International Centre for Genomic Medicine in Neuromuscular Diseases (ICGNMD)

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www.ucl.ac.uk/icgnmd

48 month Progress Report (June 2022-May 2023)

International Centre for
Genomic Medicine in
Neuromuscular Diseases

ICGNMD Year 4 Report

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1. Director's Summary

At year 4 of 5 we have already recruited 7,574 participants from diverse global populations which are currently heavily under-represented in Neuromuscular Diseases (NMD) and wider genomic research. Indeed ~90% of all existing human genomic data is derived from European ancestry and this programme is building a valuable genetically diverse cohort. Our cohort progress has significantly exceeded our original MRC-agreed 5 year target by over 2,500 participants, with 12 months still remaining. This is despite 12 months of COVID-related delays and demonstrates the effectiveness of the global networked team of NMD experts we created. We anticipate we will achieve at least 10,000 vs a 5000 target at year 5. We believe that this represents the world's largest diverse cohort of NMD patients with both detailed phenotype and correlated genotype data.

I am delighted to report an important recent publication in press in Brain 2023 which describes the first 6001 participants along with the detailed results of the first 1,400 genetic analyses.

Comprehensive analysis of the dataset demonstrates that the platform we established has resulted in ~56% "solved/possibly solved" rate for probands across all NMD disease categories. Importantly 29% of variants considered pathogenic are novel underlining that this diverse cohort is yielding new genetic knowledge. More importantly we continue to build a large highly phenotyped cohort that do not have a genetic cause despite whole exome analysis. Arguably, this group is especially important and we are now undertaking whole genome analysis in partnership with Illumina. We have detected population-specific differences in the genetic causes of NMD, with implications for diagnostics and applicability of current and future therapeutics.

Our ICGNMD clinical fellows cohort are making excellent progress with extensive publications and have secured onward funding or faculty positions. As we enter our final year, we are working across partners to continue to deliver outputs in future years and plan applications for further funding.

Only with this MRC strategic award support have we been able to build this diverse LMIC-based cohort. In the next phase, which we are now planning, we are in a very strong position to focus on a new direction of genetic discovery research: building on our diverse unsolved discovery cohort and harder-to-reach UK patients from minority ethnic groups which we know have much lower solve rates compared to UK patients of European ancestry.

We will harness this new diverse cohort, alongside UK ~10,000 cohort we previously built in our MRC Centre 2012-2020 to identify new genes /genetic mechanisms, to study ethnicity-specific gene variations, and to evaluate potential of variants for RNA therapies. The new programme will close the current equality gap by genetically/mechanistically solving, and therefore enhancing diagnostic rates, within UK families of non-European ancestry, as well as globally.

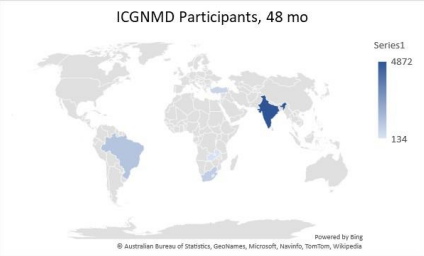

Figure: Recruitment by country at 48 months

Professor Michael G Hanna, ICGNMD Centre Director

2. ICGNMD Overview

(i) ICGNMD Aims and Objectives

The International Centre for Genomic Medicine in Neuromuscular Diseases (ICGNMD) launched in June 2019. Its mission is to create a transcontinental genomics research and capacity building partnership between the UK and multiple low-to-middle income partner sites, and to use this platform to build ethnically diverse genetic data, accurately define clinical phenotypes and study the genetic architecture of neuromuscular diseases. Approximately 90% of all existing genomic data is derived from European ancestry and this inequality is a missed opportunity to use diversity to fully understand gene function. Our work will enable us to more fully-understand the global genetic architecture of inherited neuromuscular disease with many benefits including scientific, diagnostic and ultimately therapeutic. Through developing an increasing number of genetically defined patients it will underpin “trial-ready” cohorts of patients around the world and ultimately improve the health outcomes for patients with this unmet health need, drawn from a combined population of over 1.5 billion people. The cohort of fellows we train are intended to deliver a locally enduring legacy of expertise to sustain outcomes well-beyond the award itself.

ICGNMD is comprised of 3 UK sites (UCL London, University of Newcastle and Cambridge University), 14 low-to-middle income partner sites in Brazil, India, Turkey, South Africa and Zambia and a specialist testing site for facioscapulohumeral dystrophy (FSHD) in Leiden, the Netherlands.

We defined seven Objectives:

1. Establish an international fellowship training programme to generate a group of highly trained clinical academics who will be a legacy of leaders in personalised genomic medicine in their countries. Fellows will establish enduring mutually beneficial collaborative scientific links with the UK NMD clinical and genetic expertise based in University College London (UCL), The University of Cambridge and Newcastle University.

2. Establish a core international bioinformatics platform, pipeline and cloud-based clinical and genetic database. Openly share phenotypic and genetic data, and encourage other LMICs to join this collaborative programme.

3. Build ethnically diverse genetic discovery and 'trial-ready' cohorts of children and adults with NMDs which we will deeply phenotype and comprehensively genotype.

4. Identify known and new disease genes, and assess comparative genetic architecture of NMDs across four continents. Use this knowledge to understand phenotypic variability, disease progression and disease mechanisms.

5. Increase the number of patients in LMICs with a precise genetic diagnosis enabling delivery of a personalised disease management plan, based on care guidelines to identify and manage complications using widely available and cheap interventions to improve health outcomes.

6. Enhance genetic diagnostics in UK-based Indian, Brazilian, Turkish & African NMD patients.

7. Build sustainability after five years, through retention of trained fellows in LMICs and through collaboration with host institutions, patient organisations, and local healthcare providers.

(ii) ICGNMD Leadership Team & Changes in-year (*)

The ICGNMD benefits from the expertise built during the 10-year MRC Centre for Neuromuscular Diseases (MRC CNMD, 2009-2019).

Principal Investigator: Professor Mike Hanna, UCL

UK Co-Investigators and additional linked, collaborating staff:

UCL – Institute of Neurology	
Professor Mike Hanna	Principal Investigator, UCL Lead ICGNMD Director
Professor Mary Reilly	Co-Investigator, Fellowships Lead ICGNMD Co-Director
Professor Henry Houlden	Co-Investigator, Clinical Genetics Lead ICGNMD Co-Director
Dr Rob Pitceathly	Co-investigator, Fellows Tutor & Database Design
Dr Alex Rossor, Dr Matilde Laura, Dr Ainara Salazar-Villacorta, Dr Francesca Magrinelli	Provides clinical expertise in neuropathies
Dr Enrico Bugiardini	Provides clinical expertise in muscle disorders
Dr Vinojini Vivekanandam, Dr Dipa Jayaseelan*, Dr Chiara Pizzamiglio, Professor Antonella Spinazzola	Provide clinical expertise in mitochondrial disorders and channelopathies
Dr Jana Vandrovcova	Lead Informatician
Dr Lindsay Wilson	Research Centre Manager
Dr Hallgeir Jonvik	Data Manager
Dr Heba Morsy	Informatician & Senior Lab Technician
Dr Stephanie Efthymiou	Informatician & Senior Lab Technician
Christine Oldfield	Administrative, web and events support
Zoe Scott	Administrative, web and events support
Natalia Dominik	Research Technician
UCL – Great Ormond Street Institute of Child Health (GOSH ICH)	
Professor Francesco Muntoni	Co-investigator, GOSH Lead ICGNMD Co-Director (UCL GOSH ICH)
Professor Thomas Voit	Co-investigator
Dr Anna Sarkozy	Co-investigator
Cambridge	
Professor Patrick Chinnery	Co-investigator, Cambridge Lead ICGNMD Co-Director (Cambridge)
Professor Rita Horvath	Co-investigator
Dr Fei Gao	Informatician
Newcastle	
Professor Volker Straub	Co-investigator, Newcastle Lead ICGNMD Co-Director (Newcastle)
Professor Robert McFarland	Co-investigator
Professor Robert Taylor	Co-investigator
Dr Ana Topf	Provides clinical expertise in muscle disorders
Dr Krutik Patel	Informatician
International Co-Investigators – Country/Site PIs	

India	
Professor K Thangaraj*	(Lead, India): Centre for Cellular and Molecular Biology (CCMB) & Director, Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad
Dr K Tallapaka	CCMB Hyderabad
Dr Ashwin Dalal	Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad
Dr P Govinderaj	CDFD, Hyderabad
Dr Sireesha Yareeda	Nizam's Institute of Medical Sciences (NIMS), Hyderabad
Professor Gayathri	National Institute of Mental Health & Neurosciences (NIMHANS), Bangalore
Dr BN Nandeesh	National Institute of Mental Health & Neurosciences (NIMHANS), Bangalore
Professor Nalini	NIMHANS, Bangalore
Professor Rohit Bhatia	All India Institute of Medical Sciences (AIIMS), Delhi
Professor Padma Srivastama	AIIMS, Delhi
Turkey	
Dr Yavuz Oktay	Izmir Biomedicine & Genome Centre (IBG), Izmir
Professor Haluk Topoloğlu*	Activities at Ankara City Hospital
Professor Uluç Yis	Dokuz Eylül University, Izmir
Professor Ayşe Semra Hız	IBG & Dokuz Eylül University, Izmir
Professor C Nur Semerci Gündüz	Ankara Bilkent City Hospital & Ankara Yıldırım Beyazıt University
Dr Gülay Güleç Ceylan	Ankara Bilkent City Hospital & Ankara Yıldırım Beyazıt University
Dr Ahmet Cevdet Ceylan	Ankara Bilkent City Hospital & Ankara Yıldırım Beyazıt University
Dr Büşranur Çavdarlı	Ankara Bilkent City Hospital
Brazil	
Professor Wilson Marques Junior*	University of Sao Paolo
South Africa	
Professor Francois van der Westhuizen*	North Western University, Potchefstroom
Professor Jo Wilmshurst	University of Cape Town
Professor Jeannine Heckmann	University of Cape Town
Professor Izelle Smuts	University of Pretoria
Dr Franclo Henning	Stellenbosch University
Zambia (University Teaching Hospital (UTH)	
Dr David Bearden*	University Teaching Hospital (UTH) Lusaka / Rochester USA
Dr Michelle Kvalsund	UTH Lusaka / Rochester USA (*2022 move from Michigan MSU*)
Dr Somwe Wa Somwe	UTH Lusaka
Dr Sylvia Mwanza- Kabaghe	UTH Lusaka
The Netherlands	
Dr Richard Lemmers & Professor Silvère van der Maarel	Leiden University Medical Centre (LUMC)

** Black asterisk = Designated Country Lead*

Work is currently underway to connect to expert teams at King's College London and University of Helsinki for collaboration on NMDs caused by *Titin* gene mutations.

3. Progress against funder milestones

Milestone Target 1 (**Objectives 2 & 3**)**:** Secure all required ethics approvals

Outcome: MET

By the end of Year 1, all local and national ethics were in place, with the exception of the all-India, overarching ethics clearance from the Indian Health Ministry Screening Committee (HMSC). This was required to enable entry of samples into the ICGNMD database and sharing of data internationally, even though all local Indian site ethics were in place.

The joint HMSC application was finalised in February 2020 and submitted just before lockdown, when HMSC's activities were suspended due to COVID-19. Its activities resumed in late 2020, with COVID-related applications given priority. Through the tenacity of our colleagues in AIIMS and CCMB, HMSC approval was obtained in March 2021. Since then, our Indian site partners have been working hard to upload recruited participant data into the REDCap database and subsequently ship samples to the Indian testing sites at CDFD and CCMB in Hyderabad for DNA extraction and testing.

Update: We recently reviewed all partner site ethics for confirmation that approvals remain valid and up-to-date.

Milestone Target 2 (**Objectives 1 & 7**)**:** Recruit 5 UK-based clinical fellows and 9 international clinical fellows

Outcome: EXCEEDED (6 UK Fellows & 11 International Fellows recruited as of June 2021)

2i: UK Fellows:

The UK Fellows meet weekly and have strong links to ICGNMD International Fellows with excellent peer to peer support. The UK Fellows partner with international Fellows to discuss (i) which is the best genetic analysis for the participants recruited and (ii) the significance of genetic variants detected.

(i) List of UK fellows

Site	Fellow & *Supervisors*	Details
UCL Institute of Neurology	Dr William Macken ***Sup:*** *R Pitceathly, M Hanna, J Vandrovcova*	PhD "*International Clinical Genomics & Mitochondrial Disorders*"
UCL Institute of Neurology	Dr Christopher Record ***Sup:*** *M Reilly, A Rossor, A Cortese*	PhD "*International Clinical Genomics and inherited neuropathies*"
UCL Institute of Child Health, GOSH	Dr Luke Perry ***Sup:*** *F Muntoni, A Sarkozy*	PhD "*Deep phenotyping and genotyping of rare and ultra-rare paediatric neuromuscular diseases; defining natural history and Identifying novel biomarkers with implications for diagnostic and translational purposes*"
Newcastle University	Dr Mahmood Fassad ***Sup:*** *R McFarland, R Taylor*	Post-doc in Neuromuscular Clinical Genomics
Newcastle University	Dr Christina Trainor ***Sup:*** *V Straub*	1 year Fellowship in Neuromuscular Disorders
Cambridge University	Dr Katherine Schon ***Sup:*** *P Chinnery, R Horvath*	PhD: "*Clinical and Genetic Aspects of Mitochondrial Disease*"

In-year changes to UK Fellows:

Dr William Macken, UCL: PhD completed June 2023

Dr Katherine Schon, Cambridge: PhD completed July 2023

Dr Luke Perry, UCL GOSH: PhD in submission by July 2023

Dr Chris Record, UCL: PhD in submission by July 2023

Dr Mahmoud Fassad, Newcastle: post-doctoral position ended. Dr Fassad is now developing his career in clinical genomics at University of Hospitals Leicester NHS Trust while maintaining ICGNMD activity via an honorary contract with Newcastle University.

Dr Christina Trainor, Newcastle: Dr Trainor completed a 1 year Fellowship before returning to complete her clinical training. ICGNMD activity at Newcastle continues to be delivered by the PIs with input from Senior Research Associate Dr Ana Topf.

2ii: International Fellows:

By June 2020 we had appointed 11 talented and enthusiastic international fellows (9 x MRC & 2 x funded by Guarantors of Brain), including 7 female international fellows. This year we have welcomed a new individual at **Cape Town: Dr Niki Floudiotis** has been awarded local funding for a Fellowship at Cape Town and her work will include ICGNMD cohort engagement.

As discussed in our Year 1 report, a range of bespoke Fellowship options have been developed with our partners to reflect the very different career structures in the different ICGNMD countries.

The Fellows have evolved genetic data interpretation and case discussion activity to include monthly “complex case” discussions that all Fellows (both UK and international) attend. The Chair rotates between different fellows each month. Fellows are also taking an increasing “leadership role” in conceiving, designing and delivering formal peer-peer training on specific topics.

(i) List of international ICGNMD fellows

	Country	Fellow	Details	Output
1	Brazil – Sao Paolo	R Frezatti	4-year Guarantors of Brain PhD fellow	*Clinical, electrophysiological and molecular characterization of patients with proximal non-5Q spinal amyotrophy.*
2	Brazil – Sao Paolo	P Tomaselli	4-year fellow (Faculty Fellow)	*Research outputs across a range of NMDs*
3	Turkey – IBG Izmir	I Polat	4-year PhD fellow	*Etiological factors & phenotyping-genotyping of congenital arthrogryposis*
4	Turkey – Ankara	O Koken	4-year PhD fellow	*The Mitochondrial Aminoacyl tRNA Synthetases and Their Diseases: The clinical and genomic landscape of Turkey*
5	Zambia – UTH	M Kapapa	3- year Physiotherapist PhD fellow	*Developing an evidence based Genetic Neuromuscular Diseases Management protocol for physiotherapists focused on physiotherapy practices, patients and family experiences in Zambia*
6	S Africa – Pretoria	I Smuts	(Senior Faculty Fellow/Co-I)	*Research outputs across a range of NMDs*
7	S Africa Cape Town	S Raga	4-year, 0.5 FTE PhD Fellow	*Centronuclear myopathy in Southern Africa*
8	S Africa – Stellenbosch	K Naidu	3-year, Guarantors of Brain PhD fellow	*Cohort characterisation of inherited myopathies in South Africa*
9	India – AIIMS	Vishnu V Y	4-year Faculty Fellow/Co-I	*Research outputs across a range of NMDs*
10	India – NIMHANS	M Nagappa	4-year fellow (Faculty Fellow)	*Research outputs across a range of NMDs: focus on neuropathies*
11	India - NIMHANS	BN Nandeesh	4-year Faculty Fellow/Co-I	*Research outputs across a range of NMDs: focus on mitochondrial disorders*

In-Year training & supervision for ICGNMD Fellows

Supervision:

Each International PhD Fellow has maintained:-

- **quarterly supervision meetings** with their local and UK-linked supervisors;
- **alternate supervision meetings** joined by Fellows' Tutor Dr Rob Pitceathly;
- **every 4th meeting** joined by Fellowships Director Professor Mary Reilly.

