31 GIORNATE CARDIOLOGICHE TORINESI

TURIN
October
24th-26th
2019

Heart and Neuromuscolar Diseases

- Genetic and Diagnosis -

Daniela Giachino MD PhD - Medical Genetics
University of Torino
AOU San Luigi Gonzaga – Orbassano (TO)

Inherited NMDs

- ✓ Inherited NMDs are genetic disorders typically caused by a mutation in a single gene that affects striated muscle and results in progressive weakness in affected individuals from degenerative muscle pathology

- ✓ In addition to skeletal muscle, cardiac muscle can also be affected (1) cardiomyopathy and (2) conduction defects with arrhythmias.

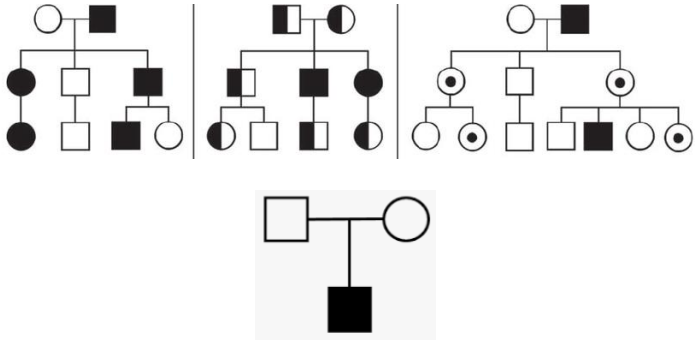
- ✓ Genotypic identification of NMD pathogeneses is a relatively recent phenomenon, and genotype-phenotype correlations continue to evolve

Feingold, AHA Scientific Statement *Circulation* 2017

The importance of genetic testing

1. A significant **phenotypic overlap** among NMDs at initial presentation,

- genetic testing is crucial to the diagnostic workup of NMDs allowing for a definitive diagnosis



