

Biomarkers for clinical trials

BIO-NMD - *an EU funded FP7 project*



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Action Duchenne Conference 2011

BIO NMD  Funded by



Topics

- What is BIO-NMD?
- What is a biomarker?
- Background: Genes and Protein and DMD
- Examples:

Exome sequencing and candidate gene analysis

- BIO-NMD pipeline and on-going work
- How you could help us

What is BIO-NMD?

- BIO-NMD is an **EU funded** research project focusing on:
 - **Duchenne** muscular dystrophy;
 - **Becker** muscular dystrophy;
 - **Ullrich congenital muscular dystrophy**;
 - **Bethlem myopathy**
- The project is searching for **biomarkers** in people with these conditions

Why biomarkers?

- Biomarkers are substances in the body that offer a way to measure normal or abnormal processes.
- A biomarker may be a protein found in bodily fluids such as the blood or urine, or in tissues such as the muscles. Alternatively, a particular gene could be a biomarker.
- They can be useful as surrogate endpoints in **clinical trials** thus speeding up, reducing the cost and improving sensitivity of trials.
- Serum creatine kinase (CK) is a bad biomarker
- Example: CK high in Becker and Duchenne Muscular dystrophy and dependent on environmental factors

Other benefits of biomarkers

- Disease progression can be accurately measured allowing better **clinical management of symptoms**
- Existing treatments can be adjusted to precisely meet the needs of individual patients and ensure they get the **maximum benefit**
- **Example prediction of Steroid response and side effects**
- Identifying pre-clinical biomarkers (both genomic and proteomic) in DMD to be used as markers
 - for disease progression and prognosis (**diagnostic biomarkers**)
 - drug response (**pharmacodynamic biomarkers**)
 - therapy monitoring (**pharmacokinetic and safety biomarkers**)
- Focusing on non-invasive biomarkers (e.g. blood, urine)

What is a gene?

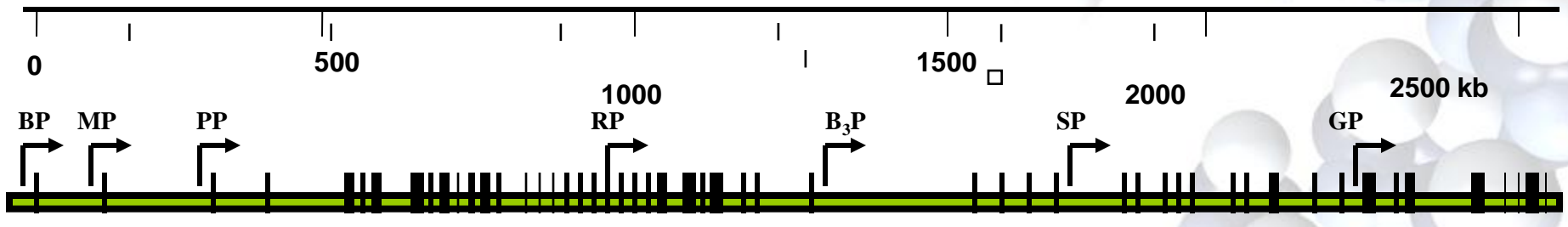
- A gene contains the instructions or **code** to make a **protein**
- The genes are located along strands of **DNA** in the nuclei of cells
- Each gene is made up of nucleotides or **bases** in a set order which cause particular **amino acids** to be made
- These amino acids are the **building blocks** of the **proteins**

From gene to protein

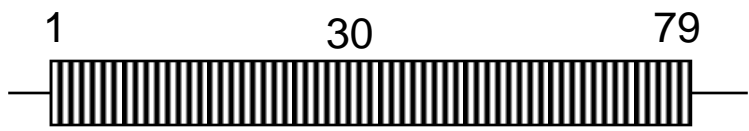
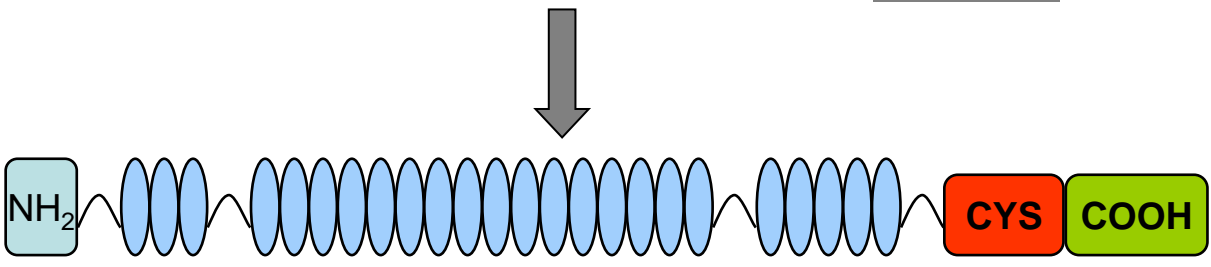
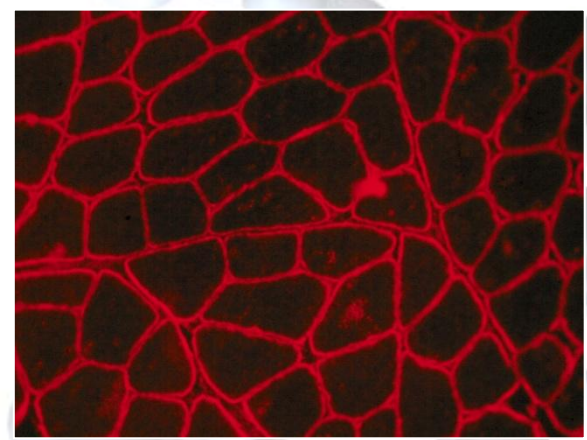
- To get from a gene to a protein, the sequence of bases needs to be **copied**
- **RNA** does the copying – it makes a **mirror image** of the sequence and carries it to the protein making parts of the cell – the **ribosome**.
- Here, the special order of the bases binds to **specific amino acids** so they are now arranged in the order needed to make the protein

