ISSUE 2 JULY 2011

BIO-NMD newsletter

Introduction

This is the second newsletter from the BIO-NMD project. If you didn't get a copy of the first issue, which focused on the project's background and had a feature on biobanking, you can still find it now at: http://www.bio-nmd.eu/news/bio-nmd-newsletter/

This second edition contains a progress update as well as a brief article about how samples are collected for use in BIO-NMD and why it is hoped that the project will have such a positive impact on the diagnosis and treatment of patients with neuromuscular disease.
You will also find a diary of events and other news from related projects and organisations.

Any comments or feedback you have about this newsletter would be most appreciated – please let us know what is useful, what is not and what you feel is missing.

Thank you for your interest in BIO-NMD!

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Inside This Issue

Send us your feedback via:
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BIO-NMD is supported by FP7 under contract number
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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 6-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

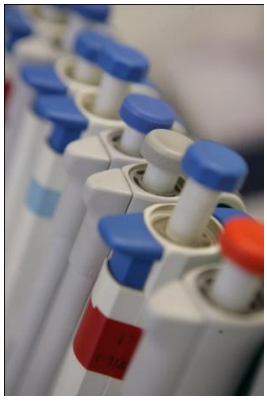
The project is searching for 'biomarkers' in people with these conditions. Biomarkers are substances in the body that offer a way to measure normal or abnormal processes.

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 **replace the use of muscle biopsies**
- **Diagnosis can happen earlier** because testing for biomarkers is quicker and easier than genetic testing
- **Disease progression can be accurately measured** allowing 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

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Who is involved in BIO-NMD?

Professor Alessandra Ferlini at the University of Ferrara coordinates the project

There are 12 European partners involved in the BIO-NMD project coordinated by Professor Ferlini at the University of Ferrara, Italy. There is also a Patient Association Committee whose job it is to make sure patients' interests are represented and that they are kept informed about BIO-NMD's progress.

The project's website has full details about the 12 partners and the patients' committee at **www.bio-nmd.eu**

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Muscular Dystrophy Campaign (UK) represents patients at the BIO-NMD progress meeting in Freiburg

Alternative ways of testing the benefits of a potential treatment has great support

In June 2011 representatives from each of the BIO-NMD partners attended a progress and planning meeting in Freiburg, Germany. Partners presented details of the progress that has been made by the consortium towards the discovery and validation of biomarkers for Duchenne, Becker, Ullrich and Bethlem.

Representing the Patient Association Committee at the meeting was Dr Marita Pohlschmidt from the Muscular Dystrophy Campaign, UK. Marita told us how she felt that the project was important for patients:

"Being involved in an initiative such as BIO-NMD is a great chance for a patient organization to be where cutting edge science is happening and also makes it easier to update our families on any new advances. It is crucial to keep the dialogue going between the scientific community and those directly affected to ensure their interests and needs are considered.

BIO-NMD is an exciting project because finding biomarkers enables our scientists to understand muscle disease better and ultimately these might replace painful biopsies that sometimes are part of clinical trials. I very often get feedback from our families that research into alternative ways of testing the benefits of a potential treatment has great support as it would make participation in clinical studies much easier" **Dr Marita Pohlschmidt, Director of Research, Muscular Dystrophy Campaign, UK.**

It is hoped that Patient Association Committee members (who also include Elizabeth Vroom, UPPMD and Anna Ambrosini, Telethon Italy) will be able to attend all future BIO-NMD meetings too. In the meantime, BIO-NMD is discussing plans to have representatives of the consortium come to patient organisations' national conferences in order to discuss the project in more detail with patients and their families.

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Encouraging results are presented at progress meeting.

At the recent meeting in Freiburg, a great deal of progress was reported. A (brief!) summary of the work from each partner is summarized below.

The University of Ferrara, Italy have shared samples of biomaterials with other partners for analysis using "OMICs" techniques - these are high tech approaches for analysing a wide range of data, in this case all the DNA and RNA in a sample. For example, they have sent blood samples (plasma and serum) from neuromuscular patients to the Royal Institute of Technology, Sweden in order that they can identify unusual sets of proteins which may be useful as biomarkers.

