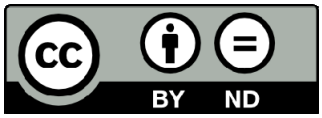
UNIVERSITAT DE BARCELONA

Identificación de predictores de arritmias cardíacas y muerte súbita en pacientes pediátricos afectos de enfermedades neuromusculares

Sergio César Diaz



Identificación de predictores de arritmias cardíacas y muerte súbita en pacientes pediátricos afectos de enfermedades neuromusculares

Memoria de tesis doctoral presentada por

Sergio César Díaz

para optar al grado de Doctor en Medicina

por la Universidad de Barcelona

*Trabajo realizado bajo la dirección y tutorización del **Dr Josep Brugada Terradellas** (Servicio de Cardiología, Hospital Clínic, Universidad de Barcelona) y bajo la dirección de **Dra. Georgia Sarquella Brugada** (Unidad de Arritmias pediátricas del Hospital Sant Joan de Déu, Universidad de Barcelona).*

Línea de investigación:

Fisiopatología de las Enfermedades Pediátricas

(Medicina e Investigación Traslacional).

Facultad de Medicina y Ciencias de la Salud. Universidad de Barcelona.

Septiembre, 2024

A veces se nos olvida
que lo más importante que nos llevaremos
es el tiempo que sonreímos y que somos felices,
rodeados de nuestra gente.
A veces se nos olvida la empatía,
ayudar más y mejor a los demás, hoy.
A veces se nos olvida que quizás mañana sea tarde,
que tenemos abrazar y besar, ahora.
A veces se nos olvida vivir en la propia vida,
pues esta no retorna
y el tiempo no se nos devuelve.

S Cesar, 2024

A mi familia y amigos

A mi Billy

Agradecimientos

Un día me hicieron la pregunta sobre qué fue lo que despertó mi interés en investigar las enfermedades neuromusculares. Bien, pues fue Carlota, una paciente. “La” paciente. Y estoy seguro de que, tanto el resto de los pacientes como Georgia y yo, le debemos a Carlota su espíritu crítico y luchador. Lo que empezó siendo un reto para todo el equipo de la unidad de arritmias ha acabado, tras varios años después y largas horas de esfuerzo y dedicación por parte de todos, en esta tesis doctoral que debíamos a los pacientes y, en especial, a Carlota. Pero Carlota no fue la única, tan sólo fue la primera. Pudimos ver la misma fortaleza en la mirada de Andrés y el mismo tesón en los ojos de Álvaro y de todos y cada uno de los pacientes. Y quizás pueda resumir en una tesis parte de lo que hemos podido investigar en los pacientes con enfermedades neuromusculares, pero no hay suficiente papel para escribir todo lo que ellos nos han enseñado: lecciones de vida.

A mis directores de tesis, el Dr. Josep Brugada y la Dra. Georgia Sarquella, gracias por las incalculables horas dedicadas a los pacientes y a la supervisión de todo el trabajo y por despertar mi interés en el apasionante mundo de las arritmias. Aún recuerdo el momento de pasar visita en un despacho improvisado al lado de la antigua sala de electrofisiología con Josep, de las preguntas sobre trazados de electrocardiograma que tan sólo él era capaz de ver pero que, con eterna paciencia, hacía que me pusiera las “gafas” de electrofisiólogo para comprender el trazado y me hacía ver que era más fácil de lo que parecía al principio (hasta el siguiente trazado, que volvía a pasar lo mismo). A Georgia, por confiar en mí ese día mientras comíamos un bocata en las escaleras antiguas del hospital, cuando todo parecía perdido y sin rumbo pusiste un timón y una vela allí donde se necesitaba. Y el diseño del barco, que era la unidad de arritmias, necesitaba de tu tenacidad para ser construido y tomar un buen rumbo. Gracias por recordarme que la medicina es algo más que protocolos: es pasión, es saber parar y escuchar, es sentir y abrazar, es sonreír y celebrar, es acompañar y ofrecer ayuda, es saber despedirse y decir adiós. La unidad de arritmias ha ido creciendo gracias a todo ese esfuerzo de tantos años, y tengo

que agradecer también a Oscar Campuzano, por estar disponible incondicionalmente como un padre clínico y ser un referente para mí, a José Cruzalegui, por tenderme siempre una mano y por su espíritu trabajador incansable, a Fredy Chipa por tu amabilidad atendiendo siempre todas mis dudas sobre los trazados, a Irene, por estar siempre hilando las emociones de todos, tanto las nuestras como la de los pacientes, a Estefi, por su sonrisa y por estar siempre dispuesta ayudar, a Andrea, por tu dinamismo, ganas de aprender y ayudar en todo lo que sea, a Nuria, por tus abrazos sinceros y tu ironía que tanto me gusta, y a Patricia, por tu templanza y cariño conmigo y con todos los compañeros.

Al Dr. Andrés Nascimento, Dr. Carlos Ortez, Dr. Daniel Natera, Dra. Jessica Expósito, Dra. Laura Carrera y a todo el equipo integrante de la UTIN, por confiarme a sus pacientes tan especiales y por poder ser miembro de este fantástico equipo. Gracias de corazón.

A la Fundación Andrés Marcio, gracias por el infinito soporte y la confianza en nuestro equipo. Recuerdo, hace ya algunos años, la primera reunión con Georgia para poder ayudar a los niños con laminopatía. Esto es lo que ocurre cuando la pasión por algo y las ganas de ayudar se juntan: nace un proyecto maravilloso por y para los pacientes.

A la Fundación Daniel Bravo, gracias por confiar en nuestro equipo y darme la oportunidad de ayudar a estos y a otros pacientes. Personas como el señor Daniel Bravo son las que mejoran el mundo y sirven de fuente de inspiración para llegar a ser mejores personas y profesionales.

A Victori Fiol, gracias por tantas cosas… Llegaste como gestora de pacientes a la unidad de arritmias, pero lo que no sabía es que eras tan buena persona y creo que a los cinco minutos ya fuiste amiga. Siempre me has tendido una mano y has tenido un abrazo para mí, tanto dentro como fuera del hospital. Gracias por querer bien y tanto a Billy. Algunas veces tengo la fantasía de que vuelves otra vez, otras veces que me voy yo a Mallorca contigo. Tal vez, por el momento, tengamos que disfrutar el uno del otro reservando esos días tan esperados y

disfrutando en el jardín que cuida tu madre, comiendo la deliciosa paella de tu padre o las cocas de trampó de tu madre, pasando momentos inolvidables con tus gatas Blanca y Dolça, tomando un helado en el San Remo con Pedro y enseñándome tu isla poco a poco, sin prisas, sin fronteras. Esta tesis también es tuya, por supuesto.

Al Dr. Joan Sánchez por su confianza en todo lo que hago, Dr. Juan Carretero, por haberme enseñado y haber tenido tanta paciencia conmigo, al Dr. Fredy Prada, por su templanza y consejos, al Dr. Quim Bartrons, por sus enseñanzas y clases magistrales, a la Dra. Carin Walter, por tu paciencia y confianza conmigo dentro del hospital y también fuera en alguno de nuestros viajes más inesperados, al Dr. Alex Pérez, por decir siempre un sí cuando se te necesita, a la Dra. Esther Aurensanz, por tu dinamismo inigualable, a María José, a Raquel, a Natalia, a Fabi, a Diana, a Isaac, a Maite, a Silvia, a Eva, a Andrea… y a todo el resto de compañeros cardiología pediátrica que han estado siempre a mi lado y me han enseñado tantísimo.

A Devyani Chowdhury, a su equipo del CCC, a Christine Pascua y a todos los profesionales de la Clinic for Special Children en Pensilvania, que me hicieron sentir un poco más como en casa mientras aprendía sobre el fascinante mundo del análisis ecocardiográfico avanzado de los corazones de la comunidad *Amish.*

A María Clara, por tu sonrisa, abrazos y por saber estar siempre para escucharme. Sabes que si te marchas cinco minutos de tu silla ya te echo de menos. De verdad, gracias por estar ahí siempre.

A Eli, porque siempre estás ahí para mí y estar contigo un ratito en el 331, a pesar de las interrupciones, hace que el trabajo sea menos trabajo. No soporto la idea que digas la palabra jubilación y haré lo posible para engatusarte y te quedes un poquito más con nosotros. Esta tesis también es gracias a ti.

A las catalanizadas argentinas Lucía y Paula, porque sé que siempre estáis ahí para escucharme. Gracias por los paseos y las quedadas (también las

internacionales con Stef y resto de amigos), momentos que siempre guardaré en mi memoria más allá de los recuerdos de la actividad frenética del hospital.

A mis amigos Raúl, Nathalia y Noelia. La vida es lo que pasa mientas escribes el doctorado, pero con quién se pasa la vida y saber elegir a cada quién es trascendental. Gracias por estar ahí siempre, sin condiciones, por hacer un hueco cada vez que necesitaba, por los achuchones, por los paseos con los *perrijos* y por, sin duda, escucharme sin juicios y apoyarme sin condiciones. Esta tesis también es gracias a vosotros.

A mi amigo Gerard, por compartir conmigo parte de esta vida haciendo que sea más amena sea haciendo una paella *ebrenca*, una ruta *conejil*, ir a un concierto *indie*, o teniendo charlas interminables sobre tu tesis, la mía y sobre todos los temas opinables. Gracias por recibirme en tu mundo y por invitarme a desconectar en los eternos arrozales sea cual sea la estación del año. Y a su compañera Laura, por ofrecerme su ayuda y arrojar su luz en momentos en los que lo necesitaba. Esta tesis es, también, gracias a vosotros.

A mis abuelos, gracias a los cuatro por enseñarme tantas cosas durante todos aquellos veranos en el pueblo. Seguro que, desde ese limonero y ese jazmín, os debéis sentir orgullosos de mí, no me cabe la menor duda.

A mi Billy, por haberme acompañado durante todos estos años. Eras mejor persona que muchas personas y sólo sabías dar amor y cariño, sin rencor ni condiciones. Gracias por acompañarme en esta aventura y haber sido parte de mi vida.

A mi hermano Miguel, porque no hay carretera en el mundo que se le resista para llegar a mí cuando más lo necesito. Por darme soporte vital y saber decir las palabras que necesito oír en cada momento y darme otras perspectivas cuando tan sólo una se apodera de la mente. Esta tesis también es gracias a ti.

A mis padres, estoy aquí y soy así gracias, también, a vosotros. Por apoyarme incondicionalmente todos estos años y por confiar en mi juicio. Esta tesis es también por y para vosotros.

A todos los que he nombrado y a los que no he podido,

gracias.

Financiación

Los artículos que se presentan en esta Tesis han recibido fondo de:

- La Caixa Foundation LCF/PR/GN16/50290001 y LCR/PR/GN19/50320002.
- Instituto de Salud Carlos III, Fondo de Investigación Sanitaria-FIS-(PI21/00094)
- Fundación Andrés Marcio – Niños contra la Laminopatía

Las entidades de financiación no han tenido ningún papel en el diseño del estudio ni en la recogida y análisis de los datos, ni en las decisiones sobre su publicación.

Los resultados de esta tesis han sido parcialmente presentados como comunicaciones orales en las siguientes reuniones científicas:

- Comunicación oral en el 4th International Meeting on Laminopathies:
    - Different disease progression velocity in two female monozygotic twins diagnoses with LMNA-related congenital muscular dystrophy. Congreso: 4th International Meeting on Laminopathies. 9-12 May 2023.

Índice

1. Abreviaturas y acrónimos

AAS: Ácido Acetil Salicílico

AV: aurículo-ventricular

AVC: Accidente Vascular Cerebral

ARA: antagonistas del receptor de la angiotensina

cDM1: Distrofia Miotónica tipo 1 congénita

CPK: creatinin-kinasa

CV: cardiovascular

DAI: Desfibrilador automático implantable

DM1: Distrofia miotónica tipo 1

DMD: Distrofia Muscular de Duchenne

DMB: Distrofia Muscular de Becker

DMB/D: fenotipo intermedio Becker-Duchenne

ECG: electrocardiograma

EDMD: Distrofia muscular de Emery-Dreifuss

FA: Fibrilación auricular

FEVI: fracción de eyección del ventrículo izquierdo

GLS: *strain* longitudinal global

IC: Insuficiencia cardíaca

iDM1: Distrofia Miotónica tipo 1 infantil

IECA: inhibidores del enzima convertidor de la angiotensina

jDM1: Distrofia Miotónica tipo 1 juvenil

L-CMD: Distrofia muscular congénita relacionada a *LMNA*

LGMD1B: Distrofia muscular de cinturas tipo 1B

LVEF: *left ventricular ejection fraction*

MCD: miocardiopatía dilatada

NT-proBNP: fracción N-terminal del pro-péptido natriurético cerebral

mRNA: RNA mensajero

Pre-mRNA: mRNA precursor

RMc: Resonancia Magnética

RNA: ácido ribonucleico

TA: Taquicardia auricular

TV: Taquicardia ventricular

2. Artículos de la tesis

Tesis en formato de compendio de publicaciones. La tesis consta de los siguientes artículos:

- **Cesar S**, Campuzano O, Cruzalegui J, Fiol V, Moll I, Martínez-Barrios E, Zschaeck I, Natera-de Benito D, Ortez C, Carrera L, Expósito J, Berrueco R, Bautista-Rodríguez C, Dabaj I, Gómez García-de-la-Banda M, Quijano-Roy S, Brugada J, Nascimento A and Sarquella-Brugada G. Characterization of cardiac involvement in children with *LMNA*-related muscular dystrophy. Frontiers in Cell and Developmental Biology. 2023. 11:1142937.
    - Factor de impacto: 6.081
    - Cuartil: 1

- **Cesar S**, Coll M, Fiol V, Fernandez-Falgueras A, Cruzalegui J, Iglesias A, Moll I, Perez-Serra A, Martínez-Barrios E, Ferrer-Costa C, Olmo Bd, Puigmulè M, Alcalde M, Lopez L, Pico F, Berrueco R, Brugada J, Zschaeck I, Natera-de Benito D, Carrera-García L, Exposito-Escudero J, Ortez C, Nascimento A, Brugada R, Sarquella-Brugada G and Campuzano O. *LMNA*-related muscular dystrophy: identification of variants in alternative genes and personalized clinical translation. Frontiers in Genetics. 2023 14:1135438.
    - Factor de Impacto: 4.772
    - Cuartil: 1

3. Resumen de la tesis

Título: Identificación de predictores arritmias y muerte súbita en pacientes pediátricos afectos de enfermedades neuromusculares.

Introducción: Las enfermedades neuromusculares son enfermedades raras de base genética y que pueden manifestarse desde edades muy tempranas y pudiendo tener manifestación cardíaca. Esta afectación cardíaca, cada vez más reconocida a medida que han ido mejorando las terapias de los otros órganos afectos y por la rehabilitación, ocurre por un proceso degenerativo que pone a estos pacientes a riesgo de padecer arritmias, defectos de conducción, disfunción ventricular y muerte súbita. La afectación cardíaca en enfermedades neuromusculares ha sido, clásicamente, poco estudiada. Bajo un protocolo específico de seguimiento y manejo de estas enfermedades raras podrían detectarse signos precoces de miocardiopatía y arritmias.

La afectación cardíaca en enfermedades neuromusculares es muy heterogénea y varía en función del tipo de enfermedad: desde ausente o leves manifestaciones cardíacas hasta formas severas de miocardiopatía con insuficiencia cardíaca y arritmias malignas que pueden llevar a la muerte súbita, incluso como primera manifestación de la afectación cardíaca.

Por esta razón, los pacientes pediátricos con enfermedades neuromusculares deben ser estudiados exhaustivamente a nivel cardiológico para detectar la afectación cardíaca de forma precoz y poder indicar terapias específicas preventivas de fallo cardíaco, arritmias y/o de muerte súbita.

Hipótesis: el estudio con ecocardiografía funcional y de deformación miocárdica y la monitorización electrocardiográfica de larga duración en pacientes con enfermedades neuromusculares pueden cambiar el pronóstico cardiovascular y el riesgo de muerte súbita en pacientes pediátricos.

Objetivo: identificar predictores de arritmias cardíacas y muerte súbita en pacientes pediátricos afectos de distrofia muscular y distrofinopatías, como grupos de riesgo de las enfermedades neuromusculares.

Métodos: se ha realizado un estudio prospectivo durante 5 años de pacientes con enfermedades neuromusculares (distrofinopatías, distrofia miotónica tipo 1 y laminopatías) en el Hospital Sant Joan de Déu para analizar predictores de arritmias y muerte súbita mediante el análisis de función cardíaca (mediante ecocardiografía funcional y segmentaria) y monitorización cardíaca (análisis de arritmias bajo monitorización de larga duración). Los métodos propuestos no están bajo guías clínicas pediátricas y se cree en su utilidad como herramientas fundamentales de diagnóstico precoz de afectación cardíaca en este grupo de pacientes.

Resultados principales: de la población con enfermedades neuromusculares que ha sido estudiada prospectivamente, se han detectado arritmias malignas en pacientes afectos de distrofia muscular relacionada con el gen *LMNA* en la monitorización electrocardiográfica con Holter subcutáneo, pudiendo detectar arritmias malignas en 5 pacientes en los que se ha tenido que implantar un desfibrilador para la prevención de la muerte súbita, y un marcapasos en un paciente, debido a asistolia como forma de debut cardíaco de la enfermedad. Durante el seguimiento, después de la publicación de los artículos, se han detectado también en el registro de holter subcutáneo arritmias (auriculares y ventriculares) refractarias al tratamiento farmacológico y electrofisiológico en dos pacientes gemelas, con aparición de insuficiencia cardíaca por miocardiopatía rápidamente progresiva conduciendo a la muerte de ambas pacientes.

En cuanto al grupo de pacientes con diagnóstico de Distrofia miotónica de Steinert, tras el análisis las características clínicas, ecocardiográficas y electrocardiográficas de este grupo de pacientes no ha habido diagnóstico de arritmias potencialmente malignas ni miocardiopatía en edad pediátrica, aunque se ha encontrado información cardiovascular valiosa que puede ser el sustrato para arritmias malignas y miocardiopatía en la etapa adulta.

Del grupo de pacientes con distrofinopatías (Distrofia muscular de Duchenne y Becker), a pesar de encontrar datos de miocardiopatía dilatada y/o fibrótica en edades precoces, tras el análisis prospectivo clínico, de las pruebas de imagen y de las pruebas electrofisiológicas no invasivas, no se han obtenido predictores dada la falta de arritmias potencialmente malignas ni muerte súbita. A pesar de esto, hay datos valiosos sobre la expresividad variable del fenotipo y del

deterioro precoz del *strain* miocárdico en algunos pacientes a pesar de mantener valores normales de función cardiaca, que invitan a seguir investigando predictores de severidad fenotípica.

Conclusiones:

En las enfermedades neuromusculares, a pesar de existir insuficiencia cardiaca y arritmias, es frecuente que no haya ningún síntoma cardiovascular o que éstos sean atípicos, dificultando el manejo clínico. Además, la expresión del fenotipo cardíaco y su grado de severidad son variables, incluso con una misma mutación patogénica, sugiriendo que existen modificadores genéticos que podrían representar potenciales indicadores de letalidad.

Los accidentes cerebrovasculares estuvieron presentes en las laminopatías de debut neuromuscular precoz antes de los 2 años de edad por lo que podría considerarse tratamiento anticoagulante en caso de coexistir arritmias auriculares y disfunción cardíaca.

En las laminopatías neuromusculares es frecuente encontrar, precozmente, antes de los 10 años de edad, insuficiencia cardíaca por miocardiopatía dilatada y arritmias potencialmente malignas relacionadas con la muerte súbita. A pesar de que el estudio electrofisiológico no mostró predictores de arritmias potencialmente malignas, la monitorización electrocardiográfica con dispositivos implantables (holter subcutáneo) sí fue útil, ayudó a identificar arritmias potencialmente letales y a tomar decisiones clínicas para prevenir la muerte súbita, especialmente las que debutan con clínica neuromuscular de forma precoz antes de los 2 años de edad.

En las laminopatías neuromusculares y en la distrofia muscular de Duchenne, el análisis ecocardiográfico básico y avanzado con *strain* miocárdico es útil para identificar alteraciones de la deformación cardíaca de forma más precoz que valores clásicos como la fracción de eyección. En el caso de las laminopatías, el *strain* miocárdico del ventrículo izquierdo se relaciona a un peor pronóstico cardíaco.

En la distrofia miotónica tipo 1 (enfermedad de Steinert), las arritmias potencialmente malignas y la muerte súbita son muy poco frecuentes durante la edad pediátrica, aunque existen hallazgos electrocardiográficos precoces que

pueden ser motivo de investigación en la etapa adulta para poder comprobar si son indicadores precoces de un peor pronóstico cardiovascular.

En la distrofia muscular de Duchenne, las arritmias potencialmente malignas y la muerte súbita son muy poco frecuentes durante la edad pediátrica, aunque la miocardiopatía dilatada y la insuficiencia cardíaca son progresivas durante la adolescencia y la función cardíaca se deprime significativamente.

4. Introducción

4.1. Generalidades

Las enfermedades neuromusculares son enfermedades raras de origen genético que se manifiestan clínicamente al nacimiento o en la infancia precoz. Se caracterizan por diferentes grados de hipotonía congénita, retraso del desarrollo motor, debilidad muscular progresiva, y distrofia en la biopsia muscular. Estas enfermedades neuromusculares son genética y fenotípicamente heterogéneas, y coinciden en no tener, por el momento y de forma general, un tratamiento curativo.(1)

Las enfermedades neuromusculares también pueden afectar al miocardio, que ocurre como un proceso degenerativo con fibrosis y reemplazamiento con tejido graso en el miocardio. El papel del cardiólogo es importante por el riesgo que existe de arritmias auriculares, enfermedad del tejido de conducción, bradicardia, arritmias ventriculares, y muerte súbita.

En población pediátrica, tienen un papel relevante para el cardiólogo pediátrico las distrofinopatías (distrofias musculares de Duchenne y Becker), la distrofia miotónica y las laminopatías (distrofia muscular de cinturas, distrofia muscular congénita, distrofia muscular de Emery-Dreifuss), que se caracterizan por distrofia del músculo esquelético y, en ocasiones, del músculo cardíaco, por lo que pueden manifestar grados variables de severidad de miocardiopatía dilatada, degeneración del tejido de conducción y eventos arrítmicos (2–7).

El análisis cardíaco en estos pacientes es importante por diversas razones. Primero, el claro impacto en el cuidado de estos pacientes y la prevención de la muerte súbita, detectando, previniendo y ayudando a tratar la insuficiencia cardiaca y las arritmias potencialmente letales. Hay fármacos y terapia antiarrítmica que pueden reducir la morbilidad en pacientes con disfunción ventricular izquierda asintomática.