2. Typical disease **inheritance patterns**

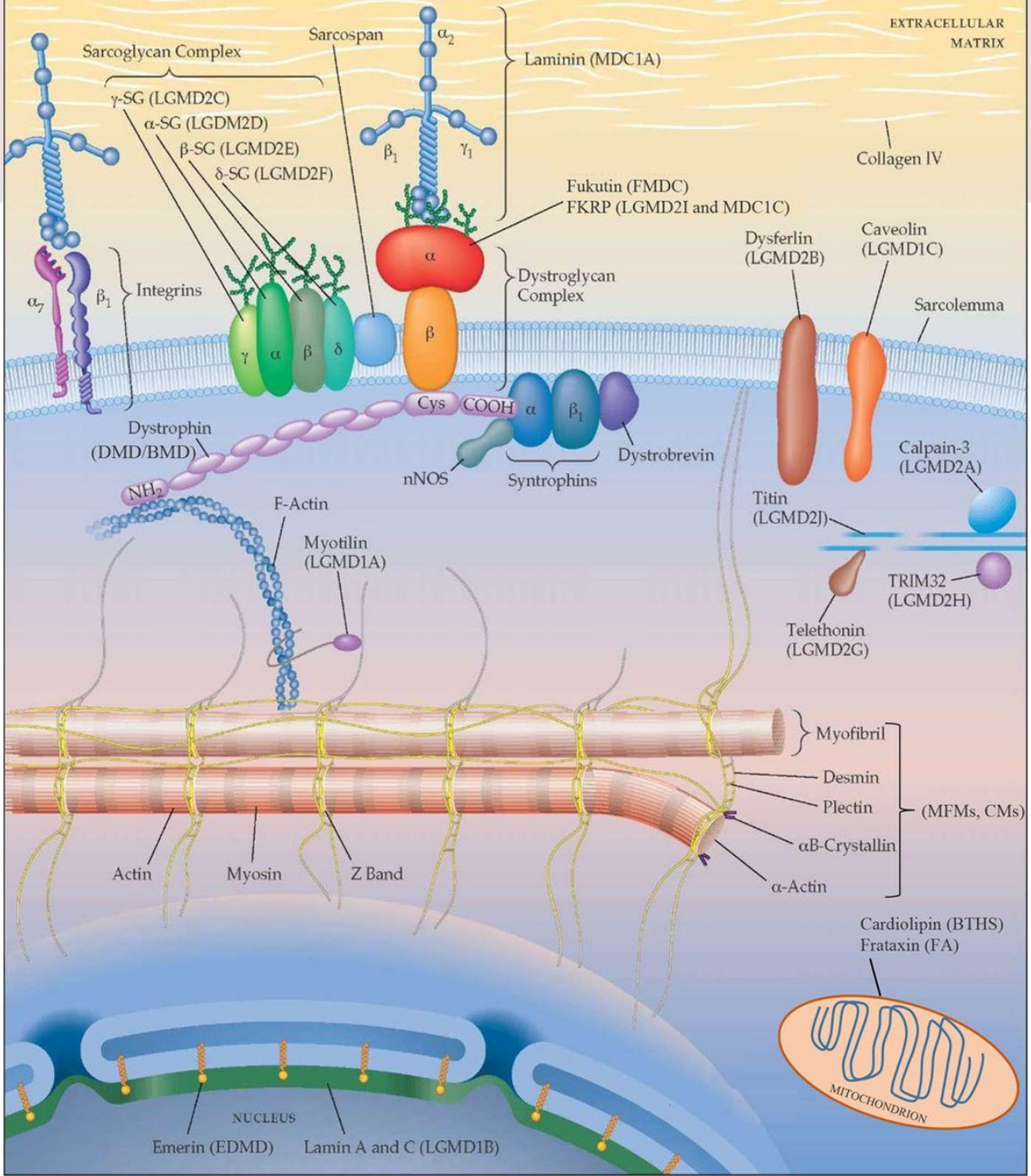
HOWEVER

- phenotypic variability within family members
- spontaneous mutations

3. Knowledge of the specific underlying disease process provides

- information about
  - clinical expectations
  - genetic counseling
  - family screening
  - prenatal diagnosis

Ref. Feingold, AHA Scientific Statement *Circulation* 2017



Ref. Feingold, AHA Scientific Statement *Circulation* 2017

Characteristics of NMDs With Cardiac Involvement

Condition	Gene Locus	Gene Product	Heritance	Cardiac Features		
				Cardiomyopathy	Arrhythmia	Conduction
X-linked recessive muscular dystrophies						
Duchenne	Xp21	Dystrophin	XLR	Common (DCM)	Common (late)	Rare (late)
Becker	Xp21	Dystrophin	XLR	Common	Common	Rare (late)
Emery-Dreifuss	Xq28	Emerin	XLR	Rare	Common	Common (SD)
Limb-girdle muscular dystrophies						
LGMD1B	1q11–q21	Lamin A and C	AD	Common (DCM)	Common (AT, VT)	Common (SD)
LGMD1C	3p25	Caveolin-3	AD	Rare (DCM)	Not reported	Rare (AVB)
LGMD1E	7q36	DNAJB6 (co-chaperone)	AD	Rare	Rare	Rare
LGMD2B	2p13	Dysferlin	AR	Rare (DCM)	Not reported	Not reported
LGMD2C	13q12	γ-Sarcoglycan	AR	Common (DCM)	Rare	Rare
LGMD2D	17q12–q21	α-Sarcoglycan	AR	Common (DCM)	Rare	Rare
LGMD2E	4q12	β-Sarcoglycan	AR	Common (DCM)	Common	Common
LGMD2F	5q33–q34	δ-Sarcoglycan	AR	Rare	Rare	Rare
LGMD2I	19q13.3	Fukutin-related protein	AR	Common (DCM)	Rare	Rare
Associated with mitochondrial dysfunction						
Barth syndrome	Xq28	Tafazzin	XLR	Common (LVNC, DCM, HCM)	Occasional	None
Friedreich ataxia	9q21.11	Frataxin	AR	Common (HCM)	Common (late)	Rare

