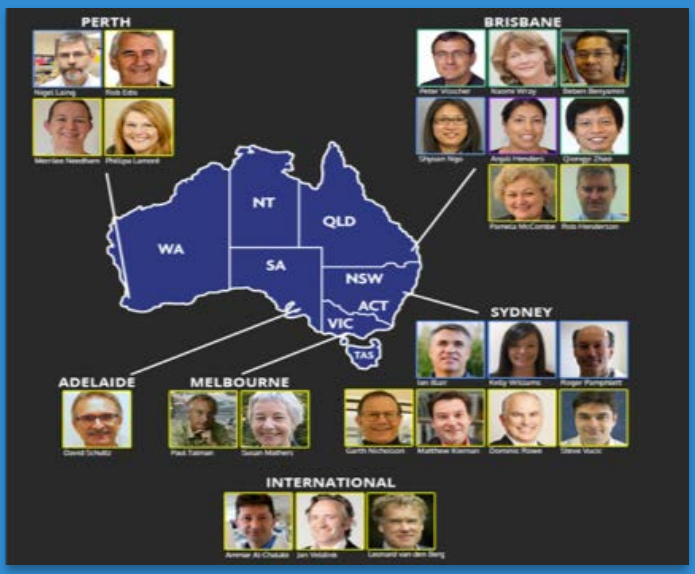
The Sporadic ALS Australian Systems Genomics Consortium



Final Report

MND Australia
Ice Bucket Challenge Grant
2015-2019

Supported By:

Institutions:

Royal Brisbane and Women's Hospital
Metro North Hospital and Health Service

The University of Queensland Australia | Create Change

Macquarie University

MND Australia Ice Bucket Challenge Grant 2015-2019:
The Sporadic ALS Australia Systems Genomics Consortium

Final Report Executive Summary

There are many things that we don't understand about MND/ALS:

- What are the causes of MND/ALS?
- Why are do some people progress faster/slower?
- Why is there such a diversity in clinical presentation such as site of onset?
- Why do some people tolerate riluzole better than others?

These are difficult questions, and data - clinical, lifestyle and biological - are the key to generating evidenced-based answers. Data can only be accumulated if individuals, both those with and without MND/ALS, provide specific consent to participate in research.

For many other diseases and disorders there are already large international data resources of clinical information and biological samples. Blood samples are easy to collect and can be very informative. From blood we can measure:

- genetic risk factors
- epigenetic risk factors, which might show signatures of environmental exposures
- biomarkers - gene expression, lipids, proteins, cell types, inflammatory markers.

Prior to 2015, less than one-third of those with MND/ALS in Australia contributed to a long-term linked clinical data and biological data resource. Such a resource has the potential to underpin many current and future research projects. Moreover, this data resource and data capture is needed to make Australia attractive for future international clinical trials.

The MND Australia Ice Bucket Challenge Grant administered by the Motor Neurone Disease Research Institute Australia (MINDRIA) funded the establishment of the Sporadic ALS Australia Systems Genomics Consortium. Building on the strong research base and collaborations that already existed, SALSA-SGC brought together the seven major MND clinics to establish consistent collection of longitudinal clinical information and biological samples across all major clinics in Australia. The SALSA-SGC has successfully reached the milestones of the research proposal, and has been contributed to attracting > $1.5M additional funding for ALS/MND research. The strong foundations laid down will contribute to new research for decades to come, although additional long-term funding is needed to deliver its full potential.

SALSA Consortium

1 April 2016 - 31 May 2019

Total Cases: 596 Total Controls: 223

	QLD	VIC	SA	WA	MQC	BMC	WMD
	Cases 131 / Controls 80	Cases 170 / Controls 0	Cases 33 / Controls 0	Cases 111 / Controls 0	Cases 132 / Controls 143	Cases 16 / Controls 0	Cases 3 / Controls 0
VISITS	LAUNCHED 06/04/2016 Primary Visits 131 Subsequent Visits 278	LAUNCHED 02/11/2016 Primary Visits 170 Subsequent Visits 202	LAUNCHED 30/01/2018 Primary Visits 33 Subsequent Visits 29	LAUNCHED 04/05/2016 Primary Visits 111 Subsequent Visits 217	LAUNCHED 01/04/2016 Primary Visits 132 Subsequent Visits ~290	LAUNCHED 08/05/2018 Primary Visits 0 Subsequent Visits 0	LAUNCHED 03/12/2018 Primary Visits 3 Subsequent Visits 0
BIOLOGICAL SAMPLES	Cases 109 / Controls 80 Total Collns 305 / Total Collns 84	Cases 165 / Controls 0 Total Collns 354 / Total Collns 0	Cases 32 / Controls 0 Total Collns 60 / Total Collns 0	Cases 109 / Controls 0 Total Collns 294 / Total Collns 0	Cases 132 / Controls 143 Total Collns 428 / Total Collns 286	Cases 16 / Controls 0 Total Collns 23 / Total Collns 0	Cases 3 / Controls 0 Total Collns 3 / Total Collns 0
GENOMIC DATA	Cases: GWAS: 53, MWAS: 54, WGS: 65, Mutation Panel: 103 Controls: 36, 37, 41, 36	Cases: GWAS: 129, MWAS: 30, WGS: 0, Mutation Panel: 163 Controls: 0, 0, 0, 0	Cases: GWAS: 17, MWAS: 0, WGS: 0, Mutation Panel: 28 Controls: 0, 0, 0, 0	Cases: GWAS: 78, MWAS: 10, WGS: 0, Mutation Panel: 95 Controls: 0, 0, 0, 0	Cases: GWAS: 0, MWAS: 0, WGS: 0, Mutation Panel: 0 Controls: 0, 0, 0, 0	Cases: GWAS: 14, MWAS: 0, WGS: 0, Mutation Panel: 16 Controls: 0, 0, 0, 0	Cases: GWAS: 1, MWAS: 0, WGS: 0, Mutation Panel: 2 Controls: 0, 0, 0, 0

