INTRODUCTION

Motor Neurone Disease (MND) is a fatal, rapidly progressing neurodegenerative disease that affects the brain and spinal cord. It kills a third of people within a year and more than half within two years of diagnosis. Six people are diagnosed every day and there is currently no cure and the only approved drug in the UK works only for up to 12 months.

The UK MND Collections is an internationally recognized and unique resource set up in 2003 to assist researchers in finding the causes behind MND. >3000 people with living MND/lausie controls provided blood samples (DNA was extracted using Nucleic BAC protocols) along with clinical/phenotypic data, between 2003 and 2012.

Lymphoblastoid cell lines (LCLs) were also produced from the white blood cells of the majority of these samples.

An epidemiology survey was conducted on 200 participants, and 200 separately matched controls. Data examples include: motoric health, employment and environmental exposures.

Sample collection concluded and the resource became fully accessible in 2012 with >50 papers published to date and data/samples shared with over 20 countries across the world.

OBJECTIVES

- All biological samples have a minimum dataset: age at sample taken, gender, affection status, disease duration CE/Stat status and age of onset and survival years where available. Access to additional clinical/laboratory/pathological data is via application.

- Epidemiology study data collected by a self-report questionnaire, followed by a telephone interview with a research nurse.

- Samples/data are free to access for academic/nor for profit organisations (a small admin and shipping charge from CSAM and ECACC applies).

- Samples were anonymised and an ID code allocated (which is used in our database) on the patient notes (stored securely at our centres).

- CSAM operates under IS09000:2000 standards. DNA normalized to stocks of 100ng/g and stored at -80°C.

- ECACC operates under ISO 9001:2005 standards. Collected under HS licence 12114. Cell lines are split into a familial (inherited) range (available on the ECACC website) and a sporadic (no familial range) multiple. Aliquots of each sample were produced. Master and working cell banks for the familial range were produced to prevent phenotypic drift. These have been used to create induced pluripotent stem cells (iPSCs) (Poster 2).

DATA ENRICHMENT AND DEVELOPMENT

- Original samples and data have been further added to by projects using Genome Wide Association Studies (GWAS), Whole Genome Sequencing (WGS), Exome Sequencing, methylation, SHP and MicroRNA analysis.

- Samples can be used in projects of related conditions, such as fronto-temporal dementia (FTD).

- Participant consent allowed for commercial use (unusual for MND samples), meaning international companies approach us for sample use.

- All data produced must be made available by the researcher on a publicly accessible database -6 months after publishing. Some types of data e.g. gene mutations present in our samples, are sent back to us for other researchers to access

- Governance – the MND Association acts as custodian for the Collections, access decisions are made by our Biomedical Research Advisory Panel (BRAP) and the principal investigators of the Collections, no researcher may attempt to contact any participants, full T&Cs available

- We have a dedicated section of our website with details of samples/data and the application process, as well as an email address for enquiries (mndcollections@mdna.org).

- Sample/data access is achieved usually between 3 weeks and 3 months after the successful submission and approval of a completed application form

ACKNOWLEDGEMENTS AND REFERENCES

The setup and running of the Collections is funded by the MND Association (including anonymous donors) with additional funding provided by the Wellcome Trust. We would like to thank people with MND and their families for donating samples and associated data, as well as all researchers and colleagues included in the set up and running of the UK MND Collections.


CASE STUDY 2

Treatment and Characterisation of Induced Pluripotent Stem Cells from MND

INTRODUCTION

A target of Three Peripheral Blood Lymphocytes (PBL) samples were made per patient blood sample (during original sample collection phase), one PBL was converted into a lymphoblastoid Cell Line (LCL) to ensure an everlasting supply of DNA for the UK MND Collections. The remaining two PBLs were stored as back ups for any issues with the cell lines.

In 2012, Sir John B. Gurdon and Shinya Yamanaka were awarded the Nobel Prize in Physiology or Medicine “for the discovery that mature cells can be reprogrammed to become pluripotent.”

This has opened the door for a large numbers of researchers in many different diseases to be able to look at a model of their disease in the tissue that the disease affects, directly from patients who have the disease. This acts to not only reduce the number of animal models that are used, but also provides the ideal model for looking at the disease instead of using animal models as a proxy.

Professor Chris Shaw’s team at King’s College London are using the Lymphoblastoid Cell Lines (LCLs) from the MND Collections to create Induced Pluripotent Stem Cells (iPSCs) to model motor neurone disease. As part of the approval for their application to use the LCL, the MND Association’s Biomedical Research Advisory Panel (BRAP) said they were confident that Professor Shaw’s team also create iPSCs directly from PBLs of the same patient samples for a number of the LCLs to provide a direct comparison between the two techniques.

OBJECTIVES

- To create iPSC lines from 30 LCLs with known genetic mutations from within the MND Collections. These are able to be converted into MotorNeurons (MNs) and SupportCells that act as a model to understand what is killing the motor neurones, as well as a model for testing new therapeutic drugs directly on motor neurones that are affected by MND.

RESULTS SO FAR

iPSC lines have been successfully made from 29 (LCL) and 5 PBLs (this is one of the first times that iPSCs have been created from PBLs for MND sourced from the MND Collections. We believe that this is the largest collection of iPSCs created from blood cell lines in the UK as fibroblasts are more commonly used and the only collection to have metting genomic DNA and extensive clinical data for that sample.

ENGAGEMENT AND DISSEMINATION

The iPSC collection has been presented at:

- a number of internal meetings (both at King’s College London and at the MND Association)
- as a poster at two KCL Dementia Institute Research Symposium
- as a poster at the 2IP internship Symposium on MNDRALS in Glasgow in 2018
- as a poster at the ENALS meeting in Edinburgh in 2018

A further update on the project and iPSC collection will be presented as a poster at the 3IP international Symposium on MNDRALS in Perth, Australia later this year.

NEXT STEPS - RESEARCH AND FUNDING

The protocols used for the creation of the iPSCs have been modified from already published protocols, and a paper is currently being drafted to discuss the iPSC collection, their characterisation and the modified protocols.

Work is being conducted to compare the iPSCs created from LCLs and PBLs with a paper being drafted on the work to date. The success of being able to create iPSCs direct from PBLs has potential for future clinical applications.

The work contributed to several successful funding applications, including support from the My Name’s Doddie Foundation. Future work on the iPSCs will look at pathways related to MN death and drug testing directly on MNs.

The project contributed the majority of work as a PhD for a student in Prof Shaw’s lab.

FUTURE COLLABORATION

Professor Shaw’s team are currently collaborating with a private company in America who are accessing some of the MND Collections LCLS to create iPSCs. They have been able to share all their relevant protocols to assist the company and to avoid duplication of effort, Professor Shaw’s team have also agreed to supply control PCLs that they have already created. This application was approved via the MND Association and represented the first time the MND Collections has been used by a nonacademic organisation. This provided the opportunity for us to create a template structure and documents for future commercial interest.

Professor Shaw’s team are collaborating with a researcher from Huddersfield who wishes to use the iPSCs; this is also the subject of a current research grant application to the MND Association which Professor Shaw’s team are also assisting with.

Discussions are underway for a number of collaborations for the use of the iPSCs between Professor Shaw’s team and other research teams at King’s College London.

All of the iPSC lines created as part of this project will be deposited at ECACC, 2 lines have already been deposited (and characterisation datasheets provided), with the remainder being deposited by the end of this year. All lines will be available to international researchers via the MND Collections application process by January 2021

iMotor Neurones and iSupport Cells

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