La genetica e la clinica: un imprescindibile connubio nella diagnosi delle malattie muscolari complesse

Il punto di vista del genetista

Roma, 1-2 Marzo 2019

FERNANDA FORTUNATO, MD
DIPARTIMENTO DI SCIENZE MEDICHE,
DIPARTIMENTO INTERAZIENDALE MATERNO-INFANTILE UO GENETICA MEDICA
AOU SANT'ANNA FERRARA

frtfnn@unife.it

TOPICS

- Rare neurological and neuromuscular diseases (NNMD): concepts
- Genetic diagnosis: brief summary
- New techniques for genetic testing
- Prevention
- Why genetic diagnosis is necessary

NEUROLOGICAL AND NEUROMUSCULAR DISEASES (NNMD)

Neurodegenerative (ND) and neuromuscular (NM) diseases are amongst the most frequent classes of rare diseases, affecting life and mobility of **500.000 patients** in Europe and millions of their caregivers, family members and employers

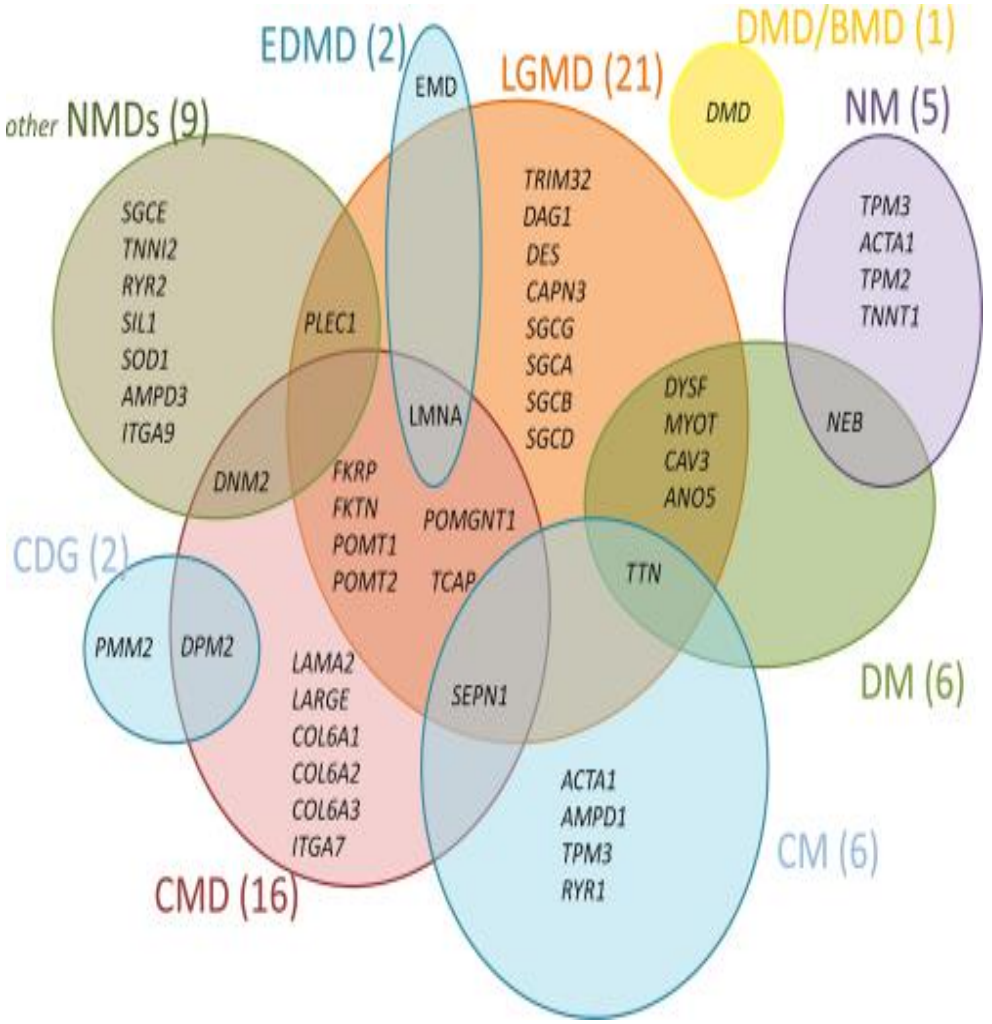
Disease	Prevalence	Patients in Europe*
FTLD	3-10 per 100,000	15,000-50,000
HD	3 - 7 in 100,000	35,000
Ataxias	20 in 100,000	200,000
HSP	2-10 in 100,000	10,000-50,000
SMA/LMND	5-10 in 100,000	20,000-40,000
HMN	1 in 2,500	200,000
CMS	1 - 10 in 1 Mio	500 – 5,000
CMD	7 - 12 in 100,000	40,000
D(B)MD	1 in 20,000	25,000
Dysferlinopathies	1 in 50,000	10,000
FKRP	0.5 in 100,000	2,000
MCP	0.5 to 1 in 40,000	6,000-12,000

***Patients numbers are estimated from numbers of single countries and extrapolated to the EU, or are calculated from heterozygote frequencies**

NEUROLOGICAL AND NEUROMUSCULAR DISEASES

NDDs/NMDs are characterized by:

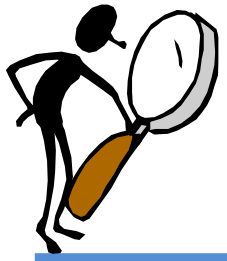
- **High genetically heterogeneity:** for NMDs more than **500 genes** are implicated on disease's pathogenesis
- **Mutations occurring in largest genes:** these genes sometimes are not fully tested in diagnostic but have been analyzed firstly for mutation in hot spot regions
- **Clinical heterogeneity: overlap of symptoms** in different NDDs/NMDs diseases
- **Unidentified genes**

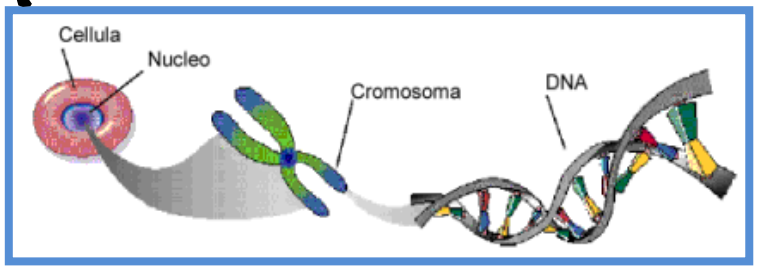


As a result, between 30-80% of patients with NDDs/NMDs remain undiagnosed

NDDs/NMDs: GENETIC VIEW

- **Inheritance: all types**
  - **Autosomal Dominant**
  - **Autosomal Recessive**
  - **X-linked**
  - **Matrilinear**





- **Functional/anatomic compartments:**
  - All **CNS**, **cerebellum**, **compartments**
  - **Spinal cord**
  - **Peripheral nerves**
  - **Skeletal muscle**
  - **Motoneurons**
  - **Neuromuscular junctions**

- **Mutations: all types**
  - **Large rearrangements** (deletions, duplications)
  - **Small mutations** (nonsense, missense, frameshifting, splicing)
  - (-100% mutations detected, method dependent)
  - (all possible mutations identified, **multiple methods often needed**)
  - **Specific disease diagnosed**

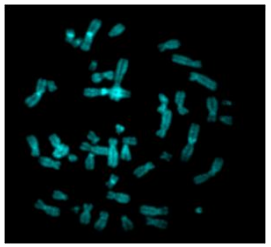
GENETIC DIAGNOSIS: THE DIAGNOSIS ON GENOTYPE
Types and Timing

