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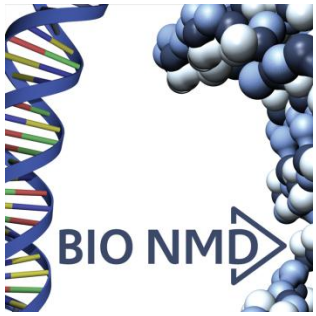
BIO-NMD newsletter



Inside This Issue

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Welcome!

Welcome to the BIO-NMD newsletter. Whether this is the first time you have read about BIO-NMD, or whether you are already familiar with the project, we hope that you will find the information here of use and interest.

The BIO-NMD project has been conducting research into biomarkers for neuromuscular disease. It has been generously funded by an EU FP7 grant over 3 years. This funding has now come to an end and therefore, this newsletter gives a summary of the background and successes of the project.

You will find here, a general overview and explanation of the BIO-NMD project, who has been involved, a summary of the project results and an outline of some plans for the future.

Thank you for your interest in BIO-NMD!

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SEVENTH FRAMEWORK PROGRAMME

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 Alliance (www.treat-nmd.eu) and was borne from collaborations and research ideas within that network.

The project has been searching for 'biomarkers' in people with these conditions. **Biomarkers** are substances (like proteins) in the body that offer a way to measure normal or abnormal processes and to monitor any changes in those processes.

Visit the project's website at

www.bio-nmd.eu

Why biomarkers?

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 biomarkers were identified in patients' blood, saliva, skin or urine, samples of these could be taken throughout clinical trials. Measuring the levels of these biomarkers would show researchers clearly and accurately whether the drug being tested was having 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 invasive muscle biopsies which can be a particular burden on people with a muscle-wasting disease
- Diagnosis can happen earlier because testing for biomarkers may be quicker and easier than genetic testing in some cases
- Disease progression can be more 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
- Patients' likely response to a particular type of therapy may be predicted, ensuring that the best drug is given to each patient whilst minimizing the chances of unpleasant or even dangerous side effects.

Why were these diseases chosen for study?

Neuromuscular disorders include a large number of different conditions so studying them all at once would be impractical and expensive. The BIO-NMD researchers therefore chose **Duchenne** and **Becker** muscular dystrophy which are both caused by changes in the **dystrophin gene** and **Ullrich congenital muscular dystrophy** and **Bethlem myopathy** which are both caused by changes to **collagen VI genes** as a starting point. They chose these conditions 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.

These conditions are also representative of many others because they include early and late onset forms, fast and slow progression and both severe and mild symptoms. In addition, they affect two different parts of muscle cells which are also often affected in other conditions, so any biomarkers found may be more **widely applicable**.

By focusing on a small number of conditions, efforts can be intensified to find biomarkers which can then be **applied to other conditions**. This was true for example during research into biomarkers for breast cancer which have then proved useful for other cancer types.

More about the conditions studied

Duchenne and Becker Muscular Dystrophies (DMD & BMD) are X-linked (usually only boys are affected) recessive neuromuscular disorders affecting approximately 1 in 3,500 and 1 in 30,000 live male births, respectively. Both are characterized by progressive symmetrical muscular weakness. DMD symptoms typically present before age five whereas the milder BMD may not be diagnosed until later.

DMD and BMD are caused by mutations in the dystrophin gene. The human dystrophin gene is the largest human gene, found on the X chromosome and occupying roughly 0.1% of the genome.

Bethlem myopathy is a relatively unknown NMD and is caused by a fault in one of the genes that codes for the protein, collagen VI. It can be diagnosed in infancy right through to adulthood as symptoms can be so varied.

The condition is usually inherited via a dominant gene - this means that if a parent has the condition, there is a 50% chance that their child will inherit it. However, sometimes Bethlem myopathy occurs because of a spontaneous mutation and there is no family history of the condition. It is not X-linked so it affects both boys and girls.

Ullrich congenital muscular dystrophy is also caused by a mutation in one of the genes which codes for collagen VI. It is not X-linked. It is usually a recessive condition which means that the mutated gene is inherited from both parents. Those with just one copy are carriers. Sometimes, the condition is not inherited but occurs because of a spontaneous mutation.

More information can be found via a number of different resources, but the **Muscular Dystrophy Campaign** (UK) is a good starting point for explanations in English. Their website can be found at www.muscular-dystrophy.org/

Who is involved in BIO-NMD?

