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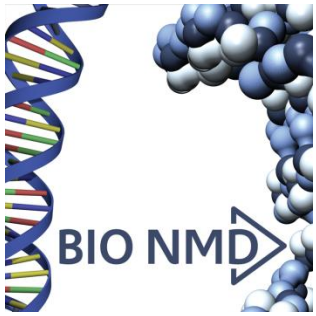
BIO-NMD newsletter



Inside This Issue

Introduction

This is the third and penultimate newsletter from the BIO-NMD project. If you didn't get a copy of the first two issues, which focus on the project's background, a feature on biobanking and on sample collection, you can still find them now at: http://www.bio-nmd.eu/news/bio-nmd-newsletter/

This edition forms a **progress update** to let you know what has been happening in the BIO-NMD project. You will also find a diary of events and other news from related projects and organisations.

Thank you for your interest in BIO-NMD! Please do not hesitate to get in touch with any comments, questions or requests.

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Send us your feedback via:
www.bio-nmd.eu/forPatients/
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What is BIO-NMD?

BIO-NMD is a 3 year EU-funded research project focusing on Duchenne and Becker muscular dystrophies and collagen VI-related myopathies (which includes Ullrich congential muscular dystrophy and Bethlem myopathy). It is closely linked to the TREAT-NMD network of excellence and was born from collaborations and research ideas within that network.

Visit the project's website at

www.bio-nmd.eu

The project is searching for 'biomarkers' in people with these conditions. Biomarkers are substances in the body that offer a way to measure normal or abnormal processes.

A major application of new biomarkers is in clinical trials. At the moment, when a new drug is being tested, researchers use a variety of ways to measure whether the drug has had a positive effect. However, these measures are not always very good at showing small changes and improvements in a patient's symptoms, especially when the drugs are only being tested for a short time.

However, if the BIO-NMD project can find biomarkers in patients' blood or urine, samples of these can be taken throughout clinical trials. Measuring the levels of these biomarkers will show researchers clearly and accurately whether the drug being tested has had an effect or not.

Biomarkers offer a way to measure processes in the body

There will be other benefits in discovering biomarkers for neuromuscular diseases:

- Blood and urine testing may be able to **reduce the use of muscle biopsies**
- **Diagnosis can happen earlier and with less expense** because testing for biomarkers may be quicker and easier than genetic testing
- **Disease progression can be accurately measured** aiding better clinical management of symptoms
- Existing treatments (including drug dosage) can be adjusted to **precisely meet the needs of individual patients** to ensure they get the maximum benefit

The latest results and activity from BIO-NMD partners.

The **University of Ferrara (UNIFE)** has analysed the coding regions (exomes) of DNA samples from collagen 6 and DMD patients. This analysis has identified changes called SNPs (single nucleotide polymorphisms) which may be useful biomarkers in these diseases. A SNP is where one letter in the DNA code of a gene is altered. This may have no effect, it may cause a different protein to be made or it may cause the gene to stop being copied too early. 10 possible SNP biomarkers have been identified for collagen 6 myopathy (Bethlem) and 32 SNPs have been found for dystrophinopathy (Duchenne and Becker). All these SNPs are being checked (validated) in larger patient populations.

The DNA from Duchenne and Becker patients who show different responses to corticosteroid treatment and from collagen 6 patients who have received ciclosporin A treatment have been fully analysed by **LifeTechnology** and **UNIFE**. That analysis revealed 34 possible SNPs in DMD/BMD patients which seem to be linked to a difference in response to steroids. In collagen 6 patients the analysis is still in progress.

Analysis of the DMD gene identified 5 regions involved in how dystrophin is copied by RNA (transcription), particularly related to the way this transcription is controlled. This will help to understand how expression of the dystrophin gene is regulated.

Leiden University Medical Center (LUMC) has been identifying new protein biomarkers for muscular dystrophies in blood serum samples. The method pre-treats the samples to prevent proteins which are present in high levels in the blood from hiding potential biomarkers found in lower concentrations. Then, the proteins of interest are separated, broken down into smaller fragments, analyzed by sophisticated machines and identified with the help of a database search. From these studies some proteins with biomarker potential for muscular dystrophies have been found but the analysis is still ongoing.