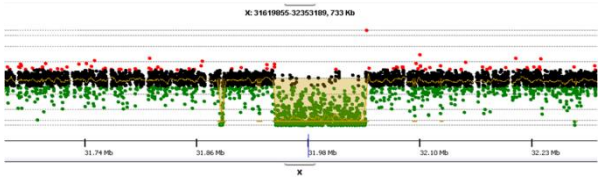
- **Definition**

The analysis of human DNA, RNA, chromosomes in order to detect heritable disease- related genotypes/ mutation for clinical purposes

- **Genome testing**

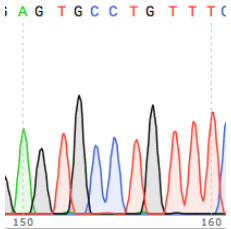
karyotype, CGH, Whole genome, RNAseq





- **Gene (single-multi) testing**

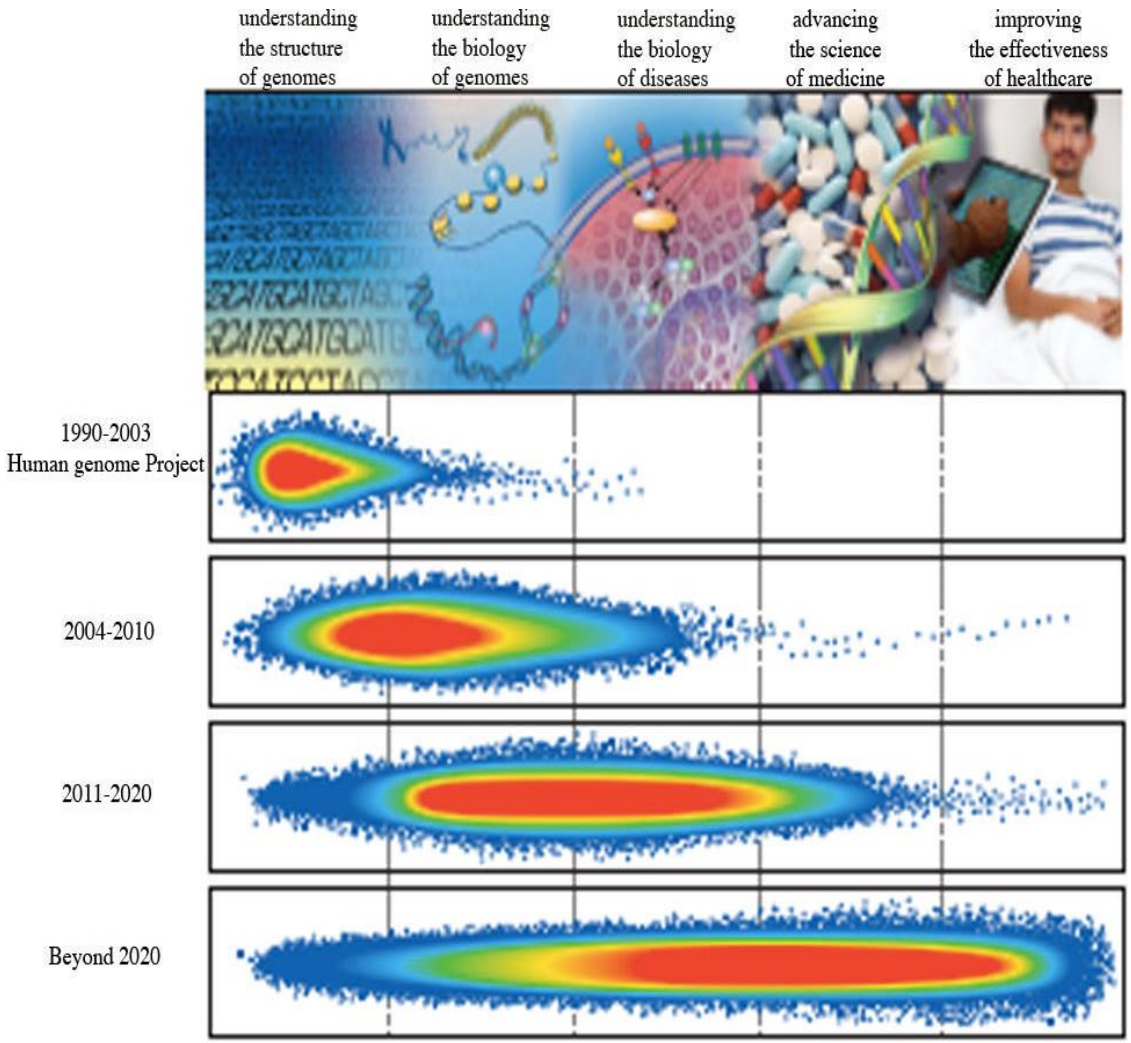
PCR, Sanger sequencing, MLPA, panels, WES, etc



- **Prenatal**
  - **foetal tissues/fluids**
- **Couple testing**
  - **Preconceptional** and **preimplantation or PGD**
- **Perinatal (screening)**
  - **Newborn screening**
- **Postnatal**
  - **Diagnostic** (disease phenotype)
  - **Presymptomatic** (risk of diseased phenotype)
  - **Predictive** (susceptibility for a diseased phenotpe)

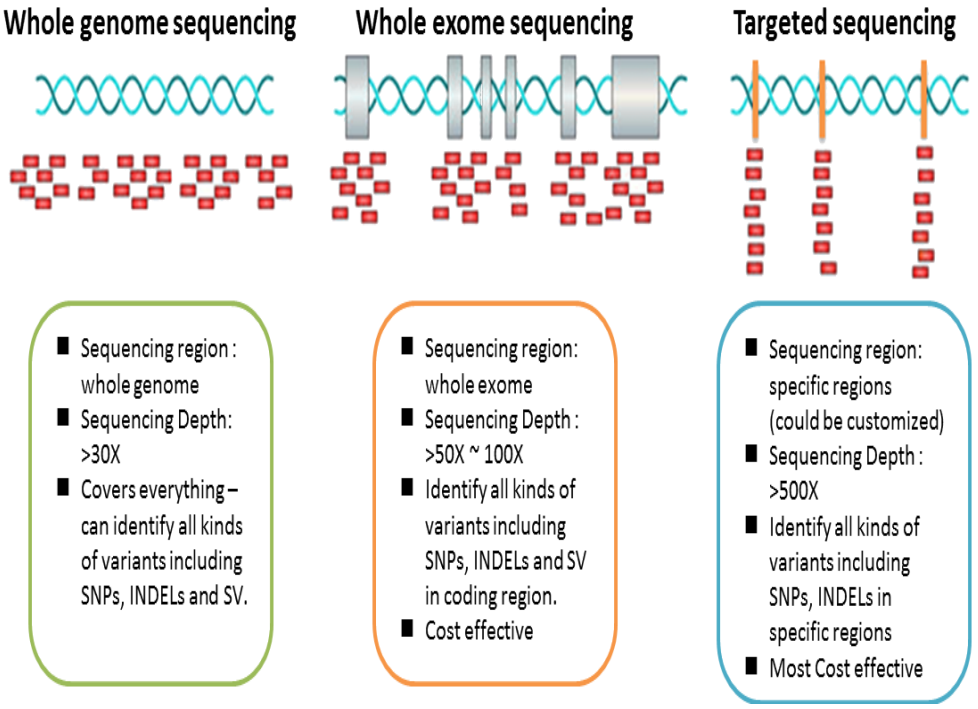
GENETIC TESTING MUST HAVE CLINICAL VALIDITY TO BE TRANSLATED INTO CLINICAL PRACTICE