Written reports produced for every 2nd meeting, with contributions from Fellow and supervisors.

The International Faculty Fellows have **quarterly meetings** with Professor Reilly and Dr Pitceathly, to discuss overall and individual progress.

Training:

International Webinars: All Fellows continue to attend and present at quarterly ICGNMD "**International Grand Round Webinars**". Here, two ICGNMD Fellows present a consented NMD case of interest to other Fellows, ICGNMD Investigators and wider teams, as well as UCL trainees from across the Institute of Neurology, and the possible genetic diagnosis is discussed in depth.

The 2022/2023 schedule is below:

Date	**June 22**	**Sep 22**	**Dec 22**	**Mar 23**
Presenter 1	K Schon (Cambridge)	P Tomaselli (Brazil)	K Naidu (Stellenbosch)	M Kapapa (Zambia)
Presenter 2	Vishnu VY (AIIMS)	C Record (UCL)	I Polat (IBG Izmir)	R Frezatti (Brazil)

International Genetic Analysis Meetings: All Fellows and wider ICGNMD International Teams attend/present at ICGNMD "**International Genetic Test Planning Meetings**". When 15-30 probands accumulate in the ICGNMD database, this triggers the Fellows to prepare 1 powerpoint slide per proband, with summary data and proposed genetic test. The local team and a selection of UK ICGNMD Fellows & PIs then attend a 2 hour online meeting to discuss these cases and agree or suggest other testing for the proband. Later, when genetic test results are returned, similar "**International Genetic Analysis Meetings**" evaluate genetic variants detected, to determine if these have "solved" a case and/or have follow-on research value (e.g. via cross-site cohort analysis of a defined group of variants, or functional analysis of a specific variant's impact).

These meetings are a true ICGNMD "USP": they allow junior staff to present cases to local and international NMD world experts and have been very interesting and successful. The international PIs bring critical insight into local prevalence of different pathogenic variants and phenotypic variation, while disease-specific world experts provide insights into subtle phenotypic hints that a particular genetic variant may be responsible, as well as advising on any additional, non-genetic testing/phenotyping that may help to confirm genetic cause. Examples of some early outcomes of these discussions are listed above.

Training attended by ICGNMD Fellows and wider ICGNMD teams:

Event Type	Date
How to close cases in the Study database (1 afternoon)	27 July 2022
Using the Integrative Genome Viewer for in-depth variant checks (2 hours)	10 October 2022
WMS Congress, Halifax	12 October 2022: conference for Fellows Dr Vishny VY, Dr P Tomaselli, Dr R Frezatti and Dr Ozlem Koken, sponsored by World Muscle Society with linked interviews and social media
ICGNMD International Meeting 2023	28 March 2023, in-person meeting for ICGNMD teams to showcase progress cross site and plan future activities
ICGNMD PhD Fellows Retreat (pm/eve)	28 March 2023, PhD Fellows engaged in group activities and the following presentations: • **Drug Discovery** Prof Fiona Randall, UCL Drug Discovery Institute • **Media Engagement** Prof Giles Yeo, University of Cambridge • **Applying for grants** Dr Mariana Defino-Machin, MRC • **Career Development** Prof Francesco Tedesco – UCL and Crick Institute • **Exploring the diagnosis of rare conditions in the context of genetic ancestry** Dr Sam Tallman, Genomics England • **How to publish a paper** Dr Aisling Carr, UCL
CNMD Neuromuscular Translational Research Conference 2023 (#16)	29-30 March 2023: 2 day in person conference, with all Fellows submitting abstracts and invited speakers including ICGNMD PIs Professor Izelle Smuts (Pretoria) & Dr Richard Lemmers (Leiden)

2iii. Fellows' in-year publications:

See **Section 5** of this report for a list of Fellows' publications in the reporting year.

2iv. Wider support for international early career researchers:

We connect with, learn from, and/or train, a ***much larger cohort*** of motivated early- and mid-career clinicians. These are (i) directly involved in ICGNMD activity and (ii) invited to attend training. These currently include:-

Additional ICGNMD staff benefitting from Fellows' activities	
ICGNMD Partner	**Engaged in ICGNMD activity & attending Fellows' training**
NIMHANS, Bangalore	Dr P Govinderaj
NIMHANS, Bangalore	Ms Shivani Sharma
NIMHANS, Bangalore	Dr AB Taly
NIMHANS, Bangalore	Dr Yasha TC
NIMHANS, Bangalore	Dr Seena Vengalil
NIMHANS, Bangalore	Dr Nashi Saraswati
NIMHANS, Bangalore	Dr Vidhatri Ramaka
NIMHANS, Bangalore	Dr Kosha Srivastava
NIMS, Hyderabad	Dr Naveena Naeem
AIIMS, Delhi	Ms Alisha Reyaz
AIIMS, Delhi	Dr Rinkle Mishra
CCMB, Hyderabad	Dr Karthik Tallapaka
Ankara, Turkey	Dr Didem Ardicli
University of Pretoria, South Africa	Dr Ronel Human
University of Pretoria, South Africa	Dr Elsa Lubbe
University of Pretoria, South Africa	Dr Malebo Nonyane
University of Cape Town, South Africa	Ms Kaitlyn Sparks
University of Cape Town, South Africa	Mr Imraan Dixon
University of Cape Town, South Africa	Dr Niki Floudiotis
NWU, South Africa	Ms Michelle Mereis

Abbreviations: NIMHANS: National Institute of Mental Health & Neurosciences; AIIMS: All India Institute of Medical Sciences, NWU: North-West University

Milestone Target 3 (Objective 2 & 3): Create a bioinformatics platform, etc

3(i): Appoint 4 x ICGNMD bioinformaticians:

OUTCOME: MET (4 appointed)

We also connect with **international informaticians** (non-MRC funded) to assist clinical colleagues' data analysis. Those linking since the award's start are listed below and have attended ICGNMD meetings in addition to supporting informatics work.

Dr Melissa Nel's contribution at Cape Town has been especially impressive, implementing aligned analytical pathways within Cape Town and this year she was awarded a prestigious Wellcome CDA award for her project "*Harnessing the diversity of African genomes to drive novel disease gene and pathway discovery for Amyotrophic Lateral Sclerosis (ALS) Spectrum Disorders*". Her time during this award will be used to fully-establish a Neurogenomics lab within the University of Cape Town Neurosciences Institute. We are fortunate to have her input and experience to support ICGNMD Cape Town data analysis.

Newly-engaged international bioinformaticians	
NWU, South Africa	Dr Maryke Schoonen
NWU, South Africa	Dr Surita Meldau
Cape Town, South Africa	Dr Melissa Nel
Cape Town, South Africa	Ms Amokelani Mahungu
CCMB/NIMHANS	Dr P Govinderaj

3(ii): Informaticians engage with RD-Connect & attend training

OUTCOME: MET

See Year 1 report.

3(iii): Secure all agreements and ethics to enable clinical and genetic data capture in each LMIC.

OUTCOME: MET

Please refer to Year 1 and 2 reports. We are currently preparing data sharing agreements to bring teams at King's College London and University of Helsinki into ICGNMD for the purposes of collaborations around titinopathies and disorders linked to Nebulin.

3(iv): Implement SOP for all blood/DNA collection and processing in each LMIC.
3(v): Implement SOP for genetic analysis according to pipeline detailed in the case for support.

OUTCOME: MET

Please refer to the Year 1 report.

3(vi): Implement bioinformatics algorithm in line with RD connect system as outlined in case for support.

OUTCOME: MET in Year 1 with new, bespoke pipeline

Update: in year pipeline updates: This year we have refined the bioinformatics pipeline as follows:

(i) Added more **control population data** to the pipeline to improve variant frequency estimates from relevant populations;
(ii) Implemented improved **homozygosity mapping** as well as **splice site variant** and **UTR variant** detection;
(iii) **Dr Fei Gao** and **Dr Jana Vandrovcova** have implemented a "**bespoke Mitochondrial analysis**" option for Mitochondrial Disease participants with exome data, where mutation in the mitochondrial genome is suspected. This aims to provide enriched data on the presence and penetrance of mitochondrial DNA mutations;
(iv) **Dr Krutik Patel** and **Dr Jana Vandrovcova** are finalising "**bespoke SV/CNV analysis**" option for cases (especially complex disorders) with exome data, where structural variants or copy number variants are suspected.
(v) **Dr Heba Morsy** is finalising a bespoke pipeline for "intronic variant detection and prioritisation" to apply across the unsolved dataset, for example to detect "2nd hits" in recessive disorders and splice variants with predicted moderate or high functional impact.

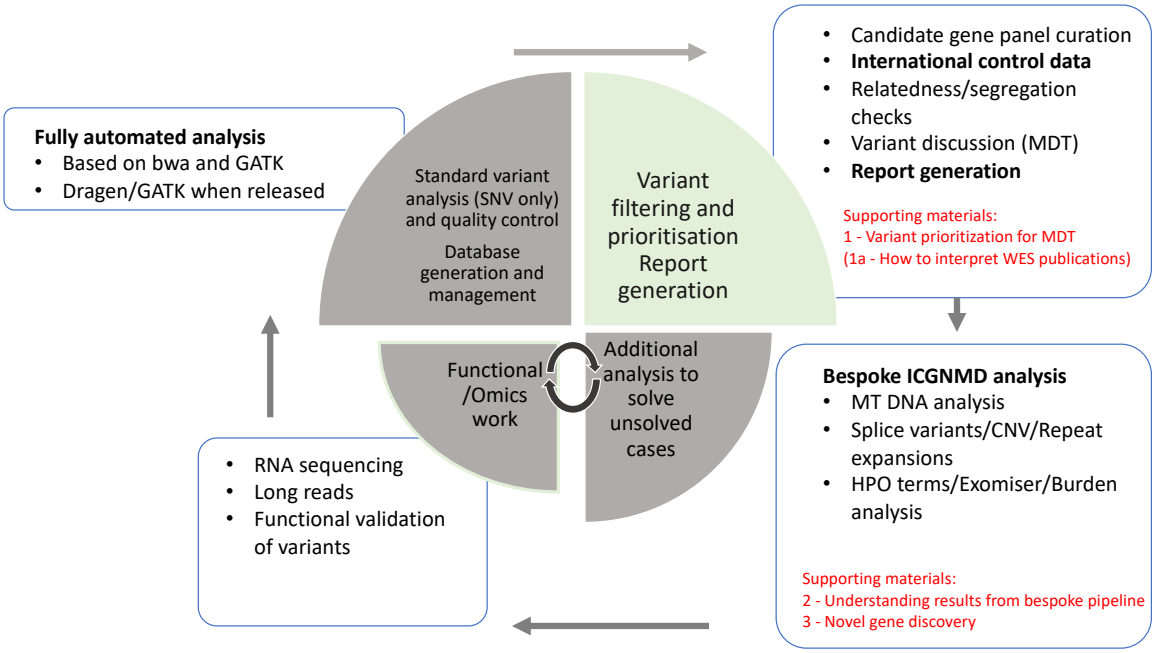

Figure summarising ICGNMD Informatics Strategy: please note that while functional/omics work listed is largely outside the scope of current ICGNMD funding, we will seek additional international funds to support.

3(vii): Commence clinical and genetic data capture, storage, sharing

OUTCOME: MET

Please refer to Year 1 report.

Update: in year data storage and sharing developments: We are semantically tagging each partner sites' genomic data files with usage restrictions (using "DUO codes") as part of our on-going work to archive data in the EMBL-EBI EGA. These terms will follow restrictions defined in local site Study Ethics documents.

We have also developed the framework for our ICGNMD Data Access Committee, which will review applications from the wider research community to access ICGNMD genomic and summary phenotypic data after the embargo period for internal research and IP exploration.

Milestone Target 4 (Objective 3): Recruitment of Study Participants

OUTCOME 4: EXCEEDED

	Annual Target	Accrued Target	Actual Accrued	Recruitment over Target
Year 1	200	200	**251**	51
Year 2	1100	1300	**1368**	68
Year 3	1200	2500	**4718**	2218
Year 4	1250	3750	**7574**	3824
Year 5	1250	5000	n/a	n/a

Breakdown of recruitment by site (as measured by database entries) is below:

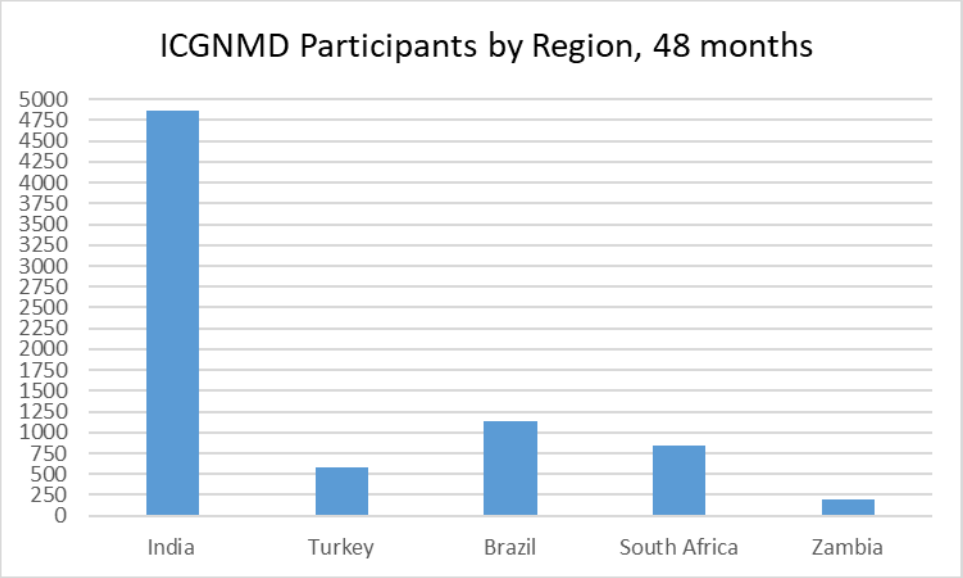

Figure 1: recruitment to date by country as of end-May 2023: only participants with completed REDCap data entries are counted

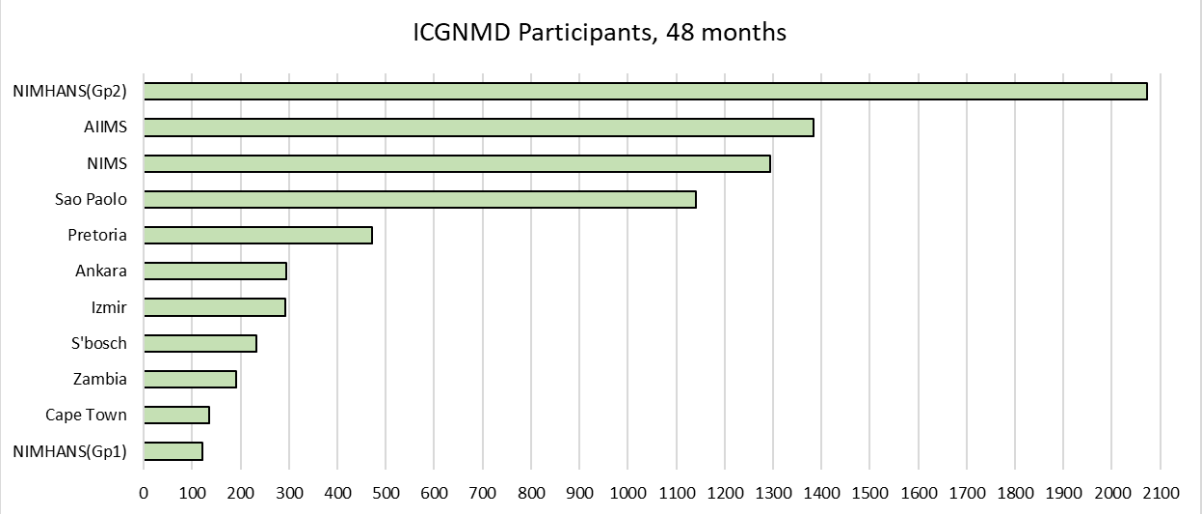

Figure 2: recruitment by site at end-May 2023. **Sites (top to bottom):** NIMHANS (India), Prof Nalini Group, AIIMS (India), NIMS (India), Sao Paolo (Brazil), Pretoria (S Africa), IBG Izmir (Turkey), Ankara (Turkey), Stellenbosch (S Africa), UTH Lusaka (Zambia), Cape Town (S Africa), NIMHANS (India), Dr Nandeesh Group.

