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NON-TECHNICAL SUMMARY

Epigenetic inheritance and the control of developmental and physiological processes

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

genes, gene regulation, developmental disorders, genetics, epigenetics

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

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 understand the function and regulation of genes that are important for animal body function and development throughout the life-course.

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?

Our study has important implications for the health and well being of the individual and their offspring. Epigenetics refers to a range of factors that regulate the activity of genes, a controlled process that is important for ensuring that genes are expressed properly in the right cells at the right time.

When epigenetic processes do not work properly, cells behave abnormally leading to diseases such as growth abnormalities, disorders of the nervous system, metabolic diseases such as obesity and diabetes and cancer *. For example, Angelman syndrome, an imprinting disorder leading to severe intellectual disability affects 1 in 12,000-1 in 20,000 people worldwide, and Prader-Willi syndrome, another imprinting disorder linked to intellectual disability, compulsive eating and obesity affects 1 in 10,000-1 in 25,000 people worldwide.

Epigenetic marks are set early in development and altered over time. While much can be learned about epigenetics from studying cells in a dish, information about the ways epigenetic changes are regulated can only be understood by investigating the whole body throughout its life.

* https://www.nature.com/scitable/content/epigenetic-diseases-and-their-causes-and-symptoms-37397/

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

We will generate new knowledge, to be shared with others, which will lead to the discovery of important factors that change gene expression without altering the genetic code in the form of DNA, this field is known as epigenetics. Specifically, processes in the contexts of the nervous system, growth and development and lifestyle-related diseases, for the benefit of humanity.

We will also understand fundamental processes associated with health and wellbeing in parents and the effect on offspring when these are perturbed genetically or environmentally. This has the potential to lead, in the long term, to new ways to detect diseases, predict their development and identify new ways to design medications for treatment.

Our findings will be published in scientific journals which are accessible to all, and our data will be placed in publicly available online resources. These published data will support future funding applications and has the potential to take the scientific discoveries to the clinic and the medical field.

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

During the 5 year course of the licence:

Our research in epigenetics will have an impact on our understanding of genetic diseases by identifying regions of the DNA where abnormal changes might lead to developmental and life-style related disorders, and cancer.

Our research on nutrition, before and after birth, will help understand more about why babies can be born with an abnormal weight. Additionally, our findings will look at the long-term impact nutrition has on their health and wellbeing over the lifecourse. This work will also have an impact on our understanding the benefits of breastfeeding for babies and mothers.

Our research on epigenetic processes being passed from parent to offspring, will help us understand whether and how environmental changes influence our DNA. We will investigate the extent of these environmental influences and how or if they can be inherited from parent to offspring and even to the next generations.

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

Our research is highly collaborative and we will actively seek to contribute our expertise, technology and materials, positive and negative data to others and are often sought out by colleagues who wish to collaborate with us. We have a good track record in this, as evident from the publications that we co-author with others.

We will continue to establish and participate in national and international scientific organisations to maximise the impact of our research and add value altogether. We will continue to present our work prior to publication at conferences, seminars and lectures.

We actively collaborate with scientific companies to screen for the best tools to be used in the research community and we regularly report successful and unsuccessful results.

We are obliged by our funding agencies to publish our studies only in peer-reviewed open access journals.

Species and numbers of animals expected to be used

- Mice: 25,550
- Zebra fish (Danio rerio): 11,500

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.

Mice and zebrafish are used to investigate aspects of our studies. An extensive catalogue of genes already exists for these organisms and they are widely used across the scientific community which allows for accurate comparisons between data. Also, well-established procedures already exist that are not harmful for the animals which can be used to mutate the genes or change their regulation. Many of the genes found in mice and zebrafish are found in humans too.

We study developmental processes therefore it is necessary to study all life stages.

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

For the vast majority of our work animals will be mated and material collected, post mortem (after death), at various life stages.

To study embryonic development, female mice will typically be injected into the abdomen, with a hormone to stimulate egg production, this is done twice in an animal's life and eggs are collected post mortem.

To assess metabolic processes animals may receive an altered diet, and substances such as glucose or insulin, will typically be administered once into the abdomen, followed by blood sampling of one drop, using a blood sugar monitor similar to that used by diabetics. Up to 6 blood samples will be taken from the tail vein over 4 hours. Typically, a mouse will experience each of these above procedures.

Some animals will undergo 1, 2 or sometimes 3 behavioural tests typically to test motor skills, anxiety and spatial navigation.

To evaluate mouse milk the mothers will be separated from the pups for up to 4 hours to allow milk to accumulate in the mammary glands. After this 4-hour period of separation, mothers will be anaesthetised and will have an injection of hormone to stimulate milk production. The nipple will be gently squeezed to release the milk. Mothers will then recover on a heated pad, and then returned to the pups that will remain in the nest undisturbed in a heated cabinet.