Muchos de estos pacientes tienen una disfunción ventricular y, por ende, una insuficiencia cardíaca crónica. Hasta la fecha hay muy pocos estudios ecocardiográficos en pacientes pediátricos con enfermedad muscular esquelética, con poca población de estudio y con marcadores ecocardiográficos y electrofisiológicos aún por definir.(8)

Tal y como se ha descrito, algunos de estos pacientes pueden manifestarse como muerte súbita antes de que las alteraciones en la ecocardiografía básica puedan ser detectadas, por lo que su estudio es necesario desde edades precoces. A pesar de esto, la afectación cardíaca en pacientes con afectación del músculo esquelético ha sido escasamente estudiada durante la vida pediátrica.(6,9–13)

4.2. Fenotipos neuromusculares con expresión cardíaca

De las enfermedades neuromusculares con más expresión cardíaca se han descrito las laminopatías neuromusculares, las distrofinopatías (Distrofia muscular de Duchenne y Becker), y las distrofias miotónicas. A continuación, se describen y analizan por separado.

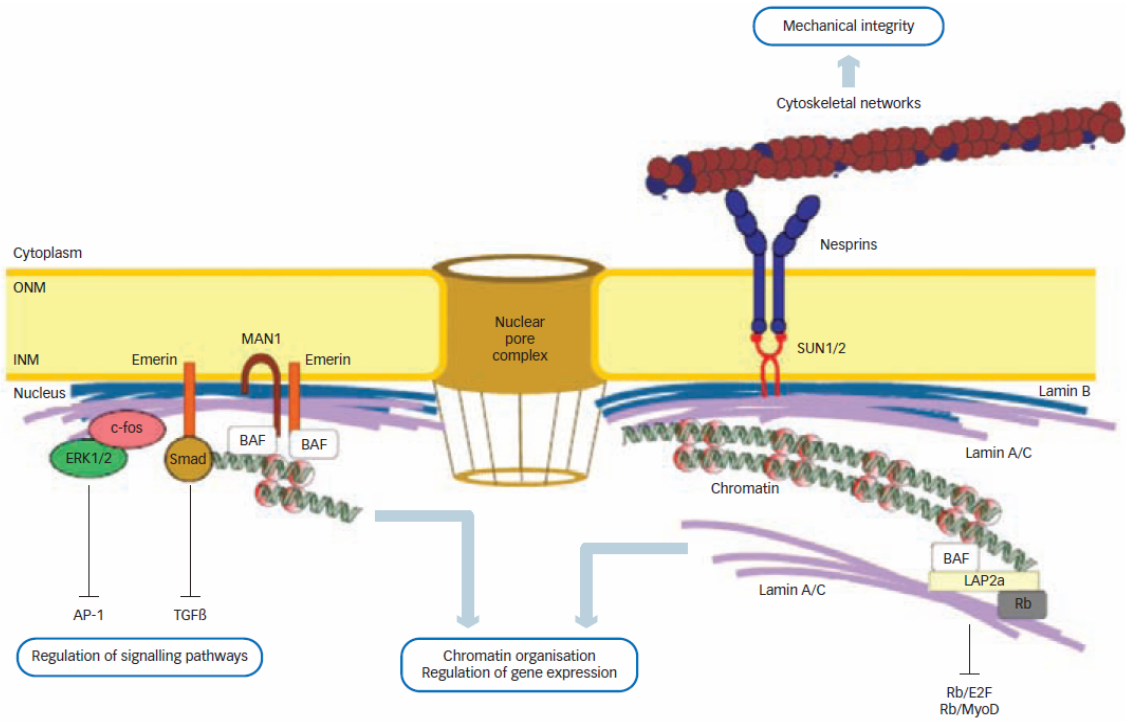
4.2.1. Laminopatías neuromusculares

Las distrofias musculares por mutación en el gen LMNA (Laminopatías) son una entidad rara (0.5/100.000) y que se caracteriza por debilidad muscular axial, escapulo-peroneal, contracturas y escoliosis. Suele encontrarse un patrón de distrofia en la biopsia muscular y una elevación de la creatinin-kinasa (CPK). (14) La expresión puede ser muy precoz, incluyendo la época fetal caracterizado por una disminución de movimientos fetales, que se traducen en la época del lactante con datos de retraso psicomotor y debilidad muscular. En ocasiones, esta expresión se focaliza en la pérdida de sostén cefálico (*Dropped-head Syndrome*), e incluso pueden perder, de forma progresiva, la habilidad de sentarse o caminar. Además, debido a esta debilidad muscular existe un riesgo incrementado de insuficiencia respiratoria, deformidades espinales y articulares, y afectación cardíaca.(14,15)

Base genética

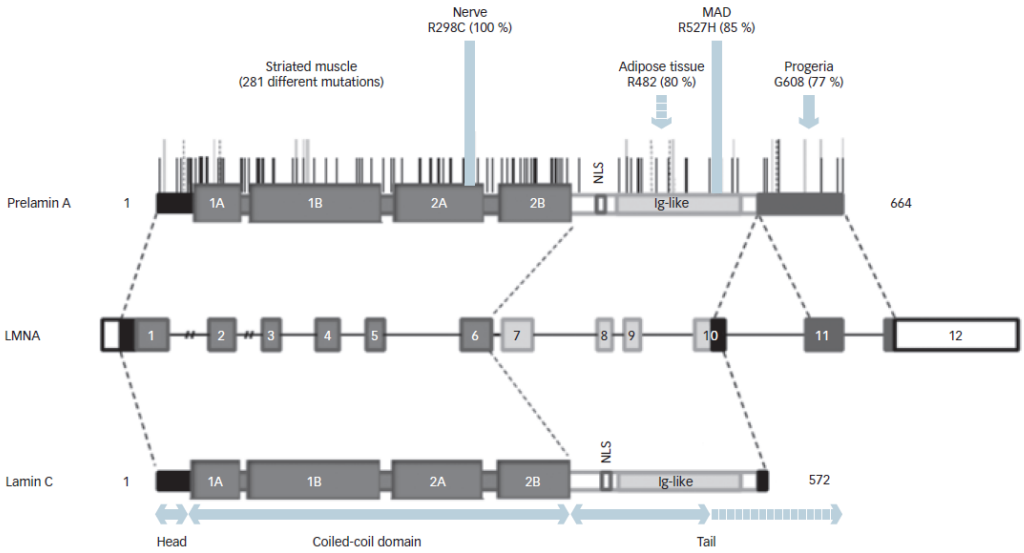
El gen LMNA, localizado en el cromosoma 1 en q21.1-21.3, de 12 exones, codifica las proteínas lámina A/C, que son filamentos intermedios importantes para el desarrollo y diferenciación celular (16), además de facilitar la señal entre el citoesqueleto y núcleo (17,18) y proporcionando estabilidad y modulando la organización de la cromatina y la expresión

génica (19–24). El esquema de la localización de la proteína en la célula, el esquema del gen y de la proteína se describen en las Figuras 1 y 2.



Lamins A/C interact with several transcription factors (c-fos, Rb, etc.) and regulate several important signalling pathways such as differentiating pathways Rb/E2F or Rb/MyoD and proliferating pathways AP-1 and TGFβ for example. Via their interactions with SUN1/2 and Nesprins, A-type lamins form nucleocytoskeletal networks with actin microfilaments, microtubules and cytoplasmic intermediate filaments, providing mechanical resistance to the cell. AP-1 = activator protein 1; BAF = barrier to auto-integrative factor; INM = inner nuclear membrane; LAP = lamin associated protein; ONM = outer nuclear membrane; Rb = retinoblastoma protein; TGFβ = transforming growth factor β.

Figura 1: esquema de la interacción de la lámina A/C con las proteínas de la membrana interna nuclear y proteínas nucleoplásmicas y sus funciones. Figura publicada por Charron et al. (146)



Schematic representation of LMNA gene and the two main isoforms: prelamin A and lamin C. The 281 LMNA mutations associated with striated muscle are depicted by black lines and are located along the molecules. LMNA mutations leading specifically to adipose tissue defects are depicted by dotted lines and are essentially located in the N- and C-terminal domains, with a hotspot in the Ig-like domain at Arg482 (80 % of the patients). Mutations associated with premature aging syndromes (progeria) are depicted in light grey. They are also essentially located in the N- and C-terminal domains, with a hotspot at position 608 (77 % of HGPS patients) and at position 527 (85 % of MAD, or mandibuloacral dysplasia, patients). The position of the unique LMNA mutation, p.R298C, leading to axonal neuropathy, is also indicated.

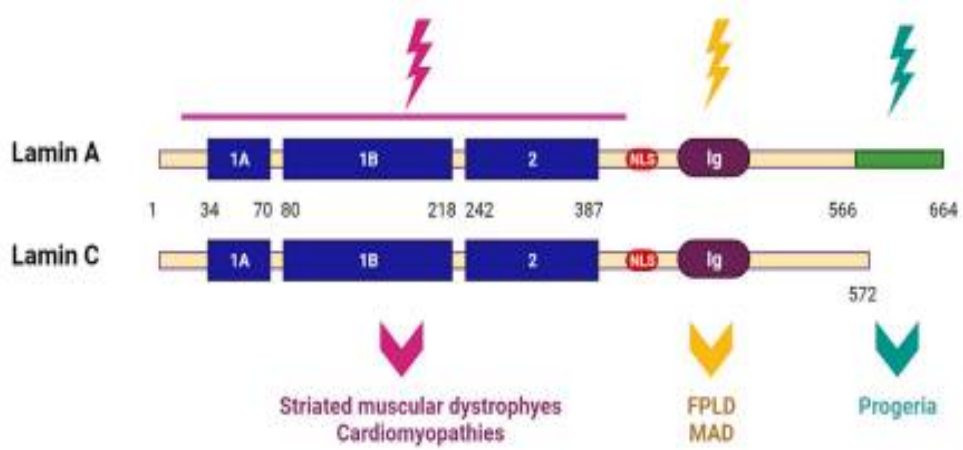


Figura 2: figura superior, distribución de las mutaciones en el gen LMNA, publicado por Charron et al (146) y figura inferior, esquema de la lámina A y C publicado por Malashicheva et al (100). FPLD: Lipodistrofia familiar parcial. MAD: Displasia mandíbulo-acral.

Las mutaciones más frecuentemente identificadas en la literatura son no truncantes e, hipotéticamente, la combinación de diferentes grados de haploinsuficiencia los efectos de un mecanismo dominante negativo contribuyen a la severidad de la enfermedad dado que permitiría una producción de síntesis de proteína completa o casi completa, pero disfuncionante con más expresión en músculo estriado que las mutaciones truncantes.(25–28)

Existen algunas hipótesis para justificar los mecanismos subyacentes responsables de la fisiopatología de las laminopatías con expresión cardíaca. Entre estas hipótesis se hallan: defectos de la estructura nuclear, alteración estructural de la arquitectura del tejido contráctil y alteraciones de la transcripción de los factores de regulación y de la unión a la cromatina (ver figura 3). (29)

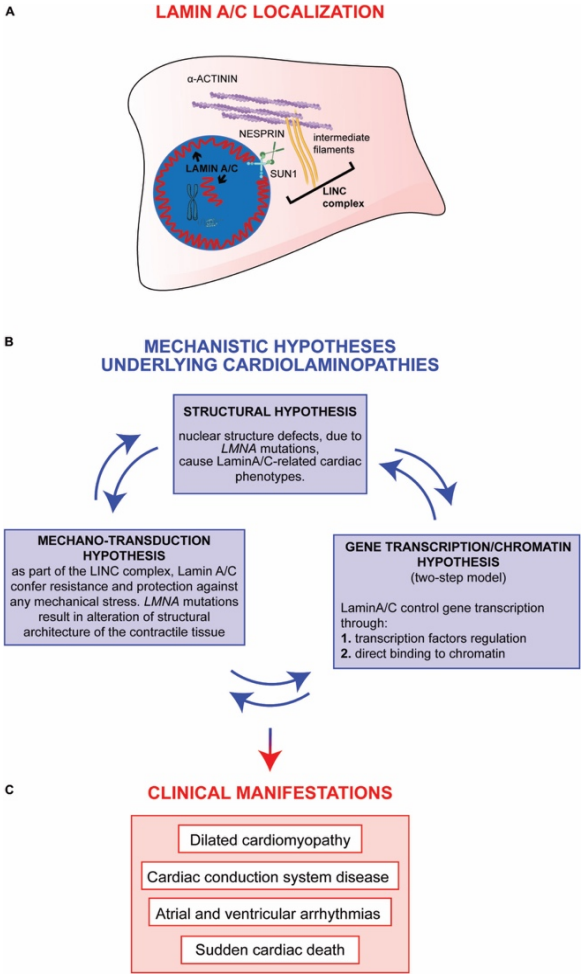


Figura 3. Figura publicada por Crasto et al. (29) Representación gráfica de la localización de la proteína lámina A/C y de las hipótesis de los mecanismos que pueden existir detrás de la afectación cardíaca.

Específicamente, en la miocardiopatía por alteración en LMNA, existe una desestabilización de la red de microtúbulos. El transporte de proteínas por los microtúbulos establece, mantiene y remodela componentes celulares importantes en los miocardiocitos incluyendo el túbulo transverso, retículo sarcoplasmático y discos intercalados. Es en estos discos intercalados donde existen regiones para la unión a cardiomiocitos adyacentes y son más ricos es desmosomas, entre otros. Los microtúbulos están unidos al núcleo mediante una conexión directa con la lamina nuclear mediante el complejo LINC. La inestabilidad de los microtúbulos en la miocardiopatía por LMNA causada por la expresión disminuida de la alfa-tubulina acetilada causa un remodelado de Cx43 y altera la comunicación entre

miocardiocitos, responsable finalmente de las alteraciones cardíacas. Este mecanismo de alteración citoesquelética se representa en la figura 4.

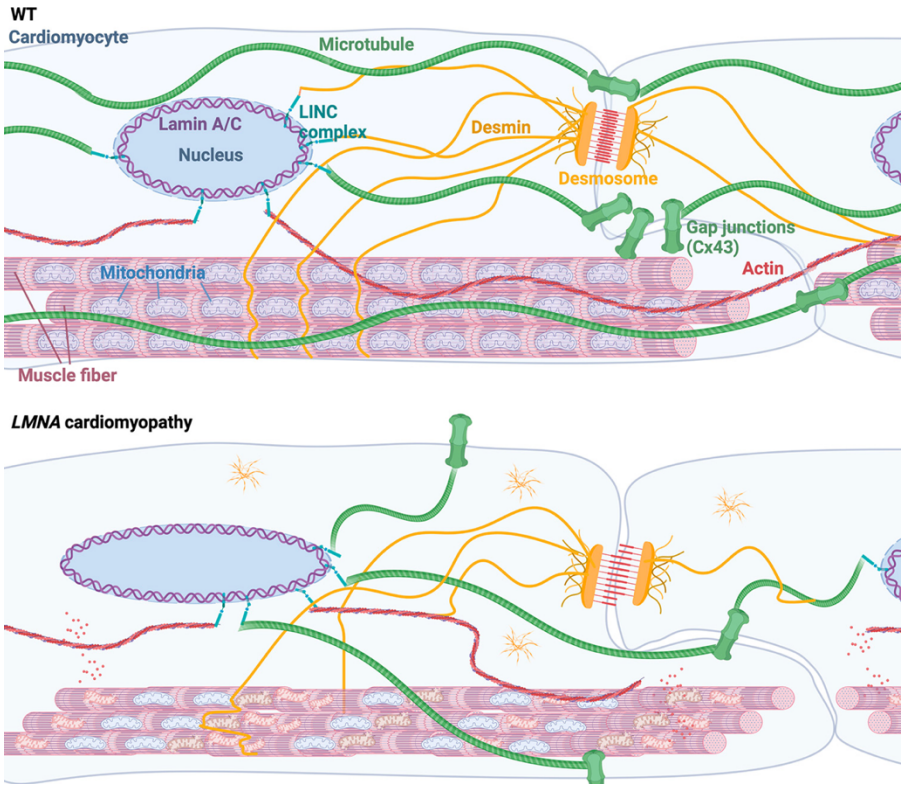


Figura 4, de la publicación original de Chatzifrangkeskou et al. (30) En ella se representa la figura esquemática de la alteración citoesquelética en la miocardiopatía por LMNA.

Además de esta alteración, existen otras anomalías a nivel de la organización del filamento intermedio (entre otros, la desmina) que provocaría, entre otros, una disrupción de la desmina al núcleo, que resulta en alteraciones en la interacción entre lamina y cromatina y en, finalmente, una disfunción del miocardiocito ventricular. (30)

Fenotipos

Existe una expresión fenotípica muy diversa descrita en la literatura, incluyendo la neuromuscular, la cardíaca con trastorno de la conducción y trastornos metabólicos. Los fenotipos se resumen en la figura 5.

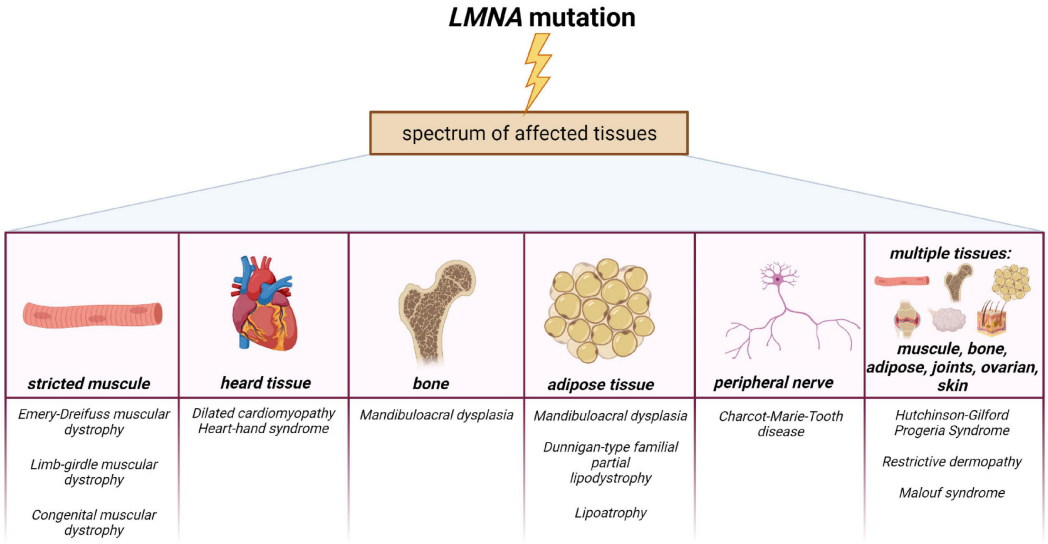


Figura 5. Espectro fenotípico de las laminopatías y los diferentes tejidos que pueden afectarse, figura extraída del artículo de Malashicheva et al (100).

Dentro de las laminopatías neuromusculares por mutación en el gen *LMNA*, se describen la **distrofia muscular congénita o *LMNA*-related congenital muscular dystrophy** (L-CMD, OMIM 613205), la **distrofia muscular de Emery-Dreifuss autosómica dominante o Emery-Dreifuss muscular dystrophy** (EDMD, OMIM 181350) y la **distrofia muscular de cinturas o Limb-girdle muscular dystrophy 1B** (LGMD-1B). (25,31–33) Es difícil establecer un pronóstico o una predicción tan sólo con la variante patogénica en el gen LMNA ya que se ha reportado ampliamente en la literatura diferentes fenotipos con una misma variante patogénica, por lo que hay otros factores que colaboran en la expresión fenotípica y en el grado de severidad de la laminopatía. (34)

Los pacientes con L-CMD presentan problemas motores severos y debilidad muscular de forma muy precoz antes de los 2 años de edad. Aunque clásicamente no se ha relacionado de forma inicial con el compromiso cardíaco, sí que se han publicado casos de muerte súbita.(14) Los pacientes con EDMD pueden tener complicaciones cardíacas incluyendo la insuficiencia cardíaca, trastornos de la conducción, accidente vascular cerebral y muerte súbita. Aunque la miocardiopatía dilatada suele presentarse a una edad más avanzada, las

arritmias (bloqueo AV, silencio auricular, taquicardia auricular, fibrilación auricular y TV) pueden ser más precoces. En el caso de la LGMD1B, la debilidad suele ser de cinturas y de inicio antes en extremidades inferiores que las superiores.(6,35–37) Se ha relacionado también con arritmias y defectos de la conducción, aunque la miocardiopatía dilatada sería de aparición muy tardía en el adulto, en caso de presentarse, al igual que EDMD. (37)

Expresión neuromuscular

La expresión fenotípica del grupo de pacientes con diagnóstico de EDMD y LGM1B suele ser más tardía que en el caso de L-CMD (antes de los 2 años de edad), aunque puede existir solapamiento en la expresión fenotípica que dificulte la clasificación clínica.(25,38,39) Los pacientes con L-CMD se han relacionado con complicaciones precoces a nivel respiratorio, cardíaco y nutricional, así como escoliosis severa con afectación respiratoria secundaria. (34,38) En este subgrupo de pacientes es muy llamativa la precocidad de la debilidad axial, el retraso motor y la pérdida motora rápidamente progresiva con la característica debilidad del cuello (*Dropped head*). (14,25,32) Además, estos pacientes presentan una pérdida de la deambulación precoz con necesidad de ventilación invasiva o no invasiva por insuficiencia respiratoria. En revisiones retrospectivas se ha postulado que cuanto antes es la pérdida de la deambulación o no haber conseguido nunca deambular, peor suele ser el pronóstico respiratorio y cardíaco. (25)

En la figura 6 se representa gráficamente los diferentes fenotipos neuromusculares de la laminopatía.