SALSA Consortium

DATA:

Clinical N (Unique Cases) = 596

BIOLOGICAL RESOURCE:

CASES: DNA, Plasma, Serum, Buffy Coat N (Unique Cases) = 527

CONTROLS: DNA, Plasma, Serum, Buffy Coat N (Unique Controls) = 199

SYSTEM GENOMICS DATA:

GWAS N = 328 / MWAS N = 131 / WGS N = 106 / ALS Mutation Panel N = 443

Site Legend		
RBWH	QLD	Royal Brisbane and Women's Hospital
CHCB	VIC	Calvary Health Care Bethlehem
FMC	SA	Flinders Medical Centre
SCGH	WA	Harry Perkins Institute for Medical Research
FSH	WA	Fiona Stanley Hospital
MQC	NSW	Macquarie University
BMC	NSW	Brain and Mind Centre
WMD	NSW	Westmead Hospital

GWAS (genome-wide association study); MWAS (DNA methylation-wide association study)

* GWAS and MWAS were completed for 406 MQC individuals who were recruited prior to the commencement of SALSA.

Introduction 3

SALSA-SGC 3

Establishing an Australia-wide data collection and data reporting platform 4

Establishing a consistent collection of biological samples 5

The important role of research nurses 6

What is systems genomics in the SALSA-systems genomics consortium? 7

Sample collection – what has been achieved? 7

Integration with Australian MND Registry (AMNDR) 8

Genomic data 8

Additional Funding 9

Research results 11

Future Plans 12

Outcomes 12

APPENDIX 14

1. THE TEAM 14
2. BUDGET 17
3. RESOURCES 18

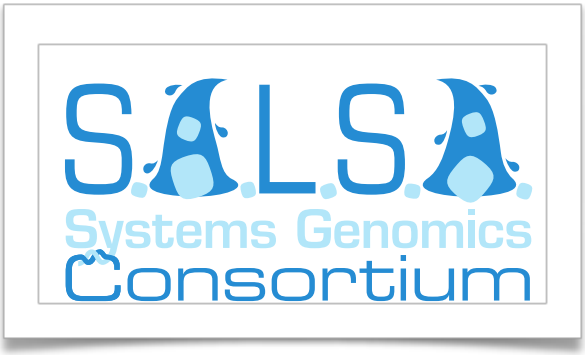
Introduction

In August 2014 the ALS/MND Ice Bucket Challenge swept the world to raise awareness of motor neurone disease (MND) and to raise funds for research. More than 2.4 million tagged videos circulated on Facebook featuring an assortment of people gasping in the wake of a bucket of ice and water dumped over their heads. In Australia, over $3 million was donated to support MND research and care. Donations from 30,000 Australians generated $1.05 million for MND research administered through the MND Research Institute of Australia (MNDRIA). MNDRIA decided to award a single grant – their largest-ever single award – to a multi-site project that was to focus on sporadic ALS/MND conducting research in humans. This approach was forward-thinking in three ways. First, it recognised that biological research of ALS/MND to date focuses on the clues derived from the families with multiple affected members for which causal gene mutations have been identified (such as *SOD1* or *C9orf72*). The goal, therefore, was to focus on what could be learnt about ALS from the >90% of those with ALS/MND who do not have this form of the disease. Second, it recognised that collaboration is a key to successful human research, and so this grant could facilitate Australia's role in growing international consortia and collaborative studies. Third, it recognised that research in people is costly, and that to make a long-term disruptive difference to research in Australia requires investment into strong foundations for future research to build on.

SALSA-SGC

(the Sporadic ALS Australian Systems Genomics Consortium)

The MND Australia Ice Bucket Challenge Grant was awarded to establish the Sporadic ALS Australian Systems Genomics Consortium. Building on the strong research base and collaborations that already existed, SALSA-SGC brought together the seven major MND clinics to establish consistent collection of longitudinal clinical information and biological samples across all major clinics in Australia. From the outset SALSA-SGC had a long-term vision. The goal was to set up a foundational infrastructure that would underpin human MND research in Australia, and facilitate our role in international studies, for the next decade or more. MND is a heterogeneous disorder with unknown causes for more than 90% of those diagnosed. Site and age of onset and rate of disease progression all vary between people. We live in an era of "big data" where patterns of factors can be extracted from large datasets which have meaningful interpretation. In some countries, big data resources for MND had already been established. The goal of SALSA-SGC was to enable

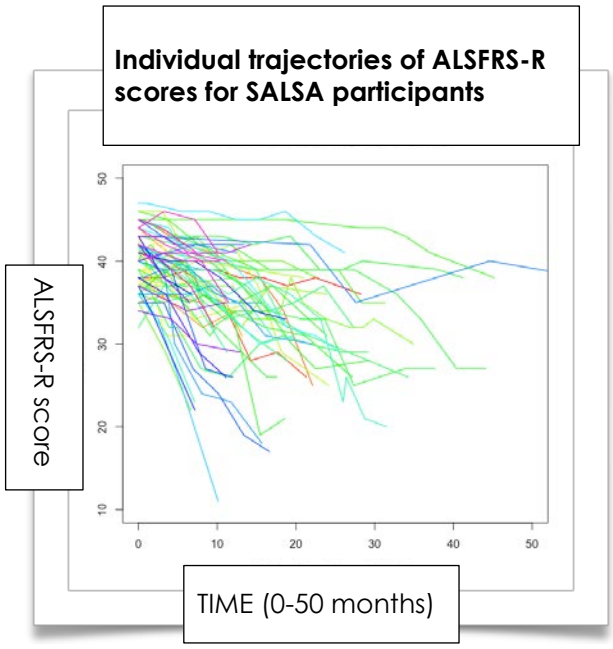


all Australians with MND to contribute to a big-data research resource that integrates clinical, lifestyle and biological information.