A**Chromosome**

Xp21

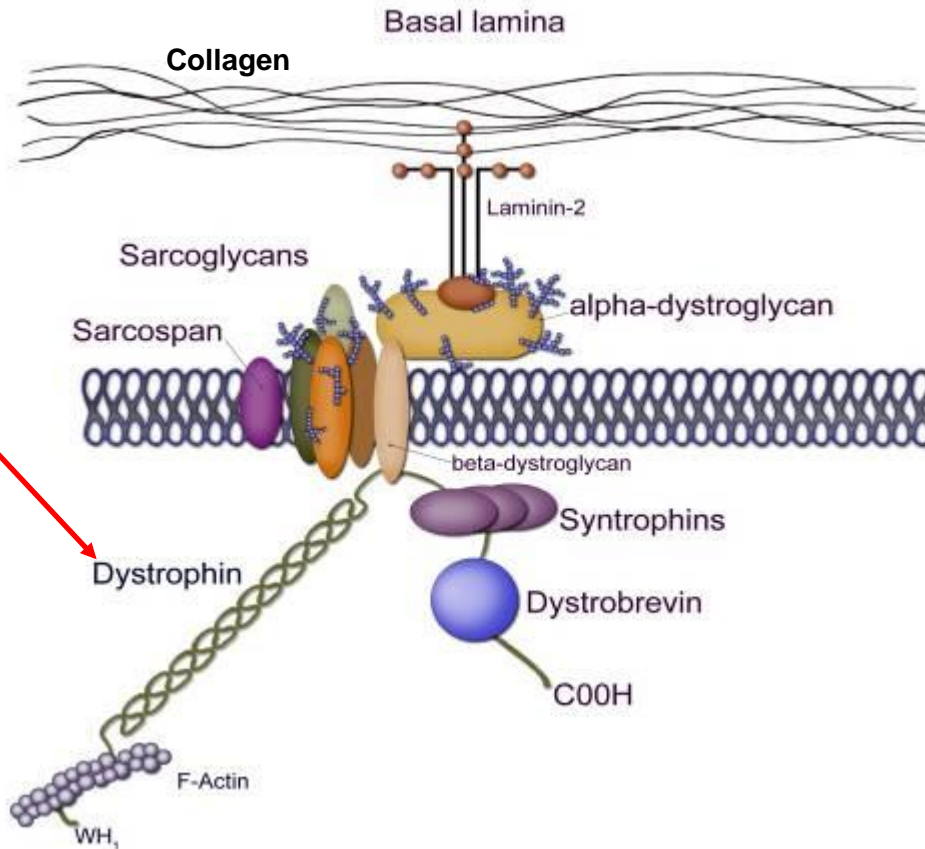


Dp427B
Dp427M
Dp427P
Dp260
Dp140
Dp116
Dp71

DNA**mRNA****Dystrophin protein**

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Dystrophin: the gene and the protein



Protein function: dystrophin belongs to the protein system of force transduction in muscle membrane and it acts as elastic bridge between the cell cytoskeleton and the extracellular matrix.

Dystrophin gene mutations are responsible for genetic diseases inherited as **X-linked recessive disorders**.