NEXT GENERATION SEQUENCING
The NGS revolution



NEXT GENERATION SEQUENCING
The NGS revolution

- **Gene panel analysis**: **selected genes** known to be associated to a specific disease
- **Whole Exome Sequencing (WES): entire set of exons sequencing** (~200,000 exons coding for ~20,000 genes and 1.5% of the genome)
- **Whole Genome Sequencing (WGS)**: **entire genome** (~3 billion base pairs)

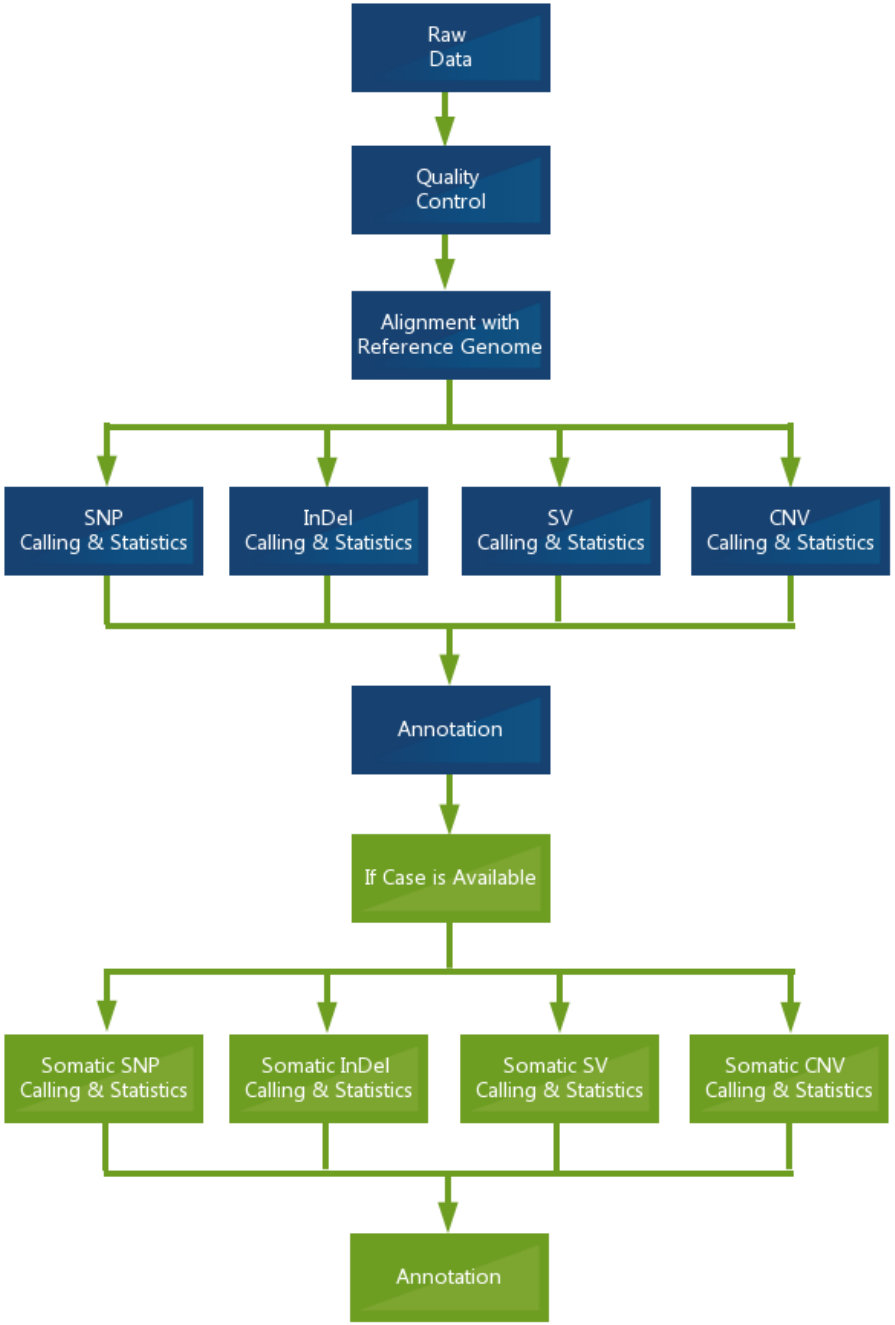


HUMAN GENOME: 3,2 Gb

NEXT GENERATION SEQUENCING
The NGS revolution

ADVANTAGES :

- Parallel sequencing of millions of nucleotides
- High throughput and many samples contemporary analysed
- High test accuracy ensured by high coverage



NGS CHALLENGING ISSUE 1: output dimension

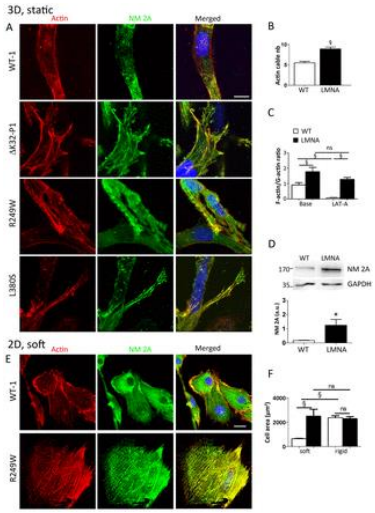
Target	Bases in the target	Median coverage	Bases to be sequenced	Expected variants	Filtering	prioritization	segregation	validation
GS	3.100.000.000	30x	>120Gb	3.000.000	Very high	Very high	yes	yes
ES	50.000.000	100x	10Gb	30.000	high	high	yes	yes
Large panel	1.500.000	200x	1Gb	1.000	medium	none	yes	IVD
Small panel	50.000	300x	0.05Gb	30	none	none	Yes/no	IVD

INCIDENTAL FINDINGS
(BRCA1 and BRCA2, others)

LOTS OF DRY LAB WORKLOAD:

- **Quality filters**
- **Variant prioritization**
- **Segregation models**
- **Variants technical validation (Sanger)**

NGS CHALLENGING ISSUE 2: variant interpretations



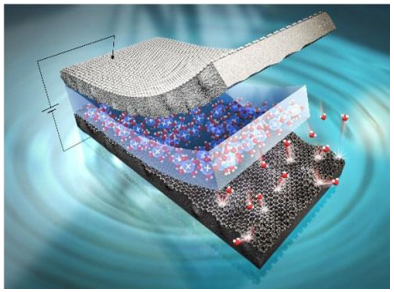
© 2014. Published by The Company of Biologists Ltd | Journal of Cell Science (2014) 127, 2873–2884 doi:10.1242/jcs.144907

RESEARCH ARTICLE

Cellular microenvironments reveal defective mechanosensing responses and elevated YAP signaling in *LMNA*-mutated muscle precursors

Anne T. Bertrand[1,2,3,4], Simindokht Ziaei[1,2,3,4], Camille Ehret[1,2,3,4], Hélène Duchemin[1,2,3,4], Kamel Mamchaoui[1,2,3,4], Anne Bigot[1,2,3,4], Michèle Mayer[5], Susana Quijano-Roy[6], Isabelle Desguerre[7], Jeanne Lainé[1,2,3,4], Rabah Ben Yaou[1,2,3,4], Gisèle Bonne[1,2,3,4,8] and Catherine Coirault[1,2,3,4,*]

Novel tools to functionally validate the genome variants



Efficient derivation and inducible differentiation of expandable skeletal myogenic cells from human ES and patient-specific iPS cells
Sara M Maffioletti, Mattia F M Gerli, Martina Ragazzi, Sumitava Dastidar, Sara Benedetti, Mariana Loperfido, Thierry VandenDriessche, Marinee K Chuah & Francesco Saverio Tedesco
Nature Protocols **10**, 941–958 (2015)

ACMG STANDARDS AND GUIDELINES | Genetics in Medicine

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,17,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[15] and Heidi L. Rehm, PhD[16]; on behalf of the ACMG Laboratory Quality Assurance Committee

ACMG STANDARDS AND GUIDELINES

	Benign		Pathogenic			
	Strong	**Supporting**	**Supporting**	**Moderate**	**Strong**	**Very strong**
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

Figure 1 Evidence framework. This chart organizes each of the criteria by the type of evidence as well as the strength of the criteria for a benign (left side) or pathogenic (right side) assertion. Evidence code descriptions can be found in **Tables 3** and **4**. BS, benign strong; BP, benign supporting; FH, family history; LOF, loss of function; MAF, minor allele frequency; path., pathogenic; PM, pathogenic moderate; PP, pathogenic supporting; PS, pathogenic strong; PVS, pathogenic very strong.