As Figures 1&2 show, sites are performing well, with exceptional performance from several Indian sites.

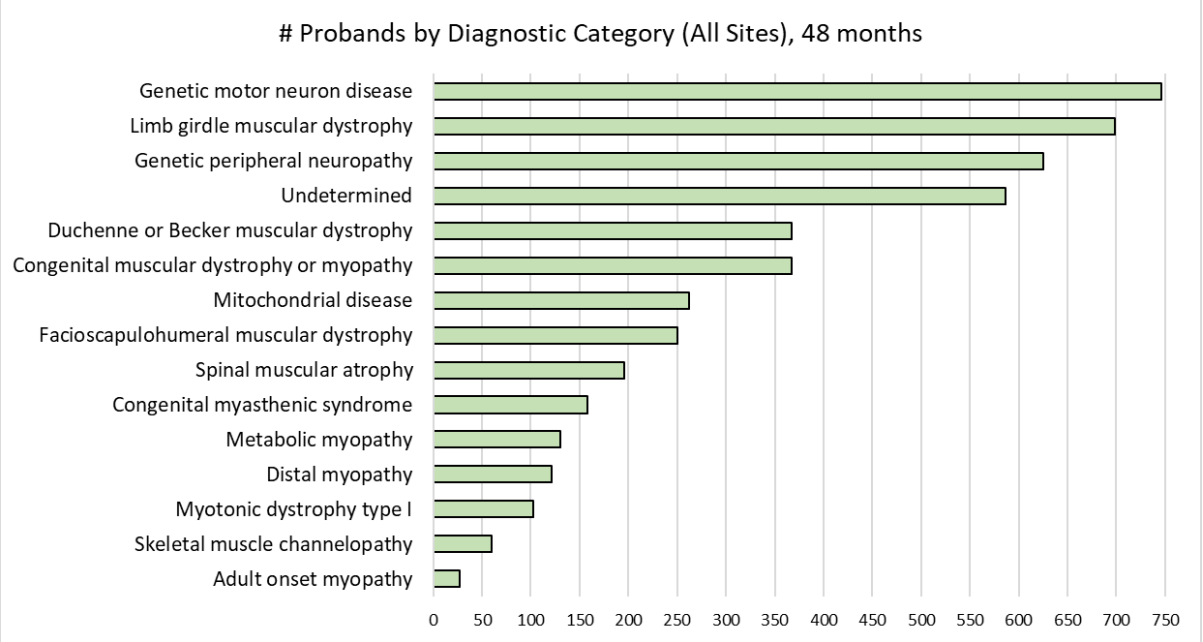

Figure 3: All ICGNMD probands, by Diagnostic Category, illustrating the range of neuromuscular diseases recruited. Recruitment at each site is not a random sample of patients attending clinic; it may be influenced by local site research foci and ICGNMD Fellow's chosen subject area.

INDIA

Since pan-Indian HMSC ethics was obtained for international data sharing in March 2021 (following a COVID-related hiatus), our Indian partners have entered consented participants into the ICGNMD REDCap database.

Indian genetic testing must take place within India as samples are not permitted to be shipped overseas at-scale. Our Indian partner testing sites (Indian government CSIR CCMB and CDFD

institutes) were obliged to halt all non-COVID related testing and research during 2020 and much of 2021: this impacted raw data output timelines but data is now being generated according to pre-pandemic plans and is starting to be processed for report generation.

AIIMS: Our AIIMS (Delhi) partner remains outstanding. During the pandemic, it established a tele-medicine service and has ethics to use this to connect with potential participants over a wide geographical area. It was the first site to recruit 1,000 participants (currently 1384) and has an excellent rate of success in recruiting family members as well as probands, important for downstream genetic analysis (including variant segregation). The ICGNMD Fellow and site Co-I, Dr Vishnu VY, has been instrumental in the site's success.

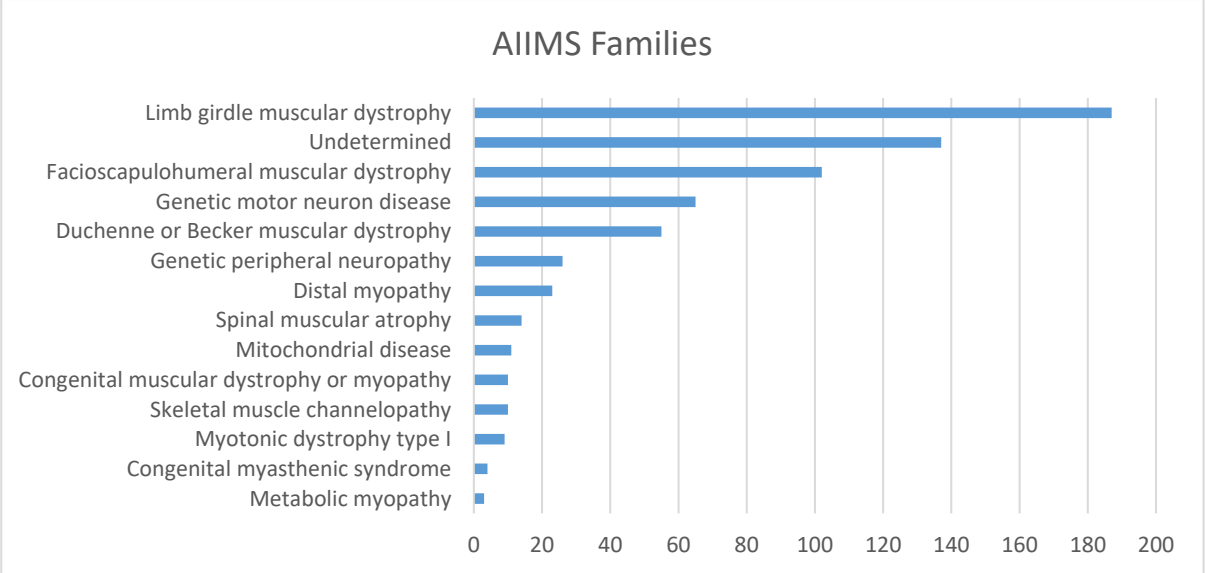

Figure 4: # AIIMS families by REDCap diagnostic category

NIMHANS: NIMHANS (Bangalore) has two recruiting groups, led by **Professor Nalini** (broad neuromuscular and neurogenetic focus with over 2000 participants recruited from a very large neurology clinic) and **Dr Nandeesh** (focus on mitochondrial disorders and inherited neuropathies recruited from a neuropathology referral service). Professor Nalini's participants include a large number (>500) of individuals who already have a genetic diagnosis: these have substantially enriched the ICGNMD cohort genetic data.

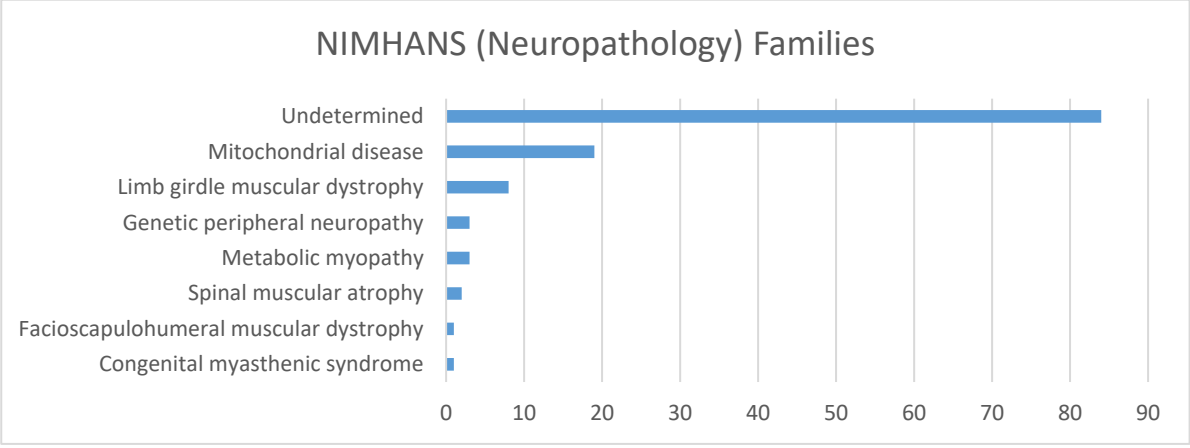

Figure 5: # NIMHANS (Dr Nandeesh group) families by REDCap diagnostic category

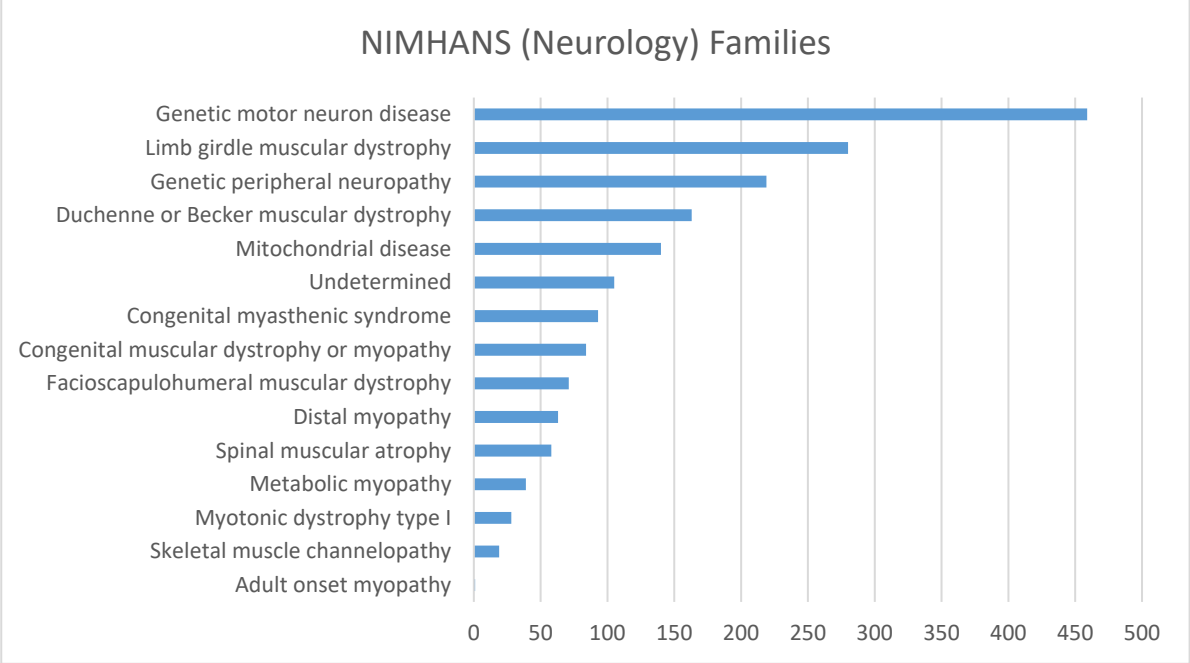

Figure 6: # NIMHANS (Professor Nalini group) families by REDCap diagnostic category

NIMS: NIMS (Hyderabad) came online in May 2021 following the very sad death of the first PI Dr Meena, and her colleague **Dr Yareeda** taking over the study. Despite missing the induction, Dr Yareeda has engaged very actively with UCL to discuss cases and meet Study protocols and data entry requirements (now over 1200 participants).

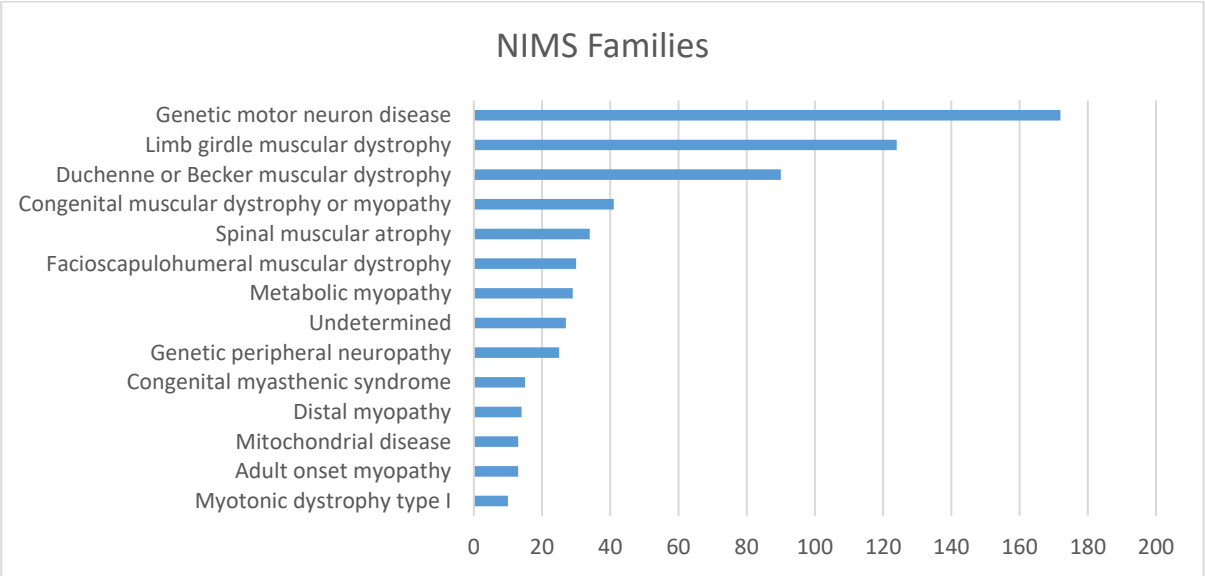

Figure 7: # NIMS families by REDCap diagnostic category

SOUTH AFRICA

Our South African partners have shown steady progress since the end of COVID-related closures, and a very positive aspect of this partnership continues to be the extent to which the sites connect regularly within the country to discuss progress and possible funding opportunities to pursue. Together they have contributed 839 participants across 475 families.

Stellenbosch University and Cape Town University recruited 232 adult participants and are increasing focused family member recruitment to aid variant identification and segregation. Ethics recruit across two sites (Groote Schuur and Tygerberg Hospitals), with our Guarantors of Brain-salaried PhD Fellow working across both. Recruitment and data analysis for the Groote Schuur site has been accelerated by local informatics analysis by Dr Melissa Nel and recruitment by PI Professor Jeannine Heckmann and Dr Elizabeth Steyn (and now by Dr Niki Floudiotis).