Ref. Feingold, AHA Scientific Statement *Circulation* 2017

Condition	Gene Locus	Gene Product	Heritance	Cardiac Features		
				Cardiomyopathy	Arrhythmia	Conduction
Myotonic dystrophies						
Myotonic dystrophy (DM) 1	19q13	Myotonin-protein kinase	AD	Occasional (DCM, HCM)	Common (AFL/AF, VT)	Common (SD)
Myotonic dystrophy (DM) 2	3q21	Zinc finger protein 9	AD	Rare in childhood	Rare in childhood	Rare in childhood
Congenital myopathies						
Central core disease	19q13.2	Ryanodine receptor	AD/AR	Rare (DCM)	Not reported	Not reported
Nemaline myopathy	1q21, 2q21–q22, 1q42.13, 19q13.4	α-Tropomyosin, nebulin, skeletal muscle α-actin, troponin T	AR/AD	DCM, HCM	Rare (long QT)	Common (mild)
Multiminicore disease	19q13.2, 1p36.13	Ryanodine receptor, selenoprotein N1	AR	Rare (HCM, RCM)	Unknown	Unknown
Centronuclear myopathy	19p13.2, 2q14, 2q31	Dynamin 2, bridging integrator 1, titin	AD/AR	Rare (DCM)	Rare	Rare
Myotubular myopathy	Xq28	Myotubularin	XLR	Not reported	Not reported	Not reported
Myosin storage myopathy	14q12	β-Myosin heavy chain	AD	Not reported	Not reported	Not reported
Congenital fiber type disproportion	1q21.2, 19q13.2, 1q42.13	α-Tropomyosin, ryanodine receptor, skeletal muscle α-actin	AR/AD	Rare (DCM)	Not reported	Not reported
Myofibrillar myopathies						
	2q35, 5q31, 10q22.3–q23.2, 11q23.1, 7q32–q35, 10q25.2–q26.2, Xq26	Desmin, myotilin, LIM domain binding protein 3, crystallin alpha B, filamin C gamma, BCL2-associated athanogene 3, four-and-a-half LIM domains 1	AD	Common	Rare (late; AF)	Rare (AVB)

Ref. Feingold, AHA Scientific Statement *Circulation* 2017

How to reach a genetic diagnosis

Identification of a patient with a genetic or even rare disease

Pretest genetic counselling
Counselling and THEN
Genetic testing
- is availabe?
- which kind?
- where?
- how long does it takes?

NO mutation

Treatment option
Surveillance

Family testing

Laboratory
> Genetic report

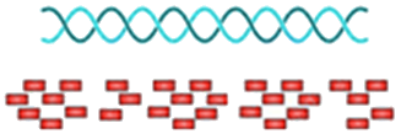
MUTATION

post test genetic counselling

Inconclusive results

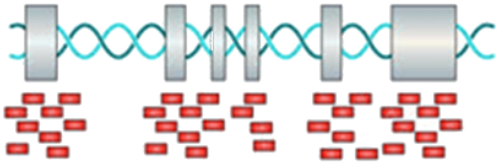
Sequencing technologies

Whole genome sequencing



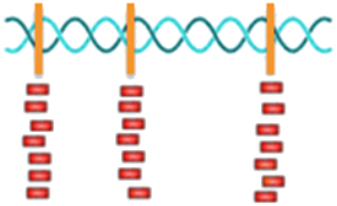
- Sequencing region : whole genome
- Sequencing Depth: >30X
- Covers everything – can identify all kinds of variants including SNPs, INDELs and SV.

Whole exome sequencing



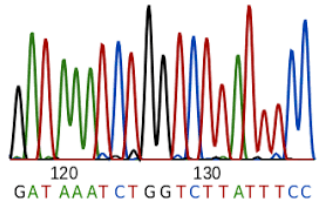
- Sequencing region: whole exome
- Sequencing Depth : >50X ~ 100X
- Identify all kinds of variants including SNPs, INDELs and SV in coding region.
- Cost effective

Targeted sequencing



- Sequencing region: specific regions (could be customized)
- Sequencing Depth : >500X
- Identify all kinds of variants including SNPs, INDELs in specific regions
- Most Cost effective

Gene by gene sequencing



- Analysis of specific gene (s)
- Laboratory with huge experience
- Better interpretation of variants
- More Cost
- Time
- Single patient analysis
- Single hit
- Genetic odyssey

Multiple hits

Sequencing results

IF the test is NEGATIVE for mutations

- Coverage
- Depth
- Type of expected mutation
  - (Copy Number Variants are not easly detect by NGS)

IF the test is POSITIVE for variants (**not yet mutation!**)

- Novel mutation
- Multiple hit
- Incidental findings
- Segregation in family (carrier affected/non-carrier healty)

Classification of DNA variants

Population data

Computational and predictive data

Functional (*in vitro* & *in vivo*) data

Segregation data

De novo data

Allelic data (AR)

© American College of Medical Genetics and Genomics ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

2015

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[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; on behalf of the ACMG Laboratory Quality Assurance Committee

NGS sequencing expands the phenotype spectrum

RESEARCH ARTICLE

De novo mutations in *FLNC* leading to early-onset restrictive cardiomyopathy and congenital myopathy

Artem Kiselev[1]* | Raquel Vaz[2]* | Anastasia Knyazeva[1] |

- FLNC: neuromuscular disorders and cardiomyopathies (HCM, DCM, ACM)
- new clinical phenotype of filaminopathy in 4 patients with early-onset restrictive cardiomyopathy (RCM) in combination with congenital myopathy
- 3 of the patients also presented with arthrogryposis.