The long-term goal is that biological samples taken at the first clinic visit (or before) could be used to predict disease sub-types, which may be a key to the success of future clinical trials of novel therapies. Understanding the complex mix of genetic and non-genetic factors that contribute to MND may be a key for prevention. For many human diseases, there is a growing recognition that more personalised approaches to clinical care are needed, responding to the biological make up of each person, but data are needed to enable generation of evidence-based decisions for such individualised treatment. The last 10 years has witnessed incredible technical progress in our ability to take measurements from biological samples. It is clear that the next few years will see further progress. A resource of biological samples allows the research of the future to be conducted on people who have MND today.

Establishing an Australia-wide data collection and data reporting platform

A key aim of the SALSA-SGC consortium was to establish consistent collection of longitudinal clinical information of people with MND in order to establish a resource for current and future research. To achieve this goal we conducted in-depth consultations with the clinical teams at each site to understand their needs. A recurring theme was that there was little interest in having a standard "data-in" research database. Instead, a "data-in" and "data-out" clinical management tool would provide a "win-win" for contributing to long-term collaborative research resource while also being useful for local research and for clinical practice. A multiple-sclerosis data platform provided an example of the sort of data system that would be useful, and more importantly, would be clinically used.



Some features of the SALSA data collection and data reporting platform are:

- Data entry via online/tablet formats.
- Viewing and download of entered data is defined by login permissions, for example, differing between research nurses and clinicians.

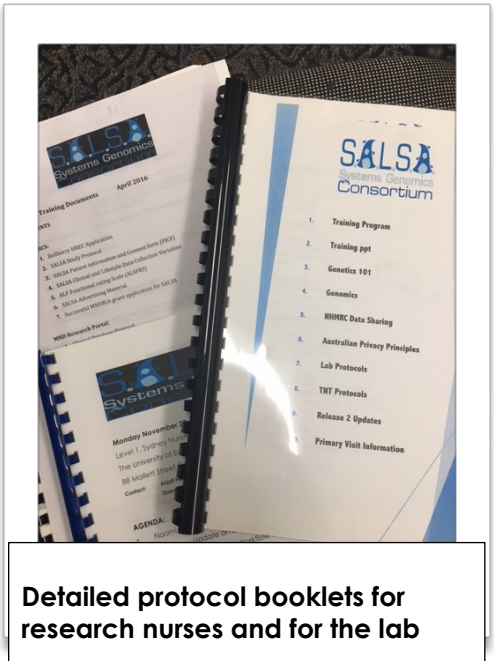
- A single system used across clinical sites, but with data access controlled by permissions, we achieve site-specific data storage and management, in line with institutional research governance requirements.
- Longitudinal tracking of clinical phenotypes for cases and asymptomatic participants.
- Longitudinal integration of biological sample collection.
- Specialist reports for individual patients including graphical tracking of ALSFRS-R.
- Specialist snapshot summary reports for individual clinical sites on demand, in real-time.
- Site specific monthly reports detailing participant recruitment and some clinical data.
- Ability to upload clinical reports in any format e.g., pdf to store with the directly entered data.
- Alignment with the Australian MND Registry (AMNDR)
- Upload of previously collected data by direct data upload.
- Ability to easily add site specific data entry for single/multiple site bespoke projects.

The Macquarie team have taken an independent instance of the SALSA Drupal framework, allowing them to embellish and bespoke the data entry, sharing their data into the joint database.

Establishing a consistent collection of biological samples

The key aim of the SALSA-SGC consortium was to establish consistent collection of biological samples from clinics across Australia that could be linked to the detailed clinical information of each subject. Several research clinics were already collecting biological samples, and so we aimed to harmonise protocols, recognising that consistent processing is a simple basic principle to ensure that future research using the samples is as unbiased as possible from technical artefacts. The long-term vision is that the SALSA-SGC will collect many types of biological samples (blood, urine, faeces, muscle), but the initial funding has allowed us to focus on blood sample collection. Processing of blood samples under standardised protocols is conducted at the University of Queensland (UQ), Harry Perkins Institute (HPI), WA and Macquarie University (MQ),NSW.

Samples collected at the Flinders Medical Centre, Calvary Health Care Bethlehem, University of Sydney Brain & Mind Centre, and Westmead Hospital are couriered to the laboratory at the University of Queensland. Retaining the link between a physical sample and database-generated clinical information is fundamental. All samples, and their daughter aliquots are barcoded which helps reduce human error associated with manual entry of sample IDs when biological measures are generated. Despite several research groups collecting biological samples prior to SALSA-SGC, barcoding of MND samples in Australia had not previously been implemented.