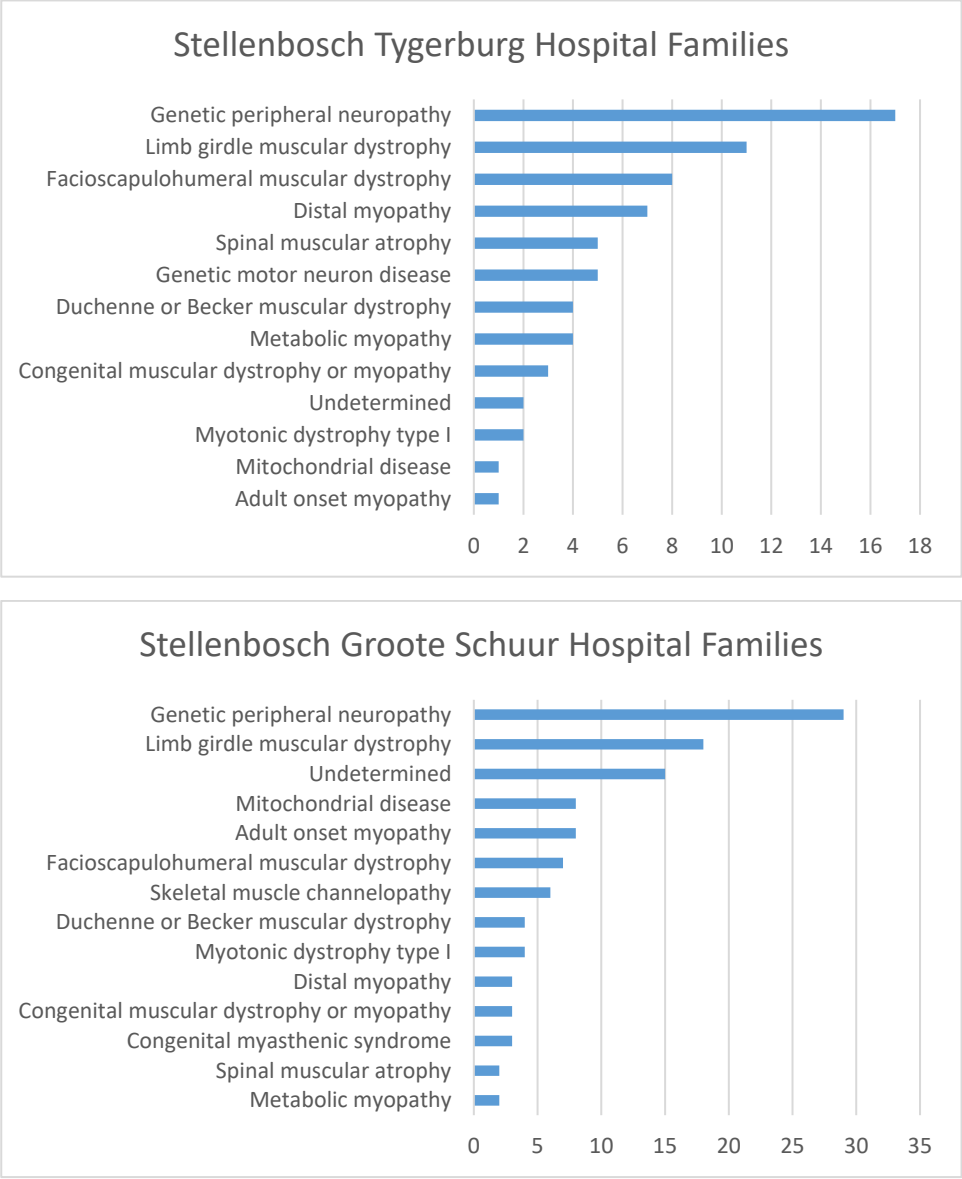

Figure 8: # Stellenbosch families by REDCap diagnostic category

The **University of Cape Town** has a part-time (0.5xFTE) PhD Fellow, Dr Sharika Raga, who is responsible for paediatric-focused recruitment with PI Professor Wilmshurst. Despite her more limited time for the project, Dr Raga has managed recruitment and REDCap data entry of 135 participants.

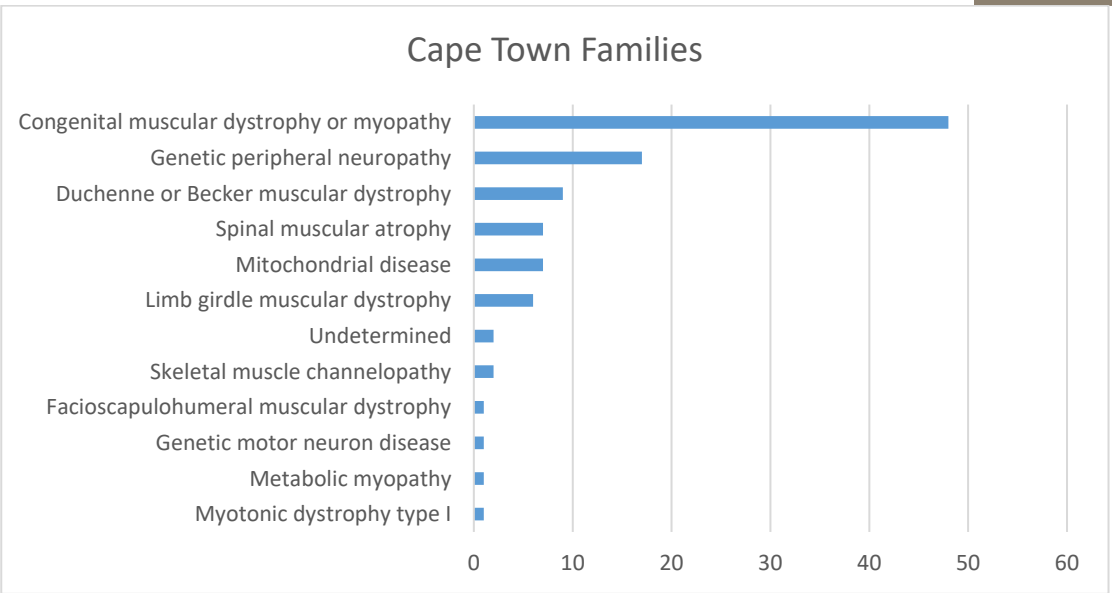

Figure 9: # Cape Town families by REDCap diagnostic category

The **University of Pretoria** (472 participants) is delivering outstanding results, particularly as the ICGNMD PhD Fellow originally appointed had to resign for personal reasons, and as the Steve Biko Academic Hospital where PI Professor Smuts works was at the epicentre of the first COVID-19 Omicron variant outbreak. Professor Smuts was unable to recruit another Fellow to post and so was appointed as a non-remunerated Faculty ICGNMD Fellow, with funds used to support am experienced part-time clinician, Dr Ronel Human, to consent participants, undertake study visits and data entry, and collect samples. Colleague Dr Else Lubbe also plays an important role. There is very strong “buy-in” from the wider team at Pretoria, with many colleagues joining case discussions and training events. Pretoria also benefits from close collaboration with ICGNMD non-clinical partner North-West University, led by Professor Francois van der Westhuizen. His post-doctoral researcher, Dr Maryke Schoonen, is supporting local evaluation of genetic test results alongside Professor Smuts. The Pretoria site is also recruiting many family members (greatly helping us to identify causative variants) and a highly ethnically diverse and ethnically well-defined cohort, with people travelling to the clinic from a very wide geographical catchment area across the north of the country.

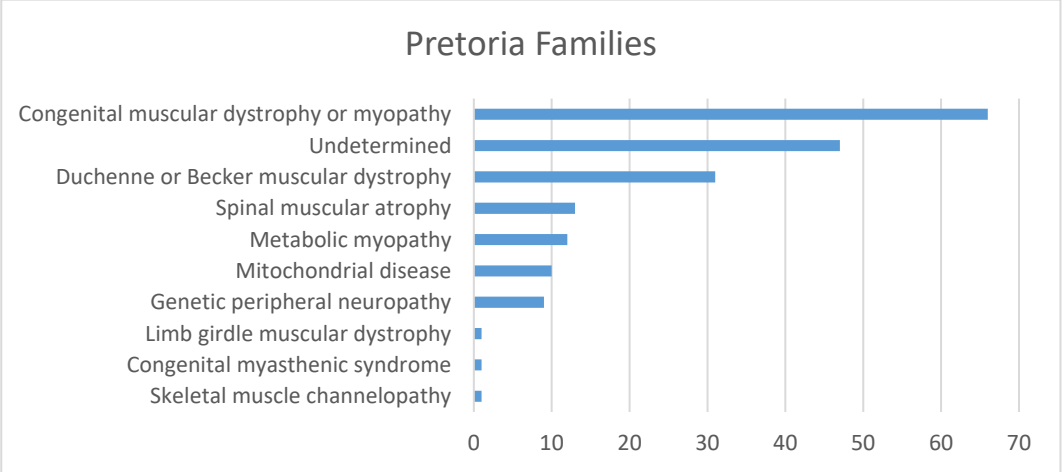

Figure 10: # Pretoria families by REDCap diagnostic category

ZAMBIA

The **University Teaching Hospital (UTH)** (Lusaka) has made steady progress this year. Close to 200 participants have now been recruited, first genetic discussions have taken place, with some early outputs and other samples currently at Macrogen for exome, or UCL for SMA MLPA and other single gene tests. One of the many logistical challenges we continue to face for Zambia is sample transport, which is very costly. To resolve this challenge, site PI Dr Kvalsund has twice broken up international travel with a change in London to deliver samples. The cost of flights, overnight accommodation and transport in London for each delivery was less than shipping samples and also enabled very productive in-person meetings.

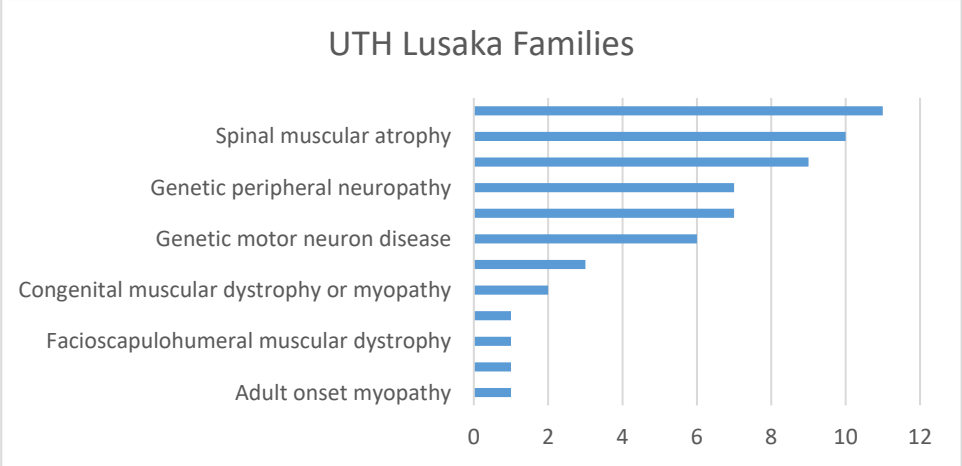

Figure 11: # UTH Zambia families by REDCap diagnostic category

TURKEY

Our two Turkish partners continue progress recruitment and genetic analysis with recruitment primarily undertaken by the ICGNMD PhD Fellows.

IBG Izmir: The IBG centre is focused on paediatric cases (293 participants across 96 families) with particularly good representation of muscle disorders and arthrogryposis. Samples are sent to Ankara for testing in the ICGNMD pathway.

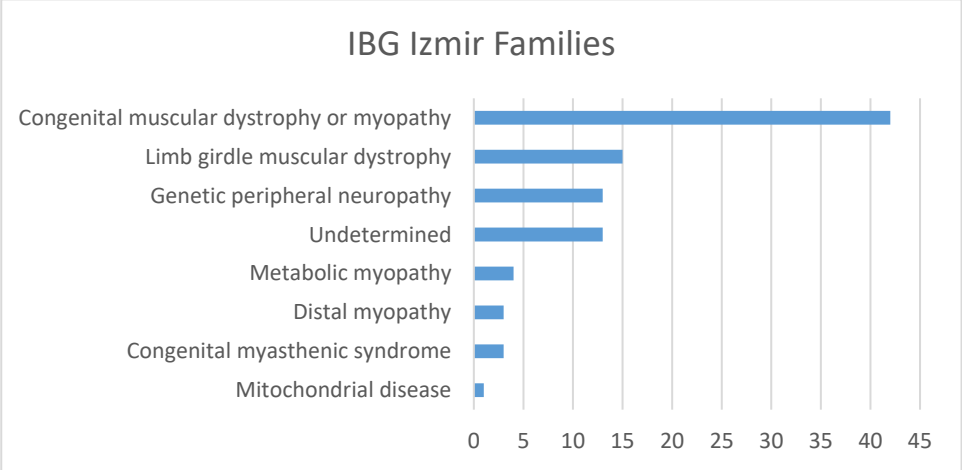

Figure 12: # IBG Izmir families by REDCap diagnostic category

Ankara City Hospital: The Ankara centre is focused on paediatric cases (295 participants across 89 families) with a good representation of dystrophies. The Fellow responsible for the bulk of recruitment has good face-face access and engagement with the Ankara City Hospital Genetics Team, who assist with variant interpretation and planning tests, and whose staff also attend ICGNMD online events to provide their expertise and share local experience and perspectives.

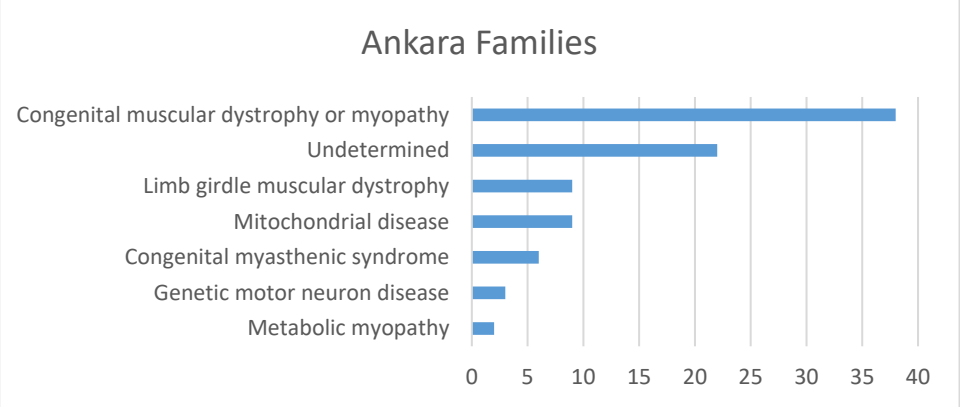

Figure 13: # Ankara City Hospital families by REDCap diagnostic category

BRAZIL

FAEPA, University of Sao Paolo: Brazil continues to recruit large numbers of participants with high-quality data entry (1141 participants across 745 families by Year 3 end on REDCap, with another 500 recruited and awaiting REDCap entry). Cases include an exciting cohort of non-5q Spinal Muscular Atrophy patients.

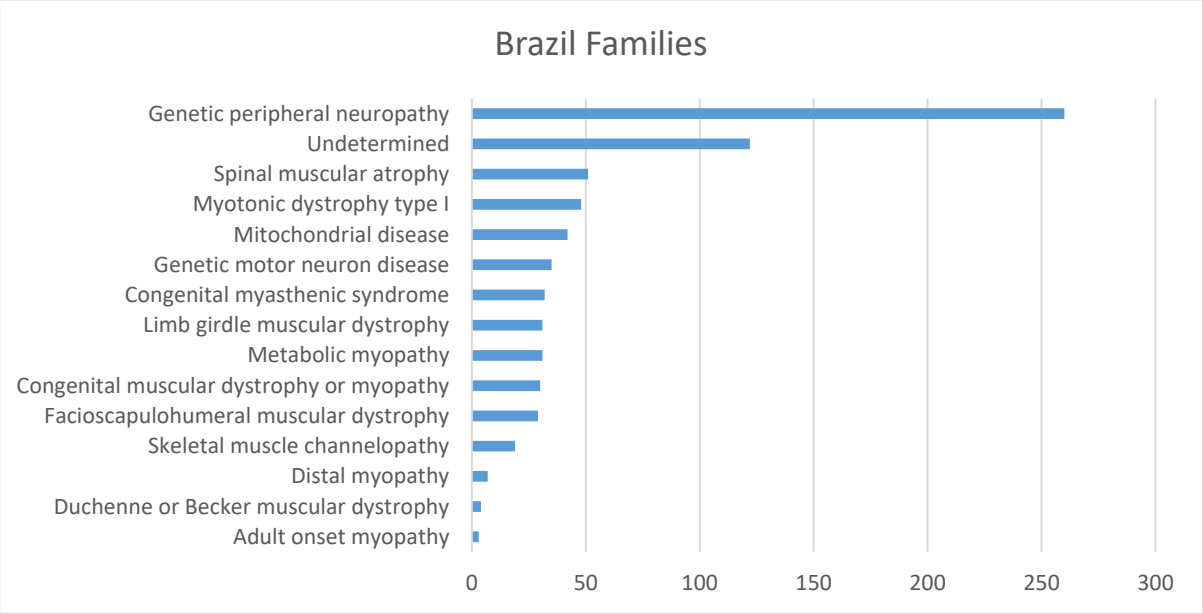

Figure 14: # FAEPA, Sao Paolo, families by REDCap diagnostic category

Milestone 3 & 4 (Objectives 4, 5, 6): Commence genetic diagnosis of LMIC patients.

