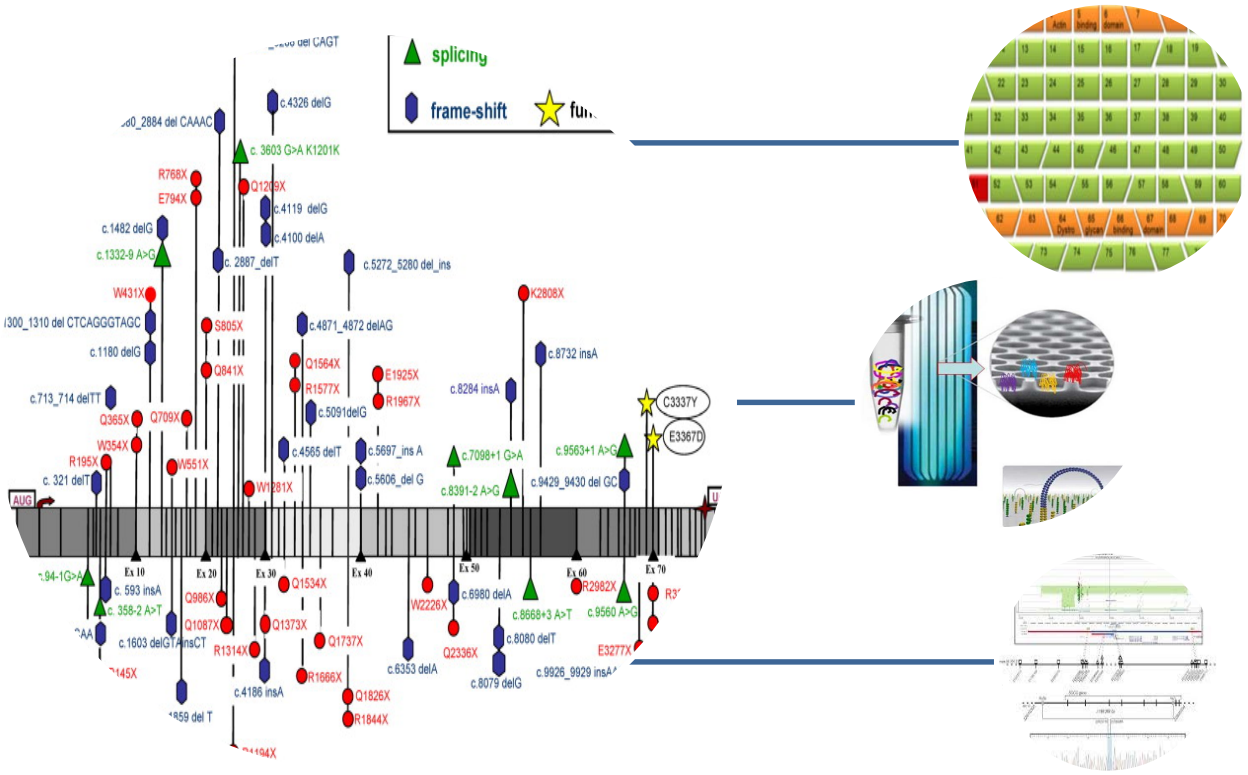
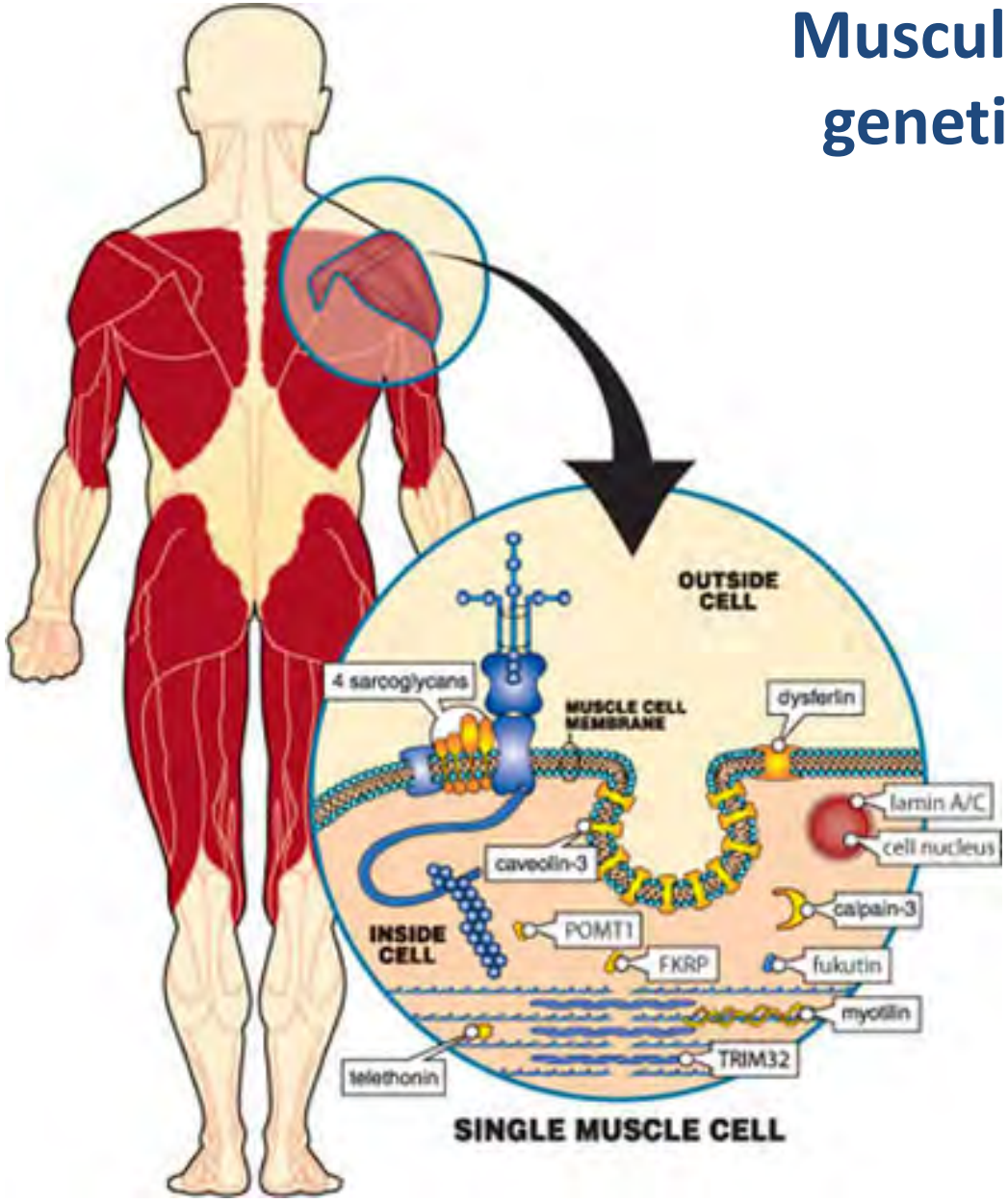
Basi genetiche della distrofia muscolare dei cingoli da deficit di Calpaina 3

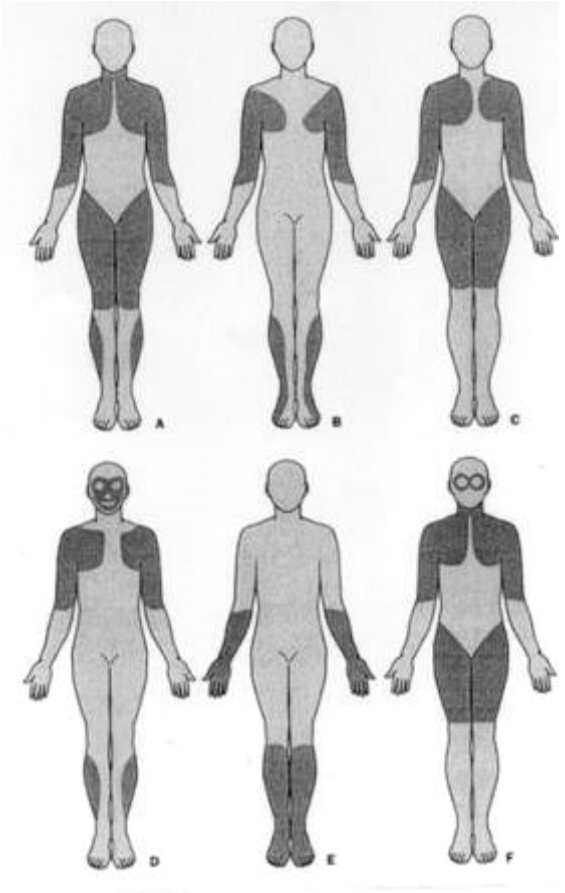
Vincenzo Nigro, TIGEM e Seconda Università di Napoli

Bosisio Parini, 14 novembre 2015



Muscular dystrophies are genetically and clinically heterogeneous





INVITED REVIEW

Genetic basis of limb-girdle muscular dystrophies: the 2014 update

VINCENZO NIGRO AND MARCO SAVARESE

Dipartimento di Biochimica, Biofisica e Patologia Generale, Seconda Università degli Studi di Napoli and Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy

nant and 23 autosomal recessive. The dominant forms (LGMD1) are: LGMD1A (myotilin), LGMD1B (lamin A/C), LGMD1C (caveolin 3), LGMD1D (DNAJB6), LGMD1E (desmin), LGMD1F (transportin 3), LGMD1G (HNRPDL), LGMD1H (chr. 3). The autosomal recessive forms (LGMD2) are: LGMD2A (calpain 3), LGMD2B (dysferlin), LGMD2C (γ sarcoglycan), LGMD2D (α sarcoglycan), LGMD2E (β sarcoglycan), LGMD2F (δ sarcoglycan), LGMD2G (telethonin), LGMD2H (TRIM32), LGMD2I (FKRP), LGMD2J (titin), LGMD2K (POMT1), LGMD2L (anoctamin 5), LGMD2M (fukutin), LGMD2N (POMT2), LGMD2O (POMTnG1), LGMD2P (dystroglycan), LGMD2Q (plectin), LGMD2R (desmin), LGMD2S (TRAPPC11), LGMD2T (GMPPB), LGMD2U (ISPD), LGMD2V (Glucosidase, alpha), LGMD2W (PINCH2).

One disease < many genes

which LGMD form?