They have also been investigating specialised cells in the blood (called macrophages) which produce and secrete a protein called collagen 6 - a major component of the connective tissue between muscle fibres. Results were found to mirror those obtained from muscle biopsies. This suggests that these macrophages may offer a reliable, minimally invasive biomarker to replace muscle biopsies in the diagnosis and monitoring of Bethlem myopathy and Ullrich congenital muscular dystrophy. This work has now been published (Gualandi, *Muscle and Nerve*, 2011).

At the **Leiden University Medical Center** (LUMC), partners have been using mass spectrometry to identify potential new biomarkers in blood serum. They will soon be analysing these proteins to discover how they are constructed from their peptide building blocks (peptide analysis).

Newcastle University has been coordinating exchange of patient samples (serum, plasma, DNA and biopsies) between partners as well as identifying where further samples are needed and requesting these from the EuroBiobank. They have also been involved in collecting new samples from patients. However, no new biopsies have been requested for BIO-NMD – any biopsies used were taken previously, usually for the purpose of diagnosis.

Newcastle have sent the (anonymised) clinical data which accompanies samples to INSERM so that they can build the database required by the project.

They have also been increasing the visibility of the project through newsletters, websites, academic and industry events and press releases to the scientific media. BIO-NMD's website (www.bio-nmd.eu) is updated from Newcastle and provides a central point for all information about the project.

The **University of Padova** have recently discovered several potential novel biomarkers for collagen 6 (COLVI) disease through their studies on autophagy – the process by which a cell degrades its old, damaged or no longer required components. When this process is interrupted, the build up of these defective components can lead to cell (and therefore muscle) damage.

They found that the autophagic process is impaired in muscles from both the COLVI mouse model and patients affected by collagen 6 diseases. Therefore, components involved in this process could serve as biomarkers of these conditions. They also found that reactivation of the defective autophagy was able to restore muscle cell survival with structural and functional improvement in COLVI mice.

Partners at the **University College London** have been sequencing the protein-coding parts of DNA (the exome) in samples from 2 distinct groups of Duchenne patients:

- Those who have either early or late loss of ambulation (ability to walk)
- Those who have a longer or shorter than typical survival

They are looking for genetic biomarkers that affect the severity of Duchenne muscular dystrophy. Data from this work is still under analysis.

UCL have also been involved in the exchange of samples coordinated by Newcastle.

University of Rome Tor Vergata has been keeping up to date with the latest regulatory guidelines of the European Medicines Agency (EMA) which govern the qualification procedures of new biomarkers, also in relation to drug response. It has kept other partners in the consortium informed of any developments and issues through regular updates.

INSERM in France has continued with the development of software (known as UMD-HTS) crucial to the success of the BIO-NMD project. This system can predict the pathogenicity (ability to cause disease) of mutations within different parts of the DNA. It could therefore be used to select the most relevant mutations likely to be useful as new biomarkers. The software is now ready for initial use by the other partners.

The **University of Milan** have been looking at the effects of cyclosporine A treatment on collagen 6 disease in the mouse model. They have now identified molecules linked to partial muscle recovery after cyclosporine A treatment which may, if validated, act as biomarkers to monitor the effectiveness of cyclosporine A treatment in patients.

KTH Biotechnology in Stockholm have selected a large set of antibodies which bind specifically to potential biomarkers. These antibodies can be used to analyze, detect or capture these proteins and eventually identify new protein biomarkers in blood samples which give useful information about a patient's likely prognosis.

Applied Biosystems have been working with The University of Ferrara to develop new methods to sequence a panel of neuromuscular disease related candidate genes identified using Ariadne's database.

Ariadne have completed an audit and analysis of publicly available data in the literature on biomarkers for Duchenne muscular dystrophy disease progression. Partners' data on new potential biomarkers have also been incorporated to provide a valuable database of knowledge for use by partners in the BIO-NMD project.