MET

(i) Genetic analysis snapshot at Year 4 end

To recap, Zambia, South Africa and Brazil can ship samples to the UK for testing, while Indian and Turkish partners must test in-country.

At Year 4 end, the majority of DNA has been collected and delivered to the relevant testing site, 1039 genetic tests (spanning whole exome, genome, single gene tests, and FSHD familial analysis) are underway, with 1588 genetic tests completed and a result issued. These metrics include 628 WES in progress and 1011 WES completed (**Table 1**).

These metrics do not include ca 50 cases where the ICGNMD pipeline was applied to reanalyse genetic data generated outside of the study: results are also included in our analyses and collaborative research. We are working towards augmenting Indian ICGNMD outputs this year with a sub-project at NIMHANS (Professor Nalini's Neurology Group) to identify and recruit over 400 individuals with existing exome data. These are a mix of "genetically solved", "possibly solved" and "unsolved" condition participants: those who remain unsolved will have their existing data reanalysed in the ICGNMD pipeline to try to identify potential genetic cases of their conditions (e.g. 2nd hits in recessive disorders or difficult to capture structural variants). Those who have an existing genetic diagnosis will become part of emerging sub-cohorts for specific NMDs, with particularly strong representation of Limb-Girdle Muscular Dystrophies.

We continue to try to maintain a balance of testing across diagnostic categories and regions, with resources distributed in a manner likely to deliver broadest insights.

(ii) Discussing & Closing Cases:

Under the ICGNMD pathway, results of each case is discussed together by the Partner Site and their linked UK team **before a final case conclusion**. After that, a final research report is generated, and signed off by Professor Hanna, Professor Houlden or another nominated member of the ICGNMD Leadership team. Wider, monthly meetings discuss more complex cases as a group.

This process takes time as the International and UK Fellows must review the case and assign ACMG criteria to any likely causative variants.

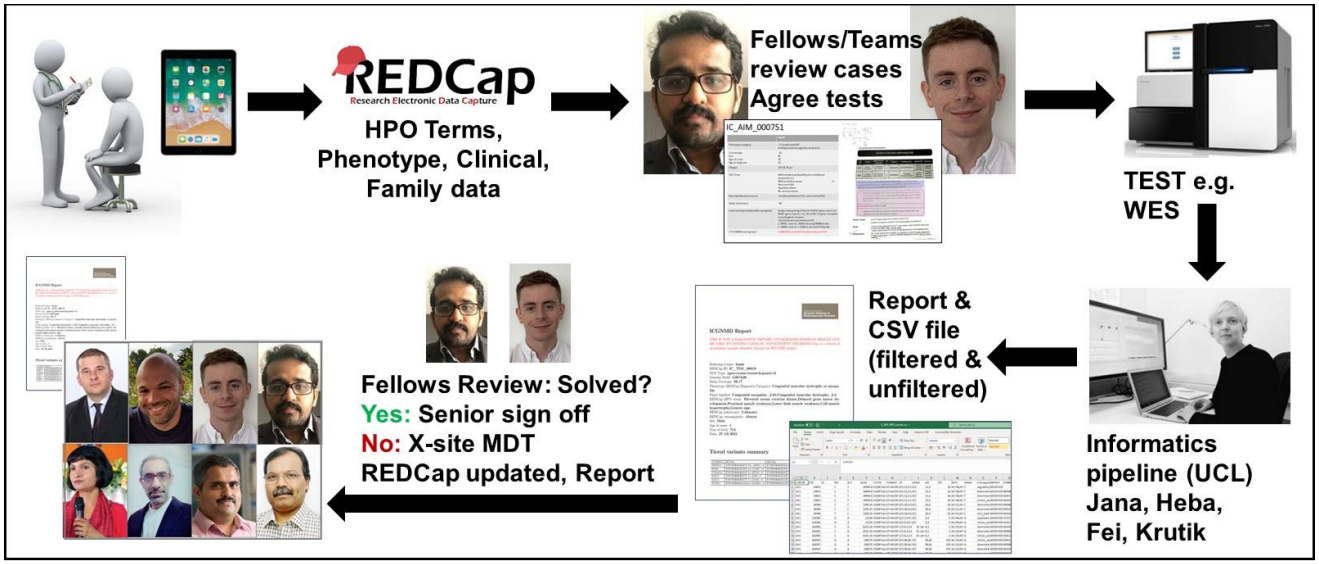

Figure 15: ICGNMD test pathway for AIIMS, Delhi site, from recruitment to final report. The responsible Fellows for this site are Dr Vishnu VY (AIIMS) and Dr Will Macken (UCL).

(iii) Rates of Genetic Diagnosis Success

From November 2022 to January 2023, all UK and International Fellows and PIs worked together to review all data outputs to date, to generate metrics on cases that are “solved”, “possibly solved” and “unsolved” by genetic analysis. The outcome of this analysis is now part of our first “all ICGNMD” publication, currently in submission to the journal Brain.

Outcomes of January 2023 review:

Our cohort has a broad spread of neuromuscular diseases, with the most common clinical categories in line with known relative prevalence. The four most common neuromuscular conditions by initial clinical diagnosis (excluding conditions not assignable to a category) are: Limb Girdle Muscular Dystrophy (LGMD; 18.1%), Genetic Peripheral Neuropathies (PN; 15.5%), Congenital Muscular Dystrophy or Myopathy (CM(D); 9.44%) and Duchenne or Becker Muscular Dystrophy (DMD/BMD; 8.6%). Together, these four categories comprise just over half of the ICGNMD cohort. The age of participants ranges from newborns to over 80 years old, with median age of 26 years old. Around one third (35%) of cohort participants are aged 18 or under at recruitment. Using 1000 Genomes populations as a background for ancestry estimation, 82% percent of individuals tested by WES were of non-European ancestry.

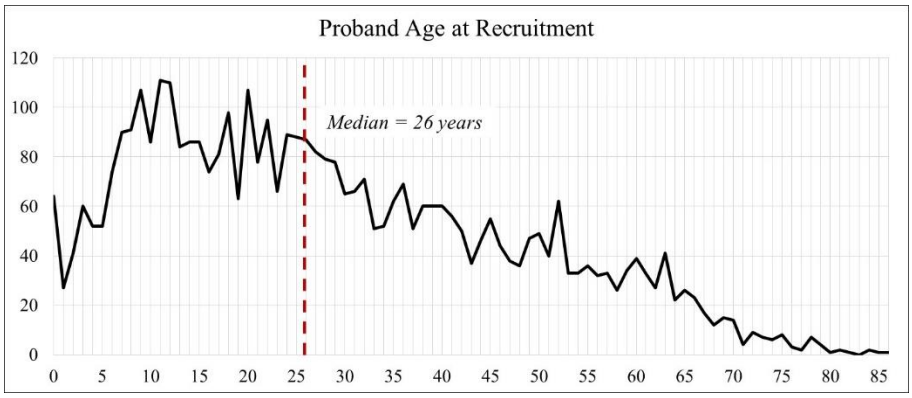

Figure 16: Proband "Age at Recruitment" (relatives not shown), with median age shown (26 years).

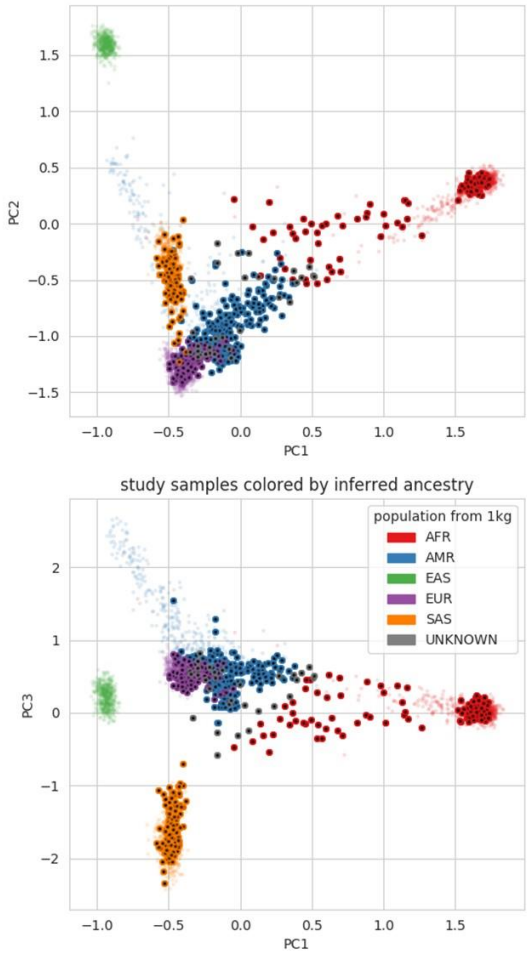

Figure 17: Principal component analysis (PCA) plot of predicted ethnicity of the ICGNMD cohort. PCA clusters generated for 611 exome samples using 1000 Genomes (1kg) population data as implemented in Peddy. The plot shows the majority of ICGNMD samples are of non-European ancestry and admixed. Dots with black centre represent ICGNMD samples. Faint background colour dots show 1000 Genomes samples. AFR=Africans; AMR=Admixed Americans; EAS=East Asians; EUR=Europeans; SAS=South Asians.

We reviewed 611 WES reports, 274 single gene tests and 101 FSHD family analyses. The average solved rate across the cohort was approximately 56%. In-depth review of the four most common clinical categories (LGMD, PN, CM(D), DMD/BMD) yielded diagnostic uplift, with 59% "solved" and

13% “possibly solved” (outcome classed as ‘possibly solved’ if a strong candidate variant identified ((two variants/homozygous recessive disorders)) based on population frequency (<0.01% frequency), bioinformatic predictions and clinical phenotype, but at least one variant is classified as a VUS according to ACMG criteria). Almost 29% of variants identified as causing, or very likely to cause, the disease, were novel, increasing diverse pathogenic variant knowledge. The unsolved participants are now being curated for further testing (including WGS and arrays to look for copy number/structural variants) and represent an important new, global discovery cohort. Overall, the review demonstrated that the ICGNMD cohort provides a large resource from under-represented populations for genetic and translational research.

We plan to run another cross-cohort review in Autumn 2023.

Test	**Total tested**	**Solved**	**Unsolved**	**% Solved**
Myotonic Dystrophy Type I (*DMPK* Triplet Repeat Primed PCR)	20	19	1	95.0
Duchenne Muscular Dystrophy (Dystrophin (*DMD*) MLPA)	12	5	7	41.7
Friedreich's Ataxia (Frataxin (*FXN*) Triplet Repeat Primed PCR)	3	2	1	66.7
Spinal and bulbar muscular atrophy (SBMA; Kennedy's Disease) (androgen receptor (*AR*) repeat expansion (fluorescently labelled PCR)	6	3	3	50.0
Oculopharyngeal muscular dystrophy (OPMD) (PCR)	3	1	2	33.3
Spinal Muscular Atrophy (SMA) (*SMN* MLPA)	8	6	2	75.0
India CMT1 (MLPA: *PMP22/MPZ/GJB1* dup/del)	9	1 *PMP22* deletion	8	11.1
Brazil CMT1 (*PMP22* dosage analysis)	55	14 *PMP22* duplications	41	25.5
Brazil CMT1 (*PMP22/GJB1/MPZ* PCRs)	60	26	34	43.3
Facioscapulohumeral Muscular Dystrophy (FSHD) (Southern Blot & Methylation analysis)	98	60	38	61.2
Totals	**274**	**137**	**137**	**50.0**

Table 2: Summary of single gene test (incl. FSHD) outcomes, from January 2023 cross-site data review.

Disease category	Gene	Variant	ACMG classification
Neuropathy	*SBF2*	ENST00000256190.13:c.2100+1G>A	Pathogenic
Neuropathy	*NEFL*	ENST00000610854.2:c.796G>T	Pathogenic
Neuropathy	*HSPB1*	ENST00000248553.7:c.504del	LP
Neuropathy	*SH3TC2*	ENST00000504517.5:c.321G>A	LP
Neuropathy	*IGHMBP2*	ENST00000255078.8:c.449+2T>A	LP
Neuropathy	*GAN*	ENST00000648994.2:c.280C>T	LP
Neuropathy	*MPZ*	ENST00000463290.5:c.620_623dup	LP
Neuropathy	*PMP22*	ENST00000312280.9:c.448_449delGGinsTT	LP
Neuropathy	*NEFL*	ENST00000610854.2:c.65_68delCCCGinsT	LP
Neuropathy	*VRK1*	ENST00000216639.8:c.1012A>T	LP
Neuropathy	*MPV17*	ENST00000233545.6:c.176_181del	LP
Neuropathy	*PRX*	ENST00000324001.8:c.1560_1562del	LP
Neuropathy	*PMP2*	ENST00000256103.3:c.19G>C	LP
Neuropathy	*MPZ*	ENST00000463290.5:c.212A>G	VUS
Neuropathy	*MPZ*	ENST00000672602.2:c.772C>G	VUS
Neuropathy	*ATP7A*	ENST00000341514.11:c.2083C>T	VUS
Neuropathy	*SCN11A*	ENST00000302328.9:c.1101G>T	VUS
Neuropathy	*AIFM1*	ENST00000287295.8:c.512T>C	VUS
Neuropathy	*ATL1*	ENST00000358385.12:c.1208G>C	VUS
Neuropathy	*KIF1A*	ENST00000648047.1:c.368A>G	VUS
Neuropathy	*KMT2C*	ENST00000262189.11:c.1013C>T	VUS
Neuropathy	*MPZ*	ENST00000672602.2:c.772C>G	VUS
CM/CMD	*NEB*	ENST00000397345.8:c.17502_17510dup	LP
CM/CMD	*LAMA2*	ENST00000421865.3:c.4127T>A	LP
CM/CMD	*RYR1*	ENST00000355481.8:c.6175_6187del	LP
CM/CMD	*MSTO1*	ENST00000245564.2:c.1678C>T	LP
CM/CMD	*PIEZ02*	ENST00000302079.10:c.1345C>T	LP
CM/CMD	*PIEZ02*	ENST00000302079.10:c.5082+2T>C	LP
CM/CMD	*CHCHD10*	ENST00000484558.2:c.262-1_262dup	LP
CM/CMD	*MMF*	ENST00000304593.14:c.744+1G>A	LP
CM/CMD	*NEB*	ENST00000397337.6:c.736dup	LP
CM/CMD	*NEB*	ENST00000397345.8:c.23310del	LP
CM/CMD	*LAMA2*	ENST00000421865.3:c.1170C>A	LP
CM/CMD	*TPM3*	ENST00000271850.11:c.734G>C	LP
CM/CMD	*RYR1*	ENST00000355481.8:c.12323G>A	VUS
CM/CMD	*BICD2*	ENST00000356884.11:c.1993_1998dup	VUS
CM/CMD	*ACTA1*	ENST00000366683.3:c.182A>G	VUS
CM/CMD	*MYH2*	ENST00000245503.10:c.4809G>A	VUS
CM/CMD	*GBE1*	ENST00000429644.7:c.602A>G	VUS
CM/CMD	*RYR1*	ENST00000355481.8:c.9678G>T	VUS
CM/CMD	*MSTO1*	ENST00000245564.8:c.49G>C	VUS
CM/CMD	*FKRP*	ENST00000318584.10:c.1034G>T	VUS
CM/CMD	*PLOD1*	ENST00000196061.5:c.1285G>C	VUS

LGMD	*GNE*	ENST00000396594.8:c.1057C>T	Pathogenic
LGMD	*DYSF*	ENST00000258104.7:c.4558del	Pathogenic
LGMD	*DYSF*	ENST00000258104.7:c.3496_3508del	LP
LGMD	*GNE*	ENST00000396594.8:c.2196G>C	LP
LGMD	*GNE*	ENST00000396594.8:c.1000dup	LP
LGMD	*CAV3*	ENST00000343849.3:c.262T>G	LP
LGMD	*DYSF*	ENST00000258104.7:c.856-1G>A	LP
LGMD	*HSPG2*	ENST00000374676.4:c.14C>T	VUS
LGMD	*HSPG2*	ENST00000374676.4:c.14C>T	VUS
LGMD	*SYNE2*	ENST00000344113.8:c.18212G>A	VUS
LGMD	*DYSF*	ENST00000258104.7:c.1781T>C	VUS
LGMD	*DYSF*	ENST00000258104.7:c.5388dup	VUS
LGMD	*KIF5A*	ENST00000286452.5:c.839G>T	VUS
LGMD	*MYH3*	ENST00000583535.6:c.3131A>T	VUS
LGMD	*RYR1*	ENST00000355481.8: c.2321 G>A	VUS
LGMD	*DMD*	ENST00000343523.7:c.1859A>T	VUS
DMD/BMD	*DMD*	ENST00000357033.9:c.2381-1G>C	LP
DMD/BMD	*DMD*	ENST00000357033.9:c.4575_4579del	LP

Table 3: Novel genetic variants identified in inherited peripheral neuropathy, congenital myopathy/muscular dystrophy (CM/CMD), limb-girdle muscular dystrophy (LGMD) and Duchenne/Becker muscular dystrophy (DMD/BMD) cohorts in Jan 2023. Abbreviations: LP (Likely Pathogenic), Variant of uncertain significance (VUS). ACMG (American College of Medical Genetics).