In rare diseases, patient numbers are low so sharing samples and data is even more important

For projects like this to be successful, the collaboration of many professionals with different skills is required. For research into rare disease, international cooperation is especially important because patient numbers are so very small. This means that sharing resources such as samples, expertise and data between centres around the world is critical to ensure that there are enough patients for any study to be valid. There are 12 European partners involved in the BIO-NMD project coordinated by Professor Ferlini at the University of Ferrara, Italy. For example, some are well placed to collect and distribute patient samples and others are experts in studying genes or proteins, data analysis, animal models or project management. This collaboration of geographically diverse scientists also allows access to a larger cohort of patients with a particular condition or a specific mutation. This is very important when studying such rare diseases.

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**

Instituts thématiques Inserm Institut national de la santé et de la recherche médicale

UNIFE

Muscular Dystrophy Campaign

LUMC

UCL

KTH VETENSKAP OCH KONST KTH Biotechnology

Newcastle University

United Parent Projects Muscular Dystrophy

ARIADNE UNIVERSITÀ TORINO

ACIES Conseil en Management et Valorisation de la Recherche

Applied Biosystems part of life technologies

TeleThon COMBATTI LA DISTROFIA MUSCOLARE E LE ALTRE MALATTIE GENETICHE

UNIVERSITÀ DEGLI STUDI DI MILANO

BIO-NMD is a Translational Research Project – What does this mean?

Translational research bridges the gap between laboratory and patient

Scientists are constantly discovering more and more about the human body. We now understand more than ever the processes which keep us alive and well along with some of the things that go wrong causing disease or ill-health. Much of new discovery and technology comes from basic research which takes place in the laboratories of academic institutions or companies around the world. However, harnessing this knowledge and turning new discoveries into useful, marketable treatments of direct benefit to human health is much more difficult. **Translational research** aims to overcome this problem. It is research which turns new knowledge and understanding gained in the laboratory into meaningful therapies, drugs or preventative measures which are of direct benefit to patients. In short, it **bridges the gap between laboratory and patient**.

BIO-NMD has done this by trying to identify biomarkers which can be measured in patients' blood, skin or urine to tell doctors more about the type of neuromuscular disease they have and the best treatment programme for them. Biomarkers will also speed up the testing of new drugs which may be of direct benefit to patients' health.

Some important results and outcomes

The success of BIO-NMD has been due to the collaborative nature of this project. The EU FP7 funding has allowed world-leading experts in different aspects of biomarker discovery to work together, sharing resources and results in order to achieve one common goal.

Collaboration between partners has led to success

We have been able, for example, to share over 1000 precious **patient samples** between partners for different kinds of analysis. Most of these samples have been held in partners' own institutions but the consortium was also able to request additional samples from the **EuroBiobank** (http://www.eurobiobank.org) – especially muscle biopsies – where they were needed.

Members of the BIO-NMD consortium met, in person, every 6 months. This allowed the leading researchers from each partner – known as principal investigators or PIs – to get together along with the full research teams, including laboratory scientists, clinicians, bioethics experts, patient representatives, project managers and biological data experts, in order to discuss and present progress, share ideas and plan the next steps.

At the end of the 3 year funding period, this has resulted in some promising and exciting outcomes. A table of the most significant results from the BIO-NMD project is given below along with references to the detailed methodology and results published in the academic press.

It can be seen that there are several potential biomarkers identified as part of the research. **MMP-9**[1] is a protein whose levels in the blood appear to be directly linked to the progression of DMD. Further work is underway in order to 'validate' (or prove) that MMP-9 levels are reliably and accurately linked to disease progression.

Epidermal **melanocytes**[2] (cells found in the skin) have been shown, for the first time, to express dystrophin. As melanocytes can be cultured (grown) from cells taken by skin biopsy, they offer a potential cellular model for the study and monitoring of DMD, BMD and other dystrophinopathies, **without the need for muscle biopsies**.

A range of promising, potential biomarkers have been identified

Autophagy is an essential process where a cell's components, such as mitochondria which use glucose to release energy, are broken down and recycled in order to repair damage and remove dysfunctional parts from cells. Autophagy has been found to be impaired in collagen VI disease leading to the accumulation of dysfunctional mitochondria and other components, resulting in the degeneration of muscle fibers. Monitoring autophagy through measuring the **accumulation of these damaged components** may therefore be a way to track disease progression in Ullrich congenital muscular dystrophy and Bethlem myopathy[6].

Ariadne DX are the BIO-NMD partners who are experts in **bioinformatics** – ie handling, processing and analyzing large amounts of biological data using sophisticated software. They have used the data generated by the BIO-NMD project, along with input from other partners to select a list of **12 genes** which may be potential **drug targets for NMDs**[4]. The expression of these genes could be useful as biomarkers to monitor the efficacy of any new drugs acting on those targets. Ariadne DX has also ranked the **DMD biomarkers** identified by other partners in order of significance for further study.

