Welfare Assessment and Phenotype Characterisation of Transgenic Mice

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Summary

Induced mutations can cause new and unpredictable phenotypes and may impact the health and welfare of animals. Impairments may arise within normal husbandry and breeding regimes i.e. before starting to do experiments. In order to apply the 3R principles and to use transgenic animals under high scientific and welfare standards, two structured forms for individual health monitoring and strain characterisation have been developed. They are available at: www.vu-wien.ac.at/labortierkunde or www.altex.ch

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Background Information

Transgenic animals in animal experimentation

Variations from the mean or norm have always been interesting to biomedical research in providing clues as to the normal and pathological functions of living beings. Genetic mutations are particularly useful because they can be reproduced by breeding; thus they are accessible to a thorough investigation. With the advent of transgenic technology and its routine application in many laboratories around the world, the generation and use of genetically modified animals has increased dramatically in biomedical and pharmaceutical research and safety testing. This development has been additionally accelerated by the decoding of the genome of man, mouse and rat; currently about 10% of the mouse's genes have been modified experimentally and incorporated into genetically engineered organisms (Austin et al., 2004). In 2005, 94.000 such animals - mainly mice - were used in Switzerland for experimentation, an increase of 16% over the previous year; 22% of all mice used were genetically engineered. In the period 1997 to 2004, 5.000 transgenic mouse strains were kept in Swiss laboratories (Bundesamt für Veterinärwesen, 2006) and new strains are being added continuously. However, transgenic animals have a potential for genetically derived health impairments and other welfare problems (e.g. developmental difficulties, behavioural abnormalities, problems of reproduction); in fact, 7% of all transgenic strains kept in Switzerland show a mild degree of innate suffering (severity score 1), 5% have a middle or high degree of suffering (severity scores 2 and 3; Bundesamt für Veterinärwesen, 2006). This needs to be addressed systematically in order to adopt 3R measures of refinement and to take ethical decisions about the continuation of heavily loaded strains.

Is there a particular health and welfare problem with transgenic animals?

Transgenic mice are valued models in biomedical research. Several strains are used to investigate human congenital diseases and disorders; these strains carry a genetic defect that may or may not be clinically apparent in the animals. In practice, altering the genotype has not necessarily an impact on the health of transgenic strains. However, the resulting phenotypic consequences can not be predicted in detail and a reduced viability or impaired health at the phenotypic level may be expected in several cases (Bundesamt für Veterinärwesen, 1998; 2006). The impairment is also present even if the animals are not used for experimentation, and is transmitted from one generation to the next by normal breeding. The degree of impairment and the symptoms of suffering will vary from minor to severe and may show up at various stages of ontogenesis in different strains.

Within a single strain, the degree of suffering will depend primarily upon the genetic alteration, on the genotype (the alleles at a specific locus) of each individual and the genetic background, but can also be strongly influenced by environmental conditions.

It may or may not be possible to alleviate suffering by specific measures. In any case, each newly created transgenic strain has the potential to cause poor health and suffering in the animals. In this regard, transgenic technology poses a challenge for the 3R goal of "refinement": if a mouse strain is to carry certain dysfunctions at the genetic level for scientific reasons, everything has to be undertaken to minimise animal suffering.

Can transgenic animals offer a particular contribution to the goals of 3R?

In spite of the problems listed above, transgenic animals may represent a refinement in comparison to some other traditional

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experimental models of disease in which the animals bear a heavy load of suffering. It seems possible to create "elegant" models where – in the best case – a genotype is an excellent model of disease for selected body functions at the molecular or cellular level while the corresponding phenotype is completely healthy. However, in order to ensure that the well-being of a newly created transgenic strain is unaffected, a careful and comprehensive examination is indispensable.

Given these two problems and tasks at the level of the animal strain, i.e. (i) proof of absence of health problems due to a modified genotype and (ii) recognition, prevention, minimisation and therapy of reduced welfare, the first few generations of newly created mutant strains (founders, F1 and F2) need to be characterised with respect to their phenotype using health and welfare assessment guidelines.