(iv) Some notable findings to-date:

The January 2023 review was an opportunity to understand emerging trends and notable findings, some of which are summarised below.

Example 1: FSHD: *More severe repeat contractions appear to be required to manifest FSHD in Indian populations, compared to European populations, with implications for familial diagnostic testing in labs around the world. This is timely as new testing methods are increasing opportunities for FSHD genetic testing in low-middle income settings. These results are currently under review for publication.*

Example 2: Congenital Myopathy in South Africa: *Emerging data suggests that Stac3 may be responsible for more cases of congenital myopathy in the north of South Africa than in the South of the country, with RYR1 mutations predominating there, and that a Founder population may present with a specific phenotype. These results are currently under review for publication.*

Example 3: A ETFDH Founder mutation causing multiple acyl-CoA dehydrogenase deficiency (MADD) in the Afrikaans sub-population. *These results are currently under review for publication.*

Example 4: A neuropathy unique to black South African populations caused by compound heterozygous MPV17 variants. *These results are currently part of a grant-funding application.*

Example 5: Population-specific trends in the genetic causes of Duchenne Muscular Dystrophy (DMD) with implications for applicability of emerging nucleic acid therapies. Duchenne Muscular Dystrophy is caused most often by large *DMD* gene deletions of varying size, and less often by point mutations (in our cohort, MLPA and WES contribute 86% and 14% of

diagnoses respectively). By analysing the spread of genetic causes across different ICGNMD partner countries, we can observe clear differences in the genetic picture of DMD across populations. Comparison between Indian and South African cohorts identifies differences in both type of genetic variants (deletions, duplications, nonsense, splice variants) and their distribution (including intronic breakpoints) within the DMD gene. The South African cohort demonstrates a higher number of duplications and nonsense mutations and a higher proportion of intronic breakpoints in the proximal 5' end of the gene. Further work is underway to explore if this could be due to differences in cohort size and variation in patient recruitment (e.g. locally solved via MLPA vs unsolved patients) however a comparatively low proportion of large deletions in South African populations has been reported before. It will be important to understand the reason(s) for this observation as, if they reflects genuine variation between populations, there may be implications for applicability of exon-skipping therapies.

Example 6: A lower than expected number of COL6A1 and TTN-related myopathy and muscular dystrophy cases in the ICGNMD cohort. *These results are currently under review as we collate more data for robust metrics.*

Example 7: New comparative data on mitochondrial disease variants. Our ICGNMD UK teams in Cambridge and UCL have published important work on the frequency and phenotype of mitochondrial disease variants in the 100,000 Genomes Project. They have extended this work to compare Genomics England data findings to the ICGNMD cohort and find similarities in terms of (i) the very mixed phenotypes reported, and (ii) a high number of mitochondrial disease mimics (41% in ICGNMD *versus* 62.5% in Genomics England). *Early, observed differences under review as we collate more data.*

Example 8: Possible **CAPN3 Indian Founder limb-girdle muscular dystrophy population in Delhi**. *These results are currently under review as we collate more data.*

Some notable case studies to date:

ICGNMD teams across **UCL** (Dr Pitceathly, Professor Houlden & Professor Hanna), **Cambridge** (Professor Horvath), and **IBG Izmir** (Dr Oktay), working closely with colleagues in the UK and internationally, have identified novel variants in the ***PTPMT1*** **mitochondrial gene** across a group of unrelated probands. This gene's enzymatic product helps produce the phospholipid cardiolipin in the mitochondrial inner membrane. This work is important because it builds on Dr Pitceathly's MRC Clinical Fellowship work to investigate the role of the phospholipid Cardiolipin in mitochondrial disease. This work is currently under review for publication and is a good example of the need in rare disease research to collate cases globally to obtain insights of potential therapeutic relevance.

The Brazil Team has identified an extremely rare adult-onset example of **Krabbe's disease** in a participant of African ancestry. This has now been confirmed with enzymatic analysis, histopathology and MRI and is being written up.

Professor Wilmshurst and ICGNMD Fellow **Dr Sharika Raga** at Cape Town have published details of an ICGNMD study family who had private Invitae exome testing after their first child experienced severe respiratory compromise and developmental milestone regression: the child was diagnosed with a rare neurometabolic disorder called **riboflavin transporter deficiency**, and symptoms improved with riboflavin supplementation therapy. A younger sibling had antenatal genetic testing which showed the same variant. This allowed riboflavin supplementation to be started in utero and continued from birth. The publication records that the child remains completely clinically asymptomatic and conforms this supplementation as a safe method to prevent symptoms of riboflavin transporter deficiency. This has now been published in Pediatric Neurology (DOI: 10.1016/j.pediatrneurol.2023.04.004).

A **novel chloride channel CLCN1 variant** has been detected in an Indian participant with **myotonia congenita**. CLCN1 is expressed only in skeletal muscle and controls the flow of chloride ions into cells to stabilize their electrical charge and prevent muscles from contracting abnormally. This new variant adds to what is known about the genetic causes of this disease. Frog oocyte expression analysis is underway at UCL to confirm the variant impacts on channel activity as expected: this data will be published as a case study.

A Brazilian adult participant with **progressive ataxia** was found to carry a **novel potassium channel KCNA1 variant**. Potassium channels are essential for transmission of electrical signals in the brain's nerve cells, regulating communication between them. The variant is in a region expected to affect channel function and an analogous mutation in *Drosophila* showed a large shift in the voltage dependence of channel activation, such that at physiological voltages, the channel is inactive. Such loss of function is typically associated with ataxia. UCL has completed frog oocyte expression analysis (variant co-expressed with WT mRNA) for this novel variant and shown current amplitude to be significantly suppressed relative to wildtype, suggesting strong dominant negative effects of the variant in the channel tetramer. Further validation is underway so this case can be published.

The **Stellenbosch** team has identified several families with the same Nebulin deletion and this is being prepared for publication.

The team at **AIIMS** is planning a case series of Indian population **Kennedy's patients**, confirmed via ICGNMD single gene testing at CDFD, Hyderabad. Only two Indian cases have been published to date.

The team at **AIIMS** identified an extremely rare form of inherited inflammatory multisystem disease **DADA2**, caused by ADA2 c.13G>C (p.G5R). The participant's phenotype resembled polyarteritis nodosum and manifesting with mononeuritis multiplex and stroke, and was published in Practical Neurology in 2021.

Two ICGNMD participants with **acute intermittent porphyria** (AIP) due to HMBS variants from **Brazil** and **Pretoria** highlight the need for awareness for inherited neuropathies in acute and relapsing conditions.

Homozygous **SORD** variants in a **Brazil** ICGNMD participant with hereditary neuropathy illustrate the need for constant update of virtual panels; this common cause of Charcot-Marie-Tooth disease (CMT) was only first published in 2020.

Although **PMP2** has been a gene on the neuropathy panel for many years, a novel heterozygous variant found in **Brazil** will add to the few reports in the literature.

The **Stellenbosch** team has identified an unusual PEX11B neuropathy phenotype similar to **peroxisome biogenesis disorder 14B** and a publication is being prepared.

4. In-year achievements & publications

Activities, achievements and publications below are relevant to:

Objective 1: *train Fellows and establish links between international Fellows and UK sites;*

Objective 4: *identify known and new diseases genes and assess global genetic architecture of NMDs;*

Objective 5: *increase number of patients in LMICs with a precise genetic diagnosis;*

Objective 7: *sustainability of activities.*

- March 2023 was the first opportunity for all ICGNMD PIs and Fellows to meet in London since pandemic travel restrictions were lifted. With additional financial support from UCL's Global Engagement Team, we were able to bring staff to UCL for a 1.5-day **ICGNMD International Meeting**, followed by attendance at the annual **UK Neuromuscular Translation Research Conference** originally a key part of the ICGNMD's long-running precursor, the MRC UK Centre for Neuromuscular Diseases. This was the first in-person meeting possible since ICGNMD's Jan 2020 induction.
- The 3 days together were an excellent opportunity to review and compare data and plan next steps, including publications. The ICGNMD PhD Fellows had a retreat afternoon of activities and were delighted to welcome MRC's **Dr Mariana Delfino-Machin** to speak about applying for fellowships and grants.
- **Illumina:UCL:ICGNMD parter collaborations.** We have now fully-executed all contracts for a new Illumina UK partnership to access **very low-cost whole genome sequencing** for up to 1,000 ICGNMD participants initially. This is a very important development as it will greatly enhance opportunities for diagnostics and discovery of novel intronic variants, including modifiers of NMD severity that could give new insights for potential therapeutics. The terms of the subcontract required careful wording to ensure compatibility with local ethics committee and university requirements and we undertook extensive preliminary consultation with our partners to increase uptake of this opportunity. **We have now completed tripartite agreements for all 3 South African recruitment sites and Brazil and first data is starting to emerge.**
- UCL's **Dr Rob Pitceathly** was invited to travel to South Africa by ICGNMD South African PIs to present at the **South African Neurology Congress 2023** his research in mitochondrial disease and the work of the UCL Queen Square Institute of Neurology Highly Specialised Services in Channelopathies and Mitochondrial Disease.
- ICGNMD's international Fellows were invited to apply for places to attend either the 2022 or **2023 World Muscle Society Congress**, with the generous support of the WMS. There were 5 successful applications: 3 Fellows presented posters at the 2022 Congress in Halifax, Canada (*Dr Naidu and Dr Raga will attend in 2023*). ICGNMD AIIMS Fellow Dr Vishnu VY was invited to become a member of the organising committee for the 2023 Congress.
    - **Dr Pedro Tomaselli**, FAEPA, Sao Paolo: *A Brazilian cohort study of limb-girdle muscular dystrophy*
    - **Dr Rodrigo Frezatti**, FAEPA, Sao Paolo: *A Brazilian cohort study of non5Q SMA*

- **Dr Vishnu VY**, AIIMS, Delhi: *A cross-sectional study of a genetically-confirmed cohort of facioscapulohumeral muscular dystrophy (FSHD) in the Indian population* (this won a prize)
- **Dr Ozlem Koken**, Ankara: *The molecular landscape of CAPN3 mutations in limb-girdle muscular dystrophy: experience of a tertiary centre in Turkey*

- **Dr Luke Perry** (GOSH ICGNMD Fellow) has joined with Cape Town ICGNMD fellow to involve the South African site in **a study on salbutamol/pyridostigmine therapy** in genetically characterized patients with fatigable weakness/NMJ transmission dysfunction.
- **Dr Chris Record** (UCL ICGNMD Fellow) presented a poster and delivered a talk at the Anglo-French BPNS/SFNP annual meeting in December 2022 and won the poster prize.
- **Dr Will Macken & Dr Katherine Schon** were invited to give oral presentations of their posters at the 2023 EUROMIT summit in Bologna in relation to their in-year publications below.
- **Dr Stephanie Efthymiou** is the post-doctoral research fellow in Professor Houlden's lab supporting ICGNMD testing and data analysis. She was selected to give an oral presentation of her poster at the 2023 UK Brain conference titled "*Optical Genome Mapping for the Molecular Diagnosis of Facioscapulohumeral Muscular Dystrophy: advancement and challenges*" and also to give an oral presentation of a different poster titled "*Biallelic variants in ARHGAP19 cause mixed demyelinating and axonal polyneuropathy*" at EPNS 2023.

Fellows' Publications (*full list of publications acknowledging the MRC ICGNMD Strategic Award in Annex*):

All ICGNMD Fellows are supported to produce publications as part of their Fellowship, to meet Objectives related to training and capacity building.

Dr Chris Record, ICGNMD PhD Fellow UCL:

- *Genetic analysis and natural history of Charcot-Marie-Tooth disease CMTX1 due to* GJB1 *variants*, Brain, 2023 (doi: 10.1093/brain/awad187)
- *Beware next-generation sequencing gene panels as the first-line genetic test in Charcot-Marie-Tooth disease*. J Neurol Neurosurg Psychiatry. 2022 (doi: 10.1136/jnnp-2022-330223)
- *Unusual upper limb features in SORD neuropathy*. J Peripher Nerv Syst. 2022 (DOI: 10.1111/jns.12492)
- *Severe distinct dysautonomia in RFC1-related disease associated with Parkinsonism*. J Peripher Nerv Syst. 2022 (doi: 10.1111/jns.12515.)
- *Genetic pain loss disorders*. Nat Rev Dis Primers. 2022 (doi: 10.1038/s41572-022-00365-7)

Dr William Macken, ICGNMD PhD Fellow UCL:

- *Specialist multidisciplinary input maximises rare disease diagnoses from whole genome sequencing*. Nat Commun. 2022 (doi: 10.1038/s41467-022-32908-7)
- *Discussion of off-target and tentative genomic findings may sometimes be necessary to allow evaluation of their clinical significance*. Journal of Medical Ethics 2023 (accepted for publication)

Dr Katherine Schon, ICGNMD PhD Fellow Cambridge:

- *Nuclear-embedded mitochondrial DNA sequences in 66,083 human genomes*. Nature, 2022 (doi: 10.1038/s41586-022-05288-7)
- *Whole-genome sequencing for mitochondrial disorders identifies unexpected mimics*. Pract Neurol. 2023 (doi: 10.1136/pn-2022-003570)
- *Multi-system pathology in McLeod Syndrome, Neuropathology* (accepted June 2023)

Dr Luke Perry, ICGNMD PhD Fellow GOSH:

- *GGPS1-associated muscular dystrophy with and without hearing loss*. Ann Clin. Transl. Neurol. 2022 (doi: 10.1002/acn3.51633)
- *Muscle magnetic resonance imaging involvement patterns in nemaline myopathies*. Ann Clin. Transl. Neurol. 2023 (doi: 10.1002/acn3.51816)

Dr Musambo Kapapa, ICGNMD PhD Fellow Lusaka:

- *Genetic Neuromuscular Disorders in Zambia: Health Services Access, Utilization, and Needs*. Pediatric Neurology, Accepted for publication