- difficult diagnosis due to:
  - genetic heterogeneity
  - overlapping phenotypes
  - interfamilial and intrafamilial variability

Prevalence of calpainopathies

- Veneto (IT) 1:156,000 (Fanin et al. 2005)
- Friuli (IT) 1:60,000 (Fanin et al. 2005)
- Our re-calculation set the prevalence to 1:42,700 with a carrier frequency 1:103
- LGMD2A is the most common form of AR muscular dystrophy
- Additional mutations are carried by atypical patients or subjects with high CK, most of which **are not recruited for genetic studies**

Rev 7.51n/W (Jan 20 2003)
Journal of Medical Genetics mg28738 Module 1 2/3/05 13:59:29 Topics: 11; 251
1

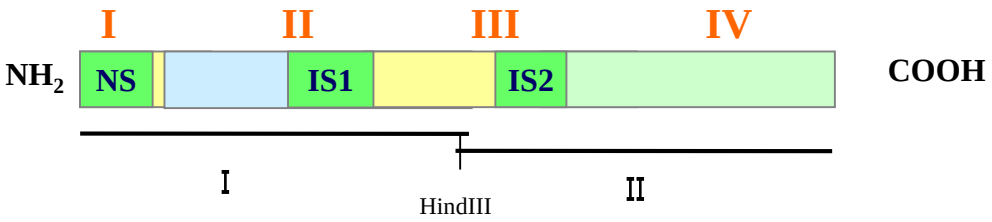
ORIGINAL ARTICLE

Extensive scanning of the calpain-3 gene broadens the spectrum of LGMD2A phenotypes

G Piluso*, L Politano*, S Aurino, M Fanin, E Ricci, V M Ventriglia, A Belsito, A Totaro, V Saccone, H Topaloglu, A C Nascimbeni, L Fulizio, A Broccolini, N Canki-Klain, L Ines Comi, G Nigro, C Angelini, V Nigro

J Med Genet 2005;000:1–9. doi: 10.1136/jmg.2004.028738

Calpain 3



- 2466bp, 821AA, 94kDa protein

- Domain II is a protease module
- Domain IV is a Ca^{2+} BD
- NS, IS1 and IS2 are calpain3 muscle specific sequences

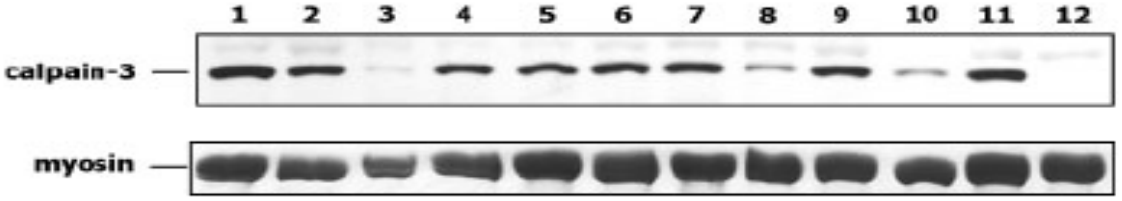
- LGMD2A [MIM 253600] is the most prevalent forms of autosomal recessive muscular dystrophy in Italy and Brasil and is caused by loss-of-function mutations in the CAPN3 gene

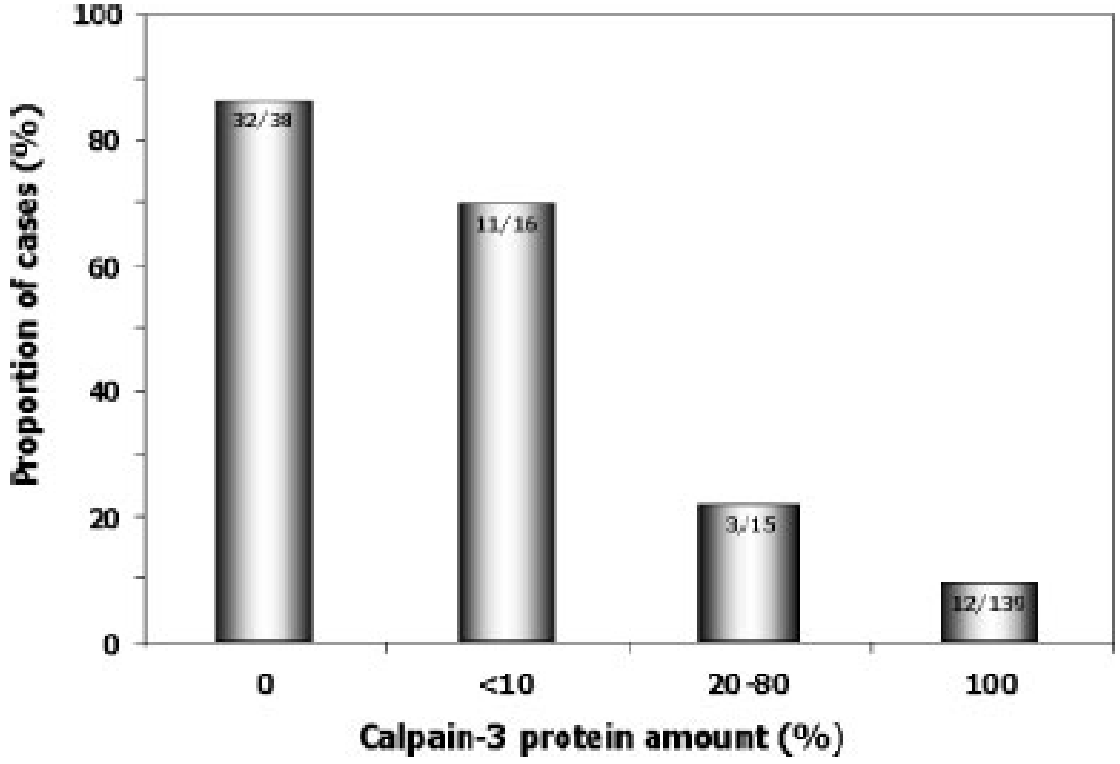
Western blot study of LGMD2A

WB usually precedes CAPN3 gene testing

- commercially available antibodies only work on WB and not on tissue sections
- WB analysis alone could give both to false positive (low specificity) and false negative results (low sensitivity)
- there are patients with some missense mutations who do not show protein loss by WB
- The relationship between dysferlin and calpain-3 seems to be bidirectional: combined protein deficiency in LGMD2A patients, but also in LGMD2B patients

The likelihood to find a mutation *(from Fanin et al.)*



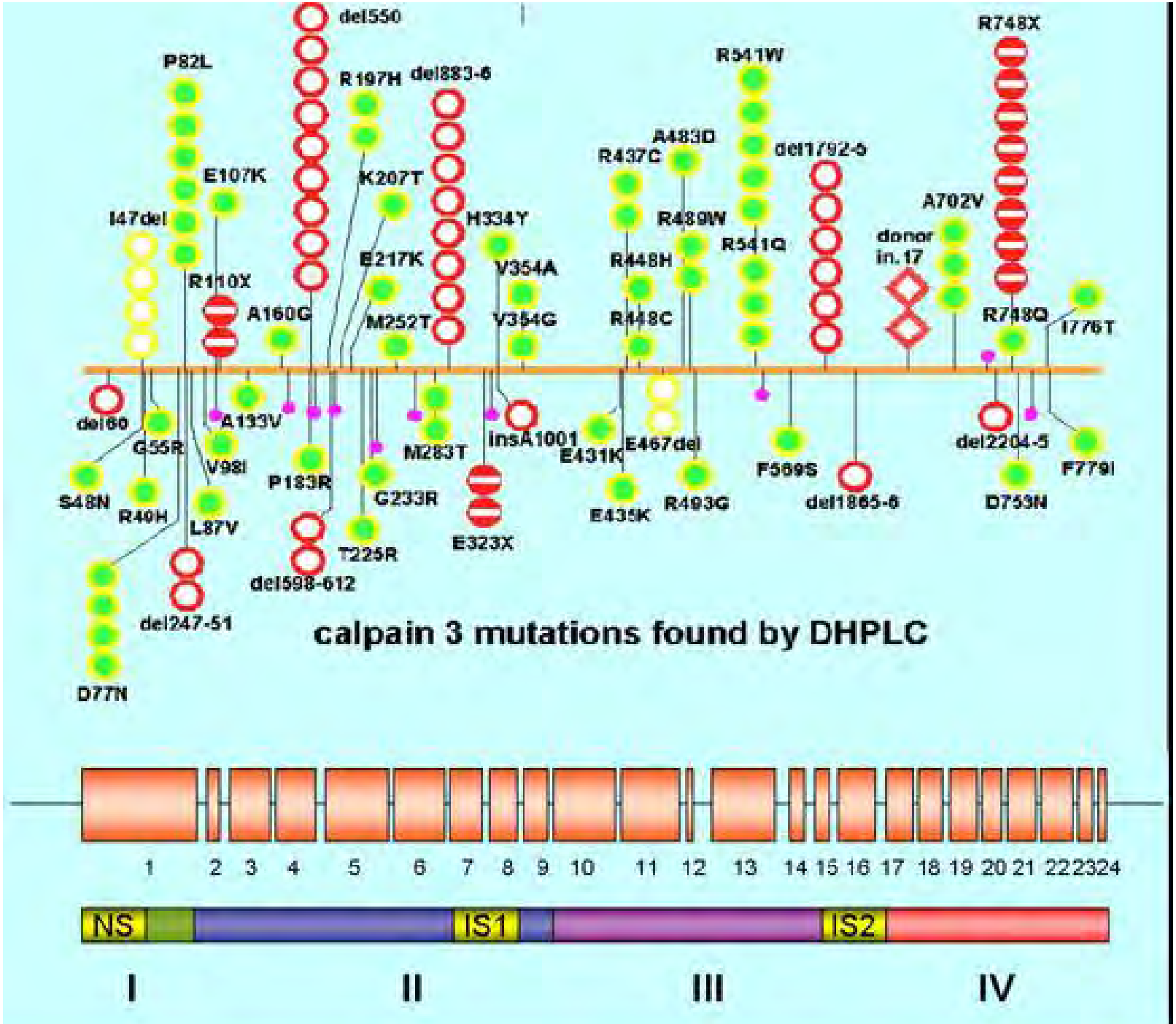


LOVD: 482 unique CAPN3 variants in 1,739 LGMD2A individuals

variant	number	location			
		5'start	coding	intron	3'stop
substitutions	1855	18	1315	518	4
deletions	639	1	586	51	1
duplications	51	0	43	8	0
insertions	4	0	3	1	0
insertion/deletions	268	0	268	0	0
totals	2831	19	2215	578	5

LGMD2A by calpain 3 gene mutations

- By DHPLC we studied 530 subjects with different grades of symptoms and 300 controls
- We identified 141 LGMD2A cases, carrying 82 different CAPN3 mutations (45 novel), along with 18 novel polymorphisms

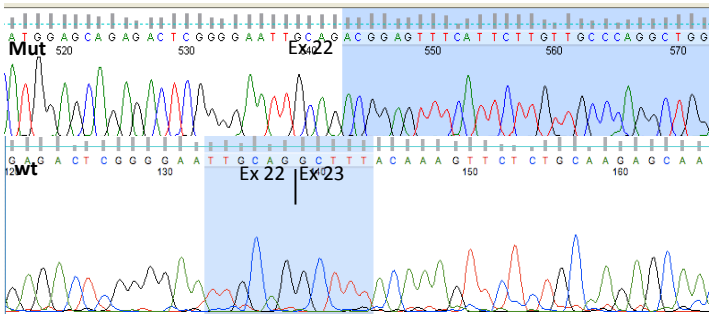

del550
P82L
R197H
del883-6
R541W
R748X
A483D
R437C
del1792-5
E107K
K207T
H334Y
R489W
A702V
I47del
E217K
V354A
R541Q
donor
in.17
R448H
R110X
M252T
V354G
R748Q
I776T
A160G
R448C
del60
A133V
G55R
M283T
insA1001
E431K
E467del
del2204-5
F779I
V98I
P183R
F569S
S48N
G233R
E435K
R493G
del1865-6
D753N
R40H
L87V
T225R
E323X
del598-612
del247-51
D77N
calpain 3 mutations found by DHPLC
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 222324
NS
IS1
IS2
I
II
III
IV

CAPN3 mutations identified

group	patients n	CAPN3 mut %	2° Allele %
A(severe)	123	39,8	93.9
B (intermediate)	142	31.0	86.4
C (mild)	158	18.4	50.0
D (isolated CK↑)	87	12.6	27.7
P (presympt.)	20	40.0	25.0

Nei casi non risolti, il sequenziamento dell'mRNA da biopsia muscolare porta la sensibilità diagnostica al 99%

Caso#3: c. 2949+964 g>a; r. 2949+889_2949+1147ins

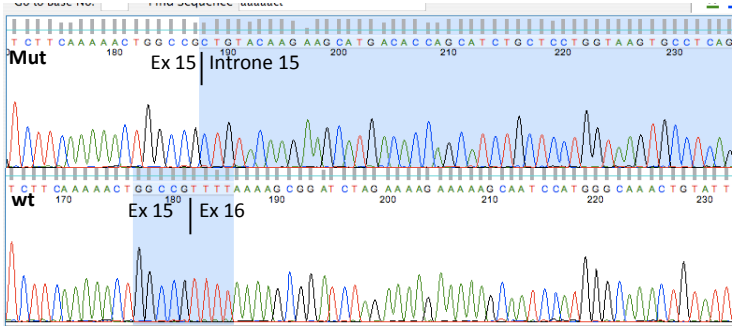


wt GAATTGCAG | GCTTTACAAAGTT
(Ex 22 | Ex 23)

mut TGCAG|acggag..ctcccggg..attacagt|GCTTT
(Ex 22 | Intr 22 | Ex 23)

c.2949+889 cryptic splice site c.2949+1147
tttagacggag…. ctcccgga..attacaggtgt
wt: g