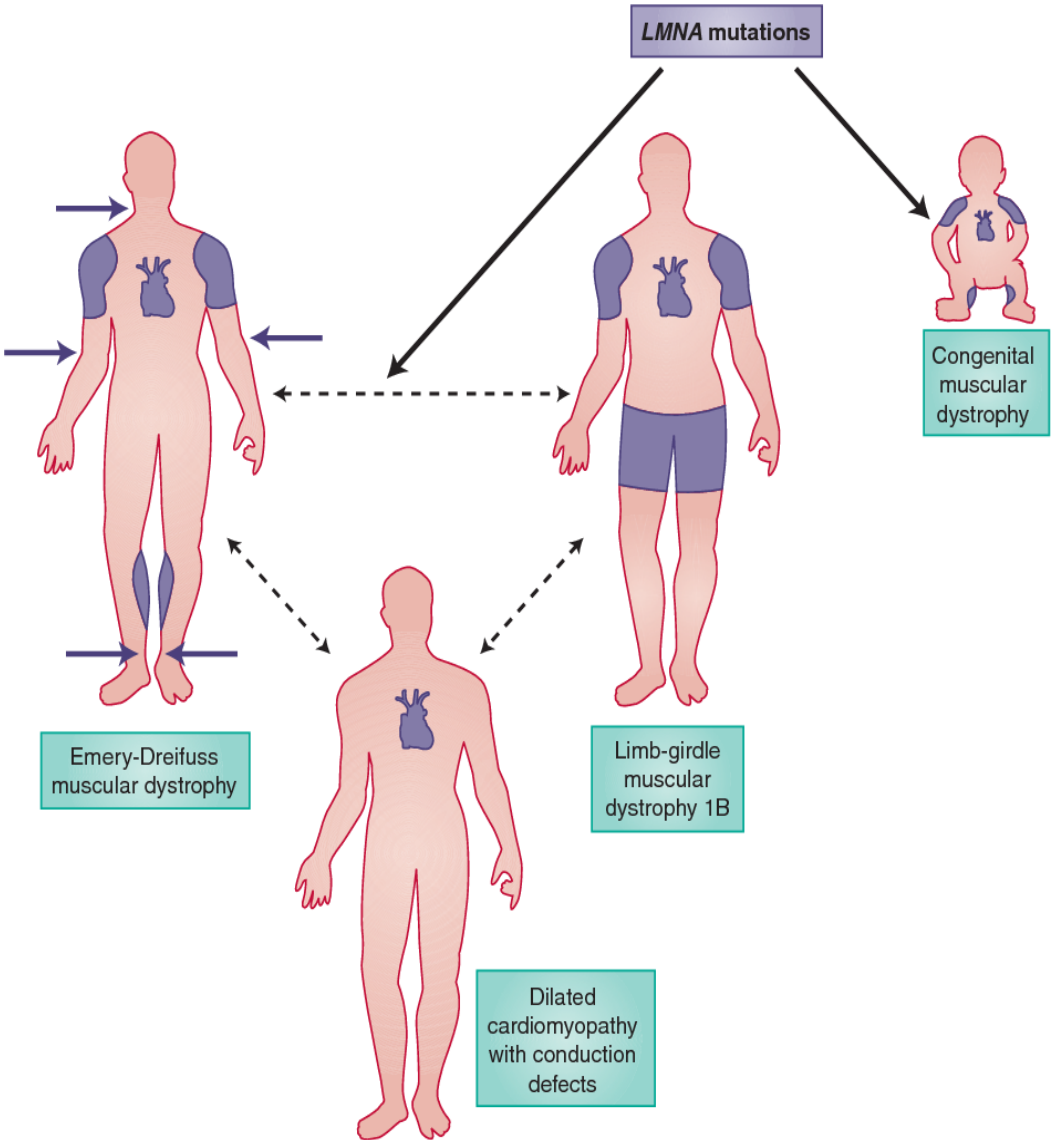


Figura 6, extraída del artículo de Lu et al.(36), donde se representan los fenotipos por laminopatía: EDMD (Distrofia muscular de Emery-Dreifuss), LGMD1B (Distrofia muscular de cinturas 1B), L-CMD (distrofia muscular congénita) y MCD (miocardiopatía dilatada) con defectos de la conducción sin alteración neuromuscular.

Expresión cardíaca

La miocardiopatía dilatada con arritmias, trastornos de la conducción y muerte súbita están descritas en las laminopatías neuromusculares, tanto en la infancia como en los adultos, haciendo de esta enfermedad un desafío para el cardiólogo dada la amplia expresividad fenotípica y los escasos estudios prospectivos especialmente en edad pediátrica.(2,4,25,36–38,40–48). La evolución cardiológica que pueden presentar se representa en la figura 7.

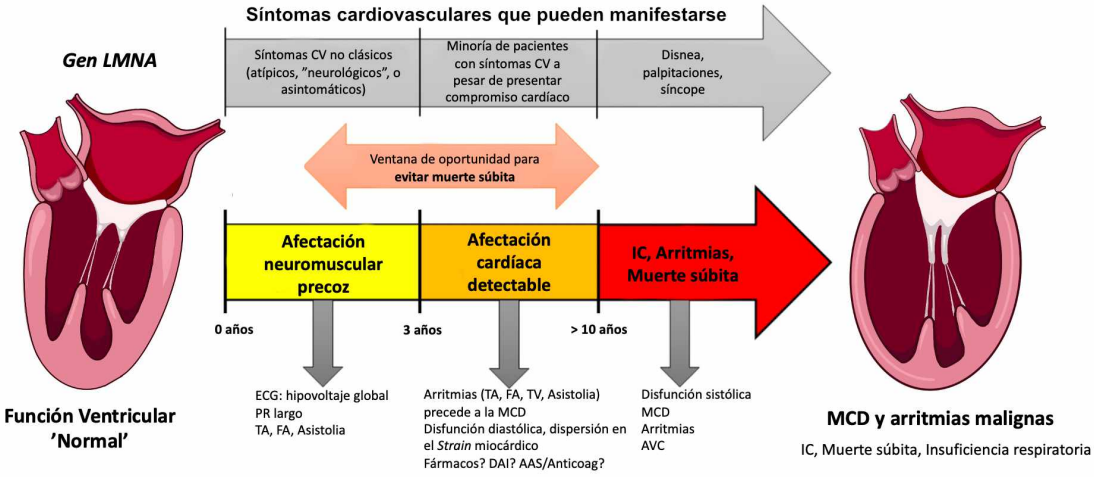


Figura 7 (creación propia). Implicación y evolución cardiovascular en la laminopatía neuromuscular en edad pediátrica. *CV: cardiovascular; ECG: electrocardiograma. TA: taquicardia auricular. FA: fibrilación auricular; TV: taquicardia ventricular. MCD: miocardiopatía dilatada. DAI: desfibrilador automático implantable; AAS: ácido acetilsalicílico; AVC: accidente vascular cerebral; IC: insuficiencia cardiaca.*

Los síntomas cardiovasculares están infraestimados en esta población ya que cuanto más severo es el fenotipo neuromuscular, más se confunden los síntomas cardiovasculares con otros síntomas y más atípicos suelen ser éstos. (49,50)

Clásicamente, la evaluación cardiológica en la laminopatía neuromuscular no se ha establecido de forma sistemática en la edad pediátrica, reservándose los controles sólo cuando los pacientes estaban sintomáticos, siendo en muchos casos una situación tardía con grave repercusión cardíaca. A pesar de series retrospectivas amplias que ponen de manifiesto la expresividad cardíaca en laminopatías congénitas con

presencia de alteraciones cardiológicas precoces, necesidad de DAI y marcapasos, existen aún muchos interrogantes aún sobre la causa exacta de mortalidad en edad pediátrica. (25)

A nivel ecocardiográfico, no hay datos publicados específicos sobre el funcionalismo cardíaco o de deformación cardiaca (*strain* miocárdico) en las laminopatías neuromusculares en edad pediátrica, restringiéndose los datos de la miocardiopatía dilatada y *strain* miocárdico a aquellos recogidos en miocardiopatía dilatada en otros contextos genéticos o bien en series de adultos con mutación en el gen de lamina A/C. (44,51,52)

En cuanto al control clínico para determinar la existencia de arritmias potencialmente malignas, tampoco hay protocolos pediátricos al respecto, ajustándose el seguimiento al que se realiza de forma general en otras entidades neuromusculares (ECG, Holter 24h y estudio electrofisiológico según indicaciones generales de síntomas o hallazgos de TV). En el último consenso para evaluación y manejo de arritmias en enfermedades neuromusculares para población adulta, se incluye la valoración de EDMD y LGMD1B pero no de la L-CMD, sugiriendo que esta población tiene un mayor riesgo de arritmias (taquiarritmias ventriculares, bradiarritmias) y muerte súbita, que puede manifestarse en cualquier edad e independiente del grado de afectación neuromuscular, y que suele ser más precoz en EDMD que en LGMD1B. (37) Es por este motivo que un holter implantable incrementa la tasa de éxito diagnóstico de arritmias en adultos, tal y como se sugiere, pudiendo beneficiarse del implante de un dispositivo como un DAI o un marcapasos. (37)

Muchos de los pacientes son poco o no deambulantes, presentar comorbilidades y deformidades torácicas, con lo que se infraestiman síntomas como las palpitaciones y el dolor torácico de origen cardíaco. (49,50)

También está descrito que los pacientes con EDMD y LGMD1B pueden presentar a lo largo de su vida mayor riesgo de fibrilación auricular, *flutter* auricular, y silencio auricular, y puede que el accidente vascular cerebral sea la primera manifestación incluso en jóvenes por lo que se sugiere anticoagulación independientemente de los riesgos tradicionales calculados por CHAxDSx-VASc. (37)

A nivel genético, existen numerosos reportes en la literatura que recogen los tipos de variantes patogénicas más frecuentemente identificadas en esta población. Específicamente, en la laminopatía congénita se recogen muchas variantes patogénicas no truncantes, sugiriendo un probable mecanismo dominante negativo con la producción de una proteína completa o casi completa de lamina A/C, mientras que las variantes truncantes podrían estar más relacionadas con formas menos severa de la enfermedad o con afectación cardíaca aislada. (25,26)

Pronóstico y factores de riesgo

El corazón puede verse afectado en cualquiera de las tres manifestaciones fenotípicas descritas, y puede preceder a la clínica neuromuscular y suelen tener peor pronóstico las formas con debut más precoz y/o congénitas.(25,31–33) A pesar de que existen protocolos en adultos con calculadoras de riesgo para taquicardias ventriculares para la laminopatía cardíaca, no hay publicaciones al respecto en pacientes con laminopatía neuromuscular en edad pediátrica para prevenir las arritmias malignas y muerte súbita. (25,28) Factores de riesgo como un intervalo PR >240ms, un bloqueo de rama izquierda y/o un intervalo HV prolongado se han asociado a mayor riesgo de arritmias ventriculares en pacientes con EDMD y LGMD1B en etapa adulta, pero no en niños ni tampoco se incluyen valoraciones de pacientes con fenotipo L-CMD. (37)

Además, no hay una clara correlación fenotipo-genotipo, por lo que se ha sugerido que existen modificadores epigenéticos, ambientales y, probablemente, genéticos que sean responsables de la heterogeneidad alélica (31).

Tratamiento

No hay un tratamiento específico para la insuficiencia cardíaca ni las arritmias en pacientes con laminopatía neuromuscular. Habitualmente, se utilizan los fármacos para insuficiencia cardíaca como antagonistas neurohormonales, diuréticos y vasodilatadores. Para las arritmias tampoco hay tratamientos específicos más allá de los antiarrítmicos, así como los dispositivos de estimulación cardíaca, cuando están indicados,

como el marcapasos, la terapia de resincronización cardíaca y el desfibrilador. (7,53–56)

4.2.2. Distrofia miotónica tipo 1 (Enfermedad de Steinert)

La distrofia miotónica tipo 1 (DM1), o enfermedad de Steinert, es la distrofia neuromuscular más prevalente en adultos (1:8000), aunque puede diferir según regiones geográficas (1:600 en Canadá por efecto fundador). (57,58)

Base genética

La base genética de la DM1 se halla en el cromosoma 10q13.3, en el que existe una expansión patológica autosómico dominante de repeticiones CTG en el gen DMPK. A nivel molecular, existe una ganancia de función tóxica de RNA, que conlleva a una retención nuclear de las repeticiones de CTG y causa desregulación de *splicing* alternativo de un subconjunto de pre-mRNAs.(57) Esto podría estar en relación con las arritmias detectadas en algunos pacientes con DM1, debido a un *splicing* anómalo del gen SCN5A. (57,59)

En las generaciones consecutivas hay un fenómeno de anticipación, que consta en una aparición más precoz y severa de la clínica. (60,61) En población sana, el número de repeticiones CTG normales varía de 5 a 37. (62)

Clínica y presentación

La aparición de la clínica puede ser variable, desde prenatal hasta la etapa adulta. Además, existe variabilidad clínica que puede ser explicada por la expansión inestable de CTG a lo largo de la vida en células somáticas.(62,63) Según el momento del debut y de la severidad clínica en edad pediátrica, se puede categorizar en forma congénita (cDM1), infantil (iDM1) y juvenil (jDM1).(64)

La forma cDM1 puede ya ser detectada en época fetal por falta de movimientos fetales y por polihidramnios, y acostumbra a corresponderse con >1000 repeticiones CTG. Además, puede asociar pie equino-varo y ventriculomegalia. Asocia una mortalidad que puede llegar al 18% según

las series. Es el grupo de pacientes más afecto a nivel sistémico, ya que suele haber retraso del desarrollo, del lenguaje, hipotonía, disfagia e insuficiencia respiratoria. (62,65,66)

La forma iDM1 se caracteriza por presentar síntomas antes de los 10 años de edad, y predominan los trastornos cognitivos y del comportamiento, así como trastorno de la atención, autismo y problemas psiquiátricos. (62)

La forma jDM1 ocurre más tardíamente a partir de los 10 años de edad y se asemeja más a la forma clásica de DM1. El síntoma más común es la miotonía, que suele ser más pronunciada después del descanso y mejora con la actividad muscular. Los grupos musculares afectados incluyen lo del antebrazo, mano, lengua y mandíbula. Esto se traduce en una distribución muscular preferente craneal (incluyendo ptosis), tronco y parte distal de extremidades. Evolutivamente, pueden afectarse grupos musculares más proximales. (62)

Afectación cardíaca

Las arritmias por trastornos de la conducción son la presentación clínica cardiovascular más habitual en la DM1 en el adulto. El bloqueo AV es la segunda causa de mortalidad después de la insuficiencia respiratoria, aunque puede variar según las series y las formas de presentación de la DM1. (62,67)

Los trastornos de la conducción y arritmias son frecuentes en la etapa adulta e incluyen el bloqueo AV de primer grado (28-45%), el bloqueo de rama (16-19%), arritmias supraventriculares (5-12.5%) y arritmias ventriculares (taquicardia ventricular entre el 1 y 4%). (57,67) La fibrosis infiltración grasa y retrasos en la conducción en el sistema His-Purkinje pueden llevar a la presentación de las arritmias ventriculares. (57) También han sido descritos: bradicardia, pausas sinusales o asistolia, *flutter* y fibrilación auricular. La miocardiopatía con disfunción ventricular puede estar presente a partir de la tercera o cuarta década de la vida, siendo raro que aparezca antes de esa edad.(57)

En cuanto a la miocardiopatía y/o disfunción cardíaca, se ha descrito esencialmente en adultos entre 10-20% de los pacientes con DM1, con fenotipos como la miocardiopatía hipertrófica y dilatada, con o sin

disfunción sisto-diastólica, y también casos de prolapso valvular mitral. (68,69) En algunos casos, la disfunción sistólica ventricular en adultos se ha podido asociar a los defectos de la conducción (bloqueo de rama izquierda) por lo que se sugiere que puede haber una relación electromecánica de disincronía o asincronía que colabore o sea responsable de la disfunción sistólica y que además pueda ser responsable de una deformidad cardíaca alterada de forma precoz a pesar de no presentar datos de disfunción cardiaca. (69,70)

En la figura 8 se resumen los grupos y el compromiso cardiovascular que puede presentarse según la edad.

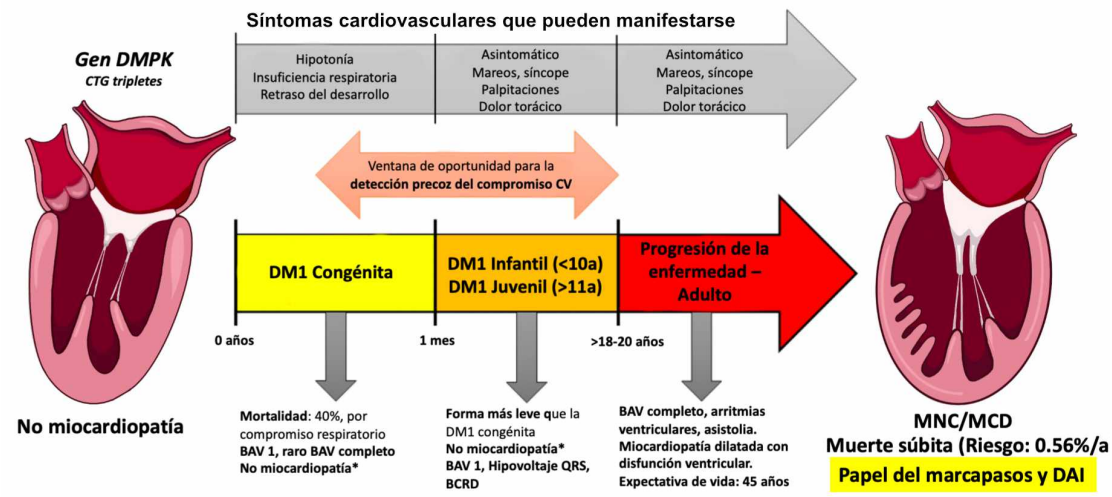


Figura 8 (creación propia). Resumen gráfico del compromiso cardiovascular en la DM1 según subgrupo de DM1 congénita, infantil o juvenil. La etapa del adulto representada en rojo es la de mayor riesgo cardiovascular descrito en la literatura.

Otras manifestaciones sistémicas

Otras manifestaciones sistémicas que justifican el manejo multidisciplinar son las oculares (cataratas), neuropsiquiátricas, cognitivas, trastorno del comportamiento, gastrointestinales (colelitiasis, estreñimiento, diarrea), endocrinológicas (hipogonadismo, hipercolesterolemia, hipertrigliceridemia), disfunción hepática (aumento de enzimas hepáticos), entre otras.

Manejo y tratamiento

No hay un tratamiento específico para la DM1 que altere el curso o que sea curativo. Hay algunos fármacos que pueden mejorar la miotonía (anticomiciales, antiarrítmicos) aunque debe tenerse en cuenta el efecto proarrítmico que puede representar ya que algunos de ellos son bloqueantes de canales de sodio. (57) Se recomienda un manejo multidisciplinar dada la afectación multisistémica en la DM1.(57,62) A nivel respiratorio se recomienda el seguimiento evolutivo para detectar factores contribuyentes a la hipoventilación y comprobar indicación de una ventilación no invasiva o, en caso necesario, ventilación con traqueostomía.(62)

En cuanto a las posibles dianas terapéuticas basadas en los mecanismos fisiopatológicos, hay revisiones que sugieren posibles líneas de investigación, pero al tratarse de una manifestación multisistémica aún no hay ninguna evidencia al respecto (ver figura 9). (71)

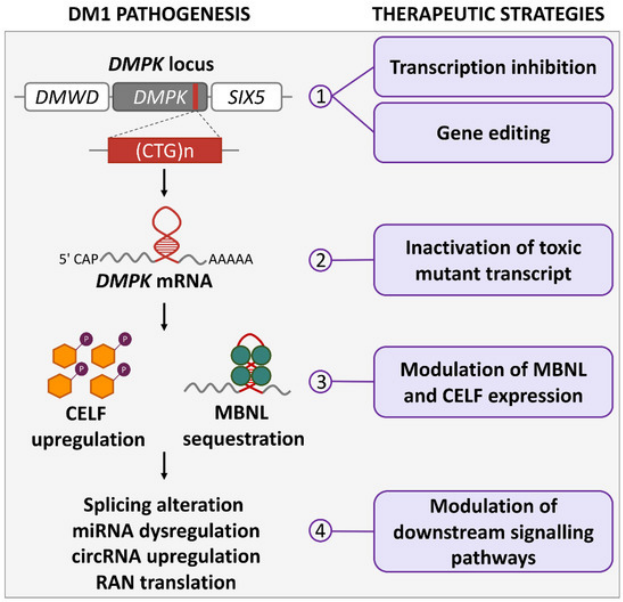


Figura 9, figura publicada por Izzo et al (71). Posibles dianas terapéuticas basadas en los mecanismos fisiopatológicos en la distrofia miotónica tipo 1.

Predictores de muerte súbita

La incidencia anual de muerte súbita en la DM1 en adultos se estima entre 0.53% y 1.15%, frecuentemente atribuida a bradiarritmias por la enfermedad avanzada del tejido de conducción. Otras arritmias involucradas pueden ser la taquicardia ventricular, taquicardia ventricular rama-rama y otras causas no cardiológicas como el tromboembolismo pulmonar. (57,72,73)

En las recomendaciones para adultos recientemente publicadas se indican como predictores de muerte súbita tener un PR ≥ 240ms o un QRS ≥ 120, y la presencia de bloqueo de rama izquierda. (57,74) En el estudio electrofisiológico se ha encontrado un riesgo de bloqueo AV completo y de muerte súbita aquellos pacientes con un intervalo HV 70 y una prolongación media del intervalo HV de 1.2ms/año. (57) Estos predictores se traducen en las recomendaciones publicadas recientemente para valorar la implantación de un marcapasos o un desfibrilador, según la indicación, o una terapia de resincronización. (57) En la figura 10 se muestra el algoritmo cardiovascular en el manejo de la DM1 según las recomendaciones para adultos.

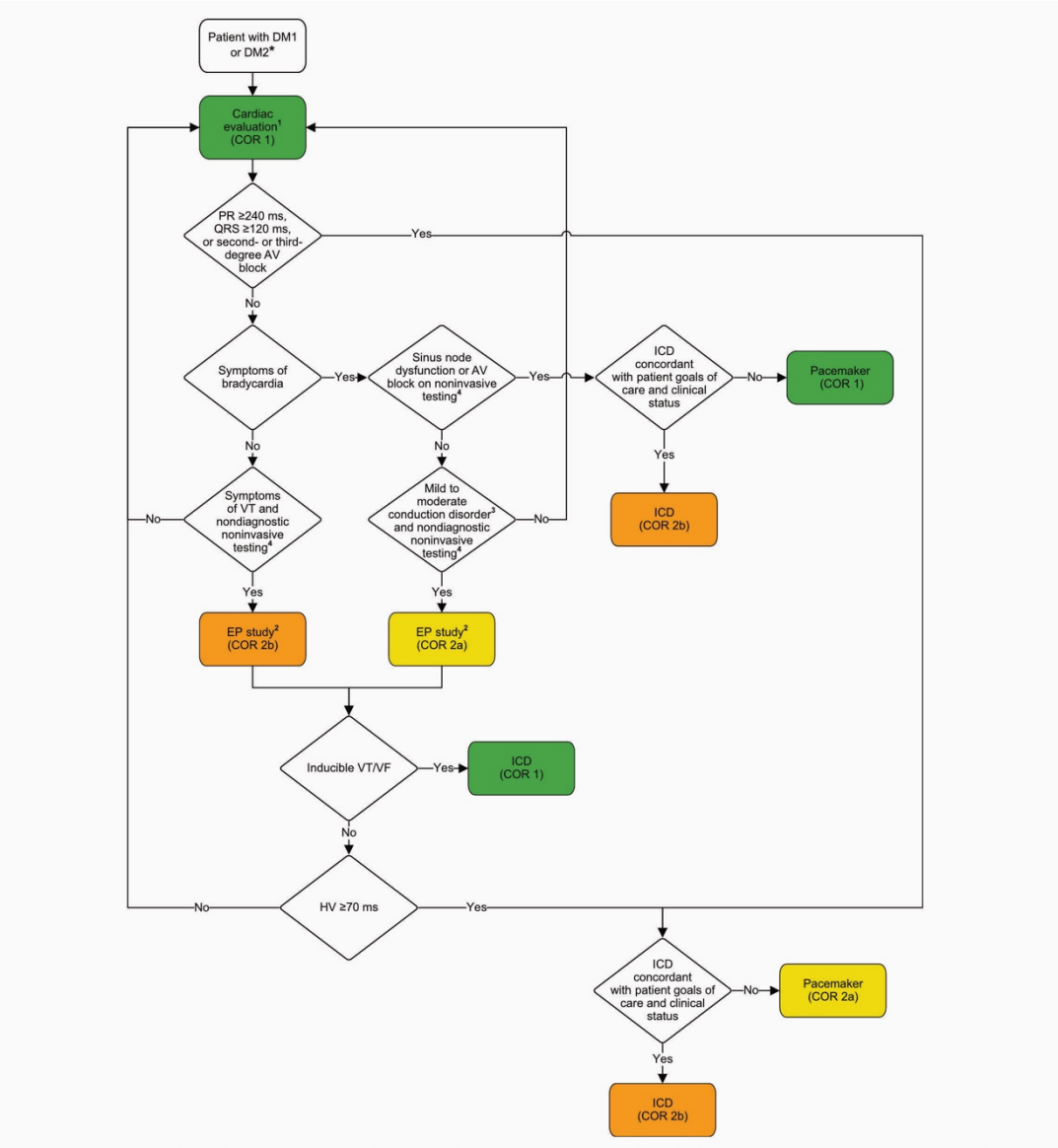


Figura 10. Imagen extraída de Groh et al (57). HRS Evaluation and Management of Arrhythmic Risk in Neuromuscular Disorders).