Dr Sharika Raga, ICGNMD PhD Fellow Cape Town:

- *Normal Outcome with Prenatal Intervention for Riboflavin Transporter Defect*. Pediatr Neurol 2023 (doi: 10.1016/j.pediatrneurol.2023.04.004)
- *A case for genomic medicine in South African paediatric patients with neuromuscular disease*. Front Pediatr. 2022 (doi: 10.3389/fped.2022.1033299)
- *Epileptic spasms: A South African overview of aetiologies, interventions, and outcomes*. Dev Med Child Neurol. 2023 (doi: 10.1111/dmcn.15433)

Dr Rodrigo Frezatti, ICGNMD PhD Fellow Sao Paolo:

- *Conduction block and temporal dispersion in a SIGMAR1-related neuropathy*. J Peripher Nerv Syst. 2022 (doi: 10.1111/jns.12517)

Dr Ozlem Koken, ICGNMD PhD Fellow Ankara:

- *High diagnostic yield of targeted next-generation sequencing panel as a first-tier molecular test for the patients with myopathy or muscular dystrophy*. Ann Hum Genet. 2023 (doi: 10.1111/ahg.12492)

Dr Vishnu VY, ICGNMD Faculty Fellow AIIMS:

- *Quantifying Quality of Life after Stroke*. Ann Indian Acad Neurol. 2022 (doi: 10.4103/aian.aian_116_22)
- *Predictors of Seizures and Associated Functional Outcome in a Cerebral Venous Thrombosis Cohort*: An Ambispective Cohort Study. Ann Indian Acad Neurol. 2022 (doi: 10.4103/aian.aian_281_22)

Dr Pedro Tomaselli, ICGNMD Faculty Fellow FAEPA Brazil:

- *Misdiagnoses in a Brazilian population with amyotrophic lateral sclerosis*. Arq Neuropsiquiatr 2022 (doi: 10.1055/s-0042-1755224)
- *Autosomal Recessive Cerebellar Ataxias in South America*: A Multicenter Study of 1338 Patients. Mov Disord. 2022 (doi: 10.1002/mds.29046)

- *Small-Expanded Allele Spinocerebellar Ataxia Type 17 Leading to Broad Movement Disorder Phenotype in a Brazilian Patient*. Cerebellum 2022 (doi: 10.1007/s12311-021-01339-3)
- *There is no shortage, but inequality: demographic evolution of neurologists in Brazil* (2010-2020). Arq Neuropsiquiatr 2023 (doi: 10.1055/s-0043-1761490)
- *Biallelic variants in COQ7 cause distal hereditary motor neuropathy with upper motor neuron signs*. Brain 2023 (doi: 10.1093/brain/awad158)

Dr Sireesha Yareeda, ICGNMD Faculty Fellow NIMS, Hyderabad:

- *Muscle spasms as presenting feature of Nivelon-Nivelon-Mabile syndrome*. Am J Med Genet A. 2023 (doi: 10.1002/ajmg.a.63000)
- *Impact of COVID-19 on Guillain-Barre Syndrome in India: A Multicenter Ambispective Cohort Study*. Ann Indian Acad Neurol. 2022 (doi: 10.4103/aian.aian_523_22)
- *Association of HLA DRB1-DQB1 Haplotypes with the Risk for Neuromyelitis Optica among South Indians*. Neurol India. 2022 (doi: 10.4103/0028-3886.355130)
- *Impact of Thymectomy on Crisis Incidence and Quality of Life amongst Generalised Myasthenia Gravis Patients*. Neurol India. 2022 (doi: 10.4103/0028-3886.364067)
- *A real world multi center study on efficacy and safety of natalizumab in Indian patients with multiple sclerosis*. Mult Scler Relat Disord. 2022 (doi: 10.1016/j.msard.2022.104059)

Dr Nandeesh, BN, ICGNMD Faculty Fellow NIMHANS Bangalore:

- *Acute fatal leukoencephalopathic presentation of CADASIL*. Can J Neurol Sci. 2023 (doi: 10.1017/cjn.2023.54.)
- *TEFM variants impair mitochondrial transcription causing childhood-onset neurological disease*. Nat Commun. 2023 (doi: 10.1038/s41467-023-36277-7)
- *Obstructive Hydrocephalus as the Solitary Manifestation of Young Onset Erdheim-Chester Disease*. Ann Indian Acad Neurol. 2023 (doi: 10.4103/aian.aian_537_22)
- *Phenotype Genotype Characterization of FKRP-related Muscular Dystrophy among Indian Patients*. J Neuromuscul Dis. 2023 (doi: 10.3233/JND-221618)
- *PET-MRI in idiopathic inflammatory myositis: a comparative study of clinical and immunological markers with imaging findings*. Neurol Res Pract. 2022 (doi: 10.1186/s42466-022-00213-9)

Notable in-year ICGNMD PI-linked publications:

The first **"all-ICGNMD" publication** presenting the project and summarising the outcomes of our Jan 2023 data review is now in as an advance article in the journal Brain (https://doi.org/10.1093/brain/awad254). In addition to presenting early findings, the aim of this paper is to provide a template for other rare disease consortia to use when establishing international collaborations.

"Factors associated with the severity of COVID-19 outcomes in people with neuromuscular diseases: Data from the International Neuromuscular COVID-19 Registry", Eur J Neurol, 2023 (doi: 10.1111/ene.15613)

> **UK and international ICGNMD staff and fellows** played a leading role in the above publication.

"*A case for genomic medicine in South African paediatric patients with neuromuscular disease*", S Raga, J Wilmshurst, I Smuts, S Meldau, S Bardien, M Schoonen & F van der Westhuizen. Frontiers in Pediatrics, November 2022 (doi.org/10.3389/fped.2022.1033299)

This welcome in-year publication from the ICGNMD Centres in South Africa introduced the ICGNMD in South Africa and was an opportunity to review the current context of neuromuscular clinical genetics in-country.

"*Genetic analysis and natural history of Charcot-Marie-Tooth disease CMTX1 due to* GJB1 *variants"*, C Record… M Reilly, Brain, 2023 (doi: 10.1093/brain/awad187)

Professor Reilly's group at UCL worked with national and international colleagues (including the ICGNMD Muntoni and Horvath PIs) in this exhaustive review of Charcot-Marie Tooth Disease caused by GJB1 variants (CMTX1). This is the second most common cause of CMT in European populations, but firm diagnoses are impacted by the high number of variants of uncertain significance (VUS). Over 380 participants underwent genotype:phenotype analysis and results helped define more variants as pathogenic or likely pathogenic, and provided stratified data for disease progression and phenotypic severity across different mutations.

"*Genotype-phenotype correlation and natural history study of dysferlinopathy: a single-centre experience from India*". Nashi S…, Thangaraj K, Nalini A, Neurogenetics 2023 (doi: 10.1007/s10048-022-00707-3)

"*Clinical, genetic profile and disease progression of sarcoglycanopathies in a large cohort from India: high prevalence of* SGCB c.544A > C". Bardhan M… Nalini A, Neurogenetics 2022 (doi: 10.1007/s10048-022-00690-9)

"MYH2*-related Myopathy: Expanding the Clinical Spectrum of Chronic Progressive External Ophthalmoplegia (CPEO)*". Baskar D, Vengalil S, Nashi S… Nalini A, J Neuromuscular Diseases 2023 (doi: 10.3233/JND-230017)

"*Phenotype Genotype Characterization of FKRP-related Muscular Dystrophy among Indian Patients*". Unnikrishnan G… Nashi S, Vengalil S… Nandeesh BN, Nalini A, J Neuromuscular Diseases 2023 (doi: 10.3233/JND-221618)

The 4 in-year publications above illustrate the important contribution of Indian **NIMHANS** ICGNMD PI **Professor Nalini** to expanding Indian NMD clinical genomic data. Co-authors **Dr S Nashi** and **Dr S Vengalil** also provide critical support to the ICGNMD study and, as described above, Professor Nalini has shared existing genetic findings and data with the ICGNMD to significantly expand our Indian cohort knowledge.

"*TEFM variants impair mitochondrial transcription causing childhood-onset neurological disease*". Van Haute L… Dominik N… Houlden, H... Vengalil S… Maroofian R… Nalini A… Horvath R, Nature Communications 2023 (doi: 10.1038/s41467-023-36277-7)

This collaboration across **NIMHANS** (Professor Nalini), **UCL** (Professors Hanna and Houlden) and **Cambridge** (Professor Horvath) provided first evidence for the role of the TEFM mitochondrial transcription elongation factor in mitochondrial respiratory chain deficiency and a diverse range of clinical phenotypes, including mitochondrial myopathy with a treatable neuromuscular transmission defect. This study was valuable in combining phenotypic and mechanistic data from human and animal models to thoroughly characterise the gene and its mutational spectrum.

"Conduction block and temporal dispersion in a SIGMAR1-related neuropathy". J Peripher Nerv Syst. 2022 (doi: 10.1111/jns.12517)

The teams at **FAEPA, Brazil** (Professor Marquez Jnr) and **UCL** (Professor Reilly) together published this first reported case of a Brazilian patient with **distal hereditary motor neuropathy** presenting with conduction block and chronic distal denervation neuropathy on electrophysiological examination (features are classically associated with acquired demyelinating neuropathies). ICGNMD exome revealed this to be caused by a homozygous nonsense pathogenic variant in sigma nonopioid intracellular receptor-1 gene (SIGMAR-1). This case study is important because it expands the neurophysiological spectrum of dHMN and also is evidence of the existence of a genetic "mimic" of acquired disease.

"*Biallelic variants in COQ7 cause distal hereditary motor neuropathy with upper motor neuron signs*" Rebelo A, Tomselli, P… Wilson Marques, Stephan Zuchner, Brain 2023 (doi: 10.1093/brain/awad158)

The **FAEPA** team also contributed to a 9 family study, with evidence of a Founder effect in 5 Brazilian families with primary coenzyme Q10 deficiency caused by COQ7 biallelic variants, each manifesting an unusual distal hereditary motor neuropathy phenotype not previously associated with COQ7.

"*Consensus Competencies for Post-Graduate Fellowship Training in Global Neurology*" Neurology 2023 (doi: 10.1212/WNL.0000000000207184)

Zambian ICGNMD PIs **Dr Kvalsund** and **Dr Bearden** contributed to developing key competencies for US neurologists undergoing advanced neurology training, helping to position them to undertake effective work and research in low- and middle-income contexts.

"Genetic Neuromuscular Disorders in Zambia: Health Services Access, Utilization, and Needs". Pediatric Neurology, Accepted for publication

The **Zambian** team awaits publication of this important paper with Fellow Musambo Kapapa as first author. This concludes that (i) physiotherapists hold a major burden of care for people with inherited NMDs in Zambia, (ii) the progressive nature of these diseases requires specialised rehabilitative approaches in some cases, and (iii) there is an urgent need to engage physiotherapists in the multidisciplinary care planning and delivery in low-income settings such as Zambia.

"*A single low-dose rituximab infusion in severe chronic refractory myasthenia gravis in resource-limited settings*" Heckmann, J, J Neurol Sci 2022 (doi: 10.1016/j.jns.2022.120394)

Cape Town PI **Professor Heckmann** published evaluation of affordable rituximab treatments in patients with refractory anti-muscle specific kinase antibody myasthenia gravis, in particular a single, low-dose infusion instead of repeated multi-dose cycles. The outcomes were sufficiently positive for this to be a reasonable first approach in resource-limited settings. Professor Heckmann has also published in-year work to highlight the need for more globally-relevant ALS clinical panels and has adapted a commonly used ALS behavioural screen (ECAS) for three South African language groups.

"*FXR1-related congenital myopathy: expansion of the clinical and genetic spectrum*" Mroczek M, … Topaloglu H,… Volker Straub, Grace Yoon, J Med Genet 2022 (doi: 10.1136/jmedgenet-2021-108341).

Turkish and **Newcastle** ICGNMD PIs were involved in this publication presenting 4 unrelated families with biallelic pathogenic variants in exon 15 of FXR1. This contributes to understanding of FXR1-related congenital myopathy as an emerging, clinical disorder.

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UK ICGNMD PIs have worked together on several significant publications, including important chapters of the **2023 edition Handbook of Clinical Neurology**, for example (i) Professor Horvath and Professor Reilly contributed to the chapter "*Peripheral Neuropathy in Mitochondrial Disease*" (doi: 10.1016/B978-0-12-821751-1.00014-2), (ii) Professor Chinnery authored the chapter "*Mitochondrial Disease in Neurology – Past, Present, and Future*", and (iii) Professor Horvath and Professor Chinnery authored the Handbook's preface (doi: 10.1016/B978-0-12-821751-1.00017-8).

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"*Prevalence of genetically confirmed skeletal muscle channelopathies in the era of next generation sequencing*", Vivekanandam V,… Hanna MG, Neuromuscul Disord. 2023, (doi: 10.1016/j.nmd.2023.01.007)

"*Andersen-Tawil syndrome: deep phenotyping reveals significant cardiac and neuromuscular morbidity*". Vivekanandam V,… Hanna MG, Matthews E, Brain 2022 (doi: 10.1093/brain/awab445)

*"*In silico *versus functional characterization of genetic variants: lessons from muscle channelopathies*", Vivekanandam V,… Hanna MG, Brain 2022 (https://doi.org/10.1093/brain/awac431)

> ICGNMD and UCL Institute of Neurology Director **Professor Michael Hanna's** published works in-year included work to refine prevalence data of a range of skeletal muscle channelopathies using available next-generation sequencing data, work to extend the phenotypic spectrum of Anderson-Tawil syndrome and a critical appraisal of the ability of a range of *in silico* tools to predict channelopathy variant pathogenicity. These each have positive impact on ICGNMD channelopathy work via improving our ability to review the relevance of variants to clinical phenotype and to evaluate the significance of results, and have led to a significantly increased channelopathy prevalence (from 1.12 cases to 1.99 cases per 100 000 population).

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"*Trials for Slowly Progressive Neurogenetic Diseases Need Surrogate Endpoints*." Reilly MM,… Shy ME. Ann Neurol. 2023 (doi: 10.1002/ana.26633)

"*Clinical and Genetic Evaluation of People with or at Risk of Hereditary ATTR Amyloidosis: An Expert Opinion and Consensus on Best Practice in Ireland and the UK*." Gillmore JD, Reilly MM,…Hawkins PN. Adv Ther. 2022 (doi: 10.1007/s12325-022-02139-9)

"*Genetic analysis and natural history of Charcot-Marie-Tooth disease CMTX1 due to GJB1 variants*." Record C,…Horvath R,…Muntoni F,…Reilly MM, Brain 2023 (doi: 10.1093/brain/awad187)

"Cerebellar ataxia, neuropathy and vestibular areflexia syndrome (CANVAS): genetic and clinical aspects." Cortese A,…Houlden H, Reilly MM. Pract Neurol. 2022 (doi: 10.1136/practneurol-2020-002822)

> ICGNMD Co-Director and Fellowships lead **Professor Reilly** has published diverse work relating to inherited nerve and neuromuscular disorders, including work to improve trial data and methodologies, and new genetic insights into diseases that include Charcot-Marie-Tooth disease and CANVAS.

"*Opportunities for mitochondrial disease gene therapy*", Viscomi C… Chinnery, PF, Nature Reviews Drug Discovery 2023 (doi: 10.1038/d41573-023-00067-z.PMID: 37106085)

"*Cell lineage-specific mitochondrial resilience during mammalian organogenesis*", Burr SP… Chinnery PF, Cell 2023 (doi: 10.1016/j.cell.2023.01.034)

"*Heteroplasmic mitochondrial DNA mutations in frontotemporal lobar degeneration*", Nie Y,… Chinnery PF, Acta Neuropathol 2022 (doi: 10.1007/s00401-022-02423-6)

Cambridge ICGNMD Lead **Professor Chinnery** continues to publish important work to extend understanding of the biology of mitochondria in all contexts, alongside trials. Example publications above include a new Cell publication which sheds light on cell-lineage specific adaptation of mitochondria which may help to explain the remarkable tissue specificity seen in mitochondrial disorders, a review of opportunities for mitochondrial disease gene therapy and new work to shed light on the role of mitochondria and mtDNA variants in frontotemporal lobar degeneration (FTLD).