To study neurogenesis in zebrafish, animals will typically be singly housed, undergo behavioural tests, may have blood taken, and tissue sampling under anaesthesia such as fin clip. The fin clip is the collection of the very tip of the tail fin under brief anaesthesia. The fin tail grows back shortly afterwards. Taking a fin clip does not interfere with swimming.

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

The vast majority of animals will undergo well-established procedures and will experience no adverse effects.

When new mouse lines are created or new crosses are bred we do not know the effects these will have on the offspring. Occasionally, these new lines may generate offspring with delayed developmental growth and the offspring may be born with a lower birth weight compared to their wild-type litter mates. Sometimes these mice with a lower birth weight regain a normal weight during adulthood.

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)?

Mice: Sub-threshold - up to 60%

Mild - up to 30%

Moderate - up to 10%

Zebrafish: Sub-threshold - up to 60%

Mild - up to 25%

Moderate - up to 15%

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

- Kept alive
- Used in other projects
- Killed

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?

We are performing experiments to assess the health of the whole organism. Our observations include multiple body systems and organs working together in a living animal, and multiple generations which we explore as part of our objectives. Such experiments cannot be performed in a dish which usually only allows studying simple systems and very few cell types. Epigenetic states in a dish are very different to their natural state in the living animal, and currently are unable to recapitulate the physiological status.

We are studying mechanisms and pathways in a developing organism in embryonic and adult stages, therefore, we need to look at different time points throughout development. As cells in a dish are exact copies of each other this means that changes occurring in developing cells cannot be seen.

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

We use non-animal alternatives as much as possible before moving on to animal models. Such alternatives include cells in a dish experiments, which allow us to answer simple questions without a live animal. We perform initial experiments in cells to test some of our hypotheses and choose the best candidate genes and pathways to focus on before moving on to animal experiments. This way, we can reduce the total number of animals we use.

In addition, we use cells to mimic as much as possible developmental processes in dishes. Such technologies make it possible to study early developmental processes, such as embryonic development.

To reduce animal work, we use publicly available sources and re-analyse existing data wherever possible with computer-based work and analysis.

Why were they not suitable?

Very often, the non-animal alternatives are not suitable. We cannot conduct experiments that address the health of an entire organism in the dish, which lacks the complexity and the interactions occurring in a physiological context.

Epigenetic states differ very significantly between cells in a dish and a living organism and to be able to conduct experiments that produce reliable and relevant data, we must use animal models.

When studying maternal-offspring interactions, there are no alternative non-animal methods that are sophisticated enough to address behavioural, hormonal, epigenetic and physiological questions which are very important aspects of our research programme.

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?

We have estimated the numbers based on our work over the past 30 years. Key members of our team have enough knowledge and experience for their research field. We estimated the numbers depending on their projects using robust statistical analysis software on data generated from initial experiments using a small number of animals or cells in a dish (pilot studies) or from past experiments to allow us to determine our sample size.

Our research requires the use of large numbers of animals. The nature of our work focuses on the differences inherited from the mother and father. In order to see such differences, we need to follow the transmission from the mother and father in separate breeding pairs, which doubles our animal numbers for every experiment.

Additionally, in many settings of breeding pairs, we only need to study either males or females, for example when studying mammary gland biology, which means we only use 50% of the animals that are born.

Moreover, many experiments only focus on a certain mutation which is only carried by 25% of the animals that are born.

For these cases when many animals that are born will not be used in such experiments, we have a sophisticated communication platform within the lab, to ensure maximal usage by other researchers, for example for breeding, pilot experiments, tissue collection.

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

We use online tools such as NC3Rs EDA https://www.nc3rs.org.uk/experimental-design-assistant-eda and PREPARE guidelines https://norecopa.no/PREPARE, and NC3Rs website https://nc3rs.org.uk/3rs-advice-project-licence-applicants-reduction . Additionally, we consult collaborators or experts when we need to design new experiments in which we need to acquire additional expertise.

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

1) efficient breeding

We work closely with animal technicians in the facility, who will advise us if breeding pairs are not producing any litters or producing fewer litters. Furthermore, all breeding information is recorded in our online colony management system, where we record all animals and their procedures. Therefore we will notice immediately if breeders have problems.

2) sharing of animals, tissue samples, and cell lines

We communicate regularly to share animals, tissue samples, and cell lines. We use shared files to summarise and share the information of stock tissue samples and cell lines. All animal information has been recorded in colony management system, therefore animal users can easily check which animals are available.

We share animals, tissues and cell lines with our collaborators worldwide. This means that others do not need to generate new mutants or samples, minimising animal numbers.

3) preservation of mouse strains

We freeze sperm or preimplantation embryos from mouse strains that are not planning to use at least for a couple of years. We have deposited some lines into The European Mouse Mutant Archive which are made available to the scientific community.