Novamen provide project management and support to all the BIO-NMD partners. This ensures the smooth running of the project within budget and to EU requirements. They have arranged the 6-monthly progress meetings, circulated minutes, coordinated the writing and submission of detailed reports on progress to the EU amongst many other BIO-NMD project management activities.

In summary, the sharing of samples between centres has been coordinated to make it as efficient as possible. These samples are being used for research to enable a better understanding of the diseases. The validation (proving that they work) of new molecules as biomarkers is in progress. Once validated, the biomarkers may be used to support diagnosis, counselling on disease progression and as tools to monitor efficacy in future clinical trials.

Some of the partners at the kick-off meeting back in January 2010

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Collection and use of samples in the BIO-NMD project

Introduction

For BIO-NMD to be successful, partners need access to samples of biomaterials from patients with the neuromuscular diseases which they are studying. However, these are extremely precious and collecting new samples of blood requires patients – the majority of whom are children – to consent to them being taken during clinics.

Because of this, it is important that partners communicate with one and other about what they need for their part of the research and what is already available in other repositories which can be shared. This means that no sample should be wasted or used unnecessarily.

It is also very important that the samples are collected, stored and transported in a way which means they are of the highest quality and that anonymised clinical data about the patient is linked to each sample.

Newcastle University is the BIO-NMD partner responsible for coordinating this process and ensuring that we get these factors right. This brief article is based partly on discussions with Amina Chaouch, a doctor responsible for this part of the BIO-NMD project at Newcastle. At University College London Sebahattin Cirak has a similar role and works closely with Amina to ensure that these goals are realised.

Which neuromuscular diseases are being studied in the BIO-NMD project?

Neuromuscular disorders include a large number of different conditions so studying them all at once would be impractical and expensive. The researchers within BIO-NMD therefore chose Duchenne and Becker muscular dystrophy and the collagen 6-related myopathies (Ullrich congenital muscular dystrophy and Bethlem myopathy) as a starting point. These conditions were chosen because the genetic defects causing them are well characterised, and there is already a body of information available on how these genetic defects cause the symptoms. They are also representative of many other conditions because they include both early and late onset forms, fast and slow progression and severe and mild symptoms. In addition, a major advantage of researching these conditions is that they affect two different compartments of muscle cells which are also often affected in other conditions, so any biomarkers found may be more widely applicable.

Sample collection

Samples are therefore needed from patients with Duchenne and Becker as well as those with Ullrich and Bethlem. Patients are approached during clinic appointments at Newcastle, Leiden, London and Ferrara to ask if they wish to provide these samples for research into neuromuscular disease. It is explained that their participation is entirely voluntary and that there is no disadvantage to them in declining. The use of their samples is discussed and, if they are in agreement, a signed consent is obtained[1].

If patients agree, the samples are collected in the form of 3 vials of blood. These vials are sent to a biobank and processed resulting in samples of plasma, serum and DNA. They are stored in biobanks under controlled temperature conditions to ensure that they are not degraded and remain useful for research.

Clinical data on patients is also collected and linked to their samples. This data includes the mutation they have and (if applicable) the age at which they stopped walking and/or needed ventilation to help with breathing. This data is extremely important because it allows the grouping of samples into useful categories, for example to compare patients who stopped walking very early or very late - as the work underway at University College London within BIO-NMD is doing; or to compare patients with the same mutation. This might allow identification of a biomarker which correlates with a particular characteristic of a disease.

The use of samples already in storage

As part of their role in BIO-NMD, Newcastle University has been identifying samples already held in biobanks and other repositories. This is especially useful for obtaining muscle samples. Whilst BIO-NMD is trying to identify non-invasive biomarkers, it is useful to have muscle biopsies during the study.