Detailed protocol booklets for research nurses and for the lab

Resources: All samples received at the UQ and HPI sites have had the following fractions collected and stored: 4 x 1.8mL EDTA plasma, 2 x buffy coats, 4 x 500µL serum. One buffy coat from the first visit collection is routinely extracted for genomics. All other samples are stored at -80C.
All samples at MQ have the following fractions collected and stored: 8 x 760µl plasma, 6 x 500µl serum, 1 x 10ml EDTA tube stored for DNA extraction, 1 x PAXgene tube stored for RNA extraction, 6 x 760µl urine.

We have developed and provided project protocol handbooks. Site audits were conducted at Macquarie and Harry Perkins labs mid-way through the project to ensure that all sites were following protocols in the same way.

Ethics

All research on human subjects is governed by guidelines overseen by human ethics research committees. The ethics considerations are complex for studies generating data from DNA. A multi-site study is particularly complex. The first HREC application presenting the basic project was submitted in April 2016. The SALSA-SGC protocol has now been approved by 8 HRECs with a further two sites (University Hospital Geelong and University of Auckland) expected to go live in the next 3 months. We have provided assistance and expertise in managing ongoing amendments and annual reporting where required.

The important role of research nurses

In all research projects the "devil is in the detail". Research nurses are the driving force of the SALSA-SGC protocol. Recruitment of participants requires detailed explanation of the participant information and consent form. High quality data is achieved through care in consistent application of the study protocol. Research nurses gathered in Brisbane in September 2016 to learn best practice. Monthly telcons with project manager, Ms Anjali Henders, helped to iron-out the problems as they arose. The care taken by the research nurses in working with the participants

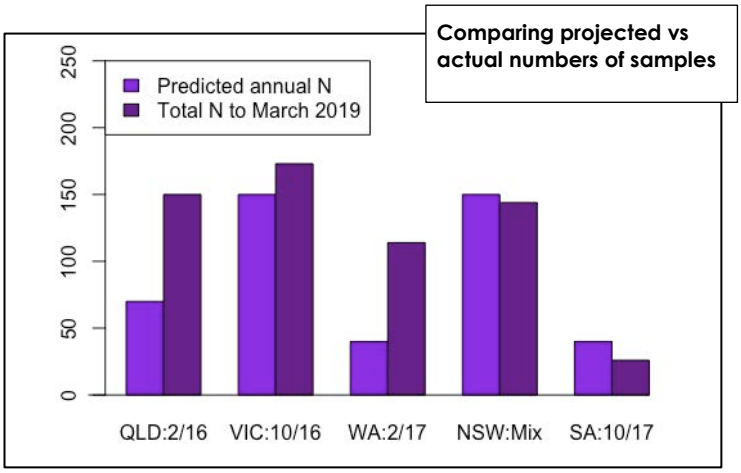
Research nurse training day

who have been willing to gift their time (and blood!) to the project have been key for success. Unfortunately, the funding of the SALSA-SGC only allowed for a 50% research nurse per state. Lack of research nurse resources accounts for the low recruitment from two research active Sydney clinics.

What is systems genomics in the SALSA-systems genomics consortium?

From the outset, the vision for the project was a resource that could generate biological measures that could be integrated with clinical measures. The vision is that the biological measures would be multiple layers of "omics" data generated for the same people. Firstly, genomics – measures of DNA variants across the whole genome – and which is the same in every cell. All other omics measures -epigenomics (measures of chemical changes superimposed on the DNA), transcriptomics (measures of expression of ~20,000 genes encoded by the genome), lipidomics (measures of up to ~650 lipids), metabolomics (measures of up to 2~,000 metabolites), proteomics (measures of proteins) differs between cell types, with age and in response to the environment. Systems genomics implies integration of these omics measures to understand how biological pathways change as a consequence of disease and to find patterns representative of different biological sub-types of disease. To build the foundations for this long-term vision, the SALSA-SGC was funded to generate genomic and DNA methylation epigenomic data from whole blood.

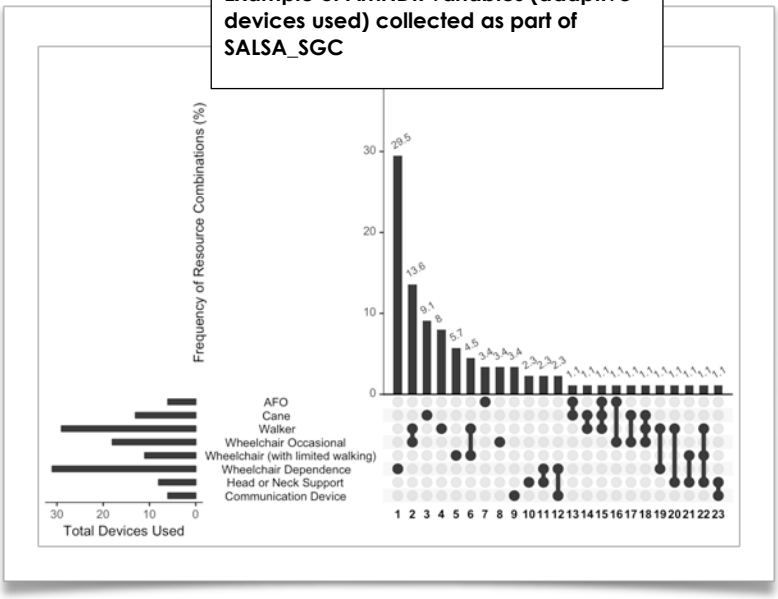
Sample collection – what has been achieved?