Caso#4: c.1812+601 a>g; r. 1812+480_1812+600ins



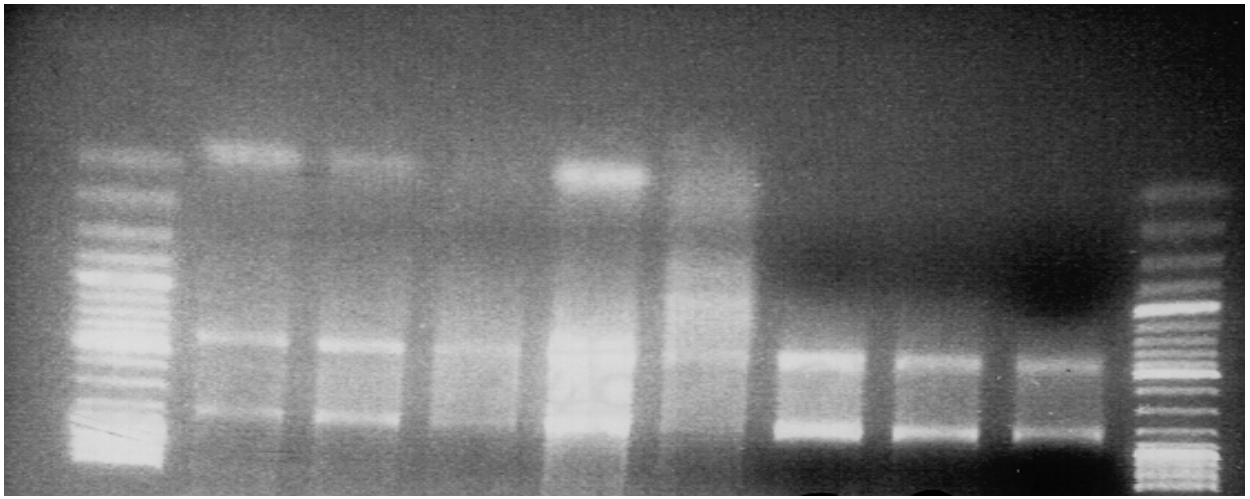
wt CAAAAACTGGCC | GTTTTAAA
(Ex 15 | Ex 16)

mut CAAAAACTGGCCgctgtacaa…agagGTTTTAAA0
(Ex 15 | Intr 15 | Ex 16)

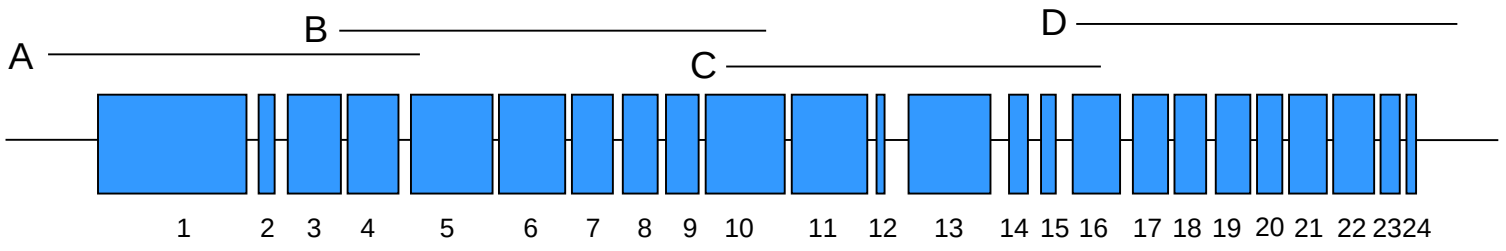
Wt: **a**
c.1812+480aggctgtacaa……. c.1812+600
agaggtaag

From blood RNA

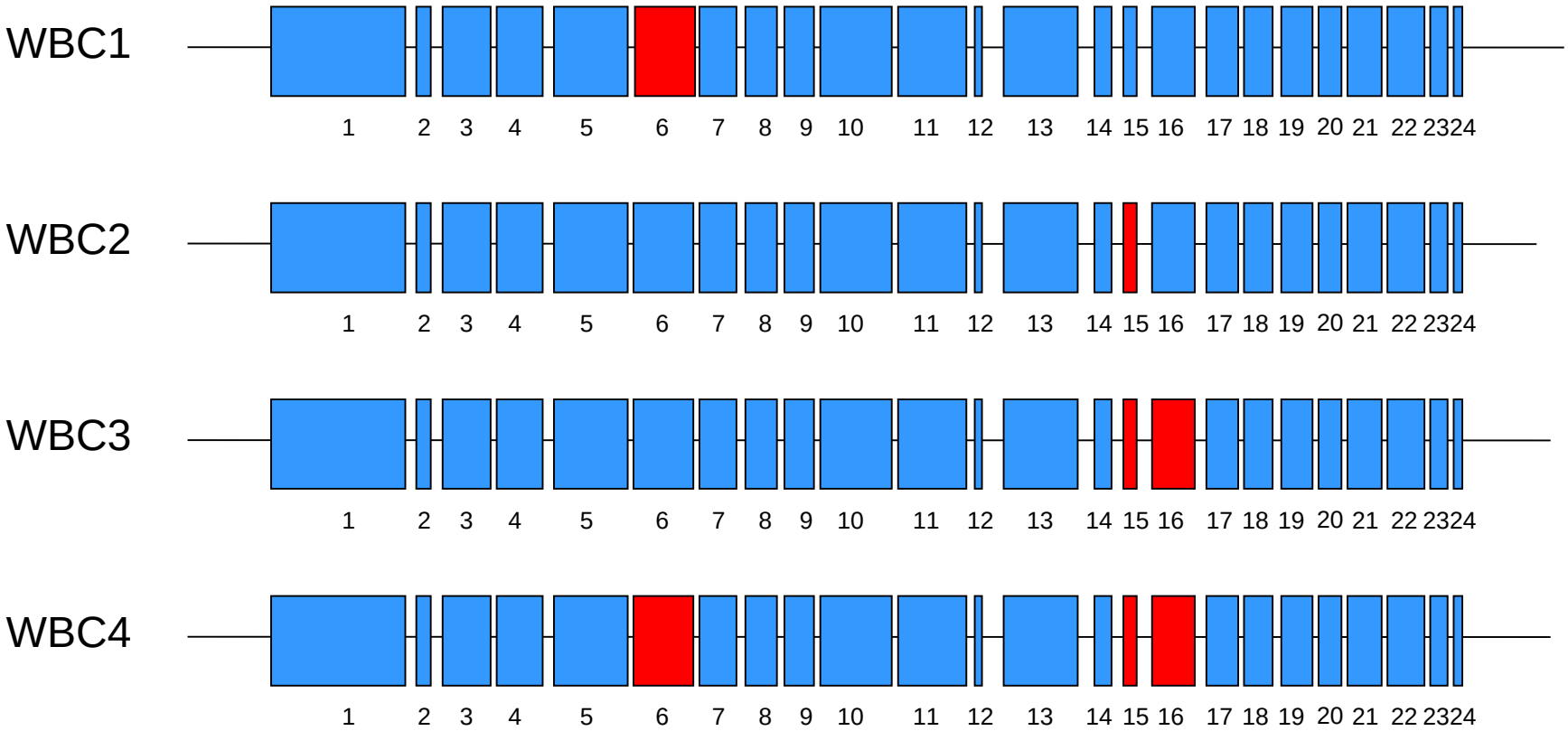
- Using the PAX RNA blood kit (Qiagen)
- RNA can be extracted immediately (better) or after a few days
- Suitable for calpain3 and dysferlin analyses



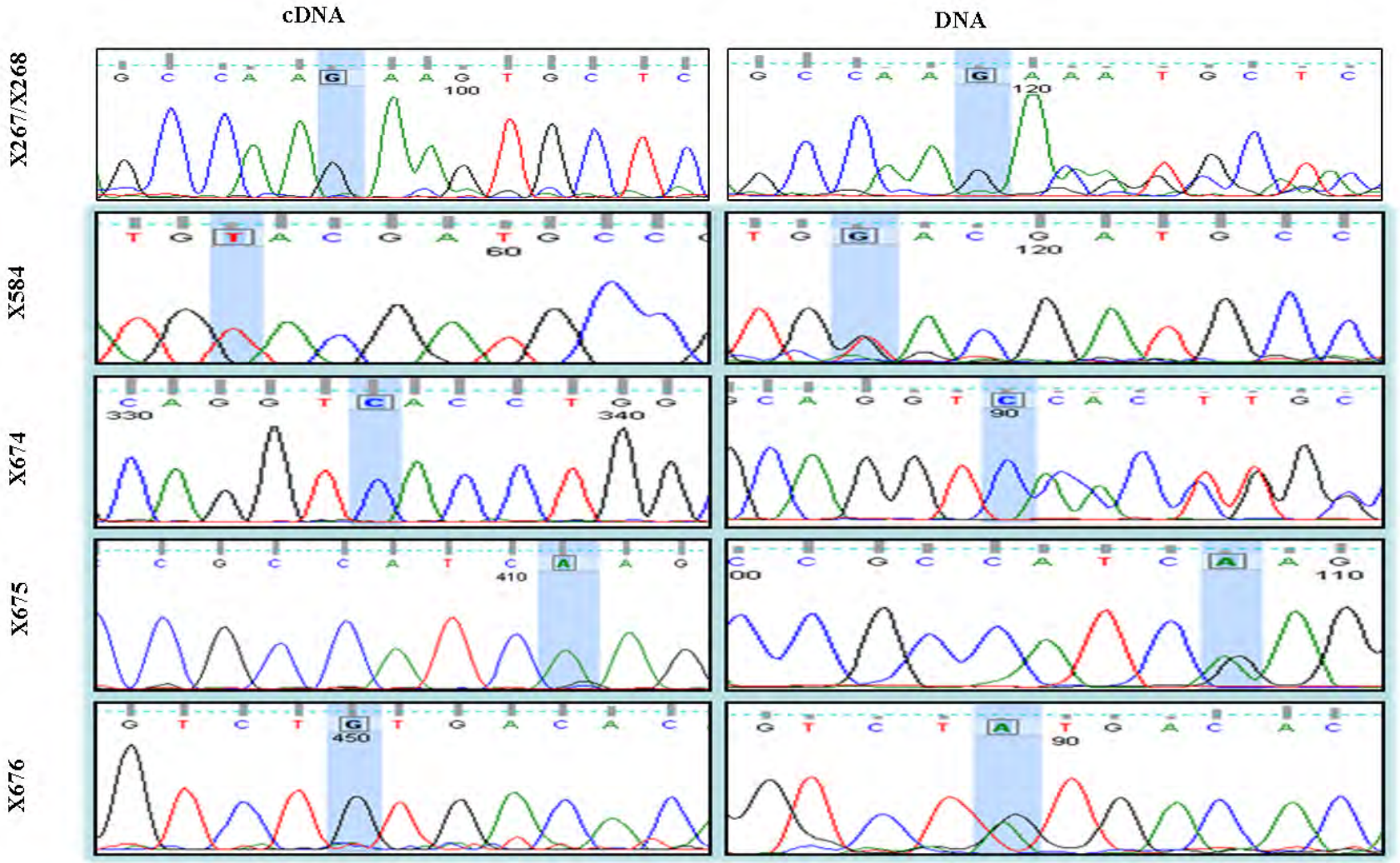
CAPN3 cDNA is amplified in 2 or 4 fragments