NGS CHALLENGING ISSUE 3: improving CNVs and dynamic mutations detection

Phased sequencing
From genotype to haplotype sequencing

Haplotype Phasing

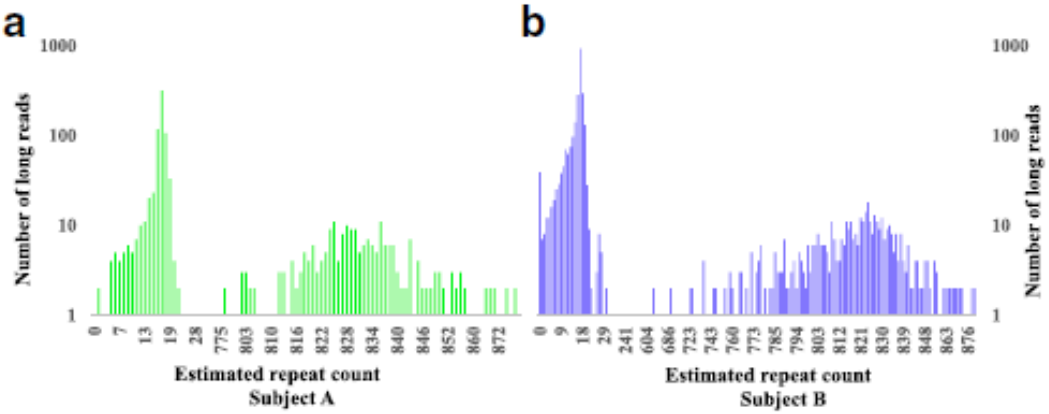
Haplotypes
ATCCGA
AGACGC

Genotype
A{T/G}{C/A}CG{C/A}

- High throughput cost effective sequencing technology gives genotypes and not haplotypes.

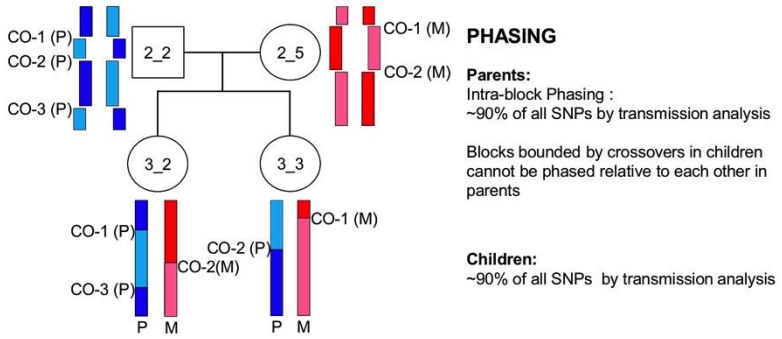
Possible phases: ATACGA AGACGA AGCCGC ATCCGC ….

Repeat counting by RepeatHMM in WGS



ADVANTAGES:

- Analyze **compound heterozygotes**
- Measure **allele-specific expression**
- Identify **variant linkage**
- **Long-reads based sequencing**
- **CNV detection** and **epigenetic changes**



THE CHANGING SCENARIO OF NMD GENETIC TESTING: mutation detection

LOW THROUGHPUT

Single gene testing

- Copy Number Variations
- Dynamic mutations
- Epigenetic changes
- Small mutations
- Mosaicisms
- Digenic inheritance or mutation load

NGS

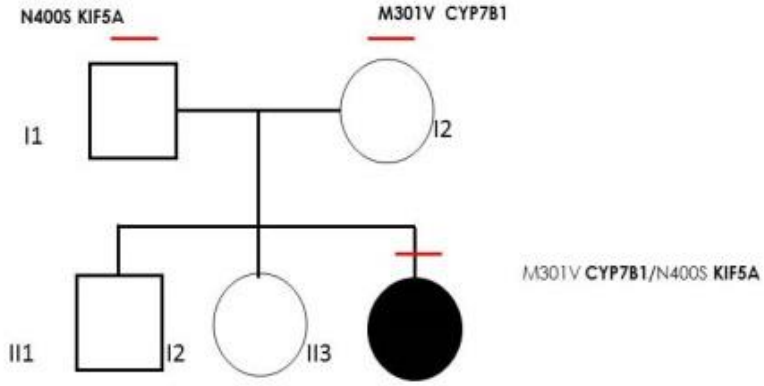
- Copy Number Variations
- Dynamic mutations
- Epigenetic changes
- Small mutations
- Mosaicisms
- Digenic inheritance or mutation load

HIGH THROUGHPUT

Pharrel William, composer. Affected with hereditary paraplegia

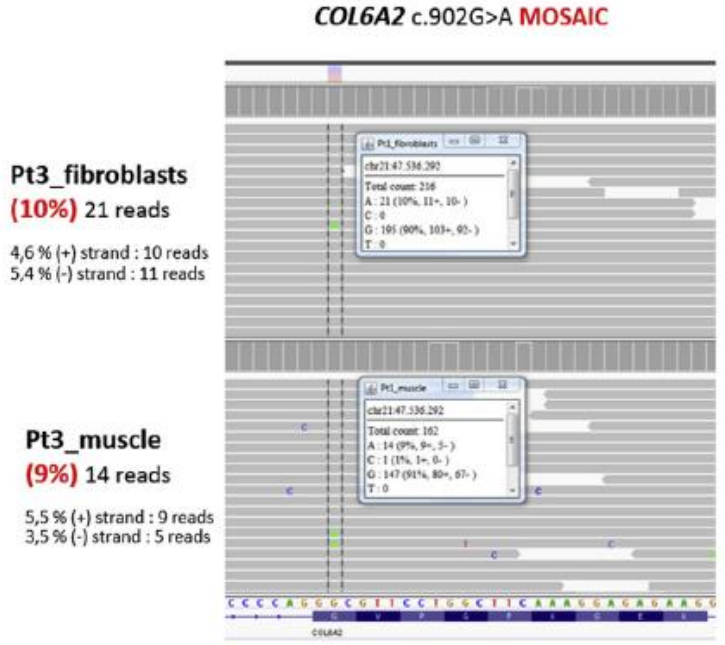
FACING NEW GENETIC PROFILES: digenic inheritance and mosaicism