A pesar de estas recomendaciones, no existen unos valores electrofisiológicos establecidos para la edad pediátrica ni tampoco guías específicas de manejo para poner de manifiesto predictores del riesgo cardiovascular durante la infancia.

4.2.3. Distrofinopatías: Distrofia muscular de Duchenne

Las distrofinopatías están caracterizadas por la debilidad muscular progresiva por degeneración de la fibra muscular. Tienen en común la ausencia, disminución o expresión anómala de la distrofina. En la figura 11 se muestra la representación gráfica de la distrofina entre el citoesqueleto y la matriz extracelular.

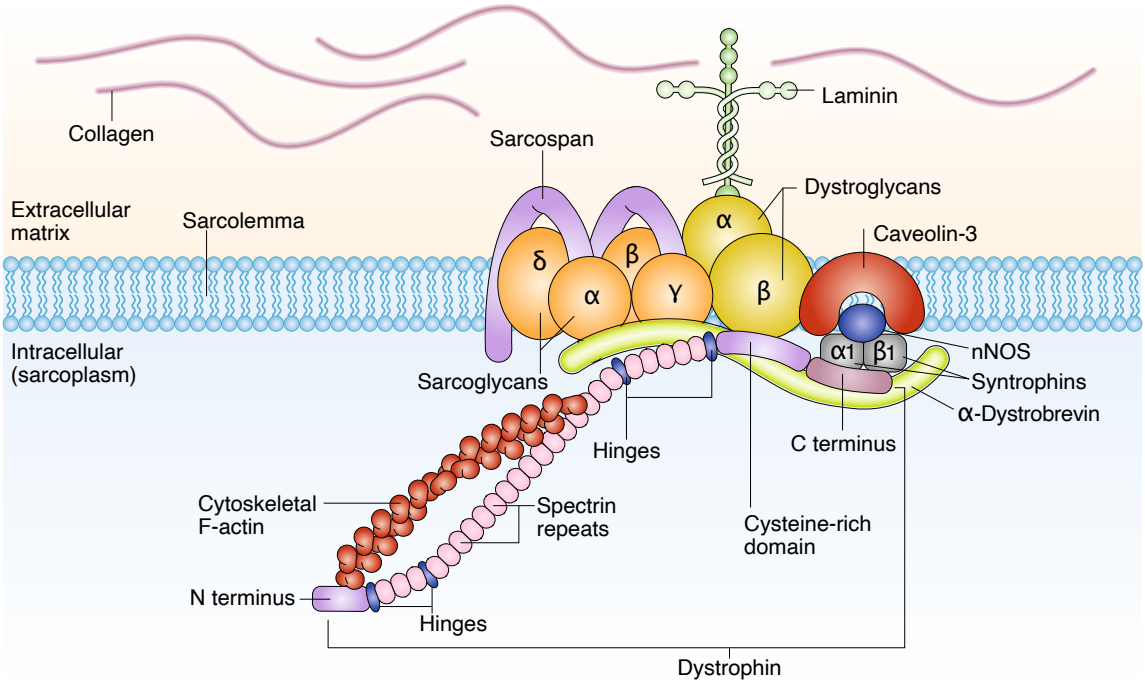


Figura 11. Representación gráfica de la distrofina, que sirve de enlace mecánico entre el citoesqueleto de la célula muscular y la matriz extracelular. Fuente de la imagen: Davies et al. (147)

Epidemiología

De los fenotipos más frecuentemente reconocidos está la distrofia muscular de Duchenne (DMD), con una prevalencia de 5/100000 varones y una incidencia de 1 de cada 3800-6300 recién nacidos varones. Existen fenotipos intermedios o más leves a nivel neuromuscular, que no están exentos de afectación cardíaca pero sí tienen una distrofina más funcional (Distrofia muscular de Becker, DMB).

Genética

Es una enfermedad ligada al cromosoma X. El gen responsable de la codificación de la distrofina, el gen DMD, se encuentra en el Cromosoma X (Xp21).(75) La estructura del gen DMD se resume en la figura 12. La mayor parte de pacientes con DMD, alrededor del 70-75%, tendrán una deleción o una duplicación, pero también existen mutaciones puntuales o deleciones/duplicaciones pequeñas hasta en un 30% de pacientes. La alteración genética provoca una ruptura del marco de lectura del ARNm que sintetiza la distrofina y que conduce a la producción de una proteína no funcional. Aunque este mecanismo puede no estar presente en el 10% de los casos, pues hay un reordenamiento secundario oculto o salto espontáneo de la lectura de un exón, algo que restablecería el marco de lectura.(76)

Un 10% de los pacientes pueden tener mutaciones puntuales *nonsense*, y que igualmente pueden ocasionar fenotipo DMD debido a que la distrofina es no funcional por un codón de parada prematura de la lectura ribosomal sin ruptura del marco de lectura. (76)

En revisiones publicadas basándose en bases de datos genéticas, se halla una mayoría de mutaciones *frame-shift* (93%). Además, se describe que el 66% de las deleciones amplias en la DMD se hallan en región *hotspot* 45-55, 14% en región *hotspot* 2-20 y 20% en regiones no *hotspot.* En cambio, en duplicaciones amplias, se halla el 15% en región *hotspot* 45-55, 50% en región *hotspot* 2-20 y el 35% en regiones no *hotspot*. (77)

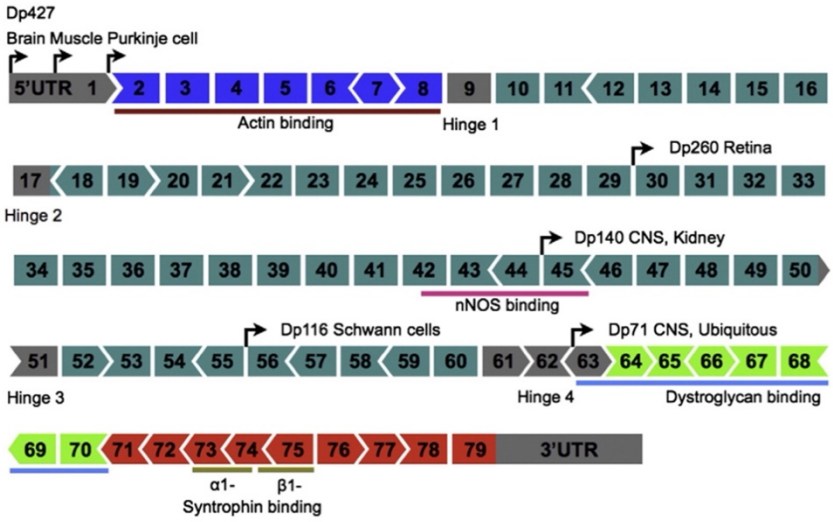


Figura 12, extraída de Douglas et al (75) Representación gráfica del gen DMD y los 79 exones representados. Los exones quedan coloreados según el dominio que codifican: en azul región proximal N-terminal, Rod domain en verde oscuro, Cysteine-rich domain en verde claro, en rojo la región C-terminal.

Clínica

En la tabla 1 se resumen los síntomas y signos clásicos y más frecuentes en la DMD.

Tabla 1. Clínica más frecuente en la DMD	
Clínica motora	**Clínica no motora**
Trastornos de la marcha	Retraso cognitivo
Pseudo-hipertrofia de gemelos	Estancamiento o retraso ponderal
Signo de *Gowers*	Trastornos de aprendizaje y atención
Caminar de puntillas	Retraso en el habla o dificultades de articulación
Retraso motor grueso	
Debilidad en el control de la cabeza	
Disminución de la resistencia al ejercicio	
Incapacidad para saltar, correr o trepar	

Dificultad para subir escaleras Pie plano Caídas frecuentes o torpeza Incapacidad para seguir a sus compañeros Pérdida de habilidades motoras Dolor muscular o calambres
Adaptación de la tabla de las Recomendaciones de la sociedad española de cardiología pediátrica y cardiopatías congénitas. **Disponible en: *https://secardioped.org/guias/***

Existen diferentes **fases** en la DMD que marcan la historia clásica o natural de la enfermedad (78,79):

- Fase presintomática (antes de los 2 años de edad): no suele haber manifestaciones clínicas, pero en ocasiones puede existir retraso global del desarrollo, del habla o dificultades de la interacción verbal.
- Fase ambulatoria (entre los 2 y los 12 años de edad):
    - Temprana (entre 2 y 5 años): debilidad muscular que se representa más en extremidades inferiores y se traduce en caídas frecuentes, dificultades para la marcha y para subir escaleras. Existe pseudo-hipertrofia de gemelos y signo de Gowers positivo.
    - Tardía (entre 5 y 12 años): adquieren habilitades motoras aunque pronto inician un deterioro progresivo con pérdida de la fuerza muscular, además de contracturas musculares, fracturas y escoliosis. Pueden asociar alteraciones conductuales, cognitivas, intelectuales y también asociar trastorno del espectro autista.
- Fase no ambulatoria (entre los 12 y los 20 años de edad):
    - Temprana (entre los 12 y 16 años): en la DMD suele representarse con una pérdida de la marcha y es este el

momento de inicio evidente de la miocardiopatía y patología respiratoria.

- Tardía (a partir de 20 años): se caracteriza por empeoramiento respiratorio, cardiológico y precisan soporte ortopédico, además de la incapacidad de mantenerse en una posición sentada.

- Variabilidad individual: la progresión y gravedad de los síntomas viene influida por factores genéticos y/o ambientales, que se traducen, entre otras, en una diferente expresividad fenotípica y funcionalidad de la distrofina, por lo que hay fenotipos más leves que no cumplirían con las etapas descritas y se comportarían más como fenotipos intermedios o DMB.

La afectación cardíaca en la distrofia muscular de Duchenne y Becker es una de las principales causas de morbimortalidad de estos pacientes, sobre todo en la segunda y cuarta década de la vida. Existe una disfunción ventricular con o sin miocardiopatía dilatada que evoluciona a la insuficiencia cardiaca y arritmias.(80)

Diagnóstico de la DMD

El diagnóstico clínico se suele sospechar en los primeros años de vida, a través del hallazgo de alteraciones motoras con o sin antecedentes de distrofia muscular en la familia. El retraso del inicio de la deambulación es un hallazgo frecuente, con dificultad para levantare del suelo, correr o saltar. La hipertrofia de los músculos gastrocnemios (gemelos) es un hallazgo frecuente en la exploración física.

A nivel del análisis de sangre, es frecuente encontrar niveles elevados de CPK y de transaminasas:

- Los niveles elevados de CPK en sangre aparecen incluso antes del inicio de los síntomas motores y pueden permanecer elevados durante años. En etapas evolucionadas de la enfermedad pueden hallarse niveles normales o bajos de CPK en sangre debido a la sustitución fibro-adiposa en el músculo.

- Los niveles elevados de transaminasas en sangre son debidos a esta miólisis y, a priori, no representa afectación hepática.

El diagnóstico de certeza viene dado por la confirmación genética y, en caso de dudas, apoyo en la biopsia muscular.

Pronóstico

Viene marcado por el estado cardiológico y/o respiratorio. En el caso de la DMD clásica es rara una supervivencia más de la tercera-cuarta décadas de la vida.

Tratamiento

Actualmente no hay un tratamiento curativo para la DMD, aunque hay diversos ensayos clínicos en desarrollo, inclusive terapia génica, para poder ofrecer un tratamiento a estos pacientes.

Clásicamente, el tratamiento en la DMD viene definido por:

- Corticoterapia: aunque se ha descrito un efecto beneficioso a nivel neuromuscular mediante la ralentización de la progresión de la debilidad muscular y retrasar la pérdida de la deambulación. Existen controversias sobre la clase de corticoide y la dosis. Los más utilizados son Prednisona 0.75mg/kg/día y Deflazacort 0.9mg/kg/día.

  El momento de su suspensión después de la pérdida de la marcha también ha suscitado controversia, y hay estudios que defienden hipótesis contrarias, sobre todo por los efectos secundarios que pueden asociar.
- Vitamina D3: es común suplementar con Vitamina D3 debido al riesgo de osteoporosis de causa multifactorial en la población con DMD, sobre todo si hay uso de corticoterapia oral a largo plazo.
- Inhibidores de la enzima convertidora de la angiotensina (IECA): en las recomendaciones actuales se aboga por el inicio de un IECA a los 10 años de edad ya que se ha visto reducción de la tasa mortalidad y hospitalización en pacientes con DMD y reducción de la función ventricular izquierda y signos de insuficiencia cardiaca. (78,81)

- Tratamiento de la insuficiencia cardíaca y de las arritmias: no hay un tratamiento específico para la insuficiencia cardiaca en pacientes con DMD. Habitualmente, se usan los fármacos habituales ya conocidos en la insuficiencia cardiaca como pueden ser: IECA, antagonistas del receptor de la angiotensina (ARA), diuréticos, beta-bloqueantes, ivabradina y sacubitrilo-valsartán. También puede haber indicación de tratamiento anticoagulante en caso de riesgo de trombo intracardiaco debido a la insuficiencia cardiaca. (78,80)

Afectación cardiológica

La distrofina se encuentra, además de en el sarcolema esquelético, en el sarcolema cardíaco. El déficit de distrofina provoca una inestabilidad en el sarcolema, provocando daño de los miocardiocitos, inflamación y fibrosis. Esto se traduce en una miocardiopatía dilatada y/o fibrótica que conduce a la insuficiencia cardíaca. (57,78)

Etapas de la miocardiopatía(80)**:**

- **Etapa preclínica**

  Alteraciones a nivel microscópico y alteraciones en el ECG incluso antes de los 10 años de edad. Normalmente no hay disfunción sistólica, pero pueden detectarse datos de disfunción diastólica incipiente.

- **Etapa clínica**

  Atrofia de los miocardiocitos, con presencia de fibrosis subendocárdica y dilatación de cavidades. Habitualmente no hay síntomas cardiovasculares claros.

- **Etapa de afectación cardiológica evidente**

  Es la etapa en la que ya existen síntomas claros a nivel cardiovascular y suele ser por encima de los 18-20 años. Los síntomas de insuficiencia cardiaca, frecuentemente, son difíciles de reconocer dado que existe una gran debilidad muscular y existe una reducción de la movilidad y/o incapacidad para deambular. Por esta razón, previo al desarrollo de guías internacionales, los

pacientes eran derivados al cardiólogo en esta etapa sintomática ya muy tardía.

En la figura 13 se representa la afectación cardiológica en las diferentes etapas de la enfermedad.

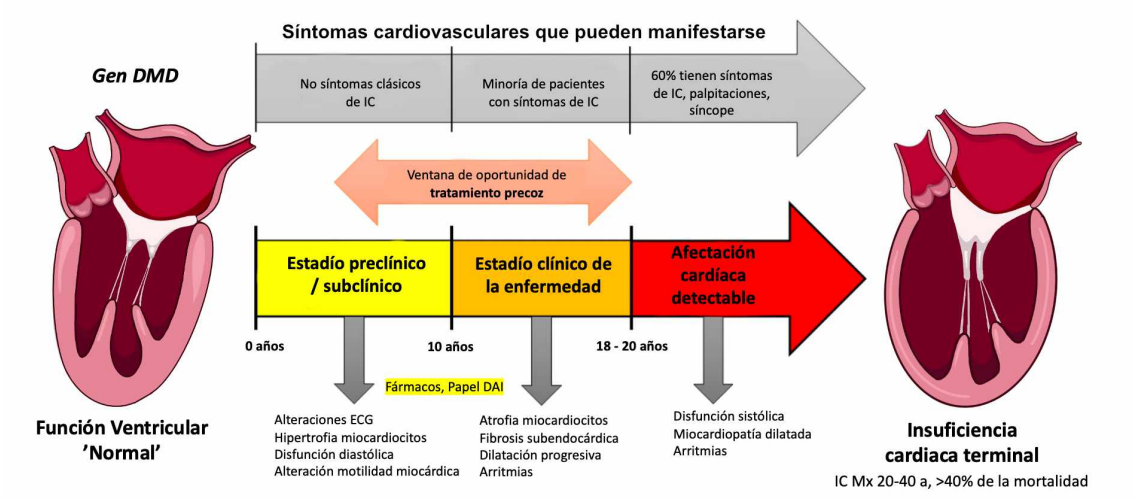


Figura 13 (creación propia). Se representa gráficamente las diferentes etapas de la afectación cardiaca en la Distrofia muscular de Duchenne.

Evaluación de la función cardíaca:

La ecocardiografía es la técnica habitual para la valoración cardiológica, aunque presenta limitaciones por la ventana acústica limitada en pacientes con DMD. La evaluación mediante ecocardiografía funcional y *Strain* miocárdico ha permitido una evaluación más precisa de la función cardíaca. El *Strain* miocárdico permite detectar alteraciones subclínicas incluso antes de que se afecte la fracción de eyección. (82–84) Además, la RM cardiaca con contraste y realce tardío de Gadolinio representa la prueba de referencia para valorar la función y la fibrosis miocárdica, aunque en edades tempranas puede precisar anestesia general para el control de la apnea para la adquisición de las imágenes. (78,85)

Para los pacientes con mutación en la región proximal N-terminal parece relacionarse con más síntomas cardiológicos. (86–90) Además, en la mutación de los exones 45 al 52 del gen DMD, se ha descrito una precocidad de la disminución de la fracción de eyección por lo que puede haber una variabilidad en la expresión fenotípica cardíaca en función de

las mutaciones patogénicas de cada paciente. Además, puede existir una variabilidad de la expresividad fenotípica entre pacientes incluso teniendo la misma mutación patogénica, como sucede con los pacientes con deleción del exón 45-55, lo que sugiere que existen otros factores modificadores del fenotipo. (91,92)

Arritmias y muerte súbita:

El riesgo de presentar arritmias ha sido ya previamente descrito, entre las que se encuentran fibrilación auricular, *flutter* auricular, taquicardia ventricular y fibrilación ventricular. Las bradiarritmias son infrecuentes en pacientes con DMD y las taquiarritmias auriculares no han sido extensamente estudiadas así como el riesgo tromboembólico.(57) En el seguimiento se ha propuesto la monitorización ambulatoria con Holter 24h o, en caso necesario, Holter subcutáneo para monitorización de larga evolución, aunque se deja a criterio del clínico. (57,78)

En adultos, el realce tardío de Gadolinio en la resonancia magnética se ha relacionado con un factor predictivo de mortalidad, eventos cardiovasculares y arritmias ventriculares. (57)

La muerte súbita ha sido también descrita en pacientes con DMD pero, aunque no se conoce con exactitud la incidencia, parece corresponderse a una incidencia baja y podría tener una causa mixta respiratoria y cardíaca. (57,93,94) En una revisión retrospectiva reciente Wittlieb-Weber et al revisó la mortalidad en DMD, y se ha postulado como factores de riesgo de mortalidad, independientemente de la causa, la pérdida precoz de la deambulación, la ausencia de tratamiento con corticoterapia, valores bajos de fracción de eyección en el último control del paciente y valores altos de BNP. La muerte súbita se dio en 5 pacientes de los 466 pacientes, aunque sin datos concluyentes en las pruebas que se disponían. (95)

No hay guías específicas para el implante de un DAI en prevención primaria en población pediátrica y la utilización en prevención secundaria es bajo criterios habituales de taquicardia ventricular o fibrilación ventricular en adultos. Hay que tener en consideración el pronóstico global del paciente, las particularidades de la caja torácica y el riesgo de más complicaciones durante el implante de dispositivos para el tratamiento del

ritmo cardiaco.(78) En las últimas guías publicadas por la ESC para el manejo de arritmias en población adulta con DMD se considera clase IIb de recomendación el implante de un DAI si el paciente tiene realce tardío de Gadolinio de forma significativa en el estudio por resonancia magnética cardiaca, aunque no hay evidencia en población pediátrica. (96) Además, en el adulto con DMD ante una fracción de eyección del ventrículo izquierdo <35% se puede considerar el implante de un DAI, individualizando el estado clínico cardiológico y global del paciente. (57)

5. Hipótesis

Hipótesis

El estudio con ecocardiografía funcional y de deformación miocárdica y la monitorización electrocardiográfica de larga duración en pacientes con enfermedades neuromusculares pueden cambiar el pronóstico cardiovascular y el riesgo de muerte súbita en pacientes pediátricos.

6. Objetivos

Objetivo principal

Identificar predictores de arritmias cardíacas y muerte súbita en pacientes pediátricos afectos de distrofia muscular y distrofinopatías, como grupos de riesgo de las enfermedades neuromusculares.

Objetivos específicos

1. Correlacionar los síntomas con los hallazgos de las exploraciones complementarias para determinar si sintomatología puede ser un factor pronóstico.
2. Realizar una correlación genotipo-fenotipo de las enfermedades neuromusculares estudiadas.
3. Determinar el subgrupo de pacientes que puedan estar a riesgo de eventos de accidente vascular cerebral debido a un origen cardíaco.
4. Identificar factores de riesgo de arritmias y muerte súbita en la cohorte con laminopatías neuromusculares.
5. Determinar el papel del uso de dispositivos implantables para la monitorización del ritmo cardíaco (holter subcutáneo) como herramienta pronóstica de detección de arritmias y muerte súbita en las laminopatías neuromusculares.
6. Determinar el papel del estudio electrofisiológico como herramienta pronóstica de arritmias y muerte súbita en las laminopatías neuromusculares.
7. Analizar el valor pronóstico del *strain* miocárdico en los pacientes con laminopatía neuromuscular y distrofia muscular de Duchenne.
8. Caracterizar el estado cardiovascular e identificar factores de riesgo de arritmias y muerte súbita de la cohorte estudiada con distrofia miotónica de Steinert y distrofia muscular de Duchenne.

7. Material, métodos y resultados

Artículo 1. Resumen estructurado

Characterization of cardiac involvement in children with *LMNA*-related muscular dystrophy.

Introducción

La distrofia muscular por LMNA es una entidad rara que se engloba dentro de las laminopatías. Dentro de estas distrofias musculares por LMNA se encuentran la Emery–Dreifuss muscular dystrophy (EDMD), Limb–girdle muscular dystrophy type 1B (LGMD1B), y *LMNA*-related congenital muscular dystrophy (L-CMD). La insuficiencia cardíaca, arritmias malignas y muerte súbita pueden estar presentes en esta población, aunque en edad pediátrica no existe un consenso de seguimiento ni protocolos específicos de manejo cardiovascular.

Objetivos

El objetivo fue llevar a cabo un seguimiento prospectivo cardiovascular en pacientes pediátricos diagnosticados de distrofia muscular por LMNA.