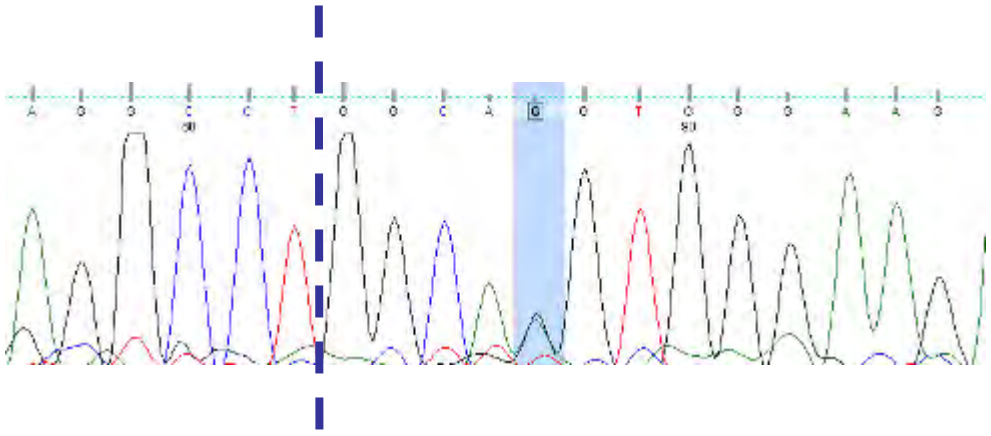
CAPN3 alternative splicing: muscle-WBC



Mutations missed by cDNA sequencing for nonsense mediated decay

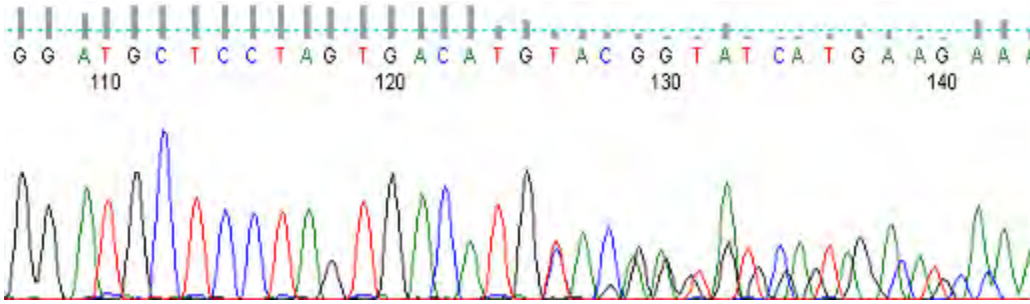


At genomic level:



G GCC TGG CAG gtgggaag WT

G GCC TG gcaAgtgggaag mutated

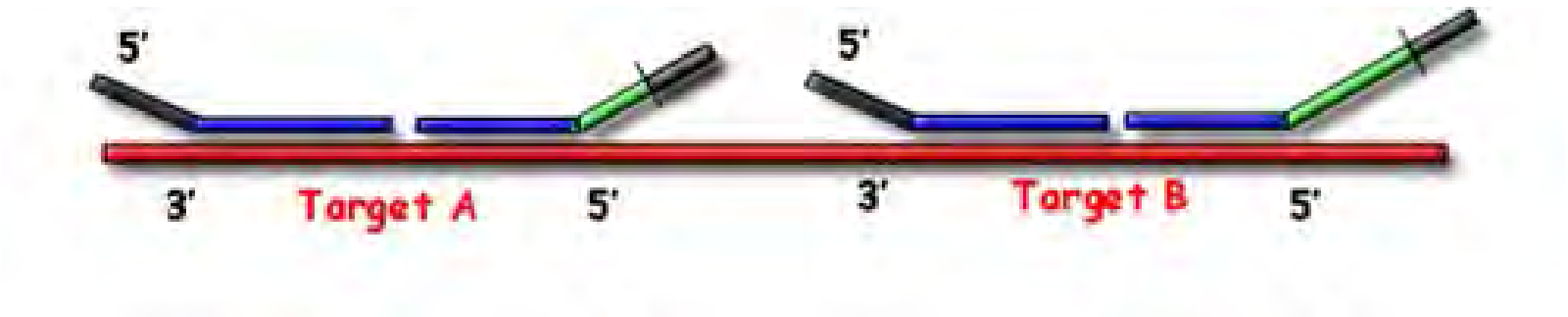


Ex20: 2184G>A

Ex5: 745_801+21del

Hybridysation

1. The MLPA probemix is added to denatured genomic DNA
2. The two parts of each probe hybridise to adjacent target sequences



Clinical Chemistry 57:11
1584–1596 (2011)

Motor Chip: A Comparative Genomic Hybridization Microarray for Copy-Number Mutations in 245 Neuromuscular Disorders

Giulio Piluso,[1] Manuela Dionisi,[1] Francesca Del Vecchio Blanco,[1] Annalaura Torella,[1] Stefania Aurino,[1,2] Marco Savarese,[1,2] Teresa Giugliano,[1] Enrico Bertini,[3] Alessandra Terracciano,[3] Mariz Vainzof,[4] Chiara Criscuolo,[5] Luisa Politano,[6] Carlo Casali,[7] Filippo Maria Santorelli,[8] and Vincenzo Nigro[1,2*]

BACKGROUND: Array-based comparative genomic hybridization (aCGH) is a reference high-throughput technology for detecting large pathogenic or polymorphic copy-number variations in the human genome; however, a number of quantitative monogenic mutations, such as smaller heterozygous deletions or duplications, are usually missed in most disease genes when proper multiplex ligation-dependent probe assays are not performed.

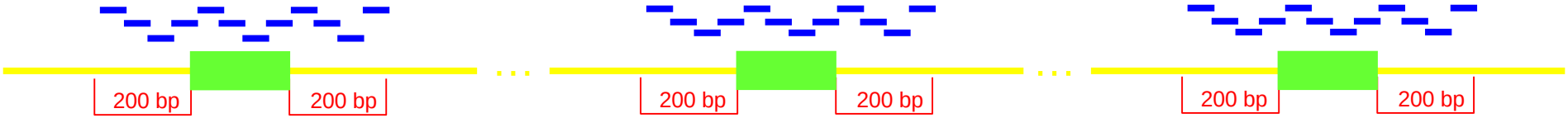
METHODS: We developed the Motor Chip, a customized CGH array with exonic coverage of 245 genes involved in neuromuscular disorders (NMDs), as well as 180 candidate disease genes. We analyzed DNA samples from 26 patients with known deletions or duplications in NMDs, 11 patients with partial molecular diagnoses, and 19 patients with a clinical diagnosis alone.

lecular diagnosis and gene investigation in neuromuscular diseases.

Neuromuscular disorders (NMDs)[9] are a highly heterogeneous group of genetically determined diseases encompassing many conditions that, directly or indirectly, impair muscle function by affecting the muscles and/or their nervous control. In the annually published gene table of NMDs (http://www.musclegenetable.org) *(1)*, 495 clinical entries and 272 distinct causative nuclear genes have been annotated to date. Genetic and clinical redundancy reflects the broad phenotypic variability included under the term "neuromuscular disorders," embracing myopathies, cardiomyopathies, and neuromyopathies. In addition, at least 99 mapped

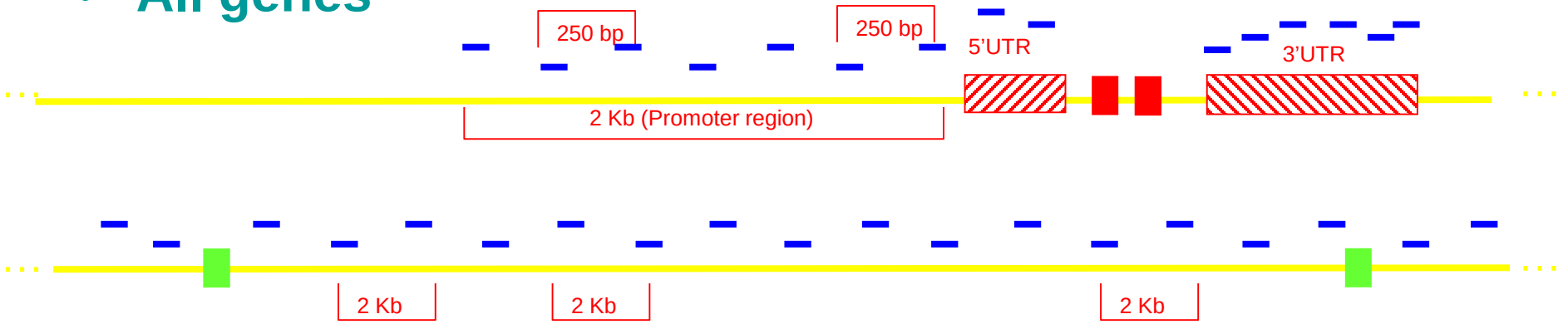
probe selection strategy

- **DMD gene**

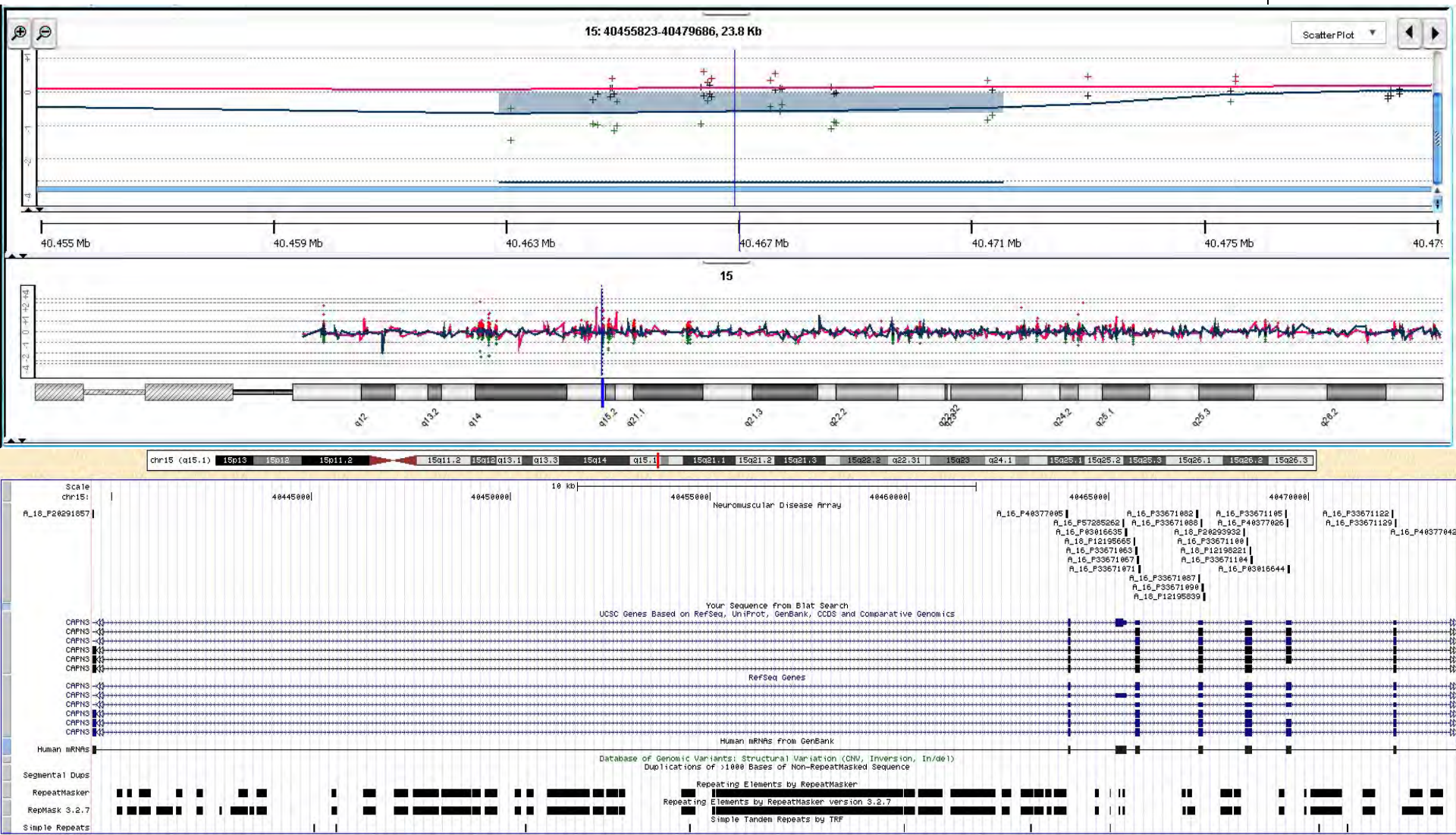


- **All the other genes**

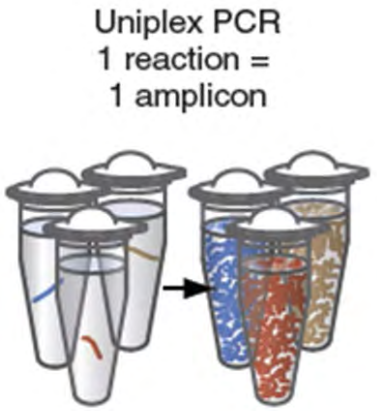
- **All genes**



X640, diagnosed as BMD, with two sisters heterozygous deletion of exons 2-8 at the CAPN3 locus + point mutation