AR Spastic paraplegia (SPG) Digenic inheritance

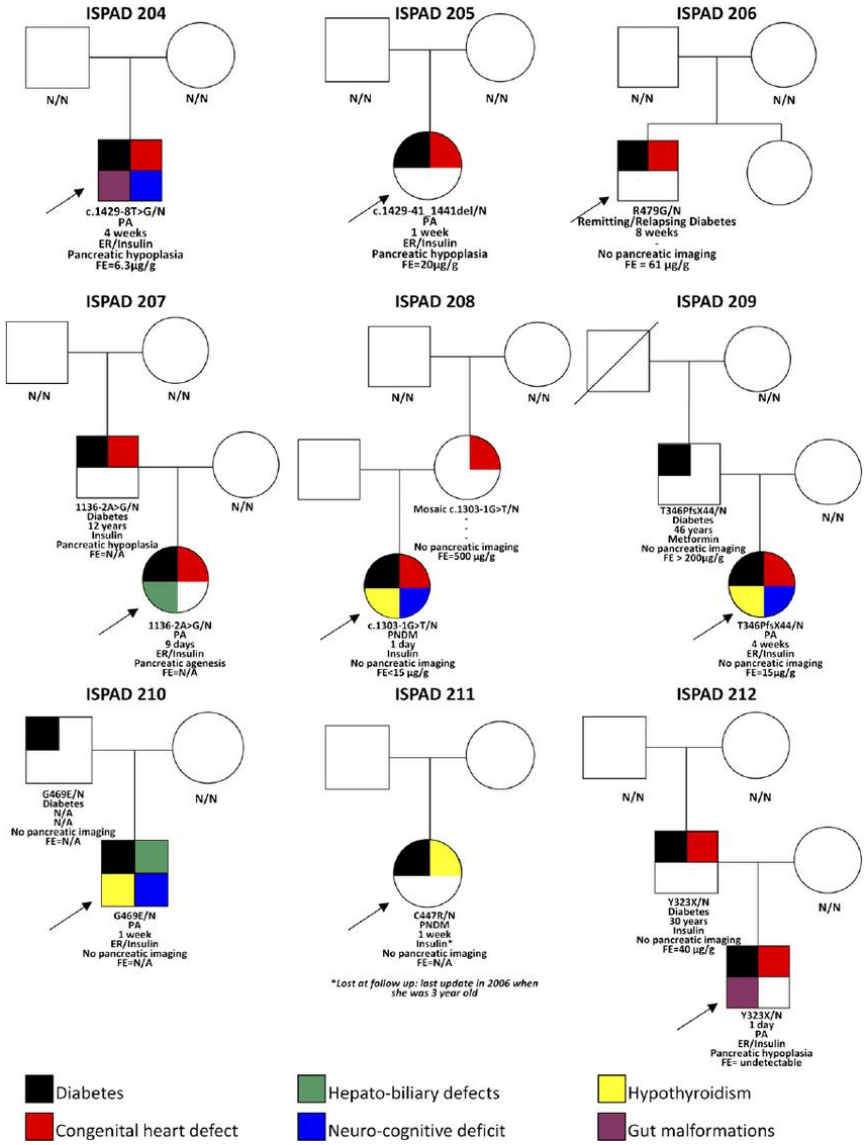


Hereditary paraplegia, HP gene panel : **digenic** inheritance
KIF5A/CYP7B1 double heterozygosis

AR Ulrich Congenital Muscular Dystrophy, COL6A1 Mosaicism



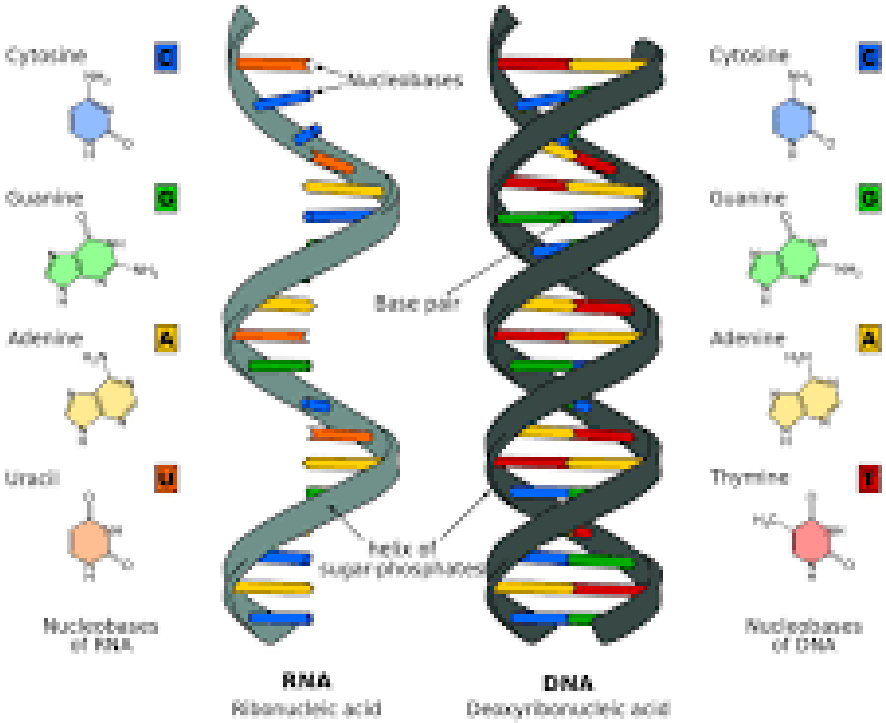
FACING NEW GENETIC PROFILES: the mutation load

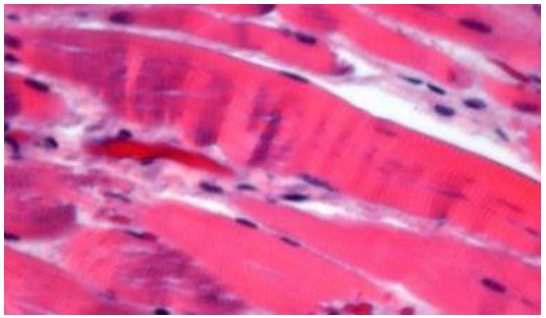


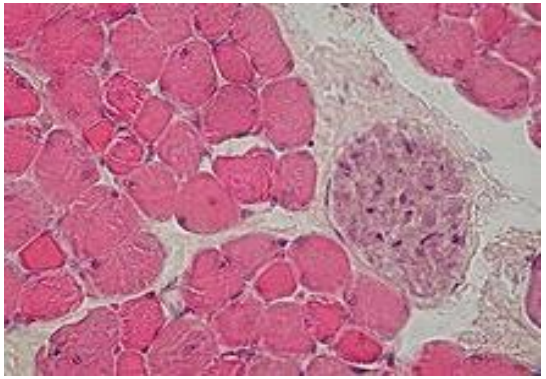
MULTIPLE GENE VARIATIONS:

- **Major gene (susceptibility)**
- **Polygenic model (multifactorial)**
- **Mutation load (mendelian)**

NEW TECHNIQUES FOR GENETIC TESTING:
RNA-based genetic diagnosis







ANNALS of Clinical and Translational Neurology — Open Access

BRIEF COMMUNICATION

RNAseq analysis for the diagnosis of muscular dystrophy

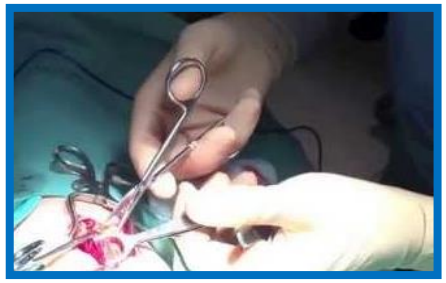
Heman Gonorazky[1,2,3,*], Minggao Liang[2,4,*], Beryl Cummings[5,6,*], Monkol Lek[5,6], Johann Micallef[2], Cynthia Hawkins[7], Raveen Basran[7], Ronald Cohn[2,3,4], Michael D. Wilson[2,4], Daniel MacArthur[5,6], Christian R. Marshall[7], Peter N. Ray[2,4,7] & James J. Dowling[1,2,3,4]