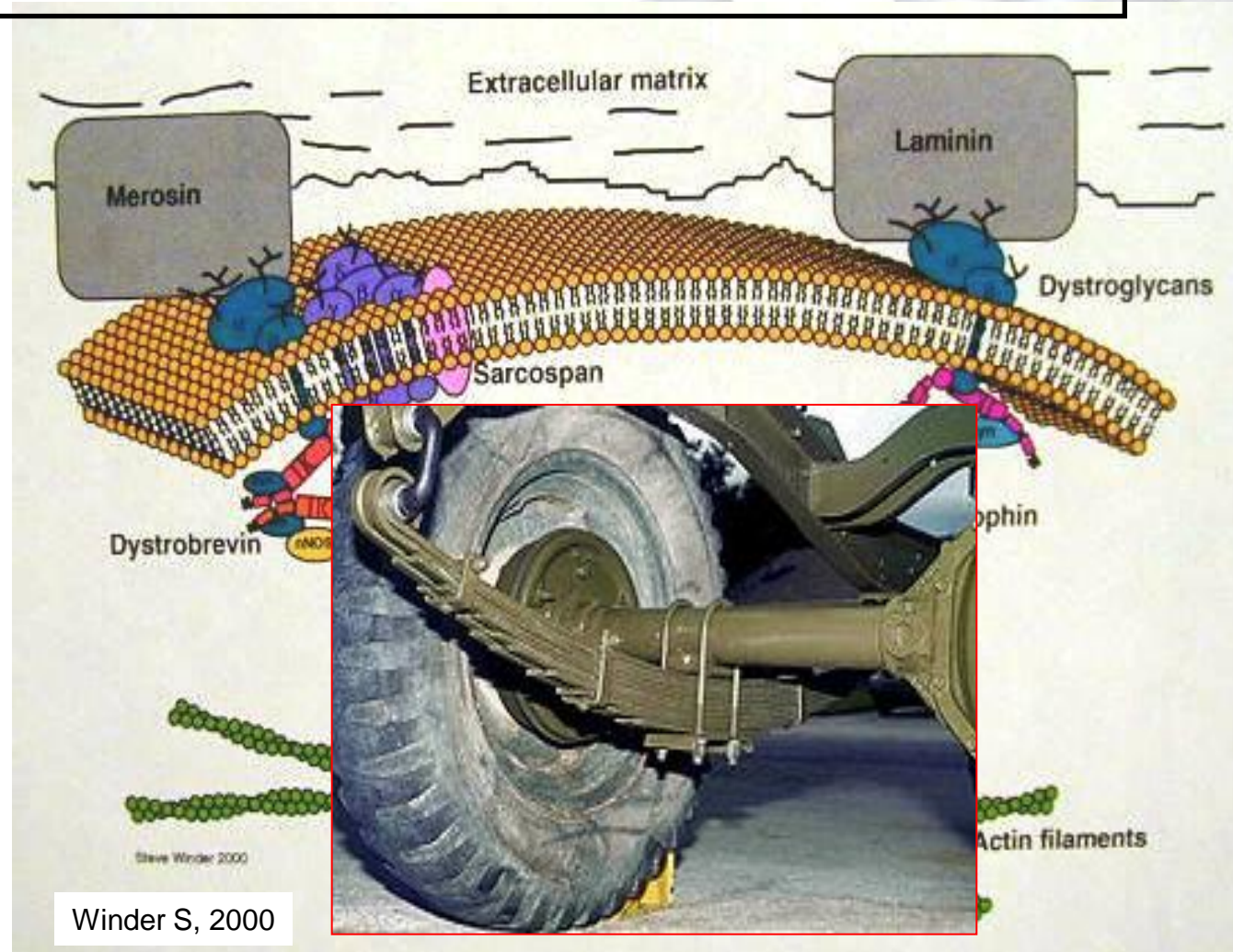
The pathology affects almost **exclusively male individuals**.

Mutations **interrupting the reading frame** cause dystrophin absence and **severe clinical features (DMD)**.

Mutations **maintaining the reading frame** cause dystrophin reduction and **BMD or milder clinical features**.