The graph on the right shows recruitment by state compared to annual recruitment as projected in the SALSA-SGC grant application. The application also recognised that there would be a phased roll-out of protocols. The month/year date next to the state name represents the date that ethics approval was received for each site. For NSW we have stated 'mix' for date of ethics approval as NSW includes three clinical sites. For the Macquarie site, the SALSA-SGC protocol was a simple addition to the existing ethics approvals, whereas ethics approvals were not achieved for the University of Sydney Brain & Mind Centre until March 2018 and Westmead Hospital until September 2018. The latter two sites have only contributed 19 samples. In total we have deposited blood samples from 651 cases, 230 controls, a total of 1967 sample collections and storage of > 22,000 sample aliquots.

In the grant application we concluded that by the end of the project we would have banked 1,200 case samples representing both historically collected cases and cases collected under the SALSA-SGC protocol. In fact, we have exceeded this target as we will have > 1500 MND participants with genomic data.

Example of AMNDR variables (adaptive devices used) collected as part of SALSA_SGC



Integration with Australian MND Registry (AMNDR)

The SALSA data portal was designed to be aligned with AMNDR so that data need only be entered once and shared with AMNDR. While AMNDR is a de-identified data collection system, SALSA retains personal identifying data (securely stored) to allow down-stream linkage with other forms of data. Analysts of data are only supplied with de-identified data.

Genomic data

The long-term vision of SALSA-SGC is to use genomic data to understand the causes and heterogeneity of MND. The grant funding was to establish the foundations of a research resource that would leverage future additional funding to continue sample collection and generate 'omic data.

SNP array data: The MND Australia Ice Bucket Challenge Grant included funding to generate genome-wide single nucleotide polymorphism data on 200 cases and 200 controls for genome-wide association studies (GWAS) analysis at a cost of ~$50/sample. A GWAS tests for differences between cases and controls of the frequency of common DNA variants, testing about 1 million independent variants. Based on experience from many diseases and disorders, we know that very large samples are needed for GWAS and so this sample would be too small as a stand-alone study. Current published GWAS comprise 20K cases, and 50K controls, and have identified six risk loci associated with sporadic ALS. The only way large well-powered GWAS are achieved is by each country contributing their small under-powered studies. Based on MND Australia Ice Bucket Challenge Grant

Illumina Global screening array measures >650K DNA

funding and previous NHMRC funding we have contributed a sample 973 cases, 759 controls for inclusion in the next international GWAS. Another 646 SALSA-SGC participants, all those recruited up to Feb 2019 are currently having GWAS data generated.

***DNA methylation data**:* The MND Australia Ice Bucket Challenge Grant included funding to generate genome-wide DNA methylation data on 200 cases and 200 controls for DNA methylation-wide association studies (MWAS) analysis, at a cost of ~$350/sample. An MWAS tests for differences in the proportion of cells that are have methylated DNA sites, considering 800,000 probes. To date, only small MWAS have been conducted for ALS. Based on MND Australia Ice Bucket Challenge Grant funding and previous NHMRC funding we have generated MWAS data on 827 cases and 730 controls. MWAS data on the 400 SALSA-SGC samples are currently being generated.

***Whole genome sequence data**:* The MND Australia Ice Bucket Challenge Grant included funding to generate whole genome sequence data on 150 cases and 50 controls for Project Mine at a cost of $1500/sample. Project Mine is an international project led by our collaborators in Utrecht that aims to generate whole genome sequence data on 15,000 MND cases and 7,500 controls and identify genes harbouring rare DNA variants associated with MND. NHMRC has funded generation of whole genomes from the MQ cohort. Since our application, the price of WGS has increased to $2000/sample. Hence, 70 cases and 40 controls were submitted to the Kinghorne Institute at the Garvan (to be consistent with MQ samples). The data were made available in May 2019 and are being submitted to the Project Mine study.

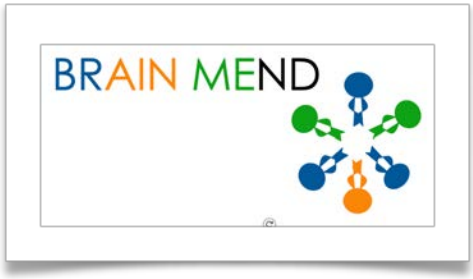
Additional Funding

Several research applications have already built on the MND Australia Ice Bucket Challenge SALSA-SGC Grant.

i) *Halpin Trust Grant*. $20K While the research focus of SALSA-SGC is genomics, we well-recognise that ALS is a multifactorial disorder and non-genetic risk factors likely play a causal role. A number of environmental risk factors have been reported for ALS, but other than military service, reported risk factors have not been replicated. It is clear that more data are needed. We were successful in our application to a UK-based charity to set up an online questionnaire to record environmental risk factors. The questionnaire has now been approved by 2 HRECs and is due to be launched in June 2019.

ii) *NHMRC-EU-JPND BRAIN-MEND,* $800K to Australian research. The Biological Resource Analysis to Identify New Mechanisms and phenotypes in Neurodegenerative Disease (BRAIN-

MEND) is an international study led by Professor Ammar Al-Chalabi (a SALSA-SGC Associate Investigator). Naomi Wray (SALSA-SGC co-PI) leads the Australian component, which focuses on shared DNAm signatures across neurodegenerative disorders. The SALSA-SGC DNAm data with contribute to this project.



iii) *NHMRC Partnership Grant* The successful application for the NHMRC Partnership Grant was directly motivated by a desire to extend the SALSA-SGC protocols beyond the lifetime of the MND Australia Ice Bucket Challenge Grant. The Partnership Grant, led by Professor Matthew Kiernan (SALSA-SGC Chief Investigator), brings together many clinicians (including all SALSA-SGC chief investigators), researchers and community groups building on many existing collaborations to use the data collected to inform future policy.