[1]Division of Neurology, Hospital for Sick Children, Toronto, Ontario, Canada M5G A04
[2]Program of Genetics and Genome Biology, Hospital for Sick Children, Toronto, Ontario, Canada M5G A04
[3]Department of Paediatrics, University of Toronto, Toronto, Ontario, Canada M5G A04
[4]Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada M5G A04
[5]Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, Massachusetts 02114
[6]Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, Massachusetts
[7]Pediatric Laboratory Medicine, Hospital for Sick Children, Toronto, Ontario, Canada M5G A04

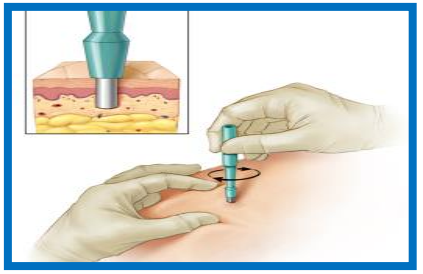
RNAseq ANALYSIS:

- **High throughput (transcriptome)**
- **RNA molecule might be used to identify all mutation types**

NEW TECHNIQUES FOR GENETIC TESTING: RNA-based genetic diagnosis



Muscle biopsy



Skin biopsy

Urine sample

RNA extraction

Myoblast cultures

RNA extraction

Fibroblast cultures

MyoD AdVΔE1 Adenoviral infection

Myogenic cells

RNA extraction

Urine-derived stem cells cultures

RNA extraction

MyoD AdVΔE1 Adenoviral infection

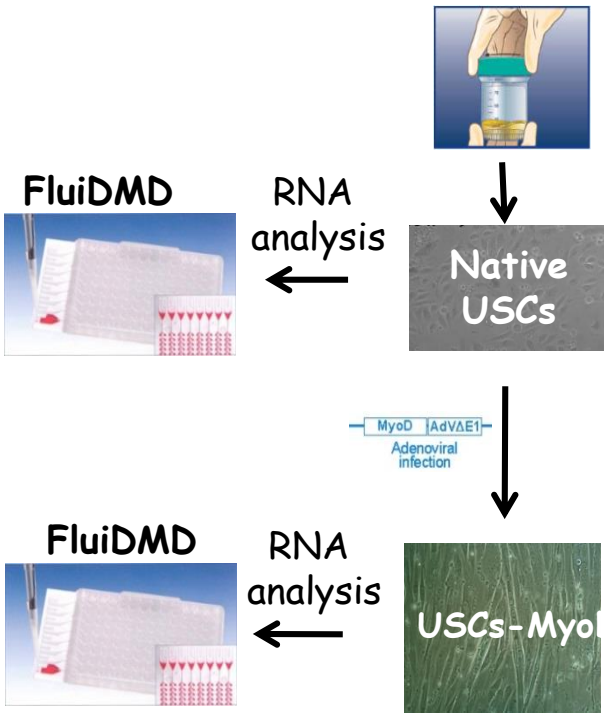
Myogenic cells

RNA extraction

RNA extraction

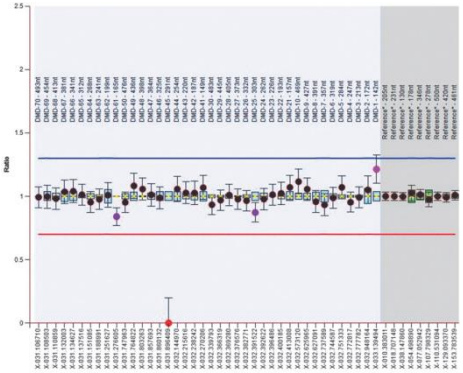
Bottleneck: reliable **RNA sources** (tissues, cells)

Single gene analysis
DMD GENE : FluiDMD card



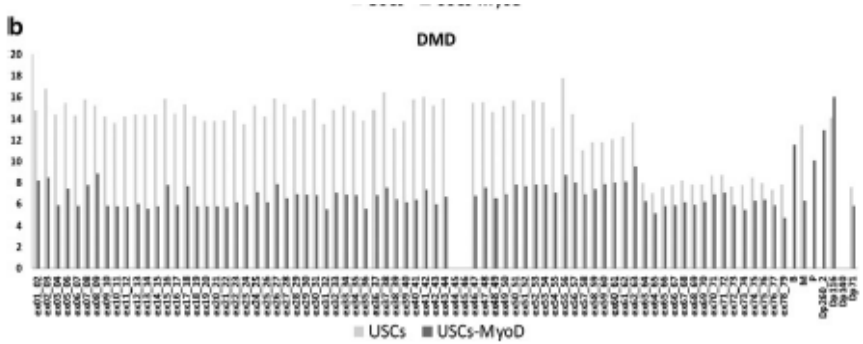
DNA:

MLPA exon 45
DELETION



RNA:

FluiDMD cards
Exon 45 omission



Duchenne Muscular Dystrophy Myogenic Cells from Urine-Derived Stem Cells Recapitulate the Dystrophin Genotype and Phenotype

Maria Sofia Falzarano,[1] Domenico D'Amario,[2] Andrea Siracusano,[2] Massimo Massetti,[2] Antonio Amodeo,[3] Federica La Neve,[2] Camilla Reina Maroni,[2] Eugenio Mercuri,[4] Hana Osman,[1] Chiara Scotton,[1] Annarita Armaroli,[1] Rachele Rossi,[1] Rita Selvatici,[1] Filippo Crea,[2] and Alessandra Ferlini[1,5,*]

Human Gene Therapy (2016) 772-83

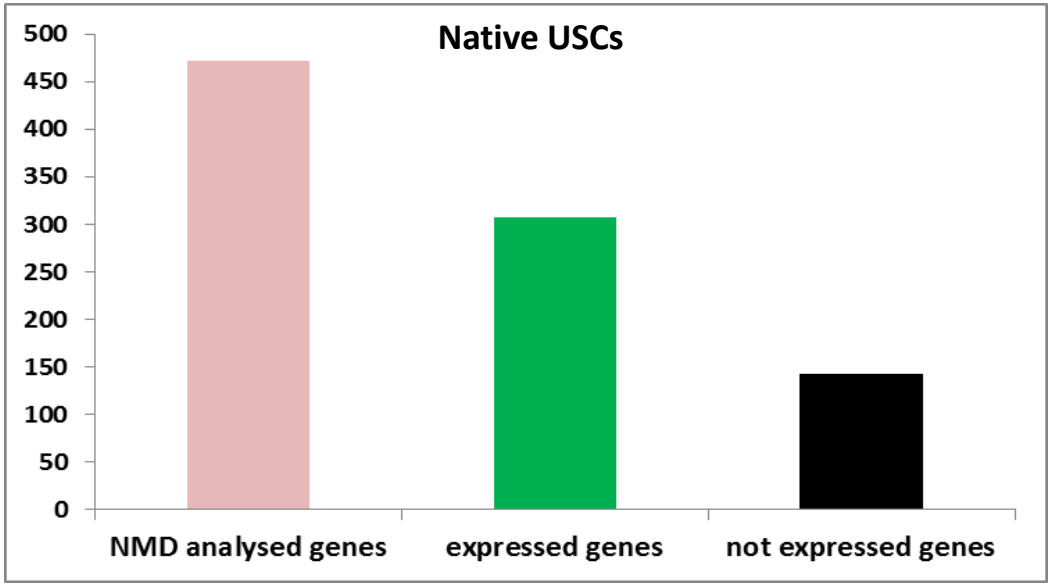
What about other NNMD genes? Testing USCs for expression of NNMD causing genes: RNA seq