Muscular dystrophy

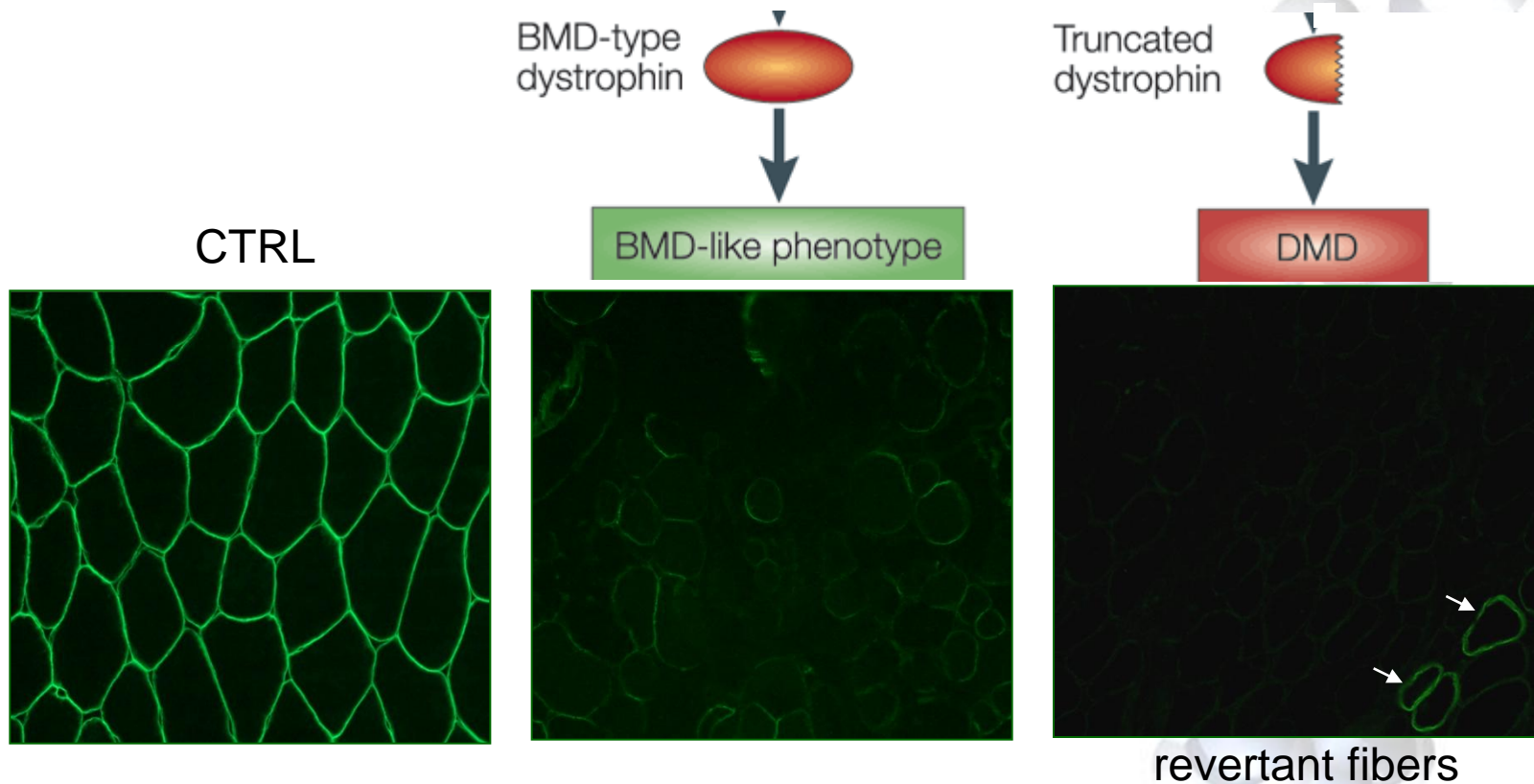
Dystrophin:
A shock absorber



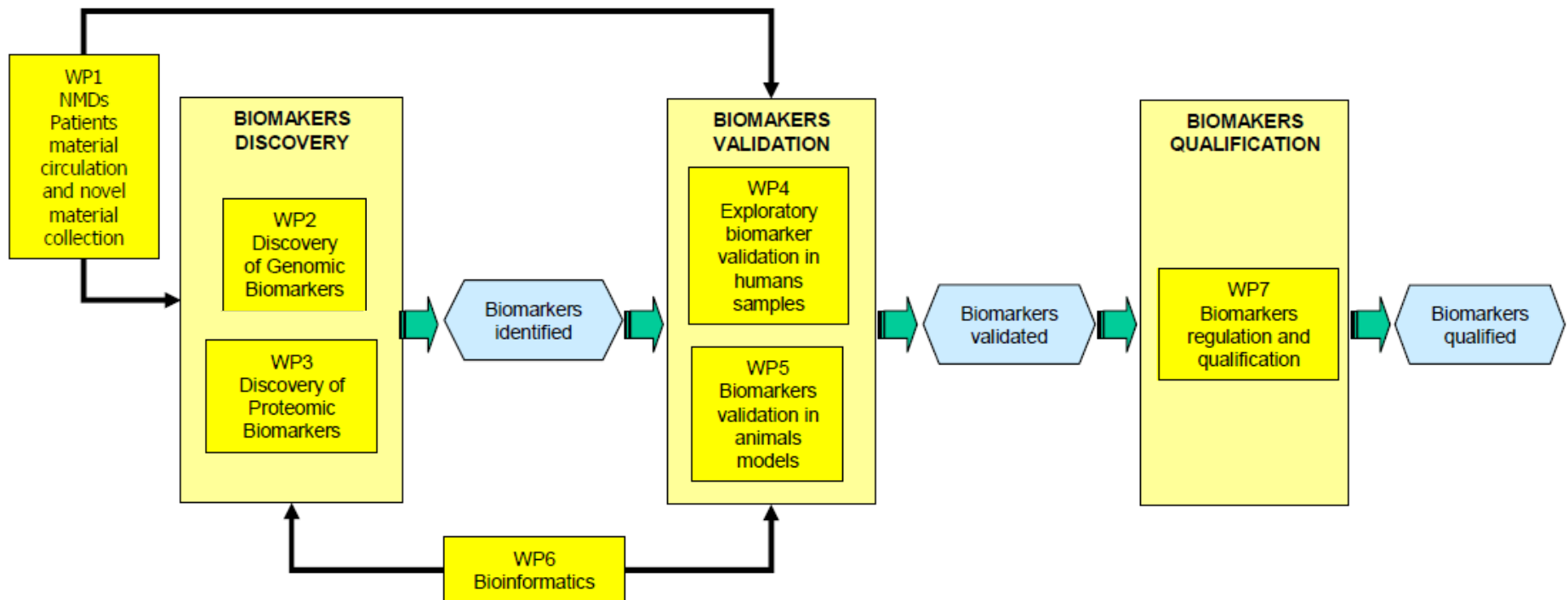
Winder S, 2000

Distinguishing DMD from BMD

- Dystrophin is virtually **absent** in skeletal muscle of **Duchenne** patients, in rare cases strongly reduced (rare revertant fibers)
- Dystrophin is **present** though qualitatively and quantitatively **reduced** in skeletal muscle of **Becker** patients