Importantly, a molecule similar to DNA, called RNA, can be extracted from muscle. When a gene is 'switched on', RNA 'photocopies' of the gene's code are made. The RNA moves outside the nucleus where it directs the manufacture of proteins. RNA can give a picture of the link between the DNA mutation of a patient and the actual protein made in their cells. RNA can therefore be the 'missing link' to explain why sometimes a patient with one particular mutation makes protein differently to another patient with the same mutation.

However, new biopsies are not collected for use in the BIO-NMD research – this would be too invasive. Partners requiring muscle samples for their work therefore rely on those already held in storage.

Distribution of samples to partners

Newcastle has coordinated and monitored the distribution of these samples as they are required by other partners. Multiple shipments have been made to Leiden and results from their work using these are very promising. Two potential biomarkers linked to disease severity have emerged and more are expected to follow (results published[2]). Other samples have been sent to London, Milan, Ferrara and Stockholm, again producing exciting and promising results.

In the future

Sample collection, storage and exchange will continue and BIO-NMD will be able to make use of these for partners' research towards their goals until the end of the project (in December 2012). Samples will also be available for similar, ethically approved research after that time.

Eurobiobank is a network of 16 biobanks from 8 EU countries which stores and distributes quality DNA, cell and tissue samples for scientists conducting research on rare diseases, including neuromuscular disorders. It will continue to be useful as a source of samples for BIO-NMD, especially muscle biopsies.

As the project progresses, longitudinal samples will be useful – this is where subsequent samples are taken from the same patients some time after the first were collected. This allows the comparison of potential biomarkers in an individual patient at different time points. Changes in levels of those biomarkers may be very informative and tell researchers more about what they mean.

Further biomarkers may be found through the study of samples of skin fibroblasts (the cells that make collagen). They may help to identify the causes of abnormal collagen production in Bethlem and Ullrich patients. This will be of great use to those partners working on the collagen 6 diseases. Skin fibroblasts are taken by removing a small cylindrical piece of skin using a local anaesthetic – the procedure is perhaps as invasive as having a blood sample taken.

Successes

The promising results and emerging new discoveries which are coming from the BIO-NMD project have only been possible because of patients giving their samples and the coordinated efforts and collaboration of all the partners involved. The sharing of resources such as human samples, the free discussion between partners about their results and ideas along with the coordinated approach to the research being undertaken has meant that efforts have not been duplicated and precious samples from patients have been used to maximum benefit.

This has all resulted in rapid progress during the first 18 months of BIO-NMD and gives extremely encouraging indications that by the end of its EU funding, the project will have been successful in its aims to improve the diagnosis, monitoring and treatment of patients with neuromuscular disease.

To comment on this article, visit www.bio-nmd.eu
or email: catherine.turner@newcastle.ac.uk

1. http://www.eurobiobank.org/en/documents/DisplayDocList.jsp%3fcatid=5.html
2. *Nadarajah VD et al., Serum matrix metalloproteinase-9 (MMP-9) as a biomarker for monitoring disease progression in Duchenne muscular dystrophy (DMD), Neuromuscul Disord (2011), doi:10.1016/j.nmd.2011.05.011*