LUMC has also started validation (checking) of some of the potential serum biomarkers that have been found in different cohorts of DMD and BMD patients. This process will show whether these biomarkers really do correlate to particular aspects of the conditions.

Newcastle University (UNEW) has been responsible for checking the availability of sufficient, high-quality, human biomaterials for the research activities of the BIO-NMD partners. They have identified and circulated a large number of samples for the project through close collaboration with partners and the EuroBiobank.

UNEW has successfully collected serum, plasma and DNA from a total of 206 patients attending its local clinics over this period. In addition, a group of DMD patients were identified whose serum samples were taken at 3 different time points, each a year apart. This is very valuable as it allows researchers in BIO-NMD to study any changes in levels of the biomarkers as time progresses and at different stages of the disease in the same patient. UNEW will coordinate further plasma and serum sample collection at other centres (UCL, LUMC and UNIFE) to make sure there are sufficient samples for the validation (checking) phase of the project.

Comprehensive clinical data, including information about ambulation, steroid treatment, cardiac and respiratory functions were compiled for each patient. The same template for this data collection has been circulated to all BIO-NMD partners to ensure that everyone gathers the same information from patients.

UNEW has continued to establish links between BIO-NMD and various events with a focus on building relationships with patient organisations. Posters have been produced and presented at the UK **Action Duchenne** and **Muscular Dystrophy Campaign** patient conferences – photos and details have been placed onto the patient orgnaisations' websites. A scientific poster was presented at **WMS** in Portugal and the **TREAT-NMD conference** in Geneva. Speaker slots have been arranged at future high quality industry symposia and further events are being explored.

In addition, the **Project Ethics Board** has met; the website has been maintained and updated; the M18 newsletter was produced and is available at: http://www.bio-nmd.eu/patient-newsletters/

University of Padova (UNIPD) has continued studies on the muscles of mice with a mutation in the Col6a1 gene to give them collagen VI disease. These animals were exercised on running wheels and treadmills. In normal mice without the mutation, physical exercise was found to activate autophagy in skeletal muscles – a physiological process where cells degrade their own aged or malfunctioning components in a controlled manner. This physical training increased the dystrophic symptoms in the mice with the collagen VI mutation. Autophagy was not activated in response to exercise in these mice and this led to a marked increase of muscle wasting. These findings indicate that proper activation of autophagy is important for muscle stability during physical activity. It may be that the proteins involved in the autophagic process will provide useful biomarkers for collagen VI disease.

UNIPD has also been investigating the regenerative potential of muscles in mice with collagen VI mutations, both in normal conditions and in response to injury. They found that collagen VI is a key component of the stem cells in adult muscles which are able to differentiate into muscle cells and involved in muscle regeneration. Lack of collagen VI leads to impaired muscle regeneration after damage.

University College London (UCL) has sequenced the coding parts of the DNA (whole exome sequencing) in 5 DMD patients with early loss of ambulation (before the age of 8.5 years) and 5 DMD patients with late loss of ambulation (after 12 years of age). Differences (variants) in these exomes were identified. Variants found in both groups, i.e. patients with early and patients with late loss of ambulation, were discounted. Those variants found in 3 or more patients and which were specific to one group or the other were considered for further analysis. 104 variants in 95 genes were specific to the early loss of ambulation group and 51 variants in 44 genes were specific to late loss of ambulation.

Further studies in a larger DMD cohort will be carried out in order to confirm the possible role of these variants as modifying genes in DMD.

University of Rome Tor Vergata (UNIROMA) has explained the guidelines of the EMA (European Medicine Agency) to partners, which may be of interest in the context of the BIO-NMD project. In particular, information has been provided on the EMA qualification process. A biomarker is 'qualified' when there is scientific consensus that it is helpful for a specific analytical or clinical use.

The EMA has recently released an opinion about qualification for a clinical biomarker related to Alzheimer's disease. The biomarker is intended to identify patients who can be recruited for clinical trials of treatments for pre-dementia Alzheimer's disease. It addresses the question of whether the use of two cerebral spinal fluid (CSF) related biomarkers are appropriate for selecting those subjects for trials into early Alzheimer's disease who have a high probability of being in the early stages of the disease. The data available consistently shows that subjects diagnosed with MCI (mild cognitive impairment) with a positive CSF biomarker profile are more likely to develop dementia in the coming 2-3 years. This qualification opinion is the first for use in humans that the Agency has issued.

