The UK MND Collections – Cell Line Samples

A biorepository of samples and clinical/epidemiology data from people living with Motor Neurone Disease

THE UK MND COLLECTIONS

MND Collections

- MND Collections DNA Bank
- MND Collections Cell lines
- MND Collections Epidemiology data

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 extends life by up to 3 months.

The UK MND Collections[1] evolved from the UK MND DNA Bank [2], which is an internationally recognised and unique resource set up in 2003 to assist researchers in finding the causes behind MND.

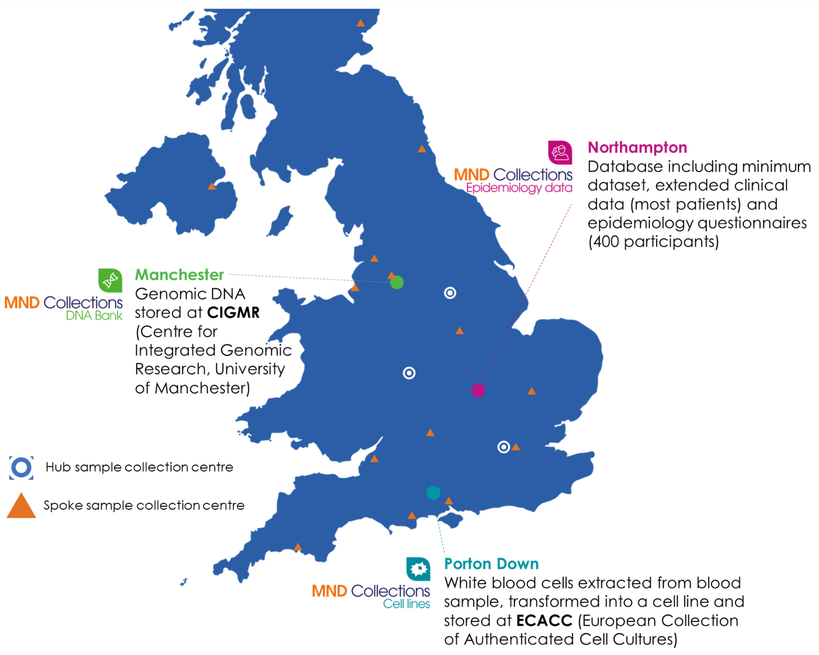
>3000 people living with MND/spouse controls provided blood samples (DNA was extracted using Nucleon BACC3 protocol), along with clinical/phenotypic data, between 2003 and 2012.

Lymphoblastoid cell lines (via Epstein Barr virus transformation) 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: lifestyle, 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.

SAMPLES AND DATA



- All biological samples have a minimum dataset: age at sample taken, gender, affectation status, diagnostic certainty (El Escorial status) and age of onset (and survival years where available). Access to additional clinical[3] and epidemiological[4] 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/not for profit organisations (a small admin and shipping charge from CIGMR and ECACC applies).
- Samples were anonymised and an ID code allocated (which is used in our database) on the patient notes (stored securely at Hub centres).
- CIGMR operates under ISO900:2000 standards. DNA normalised to stocks of 100ng/µg and stored at -80°C.
- ECACC operates under ISO 9001:2015 standards under HTA license 12114. Cell lines are split into a familial (inherited) range (searchable on the ECACC website)[5] and a sporadic (non-inherited) 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, SNPs and MicroRNA analysis.
- Samples can be used in research 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.

OBJECTIVES

- 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 participant, full T&Cs available[6].
- 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@mndassociation.org).
- Sample/data access is achieved usually between 3 weeks and 3 months after the successful submission and approval of a completed application form[7].

RECENT SAMPLE USAGE

Year of application	Number of applications received	Number of applications approved	Number of samples or data accessed
2015	4	4	4057
2016	5	5	640
2017	3	3	1215
2018	4	4	201

CASE STUDY 2 - Generation and characterisation of induced pluripotent stem cells from MND lymphoblasts to model disease pathogenesis and advance drug discovery – Professor Chris Shaw, King's College London

INTRODUCTION

A target of three Peripheral Blood Lymphocyte (PBLs) samples were made per patient blood sample (during original sample collection phase), one PBL was converted into a Lymphocyte 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.

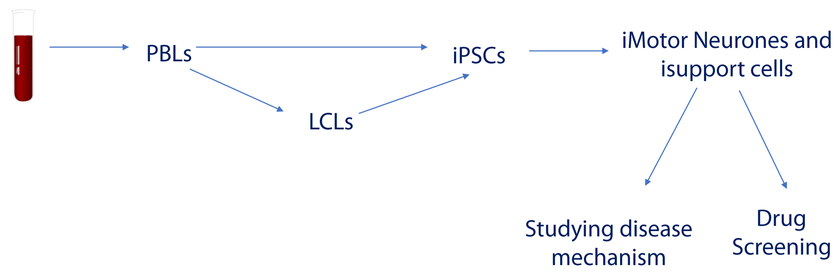
In 2012, Sir John B. Gurdon and Shinya Yamanaka were awarded the Nobel Prize in Physiology or Medicine "for the discovery that mature cells can be reprogrammed to become pluripotent."

This has opened the door for a large number 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 Lymphocyte 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 LCLs, the MND Association's Biomedical Research Advisory Panel (BRAP) requested 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 iMotor Neurones (iMNs) and iSupport Cells 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 LCLs 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 matching 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 Symposia
- as a poster at the 29th International Symposium on MND/ALS in Glasgow in 2018
- as a poster at the ENCALS meeting in Edinburgh in 2018.

A further update on the project and iPSC collection will be presented (as a poster) at the 30th International Symposium on MND/ALS 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'5 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 for a PhD for a student in Prof Shaw's lab.

FURTHER COLLABORATION

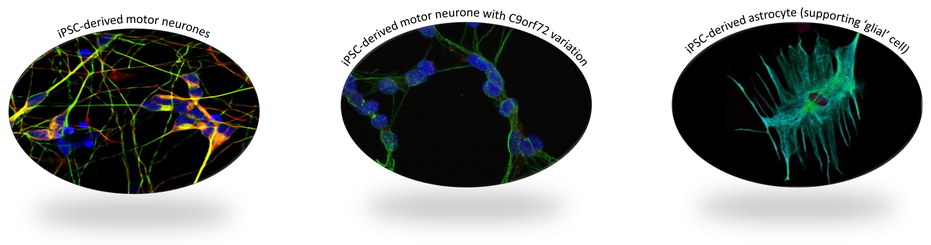
Professor Shaw's team are currently collaborating with a private company in America who are accessing some of the MND Collections LCLS to create iPSCs. They have been able to share all their relevant protocols to assist the company and to avoid duplication of effort, Professor Shaw's team have also agreed to supply control iPSCs 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 non-academic 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 teams at King's College London.

All of the iPSC lines created as part of this project will be deposited at ECACC, 6 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



ACKNOWLEDGEMENTS AND REFERENCES

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1) https://www.mndassociation.org/research/for-researchers/resources-for-researchers/ukmndcollections/, 2) Smith et al. BMC Genetics Establishing the UK DNA Bank for motor neuron disease (MND) (2015) 16:84, 3) www.mndassociation.org/extdataset, 4) www.mndassociation.org/epidataset, 5) https://www.phe-culturecollections.org.uk/products/celllines/diseaseandnormalcohortcollections/search.jsp , 6) www.mndassociation.org/collectionsterms, 7) www.mndassociation.org/applicationdna