What do we have to know about transgenic animals?

Animal care staff and scientists working with and responsible for transgenic rodents are often confronted with impairments to fitness of mutant animals. Published experimental results derived from new transgenic animals do not usually include detailed information about the requirements for breeding and maintenance of the strain. By using existing knowledge regard-

ing an efficient breeding and husbandry program of a novel line, it will be possible to reduce the overall number of animals used and to minimise the potential for affected animals to experience pain, suffering or distress.

Forms for a standardised documentation of relevant information

The literature was first reviewed to collect clinical signs described as being useful for recognising health problems and suffering in laboratory animals. This body of literature was then analysed and a relatively large set of parameters selected for the characterisation of transgenic mice. The parameters had to fulfill two criteria: (i) feasible for conducting as a screening test on a routine basis, and (ii) indicative of health or animal welfare problems at the strain level.

Next, two standardised forms were developed to characterise transgenic mice comprehensively. The form "Data Record Form" (Mertens and Rülicke, 1999; 2000b) includes 10 different score sheets for litter-wise and individual health monitoring from birth until spontaneous death or euthanasia. This information about individual animals was then structured and evaluated for the health and welfare status of the strain at two levels of detail in the form "Strain Characterisation" (Mertens and Rülicke, 2000a; 2000b). The "Basic Information" will answer

Table 1: Form for strain characterisation: general structure

I. Basic information (5 pages)	II. Detail information (13 pages)
A. Main page	F. Gen expression at molecular and cellular level; constitutive or conditional expression
B. Genotype (overview)	G. Phenotype: manifest differences in comparison with the wild type of the same genetic background
C. Phenotype (overview)	H. Additional strain specific characteristics
D. Ethical and animal welfare assessment	
E. Recommendations for housing, breeding and transport	

Table 2: Form for strain characterisation, part C: Phenotype (overview)

	no	yes	?	for details p.
1. Are there useful breeding records?	☐	☐		9
2. Are there differences between hemi-/hetero and homozygous animals?	☐	☐	☐	10
3. Are there sex specific differences?	☐	☐		11
4. Is there an increased lethality (prenatal, perinatal, postnatal)?	☐	☐		11
5. Are there abnormalities in individual development?	☐	☐		12
6. Are there apparent malfunctions and deformations?	☐	☐		13
7. Are there malformations of inner organs?	☐	☐	☐	13
8. Are there disorders in individual behavior?	☐	☐		14
9. Are there disorders in social behavior (without reproduction)	☐	☐		14
10. Are there disorders in breeding behavior?	☐	☐		15
11. Are there abnormalities in reproductive success?	☐	☐		15
12. Are there strain specific diseases?	☐	☐		16
If yes: which?				
13. Is the immune status affected?	☐	☐		17
14. Cross breeding to a different background planed or in progress?	☐	☐		17
15. Generation of double or multiple mutants planed or in progress?	☐	☐		17
16. Is there any strain specific detail information not given under pt. 1 - 15?	☐	☐		18