The BIO-NMD Pipeline

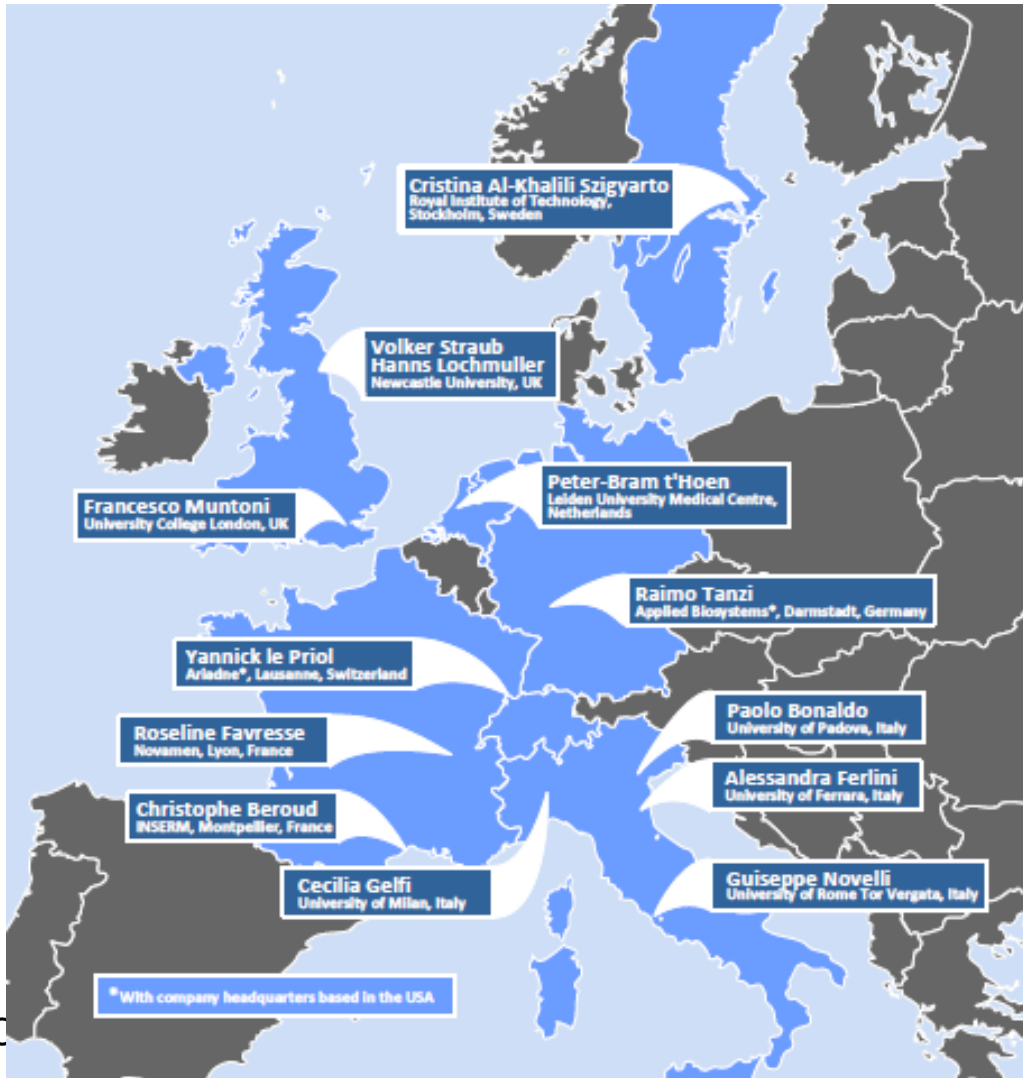


WP8 - Societal and Ethical Aspects

WP9 - Exploitation and Dissemination

WP10 - Management

Who is involved?

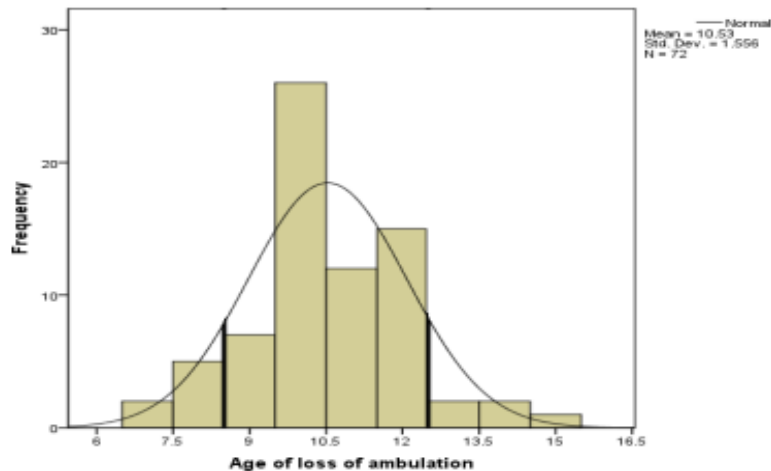


Alessandra Ferlini at the University of Ferrara co-ordinates BIO-NMD which has 12 European partners.