Sequencing DNA one-by-one (Sanger)



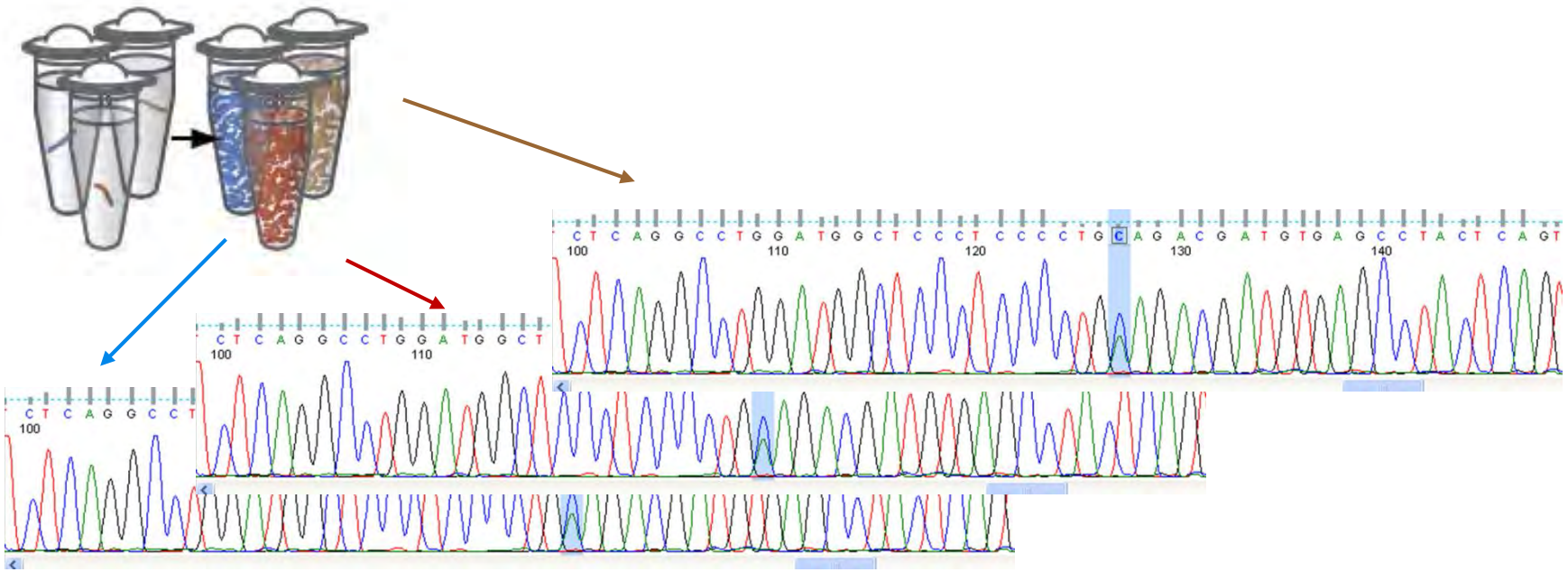
500 bases

A low fraction of dNTP is composed of ddNTPs (ddATP, ddTTP, ddGTP, ddCTP). They block the DNA polymerization

Frederick Sanger

Nobel price 1958 and 1980
born August 13 1918, died November 19 2013

Sanger sequencing
exon-by-exon, gene-by-gene

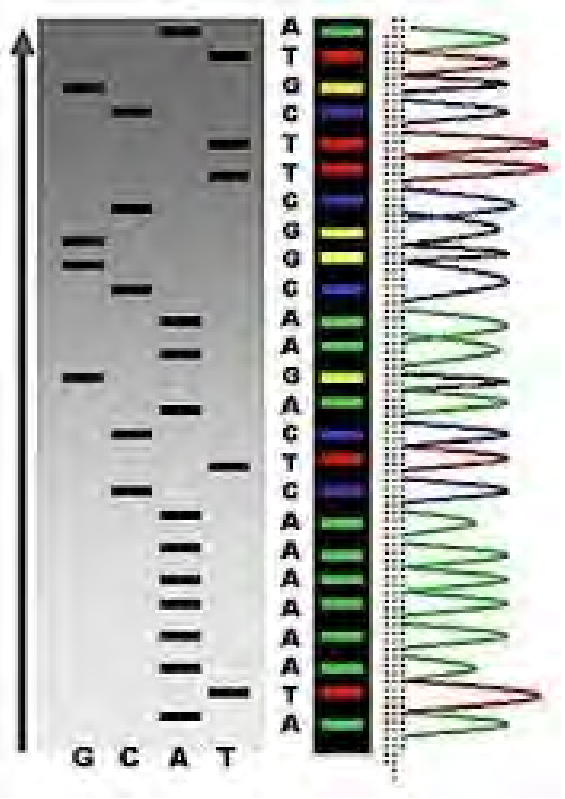


The evolution of Sanger sequencing in 1990-2000

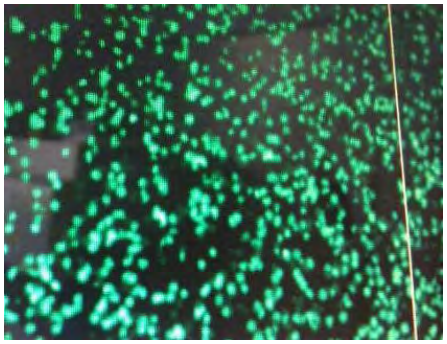
«one-by-one» **but with capillary electrophoresis**

(50,000 bases/run)

The Human Genome Project

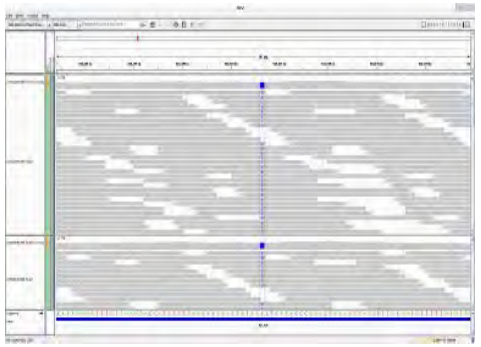


….to next generation sequencing testing



FASTQ

```
@SEQ_ID
GATTTGGGGTTAATA
+
!''*((((***+))%
```

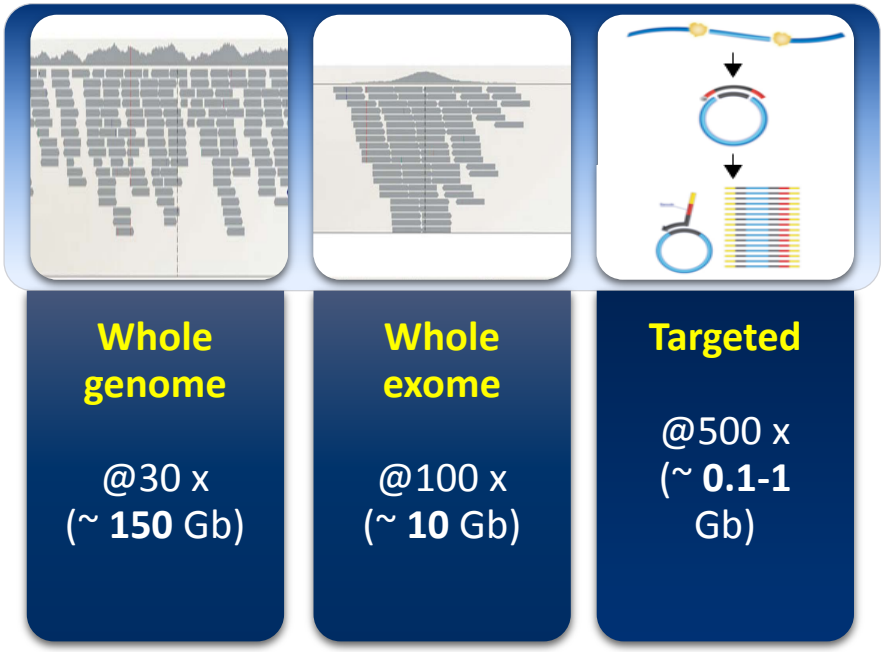


454 Sequencer (2005) 25 Millions of DNA bases/run

Illumina HiSeq X Ten (2014) 18,000,000 Millions bases/run

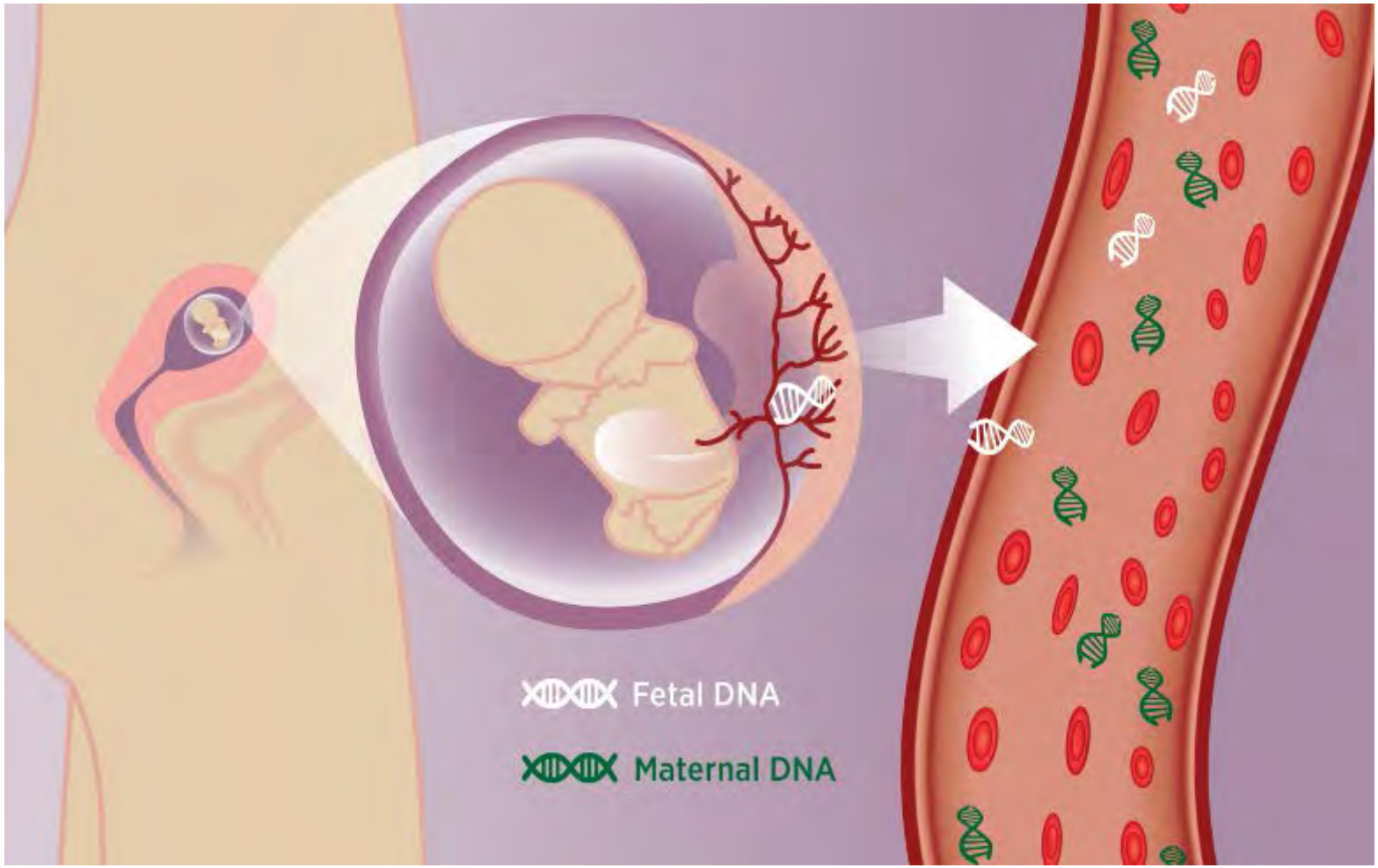
“Next Generation Sequencing”
.. To identify the cause of a genetic disorder