Each question answered with ‚yes' has to be supplemented by the corresponding form for specific information.
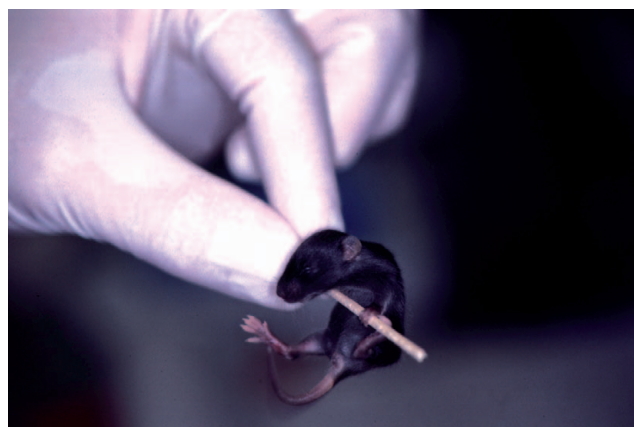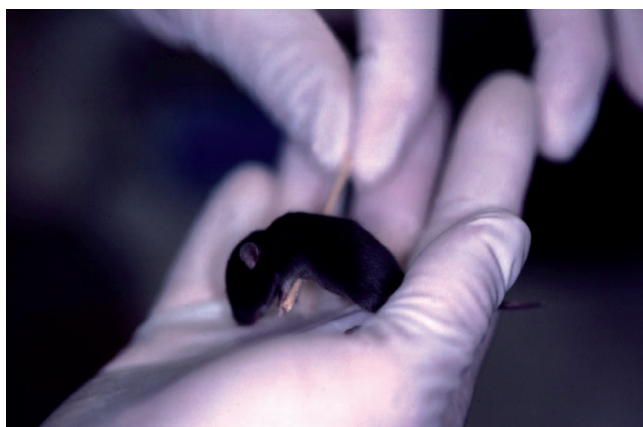
Fig. 1A, 1B: Neurobehavioral tests are easy to perform with each pup (approximately day 10-14) and indicate gross sensory-motor deficits. The pictures show the pole grasping test for motor (dis-)abilities and muscle strength.

the question if there is a welfare problem with a transgenic line (Tab. 1, Tab. 2). If so, a detailed description of the symptoms can be developed or is provided with the "Detail Information" part (Tab. 1).

Finally, the score sheets were tested for their "user-friendliness" and clarity in a pilot study that monitored and documented 106 mice from two established genetically modified strains in the first three months of life (Mertens and Rülicke, 1999). The results demonstrated the fundamental practicality of the protocols, provided that personnel were adequately instructed and able to invest additional time in the monitoring program. Meanwhile, similar recommendations for data record forms have been published by other groups (van der Meer et al., 2001; Wells et al., 2006).

Characterisation of transgenic animals improves animal welfare and science as well

A comprehensive characterisation of transgenic mice is indispensable for animal welfare, for the application of the 3R principles, for the correct interpretation of research results, and for official purposes (annual statistics). The characterisation sheet presented here is feasible for routine use as a standardised procedure. It will contribute to increased scientific accuracy and efficiency in the laboratory. Transgenic animals, whether commercially distributed, transferred to new facilities or used in animal experimentation, should always be accompanied by their score sheet and strain characterisation form. Up to now, a lack of awareness of the problem and a lack of supporting regulations has prevented such a procedure from becoming standardised. The complete forms can be downloaded as pdf.files at www.vu-wien.ac.at/labortierkunde or www.altex.ch

References

Austin, C. P., Battey, J. F., Bradley, A. et al. (2004). The knock-out mouse project. *Nature Genetics 36,* 921-924.

Bundesamt für Veterinärwesen (2006). Statistik der Tierversuche in der Schweiz 2005. www.bvet.admin.ch/tv-statistik.

Bundesamt für Veterinärwesen (1998). Gentechnisch veränderte Wirbeltiere: Anwendung der Tierschutzgesetzgebung auf Herstellung, Zucht, Haltung und Verwendung zu Versuchszwecken (www.bvet.admin.ch).

Mertens, C. and Rülicke, T. (1999). Score sheets for the monitoring of transgenic mice. *Animal Welfare 8,* 433-438.

Mertens, C. and Rülicke, T. (2000a). Umfassendes Formular zur strukturierten Charakterisierung gentechnisch veränderter Tierlinien. *ALTEX 17,* 15-21.

Mertens, C. and Rülicke, T. (2000b). Phenotype Characterization and Welfare Assessment of Transgenic Rodents (Mice). *J. Appl. Anim. Welf. Sci. (JAAWS) 3(2)*, 127-139.

Van der Meer, M., Rolls, A., Baumans, V. et al. (2001). Use of score sheets for welfare assessment of transgenic mice. *Laboratory Animals 35*, 379-389.

Wells, D. J., Playle, L. C., Enser, W. E. et al. (2006). Assessing the welfare of genetically altered mice. *Laboratory Animals 40,* 111-114 (see also www.nc3rs.org.uk).

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