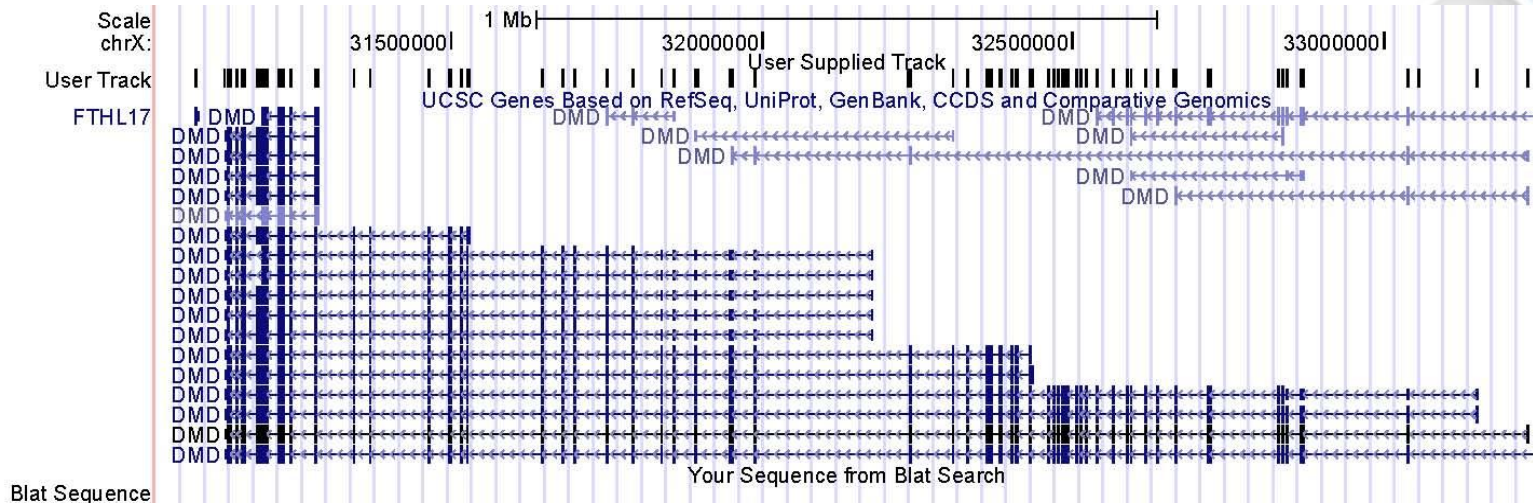
Example Exome Sequencing

- Discovery phase with focus on very mild and very severe DMD cases.



- Aim is to identify gene variants that predispose for early or late loss of ambulation. We are looking at all coding variants of the human genome by so called Exome sequencing.

Whole exome sequencing



- Protein-coding regions constitute about 1% of the human genome or about 30 megabases (Mb), split across 180,000 exons.
- Examining only 1% of the genome gives about 90-98% of the information about positions that cause changes in traits.
- Agilent SureSelect 50Mb XT- covers 99.86% human protein coding regions annotated in NCBI Consensus CDS database.

First run of Exomes...but Pipeline is working!

- Early loss of ambulation vs. late loss of ambulation

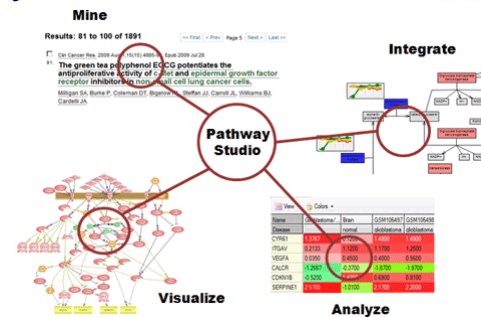
Sample ID	Age	Mutation	Ambulant	Age loss of ambulation
ES0SN	01/12/1997	DMD del48-50	No	6
ES065	09/09/2000	DMD c.2098C>T,p.Gln700X	No	7
ES005	29/01/1999	DMD del 8-12	No	7.5
ES074	22/12/1995	DMD del 10-11	No	8
ES067	02/12/1995	DMD del 46-51	No	8.5
ES028	18/06/1995	DMD del 46-49	No	12
ES050	13/03/1992	DMD del exon 45	No	12
ES075	05/12/1985	DMD del exon 51	No	12
ES045	21/07/1996	DMD del 5-7	No	15
ES0SW	12/03/1997	DMD del 42 -43	Ambulant at 14y	NA

- Currently ongoing: more samples and exome sequencing of DMD patients above the age of 30y.

Finding the needle in the haystack

- On average **85 490 gene variants** were detected in the **early loss of ambulation** group of which 77 325 were single nucleotide variants (SNVs) and 8 165 small insertion and deletions (so called indels). In the **late loss of ambulation group**, on average **85 677 gene variants** were detected - 77 390 were SNVs and 8 286 indels.
- First step checking what is unique different and never observed before did not yield in a convincing candidate variant
- BUT a gene variant in the normal population or even a combination of gene variants may be disease modifier and also a biomarker, therefore a SYSTEMSBIOLOGY approach is currently undertaken.

<http://www.ariadnegenomics.com/>



SPP1 genotype is a determinant of disease severity in Duchenne muscular dystrophy



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PhD
J.M. Devaney, PhD
C.M. McDonald, MD
On behalf of the
Cooperative
International
Neuromuscular
Research Group

ABSTRACT

Objective: Duchenne muscular dystrophy (DMD) is the most common single-gene lethal disorder. Substantial patient-patient variability in disease onset and progression and response to glucocorticoids is seen, suggesting genetic or environmental modifiers.

Methods: Two DMD cohorts were used as test and validation groups to define genetic modifiers: a Padova longitudinal cohort ($n = 106$) and the Cooperative International Neuromuscular Research Group (CINRG) cross-sectional natural history cohort ($n = 156$). Single nucleotide polymorphisms to be genotyped were selected from mRNA profiling in patients with severe vs mild DMD, and genome-wide association studies in metabolism and polymorphisms influencing muscle phenotypes in normal volunteers were studied.

Results: Effects on both disease progression and response to glucocorticoids were observed with polymorphism rs28357094 in the gene promoter of SPP1 (osteopontin). The G allele (dominant model; 35% of subjects) was associated with more rapid progression (Padova cohort log rank $p = 0.003$), and 12%-19% less grip strength (CINRG cohort $p = 0.0003$).

Conclusions: Osteopontin genotype is a genetic modifier of disease severity in Duchenne dystrophy. Inclusion of genotype data as a covariate or in inclusion criteria in DMD clinical trials would reduce intersubject variance, and increase sensitivity of the trials, particularly in older subjects.

