary relationship, e.g., in the form of research funds, consultation fees, or stock ownership. It is ethically unacceptable for researchers not to divulge the COI following the procedures specified by the journal, conference organizers, funding agency, etc. It is essential to give readers/audience the chance to consider the COI as one factor affecting their evaluation of the research. Some journals require the authors to disclose any financial arrangement[10], while others allow authors not to disclose financial relationship less than to $10,000[12]. One journal only warns the authors to use their own judgment, but to disclose anything that might prove embarrassing later if not disclosed.[13] In some cases, disclosure requirements apply retroactively to 3 years prior to the submission of manuscript[11], while in a few others, such as the New England Journal of Medicine, also require disclosure of financial interests that authors have with competitors of the company pertinent to the article.[10] For example, NEJM requires that disclosure if the author of a study on the side effects of chemotherapy holds a professorial chair endowed by a company marketing herbal medicines.

More recently, some journals require authors to disclose nonmonetary conflicts, as well, including ones from personal relationships, rivalries or religious/intellectual beliefs.[14] Thus, if the author is receiving some assistance, for example, in organizing seminars and workshops, from a pertinent company, that must be disclosed. To prevent undesirable influence of commercial companies on their researchers, some universities in the U.S. now ban any medical representatives (commonly called, “MRs”) from entering their campus.

D. Disclosure · Sharing · Offering

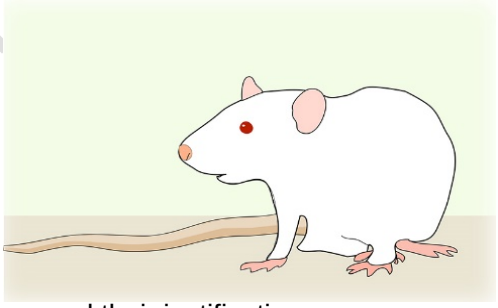
Transparency is the cornerstone of reproducibility in research; without confirmation of reproducibility by other researchers, published research cannot contribute to the progress of science. For the purpose of such confirmation, and to make the confirmation efficiently, methods, materials and tools used in a study must be presented in detail along with an unbiased description of the observations. Many journals now require authors to share information with other researchers as a condition for acceptance. Because of this requirement, in many cases researchers who intend to apply for patents must delay submission of a manuscript until their patent application has been filed.

Guidelines from Government Agencies and Journals

Government agencies in the U.S. and elsewhere have promulgated guidelines requiring disclosure of items[16-19] in each the following five categories: Many biomedical journals have also established guidelines for authors regarding these items.[10-12,14,20,21] It is the responsibility of researchers, *and no one else,* to provide their audience/readers with all required information in their publications and presentations:

1. Experimental animals
    - Species and strain
    - Gender, age and weight
    - Vendor
    - Rearing method (farm environment, food)

    When animals were an experimental model for human disease, additionally:
    - Details of the experimental conditions, as listed above, which may affect the experiment's results.
    - The human health problem that the animal model is intended to simulate.
    - The limitations of the animal model for extrapolating the results to human conditions.
2. Research Protocol
    - Reason for selection of the particular model
    - Justification of the control
    - Method of determining endpoint and its justification
    - Method of determining sample size and its justification
    - Definition of *n*, e.g., whether it represents the number of measurements or number of animals
    - Method of statistical method for data analysis and interpretation
    - Pharmacological agents and method of intervention used, dosage and the level of intervention, length of interval between doses and administrations and their justification



3. Method employed to prevent or overcome bias
    - Method of blinding or justification for not blinding in sample selection
    - Criteria for data selection
    - Method of randomization and classification
4. Results
    - The results of a reproducibility test, if applicable. Whether results are reproducible regardless of researcher, animal species of subject, strain or dosage of pharmacological agents. Relevant negative data. Dose-response curve for drug tests.
    - Method of randomization or classification
    - For drug efficiency studies, evidence that the drug reached its target
5. Discussion
    - The different interpretations for the data obtained. The limitations of data interpretation inherent in the experimental method used.
    - References to published articles in the literature for and against the results of the current study
    - The magnitude of the influence on humans if the results are to be duplicated in humans.

Most research journals set a space limit for articles, which often makes it difficult for authors to provide readers with sufficient detail in the main body of the article. Recently, however, most journals allow the publication of electronic supplements to the main article in which such details can be included. Also, it is becoming mandatory to provide a corresponding author's address so that readers can reach someone to obtain details not available in the paper. Certain "tricks" that can only be described verbally are one example of such information.