iv) *MNDRIA grant-in-aid* 2017-2018 $100K. "GWAS data for SALSA-SGC". In the MND Australia Ice Bucket Challenge Grant we focussed on collection of ALS/MND cases. However, genetic studies require comparison of cases with controls. This additional funding, allowed us to also extend our data collection focus to controls. The Halpin Trust funding allowed us to join this control sample collection with environmental questionnaire data collection and so the a major emphasis for control sample collection will begin in June 2019.

v) *MNDRIA grant-in-aid* 2017-2018 $100K. "Longitudinal assessment of behaviour and cognition in ALS through brief Online Carers' behavioural Questionnaire (OCQ)". Recognising that a long-term goal of SALSA-SGC is to develop genomic predictors of disease progression, we asked A/Professor Gail Robinson at UQ if she could develop an online version of the OCQ that forms part of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) that is usually only administered face-to-face, and hence could not be deployed at all sites. This successful grant application is allowing us to validate the OCQ, which we plan to roll out to all participating SALSA-SGC sites in the future.

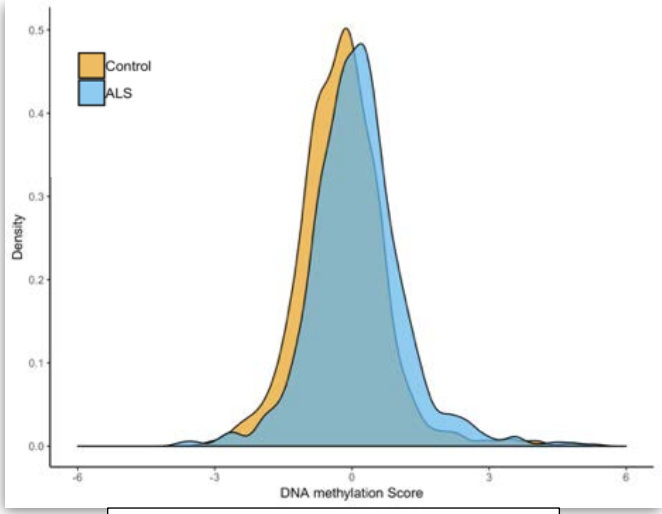
We believe that the SALSA-SGC framework has contributed indirectly to several other successful grant applications. For example, we updated the data entry portal to support the collection of muscle biopsies for the FightMND iPSC project of A/Professor Bradley Turner at Florey Institute. We also updated the data entry portal to support tracking of participation in clinical trials and we developed a mutation screening panel for identification of ALS monogenic mutations and the UNC13A variant associated with disease progression. These features were noted in the successful Medical Research Futures Fund Clinical trials application led by Professor Matthew Kiernan.

Research results

The MND Australia Ice Bucket Challenge Grant application was designed to build a long-term research resource and to generate the first genomic data sets from the data, recognising that the sample sizes that were funded were too small as stand-alone studies. Moreover, most of the data generated by the MND Australia Ice Bucket Challenge Grant have not yet been analysed. However, a small number of the SALSA-SGC samples have contributed to research that is being submitted for publication.

Genetic risk prediction: We have used the published genome-wide association study (GWAS) data to generate risk predictors which we have applied into our Australian cohort of 973 cases and 759 controls. Our results show evidence for negative selection on ALS variants, which provides important information about genetic architecture and how to design future studies. It suggests that rare genetics are likely much more important for ALS than for other common multifactorial diseases. The genetic risk predictor can be considered as a diagnostic biomarker. Our risk predictor was improved by including GWAS results from cognitive performance, demonstrating that genetic risk factors associated with reduced cognitive performance in heathy people are also associated with ALS/MND. The predictive ability is small (Area under the curve (AUC), probability of ranking a case higher than a control of 0.56) but highly significant. In future it could be combined with other biomarkers to develop a clinically useful predictive tool.

Epigenetic risk prediction. We have conducted the largest DNAm genome-wide association study (MWAS) for ALS to date, 827 cases and 730 controls. From DNAm data we are able to predict blood cell counts in cases and controls. We found evidence of an inflammation response, with high neutrophils and lower CD4-Tcells and natural killer cells in cases compared to controls. This pattern was replicated in an independent Dutch sample (from SALSA-SGC associate investigators, Professors Jan Veldink and Leonard van den Berg). In total, we achieved risk prediction from the Australian sample to the Dutch sample of AUC =0.69, demonstrating that with larger discovery samples DNAm could contribute as a biomarker to a future diagnostic prediction tool.



Differences in DNA methylation scores between Dutch cases and controls, with the scores derived from effect sizes estimated in Australian samples

Future Plans

The goals of SALSA-SGC were long-term. We hope that we have established an infra-structure that will continue. The NHMRC Partnership Grant provides some funding for research nurses to continue data entry and collection of biological samples, but realistically, additional funding (including additional funding for research nurses) is needed to maximise what can be achieved. Future infrastructure plans, which are funding dependent (unless otherwise stated) include:

- Online environmental questionnaire for those with MND and controls (June 2019)
- Formal integration of SALSA-SGC and AMNDR data collection (Partnership Grant Aim)
- Linking with the MND App being developed as part of the Partnership Grant
- Online ECAS Carers' behavioural questionnaire, made available around Australia if the funded pilot study indicates useful results.
- Mutation screening panel to identify those carrying known monogenic ALS mutations (and UNC13A rate of disease progression polymorphism), likely needed as inclusion/exclusion criteria for future clinical trials.
- Collection of biological samples other than blood (urine, faeces), including longitudinally, likely needed for better understanding of disease causes and progression.
- Update of blood collection/processing protocols to allow generation of cell lines including iPSC lines from all participants, likely needed for future precision medicine research.