Cells called **macrophages**, important in the immune system, were studied in blood samples from **collagen VI patients**. The levels of collagen VI expressed by these macrophages were compared to levels in muscle biopsies and it was found that the two mirrored each other[8]. This has led to the suggestion that macrophages in blood samples could be biomarkers to **measure disease progression or therapy efficacy** in place of muscle biopsies for patients with collagen VI related myopathies.

Biomarker	Potential use	Ref
Serum matrix metalloproteinase-9 (MMP9)	To monitor disease progression in DMD.	Nadarajah et al., 2011[1]
Melanocytes taken from skin biopsy	To monitor disease progression and response to treatment in DMD.	Pellegrini et al., 2012[2]
Long non-coding RNA (lncRNA)	May be able to indicate levels of dystrophin expression and so replace the muscle biopsies currently used for measuring disease progression or response to therapy in DMD and BMD.	Bovolenta et al., 2012[3] (open access)
12 genes selected from a list of candidates using sophisticated software	These may be genes which are potential drug targets for NMDs. Their expression could be useful to monitor the efficacy of any new drugs acting on those targets.	Kotelnikova et al., 2012[4] (open access)
TIMP-1 and Osteopontin	To monitor disease progression in DMD.	Nadarajah et al., 2011[1] van Putten et. al., 2012[5] (open access)
Some of the key proteins involved in autophagy (eg LC3, Beclin-1 and Bnip3)	To monitor disease progression in collagen VI diseases – autophagy is inactive in these conditions so, put simply, less autophagy means worsening disease.	Grumati et al. 2011[6]
FluiDMD – a method developed by partners for the analysis of the dystrophin transcript	For precise diagnosis of DMD – different transcripts (copies of the genetic material) produce different forms of dystrophin. Analysis of these transcripts gives a clear picture of the fault in the dystrophin protein.	Bovolenta et al., 2012[7]
Monocyte derived macrophages (MDMs) – mirror collagen VI expression in muscles	For diagnosis and monitoring of Bethlem myopathy and Ullrich congenital muscular dystrophy (collagen VI diseases) in place of repeated muscle biopsies.	Gualandi et al., 2011[8]

Table 1: Exploratory biomarkers for NMDs discovered as part of the BIO-NMD project

Future plans

Whilst BIO-NMD has come to the end of its EU funding period, the outcomes of the research are being taken into **new projects** and collaborations. As a result, these promising results will be explored further, built upon and used to continue progress towards the goal of having **biomarkers for NMDs** which can be used in **clinical settings**. Some specific examples of future projects are given below.

Neuromics is a large and ambitious EU funded collaboration which includes 6 partners from the BIO-NMD project. It aims to increase the number of **neuromuscular and neurodegenerative** patients receiving a genetic diagnosis by identifying new genes for these diseases. It also hopes to develop new, targeted therapies and translate these to a larger group of diseases, especially other NMDs. Part of the Neuromics research will develop biomarkers, some of which will come from the BIO-NMD project, for clinical use. In particular, these biomarkers will be focused on improving and increasing the number of successful **clinical trials** for NMD and NDD.

The Neuromics project began on 1st October 2012 and is funded for 5 years by an FP7 grant from the EU. It is coordinated by Prof. Olaf Riess at Tübingen University in Germany. A website will be launched soon and can be found at: www.rd-neuromics.eu

Neuromics will work closely with another FP7 project, **RD-Connect.** RD-Connect will develop an integrated global platform connecting registries, biobanks and clinical data for rare disease research. It aims to facilitate and expedite rare disease research in line with the recently stated IRDiRC (International Rare Diseases Research Consortium) goal of getting a **genetic diagnosis for all rare disease patients** and developing **200 new rare disease therapies** by the year 2020.

RD-Connect began on 1st November 2012 and is funded for 6 years by an FP7 grant from the EU. It is coordinated by Prof. Hanns Lochmüller at Newcastle University in the UK. The project website will be at: http://www.rd-connect.eu/

Industry involvement will be crucial to success

Other potential collaborations between BIO-NMD partners and stakeholders, including **pharmaceutical companies** are in the pipeline. At a recent BIO-NMD meeting in Rome, representatives from industry were invited to come along to hear about the results of the project and discuss ways in which they could work with partners using these results to **develop potential biomarkers further**.

Meanwhile, the BIO-NMD web-page (www.bio-nmd.eu) will remain, partners will continue to work together to exploit the project results and discussions with patient groups will go on in order that all stakeholders, including patients, remain informed and up to date about progress towards **accepted, recognized biomarkers ready for clinical use**.

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