Kiselev, A HumanMutation. 2018;39:1161–1172.

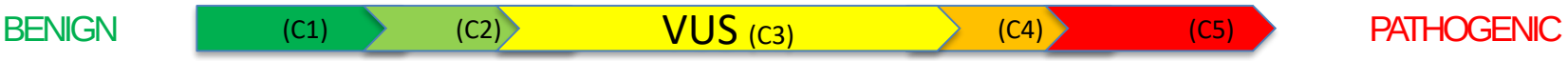
Genetic analysis

- 24 RCM patients resulted negative using a **target panel** of 108 cardiomyopathy associated genes
- Sequencing of **full exome libraries** was performed using SureSelect Human All Exon V6 r2 (60Mbp) target enrichment kit (Agilent Technologies, Santa Clara, CA, USA) with Illumina HiSeq instrument and SBSv4 chemistry
- Allignment
- Variant calling
- Variant annotation
- Average target region coverage was ~150X, 95% of all target regions were covered at least 20.X
- Variants with a maximum frequency of 0.01% in several normal population variant databases (1000G, ESP, ExAc0.2, and gnomAD) and deep intronic variants were filtered out
- Variants **in genes with a known expression in cardiac and skeletal muscle** were considered clinically relevant and confirmed by Sanger sequencing.

Kiselev, A HumanMutation. 2018;39:1161–1172.

Genetic analysis II

- Pathogenicity was assessed based on the MetaSVM predictions
- Related protein sequences from other organisms for 10th Ig-like domain of filamin-C were identified by BLASTP
- The paternity was proved using STR analysis (Loci D2S1360, D7S1517, D8S1132, D12S1064, SE-33)
- All identified genetic variants were classified according to American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al., 2015).



Kiselev, A HumanMutation. 2018;39:1161–1172.

Functional analysis

- PLASMID construct

  Wild-type (FLNCWT) and mutant (FLNCp.A1183L and FLNCp.A1186V) coding sequences of short FLNC isoform (NM_001127487.1) tagged C-terminally with EGFP (enhanced green fluorescent protein) were synthesized in vitro

- Zebrafish studies

  Overexpression of FLNCwt/p.A1183L/p.A1186V-EGFP in zebrafish was achieved by injecting the synthesized mRNAs at 2,000 ng/μl with phenol red at 0.1 μg/μl (concentration in injection solution) into 1-cell stage zebrafish embryos