Outcomes

The projected outcomes written in the MND Australia Ice Bucket Challenge Grant application are listed below in italics. For each of the four outcomes we comment on achievement.

1) Increased collaboration between ALS groups within Australia which will enable a coordinated study of sporadic ALS now and in the future. The successful application for the NHMRC Partnership Grant is an example of achieving this outcome.

2) Standard operating procedures for collection of phenotype and biological samples between groups within Australia and co-ordinated with data collection procedures implemented internationally through Project MinE. The SALSA-SGC data collection platform was designed in close collaboration with users and carefully followed data collected by international studies such as Project MinE. Five out of seven clinical sites are actively using the platform. The two sites not using the platform received very limited funding for implementation; hopefully this will change since the NHMRC Partnership Grant provides research nurse funding for all sites.

3*) Identification of new risk loci and pathways for ALS with new insights into underlying biology. Both QLD and NSW have a battery of experimental paradigms for evaluating functionality of associated variants.* This is a long-term goal, and with the samples collected to date, and with the

genomic data generated, the Ice Bucket Funded data collection will contribute to new genetic discoveries of the next decade.

4) A significant contribution to Project MinE. The cost of whole genome sequencing increased from time of application to generation of data. Instead of the 100 cases and 50 controls included in the application we were only about to generate WGS data on 70 cases and 40 controls. These have been submitted to Project MinE, and every contribution is significant in achieving the goal of 15,000 cases and 7,500 controls. Currently, Project MinE has achieved 48% of its goal.

In summary, the MND Australia Ice Bucket Challenge has achieved the outcomes set out in the 2015 application, and much more.

The data collected will continue to contribute to ALS/MND research for years to come.

A framework has been established which we hope will provide strong foundations for data collection in ALS/MND in Australia, and our role in international collaborative studies, for the next decade and beyond.

APPENDIX

1. THE TEAM

SALSA-SGC is a very large collaborative team effort. Most important are the patients and the carers who are willing to take the time to participate in research and are willing to provide biological samples, which we hope will provide a resource for research for many years.

Co-Principal Investigators

Professor Naomi Wray

Professor Ian Blair

Chief Investigators in alphabetical order

Dr Beben Benyamin, University of Queensland (now University of South Australia)

A/Professor Robert Henderson, Royal Brisbane & Women's Hospital

Professor Matthew Kiernan, University of Sydney

Professor Nigel Laing, University of Western Australia

Professor Susan Mathers, Calvary Care, Bethlehem Hospital, Melbourne, Victoria

Professor Pamela McCombe, University of Queensland Centre for Clinical Research

Professor Garth Nicholson, ANZAC Research Institute

Professor Roger Pamphlett, University of Sydney

Professor Dominic Rowe, Macquarie University

Dr David Schultz, Flinders Medical Centre

Professor Peter Visscher, University of Queensland

Professor Steve Vucic, Westmead Hospital, NSW

Dr Kelly Williams, Macquarie University

Dr Qiongyi Zhao, Queensland Brain Institute, University of Queensland

Associate Investigators

Professor Ammar Al-Chalabi, King's College London, UK

Professor Jan Veldink, Utrecht Medical Centre, Netherlands

Professor van den Berg, Utrecht Medical Centre, Netherlands

Other key researchers not named on original application

Dr Fleur Garton, University of Queensland

Dr Shyuan Ngo, University of Queensland

Dr Frederik Steyn, University of Queensland

Project Manager

Anjali Henders has been key to the success of SALSA-SGC. She has overseen the implementation of SALSA-SGC and has designed the logic of the data collection portal and supervised the

programming. She ran the training sessions for research nurses, designed the laboratory protocols, and fields the questions. Attention to detail has been the key to success.

Research Nurses

Royal Brisbane and Women's Hospital
Susan Heggie
Kathryn Thorpe
Robyn Tuffley
Calvary Health Care Bethlehem
Ruth Krasniqi
Emma Windebank
Hanne Files
Susan Caldwell
Charmaine Hovey
Yana Wu
Harry Perkins Institute
Mandi Mac Shane
Macquarie University
Susan D'Silva
Lorel Adams
Flinders Medical Centre
Marie Toubia
Jessica Zuvela
Susan Hopkins
Sydney Brain & Mind Centre, University of Sydney
Elizabeth Highton-Williamson
Nicollette Thornton
Westmead Hospital
Julie Ryder
Linda Mekhael

Laboratory Managers

University of Queensland: Leanne Wallace
Macquarie University: Sarah Furlong
Harry Perkins Institute: Elyshia McNamara

Analysts/Programmers

Madhura Bhadravathi Lokeshappa
Leonie Gough

Presentations that included reference to SALSA-SGC

International:

2017 New York University Nature Genetics, Nature Neuroscience Conference on Neurogenetics, New York, **USA**

2017 University of Pennsylvania Distinguished Seminar Series, **USA**

2018 Wellcome Trust Genomics of Brain Disorders, Cambridge, **UK**

2018 Karolinska Institute Distinguished Seminar Series, **Sweden**

2019 Cold Spring Harbour Distinguished Seminar Series, **USA**

National:

2016 MND Connect, Brisbane

2017 MND Connect, Sydney

2018 Fight MND, Melbourne

2018 Australasian Genomics Technology Association, Adelaide

2018 Melbourne Brain Meeting

2018 National MND Conference

2018 QIMR Berghofer Seminar

2. **BUDGET**

Here we reproduce the budget page provided in the grant application. The total row sums have not deviated significantly from this budget.