- **whole genome (WGS):** 3,000,000 variants and then look at (and validate) those in your genes
- **whole exome (WES):** 30,000 variants and then only analyze your genes
- **targeted sequencing:** you first **define** your genes and then sequence them **the best possible**



success	26%	30%	>40%

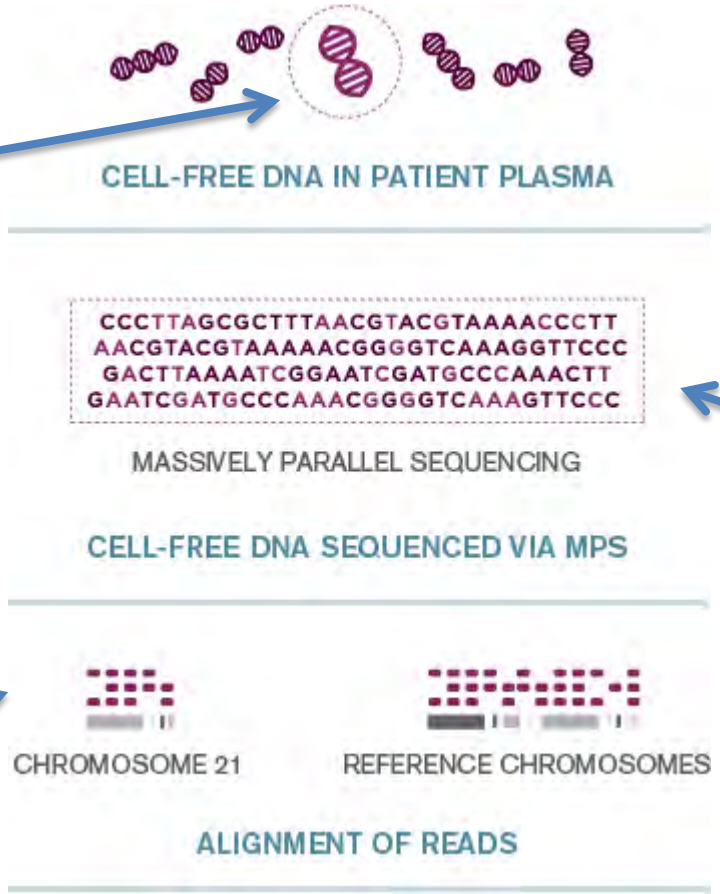
Cell Free Fetal DNA (cff DNA) in Maternal Blood



NON – INVASIVE PRENATAL TESTING

NIPT

Fetal DNA fragments in maternal blood.



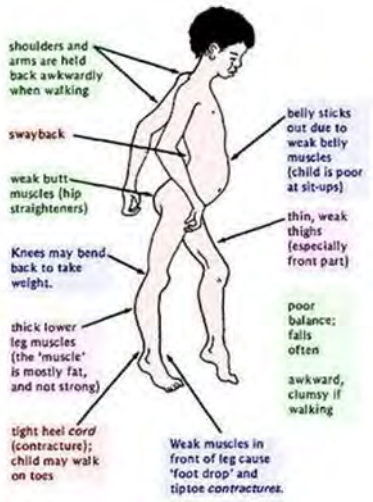
Cell free DNA fragments are then sequenced.

Compare the individual sequenced chromosomes against a reference for analysis.

MotorPlex v.5

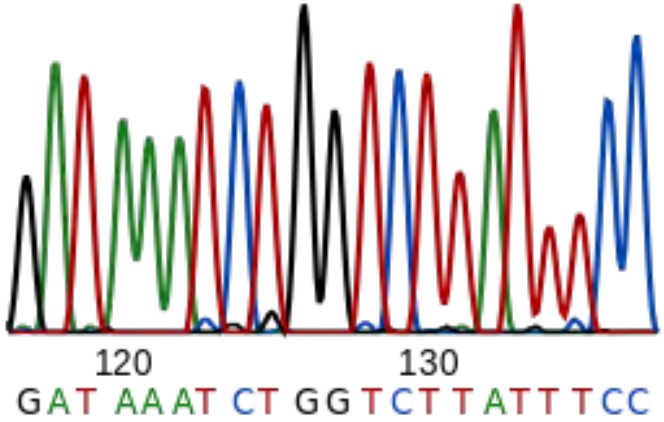
Marco Savarese, Giuseppina Di Fruscio, Annalaura Torella

- **89** muscular dystrophy genes (some very large, such as titin with 380 exons)
- 19,900 target regions corresponding to **500kbp**
- 99.8% coverage inside target regions
- **850 samples** already investigated



specificity

- we confirmed 128/128 variants (**coverage >20x**) by Sanger sequencing (100%)



93 muscle disease genes that when mutated produce overlapping phenotypes

target focused to 500,000bp to improve coverage (99.2%) and cost-effectiveness

Savarese et al. Acta Neuropathologica Communications 2014, 2:100
http://www.actaneurocomms.org/content/2/1/100

ACTA NEUROPATHOLOGICA COMMUNICATIONS

RESEARCH Open Access

MotorPlex provides accurate variant detection across large muscle genes both in single myopathic patients and in pools of DNA samples

Marco Savarese[1,2], Giuseppina Di Fruscio[1,2], Margherita Mutarelli[2], Annalaura Torella[1], Francesca Magri[3], Filippo Maria Santorelli[4], Giacomo Pietro Comi[3], Claudio Bruno[5] and Vincenzo Nigro[1,2*]

ARTICLE IN PRESS

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Available online at www.sciencedirect.com

ScienceDirect

Neuromuscular Disorders ■■ (2015) ■■–■■

NMD

www.elsevier.com/locate/nmd

Next generation sequencing on patients with LGMD and nonspecific myopathies: Findings associated with *ANO5* mutations

Marco Savarese [a,b,1], Giuseppina Di Fruscio [a,b,1], Giorgio Tasca [c], Lucia Ruggiero [d], Sandra Janssens [e], Jan De Bleecker [f], Marc Delpech [g], Olimpia Musumeci [h], Antonio Toscano [h], Corrado Angelini [i], Sabrina Sacconi [j], Lucio Santoro [d], Enzo Ricci [c], Kathleen Claes [e], Luisa Politano [k], Vincenzo Nigro [a,b,*]

OPEN ACCESS Freely available online

PLOS ONE

Next-Generation Sequencing Identifies Transportin 3 as the Causative Gene for LGMD1F

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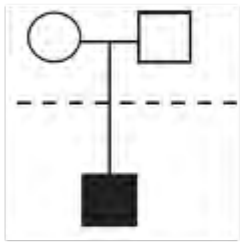
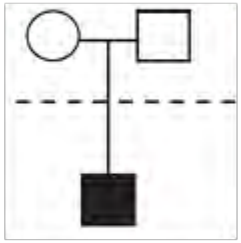
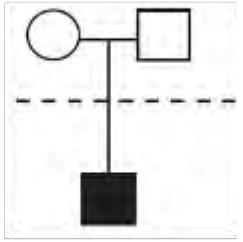
Abstract

Limb-girdle muscular dystrophies (LGMD) are genetically and clinically heterogeneous conditions. We investigated a large family with autosomal dominant transmission pattern, previously classified as LGMD1F and mapped to chromosome 7q32. Affected members are characterized by muscle weakness affecting earlier the pelvic girdle and the ileopsoas muscles. We sequenced the whole exome of four family members and identified a shared heterozygous frame-shift variant in the Transportin 3 (*TNPO3*) gene, encoding a member of the importin-β super-family. The *TNPO3* gene is mapped within the LGMD1F critical interval and its 923-amino acid human gene product is also expressed in skeletal muscle. In addition, we identified an isolated case of LGMD with a new missense mutation in the same gene. We localized the mutant TNPO3 around the nucleus, but not inside. The involvement of gene related to the nuclear transport suggests a novel disease mechanism leading to muscular dystrophy.

for NGS studies parents must be included to validate variants, discover *denovo* mutations and get phase




diagnostic yield by gene in 218/504 patients (43.2%)

from Savarese *et al* (Ms in prep)

Locus	Gene Name	patients
1q42.13	ACTA1	2
1p21	AGL	2
21q22.3	COL6A2	4
2q37	COL6A3	2
11q22.3-q23.1	CRYAB	1
Xp21.2	DMD	7
19p13.2	DNM2	5
Xq28	EMD	1
7q32	FLNC	4
17q25.2-q25.3	GLA	1
3p12	GNE	3
3p22.1	GTDC2	1
3q24	GYG1	1
12q13.2	ITGA7	2
6q22-q23	LAMA2	8
Xq28	MTM1	5
17p13.1	MYH2	1
14q12	MYH7	8
5q31	MYOT	1
2q23.3	NEB	8
11q12-q13.2	PYGM	3
20p13	RYR1	25
1p36.13	SEPN1	2
18p11.32	SMCHD1	1
6q25	SYNE1	1
14q23.2	SYNE2	2
9p13	TPM2	1
1q21.2	TPM3	2
4q35.1	TRAPPC	2

Disease	Locus	Gene Name	patients
LGMD1B	1q22	LMNA	4
LGMD1C	3p25.3	CAV3	2
LGMD2A	15q15	CAPN3	21
LGMD2B	2p13.2	DYSF	15
LGMD2C	13q12	SGCG	4
LGMD2D	17q21	SGCA	10
LGMD2E	4q12	SGCB	6
LGMD2G	17q12	TCAP	1
LGMD2H	9q33.1	TRIM32	1
LGMD2I	19q13.3	FKRP	6
LGMD2J	2q24.3	TTN	5
LGMD2K	9q34.1	POMT1	1
LGMD2L	11p13	ANO5	14
LGMD2M	9q31	FKTN	2
LGMD2N	14q24	POMT2	6
LGMD2R	2q35	DES	1
LGMD2T	3p21	GMPPB	2
LGMD2V	17q25	GAA	11

European Journal of Human Genetics (2012) 20, 999–1003

www.nature.com/ejhg

SHORT REPORT

Diagnosis by sequencing: correction of misdiagnosis from FSHD2 to LGMD2A by whole-exome analysis

Andreas Leidenroth[1], Hanne Sørmo Sorte[2], Gregor Gilfillan[2], Melanie Ehrlich[3], Robert Lyle[2] and Jane E Hewitt*,[1]

We studied and validated facioscapulohumeral muscular dystrophy (FSHD) samples from patients without a D4Z4 contraction (FSHD2 or 'phenotypic FSHD'). For this, we developed non-radioactive protocols to test D4Z4 allele constitution and DNA methylation, and applied these to samples from the Coriell Institute Cell Repository. The D4Z4 sizing showed two related subjects to have classic chromosome 4 contraction-dependent FSHD1. A third sample (GM17726) did not have a short chromosome 4 fragment, and had been assigned as non-4q FSHD (FSHD2). We tested D4Z4 haplotype and methylation for this individual but found both to be inconsistent with this diagnosis. Using exome sequencing, we identified two known pathogenic mutations in *CAPN3* (Arg490Gln and Thr184Argfs*36), indicating a case of LGMD2A rather than FSHD. Our study shows how a wrong diagnosis can easily be corrected by whole-exome sequencing by constraining the variant analysis to candidate genes after the data have been generated. This new way of 'diagnosis by sequencing' is likely to become common place in genetic diagnostic laboratories. We also publish a digoxigenin-labeled Southern protocol to test D4Z4 methylation. Our data supports hypomethylation as a good epigenetic predictor for FSHD2. The non-radioactive protocol will help to make this assay more accessible to clinical diagnostic laboratories and the wider FSHD research community.
European Journal of Human Genetics (2012) **20**, 999–1003; doi:10.1038/ejhg.2012.42; published online 29 February 2012