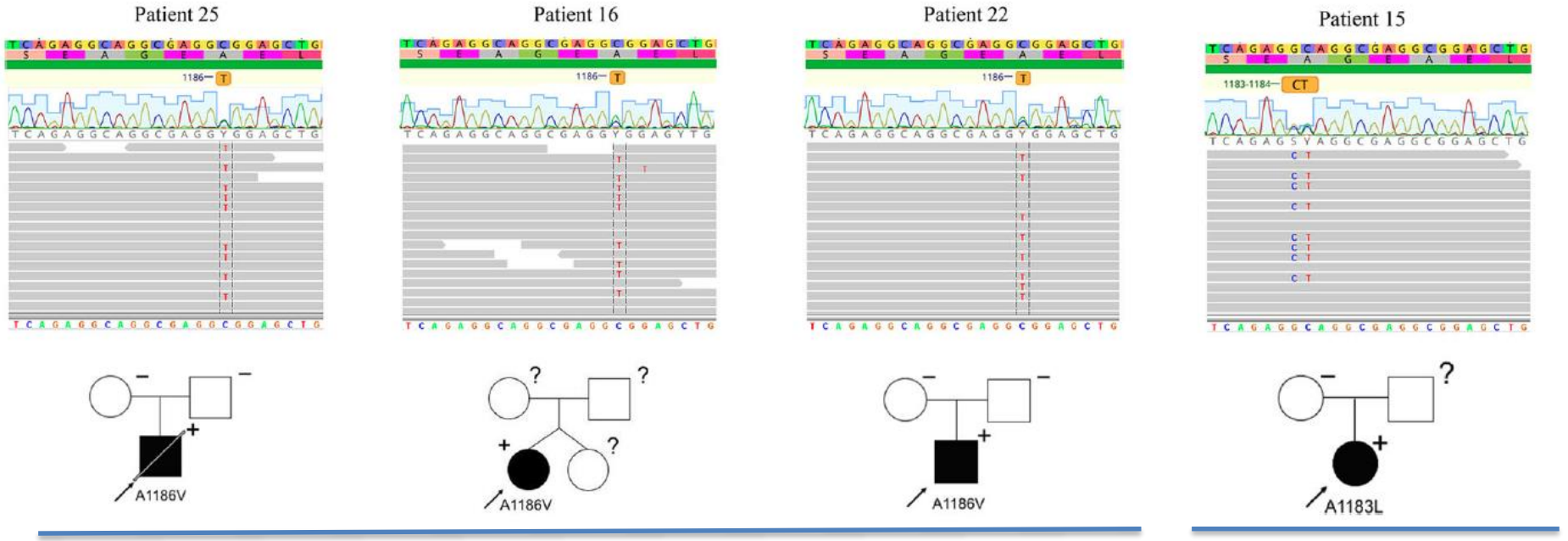
- Histology and confocal imaging

  Tissue samples from the heart and skeletal muscle, collected during the autopsy

  Zebrafish embryos at the desired developmental stage were collected