Neurology® 2011;76:219-226

Direct analysis of candidate genes



Available online at www.sciencedirect.com



Neuromuscular Disorders 21 (2011) 569–578



www.elsevier.com/locate/nmd

Serum matrix metalloproteinase-9 (MMP-9) as a biomarker for monitoring disease progression in Duchenne muscular dystrophy (DMD)

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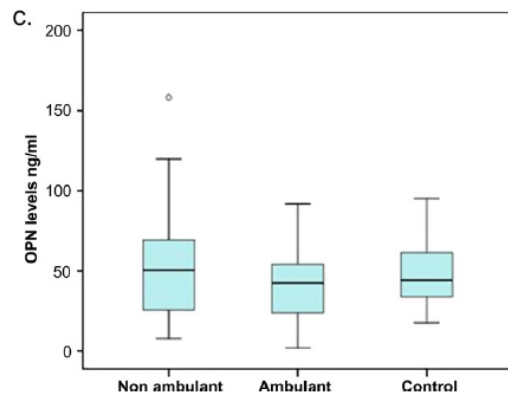
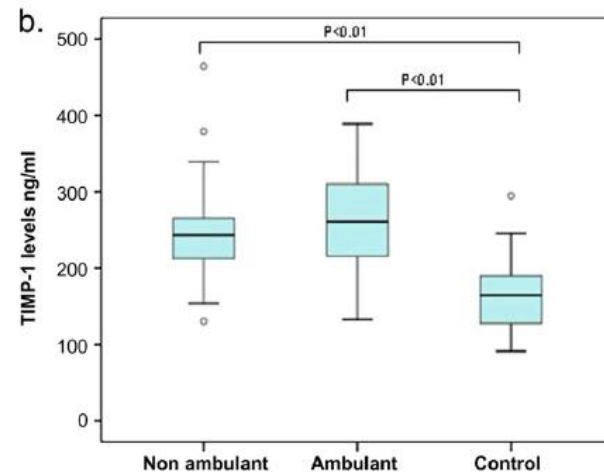
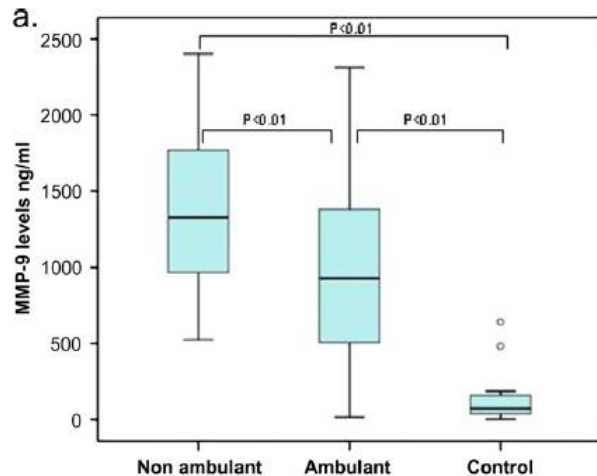
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MMP-9 as a marker for disease progression



V.D. Nadarajah et al. / Neuromuscular Disorders 21 (2011) 569–578

Fig. 4. Serum levels of (a) MMP-9, (b) TIMP-1, (c) OPN in nonambulant, ambulant and control samples. The median levels are indicated by the horizontal lines bisecting the box plot, which shows the interquartile range. The upper and lower limit of the bars show the maximum and minimum values considered while the extreme values are indicated by \circ . One way ANOVA was used to determine differences between groups and the Student's *t*-test was applied to determine significant difference between two groups.

miRNA- a new candidate

- Some of the RNA does not carry a message to code for protein – this is **non-coding RNA** (ncRNA)
- Much of this ncRNA is known as **micro RNA** (miRNA)
- These are short sequences that can **bind** to the coding RNA and cause them to be switched off – or **silenced**, preventing the protein they code for being made
- It may be that some miRNA can turn genes on but less is known about this

BIO-NMD project:
produces validated
pre-clinical biomarkers
for dystrophinopathies
and COL VI myopathies

Transfer biomarkers
to clinical practice

Pharmacokinetic biomarkers:
monitor how a particular drug
is distributed and broken down
in the body