Male , 51 years

- Aspecific mild myopathy, negative familiarity
- CK max 1800 Aspecific histological features
- IF/Western blot analyses: normal
- Genetic testing negative for Kennedy, PROMM, lamin A/C
- "*next generation sequencing*" of 98 genes Motor Haloplex v.3 in pools 5+16

Gene (simbolo)	Sequenza di riferimento	Variazione del cDNA	Variazione della proteina	Frequenza allelica max	validazione
DMD	NM_004007	c.C2708G	p.A903G	0,01	90% NGS
DPM3	NM_018973	c.C268T	**p.R90C**	0	90% NGS
PLEC	NM_201378	c.C3985T	p.R1329C	0,01	90% NGS
PLEC	NM_201378	c.G1664A	p.R555Q	0,02	90% NGS

CAPN3 **NM_000070: [c.1250C>T p.Thr417Met] + [c.1250C>T p.Thr417Met]**

Female , 43 years

- Aspecific mild proximal myopathy, negative familiarity
- CK max 1000 Aspecific histological features
- "*next generation sequencing*" of 98 genes Motor Haloplex v.3 in pools 5+16

Gene (simbolo)	Sequenza di riferimento	Variazione del cDNA	Variazione della proteina	Frequenza allelica max	validazione
DPM2	NM_003863	c.T127C	p.Y43H	0	90% solo NGS
FLNC	NM_001458	c.G5221A	p.E1741K	0.00024	100% Sanger eterozigosi
TTN	NM_133379	c.G11283C	p.K3761N	0.000539	90% solo NGS

CAPN3 **[c.1468C>T p.Arg490Trp (esone 11)] + [c.2242C>T p.Arg748Stop (esone 21)]**

List of the CAPN3 variations found in 173 myopathic patients

CAPN3	chr15:42652014	c.11T>G	p.V4G	M16 (X1356, X1434, X1469, X1527, 6249)	20% NGS		
CAPN3	chr15:42652065	c.62G>A	p.G21E	X863 (het)	100% Sanger		
CAPN3	chr15:42652076	c.73C>T	p.H25Y	X1686	95%NGS		
CAPN3	chr15:42652080	c.77C>T	p.P26L	M11 (X1343, X1405, X1439, X1498. X1236)	20% NGS		
CAPN3	chr15:42652082	c.79G>A	p.A27T	M9 (X1341, X1390, X1436, X1472, X474)	20% NGS		
CAPN3	chr15:42652160	c.157A>G	p.I53V	X1263 (het)	100% Sanger		
CAPN3	chr15:42652166	c.163G>A	p.G55R	M9 (X1341, X1390, X1436, X1472, X474)	20% NGS		
CAPN3	chr15:42676690	c.319G>A	p.E107K	X1742 (het)	100% Sanger		
CAPN3	chr15:42680002	c.550delA	p.T184RfsX36	X1245 (het)	100% Sanger		
CAPN3	chr15:42680036	c.584A>C	p.N195T	X1431 (het)	100% Sanger		
CAPN3	chr15:42681142	c.649G>A	p.E217K	X1520 (het)	100% Sanger		
CAPN3	chr15:42681187	c.694A>C	p.T232P	X1529 (het)	100% Sanger		
CAPN3	chr15:42682219	p.870G>A	p.M290I	X589 (het)	100% Sanger		
CAPN3	chr15:42682271	c.922G>A	p.G308S	X1576 (het)	100% Sanger		
CAPN3	chr15:42684875	c.984C>T	splicing	X508 (het)	100% Sanger	X1278 (het)	100% Sanger
CAPN3	chr15:42686458	c.1034C>T	p.P345L	M11 (X1343, X1405, X1439, X1498. X1236)	20% NGS		
CAPN3	chr15:42686485	c.1061T>G	p.V354G	X1550 (het)	100% Sanger		
CAPN3	chr15:42686487	c.1063C>T	p.R355W	M13 (X1348, X1428, X1442, X1517, X1229)	20% NGS		
CAPN3	chr15:42689077	c.1193+2T>C	splicing	X1550 (het)	100% Sanger		
CAPN3	chr15:42691746	c.1250C>T	p.T417M	X1668 (hom)	100% Sanger	X1227 (het)	100% Sanger
CAPN3	chr15:42691799	c.1303G>A	p.E435K	X1520 (het)	100% Sanger		
CAPN3	chr15:42691806	c.1310G>T	p.R437L	M14 (X1349, X1431, X1458, X1519, X1206)	20% NGS		
CAPN3	chr15:42693952	c.1468C>T	p.R490W	X1303 (het)	100% Sanger		
CAPN3	chr15:42694325	c.1528C>T	p.P510S	X1676	95%NGS		
CAPN3	chr15:42695076	c.1621C>T	p.R541W	X1302 (het)	100% Sanger		
CAPN3	chr15:42695076	c.1621C>G	p.R541G	X1702 (het)	100% Sanger		
CAPN3	chr15:42695161	c.1706T>C	p.F569S	X1203 (het)	100% Sanger	X1206 (het)	100%Sanger
CAPN3	chr15:42695170	c.1715G>C	p.R572Q	R3 (hom)	100% Sanger		
CAPN3	chr15:42695919	c.1746-20C>G	p.E583CfsX9	X1237 (het)	100% Sanger		
CAPN3	chr15:42701564	c.442C>G	p.Q654E	X918 (het)	100% Sanger		
CAPN3	chr15:42701984	c.1993-1G>A	splicing	X1181 (hom)	100% Sanger		
CAPN3	chr15:42702843	c.C2242T	p.R748X	X1303 (het)	100% Sanger		
CAPN3	chr15:42682142	c.802-9G>A	splicing	X1302 (het)	100% Sanger		

European Journal of Human Genetics (2015), 1–5

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ARTICLE

Are all the previously reported genetic variants in limb girdle muscular dystrophy genes pathogenic?

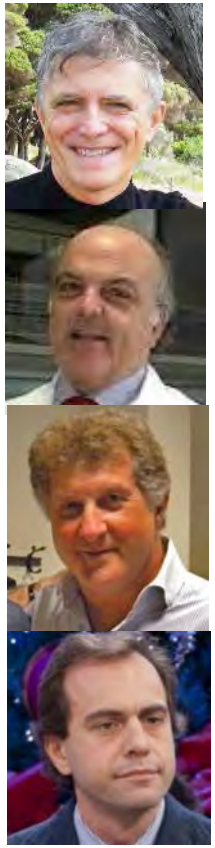
Giuseppina Di Fruscio[1,2], Arcomaria Garofalo[1,2], Margherita Mutarelli[2], Marco Savarese[1,2] and Vincenzo Nigro[1,2]

Hundreds of variants in autosomal genes associated with the limb girdle muscular dystrophies (LGMDs) have been reported as being causative. However, in most cases the proof of pathogenicity derives from their non-occurrence in hundreds of healthy controls and/or from segregation studies in small families. The limited statistics of the genetic variations in the general population may hamper a correct interpretation of the effect of variants on the protein. To clarify the meaning of low-frequency variants in LGMD genes, we have selected all variants described as causative in the Leiden Open Variation Database and the Human Gene Mutation Database. We have systematically searched for their frequency in the NHLBI GO Exome Sequencing Project (ESP) and in our internal database. Surprisingly, the ESP contains about 4% of the variants previously associated with a dominant inheritance and about 9% of those associated with a recessive inheritance. The putative disease alleles are much more frequent than those estimated considering the disease prevalence. In conclusion, we hypothesize that a number of disease-associated variants are non-pathogenic and that other variations are not fully penetrant, even if they affect the protein function, suggesting a more complex genetic mechanisms for such heterogeneous disorders.
European Journal of Human Genetics advance online publication, 22 April 2015; doi:10.1038/ejhg.2015.76

Table 5 ***In silico*** **prediction of identified variants in** ***LGMD2*** **genes**

		LGMD2		
Gene	*Benign*	*Damaging*	*Unknown*	*Total*
ANO5	3	16	10	29
CAPN3	12	201	76	289
DAG	0	1	1	2
DYSF	10	95	108	213
FKRP	7	75	13	95
FKTN	0	12	9	21
POMGNT1	1	19	15	35
POMT1	2	18	11	31
POMT2	0	20	10	30
SGCA	9	41	13	63
SGCB	1	19	11	31
SGCD	0	5	3	8
SGCG	0	10	7	17
TCAP	0	0	3	3
TRIM32	1	2	0	3
TTN	1	7	3	11
Total	47	541	293	881

UDP15001
TELETHON UNDIAGNOSED DISEASES PROGRAM 2015
three clinical sites and a coordination center



- ***Coordinator***
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  Sandro Banfi
- ***Partner 1***
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- ***Partner 2***
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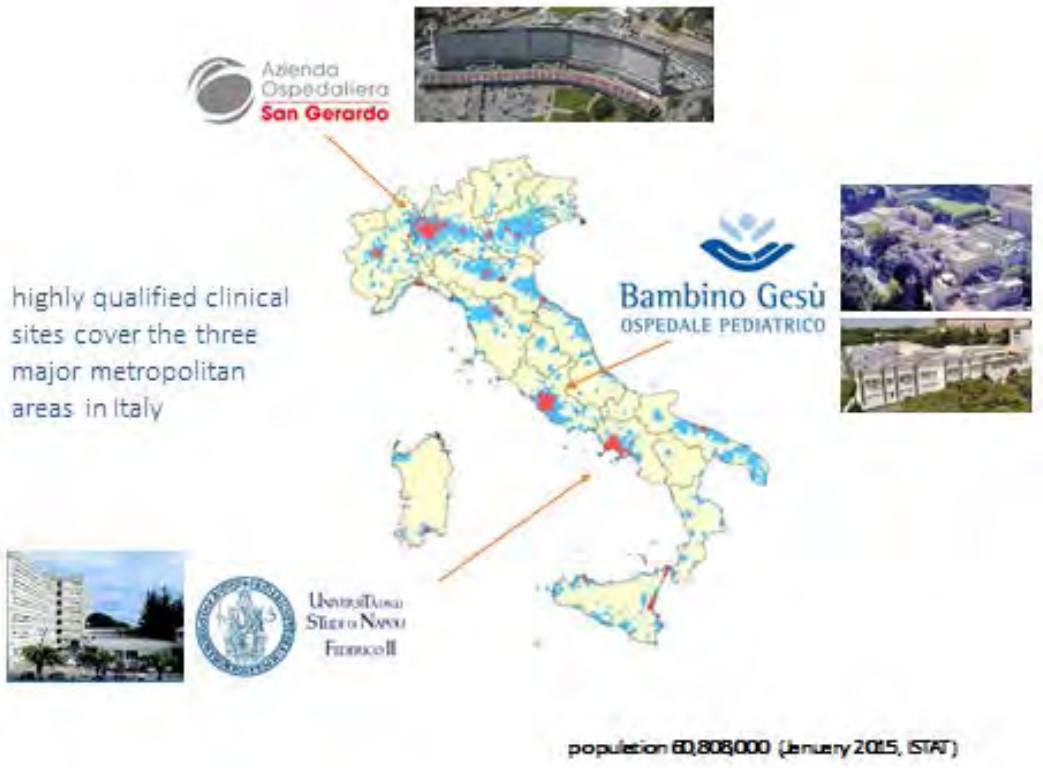
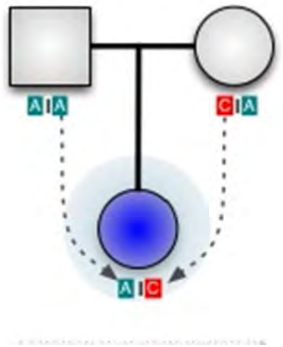


This program is an **intramural effort** of Telethon centered on the Telethon Institute of Genetics and Medicine (**TIGEM**) where NGS activities will be converged and will rely on a **core network of three centers** with great expertise in clinical genetics and pediatrics

.....children affected by an orphan genetic disease may wait indefinitely for a diagnosis....