In particular we point out that the whole project was achieved with only ~$75K coming to the leading UQ site for project management and the development of the data collection tool, ethics applications, logistics, training of research nurses and general support on a day-to-day basis. The project has benefited from significant in-kind support from UQ in leading SALSA-SGC. Moreover, each site has contributed in-kind support to achieve the reported outcomes. The project represents excellent value-for-money.

	YEAR 1	**YEAR 2**	**YEAR 3**
Senior Project Manager 0.2 FTE	$23,709	$24,420	$25,132
Research Nurse (QLD): Y1: 0.5, Y2 & Y3: 0.25 FTE	$43,122	$22,208	$22,855
Research Nurse (NSW): 0.5 FTE	$43,122	$44,416	$45,710
Research Nurse (WA): Y1: 0.5, Y2 & Y3: 0.25 FTE	$43,122	$22,208	$22,855
Research Nurse (VIC): Y2 & Y3: 0.5 FTE		$44,416	$45,710
Research Nurse (SA): Y2: 0.5 & Y3: 0.25 FTE		$44,416	$22,855
Data collection consumables	$4,950	$2,294	$2,812
Data collection consumables (NSW)	$7,425	$3,441	$4,218
Data collection consumables (WA)	$4,950	$2,294	$2,881
Data collection consumables (VIC)		$6,953	$4,703
Data collection consumables (SA)		$4,635	$3,136
Travel between sites	$5,200	$8,240	$8,268
Attend International Motor Neurone Disease Meeting	$4,000	$4,000	$4,000
Project Implementation costs	$10,400	$183	$15,616
Generation of genomic data	$160,000	$115,875	$119,250
Total	$350,000	$350,000	$350,000

All salaries include 27% oncosts and expenses increment at 3% inflation/year

Administration of funds. Funding will be received by the host institution of CIA, The University of Queensland (UQ). A multi-institution agreement will be signed by 4 CIs (NSW: CIB Blair, WA CI Laing, VIC: CI Mathers, SA: CI Schultz). Funds will be disseminated to these CIs each year (as indicated by the state acronyms in the table) when funds are received by UQ. Dissemination of funds within each state will be co-ordinated by these CIs particularly in support of collection at clinics that are not currently biobanking or are expanding collections.

Justification *Senior Project Manager (SPM)* FTE salary Yr1: $118,545, Yr2: $122,101, Yr3: $125,658

SPM reports directly to CIA Wray, CIB Blair & AI Henders, will be based at UQ and will be responsible for preparation of the standard operating procedures for phenotype & biological sample collection.

***Research Nurses* FTE salary Yr1: $86,244, Yr2: $88,831, Yr3: 91,419**

We have allocated a 0.5 FTE to each centre in their set-up year (Yr1 for QLD, NSW and WA, Yr 2 for VIC and SA). We have reduced these positions to 0.25FTE for smaller centres (QLD, WA, SA) after their set-up year. These research nurses will report to their local CI and to the senior project manager, they will be responsible for local implantation of the standard operating procedures

Data collection consumables includes direct input devices for setup years, biological sampling consumables and basic sample processing prior to biobanking and/or shipment to NSW Macquarie biobank. Costs for Yr2 have been set at 0.9 of funds required to keep the total budget within the funding limit, additional costs will be contributed in kind by each site.

Travel between sites. We have budgeted for travel of the SPM & CIA/B to travel to each site in Yrs 1 & 3 to check implementation of the standard operating procedures. At the beginning of Yr 2 all research nurses will be brought together for a meeting at UQ so that new sites in Yr2 can learn from experience of Yr1 sites.

***Project Implementation costs*:** Costs needed to establish standard operating procedures in Yr 1. In order to retain Yr2 total costs within budget, Yr2 costs have been included into the Yr3 budget as UQ will allow small negative carryover between years.

Generation of genomic data. We have costed for genomewide SNP and DNA methylation typing of 200 cases and 200 controls in Year 1, assuming $400/sample as quoted by the UQ Centre for Clinical Genomics. In Year 2 and 3 we have costed for WGS of 75 samples/year, currently quoted at $1500 (inflated by 3%/yr).

Contributions in kind:

QLD and NSW will provide in-kind support for computerisation of data collection in collaboration with the AMNDR (Australian MND Registry) system.

NSW will contribute biobanking of samples collected at clinics who do not wish to self-biobank.

QLD will contribute in kind analysis of genotype and methylation data collected in Year 1.

QLD will contribute in kind analysis of WGS data collected in Year 2 and 3.

QLD will contribute in kind generation of new methods and software that may result from analysis of methylation and WES data.

QLD will contribute state of the art computer facilities and data storage (not trivial for WGS).

Each centre will contribute storage facilities for biological samples (-80C freezers) and consumables for collection of biological samples and in-house IT support to host the data collection software.

3. **RESOURCES**

https://hsu.imb.uq.edu.au/

http://cnsgenomics.com/salsa.html

https://www.neurodegenerationresearch.eu/wp-content/uploads/2017/10/JPND-Project-Fact-Sheet_2017_BRAIN-MEND.pdf


MACQUARIE UNIVERSITY
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THE AUSTRALIAN SCHOOL OF ADVANCED MEDICINE
MMI
Main Entry
Parking P

June 2019