Safety biomarkers: indicate
toxic or adverse side effects of
a particular drug

**Pharmacodynamic
biomarkers:** monitor what
effect a particular drug is
having on the body

**Diagnostic/Prognostic
biomarkers:** identify the
disease, its stage and the likely
outcome with no therapy

Improving clinical trials
–potentially bringing
more drugs to market

Improving patient health

Improving
measurement of
disease progression

Discovery of
biomarkers for
other NMDs

Personalised treatment



Reducing the need
for biopsies

Speeding up diagnosis

Diagnosis
screening in wider
population

Developing
around 1000
immunoassays
for plasma-
serum
screening

Collecting non-
invasive
samples from
other NMDs

Validation
process for
other NMDs



Overall omics strategies for biomarker discovery

Proteomic Biomarkers

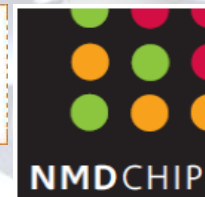
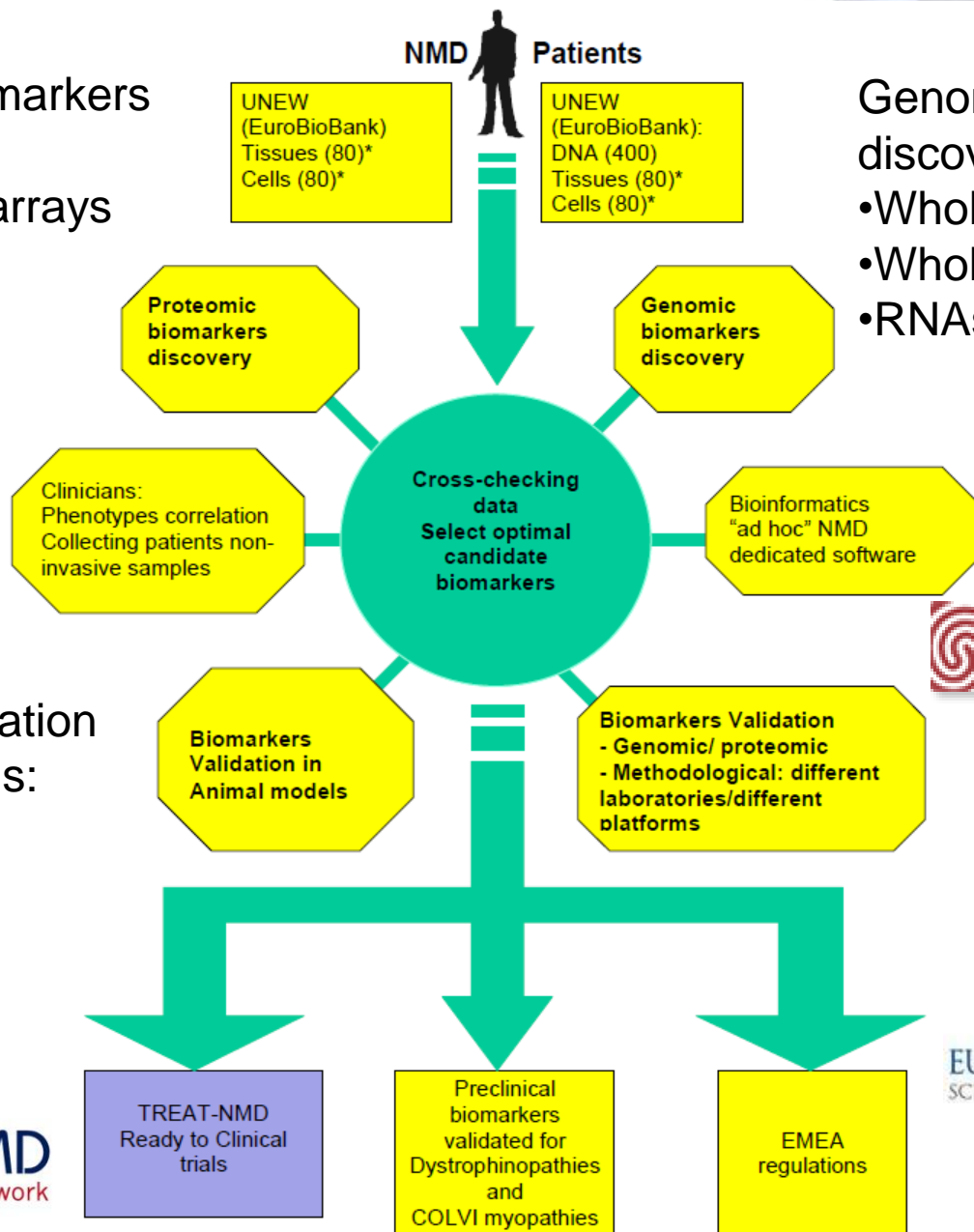
Discovery:

- Protein microarrays
- LC/MS/MS
- FTICR
- 2D-DIGE

Genomic Biomarkers

discovery:

- Whole exome sequencing
- Whole genome CNVs
- RNAseq and miRNAseq



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Biomarker validation in animal models:

mdx
mdx Utrn -/-
Col6a1 -/-

...

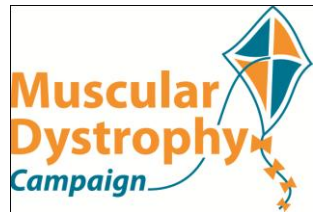


How you could support us

- Please donate a blood sample and an urine sample at the next clinic visit!
- Consent for the research use of stored diagnostic muscle biopsy samples.

Involving patients

- BIO-NMD is working with representatives from the patient community in the form of a **Patient Association Committee**
- This committee can be contacted through the BIO-NMD website at www.bio-nmd.eu
- The project's communications officer, **Cathy Turner** at Newcastle University, can be reached on **catherine.turner@ncl.ac.uk**
- **BIO-NMD's Patient Association Committee:**
 - **Marita Pohlschmidt**, Muscular Dystrophy Campaign UK
 - **Elizabeth Vroom**, United Parent Project Muscular Dystrophy
 - **Anna Ambrosini**, Telethon Italy



United Parent Projects
Muscular Dystrophy



Acknowledgements to the BIO-NMD Teams

UCL, Dubowitz Neuromuscular Centre

- **Irina Zaharieva**
- Lucy Feng
- Darren Chambers
- Jenny Morgan
- Francesco Muntoni

Newcastle University

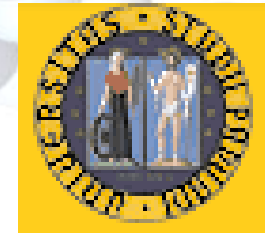
- **Cathy Turner, extra special thanks for helping in preparing this session**

LUMC

- Michiel van Galen
- Michel Villerius
- **Peter A.C. 't Hoen**

BIO-NMD partners

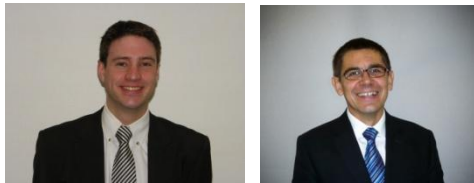
THE BIO-NMD TEAM (Together Everyone Achieves More)



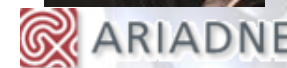
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