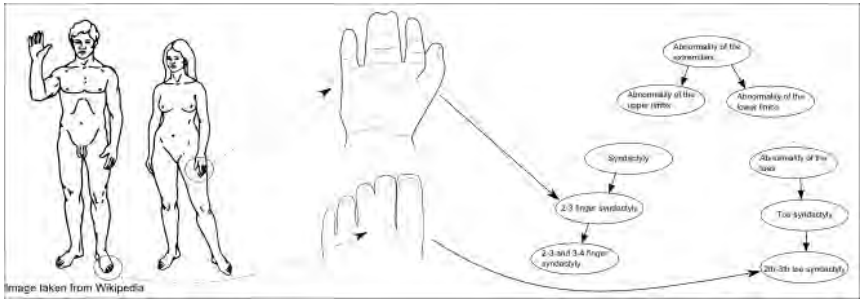
UDP15001 proposes two major aims:

- to establish a **shared** and **standardized** clinical selection of undiagnosed patients through comprehensive phenotyping
- to carry out **High-Coverage** whole exome sequencing (HC-WES) in patients and parents (1,200-1,500 subjects). This strategy will be useful
  - to improve variant calling and correct errors
  - to discover *de novo* variants
  - to get phase and haplotype

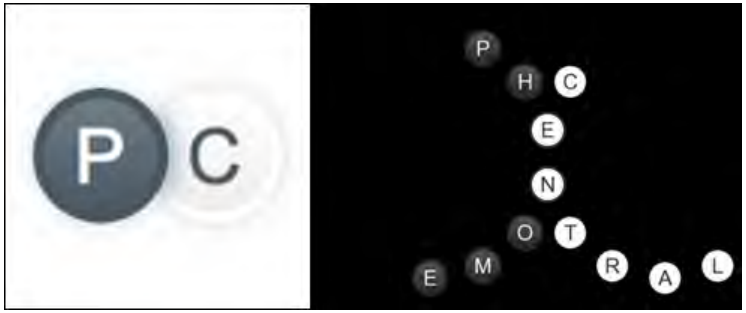
standardization and data sharing

…cooperation and collaboration, on both national and international levels, are critical factors for success in the study of rare disease…

Phenotypic data capture and storage using the standardized vocabulary defined by the **Human Phenotype Ontology** (HPO)

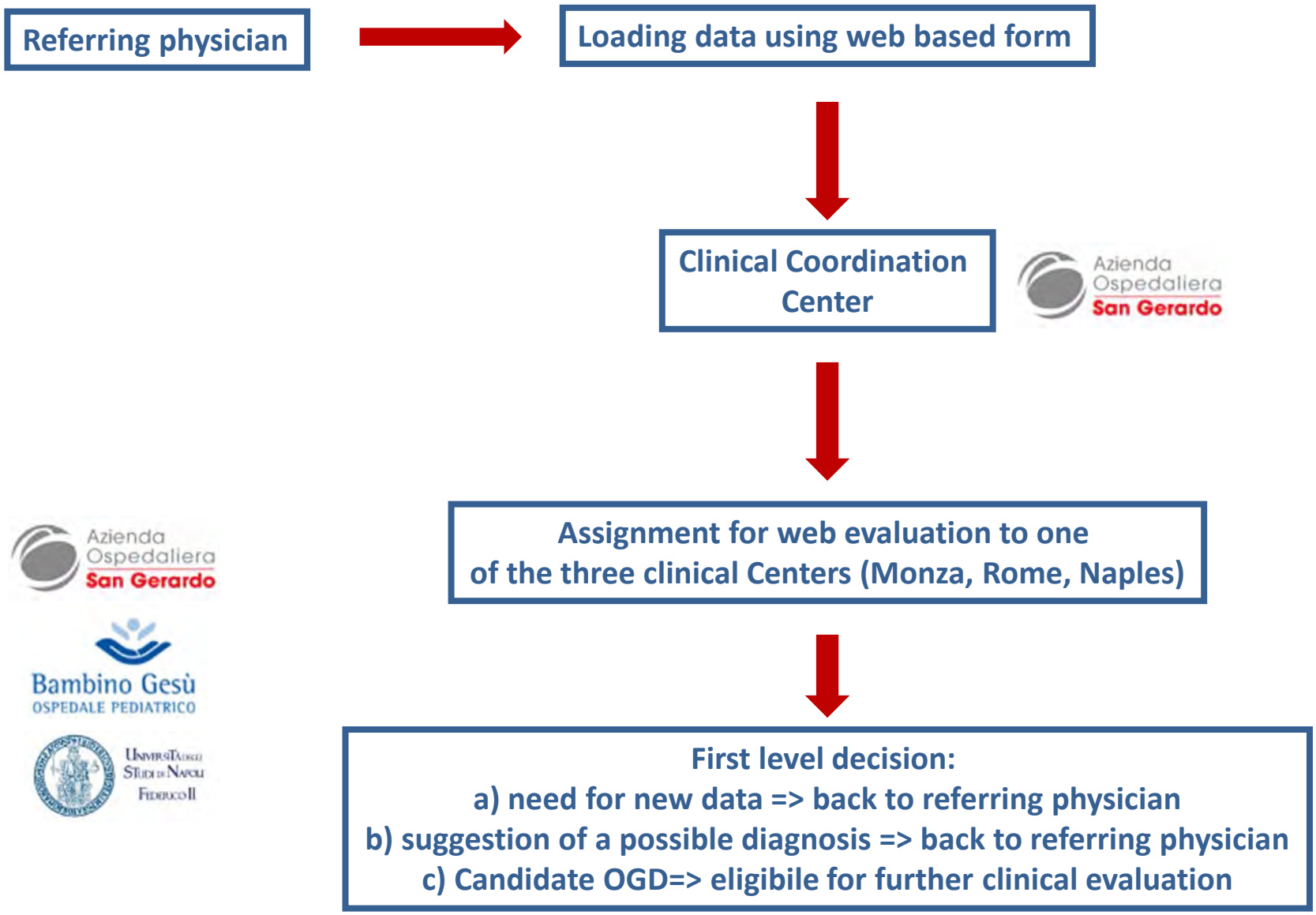


Data will be shared through **PhenomeCentral** (http://phenomecentral.org), a portal for rare disorder case sharing that is used by >350 users worldwide, Care for Rare, US Undiagnosed Diseases Network and European (Neuromics, AnDDIrare) rare disease sequencing projects

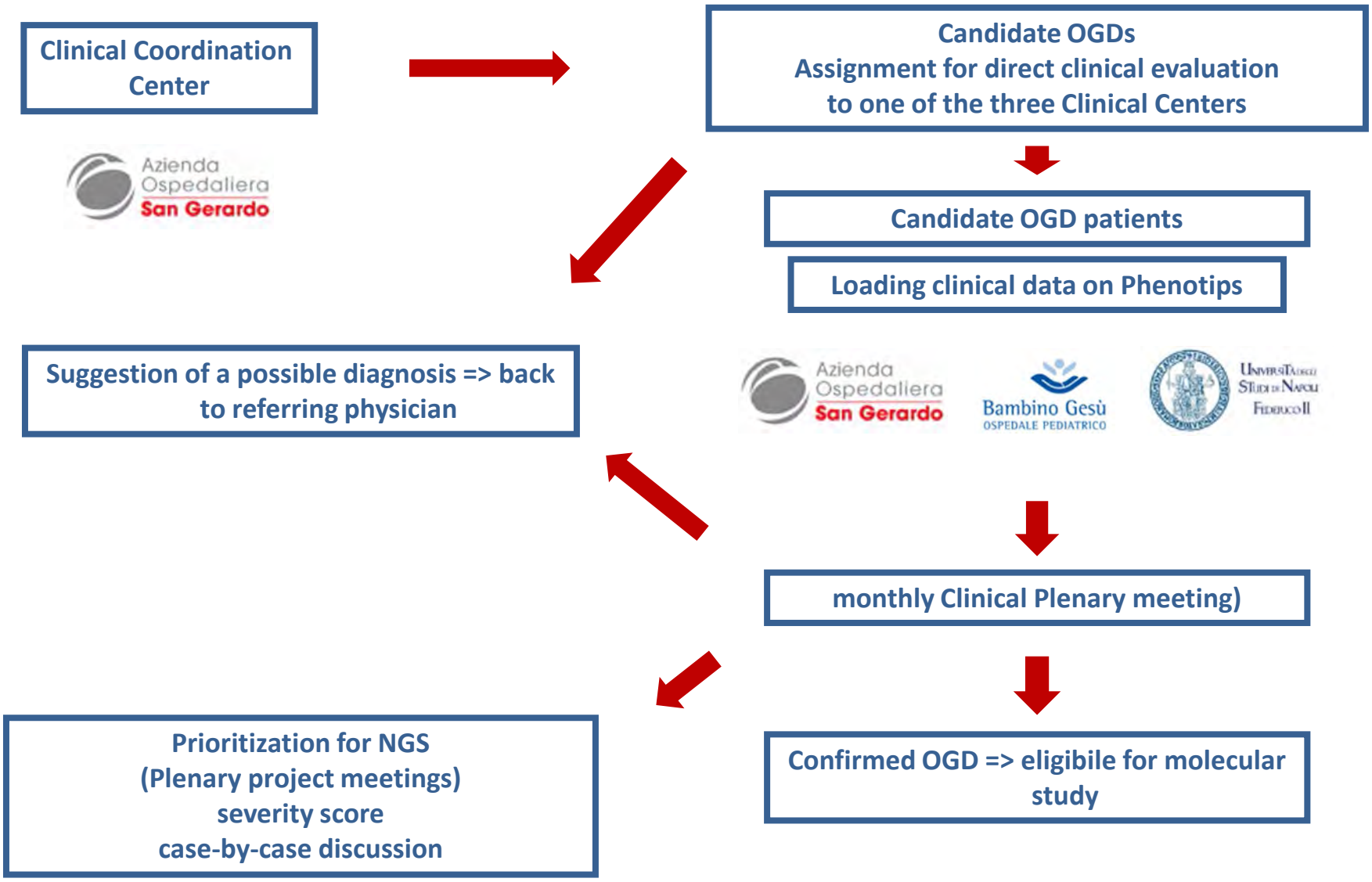


This has great potential for identifying **additional cases** of phenotypically similar patients required to validate the identified putative disease causative variant(s)

Clinical evaluation step 1



Clinical evaluation step 2



NGS analysis

Prioritized OGD (trios)

DNA extraction
Library preparation
Whole exome sequencing

Bioinformatic analysis

Candidate variants identified and data loading Phenome Central

Novel candidate gene

Searching for additional cases with variants in the same gene through international collaborations

Known gene

Re-phenotyping based on candidate gene variants

Genotype-phenotype correlations

Communication to referring physicians and family

No candidate gene

Negative results of molecular test
a) Communication to referring physicians and family
b) DNA banking (possible recall)

ethical guidelines

Informed consent for analyzing whole exome sequence data from all family members and for sharing anonymous results with other Undiagnosed Diseases networks

*…we could **incidentally** discover a variant already known (or more than one) to cause a **second genetic disease**, with no relationship to your main genetic disease. In the absence of your explicit opposition, we will confirm these unexpected DNA variants with another sequencing technique (validation)*

*We will then discuss the results with the reference centers and send these data to the referring doctor who will present with you the significance in **genetic counseling**….*

- **Seconda Università di Napoli, Italy**
  Marco Savarese, Annalaura Torella, Giuseppina Di Fruscio
  Ombretta De Concilio, Teresa Giugliano, Michele Iacomino, Arca Garofalo, Stefania Aurino, Francesca Del Vecchio Blanco, Giulio Piluso
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  Corrado Angelini, Marina Mora, Marina Mora, Lucia Morandi, Antonio Toscano, Adele D'Amico, Enrico Bertini, Giorgio Tasca, Eugenio Mercuri, Tiziana Mongini, Olimpia Musumeci, Antonio Toscano, Gabriele Siciliano, Elena Pegoraro, Enzo Ricci, Giulia Ricci, Chiara Fiorillo, Angela Berardinelli, Lucio Santoro, Lucia Ruggiero, Sonia Messina, Maurizio Moggio, Rossella Tupler, Filippo Maria Santorelli