Kiselev, A HumanMutation. 2018;39:1161–1172.

FLNC mutations identified



c.3557C>T resulted in p.Ala1186Val

c.[3547G>C;3548C>T] resulted in p.Ala1183Leu

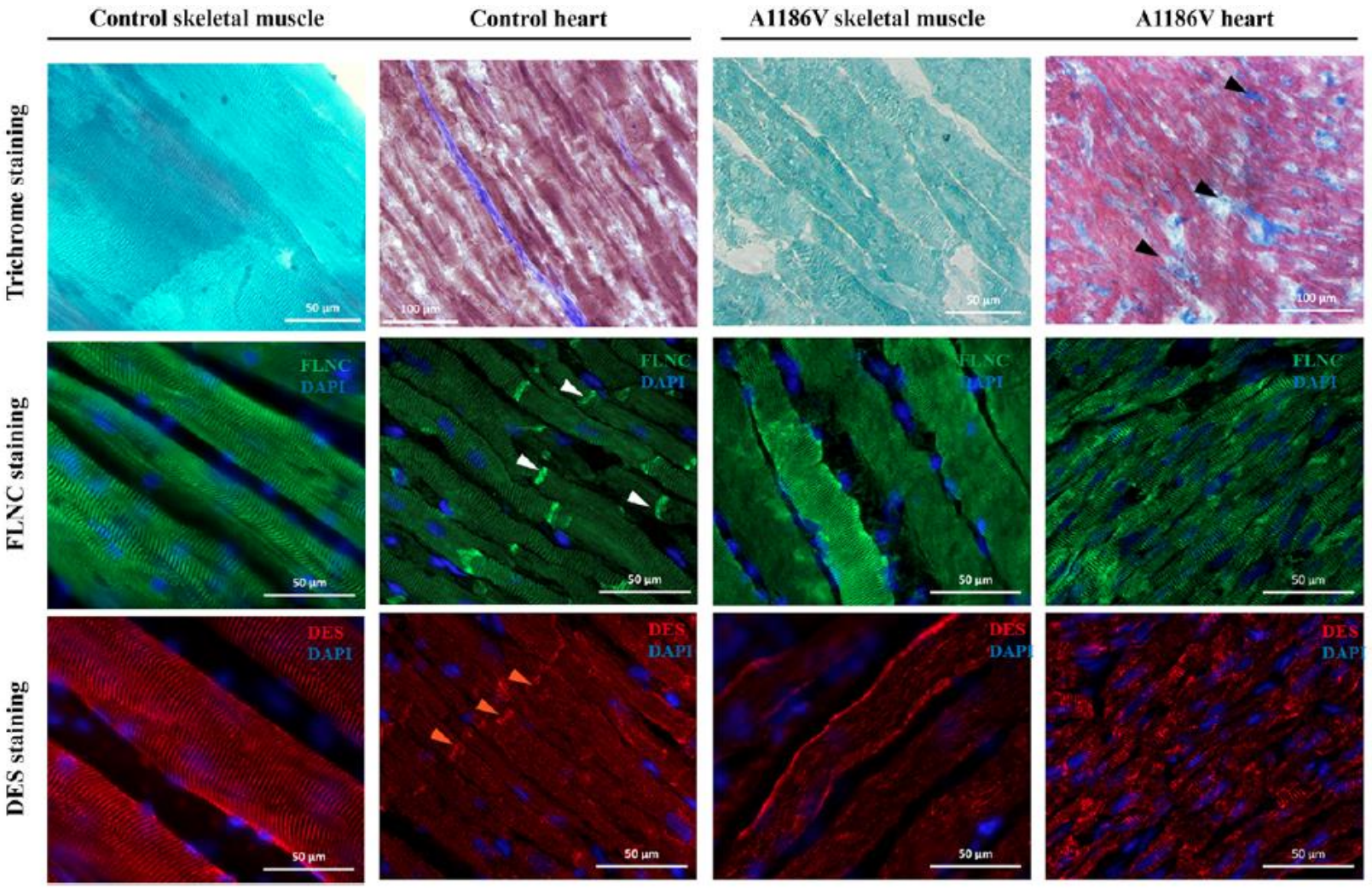
Kiselev, A HumanMutation. 2018;39:1161–1172.