4) pilot studies

Where possible we conduct initial studies on cells in a dish or with a small number of animals to determine whether experiments are then required in larger numbers. Occasionally our scientific questions can be answered by laboratory based experiments alone.

We optimise protocols with small-scale experiments with wild-type samples before conducting experiments using mutants.

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 work on mice as it is a well-established system to genetically manipulate this organism as well as to maintain controlled breeding. It is also possible to carry out phenotypic analysis of mice. Due to the genetically homozygous background of laboratory mice, genetic background effects do not affect analysis and make it easier to interpret results with fewer animals being required. The mouse genome has been well studied and characterised, therefore protocols for embryo and reproductive manipulations are well-established and safe for the mouse.

Many of our new mouse lines have been generated using CRISPR technology, which can generate multiple mutations using a single targeted event. This therefore requires the use of less mice and speeds up the generation of new mouse models. Mutations can then be selected for and an extensive breeding scheme can be avoided.

We also work on zebrafish which share a high degree of genetic and tissue similarities to mammals. As zebrafish lay as many as 200-300 eggs per mating, fewer animals are required per breeding programme. The eggs are laid outside the body therefore offspring can be studied whilst avoiding harm to the mother.

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

Our research aims to assess mammalian gene function therefore the mouse is the best model for our work. Due to the lack of imprinting in species such as the zebrafish, we cannot use alternative species for most of our research. Our studies aim to look at the function of imprinted genes throughout development so immature life stages cannot be studied alone.

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

We will use well established protocols and will carry out pilot studies before starting procedures. Procedures will be discussed with experienced collaborators to minimise welfare costs for the animals. Any animal that has been through a procedure will continuously be monitored at regular intervals examples include observing changes in locomotion (zebrafish), signs of gasping at the surface of water (zebrafish), sitting at the bottom of the tank (zebrafish), scoring sheets during procedural changes,

weighing (mice), heat mats (mice) and providing post operative bedding (mice). When appropriate, pain management such as the use of medicated palatable substances for voluntary treatment, e.g. flavoured jelly, paste or milk shake liquid will be given to mice or appropriate pain-relief drugs will be dissolved in the water for zebrafish.

Mice are typically group housed with enrichments such as chew blocks, tunnels, mouse houses and nesting material. Zebrafish are typically group tanked and provided with enrichment if needed. We are always open to new suggestions by the NACWO for enrichments, such as running wheels etc.

If possible, for any new mouse lines, inducible constructs will be used so that mice only display a phenotype (visible physical characteristics such as behaviour or growth and appearance) once the deletion is induced. We will refine the humane end point for new mouse lines and will seek advice from the Vet and the Home Office Inspector if needed.

In the case of cross-fostering (protocols 7 and 8), we identified a phenotype that presents as a hunched posture. Normally, a hunched posture may indicate that a mouse is ill or in distress. However, we have noted a gradual appearance of a hunched posture as a result of feeding abnormal milk composition. Therefore, we have excluded hunched posture as a humane endpoint and added extra monitoring and support in case of additional clinical signs.

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

Our lab has been conducting embryological work over the last 30 years, therefore we have published protocols which ensure experiments are conducted in the most refined way. For any new procedures we will review published protocols from experienced collaborators before conducting any experiments.

We use ARRIVE guidelines to ensure our experiments are performed in the most refined way, and for the responsible reporting of results. This includes a thorough study design, sample size, inclusion and exclusion criteria, randomisation, blinding (where possible), outcome measures and state of the art statistical methods.

We use PREPARE guidelines which includes three areas that determine the quality of the preparation of animal studies:

1. Formulation of the study (literature, ethics, design etc)

2. Discussions between us and the animal facility (objectives, time-scale, facility evaluation, training and health assessment)

3. Methods (test substances, quarantine and monitoring, housing and husbandry, procedures and humane end point)

Specifically, for zebrafish we will ensure that our experiments are conducted in the most refined way, according to NC3Rs https://nc3rs.org.uk/zebrafish-welfare and enrichment provided if needed such as artificial plants as recommended by the Zebrafish Husbandry Association https://zhaonline.org/.

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

We will regularly discuss with the Named Persons at our institution on the best practices and new approaches in the 3Rs, including using the NC3Rs website pages https://nc3rs.org.uk/resource-hubs. We have subscribed to the NC3Rs e-newsletter to receive information on the latest 3Rs developments as well as information on NC3Rs events and workshops, which we can attend. We consider the latest practical guidance from Laboratory Animal Science Association (LASA), and the Royal Society for the Prevention of Cruelty to Animals (RSPCA). Additionally, we use external resources such as Jackson labs database https://resources.jax.org/. Lastly, we will maintain contact with our local NC3Rs Regional Programme Manager for advice on implementing advances in the 3Rs.