In addition to information about methods and results, materials and tools that are comparable, if not identical, to those used, must be available for other researchers to reproduce the results. If these are not readily available on the open market the authors should indicate in their publication that they will be provided upon request at a reasonable cost. Such materials include antibody, virus, bacteria, animal strains and DNA clones. The author's willingness to agree to such provision can be a necessary condition for acceptance of manuscripts for publication. In extreme cases it may be essential to clear innocent authors who are accused of research misconduct by allowing replication by independent investigators.

In order to make as much information and material as possible available to as many researchers as possible, some journals list a few repository sites for the authors to deposit those items.

Cross Border Collaboration

In the past, most collaboration took place among researchers within the same institution or within the same disciplines. In recent years, as research fields have been progressively diversified and their methodologies specialized, it has become necessary for researchers to collaborate, crossing the borders of distinct research arenas and those of different countries. For example, researchers in engineering might collaborate with sociologist to study a new transportation, while biomedical researchers might collaborate with experts in economics to improve the national health insurance system. As government agencies, research institutions and various scientific societies, are all concerned about the potential damage from research misconduct, they have established laws and guidelines with which researchers must comply. Although such laws and guidelines share many general features, there are important differences in the specifics. Researchers, therefore, must be aware that their collaborators may have to comply with rules somewhat different from their own. It therefore is important for all of the collaborating researchers to share information on the respective rules before and throughout their collaboration.

The Importance of discussing among collaborators is in part because, depending on the journal chosen, each collaborator's work can draw varying degrees of attention from his/her research community. But also, each journal requires authors to take varying steps toward better reproducibility. Some journals mandate authors to confirm their conclusion in multiple ways, which is the tendency seen in major journals. Recently some questionable journals are geared only to profit making by heavily charging authors have appeared. These journals are surviving due to some researchers' willingness to publish at all costs, sacrificing quality and reproducibility in the process. However, there are also some borderline journals which have insufficient concern about reproducibility. Such borderline journals may allow publishing hypotheses with no data presentation is required; reproducibility is not an issue for such papers. All scientific journals fall somewhere between best practice and worst, and researchers must choose where they will publish. Each member of a collaboration may be shooting for a different level of reproducibility, which is another reason for the importance of discussion among collaborators before starting their work and before submitting their work for publication.

Role of the Government and Research Institutions

It is important that existing results can be used effectively, so that the generation of research can be based on recent published results. Government agencies in the U.S. and elsewhere have issued guidelines to improve reproducibility so that other researchers do not waste resources on fruitless attempts to replicate irreproducible studies.[16-19] The reason that particular attention is being paid to guidelines of research activities is that lack of reproducibility in such research can cause much greater harm to the public than irreproducible research in other fields[23-25]. As each clinical trial can cost millions of dollars and a great number of clinical trials are currently under way[22], improvements are obviously desirable.

Under these circumstances, many international science journals recently revised their instructions to authors, discussing more specifics in more detail in order to preserve the reliability of their publications.[10-12,14,20,21] In contract, government guidelines often intend to be less specific as they are intended to prevent misconducts of the nature that characterizes misconducts of the past.

It is important to recognize that publication is the final step that makes research "unreproducible" by others, causing unnecessary waste of resources. It is therefore important for government agencies and institutions to establish guidelines for research integrity that those are in good agreement with the current guidelines of leading journals.

Summary

Researchers should strive improve the reproducibility of their work by taking the following steps:

- ✓ Establishing experimental protocols free of bias.
- ✓ Rigorously adhering to the established protocol during their experiments.
- ✓ If the reproducibility has been somewhat compromised due to shortcomings in the above items, the author should preserve the integrity of the publication by explicitly describing the problems and by providing accurate data from appropriate statistical analysis.

For researchers to improve the reproducibility of their studies, it is important first that they establish appropriate experimental protocols, prior to the onset of study, that are bias-free; second that they adhere to these protocols with rigor; and third that they are vigilant about transparency, i.e., providing readers with details of the methods and results, and also the materials used so that other researchers can verify or reproduce the results.

The volume of clinical trials conducted in Japan is substantially lower than the number carried out in U.S.A. and elsewhere, as is the volume of preclinical animal studies[26]. As a result, the irreproducibility of preclinical studies is currently less of a concern for its research community. However, it should be noted that many international journals require their authors to provide the details of the studies for the sake of best possible reproducibility[27-29].

This module has been supported by AMED: Research and Development Program for Enhancement of Research Integrity, "Ethics education program on the reliability of research that medical international journal norms". The names of the experts who participated in the creation and review are listed on the front page.

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