Clinical Information

	Sex	Mutation	Mutation status	Age of RCM presentation	Age of NM presentation	Signs of NM involvement	CK and serum markers	EMG	ECG	Outcome
Patient 25	M	p.A1186V	De novo	1.4 year	At birth	Arthrogryposis at birth Limb-girdle muscle weakness Facial weakness No signs of distal muscle weakness Sparing Joint contractions Pyramid insufficiency Clonic seizures inability to obtain a sitting position independently	↑CK × 1.5-4 ↑LDH × 1.5 ↑CK-MB × 2 -4	Diffuse myopathic pattern Neuropathy	RBBB No SVT or VT	Death at 2. years of age
Patient 15	F	p.A1183L	n/a	6 months	At birth	Arthrogryposis at birth Limb-girdle muscle weakness Joint contractions Inability to get up from squatting No signs of distal muscle weakness Sparing inability to obtain a sitting position independently	Normal CK ↑LDH × 1.5 ↑CK-MB × 2	Diffuse myopathic pattern	RBBB No SVT or VT	Listed for 'Hx at 3 y.o.
Patient 16	F	p.A1186V	n/a	3 years	During first year	Limb-girdle muscle weakness Inability to get up from squatting Inability to obtain a sitting position independently Facial weakness	↑CK × 1.2 ↑LDH × 1.5 ↑CK-MB × 2	Diffuse myopathic pattern	RBBB No SVT or VT	Alive, 7 y.o.
Patient 22	F	p.A1186V	De novo	15 years of age	At birth	Arthrogryposis at birth Mild muscle weakness in limb-girdle muscles No signs of distal muscle weakness	CK normal CK-MBx 1.5	Normal	AVB I RAE, LAE No SVT or VT	Hx at 19 y.o

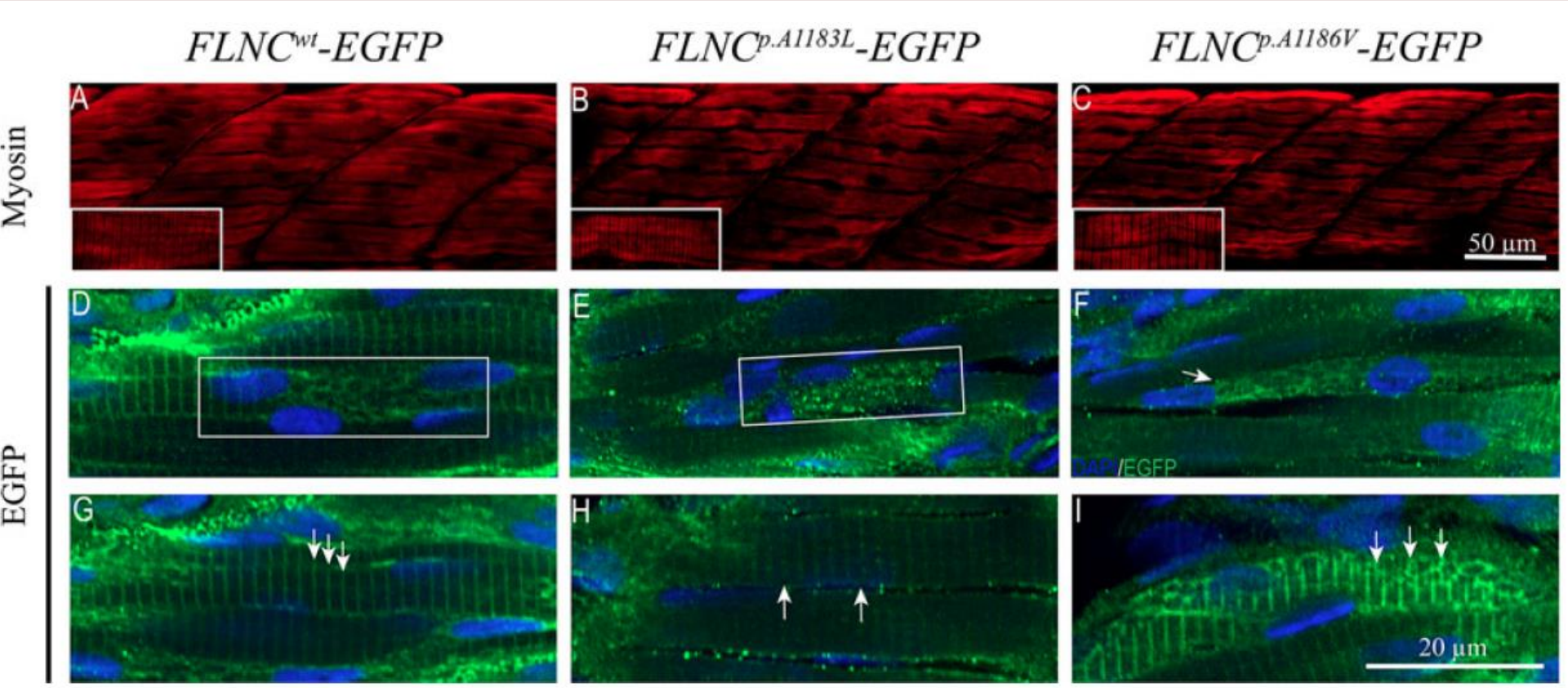
Kiselev, A HumanMutation. 2018;39:1161–1172.