Even though it does not refer specifically to NMDs, it is very important to consider the criteria used by the EMA in order to apply them to possible qualification applications for NMD biomarkers.

INSERM INSERM has been involved in the creation of bioinformatics tools (software) for linking and analyzing data in the BIO-NMD project. They have also been working on the development and implementation of *in silico* tools for identifying *in vivo* biochemical pathways and potential biomarkers.

During the first 24 months INSERM has designed the BIO-NMD database using feedback on its content from partners, received data from partners which has been input into the system and developed a new system called UMD-HTS® that can handle the large sets of data generated by sequencing work and that can predict the effect of some of the mutations discovered.

University of Milan (UNIMI) Using the human muscle samples from the **Telethon** Network of Genetic Biobanks in Italy, UNIMI carried out a study to identify protein differences between DMD and BMD patients and differences in disease progression. To reduce variability due to age, each group was divided into three sub-groups of 5 patients each:

DMD1: patients ranging from 9 months to 2 years old
DMD2: patients ranging from 2 to 5 years old
DMD3: patients ranging from 5 to 8 years old
BMD1: patients ranging from 3 months to 3 years old
BMD2: patients ranging from 3 to 6 years old
BMD3: patients ranging from 6 to 11 years old

The proteins from each individual muscle biopsy sample were then separated. After detection, the differentially expressed proteins were identified.

Differences between DMD and BMD sub-groups were assessed. Among differentially expressed proteins the most significant were contractile and metabolic. In particular, metabolic proteins were less abundant in DMD patients. Contractile proteins were also less abundant, whereas proteins involved in regeneration were more abundant. This study identifies a set of molecules differentially abundant and which can discriminate DMD from BMD. These could be candidates as markers of disease progression in DMD versus BMD patients. Further studies are underway on other samples provided by partners and results will be presented at the next meeting in May 2012.

KTH Biotechnology has been working with the list of possible (candidate) biomarkers generated by the BIO-NMD project. A list of target genes has been assembled and prioritized based on suggestions from partners. For each target, the availability of antibodies was checked in the Human Protein Atlas repository and antibodies selected. Where available, two antibodies were selected against the same target. The antibodies were then used to investigate the differential expression of these targets in patients with neuromuscular dystrophies and in controls.

The samples analyzed were kindly provided by the **UNEW**, **LUMC** and **UNIFE** partners and included both serum and plasma. The samples allowed analysis of differential protein expression in patients with Duchenne Muscular Dystrophy and Becker Muscular Dystrophy and enabled the linking of disease severity in these conditions with protein expression.

Ariadne have focused on the analysis of data generated by partners for discovery and validation of different types of candidate biomarkers. They analysed two datasets:

- gene expression data from an experiment comparing mice which have collagen 6 disease with wild-type (healthy) mice and their response to ciclosporin A treatment;
- data from an RNA-sequencing experiment comparing collagen 6 patients before and after one month of ciclosporin A treatment.

During the second half of the project, **Ariadne** will continue to interpret data generated by partners using the BIO-NMD knowledgebase (a sophisticated data repository).

Applied Biosystems (AB) completed the analysis of mutations in 20 samples from 3 cohorts;

i) DMD low response to steroid treatment;
ii) DMD high response to steroid treatment;
iii) Collagen 6 patients treated with Ciclosporin A.

They isolated the DNA sections of interest in each sample and analysed them by sequencing 230 candidate genes selected by **Ariadne** using their pathway analysis software. 18 mutations (SNPs) were found to show a significant difference in occurrence between the two DMD groups. Meanwhile, 54 mutations were found to occur in at least 3 of the 4 collagen 6 samples.

Copy number variation (a difference in the number of times a section of a gene is repeated) analysis of this data is still ongoing.

Novamen has supported partners in organising project meetings and releasing reports to the EC. They have overseen the finalisation of the Consortium Agreement, ensuring that all partners are well protected with regard to intellectual property issues.

Bi-annual meetings remain a crucial opportunity for partners to present work progress and to brainstorm. The organization of the final two meetings of the BIO-NMD steering committee has been planned. The management team has also supported the BIO-NMD partners in their work on a daily basis.