RNAseq in wild type, native UCSs

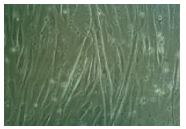
472 NNMD transcripts interrogated

308 expressed genes (FPKM>3)

164 genes not expressed



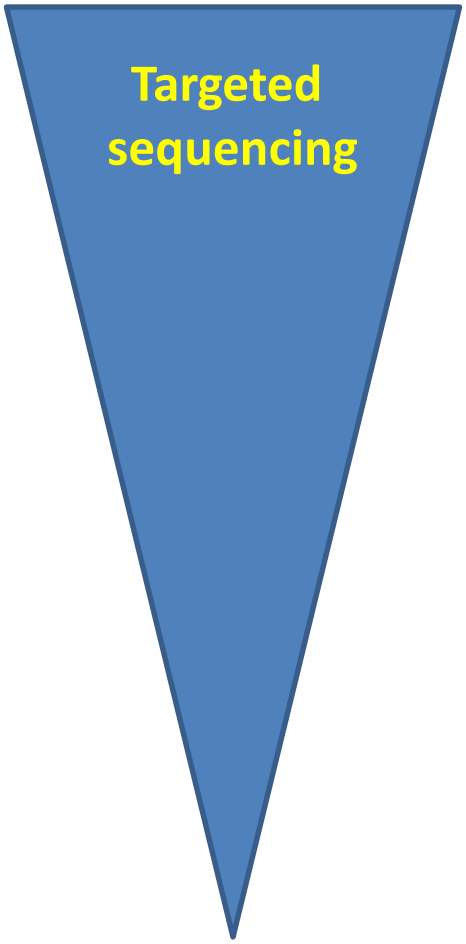
Falzarano et al., submitted



USCs

NMD Disease groups	% of NMD expressed genes
Muscular dystrophies	65
Congenital muscular dystrophies	79
Congenital myopathies	38
Distal myopathies	50
Hereditary ataxias	53
Hereditary paraplegias	75
Hereditary neuropathies	74
Hereditary cardiomyopathies	44
Motor neuron diseases	79
Myotonic syndromes	33
Other myopathies	54
Other neurological disorders	74
Congenital myasthenic syndromes	50
Metabolic myopathies	89

SCENARIOS



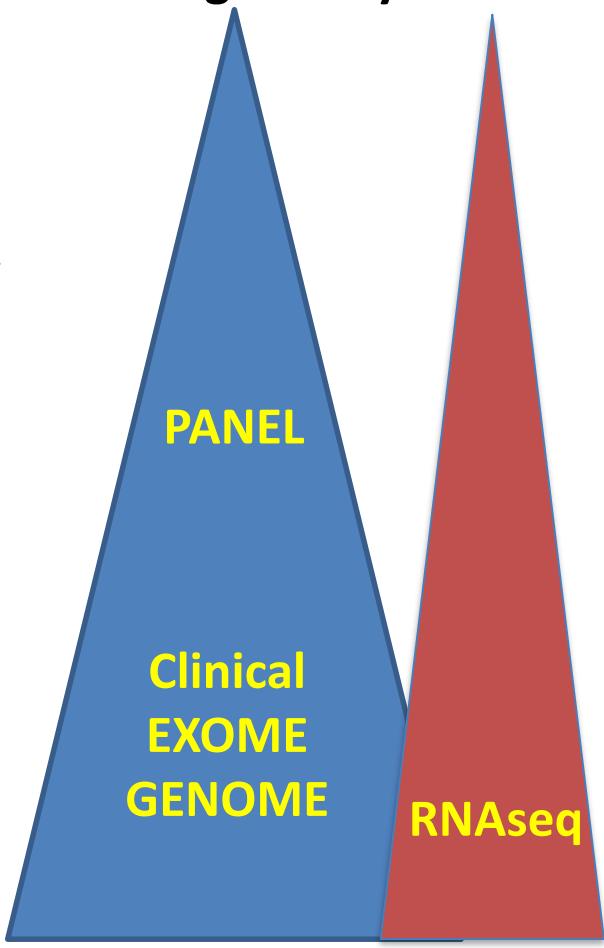
Diagnostic yields

Single gene disorders
low genetic heterogeneity,
etiological therapies available → DMD, SMA

Multiple gene disorders,
high allelic –high genetic heterogeneity
high detection rate (>50%) → LGMD, neuropathy,

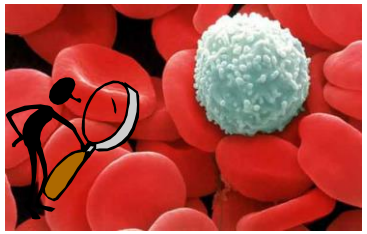
Ultra-rare NMDs
low detection rate (<50%)rate
Undiagnosed cases → Metabolic diseases

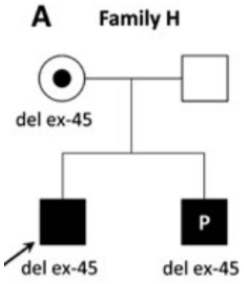
Diagnostic yields

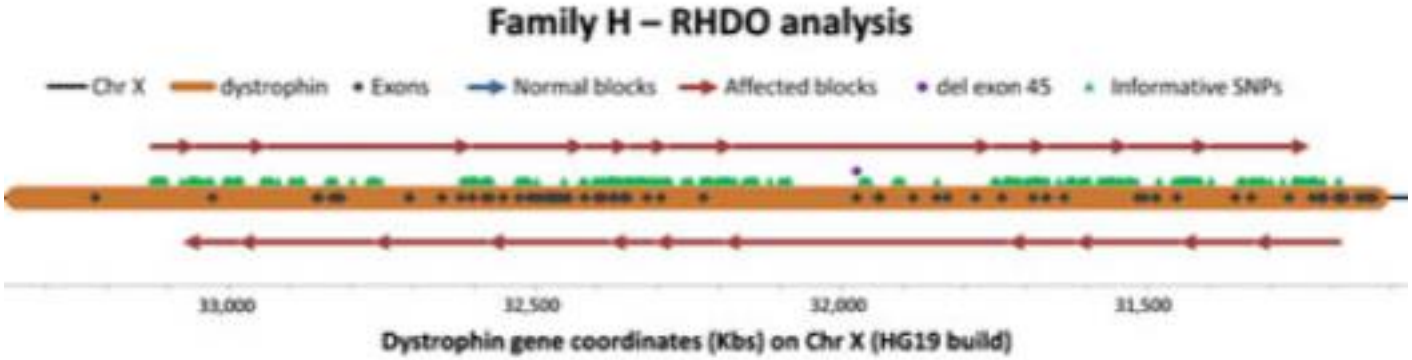


PREVENTION: NNMDs prenatal diagnosis

- **Non invasive prenatal diagnosis (NIPD)**
- **Early prenatal low invasive procedure on fetal DNA in maternal blood**