Pathogenicity assessment I



Kiselev, A HumanMutation. 2018;39:1161–1172

Pathogenicity assessment II

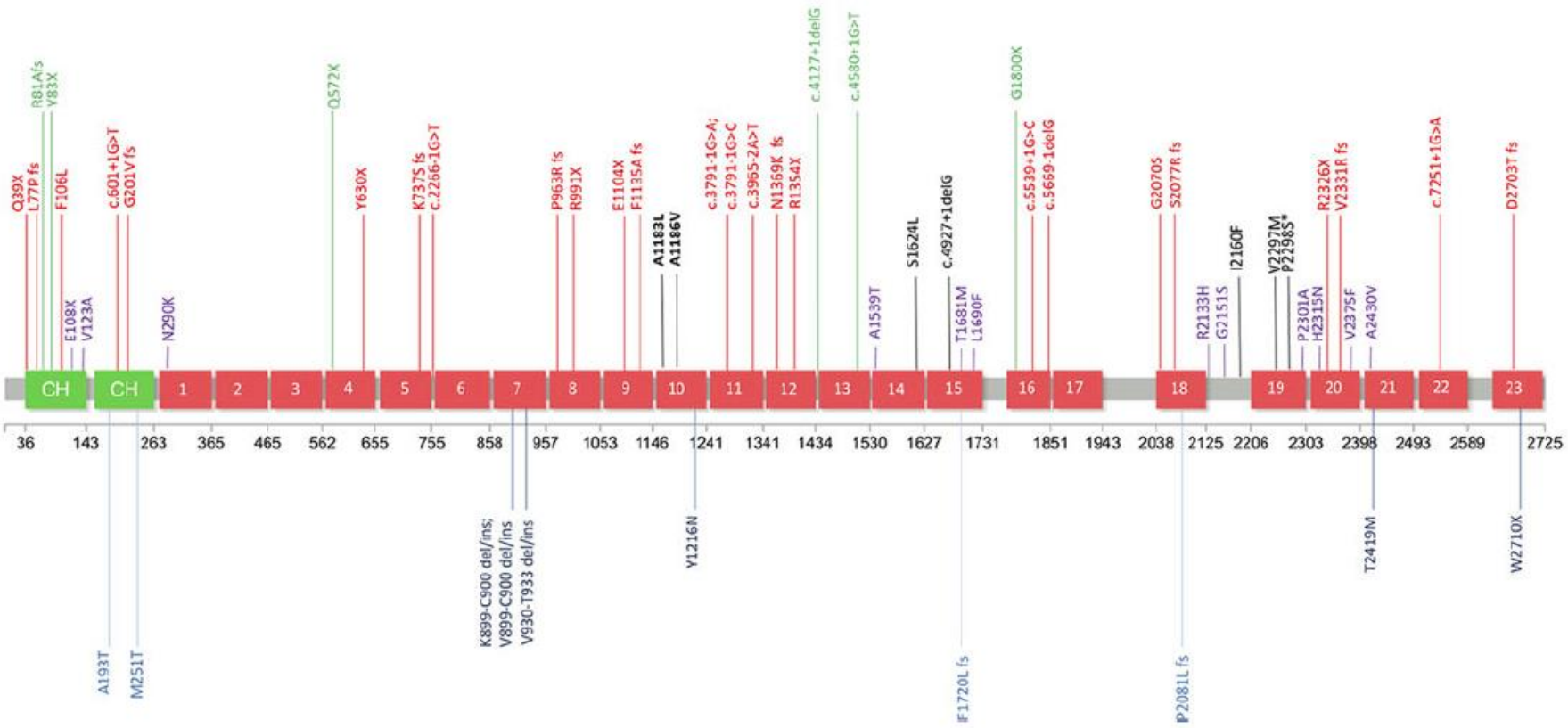


- Aggregates in the muscle of FLNCp.A1183L–EGFP, especially in the perinuclear region (E, box)
- Analysis of Z-discs in injected embryos revealed Z-discs streaming (H, arrows), or disruption of the striated pattern (I, arrows) in the presence of mutant FLNC

Kiselev, A HumanMutation. 2018;39:1161–1172

Localization of mutations in FLNC

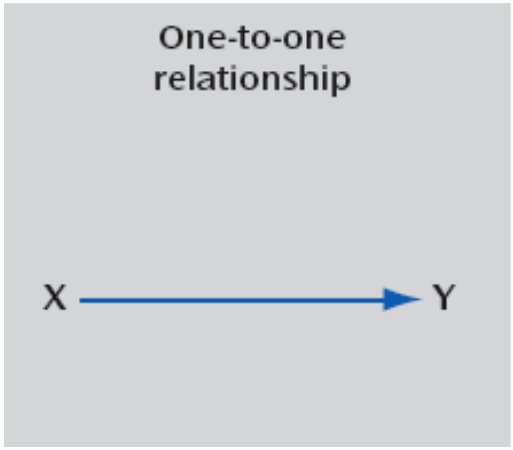
Hypertrophic CM Restrictive CM Dilated CM Arrhythmic CM

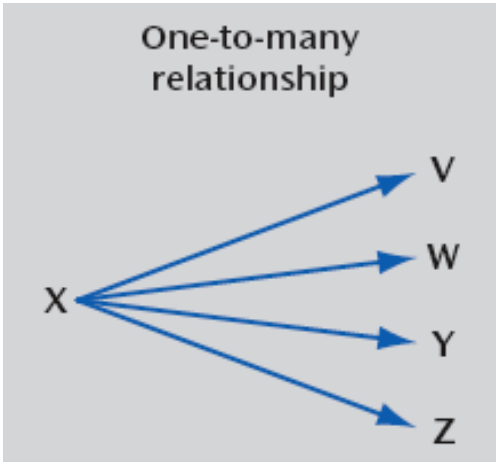


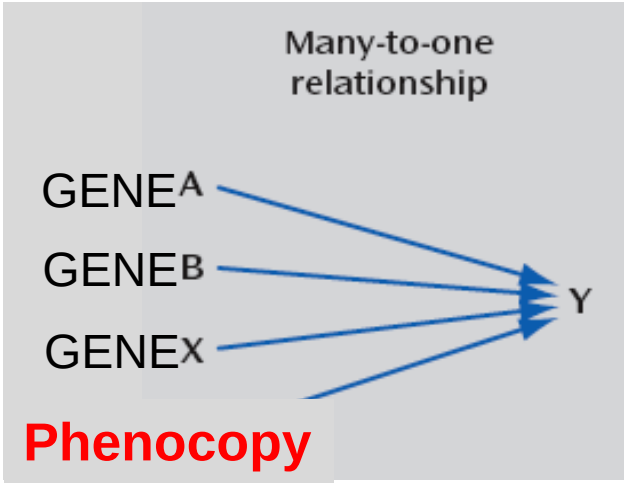
Distal myopathy Miofibrillar myopathy

Kiselev, A HumanMutation. 2018;39:1161–1172

Gene-Phenotype relationships







. . . when it comes to
reading the genome,
we are little better than
a two-year-old
trying to make sense of the
Encyclopedia Britannica

Grody, *Genetics in Medicine* **2019**

Thanks!