Welfare assessment of genetically altered mice in Phenomin, a French multi-site phenogenomics infrastructure

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Introduction:

Since adoption of Directive 2010/063 EU, many specific provisions had to be implemented to improve protection and welfare of animals used for scientific purposes. Among them, welfare assessment of newly created genetically altered (GA) mice has to be considered, as their use in research is increasing.

Beyond **regulatory reasons**, welfare assessment has to be set up first**, for ethical reasons**, such as refinement in case of detection of a harmful phenotype and sharing this information through a passport and, secondly, for **scientific reasons**. Indeed, 80% of GA lines have a phenotype *(de Angellis, 2015*) and 10% of them are subviable *(www.mousephenotype.org/data/embryo)*. Thus, it is of scientific interest to provide an early detection of phenotypes and to observe young animals to help understanding neonatal death.

PHENOMIN is a multi-site research infrastructure of excellence for translational research and functional genomics. It provides a comprehensive set of specialized services to academic and industrial users by combining the capacity of generating GA mice on a large scale with a high-throughput and comprehensive phenotypic analysis of the animals. The international Mouse Phenotyping Consortium (IMPC) is one the biggest scientific effort to understand mammalian gene function with GA mice where Phenomin is also actively involved through the generation of 235 GA lines. 3 French Institutes are involved in this project.

After the French transposition of the European Directive in 2013, a Phenomin working group, mostly composed of animal facility managers and veterinarians, has been set up in order to establish a common process of welfare assessment on our new mouse lines.

We present here how we've implemented welfare assessment in our facilities.

RECORDING DATA

We have created a first tool for animal caretakers to record their daily observations easily in an Excel spreadsheet with some Macros, following the European Working Group on severity assessment recommendations on how to perform this evalulation:

- When the line is established (From F2 onwards)
- At 3 key time-points
- On 7 mutant animals per gender, per genotype, from 2 different litters as a minimum.

Criteria for Neonate assessment:

- Number of pups on the day of evaluation and one week later (check neonatal death)
- Abnormal color of the skin : anemia, icter, blood circulation defect
- Activity of pups (e.g. reduced wriggling)
- Absence of milk spot / sign of mismothering

Criteria for Weaning and adult assessment:

- Appearance : signs of abnormal morphology (e.g. skull, tail, …)
- Coat condition
- Posture, gait and activity
- Clinical signs without handling (discharge, seizures, breathing rate)
- Clinical signs with handling (tumors, …)
- Relative size compared to WT

Reporting – Vet. + project managers

New borns
- When: 1st week
- Simple criteria: skin color/activity/feeding
- No contact needed (avoid cannibalisme)

10% neonatal death

Weanlings
- When: at weaning (3-4 weeks) report if weaning is postponed
- Several and precise criteria
- Compare mutants and WT

Adults
- When: around 8 weeks
- Several and precise criteria
- Compare mutants and WT

Cohort phenotyping (IMPC)

Phenotype scoring
- Classification: Harmful / non harmful
- Severity: light, medium, severe (possible refinement)
- Passport writing

PHENOTYPE SCORING

A second tool, scoring sheets, is used to interpret those observations and score the phenotype, The evaluation is made for each line as team approach because expertise is needed to :

- Differenciate a phenotype from a spontaneous disease linked to genetic background
- Evaluate fertility and genotype ratios (homo/het/WT)

Categories	Variables	Points
Body score condition (BSC) / relative size (RS)	0 Normal, BSC=3, RS = 1 BSC=4, RS slightly different 2 BSC=2 ou 5, RS moderately different modérément différente 3 BSC=1, RS higly different	
Appearance	0 Normal 1 No grooming 2 Rough coat 3 Highly rough coat	
Behavior from a distance	0 Normal 1 Minor modifications, hyperactivity 2 Abnormal, reduced mobility, inactivity, lethargy 3 Selfmutilation, prostration	
Behavior at manipulation	0 Normal 1 Short prostration, hyperactivity, agressivness 2 Intermittent tremors or seizures, escape attempts 3 Persisting tremors or seizures, selfmutilation, coma, no escape attempt	
		TOTAL

Score	Phenotype severity
0 - 3	Non-harmful
4 - 6	Light
7 - 9	Medium
10 - 12	Severe

Abnormality frequency (nb of mice)	Impact on severity
1 or 2	None (rare case)
3 to 9	light (can happen but not frequently)
10 or more	important (is part of the phenotype)

The observations are recorded in the green table to get a score.
That score is read in the purple table to evaluate the severity of the phenotype.
The frequency of abnormalities in the different animals (blue table) is used to describe the phenotype.

SHARING DATA

After scoring, data is compiled in a passport, a document which is to follow mice when the lines are distributed worldwide.
This passport will ensure that specific information related to animal welfare is accessible to whoever will care for these lines:

- Genetic information on the line : genetic modification, GMO classification etc.
- Ethics and welfare information:
  - Zootechnic characteristics (breeding, nutrition)
  - Severity of the phenotype
  - Phenotype description
  - Means to refine the phenotype
  - Ethical endpoints to use while breeding

CONLUSIONS : A TEAM APPROACH

Genetic engineering
Import-export service
- Creation of a new line
- Importation

→ **Breeding facility supervisor**
- Opening of a passport and an Excel sheet for the new line

→ **Animal caretakers**
- Welfare assessment
- Data recording

→ **Animal caretaker, Principal investigator /project manager, veterinarian**
- Data compilation
- Phenotype scoring
- Passport
- Project authorization form

Conclusion:

In order to implement such an assessment process in all our animal facilities, we have faced some difficulties :

- the time-consuming aspect of the task : Record and data analysis
- the need to involve a lot of staff : caretakers, vet, Animal Welfare Body, Principal investigators, project managers.

But this work had some advantages:

- a better surveillance of our mice improving daily refinement
- better traceability of information (eg. genotype ratios)
- more constructive exchanges between caretakers and researchers about expected or unexpected phenotypes.

The implementation will be over when our tool will be included in our facility management software, which is the next step of our work in Phenomin-ICS.

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References: *Wells D et al. (2006) Assessing the welfare of genetically altered mice. Laboratory Animals 40: 111-114; European Working Group on Severity Assessment Framework, Working document on a severity framework, [on line] http://ec.europa.eu/environment/chemicals/lab_animals/pdf/guidance/severity/en.pdf (Page consulted on Jan. 29, 2016); C. Brayton, Spontaneous Diseases in Commonly Used Mouse Strains / Stocks, 2009.; M. Hrabe de Angelis et al. Analysis of mammalian gene function through broad-based phenotypic screens across a consortium of mouse clinics, Nat. gen. 47.969-978 (2015).*

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