News and Events

Global TREAT-NMD conference in Geneva: registration open

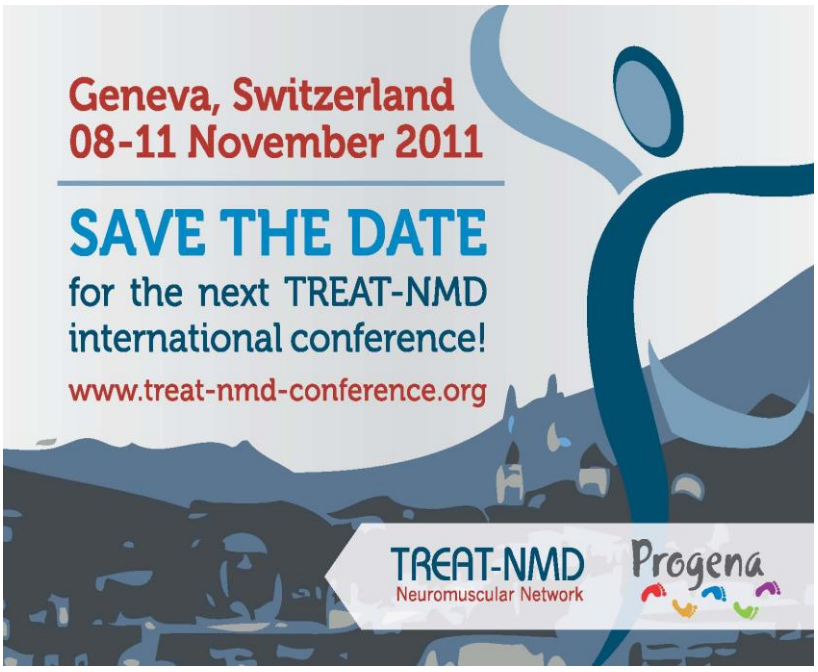
8-11th November 2011

This international conference provides a unique focus on the challenges facing therapy development for inherited neuromuscular diseases.

TREAT-NMD is an international network currently funded by the EU that aims to advance diagnosis, care and therapy development in NMDs for the benefit of patients and families. It links scientists and healthcare professionals, the pharmaceutical industry, regulators and patient groups all over the world.

Progena foundation, local host and co-organiser of this conference, is a Swiss patient organisation raising funds for the research against rare genetic diseases and providing support for families.

Further details, a conference programme, registration and abstract submission information can be found now at: **http://www.treat-nmd-conference.org/**



BIO-NMD partners are represented at big industry events

Dr Matteo Bovolenta (University of Ferrara) attended and presented at the Companion Diagnostics Summit Europe in Frankfurt on 22-24 March 2011.

Matteo began by explaining why biomarkers are needed in neuromuscular disorders. He gave the rationale for the BIO-NMD project, showing how the work was organised and the role of each partner within the EU funded project. He outlined some preliminary data from the RNA research at Ferrara on collagen 6 patients treated with Cyclosporin A.

In the last part of the presentation, Matteo discussed data from recent papers on exploratory biomarkers in Duchenne muscular dystrophy and collagen 6 disorders.

Matteo can be contacted at bvlmtt@unife.it

Dr Sebahattin Cirak (University College London) also outlined the BIO-NMD project and preliminary results at Biomarkers World Europe in London on 17th May 2011.

Further presentations and sessions dedicated to informing industry about BIO-NMD are planned for 2012.

Please submit news items for future issues to:
catherine.turner@newcastle.ac.uk

Diary dates

23rd July 2011
CMD Family Conference
Philadelphia, USA

13th August 2011
Becker Muscular Dystrophy Conference
Los Angeles, USA

Visit the BIO-NMD website for more details of these events

www.bio-nmd.eu/meetings-and-events/scheduled-meetings/

8th September 2011
TREAT-NMD/ENMC Training Course: Best practice implementation in the management of patients with NMDs
Prague, Czech Republic

8th -11th September 2011
EAMDA 41st AGM
Prague, Czech Republic

17th September 2011
Duchenne Family Support Group Annual Conference
Stratford upon Avon, UK

1st October 2011
Muscular Dystrophy Campaign Scottish Conference
Glasgow, UK

15th October 2011
Muscular Dystrophy Campaign Annual Conference
Nottingham, UK

18th – 20th October 2011
World Muscle Society International Congress
Algarve, Portugal

4th – 5th November 2011
Action Duchenne 9th Annual Conference
London, UK

8th – 11th November 2011
Global TREAT-NMD Conference
Geneva, Switzerland

28th – 30th November 2011
BIO-NMD 6 monthly progress meeting
For Partners and the Patient Association Committee
Ferrara, Italy

21st-22nd February 2012
7th Annual Biomarkers Conference
Manchester, UK

29th February 2012
Rare Disease Day
Worldwide

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

27th June – 1st July 2012
PPMD Conference
Fort Lauderdale, Florida, USA

Please submit future dates for the Diary to:
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