Other News and Events

TREAT-NMD Alliance

The TREAT-NMD Network of Excellence which was established in 2006 with the help of a 5 year, EU FP6 grant is set to continue as the TREAT-NMD Alliance. The aim is to ensure that the collaborative approach which has enabled huge achievements over the past 5 years, is set to continue. The TREAT-NMD Alliance will be coordinated by a 12 member Executive Committee with 9 academic members (scientists and clinicians) and 3 members from patient organisations. More information on the new structure can be found at http://www.treat-nmd.eu/about/governance/future-plans/ whilst TREAT-NMD's tools, services and achievements within the NMD community can be explored further at http://www.treat-nmd.eu/

The attendees of the last TREAT-NMD governing board meeting pictured in Geneva in November 2011

BIO-NMD partners are represented at patient events

BIO-NMD took posters to two UK patient organization events in 2011. They were on display at the Muscular Dystrophy Campaign's annual conference in Nottingham (October) and at Action Duchenne's event in London (November).

These were great opportunities to meet other researchers, patients, families and carers and to hear about what the priorities are for research into NMDs from different perspectives. Much of the feedback on the BIO-NMD project was that the possibility of reducing the need for invasive muscle biopsies is extremely important and a desirable goal for most patients and their families. The idea that biomarkers in body fluids may be found to monitor disease progression, check the efficacy of drugs, identify those likely to respond to a particular treatment and help with a speedier diagnosis was of great interest to those people who came to view the BIO-NMD poster.

A copy of the poster itself can be found at: http://www.bio-nmd.eu/forPatients/poster-presentation/

Grants from PPMD and MDA Fund Work by Tivorsan Pharmaceuticals on Recombinant Biglycan for the Possible Treatment of Duchenne Muscular Dystrophy

Parent Project Muscular Dystrophy (PPMD) will award Tivorsan Pharmaceuticals a $500,000 grant to develop the company's biglycan therapeutic candidate for Duchenne muscular dystrophy. The Muscular Dystrophy Association (MDA) has awarded the same team $1 million from the MDA Venture Philanthropy (MVP), a part of MDA's translational research program.

Building on previously funded work, Dr. Justin Fallon (Brown University) and the Tivorsan team seeks to use recombinant human biglycan (rh-BGN) to increase utrophin at the muscle cell membrane, resulting in reduced muscle damage and improved function. Utrophin is a molecule that is related to dystrophin in structure and form and can "stand in" for dystrophin when present in greater than normal quantities. Biglycan is a naturally occurring protein made up of amino acids and carbohydrate chains that is found in large amounts on the outside of developing and regenerating muscle cells. The form of biglycan that is active therapeutically contains only simple carbohydrate side chains. For this reason, rh-BGN is straightforward to manufacture.

Valerie Cwik, M.D., executive vice president of research and medical director for the Muscular Dystrophy Association said "MDA is excited about enabling Tivorsan to complete vital preclinical activities. Tivorsan's biglycan drug has the potential to effectively attract the utrophin protein to where it could have a clinically beneficial effect in muscle cells." PPMD President and CEO Pat Furlong believes biglycan is a promising therapy candidate in Duchenne and is happy to again support this project as Tivorsan prepares to take it to human clinical trials.

More details about this and other research funded by PPMD and MDA can be found at: www.parentprojectmd.org and http://www.mda.org/

Italian Duchenne Parent Project Conference 2012

Alessandra Ferlini, BIO-NMD coordinator, presented some "News & Views" from the BIO-NMD project at the Italian Duchenne Parent Project Conference, held in Rome (17-19 of February 2012).
The event saw speakers, parents, children and other DMD stakeholders coming from the whole Italy. Many representatives of the TREAT-NMD and BIO-NMD projects attended together with several pharma- companies.
As part of the programme, a scientific working group was organized, chaired by Carrie Miceli and Annemieke Aartsma-Rus. Researchers presented and discussed new, unpublished results with a focus on novel therapies (e.g. exon skipping, cell therapy and drugs) and the effect of steroid treatment.

Biomarkers and genetic modifiers were also discussed. This working group was an effort to further improve communication between clinicians, scientists, industry and patient associations.

