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Study identifies unexpected mutation in commonly used research mice

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A strain of inbred mice commonly used for the creation of so-called knockout animals has been found to carry a previously undetected

mutation that could affect the results of immune system research studies. In paper receiving online publication in *Cell Reports*, a team based at the Ragon Institute of MGH, MIT and Harvard describe finding a chromosome 11 mutation in a strain of C57BL/6—often called "black 6" or B6—mice that they traced back to a specific commercial supplier. Their findings suggest that the results of previous studies using this subline of C57BL/6 need to be re-checked to make sure that this newly identified mutation was not responsible for observed results.

"We found an unexpected mutations with potentially important consequences in strains of mice that had been separately engineered in labs in California and Japan, and after some detailed detective work, we traced the problem to a subline of B6 mice from one specific company that have been sold in Asia, North America, Europe and Israel," says Shiv Pillai, MD, PhD, a member of the Ragon Institute and program director of the Autoimmune Center of Excellence at Massachusetts General Hospital. "We have notified this company—Harlan Laboratories, which is now part of Envigo—of our findings, and they have been very responsive and will use approaches we have provided to check all of their colonies."

Individual inbred mouse strains used for research differ from each other at many gene sites, which can result in differences in their immune responses. In immunological studies conducted across the world, mutant mice are "backcrossed" for many generations into B6 mice so that all genes other than the mutant gene are derived from the B6 strain. This allows the comparison of data from laboratories in different parts of the world and simplifies creating mice with mutations in several genes.

A 2009 study out of Pillai's lab had found that two strains of mice engineered to lack the genes Siae or Cmah—both of which code for enzymes involved with a family of proteins called sialic acids - also had significant defects in the development of B cells, which were assumed to

be the result of the knockout genes. But when Siae-deficient mice were further backcrossed with a different group of C57BL/6 mice, the result was a strain of Siae-knockout mice that did not have the B cell development defects. A newly engineered strain of mice with a different Siae mutation also was found to have normal B cell development.

Detailed genetic sequencing of the first knockout line identified a previously unsuspected mutation in a gene called Dock2, located on a chromosome 11 instead of chromosone 9 where Siae is located. The same mutation had been previously reported in two colonies of a different knockout mouse developed in Japan. Pillai's team also found the Dock2 mutation in a completely different group of mice from the University of California, San Diego—where their Siae-mutant strain had been developed—and realized that three different engineered strains with the same unwanted mutation had probably acquired it from a common source, eventually traced back to a subline of C57BL/6 mice from Harlan/Envigo.

Since most research papers using C57BL/6 mice or other such "background" strains do not indicate the specific subline, of which there are many, the Ragon Institute researchers have no way of knowing how many studies might be affected by their findings. But they hope the publication of these results will alert other research teams to the potential need to review their results. Several commercial laboratories have instituted measures to prevent the occurrence of random mutation in inbred mouse strains, and Pillai notes, all commercial vendors should establish such programs.

"While embryonic stem cells from C57BL/6 mice have recently become available, which allows the generation of knockout strains with less backcrossing, B6 mice are used for many different kinds of experiments, including as controls," says Pillai, who is a professor of Medicine and of Health Sciences and Technology at Harvard Medical School.

"Researchers who have used them need to re-genotype the mice to look for the Dock2 mutation, and if they find it, check to see whether their results are preserved if the Dock2 mutation is bred out."

More information: *Cell Reports*, DOI: 10.1016/j.celrep.2016.04.080