Métodos

Se realizó un seguimiento cardiológico exhaustivo que consistió en recogida de datos clínicos, epidemiológicos, genéticos, ecocardiográficos y electrocardiográficos. Se implantó un holter subcutáneo a todos los pacientes incluidos en el protocolo de seguimiento y se realizó un estudio electrofisiológico.

Resultados

Se incluyeron 28 pacientes en total, cuyos fenotipos fueron: EDMD (13 pacientes), L-CMD (11 pacientes), LGMD1B (2 pacientes), y dos pacientes con debilidad leve relacionada con LMNA. Se detectó miocardiopatía dilatada en 6 pacientes y arritmias malignas en 5 pacientes (4 concomitantemente con miocardiopatía dilatada). Las arritmias malignas (20% de la cohorte) se detectaron a través del holter subcutáneo y justificaron el implante de un

desfibrilador y, en un caso, un marcapasos por detección de una asistolia. Los pacientes con fenotipo EDMD de aparición precoz mostraron peor pronóstico cardíaco y tuvieron más riesgo de miocardiopatía dilatada, mientras que las arritmias malignas se detectaron más precozmente en L-CMD, sin fenotipo de miocardiopatía dilatada al menos al inicio de las arritmias. La detección de valores bajos de *strain* miocárdico, a pesar de valores normales de fracción de eyección de ventrículo izquierdo, se relacionó con peor pronóstico cardíaco por la necesidad de DAI más adelante durante el seguimiento. Un paciente tuvo un accidente vascular cerebral, probablemente relacionado con la combinación de varios factores de riesgo como fueron la situación de base (no deambulación y poca movilidad), arritmias auriculares (silencio auricular, taquicardia auricular y fibrilación auricular), y disfunción ventricular.

Conclusiones

La aparición precoz de los síntomas y signos neuromusculares, antes de los 2 años de edad, se relacionan con un peor pronóstico cardíaco en las laminopatías neuromusculares en pacientes pediátricos. A pesar de tener estudios electrofisiológicos normales, el holter subcutáneo identificó casi un 20% de arritmias malignas que justificaron el implante de desfibrilador en 5 pacientes, previniendo así la muerte súbita. Dos de estos pacientes recibieron choques apropiados durante el período de seguimiento y ningún choque inapropiado. La atipicidad o ausencia de síntomas cardiovasculares a pesar de presentar arritmias y/o insuficiencia cardíaca, hace más vulnerable a estos pacientes y justifica un seguimiento estrecho y una monitorización electrocardiográfica continua a largo plazo. Además del análisis ecocardiográfico básico, es de importancia analizar el *strain* miocárdico, pues se ha relacionado tener un *strain* miocárdico del ventrículo izquierdo alterado, a pesar de valores normales de fracción de eyección del ventrículo izquierdo, con un peor pronóstico cardíaco por la necesidad de implante de DAI debido a arritmias potencialmente letales. La combinación de poca movilidad por la debilidad muscular, sumado a arritmias auriculares y disfunción ventricular moderada/severa pueden ser factores de riesgo relacionados con el accidente vascular cerebral, por lo que debería considerarse la anticoagulación o antiagregación en estos pacientes.

Estos resultados podrían ser la base para implementar guías clínicas específicas para prevenir las arritmias y la muerte súbita en la población con laminopatía neuromuscular pediátrica.

frontiers | Frontiers in Cell and Developmental Biology

TYPE Original Research
PUBLISHED 10 March 2023
DOI 10.3389/fcell.2023.1142937

OPEN ACCESS

EDITED BY
Marc Bartoli,
Aix Marseille Université, France

REVIEWED BY
Masamichi Ito,
The University of Tokyo, Japan
Abdallah Fayssoil,
Hôpital Raymond-Poincaré, France

*CORRESPONDENCE
Sergi Cesar,
sergi.cesar@gmail.com

SPECIALTY SECTION
This article was submitted to Molecular and Cellular Pathology, a section of the journal Frontiers in Cell and Developmental Biology

RECEIVED 12 January 2023
ACCEPTED 27 February 2023
PUBLISHED 10 March 2023

CITATION
Cesar S, Campuzano O, Cruzalegui J, Fiol V, Moll I, Martínez-Barrios E, Zschaeck I, Natera-de Benito D, Ortez C, Carrera L, Expósito J, Berrueco R, Bautista-Rodriguez C, Dabaj I, Gómez García-de-la-Banda M, Quijano-Roy S, Brugada J, Nascimento A and Sarquella-Brugada G (2023), Characterization of cardiac involvement in children with *LMNA*-related muscular dystrophy.
Front. Cell Dev. Biol. 11:1142937.
doi: 10.3389/fcell.2023.1142937

Characterization of cardiac involvement in children with *LMNA*-related muscular dystrophy

Sergi Cesar[1,2,3]*, Oscar Campuzano[4,5,6], Jose Cruzalegui[1,2,3], Victori Fiol[1,2,3], Isaac Moll[1,2,3], Estefania Martínez-Barrios[1,2,3], Irene Zschaeck[1,2,3,7,8], Daniel Natera-de Benito[7,8], Carlos Ortez[7,8], Laura Carrera[7,8], Jessica Expósito[7,8], Rubén Berrueco[9], Carles Bautista-Rodriguez[10,11], Ivana Dabaj[12], Marta Gómez García-de-la-Banda[12], Susana Quijano-Roy[12], Josep Brugada[1,2,3,6,13], Andrés Nascimento[7,8,14] and Georgia Sarquella-Brugada[1,2,3]

[1]Pediatric Arrhythmias, Inherited Cardiac Diseases and Sudden Death Unit, Hospital Sant Joan de Déu, Universitat de Barcelona, Barcelona, Spain, [2]Arrítmies Pediàtriques, Cardiologia Genètica i Mort sobtada, Malalties Cardiovasculars en el Desenvolupament, Institut de Recerca Sant Joan de Déu, Esplugues de Llobregat, Barcelona, Spain, [3]European Reference Network for Rare, Low Prevalence and Complex Diseases of the Heart (ERN GUARD-Heart), Amsterdam, Netherlands, [4]Medical Science Department, School of Medicine, Universitat de Girona, Girona, Spain, [5]Cardiovascular Genetics Center, University of Girona-IDIBGI, Girona, Spain, [6]Centro de Investigación Biomédica en Red, Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain, [7]Neuromuscular Unit, Department of Neurology, Hospital Sant Joan de Déu, Barcelona, Spain, [8]Investigación Aplicada en Enfermedades Neuromusculares, Neurociències, Institut de Recerca Sant Joan de Déu, Esplugues de Llobregat, Spain, [9]Servicio de Hematología Pediátrica, Hospital Sant Joan de Déu Barcelona, Institut de Recerca Pediàtrica, Hospital Sant Joan de Déu de Barcelona (IRP-HSJD), Universitat de Barcelona, Barcelona, Spain, [10]Paediatric Cardiology Services, Royal Brompton Hospital, Guy's and St Thomas NHS Foundation Trust, London, United Kingdom, [11]National Heart and Lung Institute, Imperial College London, London, United Kingdom, [12]Neuromuscular Unit, Pediatric Neurology and ICU Department, Raymond Poincaré Hospital (UVSQ), AP-HP Université Paris-Saclay, Garches, France, [13]Arrhythmia Section, Cardiology Service, Hospital Clínic, Barcelona, Spain, [14]Instituto Nacional de Investigación Biomédica de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Madrid, España

Introduction: LMNA-related muscular dystrophy is a rare entity that produce "laminopathies" such as Emery–Dreifuss muscular dystrophy (EDMD), limb–girdle muscular dystrophy type 1B (LGMD1B), and LMNA-related congenital muscular dystrophy (L-CMD). Heart failure, malignant arrhythmias, and sudden death may occur. No consensus exists on cardiovascular management in pediatric

Abbreviations: AV, atrioventricular; AVB, atrioventricular block; CHD, congenital heart disease; CMRI, cardiac magnetic resonance imaging; DCM, dilated cardiomyopathy; EDMD, Emery–Dreifuss muscular dystrophy; ECG, electrocardiogram; EPS, electrophysiological study; GLS, global longitudinal strain; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter defibrillator; ILR, implantable loop recorder; IQR, interquartile range; IRBBB, incomplete right bundle-branch block; LBBB, left bundle-branch block; LGMD1B, limb–girdle muscular dystrophy 1B; L-CMD, *LMNA*-related congenital muscular dystrophy; LV, left ventricle; LVEF, left ventricular ejection fraction; MAPSE, mitral annular plane systolic excursion; NIMV, non-invasive mechanical ventilation; NT proBNP, N-terminal probrain natriuretic peptide; PVC, premature ventricular contractions; RBBB, right bundle-branch block; RV, right ventricle; SCD, sudden cardiac death; TAPSE, tricuspid annular plane systolic excursion; VT, ventricular tachycardia; VF, ventricular fibrillation.

laminopathies. The aim was to perform an exhaustive cardiologic follow-up in pediatric patients diagnosed with LMNA-related muscular dystrophy.

Methods: Baseline cardiac work-up consisted of clinical assessment, transthoracic Doppler echocardiography, 12-lead electrocardiogram, electrophysiological study, and implantation of a long-term implantable cardiac loop recorder (ILR).

Results: We enrolled twenty-eight pediatric patients diagnosed with EDMD (13 patients), L-CMD (11 patients), LGMD1B (2 patients), and LMNA-related mild weakness (2 patients). Follow-up showed dilated cardiomyopathy (DCM) in six patients and malignant arrhythmias in five (four concomitant with DCM) detected by the ILR that required implantable cardioverter defibrillator (ICD) implantation. Malignant arrhythmias were detected in 20% of our cohort and early-onset EDMD showed worse cardiac prognosis.

Discussion: Patients diagnosed with early-onset EDMD are at higher risk of DCM, while potentially life-threatening arrhythmias without DCM appear earlier in L-CMD patients. Early onset neurologic symptoms could be related with worse cardiac prognosis. Specific clinical guidelines for children are needed to prevent sudden death.

KEYWORDS

laminopathies, sudden cardiac death, cardiomyopathy, A/C lamins, *LMNA*-related diseases, LMNA-related cardiomyopathy, long-term implantable loop recorder

Introduction

LMNA-related muscular dystrophy is a very rare (0.5 per 100,000) disease caused by pathogenic alterations in the *LMNA* gene. The disorder is characterized by cervical–axial weakness, scapuloperoneal weakness, joint contractures, thoracic lordosis, a dystrophic muscle biopsy, and mildly elevated creatine kinase levels (Quijano-roy et al., 2008). Children with early-onset *LMNA*-related muscular dystrophy may show decreased fetal movement and early lack of motor development since the first months of life, or later develop a loss of head and trunk control and ability to walk or sit, followed by progressive loss of axial and limb motor function. As these patients age, there is an increased risk for respiratory insufficiency, appears joint and spinal deformities and cardiac involvement. Practically all patients exhibit heart disease in long follow up studies (Dubowitz, 1999; Quijano-roy et al., 2008).

LMNA encodes the nuclear envelope proteins lamins A and C, intermediate filaments that are required during development and cell differentiation (Bonne et al., 2003). Lamins facilitate signal transduction between the cytoskeleton and the nucleus (Aebi et al., 1986; Worman and Bonne, 2007), provide genome stability and modulation of chromatin organization and gene expression (Lammerding et al., 2004; Dechat et al., 2007; Andrés and González, 2009; Dauer and Worman, 2009; Gonzalez-Suarez et al., 2009; Hutchison, 2011). Lamins consist of a globular N-terminal head domain, a central coiled-coil rod domain implicated in protein dimerization, and a C-terminal tail domain that includes an immunoglobulin-like domain where various posttranslational modifications occur (Burke and Stewart, 2012; Ho and Lammerding, 2012). *LMNA* was first identified in 1986 in humans, but it was not until 1999 that a pathogenic rare variant in the *LMNA* gene was linked to Emery–Dreifuss muscular dystrophy (EDMD) (Bonne et al., 1999). To date, over 600 disease-causing rare *LMNA* variants are characterized (Worman and Bonne, 2007; Dittmer and Misteli, 2011), and these "laminopathies" are associated with heterogeneous clinical phenotypes, including neuromuscular, cardiac, and metabolic disorders (Bertrand et al., 2011; Worman, 2012; Maggi et al., 2014). However, there is no clear correlation between genotype and phenotype, including within the same muscle, suggesting the presence of genetic modifiers and representing an example of allelic heterogeneity (Granger et al., 2011).

Muscle laminopathies may associate cardiac disease at any age and range from congenital muscular dystrophy (*LMNA*-related congenital muscular dystrophy, or L-CMD) to late-onset manifestations (limb–girdle muscular dystrophy 1B, or LGMD1B; and autosomal-dominant EDMD). L-CMD is the most severe and early phenotype, and typically presents in the first 2 years of life, either by an arrest of motor milestones before sitting or walking are acquired, or as a later presentation with a characteristic loss of head support, while sitting and or walking are still maintained (dropped head syndrome). It is a very progressive and severe disease, and shares with EDMD a recognizable scapulo-humero-peroneal pattern of muscle weakness and atrophy (predominantly proximal in upper limbs and distal in lower limbs). The heart may be involved in all three entities, and the manifestation of heart disease may precede muscle weakness or be isolated. Globally, early-onset phenotypes before 5 years of age, and specially before 2 years of age, are related with worse motor and cardiac prognosis, although heart involvement is rarely observed initially (Granger et al., 2011; Carboni et al., 2013; Maggi et al., 2014; Ben Yaou et al., 2021). Dilated cardiomyopathy (DCM) with conduction disease and sudden cardiac death (SCD) can occur in *LMNA*-related muscular dystrophies, in children and adults (Finsterer et al., 2006; Finsterer et al., 2010; Lu et al., 2011; Groh, 2012; Quarta et al., 2012; Cattin et al., 2013; Hasselberg et al., 2014; Alastalo et al., 2015; Dobrzynska et al., 2016; Finsterer and St, 2016; Heller et al., 2017; Muscogiuri, 2017; Wang et al., 2017; Groh et al., 2022). These patients have a high incidence of malignant arrhythmias at early ages, worsening prognosis and posing a clinical challenge for cardiologists, neurologists, and genetic counselors (Meune et al., 2006; Groh, 2012; Rijsingen et al.,

2012; Carboni et al., 2013; Rajdev and Groh, 2015; Kumar et al., 2016; Feingold et al., 2017; Peretto et al., 2019; Ben Yaou et al., 2021). Despite the risk prediction scores for life-threatening ventricular tachyarrhythmias (VT) have been defined in adults with laminopathies (Wahbi et al., 2019; Marchel et al., 2021a; Groh et al., 2022), there are no published guidelines for risk prediction scores in pediatric laminopathies to prevent early life-threatening VT. Given the rarity of the disease in the pediatric population, phenotype–genotype correlations are difficult to be established, and data on age of onset and course of the cardiac disease or the risk of malignant arrhythmias and SCD is scarce (Ben Yaou et al., 2021).

To bridge this knowledge gap, we performed a mid-to-long-term cardiovascular follow-up study focused on cardiomyopathies and arrhythmia during childhood and genotype–phenotype correlation in several families with *LMNA*-related muscular dystrophy.

Material and methods

Study design

We enrolled patients <18 years of age from the international reference center in neuromuscular diseases at our institution that were diagnosed with *LMNA*-related muscular dystrophy (carrying a rare pathogenic or likely pathogenic variant in the *LMNA* gene with L-CMD, EDMD, LGMD1B, and *LMNA*-related atypical phenotype with mild weakness) between 2014 and 2020. The study was motivated and promoted by the national patient association. Due to the severity of pediatric laminopathies, we analyzed in an independent category all the patients with early-onset neuromuscular phenotypes (before 2 years of age) regardless of the final diagnosed clinical phenotype (L-CMD and early-onset EDMD). At enrollment, we collected retrospective clinical data. Participants were prospectively followed from enrollment to the last follow-up evaluation according to a specific schedule. Written informed consent to participate was provided by the participants' legal guardians. Non-LMNA-related neuromuscular dystrophies were excluded from this study.

Clinical work-up and follow-up schedule

Baseline cardiac work-up consisted of a clinical evaluation, DNA samples from the patient and first-degree relatives, transthoracic Doppler echocardiography (Philips, IE33, software Intellispace Cardiovascular for standard measurements and QLAB for offline strain analysis), a 12-lead electrocardiogram (ECG), electrophysiological study (EPS), and implantation of a long-term cardiac implantable loop recorder (ILR; Medtronic Reveal LINQ) with a home monitoring system. The definition of DCM and reference values of echocardiographic measurements were adopted from the most recent pediatric guidelines and normal values published for children (Lang et al., 2005; Pettersen et al., 2008; Lee et al., 2014; Lee et al., 2017; Lipshultz et al., 2019).

EPS was performed in all patients at the time of inclusion to exclude underlying arrhythmogenic conditions and arrhythmia inducibility and to describe cardiac conduction system characteristics. The procedure was performed under mild sedation *via* the brachial or femoral vein (depending on joint retractions and grade of hyperlordosis). Baseline neuromuscular evaluation consisted of a detailed and standardized neurologic examination with diagnostic tests based on clinical indications. Retrospective clinical data from the referral center were collected. During follow-up, cardiologic and neurologic data were collected at least once a year; arrhythmia events were reviewed daily by long-term ILR software using a home monitoring system. Medical therapy and device implantation (pacemaker [PM] and implantable cardioverter defibrillator [ICD]) were indicated according to current clinical evidence.

For the echocardiogram analysis, a single observer obtained the following measurements at enrollment and during the follow-up: M-mode-derived left ventricular end-diastolic and end-systolic dimensions, left ventricular ejection fraction (LVEF; %) measured by the Simpson method, tricuspid annular plane systolic excursion (TAPSE; mm), mitral annular plane systolic excursion (MAPSE; mm), tissue doppler values from lateral and septal mitral annulus (cm/s), and spectral doppler E and A waves from inflow mitral and tricuspid filling pattern (cm/s). Global longitudinal strain (GLS [%]) was obtained from four-chamber view, and reference values (mean and p5) were obtained from published pediatric data (Koopman et al., 2019).

Cardiac events

Major cardiac events included cardiac death, heart transplant, and malignant arrhythmias, which were defined as sustained VT, ventricular fibrillation (VF), asystole, complete atrioventricular block (AVB), cardiac arrest from VT/VF (witnessed SCD occurring within 1 h of acute symptoms), or appropriate treatment (antitachycardia pacing or shock) by ICD. Minor cardiac events included worsening of heart failure, reaching DCM criteria, conduction system abnormalities (except complete AVB), supraventricular tachycardia, or any structural or functional echocardiographic abnormality according to updated definitions and references. The timing of each event was reported as age, years from clinical onset, and months from study enrollment.

Statistical analysis

Data were anonymized and stored in local institutions. We used StatCrunch (Pearson Education Inc.) for the statistical analysis. Categorical variables were expressed as numbers and percentages, and continuous variables were expressed as median and interquartile range (IQR). When appropriate, echocardiographic data were analyzed comparing enrollment and last control measurements. Because the data were not normally distributed, a Mann–Whitney *U* non-parametric test was used, as appropriate, for analysis of quantitative variables between two groups. A p-value of <0.05 was considered statistically significant. Graphical presentations of the major and minor events that occurred during follow-up were generated with GraphPad Software (Prism, version 9.1.1).

Results

Twenty-eight individuals (median age of 8.5 years at enrollment; IQR of 4–12.5 years) from 27 families were enrolled. Table 1

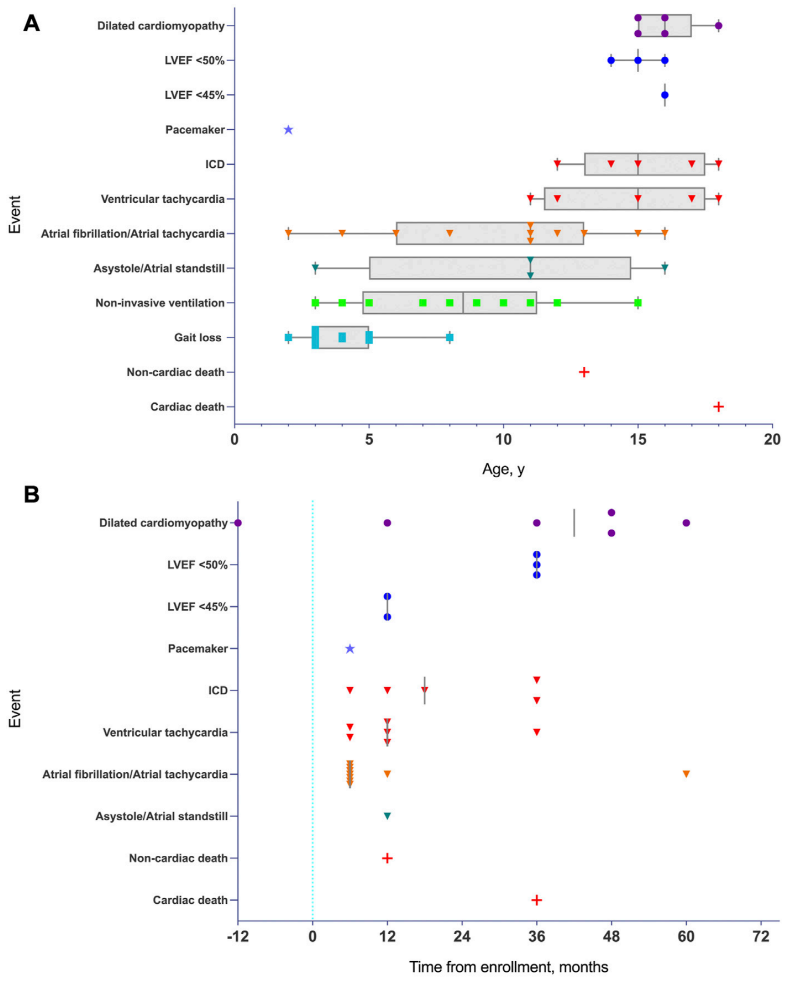


FIGURE 1
(A): Timeline of major and minor events in pediatric patients with lamin-related muscular dystrophy defined on the basis of age. Atrial arrhythmias, asystole/atrial standstill, and malignant arrhythmias preceded structural and cardiac functional abnormalities (DCM criteria or depressed LVEF). Median time values are represented by vertical grey line when required. DCM and LVEF are represented by a dot. Pacemaker are represented by a star. Arrhythmias and ICD are represented by an inverted triangle. Gait loss and NIMV are represented by a square, and death are represented by a cross. When required, minimum to maximum range age is represented in grey box and whiskers. Abbreviations: LVEF, left ventricular ejection fraction. **(B)**: Timeline of major and minor events in pediatric patients with lamin-related muscular dystrophy defined on the basis of enrollment (time 0 is the time of enrollment represented by a vertical blue dotted line). Median time values are represented by a vertical grey line. DCM and LVEF are represented by a dot, pacemaker is represented by a star, arrhythmias and ICD are represented by an inverted triangle and death is represented by a cross. Abbreviations: LVEF, left ventricular ejection fraction.

summarizes the main clinical and genetic features of our cohort (Bonne et al., 2000a; Quijano-roy et al., 2008; Chemla et al., 2010; Komaki et al., 2011; Pasqualin et al., 2014; Heller et al., 2017; Fan et al., 2020). The median age at last follow-up was 13 years (IQR of 8–17 years). All participants met clinical criteria and had confirmed *LMNA*-related neuromuscular disease. 13 (46.43%) had EDMD, 11 had L-CMD (39.28%), 2 (7.14%) had LGMD1B, and 2 (7.14%) had an *LMNA*-related atypical phenotype with mild weakness (Supplementary Figure S1). All had a pediatric onset of skeletal muscle symptoms, with most of them presenting before 2 years of age (23 of the 28 cases, 82%).