More detail and a copy of the conference programme (Italian) can be found at: www.parentproject.org/italia/

Congenital Muscle Disease Biobank at the Coriell Institute

Cure CMD and Coriell are working together to facilitate CMD research and invest in the future of CMD therapies by creating a well characterized, central source of samples from individuals with Congenital Muscle Diseases (CMD).The CMD BioBank is housed in the National Institute of General Medical Sciences Human Genetic Cell Repository at the Coriell Institute. To date, 127 samples have been submitted to the CMD Biobank. Samples in the NIGMS repository have been utilized in over 5,000 scientific publications by researchers in more than 50 countries.

Cure CMD supports the CMD BioBank by identifying and facilitating sample transfers from existing collections at other institutions. To provide scientists with a de-identified data set, CMD BioBank samples will be linked to existing profiles from sample donors in the CMD International Registry through a code number. The CMDIR staff will provide: maximal motor function achieved, breathing status, concomitant medication use and CMD subtype verification. Having detailed information regarding clinical severity assists scientists using the CMD samples in understanding therapy response.

One of the barriers to finding treatments for CMDs is a lack of available tissue and cell resources for scientists to test potential treatments. Testing medical compounds using the cells of people with CMD helps determine whether a compound may be effective. Donating blood or tissue samples to biobanks can provide scientists with the opportunity to learn about different CMD subtypes and to find therapies targeted to either CMD subtype and/or specific genetic mutation(s), if known.

To donate a sample to the CMD BioBank, you or your child must have been diagnosed with CMD (any subtype, genetic confirmation is desirable but is not required). Donations are accepted from all over the world.

For further information, please contact Tara Schmidlen MS, CGC at +1 856-757-4822 or tschmidl@coriell.org or visit the website at: www.curecmd.org/research/biobank

Diary dates

7th-8th March 2012
***Global Discovery and Development Innovation Forum**
Budapest, Hungary
***Dr Peter A. C. 't Hoen (Leiden University Medical Center) will be a key speaker, discussing the BIO-NMD project**

21st-24th March 2012
Genomic Disorders 2012
Cambridge, UK

22nd – 23rd March 2012
UK Neuromuscular Translational Research Conference
Newcastle, UK

13th-15th April 2012
PPMD's 2012 West Coast Connect Meeting
San Diego, USA

18th – 21st April 2012
Golden Helix Symposium 2012
Turin, Italy

21st-22nd April 2012
SMDF Symposium
Gothenburg, Sweden

22nd-24th April 2012
Myomatrix 2012
Reno, Nevada, USA

26th-27th April 2012
***IV Curso de Patalogia Neuromuscular en la Infancia**
Barcelona, Spain
*** Training course conducted mainly in Spanish**

8th – 11th May
Update in Neuromuscular Disorders Course 2012
London, UK

9th -10th May 2012
***BIO-NMD Steering Committee meeting**
Stockholm, Sweden
***Partners and invited attendees only**

Visit the TREAT-NMD website for more details of these events at:

http://www.treat-nmd.eu/about/events/

19th May 2012
Myotonic Dystrophy Support Group 2012 Annual Meeting
Newcastle upon Tyne, UK

7th-8th June 2012
AOMC 11th Annual Scientific Meeting
Kyoto, Japan

9th-12th June 2012
22nd meeting of the European Neurological Society
Prague, Czech Republic

17th-20th June 2012
New Directions in Biology and Disease of Muscle
New Orleans, USA

21st-24th June 2012
Families of SMA 2012 Conference
Minneapolis, USA

23rd-26th June 2012
European Human Genetics Conference
Nürnberg, Germany

27th June – 1st July 2012
PPMD's Annual Connect Conference
Fort Lauderdale, Florida, USA

30th June – 1st July 2012
Biennial FSHD International Patient and Researcher Network Meeting 2012
Atlanta, USA

7th July 2012
Jennifer Trust Annual Conference 2012
Warwick, UK

24th-26th August 2012
Canadian National Youth Conference
Calgary, Alberta, Canada

8th-11th September 2012
16th Congress of the European Federation of Neurological Societies
Stockholm, Sweden

27th-29th September 2012
Muscle Study Group Annual Meeting
Buffalo, NY, USA

5th-7th October 2012
Riding the Wave 2012
Queensland, Australia

9th – 13th October 2012
World Muscle Society 2012
Perth, Australia

9th-10th November 2012
Action Duchenne International Conference 2012
London, UK

Please submit future dates for the Diary to: catherine.turner@newcastle.ac.uk