Home Office

NON-TECHNICAL SUMMARY

Genetics of Down Syndrome

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Down syndrome, Congenital heart defects, Craniofacial development, Neuronal development, Cognitive function

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We aim to identify genes that are needed in three copies to cause specific Down syndrome phenotypes and establish the mechanisms by which they cause pathology.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Down syndrome is a human condition caused by an extra copy of chromosome 21. It is a complex disorder with many different phenotypes including leukaemia, autoimmunity, congenital heart defects, craniofacial changes, and cognitive deficits. It is not known which of the ~230 coding genes on chromosome 21 is required in three copies to cause these diverse phenotypes, and thus there are no effective treatments for any of these conditions. We aim to identify the causative genes and establish how an extra copy causes pathology. Knowing this will lay the foundations for the design of rational therapies for the phenotypes of this common genetic disorder.

What outputs do you think you will see at the end of this project?

The main outputs of this work will be knowledge about which genes cause DS phenotypes and the mechanisms by which they do so. This will be published in peer-reviewed journals, and the publications will always be open-access and thus available to all to read for free.

Who or what will benefit from these outputs, and how?

The first beneficiaries will be other researchers working on DS. More importantly, in the longer-term the work will benefit those who are working towards identifying rational therapeutic approaches to alleviate DS phenotypes. Ultimately, the beneficiaries will be people with DS who will have access to therapies that will be developed on the basis of this research.

How will you look to maximise the outputs of this work?

The main outputs from the work will be published in peer-reviewed journals, and the publications will always be open-access and thus available to all to read for free. We will also communicate our work through presentations, by giving seminars at other institutions or through seminars or poster presentations at conferences. Unsuccessful approaches will be discussed openly in appropriate venues, for example at internal meetings. We have an extensive track record of collaboration, helping other groups with more limited experience in these areas of research and sharing all our novel mouse strains, often before publication. We will continue to support such collaborative work.

Species and numbers of animals expected to be used

- Mice: 24000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The project will use embryos, neonates or juveniles to study developmental processes, e.g. heart development to investigate congenital heart defects. Adults will be used to study physiological processes typical of adult mice, e.g. cognition, locomotor function.

Typically, what will be done to an animal used in your project?

The large majority of mice in this project will be bred and then killed and tissues analysed by, imaging, cell culture, transcriptomics, proteomics flow cytometry, etc. Some mice may be treated with substances to induce gene deletion or to report cell proliferation. In some case adults will be analysed by in vivo imaging, or in tests of cognition or locomotor activity.

What are the expected impacts and/or adverse effects for the animals during your project?

For the vast majority of mice there will be no adverse effects. For some animals, there will be transient pain when being injected, but no lasting harm.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mild – 95%

Moderate – 5%

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The DS phenotypes we are studying – congenital heart defects, otitis media, craniofacial changes, learning and memory deficits, immune dysfunction and locomotor impairment – cannot be studied in

vitro. These phenotypes can only be meaningfully studied in vivo.

Which non-animal alternatives did you consider for use in this project?

We considered using human IPSCs from DS people and differentiating these into relevant cell types.

Why were they not suitable?

The use of IPSCs is possible, but very limited – essentially only individual cell types can be studied and the complexity of tissue interactions cannot be replicated in vitro.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimate is based on our current experience of carrying out similar studies under our current project licence, and the number of researchers working on this project which will remain around 5 individuals for the next 5 years.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The efficiency of animal usage will be maximised by careful control of breeding to meet research needs with respect to numbers, phenotypic uniformity and health. This has been greatly facilitated by a custom-built mouse database in which every breeding pair and every mouse born are recorded and through which we can readily monitor the numbers of mice we hold. Many experiments will require homozygous mutant animals. Littermates of these that are heterozygous or wild type will be used as age- and gender-matched controls. This allows optimal use of mouse numbers generated as well as being best scientific practice for the study of genetic alterations.

The experimental design is always based on using the smallest number of animals that are sufficient to answer the question being posed. We expect, from experience, that 6-8 animals per treatment group should be sufficient to obtain statistically robust results. For most of the quantitative experiments, sample sizes may be set using power analysis, generally using a significance level of 5%, a power of 80%, and a difference between groups of 20%. Otherwise, we will use the minimum number of animals to provide an adequate description, generally on the basis of previous experience (ours, or from the literature).

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Breeding strategies are always set up to maximise the number of useful mice from each litter. Wherever possible we will use multiple tissues and/or fluids from every animal, in order to maximise the data obtained from each mouse. Cryopreservation of gametes or zygotes will be used to preserve mouse strains, thereby obviating the need for continuous breeding and thus minimizing numbers of mice used.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use mice since these are the mammal with the best studied developmental biology and cognition.

Why can’t you use animals that are less sentient?

Many of the mice we will use will be embryos – these are most suitable for the study of developmental processes, e.g. in the heart. Since we are modelling DS phenotypes, including cognitive deficits, less sentient animals would not be suitable as they are too far away from humans.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

For all manipulations, we will adhere to the relevant guidelines that aim to minimize suffering. We examine the animals for signs of pain and discomfort (such as grimacing), providing additional analgesia if appropriate, and monitor body condition, killing the animals if the distress is likely to be more than temporary. Many of the genetic and physiological manipulations, as well as the administration of substances, including gene inducers and repressors are standard and previous refinements from the literature will be used and added to if possible. For novel types of manipulation, or where insufficient information is available, small-scale pilot experiments are conducted in order to determine the best conditions to obtain a sufficiently robust and meaningful response from the minimum dose, exposure time or treatment. These pilot experiments help to minimize any potential suffering.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Publications from the NC3Rs and the Institute for Animal Technology, as well as relevant articles in scientific journals.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We stay up to date via regular communication with animal facility staff at the host establishment, other scientists in our fields, via e-mail and other updates and publications from, and occasional attendance at meetings held by, the NC3Rs, the Institute for Animal Technology, and the International Society for Transgenic Technology, and through regular visits to their websites:

https://www.nc3rs.org.uk/3rs-resources

https://www.transtechsociety.org/

https://www.iat.org.uk/