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"*NCAM1 and GDF15 are biomarkers of Charcot-Marie-Tooth disease in patients and mice*", Jennings MJ,… Horvath RH, Brain 2022 (doi: 10.1093/brain/awac055)

"*Neuromuscular junction involvement in inherited motor neuropathies: genetic heterogeneity and effect of oral salbutamol treatment*", McMacken G,… Horvath R, J Neurol, 2023 (doi: 10.1007/s00415-023-11643-z)

Cambridge ICGNMD PI **Professor Horvath** continues to undertake diverse functional and clinical work to shed new light on neuropathies, including in animal models to define new biomarkers in Charcot-Marie-Tooth disease (of potential relevance in clinical trials) and a study extending the number of motor neuropathies with neuromuscular junction involvement. This latter study is important because it shows that a low cost, widely available β_2 adrenergic receptor agonist (Salbutamol) improved patient-reported fatigue in a much wider range of rare diseases than previously realised, with potential to benefit patients in the global South.

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"*Onasemnogene abeparvovec for presymptomatic infants with three copies of SMN2 at risk for spinal muscular atrophy: the Phase III SPR1NT trial*." Strauss KA…Muntoni F…Macek TA, Nature Medicine 2022 (doi: 10.1038/s41591-022-01867-3)

"*Dystrophin Immunity after Gene Therapy for Duchenne's Muscular Dystrophy*." Bönnemann CG, …Muntoni F. N Engl J Med. 2023 (doi: 10.1056/NEJMc2212912).

"*Targeted transcript analysis in muscles from patients with genetically diverse congenital myopathies*." Bachmann C,… Muntoni F,… Treves S. Brain Commun. 2022 (doi: 10.1093/braincomms/fcac224)

UCL GOSH PI **Professor Muntoni** continues his important research and clinical trials, including publishing notable outcomes of trials for new therapies to treat Spinal Muscular Atrophy and Duchenne's.

"*De novo KCNA6 variants with attenuated KV 1.6 channel deactivation in patients with epilepsy*." Salpietro V,… Efthymiou S,…Hanna, M,… Houlden H, Roope Mannikko, Epilepsia 2023 (doi:10.1111/epi.17455)

"*Bi-allelic LETM1 variants perturb mitochondrial ion homeostasis leading to a clinical spectrum with predominant nervous system involvement*." Kaiyrzhanov R,…, McFarland R,… Hanna M,… Taylor RW,…Houlden H, Am J Hum Genet. 2022 (doi:10.1016/j.ajhg.2022.07.007)

"*Motor neuron pathology in CANVAS due to RFC1 expansions*", Huin V… Mary M Reilly, Henry Houlden… Alexandra Durr, Brain 2022 (doi: 10.1093/brain/awab449)

> Alongside very substantial neurodegeneration work, UCL's **Professor Houlden** and his team continue to publish works on rare inherited neuromuscular disorders. Examples include a collaboration that included **Professor Hanna** linking for the first time de *novo* non-synonymous mutations in channel protein *KCNA6* to early infantile epilepsy and neurodevelopmental anomalies, and a study (also involving the ICGNMD PIs **Professor McFarland** and **Dr Taylor**) to define the clinical spectrum of patients with bi-allelic mutations in the LETM1 inner mitochondrial membrane protein, and link the observed phenotypes with defective mitochondrial Potassium ion efflux and perturbed mitochondrial osmoregulation. Professor Houlden, his team and **Professor Reilly** also continue to expand the testing, causes and phenotypic heterogeneity of **CANVAS**, a complex ataxia caused by dysfunction of the cerebellum, vestibular system and/or sensory neurones, which can be caused by bi-allelic expansions in the 2nd intron of replication factor C subunit 1 (*RFC1*) gene. We are most fortunate to be able to test samples in Professor Houlden's laboratory to diagnose CANVAS in a growing number of ICGNMD participants originally clinically diagnosed as unknown or complex neuropathy cases.

"*Defining mitochondrial protein functions through deep multi-omic profiling*." Rensvold JW,…Taylor RW,…Pagliarini D, Nature 2022 (doi: 10.1038/s41586-022-04765-3)

"*FBXL4 suppresses mitophagy by restricting the accumulation of NIX and BNIP3 mitophagy receptors*." Nguyen-Dien G,…Taylor RW,…Pagan J, *EMBO* 2022 (doi: 10.15252/embj.2022112767).

"*Pathological variants in TOP3A cause distinct disorders of mitochondrial and nuclear genome stability*." Valenzuela S,…Taylor RW,… Nicholls T, EMBO Mol Med 2022 (doi: 10.15252/emmm.202216775)

> Newcastle's **Professor Rob Taylor** and **Professor Robert McFarland** participated in highly impactful studies relevant to mitochondrial genetics, function and disease that combine in silico and lab-based approaches.

Publications in submission:

South African and **UK teams** have worked together on a paper just accepted to Frontiers in Neurology entitled "*The mutational profile in a South African cohort with inherited neuropathies and spastic paraplegia*". This combines ICGNMD data with other research data to review the spectrum genetic causes causing 60 cases of genetic neuropathy and hereditary spastic paraplegia (HSP) in South Africa. Importantly, sixty six percent of participants reported African ancestry. The study concludes that the mutational profile of black South African populations does differ substantially from the global north, most notably in the lack of CMT1A cases caused by the PMP22 gene responsible for 37% of European cohort cases. (We had previously observed this in ICGNMD data, but further work was needed to understand if this was due to Study recruitment bias.) HSP black South African participants showed an under-representation of SPG4, SPG7 and SPG11 variants common in European and Asian populations.

ICGNMD-Relevant Grants Awarded In-Year:

Professor Muntoni (UCL GOSH) was part of the UCL team that won a **UK Rare Disease Research Platform** award for the £1.2M node: "*Establishing a National Platform for the Development of Nucleic Acid Therapy for Rare Disease*" (MR/Y008405/1). We are seeking ways to connect our ICGNMD cohort to this node and its benefits.

Professor Muntoni and **Professor Hanna** received additional **UKRI funding** of £420k through his role in the £7M **TRNASNNat** (Transforming delivery, safety and efficiacy of nucleic acid therapeutics, from intracellular trafikking to targeting brain, skeletal muscle and heart) linked to NATA at Harwell.

Professor Muntoni also won industry-related funding of £670k from **Sarepta Therapeutics** to study "Telehealth and PMO (Phosphorodiamidate morpholino oligomer-mediated exon-skipping) Compassionate Use Longitudinal Study in DMD" and £1.26M from **Biogen** for a SMA Reach Light Network Grant.

Professor Muntoni was also successful in charity-linked funding, with two grants from **MDUK** (£424k to continue the UK Paediatric North Star Network & £149k as PI for the grant "Impact and Modulation of modifier genes in LAMA2 muscular dystrophy".

ICGNMD's **Professor Horvath**, **Professor Chinnery** and Cambridge colleague Dr van den Ameele in **Cambridge** won **a £4.3M 2023-2028 Wellcome Discovery Award** to explore nuclear mechanisms underpinning mitochondrial vulnerability in different cell types, which is anticipated to shed light on specific cell and tissue vulnerability in many human disorders as well as primary mitochondrial disease.

Professor Houlden now holds a **Wellcome Trust Investigator Award** "The non-coding genome in neurological disorders" (2022-2027) and also won an **Ataxia UK** PhD Studentship of £120k (2023-2026).

Professor Francois van der Westhuizen at **NWU Potchefstroom** has recently won institutional funding of £100k for a 5 year post-doctoral scientist fellowship, with the position to be applied to functional analysis and validation of Pretoria ICGNMD data.

Professor van der Westhuizen has also won additional funding of approximately £20k for a PhD studentship from the S African National Research Foundation.

Stellenbosch's Dr Franclo Henning has secured £75k funding for a 12-month neuromuscular training position from a private South African hospital consortium: he expects the new Fellow to support ICGNMD activity alongside their clinical training activities.

Professor Heckmann in **Cape Town** has secured funding from the UCT-NI Gabriel Foundation to appoint an additional part-time Fellow, **Dr Niki Floudiotis**, from April 2023, to work alongside Guarantors of Brain ICGNMD Fellow Dr K Naidu in recruitment to ICGNMD.

Dr Vishny VY and **Professor Padma** won an ICMR award of ca £80,000 to establish FSHD research capability at **AIIMS** and implement FSHD research on-site;

Dr Enrico Bugiardini won a £90,000 award for international FSHD cohort analysis using optical genome mapping at **UCL** from The Friends of FSHD charity, which he will undertake with ICGNMD researcher **Dr Stephanie Efthymiou** and in collaboration with the **Leiden** LUMC team (**Prof van der Maarel & Dr Lemmers**).

Dr Stephanie Efthymiou won a **UCL** Translational Research partnership Regenerative Medicine TIN Pilot Data Scheme grant of £10,000 to generate an organoid model of NARS1 disease. She also won a Rare Diseases Models & Mechanisms – Europe (RDMM-Europe) seeding grant of €20,000 awarded as lead investigator to functionally validate Ralgapa2 in a fruit-fly model.

5. COVID-19 Impact

Few impacts have been recorded in the last 12 months, however COVID-19 has impacted the ICGNMD since March 2020 across the following areas:-

Issue	Impact	Mitigation
Partner Outpatient Clinics closed for non-emergency cases	New recruitment halted for variable time periods	Partners using time to search Outpatient lists for potential recruits for when lockdown lifts Time used to undertake PhD research, reading and experimental design Pre-recruited Study Participants continue to be entered into the ICGNMD Database and samples prepped
Non-critical research studies placed on hold by regulatory bodies	New recruitment halted for variable time periods	Permission sought to undertake postal / telephone recruitment where no study visit needed, or to enable recruitment & records access in advance of Study visit
CCMB site for Indian exomes diverted to COVID-19 diagnostics	Delay to start of Indian WES data generation	Ramped-up testing at CCMB from June 2022 and currently establishing 2 alternative routes for testing in case of future outbreaks
Lab consumables in short supply (including ethanol)	Blood samples can't be collected and DNA can't be extracted	Samples frozen where space
International Shipping affected	Samples cannot safely be shipped between sites	Samples held in storage until safe to ship

Clinicians co-opted (to varying extents) to planning or clinical roles, before clear policies on remuneration available	Time available for research work impacted	UK clinicians are receiving NHS re-charge payments No partner country in position to access such payments
Currencies devalued in worst-hit countries against GBP and consumables costs (& need for more PPE) impacted by shortages and inflation	Fluctuating currencies can mean UCL payments fall short of actual spend in local currency	Evaluation on-going.
Planned 2021 & 2022 UK ICGNMD International Meeting in London	Networking and partnership-building opportunity	Meetings took place online successfully: now planning in-person March 2023 meeting.
Planned leadership visits (non-MRC funded) to South African and Turkey cancelled	Loss of partnership-building and training opportunities	We hope to re-schedule some visits for 2023.

Below we show impact estimates by country, developed with Partners. Figures of cases and deaths are official statistics and are of variable accuracy. The Brazilian government ceased formal reporting in June 2020.

Issue	Impact (ICGNMD activity lost)	Specific lockdown periods & Stats
India	Est. 12 months to date	Wave 1: March – May 2020: national lockdown and outpatient clinics closed to all but emergency cases for ~6 months Wave 2: April 2021 – Summer 2021: variable regional lockdown measures, most clinics closed to all but emergency cases Wave 3: a further peak Dec-March 2022 was not the cause of national lockdowns although for some of this time some Partner clinics were closed for all but emergencies When reopened, outpatient clinics show low footfall and clinicians dealing with huge backlog of delayed appointments 2nd highest number of cases reported in world, with significant under-reporting expected. Estimated 531,892 deaths

Brazil	Est. 12 months to date	Wave 1: March 2020-Oct 2020 Wave 2: March 2021-Oct 2021 (FAEPA clinics closed 01 May-11 Oct 22, with "emergency only" reopening from 11 Oct to 13Dec21. Generally high levels of COVID thereafter, no official reporting. No official lockdowns but clinics closed and staff co-opted to COVID wards for periods. DNA lab closed in 2021 and students not permitted to work in the lab. Clinical nurse co-opted to COVID care and still on secondment. 85% reduction in number of new cases and 69% reduction in number of return visits. When reopened, outpatient clinics show low footfall Highest number of cases reported in world, with significant under-reporting expected. Estimated 703,291 deaths
South Africa	Est. 12 months to date, average	Wave 1: National lockdown 16 Mar – end Apr 2020: clinics closed. Studies on hold March-Oct 2020 (7 mo) Wave 2: National lockdown Dec 2020 – 01 March 2020 (3mo): clinics closed, studies on hold. Wave 3: June 2021 – late Sept 2021 (4 mo). Wave 4: Dec 2021-April 2022 (4 mo). Omicron originates at ICGNMD partner Pretoria site: clinics closed to all but emergency cases here. While reopened, outpatient clinics show low footfall & reluctance to risk attending face-to-face study visits. Higher % of wealthy patients with "own transport" recruited to study, leading to over-representation of European-ancestry participants. Current estimate: 12 months "lost to COVID" incl. 30%-50% reduction in recruitment. Cost of DNA extractions up 30% since pandemic start. 2021 inflation rate 5.5%. Official metrics record 102,595 cumulative deaths while a Lancet Global Health paper (Bradshaw et al 2022) estimates 250,000-310,000 deaths.

Zambia	Est. 12 months to date	Wave 1: March – Oct 2020 (7 months) Study placed on hold & outpatient clinics closed to all but emergency cases March – Oct 2020 Wave 2: Jan-Feb 2021 Wave 3: June-Oct 2021 Study placed on hold June-August 2021 (3 months) Wave 4: Dec 21-Feb 22 (3 months) No study pausing but very low clinic attendance When reopened, outpatient clinics show low footfall PI Bearden based across Zambia and US could not travel to Zambia. PI Kvalsund could not leave Zambia until 2022. ~345,000 reported cases ~4,100 reported deaths (official statistics)
Turkey	Est. 6 months to date	Wave 1: National lockdown 18 Mar – 11 May 2020 Wave 2: Nov 2020-Jan 2021, limited clinic activity Wave 3: March-May 2021, shorter lockdown, limited clinic activity Wave 4: Dec 2021-March 2022 17,004,677 confirmed cases 101,419 confirmed deaths
UK	N/A	3 national lockdowns to date: • Wave 1: 26 Mar-25 Jun 2020 • Wave 2: 05 Nov – 02 Dec 2020 • Wave 3: 06 Jan – 08 Mar 2021 No lockdown in Wave 4: Dec-March 2022 24,470,262 confirmed cases 226,977 confirmed deaths Significant PI time spent on COVID work, including clinical duties and developing university and clinic/hospital responses. Significant PI and admin staff time spent monitoring COVID, adjusting invoicing, applying for related funds.

Most partners report an impact on study activity of **~12 months**. The figure is lower for Turkey, which experienced fewer clinic closures over the period. **All** clinical colleagues from around the world experienced significant stress and "burnout": their continued commitment to ICGNMD was both remarkable and humbling.

As described in previous reports, we made savings via reduced consumables spend during lockdowns. We maintained UKRI's position on its directly-funded PhD students by continuing to provide financial support, where required, for our international Fellows. This is because Fellows continued to work on their ICGNMD work in whatever ways possible, alongside any COVID-19 commitments.

We continue to monitor COVID-19 but **we hope metrics will remain at ca 12 months' impact by award-end.** The remaining significant impact is a lag in exome sequencing and single gene tests at overseas partner sites previously diverted to COVID-19 testing. We hope to make up for lost time fully by award-end in May 2024, however the **two main risks** remain: (1) large numbers of results will continue to emerge near project-end as Fellows are writing-up research and applying for new posts, reducing our capacity to review and interpret all test results, and (2) higher costs due to inflation and consumables shortages will impact testing capacity.

Inflationary pressures across partner sites continue to affect value of staff salaries and consumables costs.

6. ICGNMD Plans for Year 5 & Centre renewal

Please refer to our **MRC Programme Grant** pre-application document for details.