The median follow-up from study enrollment to last clinical evaluation was 4 years (IQR of 3–5 years) (Supplementary Figure S2). All living participants (except eight patients) had a comprehensive cardiac follow-up every 6 months that included echocardiography and remote cardiac rhythm monitoring. In the other eight patients, only clinical data and remote cardiac rhythm monitoring were available. Events by age and events by

TABLE 1 Clinical and genetic data.

Patient	Gender	Neuromuscular phenotype	Early onset	DCM	ICD/PM	Drugs	Death	LMNA de novo	LMNA c	LMNA p	Previously reported
1	M	L–CMD	Y	N	Y (ICD)	Carvedilol	N	Y	c.745C>T	p.Arg249Trp	Quijano, 2008/Komaki,2011/Pasqualin,2014/ Chemla, 2010/Heller, 2017/Fan, 2021
						ASA					
2	M	L–CMD	Y	N	Y (PM)	N	N	Y	c.745C>T	p.Arg249Trp	Quijano, 2008/Komaki,2011/Pasqualin,2014/ Chemla, 2010/Heller, 2017/Fan, 2021
3	F	EDMD	Y	Y	Y ICD)	Carvedilol Captopril Amiodarone	Y	Y	c.116A>G	p.Asn39Ser	Pasqualin, 2014/Fan,2021
						Furosemide					
						Spironolactone					
						ASA					
						LMWH					
4	F	L–CMD	Y	N	N	N	N	Y	c.91–93delGAG	p.Glu31del	Fan, 2021
5	M	EDMD	Y	Y	Y ICD)	Sotalol	N	Y	c.1358G>C	p.Arg453Pro	N
						Flecainide					
						ASA					
6	M	L–CMD	Y	N	N	N	N	Y	c.745C>T	p.Arg249Trp	Quijano, 2008/Komaki,2011/Pasqualin,2014/ Chemla, 2010/Heller, 2017/Fan, 2021
7	M	EDMD	Y	N	N	N	N	Y	c.116A>G	p.Asn39Ser	Pasqualin, 2014/Fan,2021
8	M	EDMD	N	N	N	N	N	Y	c.746G>A	p.Arg249Gln	Bonne, 2000/Komaki,2011/Fan,2021
9	M	EDMD	Y	Y	N	Carvedilol	N	Y	c.91delG	p.Glu31ArgfsTer65	N
10	F	L–CMD	Y	N	N	N	N	Y	c.89A>C	P.Gln30Pro	N
11	F	L–CMD	Y	Y	N	N	N	N	c.1487_1488+9del	–	N
12	M	EDMD	Y	N	N	N	N	Y	c.1616C>T	p.Ala539Val	N
13	M	EDMD	Y	Y	Y ICD)	Carvedilol	N	Y	c.112C>T	p.Leu38Phe	N
						Flecainide					
						ASA					
14	F	EDMD	Y	N	N	N	N	Y	c.745C>T	p.Arg249Trp	Quijano, 2008/Komaki,2011/Pasqualin,2014/ Chemla, 2010/Heller, 2017/Fan, 2021
15	M	EDMD	N	N	N	N	N	Y	c.812T>G	p.Leu271Arg	N

(Continued on following page)

TABLE 1 (Continued) Clinical and genetic data.

Patient	Gender	Neuromuscular phenotype	Early onset	DCM	ICD/PM	Drugs	Death	LMNA de novo	LMNA c	LMNA p	Previously reported
16	M	Mild Weakness	N	N	N	N	N	Y	c.879_881delCAG	p.Gln294del	N
17	M	EDMD	Y	Y	Y ICD)	Carvedilol	N	Y	c.1364G>C	p.Arg455Pro	N
18	M	Mild Weakness	N	N	N	N	N	Y	c.745C>T	p.Arg249Trp	Quijano, 2008/Komaki,2011/Pasqualin,2014/ Chemla, 2010/Heller, 2017/Fan, 2021
19	F	LGMD1B	Y	N	N	N	N	Y	c.810+ 1G>C	–	N
20	F	EDMD	Y	N	N	N	N	N	c.108G>T	p.Gln36His	N
21	M	L–CMD	Y	N	N	N	N	Y	c.104T>A	p.Leu35Gln	N
22	F	EDMD	N	N	N	N	N	Y	c.1357C>T	p.Arg453Trp	Fan, 2021/Bonne,2000
23	M	LGMD1B	Y	N	N	N	N	Y	c.1357C>T	p.Arg453Trp	Fan, 2021/Bonne,2000
24	F	EDMD	Y	N	N	Flecainide	Y	Y	c.91G>A	p.Glu31Lys	Fan, 2021
25	F	L–CMD	Y	N	N	N	N	Y (twin)	c.117T>G	p.Asn39Lys	Fan, 2021
26	F	L–CMD	Y	N	N	N	N	Y (twin)	c.117T>G	p.Asn39Lys	Fan, 2021
27	M	L–CMD	Y	N	N	N	N	Y	c.94_96delAAG	p.Lys32del	Fan, 2021
28	F	L–CMD	Y	N	N	N	N	Y	c.745C>T	p.Arg249Trp	Quijano, 2008/Komaki,2011/Pasqualin,2014/ Chemla, 2010/Heller, 2017/Fan, 2021

List of LMNA–related muscular dystrophy patients, major cardiac events, and rare LMNA variants. Abbreviations: L–CMD, LMNA–related congenital muscular dystrophy; EDMD, Emery–Dreifuss muscular dystrophy; LGMD1B, Limb–girdle muscular dystrophy 1B; DCM, dilated cardiomyopathy; ICD, implantable cardioverter defibrillator; PM, pacemaker; LMWH, low–molecular–weightheparin; ASA, acetylsalicylic acid

TABLE 2 Demographic data and overall follow-up.

Patient	Neuro muscular phenotypes	Early onset	Follow-up (time, years)	Gender	Age (y) at enroll/end follow-up	NIMV age (y)	Gait loss age (y)	LVEF simpson (%)		4-Chamber GLS (%)		ECG features during follow-up	Implantable loop recorder		DCM at end of follow-up	ICD/PM	Death	Drugs	LMNA c	LMNA p
								Enroll	Final	Enroll	Final		Atrial events	Ventricular events						
1	L-CMD	Y	4	M	11/15	12	8	63	54	−21	−20.6	Wide QRS	AT,AF	NSVT	N	Y (ICD)	N	Carvedilol	c.745C>T	p.Arg249Trp
												1st degreeAVB	Atrial standstill					ASA		
2	L-CMD	Y	6	M	2/8	N/A	3	59	57	−22	−19.5	High QRS voltage (V2–V6)	AT	–	N	Y (PM)	N	N	c.745C>T	p.Arg249Trp
3	EDMD	Y	2	F	16/18	15	3	50	26	−9	−7	1st degreeAVB	AT,AF	SVT, VF	Y	Y (ICD)	Y	Carvedilol	c.116A>G	p.Asn39Ser
												Wide QRS	Atrial standstill					Captopril		
												QS complex(V1)						Amiodarone		
												Poor R progression						Spironolactone		
												Repolarization abnormalities						ASA		
																		LMWH		
4	L-CMD	Y	3	F	9/12	5	3	54.7	69	−20.5	−17.6	N/A	AT	–	N	N	N	N	c.91–93delGAG	p.Glu31del
5	EDMD	Y	5	M	11/16	4	5	65	50	−21	−16.3	Wide QRS	AT,AF, Atrial standstill	NSVT	Y	Y ICD)	N	Sotalol	c.1358G>C	p.Arg453Pro
												IRBBB						Flecainide		
												1st degree AVB						ASA		
6	L-CMD	Y	5	M	9/14	9	5	51	N/A	−17.4	N/A	N/A	AT	–	N	N	N	N	c.745C>T	p.Arg249Trp
7	EDMD	Y	5	M	3/8	3	N/A	69	N/A	−21.5	N/A	N/A	AT	–	N	N	N	N	c.116A>G	p.Asn39Ser
8	EDMD	N	5	F	8/13	N/A	N/A	63	50	−19	−18.8	Global low voltage	–	–	N	N	N	N	c.746G>A	p.Arg249Gln
9	EDMD	Y	5	M	7/12	N/A	N/A	55	N/A	−20	N/A	N/A	AT	–	Y	N	N	Carvedilol	c.91delG	p.Glu31Alafs*65
10	L-CMD	Y	4	F	2/6	N/A	N/A	60.5	55	−26.4	−25	High QRS voltage (V2–V4)	–	–	N	N	N	N	c.89A>C	P.Gln30Pro
												Q wavesin III, V5–V6								
												Short PR segment								
11	L-CMD	Y	4	F	3/8	N/A	4	54	55	−22	−18	Normal	–	–	Y	N	N	N	c.1487_1488+9del	NA
12	EDMD	Y	3	M	15/18	N/A	N/A	59	61	−23.9	−17	Global low voltage	–	–	N	N	N	N	c.1616C>T	p.Ala539Val
												Short PR segment								
												Sinus bradycardia								
												Repolarization abnormalities								
13	EDMD	Y	4	M	15/19	10	4	58	52	−17.3	−13.7	Global low voltage	AT	NSVT	Y	Y ICD)	N	Carvedilol	c.112C>T	p.Leu38Phe
												IRBBB						Flecainide		
												Deep S wave in V3						ASA		

(Continued on following page)

TABLE 2 (Continued) Demographic data and overall follow-up.

Patient	Neuro muscular phenotypes	Early onset	Follow-up (time, years)	Gender	Age (y) at enroll/end follow-up	NIMV age (y)	Gait loss age (y)	LVEF simpson (%)		4-Chamber GLS (%)		ECG features during follow-up	Implantable loop recorder		DCM at end of follow-up	ICD/PM	Death	Drugs	LMNA c	LMNA p
								Enroll	Final	Enroll	Final		Atrial events	Ventricular events						
14	EDMD	Y	5	F	8/13	7	3	64	51.2	−23	−20.8	High QRS voltage (V4)	–	–	N	N	N	N	c.745C>T	p.Arg249Trp
15	EDMD	N	5	M	13/18	N/A	N/A	61	56.8	−19	−14.8	Global low voltage	–	–	N	N	N	N	c.812T>G	p.Leu271Arg
16	Mild Weakness	N	5	M	10/15	N/A	N/A	65	65	−21	−16.6	Normal	–	–	N	N	N	N	c.879_881delCAG	p.Gln294del
17	EDMD	Y	4	M	15/19	11	N/A	40	44	−13.6	−21	QS complex (V2)	–	NSVT	Y	Y ICD)	N	Carvedilol	c.1364C>C	p.Arg455Pro
												Poor R wave progression								
												Repolarization abnormalities								
18	Mild Weakness	N	3	M	18/21	N/A	N/A	68	N/A	−20	N/A	N/A	–	–	N	N	N	N	c.745C>T	p.Arg249Trp
19	LGMD1B	Y	5	F	11/16	N/A	N/A	61	N/A	23.8	N/A	N/A	–	–	N	N	N	N	c.810 +1G>C	N/A
20	EDMD	Y	5	F	3/8	N/A	3	64	N/A	−19	N/A	N/A	AT,AF	NSVT	N	N	N	N	c.108G>T	p.Gln36His
21	L-CMD	Y	5	M	3/8	N/A	3	63	N/A	−20	N/A	Normal	–	–	N	N	N	N	c.104T>A	p.Leu35Gln
22	EDMD	N	4	F	17/21	N/A	N/A	58.5	56.9	−19	−19.2	Global low voltage	–	–	N	N	N	N	c.1357C>T	p.Arg453Trp
23	LGMD1B	Y	3	M	5/8	N/A	3	63	60	−22	−21.7	Normal	–	–	N	N	N	N	c.1357C>T	p.Arg453Trp
24	EDMD	Y	1	F	12/13	8	N/A	57	58	−19.5	−20	Global low voltage	AT	–	N	N	Y	Flecainide	c.91G>A	p.Glu31Lys
												Repolarization abnormalities								
25	L-CMD	Y	2	F	5/7	N/A	N/A	54.5	56.7	−22.6	−23.1	Global low voltage	–	–	N	N	N	N	c.117T>G	p.Asn39Lys
												Poor R progression								
26	L-CMD	Y	2	F	5/7	N/A	N/A	61.1	59.5	−22.6	−28.8	Global low voltage	–	–	N	N	N	N	c.117T>G	p.Asn39Lys
												Poor R progression								
27	L-CMD	Y	1	M	4/5	N/A	N/A	57.2	66.9	−26.5	N/A	Poor R progression	–	–	N	N	N	N	c.94_96delAAG	p.Lys32del
28	L-CMD	Y	1	M	4/5	N/A	N/A	68	N/A	−23.5	N/A	Global low voltage	–	–	N	N	N	N	c.745C>T	p.Arg249Trp

List of LMNA-related muscular dystrophy patients, major and minor cardiac events, and rare LMNA mutations. Abbreviations: L-CMD, LMNA-related congenital muscular dystrophy; EDMD, Emery-Dreifuss muscular dystrophy; LGMD1B, Limb-girdle muscular dystrophy 1B; M, male; F, female; DCM, dilated cardiomyopathy; ICD, implantable cardioverter defibrillator; PM, pacemaker; AT, atrial tachycardia; AF, atrial fibrillation; NSVT, non-sustained ventricular tachycardia; SVT, sustained ventricular tachycardia; VF, ventricular fibrillation; GLS, global longitudinal strain; N/A, not available; AVB, atrioventricular block; IRBBB, incomplete right bundle branch block

enrollment date are represented graphically in Figure 1 (Figures 1A, B).

A total of 57.1% of participants were male. Two of the cases enrolled were female monozygotic twins. Families were originally from Spain (Bonne et al., 1999), the United Kingdom (Bonne et al., 2003), the United States (Bonne et al., 2003), Australia (Dubowitz, 1999), Canada (Quijano-roy et al., 2008), France (Quijano-roy et al., 2008), Greece (Quijano-roy et al., 2008), Russia (Quijano-roy et al., 2008), and Venezuela (Quijano-roy et al., 2008). Eleven patients (39.29%) had L-CMD, 13 (46.43%) had EDMD, 2 (7.14%) had LGMD1B, and 2 (7.14%) had an *LMNA*-related atypical phenotype with mild weakness. Early-onset skeletal muscle impairment before 2 years of age was detected in 23 of the 28 cases (82%). Table 1 summarizes the main clinical and genetic features of our cohort (Bonne et al., 2000a; Quijano-roy et al., 2008; Chemla et al., 2010; Komaki et al., 2011; Pasqualin et al., 2014; Heller et al., 2017; Fan et al., 2020). Eleven patients (39.2%; seven males) required non-invasive mechanical ventilation (NIMV) either at inclusion or during follow-up. Patients requiring NIMV had early-onset EDMD and L-CMD phenotypes. For those with gait loss, the median age for gait loss was 6 years (IQR of 5–8 years). Supplementary Table S1 compares our cohort with previous publications not focused on pediatric patients.

Major and minor cardiac events during follow-up

Cardiac function and structural heart disease

Table 2 and Supplementary Table S2 summarize major and minor cardiac events and statistical analyses of the echocardiographic data. During follow-up, rapid progression to DCM was seen in six cases (21.4%). (patient 3 [died], 5, 9, 11, 13, and 17), all showing early neuromuscular impairment before 2 years of age; five were diagnosed with early-onset EDMD, and one was diagnosed with L-CMD (patient 11).

Two of these patients showed LVEF values of less than 45% at last follow-up, and four showed DCM requiring an ICD. No right ventricle (RV) involvement was detected in our cohort, but advanced RV myocardial strain analysis could not be adequately performed because of suboptimal image quality from the RV related to chest wall deformities in the majority of patients.

LVEF was compared by the Simpson method in all 28 pediatric patients from enrollment to the end of follow-up, and a global reduction in LVEF during follow-up was found (LVEF 60.75% [IQR of 56–63.5] *versus* 56.75% [IQR of 51.6–59.75], $p < 0.05$; Figure 2A). Patients requiring an ICD (patients 1, 3, 5, 13, and 17) showed worse LVEF values at last follow-up than the rest of the cohort and were older (median age at enrollment of 15 years [IQR of 11–15] and median age at last follow-up of 18 years [IQR of 16–19]) than the rest of the cohort (median age at enrollment of 7 years [IQR of 3–11] and median age at last follow-up of 12 years [IQR of 8–15]). The median time of follow-up was 4 years in the group requiring an ICD (IQR of 4 to 4) and in the rest of the cohort (IQR of 3–5). Five patients showed LVEF values between 45% and 55% (patients 1, 5, 8, 13, and 14). No significant differences in TAPSE were observed when comparing values at enrollment with those at last follow-up (TAPSE of 19.9 mm [IQR of 15.3–22]). The value of TAPSE was ≤17 mm at last follow-up in four of five patients requiring an ICD. GLS (%) analyzed in the apical four-chamber view showed significantly lower values at enrollment than at last follow-up (−21 [IQR of −19 to −22.6] *versus* −17.6 [IQR of −16.3 to −20.6], $p = 0.01$; Figure 2B). At enrollment, GLS values below the mean (<−20.6%) were detected despite normal LVEF values (>55%) in most participants. At last follow-up, these patients showed decreasing GLS and LVEF (Figures 2A, 3B). No other significant findings were detected in the analysis of MAPSE, lateral E/E′ ratio, and septal E/E′ ratio.

Patients that finally developed DCM showed their first neuromuscular symptoms before 2 years of age (early-onset EDMD and L-CMD phenotypes) suggesting an early-onset aggressive form of laminopathy.

Arrhythmia and long-term loop recorder monitoring analysis

The ECG analysis is summarized in Table 2. Minor ECG abnormalities were present in 16 patients. Within these minor abnormalities, the following were described: first-degree AVB, wide QRS complex, short PR interval, global QRS low voltage, high QRS voltage V1–V4, QS complex in V1–V2, poor R progression, incomplete right bundle-branch block (IRBBB), abnormal Q waves, sinus bradycardia, and repolarization abnormalities. The most frequent finding (28.5%, eight patients) was global low QRS voltage that presented early; seven of eight cases had neither ventricular dysfunction nor DCM (Supplementary Figures S3A–C). The early-onset group showed the following ECG abnormalities before 2 years of age: global QRS low voltage and poor R progression.

EPS at enrollment showed seven patients (25%) with atrial conduction disorders (short non-sustained atrial tachycardia [$n = 6$] and intermittent atrial standstill [$n = 1$]) that did not merit any further treatment at that moment. No other forms of supraventricular or ventricular tachycardia were induced, and no accessory pathways were detected. HV intervals (time from the proximal His bundle to the ventricular myocardium) were within normal values. When retrospectively analyzing the EPS data in patients who presented with ventricular arrhythmia during follow-up, no abnormalities were observed.

In the ILR monitoring device analysis, malignant arrhythmia (VT/VF) was detected in five cases. (patients 1, 3 [died], 5, 13, and 17). Supplementary Table S3 summarizes the indication and the median age for device implantation. All had normal EPS results at enrollment and were never diagnosed with malignant arrhythmias at our center or from a referring hospital. In all five cases, malignant arrhythmias were detected a few months after enrollment, and they were then considered candidates for ICD implantation (Supplementary Table S3). Two of these patients that were diagnosed with VT through the ILR monitoring device had histories of intermittent pallor and fainting episodes. After ICD implantation, two patients received appropriate shocks. No inappropriate shocks were detected during follow-up. All patients carrying an ICD showed their first neuromuscular symptoms before 2 years of age.

In one participant with an L-CMD phenotype, a premature PM implantation was required because of symptomatic prolonged asystole (patient 2, 2-year-old boy). Patients 1, 3, and 5

(median age of 11 years [IQR of 11–16]) showed intermittent atrial standstill detected in traces from the ILR. During follow-up, atrial fibrillation was diagnosed in four cases (patients 1, 3, 5, and 20; median age at atrial fibrillation diagnosis of 11 years [IQR of 7.5–13.5]), and three were later diagnosed with DCM. Atrial tachycardia was diagnosed in 11 patients (patients 1–7, 9, 13, 20, and 24; median age at atrial tachycardia diagnosis of 11 years [IQR of 6–12]), and 4 were later diagnosed with DCM. Both atrial tachycardia and atrial fibrillation were present simultaneously in four patients. Example electrocardiographic traces from an ILR monitoring device are represented in Figure 4. Neither arrhythmia nor major cardiac events were detected within the patients with mild muscular impairment and late onset after 2 years old of age (patients 8, 15, 16, 18, and 22).

Demise

Two patients (2/28, 7.1%) died during follow-up. One (patient 3) was an 18-year-old female with intermittent atrial standstill diagnosed in the EPS and had an ICD due to VT/VF. The patient died because of rapidly progressive heart failure despite optimal treatment. Patient 24 was a 10-year-old female with no cardiovascular involvement who died due to respiratory infection. Both patients were early-onset EDMD, non-ambulant, required NIMV, and showed their first clinical manifestations of muscle weakness before 2 years of age.

Genetics and genotype-phenotype correlation

All 28 patients carried one rare variant in *LMNA* classified as likely pathogenic or pathogenic following American College of Medical Genetics (ACMG) guidelines and according to currently available data (Supplementary Table S4). Family segregation showed that in 26 cases (92.85%) the rare variant in *LMNA* was *de novo*. Family history of cardiac disease or neurological impairment was present in only two cases (patients 11 [c.1487_1488+9del] and 20 [p.Gln36His]). In both cases, the parents carried the same variant in *LMNA* and had mild neuromuscular impairment and malignant arrhythmias.

Twenty rare *LMNA* variants were identified (18 exonic and 2 intronic). Of all exonic rare variants, 4 were deletions, and 14 were missense. Thirteen rare variants (65%) were classified as likely pathogenic and seven (35%) as definitively pathogenic. Twelve variants were novel (two intronic, two deletions, and eight missense; Supplementary Table S4).

The most prevalent variant identified was p. Arg249Trp (six patients, 21.4%) located in exon 4 of *LMNA*. These six patients were diagnosed with L-CMD (four patients), EDMD (one patient), or lamin-related mild weakness (one patient). One patient showed malignant arrhythmias and required an ICD, and another patient needed a PM due to asystole (both L-CMD with dropped head). No DCM was detected with this rare variant. Figure 5 shows the correlation of genotype to phenotype of all 28 patients. The *LMNA* mutations identified in the six patients with DCM (patient 3 (died), 5, 9, 11, 13 and 17) were p. Asn39Ser, p. Arg453Pro, p. Glu31ArgfsTer65, c.1487_1488+9del, p. Leu38Phe, and p. Arg455Pro. In the five patients with ICDs implanted due to malignant arrhythmias (patients 1, 3 [died], 5, 13, and 17), all carried rare variants often identified in pediatric LMNA-muscular dystrophy patients (p.Arg249Trp, p. Asn39Ser, p. Arg453Pro, p. Leu38Phe and p. Arg455Pro, respectively). Four cases were diagnosed with EDMD and one with L-CMD. The two cases who died (patients 3 [with an ICD] and 24) carried also a rare variant (p.Asn39Ser and p. Glu31Lys, respectively), both located in exon 1 of *LMNA*. Both were diagnosed with early-onset EDMD and showed their first neuromuscular symptoms before 2 years of age.