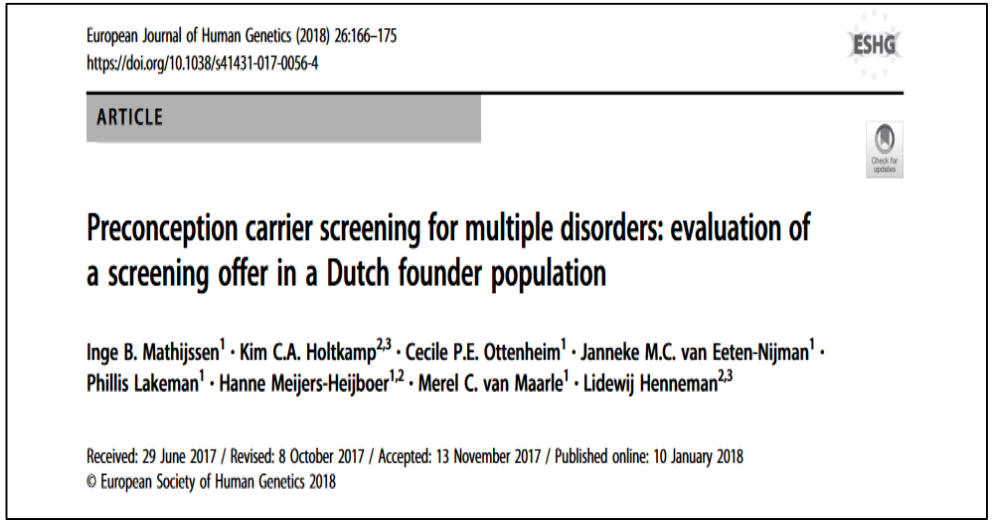
Non-invasive prenatal diagnosis of Duchenne and Becker muscular dystrophies by relative haplotype dosage†

Michael Parks*, Samantha Court, Siobhan Cleary, Samuel Clokie, Julie Hewitt, Denise Williams, Trevor Cole, Fiona MacDonald, Mike Griffiths and Stephanie Allen

West Midlands Regional Genetics Laboratory, Birmingham Women's NHS Foundation Trust, Birmingham, UK
*Correspondence to: Michael Parks. E-mail: Michael.Parks@bwnft.nhs.uk
†This study was presented orally at cfDNA 2015 conference (Copenhagen); ESHG 2015 conference (Glasgow); CNAPS IX conference (Berlin); Advancement in Molecular Prenatal Diagnostics conference (Boston).

PREVENTION: preconception carrier screening (PCS)

- Increasingly recognized critical medical component to **prevent diseases in offspring** to **reduce suffering**, **health care disparity** and **therapeutic extra cost** in future generation
- Allows couples to consider **the most complete range of reproductive options** (**PGD**, **PND**, etc)



European Journal of Human Genetics (2018) 26:166–175
https://doi.org/10.1038/s41431-017-0056-4

ESHG

ARTICLE

Preconception carrier screening for multiple disorders: evaluation of a screening offer in a Dutch founder population

Inge B. Mathijssen[1] · Kim C.A. Holtkamp[2,3] · Cecile P.E. Ottenheim[1] · Janneke M.C. van Eeten-Nijman[1] · Phillis Lakeman[1] · Hanne Meijers-Heijboer[1,2] · Merel C. van Maarle[1] · Lidewij Henneman[2,3]

Received: 29 June 2017 / Revised: 8 October 2017 / Accepted: 13 November 2017 / Published online: 10 January 2018

- PCS for multiple disorders associated to **high knowledge**, **no major psychological outcomes**, **no stigmatization**

All identified carrier couples made reproductive decisions based on their test results

PREVENTION: PCS in Australia

3 AUSTRALIAN PILOT STUDIES:

- Victoria implement screening for ≈ **100 diseases**
- preconception carrier offered for 3 years on a user pays basis
- **>12,000 individuals** have been screened

- NSW investigate PCS in consanguineous communities

- Western Australia investigate PCS panel for **>400 genes**

Courtesy of Nigel Lang Australia

RESEARCH VOLUME 197

Tay Sachs disease in Australia: reduced disease incidence despite stable carrier frequency in Australian Jews

Raelia M Lew, Anne L Proos, Leslie Burnett, Martin Delatycki, Agnes Bankier and Michael J Fietz

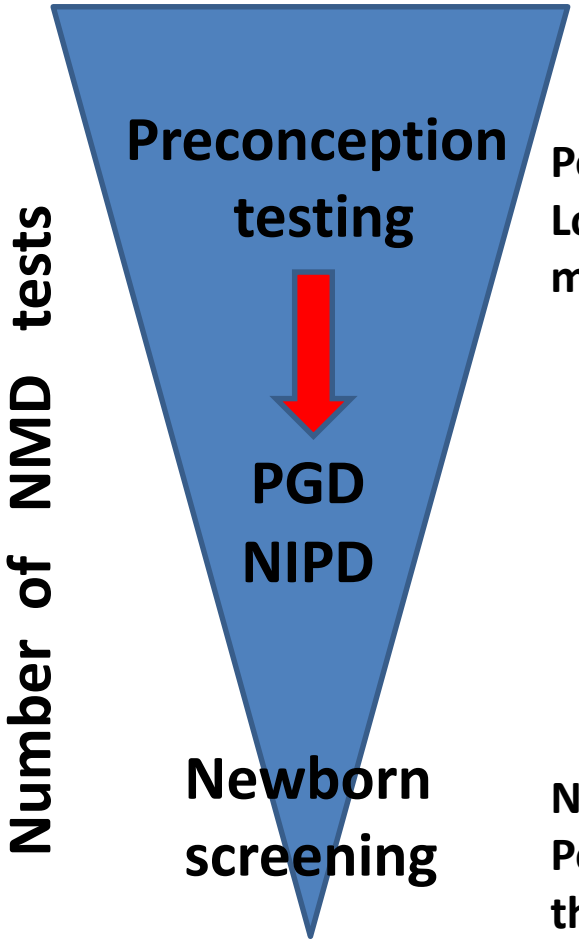
Med J Aust 2012; 197 (11): 652-654. || doi: 10.5694/mja12.11010

Published online: 10 December 2012

- **Twenty years after** the introduction of TSD carrier testing in Australia, there have been **fewer than expected Jewish TSD-affected births**

No genetic carrier identified through the screening has had a TSD-affected child

INTEGRATING PREVENTION AND THERAPY



Potentially all NMDs
Low rate of «de novo» mutation

→ Clinical Exome/genome
450 known disease genes

NMDs with approved Personalized or gene specific therapies

→ Gene specific assay

GENETIC DIAGNOSIS: WHY IS IMPORTANT

FOR THE PATIENT

1. Genetic diagnosis
2. Clinical diagnosis confirmation (disease-specific clinical follow up, natural history, phenotype-genotype correlation)
3. Differential diagnosis (phenocopies)
4. Genetic prognosis (mild to severe disease course)
5. Gold standard therapies
6. Clinical trials (personalized)

FOR THE FAMILY

1. recurrent or de novo mutations, mosaicisms (high-low risk of recurrence)
2. Carrier detections
3. Family prevention and screening
4. Prenatal testing

Cooperation Neurology and Genetics!!!!

GENETIC DIAGNOSIS: NOT TO FORGET

Counselling Informed consent and Ethical issues

Genetic testing ESHG guidelines

Genetic testing quality assessment schemes

World Health Organization

ESHG

EMQN
The European Molecular Genetics Quality Network

Università di Ferrara
fondata nel 1391

European Reference Network
for rare or low prevalence complex diseases

Network
Neuromuscular Diseases (ERN EURO-NMD)

Member
AOU di Ferrara — Italia

Azienda Ospedaliero-Universitaria S'Anna - Ferrara

Girolamo Maria Francesco Matteo Savonarola (Ferrara, 1452 – Firenze, 1498)

www.ospfe.it/medicalgenetics

SEVENTH FRAMEWORK PROGRAMME

SolveRD

NeurOmics

Duchenne Parent Project

RARER

Emilia Romagna Region Pilot Omics Project

SAREPTA THERAPEUTICS

PTC THERAPEUTICS
Translating Science • Transforming Lives