Discussion

Laminopathies are a group of ultrarare genetic diseases attributable to pathogenic rare variants in the *LMNA* gene (Worman and Bonne, 2007; Bonne and Quijano-Roy, 2013). Due to its low prevalence, few cases have been diagnosed and reported so far, and there are not enough published data about its natural history. This fact, reinforces the importance of our prospective pediatric international registry including congenital phenotypes. Other multicenter laminopathy studies and expert consensus statement on arrhythmic risk have been published for adult populations (Pasotti et al., 2008; Rijsingen et al., 2012; Haas et al., 2014; Kumar et al., 2016; Peretto et al., 2019; Groh et al., 2022) but did not focus on cardiac involvement in children, as we describe here. Additionally, reports on cardiac impairment in pediatric laminopathies with neuromuscular involvement are lacking, despite that cardiac events can be present at early stages (Benedetti et al., 2007; Maggi et al., 2014; Fan et al., 2020; Groh et al., 2022). Our study offers longer follow-up in a pediatric population and valuable information about cardiac events, neuromuscular phenotypes, and genotype–phenotype correlations.

LMNA-related diseases cause cardiac symptoms in children and young adults that gradually worsen to bradyarrhythmias and tachyarrhythmias (Groh, 2012; Rijsingen et al., 2012; Rijsingen et al., 2013; Alexandra et al., 2015; Priori et al., 2015; Groh et al., 2022). Approximately half of patients need a PM or an ICD during adulthood (Taylor et al., 2003; Priori et al., 2015). Cardiac involvement occurs in a high percentage of cases, and implantation of preventative measures, such as an ICD, is recommended in patients with malignant arrhythmias (Pasotti et al., 2008; Rijsingen et al., 2012; Maggi et al., 2014; Groh et al., 2022). Pathogenesis of *LMNA*-related cardiomyopathy remains unclear, but lamin A/C haploinsufficiency may have negative effects on the heart (Wolf et al., 2008). Both fibroblasts from L-CMD patients and myoblasts from L-CMD mouse models demonstrate increased nucleoplasmic localization of lamin A/C compared to controls and EDMD. Mislocalization of nuclear envelope proteins leads to defects in myoblast differentiation, contributing to the more severe phenotype observed in L-CMD. (Bertrand et al., 2020). Clinical recommendations for these patients were identical to those for patients with other cardiomyopathies or heart failure: pharmacologic treatment with neurohormonal antagonists, diuretics, and vasodilators and non-pharmacological treatment with ventricular device therapy, such as early PM or resynchronization therapy for progressive conduction delays and an ICD to prevent SCD (Yancy et al., 2013; Priori et al., 2015; Bozkurt et al., 2016; Yancy et al., 2016; Atalaia et al., 2021). An expert

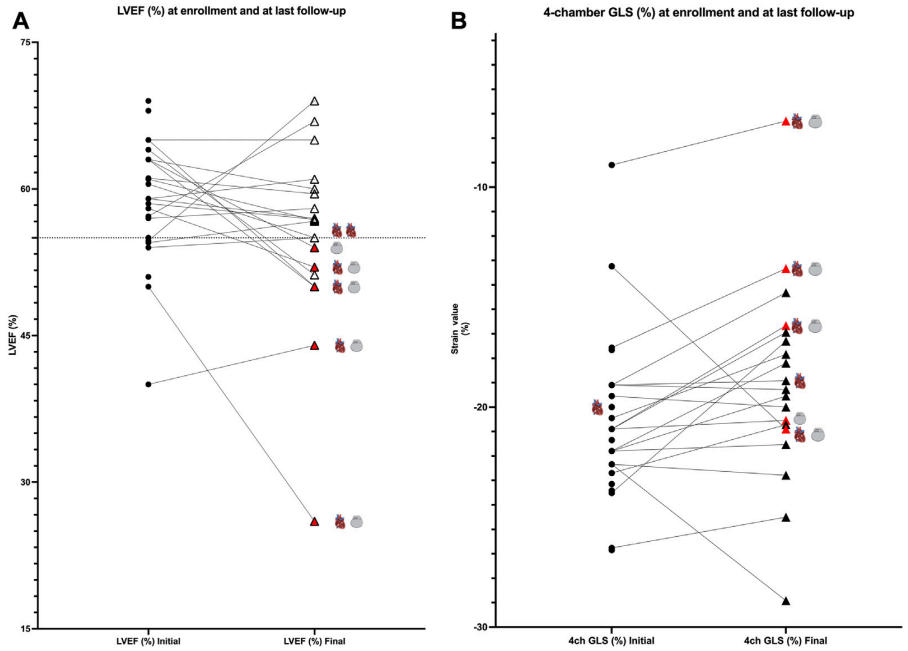


FIGURE 2
(A): comparison of values at enrollment and at last follow-up; LVEF values (%). Patients are represented as black dots at enrollment, and triangles represent patients at last follow-up. Red triangles show patients requiring an ICD (gray device represented in the graphic). Cardiomyopathy is represented by a heart. Black lines link the same patient in the two different moments of follow-up. **(B)**: comparison of values at enrollment and at last follow-up; four-chamber GLS values (%). Patients are represented as black dots at enrollment and triangles at last follow-up. Red triangles show patients requiring an ICD (gray device represented in the graphic). Cardiomyopathy is represented by a heart. Black lines link the same patient in the two different moments of follow-up.

consensus on evaluation and management of arrhythmic risk in neuromuscular disorders, published recently highlighted the especial recommendations for diagnostic testing and risk stratification in adults affected with EDMD or LGMD1B. Within the recommendations in adult population, implantable cardiac monitoring is reasonable even in the setting of a normal 12-lead ECG, ambulatory ECG monitoring and normal echocardiogram. Sustained arrhythmias, AVB and SCD are highly present in these patients and comprehensive risk stratification is needed, including EP study in select patients. Heart transplantation may be considered in certain cases with mild neuromuscular impairment (Taylor et al., 2003; Finsterer et al., 2006; Maggi et al., 2014; Pérez-Serra et al., 2016).

Six pediatric patients carrying a deleterious rare variant in *LMNA* concomitant with congenital heart disease (CHD) were previously found to have no major neuromuscular involvement (Baban et al., 2020) Most cases showed a family history of CHD and/or DCM associated with arrhythmias. Neither CHD nor aortic involvement were found in our cohort. Despite a rare deleterious variant in the *LMNA* gene, this previously reported phenotype is not similar to our study because most of our cases showed severe neuromuscular involvement, and rare *LMNA* variants are *de novo*. The wide range of phenotypes associated with rare pathogenic variants located in the *LMNA* gene are well known. During follow-up in a retrospective series of 15 pediatric patients carrying *LMNA* variants with early-onset neuromuscular symptoms, no major cardiac involvement was described (two cases of supraventricular arrhythmia, no malignant arrhythmias, no DCM, and no sudden death) or specific cardiovascular description (Jędrzejowska et al., 2021). In contrast to theses previous studies, our study characterizes the impact of LMNA variants in pediatric patients with both neuromuscular and cardiovascular involvement.

To our knowledge, no more than 10 reports have focused on pediatric laminopathies with both neuromuscular and cardiac involvement. In a retrospective study, 151 patients carrying a mutation in *LMNA* showed an early-onset phenotype, and the most frequent mutation was p. Arg249Trp; 63% of patients never acquired independent ambulation, and 37% died. Early cardiac interventions (heart medication and/or PM or ICD implantation) were usually associated with earlier respiratory interventions (intermittent positive pressure breathing, non-invasive ventilation of tracheostomy), and clinical severity was positively correlated across the triad of skeletal muscles, respiratory muscles, and myocardium. Correlation to cardiac device placement is less strongly linked with the timing of respiratory interventions and the progression of skeletal muscle weakness. Prospective natural history studies should therefore be conducted to further validate the stratification of

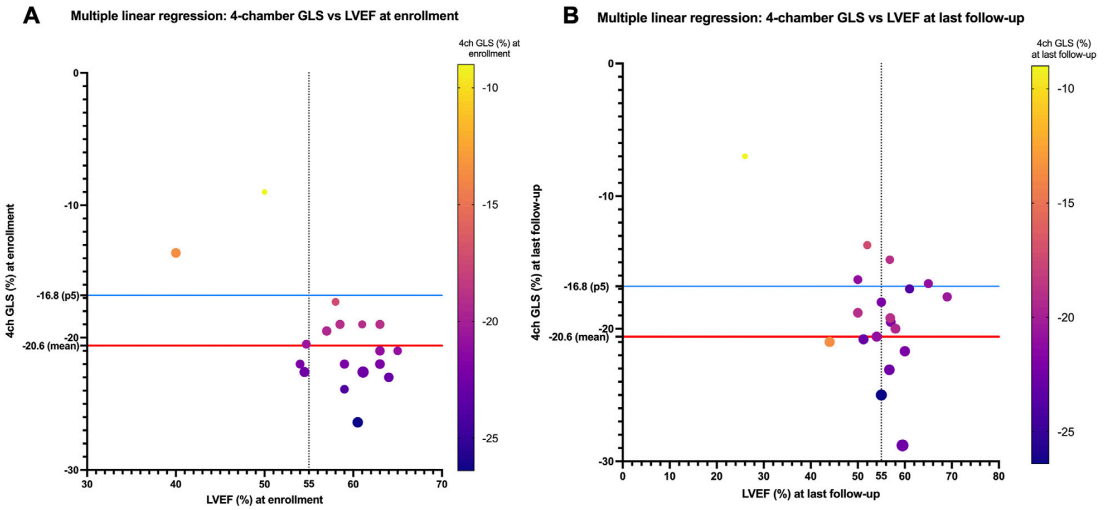


FIGURE 3
(A) Four-chamber GLS values (%) compared with LVEF values (%) for each patient at enrollment. At enrollment, despite normal LVEF (>55%), values below 20.6% are detected (20.6% is the mean normal value published in children). These LVEF values show a decreasing trend at last follow-up **(B)**.

L-CMD (Ben Yaou et al., 2021). We performed a prospective cardiac natural history of pediatric patients with *LMNA*-related muscular dystrophy.

Other studies included no more than eight patients, were retrospective, and had no adoption of preventive arrhythmogenic measures or follow-up (Bonne et al., 2000b; Komaki et al., 2011; Pasqualin et al., 2014; Parent et al., 2015; Petillo et al., 2015; Tan et al., 2015; Heller et al., 2017). In our cohort, patients 1, 2, 6, 14, 18, and 28 carried variant p. Arg249Trp, which is present in pediatric patients with arrhythmic complications without major ventricular dysfunction and could be related to neuromuscular severity (Komaki et al., 2011; Ben Yaou et al., 2021). Patient 3 and patient seven carried p. Asn39Ser, which was previously described in two patients (one died of cardiac arrest due to malignant arrhythmias, and one had premature ventricular contractions [PVCs]) (Pasqualin et al., 2014). Patient 8 carried p. Arg249Gln, which was previously reported in children with arrhythmias (Bonne et al., 2000b; Komaki et al., 2011; Heller et al., 2017).

Cardiac involvement by neuromuscular phenotype

Lamin-related congenital muscular dystrophy/ dropped head phenotype

Two groups are distinguished depending of the severity of onset, a very early form with arrest of motor development before the age of 6 months (no sitting or walking acquisition) and another with initial normal or subnormal motor milestones and subsequent loss, beginning by a characteristic presentation of loss of head support (dropped head syndrome) (Quijano-roy et al., 2008). All children have a progressive course with an initial rapid decline in cervical and axial tonus strength followed by a period of slower progression or plateau. Respiratory insufficiency is a major complication in the course of both entities, being responsible for early death in the early severe group, as early as the first 2 years of age (if no adequate ventilatory support is provided), or leading to mechanical ventilation which can evolve from non-invasive to invasive ventilation *via* tracheostomy in few years. Cardiac involvement is rarely observed initially in these children and is often subclinical in very young patients, but SCD can occur (Quijano-roy et al., 2008); therefore, routine cardiac follow-up is recommended (Quijano-roy et al., 2008; Lu et al., 2011). Our study revealed only one case (dropped head) with mild DCM with borderline LVEF values diagnosed before 10 years of age. During our follow-up, malignant arrhythmia was detected in one case (patient 1, dropped head) and an ICD was implanted at 12 years of age. Four patients showed atrial tachycardia, and one patient (patient 1, dropped head and non-ambulant) showed simultaneous atrial fibrillation and atrial tachycardia starting at 11 years of age. An ambulant child with dropped head syndrome (patient 2) had asystole and needed a PM. Both patients carried the same pathogenic rare *LMNA* variant p. Arg249Trp, which is the most frequent in pediatric squeletal laminopathies and is associated with worse clinical prognosis (Ben Yaou et al., 2021). These results support the need for close follow-up, which could show subclinical cardiac involvement in pediatric patients diagnosed with L-CMD. In a recent publication, echocardiography abnormalities were identified in 22% of L-CMD patients, and device implantation (ICD and PM) was described in 9% and 7% of the cohort, respectively (Ben Yaou et al., 2021); however, there is a lack information about the cause of death and reason for device implantation (Heller et al., 2017; Ben Yaou et al., 2021).

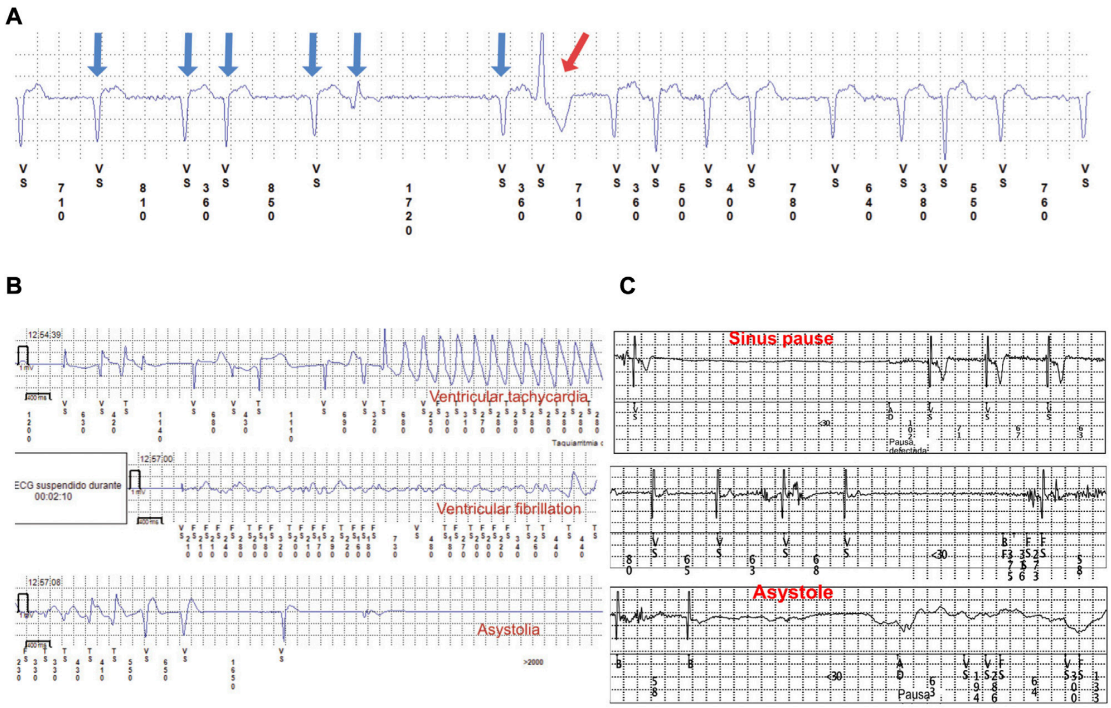


FIGURE 4
Three ECG traces from the ILR. **(A)** Atrial fibrillation (blue arrows) and a ventricular extrasystole (red arrow). **(B)** Ventricular tachycardia, VF, and asystole from a pediatric patient diagnosed with EDMD. **(C)** Sinus pause and asystole in a 3-year-old patient.

Emery-dreifuss muscular dystrophy

EDMD is the third most prevalent muscular dystrophy, and most patients present with autosomal-dominant EDMD due to *LMNA* (with higher risk of VT and DCM). *EMD* (emerin) gene is less frequent, with X-linked transmission (Bonne et al., 1999; Bonne and Quijano-Roy, 2013). However, more than 60% of EDMD cases have no deleterious variant in *EMD* or *LMNA* genes. Cardiac complications in EDMD patients can be life-threatening and lead to progressive cardiac failure, cardiac conduction disease, and SCD. DCM may occur at an advanced stage, but conduction disease is frequent (complete heart block, silent atria, atrial tachycardia, atrial fibrillation, and VT) (Figures 4B, C). Patients with EDMD are also at risk of cerebral emboli and sudden death (Bonne et al., 1999; Lu et al., 2011; Bonne and Quijano-Roy, 2013; Cattin et al., 2013; Finsterer et al., 2015; Priori et al., 2015; Marchel et al., 2021a; Marchel et al., 2021b; Groh et al., 2022). In our study, early presentation of atrial tachycardia (seven patients), atrial fibrillation (three patients), and different grades of ventricular dysfunction (six patients) that led to heart failure and malignant arrhythmias (four patients) were detected in early-onset EDMD children. Four patients required an ICD because of malignant arrhythmias (between 14 and 18 years old at ICD implantation). Surprisingly, all showed an EPS with no ventricular arrhythmia Inducibility, and the arrythmias were instead detected with the ILR. These malignant arrhythmias corresponded to sustained and non-sustained VT, and all were asymptomatic except patient 3 who exhibited pallor and seizures during VT/VF episodes. As previously described (Steckiewicz et al., 2016; Marchel et al., 2021a), the low rate of symptoms and presentation of atypical symptoms could make the global management of patients with EDMD difficult, worsening the prognosis. Antiaggregant therapy was needed in four patients because of atrial standstill, and one patient (patient 3) presented with an episode of cerebral emboli at 17 years of age, likely of multifactorial origin, before starting treatment. *LMNA* carriers have an increased risk of thromboembolic events, with an inherent risk independent of heart condition (Rijsingen et al., 2012; Rijsingen et al., 2013; Van Rijsingen et al., 2013; Groh et al., 2022) This thromboembolic risk could be related to cardiac dysfunction secondary to DCM and/or cardiac conduction disease, such as silent atria, sinus node dysfunction, sinus pauses, and atrial fibrillation (Groh et al., 2022). Long-term systemic anticoagulation therapy with a vitamin K antagonist and international normalized ratio (INR) target of 2.0–3.0 would be reasonable in DCM patients with arrhythmias, previous thromboembolic events, thrombophilic conditions, or an ejection fraction of ≤25%, but there is no evidence in pediatric laminopathies and traditional algorithms, such as CHA_2DS_2-VASc, are inappropriate for children. When silent atria or atrial fibrillation are present in children, long-term

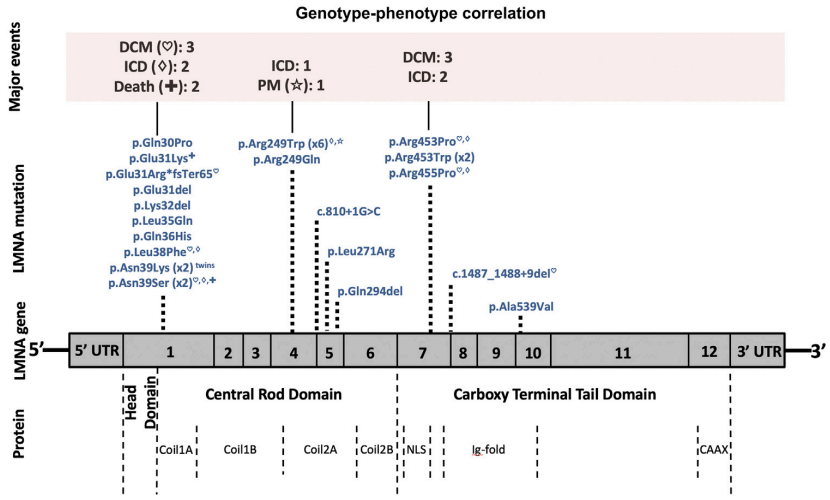


FIGURE 5
Phenotype–genotype graphic correlation. A schematic representation of the *LMNA* gene is shown. Top, major cardiac events, followed by rare pathogenic/likely pathogenic *LMNA* variants identified in our patients with the corresponding amino acid or nucleotide changes. Bottom, structural protein: the coil 1A–1B and coil 2A–2B constituting the α-helical rod domain. Abbreviations: DCM, dilated cardiomyopathy; ICD, implantable cardioverter defibrillator; PM, pacemaker; NLS, nuclear location signal; Ig, immunoglobulin.

anticoagulation therapy is only indicated when there are other risk factors (previous stroke, transient ischemic attack, hypertension, or heart failure). As atrial arrhythmias, immobilization, and heart failure are usually concomitant findings in *LMNA*-related muscular dystrophy patients, long-term systemic anticoagulation therapy may benefit higher-risk patients, for example, those with a LVEF of ≤25% and atrial arrhythmias. If patients have any contraindication to receive vitamin K antagonist or low-molecular-weight heparin, aspirin should be recommended (Monagle, 2012; Giglia et al., 2015). We observed that patients diagnosed with early-onset EDMD are at higher risk of severe cardiac involvement, mainly DCM in those older patients of our cohort, while life-threatening arrhythmias without DCM appear earlier in L-CMD patients. These findings are in agreement with previous studies (Maggi et al., 2014; Fan et al., 2020; Marchel et al., 2021b), but more studies should be performed. Arrhythmic events predict myocardial involvement in pediatric patients carrying a deleterious *LMNA* variant (Bonne et al., 2000b; Komaki et al., 2011; Pasqualin et al., 2014; Parent et al., 2015; Petillo et al., 2015; Tan et al., 2015; Heller et al., 2017; Groh et al., 2022) reinforcing the importance of investigating cardiac involvement in childhood laminopathy cohorts, even in the absence of clear neuromuscular involvement (Baban et al., 2020). Our results showed a high rate of arrhythmias at pediatric age, so early detection and treatment for each case according to guidelines is critical to preventing complications, improving prognosis, and avoiding death.

Limb-girdle muscular dystrophy type 1B

LGMD1B is a subtype of limb–girdle muscular dystrophy that presents with progressive shoulder and hip gridle weakness with prior effects on the inferior limbs *versus* the upper limbs. LGMD1B is autosomal dominant and associated with AV conduction defects, supraventricular arrhtyhmias, and ventricular arrthythmias, but late-onset DCM (Muchir et al., 2000; Lu et al., 2011) and SCD (Finsterer et al., 2015; Groh et al., 2022) have been reported. Evaluation and management of arrhythmic risk are similar to EDMD patients as published in expert consensus statement in adult population with neuromuscular disorders (Groh et al., 2022). We saw no patients of pediatric age (8 and 16 years old at last control) diagnosed with DCM and no ventricular dysfunction, and the ILR registries showed no arrhythmias.

LMNA-related atypical phenotype with mild weakness

Patients with *LMNA*-related atypical or 'undefined' phenotype were identified in this study because they did not reach phenotypic criteria for L-CMD, EDMD, and LGMD1B phenotypes. They showed an intermediate phenotype between 'dropped head' and 'early EDMD' (mild weakness, selective hypotrophy and weakness in quadriceps and elbow flexors, mild retractions in elbows, hamstrings, or paraspinal muscles, and mild weakness in neck flexors and foot extensors) as it has been showed in the literature, confirming that these phenotypes are not different entities but probably a continuum (Maggi et al., 2016). Some patients have shown DCM in the literature (Maggi et al., 2014). Our study included two patients (10 and 18 years old at enrollment, 5 and 3 years of follow-up respectively) that showed late-onset muscular manifestations, and none presented with cardiovascular involvement during follow-up at pediatric age. These findings support the idea that early-onset, severe muscular dystrophy is associated with earlier and more severe cardiac involvement.

Early-onset clinical manifestations before 2 years of age

Patients with early-onset neuromuscular impairment could show early cardiac manifestations, as published recently in a retrospective review (Ben Yaou et al., 2021). Twenty-three of the 28 patients included in our cohort showed early-onset muscle impairment before 2 years of age. Those patients diagnosed with DCM during follow-up had an early-onset phenotype (L-CMD and early-onset EDMD), and no patients with late-onset phenotypes were candidates for an ICD during follow-up. These findings suggest that earlier onset neuromuscular impairment may lead to a worse cardiac phenotype and higher risk of SCD. It would therefore be reasonable to implant an ILR device in LMNA children presenting inf the first 2 years of life with muscle weakness.

Echocardiographic analysis during follow-up

Despite a preserved LVEF, longitudinal myocardial strain analysis speckle tracking could show different patterns indicative of early abnormal segmental strain deformation (especially septal strain compared with non-septal strain), post-systolic deformation, and mechanical dispersion (Hasselberg et al., 2014; Haugaa et al., 2015; Boer et al., 2017). (Hasselberg et al., 2014; Haugaa et al., 2015; Boer et al., 2017). This myocardial strain analysis is well reported in other neuromuscular diseases, such as cardiomyopathy-related Duchenne muscular dystrophy. In our study, echocardiographic analysis during follow-up suggested that pediatric laminopathies with neuromuscular involvement are linked to relatively rapid progression to DCM and low LVEF values in a few years. Despite well-preserved LVEF values, four-chamber GLS values at an early age could identify patients that may need an ICD at pediatric age. To our knowledge, this is the first publication that suggests this relationship early during childhood (Supplementary Figure S4). The RV may be involved in *LMNA*-related cardiomyopathy with or without neuromuscular disease (Quarta et al., 2012; Forleo et al., 2015; Peretto et al., 2019; Marchel et al., 2021b). The lower TAPSE values found in patients that eventually needed an ICD could be associated with RV involvement described previously in adult series with an EDMD phenotype, (Buckley et al., 1999; Marchel et al., 2021a; Marchel et al., 2021b), but the suboptimal acoustic window did not allow for an exhaustive analysis to describe a strong trend or relationship.

Arrhythmia and death

Typical early manifestations of arrhythmia in an ECG are flat P wave, AVB, and supraventricular and ventricular arrhythmias (Maggi et al., 2016; Finocchiaro et al., 2020). Other published ECG features include LV hypertrophy data, ST depression, wide QRS complex, and P terminal force. Septal remodeling data in leads V1–V3 seem to be frequent (Ollila et al., 2017), such as Q waves in V1–V2, fragmented QRS in V2–V3, RV1>RV2, RV2>RV3, and poor R wave progression. During follow-up, 12-lead ECG and 24-h Holter monitoring are recommended at least yearly. In our study, first degree AVB, flat P wave, LV hypertrophy data, fragmented and wide QRS complex, poor R wave progression, global low QRS voltage (Supplementary Figure S3C), IRBBB, RBBB, and LBBB were detected during follow-up, in agreement with previous reports (Maggi et al., 2016; Ollila et al., 2017; Finocchiaro et al., 2020). According to our data, ILR with home monitoring is useful for early detection of potential life-threatening arrhythmias, which are usually asymptomatic (Heller et al., 2017).

LMNA carriers may experience DCM and SCD before they experience overt heart failure, as ~30% of patients will have SCD and another 30% will develop congestive heart failure. Males carrying a deleterious *LMNA* variant have worse prognosis because of malignant arrhythmias and heart failure. Laminopathies are the third neuromuscular disease in which SCD is frequently reported (Finsterer and St, 2016). In our pediatric study, and in agreement with previously published reports in adults, malignant arrhythmias are related in four/five cases to male sex, but death was related to female sex in early-onset EDMD (Marchel et al., 2021b). Our study may not be large enough to make conclusions about these trends. Atrial conduction disease and malignant arrhythmias are detected during pediatric follow-up that may lead to thromboembolism, heart failure, and SCD, especially in pediatric patients diagnosed with early-onset EDMD and L-CMD. Because there are no detailed recommendations on cardiovascular management in consensus guidelines (Wang et al., 2010), a specific protocol to detect arrhythmias is needed in this pediatric population because SCD may occur before heart failure (Berlo et al., 2005; Lu et al., 2011). We propose a tentative protocol (Supplementary Protocol S1).

The role of EPS in pediatric neuromuscular disease has not been reported; however, it would be reasonable to include it during follow-up childhood and adulthood if any symptoms (typical or atypical) occur because it could be related to potentially malignant arrhythmia. As suggested in adult population with EDMD or LGMD1B, when individuals exhibits symptoms consistent with bradycardia or VT-related symptoms or ECG shows conduction disorder, EP study may be considered for risk stratification for sustained arrhythmias, AVB and SCD (Groh et al., 2022). Our cohort includes early-onset phenotypes in the majority of patients. In our prospective cohort, early-onset patients characterized by their first neuromuscular impairment before 2 years of age seem to have a direct relationship with major and minor cardiac events, regardless of the final neuromuscular phenotype, as previously suggested (Ben Yaou et al., 2021). We therefore propose a clinical protocol for a comprehensive cardiac assessment of pediatric patients with *LMNA*-related muscular dystrophy.

Limitations

Our study has some limitations. Our hospital is an international reference center for neuromuscular diseases, and the most severe patients are referred to our institution. We had missing echocardiographic data because some patients were recruited internationally and there was no follow-up. *LMNA*-related muscular dystrophy is a very rare, often underdiagnosed, disease; therefore, all collected data are of great value.

Additionally, cardiac magnetic resonance imaging (CMRI) was not consistently available for all patients during follow-up. CMRI with late gadolinium enhancement imaging is recommended to study the presence and distribution of myocardial fibrosis. Fibrosis is frequently located along the interventricular septum, according to the worst values in the

myocardial strain analysis by speckle tracking. Underlying septal fibrosis could explain the high incidence of ventricular arrhythmias, cardiac conduction delays, and ventricular dysfunction (Holmström et al., 2011; Muscogiuri, 2017). Biomarkers may help during cardiac follow-up, such as N-terminal probrain natriuretic peptide (NT proBNP), a well-recognized biomarker that increases when ventricular function worsens (Yancy et al., 2016; Marchel et al., 2021b). In our series, NT proBNP was not homogeneously analyzed, and it might help in some cases with a high risk of ventricular dysfunction. As published previously, proteomic analysis of plasma samples could be used in the future to identify individuals with a high risk of sudden death with *LMNA*-related cardiomyopathy (Izquierdo et al., 2016). Our study also lacks analysis of other genes that may be implicated in laminopathies or related muscular diseases. In the future, whole-exome sequencing and/or whole-genome sequencing can be used to identify new alterations in any region of genome.

Conclusion

Malignant arrhythmias and SCD in *LMNA*-related muscular dystrophy occurs frequently, but no comprehensive studies focused on early identification, adoption of preventative measures, and follow-up have been performed. ILR with home monitoring identified five cases (17%) with malignant arrhythmias, and ICDs were implanted for prevention. Two cases with an ICD showed appropriate shocks. ILR may be critical for early diagnosis of life-threatening arrhythmias in laminopathies with an early-onset neuromuscular phenotype. Remote home monitoring helps for close follow-up. Echocardiographic follow-up, including myocardial strain analysis, might be helpful to identify worse prognosis in patients because DCM is present early before adulthood. Specific clinical guidelines that include management in children and emphasize the use of ILR are needed to standardize treatment and mitigate the risk of SCD, especially in those with early-onset phenotypes.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Hospital Sant Joan de Déu (Identification code: PIC-59-14). Written informed consent to participate in this study was provided by the participants and legal guardian/next of kin.

Author contributions

GS-B, SC, OC, JB, and AN developed the concept and prepared the manuscript. SC, JC, VF, CB-R, and EM-B acquired, pre-processed, and analyzed the data. GS-B, SC, OC, and JB supervised the study. IZ, DN-dB, CB-R, LC, JE, RB, ID, MG, and SQ-R contributed to manuscript revision. All authors have read and agreed to the published version of the manuscript.

Funding

This work has also been supported by Obra Social "La Caixa Foundation" (LCF/PR/GN19/50320002) and Instituto de Salud Carlos III, Fondo Investigación Sanitaria -FIS- (PI21/00094). CIBERCV is an initiative of the ISCIII, Spanish Ministry of Economy and Competitiveness.

Acknowledgments

This is the result of a hard work from patients, families and professionals working together to find answers. This long-term work could not have been possible without the unconditional support of "Fundación Andrés Marcio–Niños contra la Laminopatía" to who we are deeply thankful.

We would like to truly thank all our patients and their families, especially to Carlota who inspired us to become better doctors.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer AF declared a shared affiliation with the authors ID, MG, SQR to the handling editor at the time of review.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2023.1142937/full#supplementary-material

References

Aebi, U., Cohn, J., Buhle, L., and Gerace, L. (1986). The nuclear lamina is a meshwork of intermediate-type filaments. *Nature* 323 (6088), 560–564. Internet.

Alastalo, T-P., West, G., Li, S-P., Keinänen, A., Helenius, M., Tyni, T., et al. (2015). LMNA mutation c.917T>G (p.L306R) leads to deleterious hyper-assembly of lamin A/C and associates with severe right ventricular cardiomyopathy and premature aging. *Hum. Mutat.* 36 (7), 694–703. doi:10.1002/humu.22793

Alexandra, P., Rocío, T., Oscar, C., Paola, B., Iglesias, A., Alipio, M., et al. (2015). A novel mutation in lamin A/C causing familial dilated cardiomyopathy associated with sudden cardiac death. *J. Card. Fail* 21, 217–225. Internet. doi:10.1016/j.cardfail.2014.12.003

Andrés, V., and González, J. M. (2009). Role of A-type lamins in signaling, transcription, and chromatin organization. *J. Cell Biol.* 187 (7), 945–957. doi:10.1083/jcb.200904124

Atalaia, A., Ben Yaou, R., Wahbi, K., De Sandre-Giovannoli, A., Vigouroux, C., and Bonne, G. (2021). Laminopathies' treatments systematic review: A contribution towards a 'treatabolome. ' *J. Neuromuscul. Dis.* 8 (3), 419–439. doi:10.3233/JND-200596

Baban, A., Cicenia, M., Magliozzi, M., Gnazzo, M., Cantarutti, N., Silvetti, M. S., et al. (2020). Cardiovascular involvement in pediatric laminopathies. Report of six patients and literature revision. *Front. Pediatr.* 8, 374–379. doi:10.3389/fped.2020.00374

Ben Yaou, R., Yun, P., Dabaj, I., Norato, G., Donkervoort, S., Xiong, H., et al. (2021). International retrospective natural history study of LMNA -related congenital muscular dystrophy. *Brain Commun.* 3 (3), fcab075–15. doi:10.1093/braincomms/fcab075

Benedetti, S., Menditto, I., Degano, M., Rodolico, C., Merlini, L., D'Amico, A., et al. (2007). Phenotypic clustering of lamin A/C mutations in neuromuscular patients. *Neurol. [Internet]* 69 (12), 1285–1292.

Berlo, J. H. V., Voogt, W. G. D., Rabah, B., Yaou, B., Duboc, D., Rossenbacker, T., et al. (2005). Meta-analysis of clinical characteristics of 299 carriers of LMNA gene mutations: do lamin A/C mutations portend a high risk of sudden death. *J. Mol. Med.* 83, 79–83. doi:10.1007/s00109-004-0589-1

Bertrand, A. T., Brull, A., Azibani, F., Benarroch, L., Chikhaoui, K., Stewart, C. L., et al. (2020). Lamin A/C assembly defects in LMNA-congenital muscular dystrophy is responsible for the increased severity of the disease compared with emery-dreifuss muscular dystrophy. *Cells* 9 (4), 844. doi:10.3390/cells9040844

Bertrand, A. T., Chikhaoui, K., Yaou, R. B., and Bonne, G. (2011). Clinical and genetic heterogeneity in laminopathies. *Biochem. Soc. Trans.* 39 (6), 1687–1692. doi:10.1042/BST20110670

Boer, S. L. D., Gideon, J., Sarvaas, M., Klitsie, L. M., Iperen, G. G. V., Tanke, R. B., et al. (2017). Distribution of strain patterns in children with dilated cardiomyopathy. *Echocardiography* 34, 881–887. doi:10.1111/echo.13548

Bonne, G., Di Barletta, M. R., Varnous, S., Bécane, H. M., Hammouda, E. H., Merlini, L., et al. (1999). Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. *Nat. Genet.* 21, 285–288. doi:10.1038/6799

Bonne, G., Mercuri, E., Muchir, A., Urtizberea, A., Bécane, H. M., Recan, D., et al. (2000). Clinical and molecular genetic spectrum of autosomal dominant Emery-Dreifuss muscular dystrophy due to mutations of the lamin A/C gene. *Ann. Neurol.* 48 (2), 170–180. doi:10.1002/1531-8249(200008)48:2<170::aid-ana6>3.0.co;2-j

Bonne, G., Mercuri, E., Muchir, A., Urtizberea, A., Bécane, H. M., Recan, D., et al. (2000). Clinical and molecular genetic spectrum of autosomal dominant Emery-Dreifuss muscular dystrophy due to mutations of the lamin A/C gene. *Ann. Neurol.* 48 (2), 170–180. doi:10.1002/1531-8249(200008)48:2<170::aid-ana6>3.0.co;2-j

Bonne, G., and Quijano-Roy, S. (2013). Emery-Dreifuss muscular dystrophy, laminopathies, and other nuclear envelopathies. *Handb. Clin. Neurol.* 113, 1367–1376. doi:10.1016/B978-0-444-59565-2.00007-1

Bonne, G., Yaou, R. B., Béroud, C., Boriani, G., Brown, S., de Visser, M., et al. (2003). 108th ENMC international workshop, 3rd workshop of the MYO-CLUSTER project: EUROMEN, 7th international emery-dreifuss muscular dystrophy (EDMD) workshop, 13–15 september 2002, naarden, The Netherlands. *Neuromuscul. Disord.* 13 (6), 508–515. doi:10.1016/s0960-8966(03)00063-4

Bozkurt, B., Colvin, M., Cook, J., Cooper, L. T., Deswal, A., Fonarow, G. C., et al. (2016). Current diagnostic and treatment strategies for specific dilated cardiomyopathies: A scientific statement from the American heart association. *Circulation* 134, 579–646.

Buckley, A. E., Dean, J., and Mahy, I. R. (1999). Cardiac involvement in Emery dreifuss muscular dystrophy: A case series. *Heart* 82 (1), 105–108. doi:10.1136/hrt.82.1.105

Burke, B., and Stewart, C. L. (2012). The nuclear lamins: Flexibility in function. *Nat. Rev. Mol. Cell Biol. [Internet]* 14, 13–24. doi:10.1038/nrm3488

Carboni, N., Politano, L., Floris, M., Mateddu, A., Solla, E., Olla, S., et al. (2013). Overlapping syndromes in laminopathies: A meta-analysis of the reported literature. *Acta Myol.* 32 (5), 7–17.

Cattin, M-E., Muchir, A., and Bonne, G. (2013). State-of-the-heart" of cardiac laminopathies. *Curr. Opin. Cardiol.* 28 (3), 297–304. doi:10.1097/HCO.0b013e32835f0c79

Chemla, J. C., Kanter, R. J., Carboni, M. P., and Smith, E. C. (2010). Two children with "dropped head" syndrome due to lamin A/C mutations. *Muscle Nerve* 42 (5), 839–841. doi:10.1002/mus.21820

Dauer, W. T., and Worman, H. J. (2009). The nuclear envelope as a signaling node in development and disease. *Dev. Cell [Internet]* 17 (5), 626–638. doi:10.1016/j.devcel.2009.10.016

Dechat, T., Shimi, T., Adam, S. A., Rusinol, A. E., Andres, D. A., Spielmann, H. P., et al. (2007). Alterations in mitosis and cell cycle progression caused by a mutant lamin A known to accelerate human aging. *Proc. Natl. Acad. Sci. U. S. A. [Internet]* 104 (12), 4955–4960. doi:10.1073/pnas.0700854104

Dittmer, T., and Misteli, T. (2011). The lamin protein family. *Genome Biol.* 12 (5), 222. doi:10.1186/gb-2011-12-5-222

Dobrzynska, A., Gonzalo, S., Shanahan, C., and Askjaer, P. (2016). The nuclear lamina in health and disease. *Nucleus* 7 (3), 233–248. Internet. doi:10.1080/19491034.2016.1183848

Dubowitz, V. (1999). 68th ENMC international workshop (5th inter-national workshop) on congenital muscular dystrophy, 9-11 april 1999, naarden, The Netherlands. *Neuromuscul. Disord.* 9, 446–454. doi:10.1016/s0960-8966(99)00074-7

Fan, Y., Tan, D., Song, D., Zhang, X., Chang, X., Wang, Z., et al. (2020). Clinical spectrum and genetic variations of LMNA -related muscular dystrophies in a large cohort of Chinese patients. *J. Med. Genet.* 58, 326–333. doi:10.1136/jmedgenet-2019-106671

Feingold, B., Mahle, W. T., Auerbach, S., Clemens, P., Domenighetti, A. A., Jefferies, J. L., et al. (2017). Management of cardiac involvement associated with neuromuscular diseases: A scientific statement from the American heart association. *Circulation* 136, 200–231. doi:10.1161/CIR.0000000000000526

Finocchiaro, G., Merlo, M., Sheikh, N., De Angelis, G., Papadakis, M., Olivotto, I., et al. (2020). The electrocardiogram in the diagnosis and management of patients with dilated cardiomyopathy. *Eur. J. Heart Fail* 22 (7), 1097–1107. doi:10.1002/ejhf.1815

Finsterer, J., Ramaciotti, C., Wang, C. H., Wahbi, K., Rosenthal, D., Duboc, D., et al. (2010). Cardiac findings in congenital muscular dystrophies. *Pediatrics* 126 (3), 538–545. doi:10.1542/peds.2010-0208

Finsterer, J., and St, C. (2016). Heart disease in disorders of muscle, neuromuscular transmission, and the nerves. *Korean Circ. J.* 46, 117–134. doi:10.4070/kcj.2016.46.2.117

Finsterer, J., Sto, C., and Blazek, G. (2006). Neuromuscular implications in left ventricular hypertrabeculation/noncompaction. *Int. J. Cardiol.* 110, 288–300. doi:10.1016/j.ijcard.2005.10.028

Finsterer, J., Stöllberger, C., and Maeztu, C. (2015). Sudden cardiac death in neuromuscular disorders. *Int. J. Cardiol. [Internet]* 203, 508–515. doi:10.1016/j.ijcard.2015.10.176

Forleo, C., Carmosino, M., Resta, N., Rampazzo, A., Valecce, R., Sorrentino, S., et al. (2015). Clinical and functional characterization of a novel mutation in lamin a/C gene in a multigenerational family with arrhythmogenic cardiac laminopathy. *PLoS One* 10 (4), e0121723. doi:10.1371/journal.pone.0121723

Giglia, T. M., Dinardo, J., Ghanayem, N. S., Ichord, R., Niebler, R. A., Odegard, K. C., et al. (2015). Bleeding and thrombotic emergencies in pediatric cardiac intensive care: Unchecked balances. *World J. Pediatr. Congenit. Hear Surg.* 3 (4), 470–491. doi:10.1177/2150135112460866

Gonzalez-Suarez, I., Redwood, A. B., Perkins, S. M., Vermolen, B., Lichtensztejin, D., Grotsky, D. A., et al. (2009). Novel roles for A-type lamins in telomere biology and the DNA damage response pathway. *EMBO J. [Internet* 28 (16), 2414–2427. doi:10.1038/emboj.2009.196

Granger, B., Gueneau, L., Drouin-Garraud, V., Pedergnana, V., Gagnon, F., Ben Yaou, R., et al. (2011). Modifier locus of the skeletal muscle involvement in Emery-Dreifuss muscular dystrophy. *Hum. Genet.* 129 (2), 149–159. doi:10.1007/s00439-010-0909-1

Groh, W. J. (2012). Arrhythmias in the muscular dystrophies. *HRTHM* 9 (11), 1890–1895. Internet. doi:10.1016/j.hrthm.2012.06.038

Groh, W. J., Bhakta, D., Vice-chair, C., Tomaselli, G. F., Vice-chair, F., Aleong, R. G., et al. 2022 HRS expert consensus statement on evaluation and management of arrhythmic risk in neuromuscular disorders. *Hear Rhythm* [Internet]; 19, e61-e120. doi:10.1016/j.hrthm.2022.04.022

Haas, J., Frese, K. S., Peil, B., Kloos, W., Keller, A., Kayvanpour, E., et al. (2014). Atlas of the clinical genetics of human dilated cardiomyopathy. *Eur. Heart J.* 36, 1123–35a. doi:10.1093/eurheartj/ehu301

Hasselberg, N. E., Edvardsen, T., Petri, H., Berge, K. E., Leren, T. P., Bundgaard, H., et al. (2014). Risk prediction of ventricular arrhythmias and myocardial function in Lamin A/C mutation positive subjects. *Europace* 16 (4), 563–571. doi:10.1093/europace/eut291

Haugaa, K. H., Hasselberg, N. E., and Edvardsen, T. (2015). Mechanical dispersion by strain echocardiography: A predictor of ventricular arrhythmias in subjects with lamin A/C mutations. *JACC Cardiovasc Imaging* 8 (1), 104–106. doi:10.1016/j.jcmg.2014.04.029

Heller, F., Dabaj, I., Mah, J. K., Bergounioux, J., Essid, A., Bönnemann, C. G., et al. (2017). Cardiac manifestations of congenital LMNA-related muscular dystrophy in

children: Three case reports and recommendations for care. *Cardiol. Young* 27, 1076–1082. doi:10.1017/S1047951116002079

Ho, C. Y., and Lammerding, J. (2012). Lamins at a glance. *J. Cell Sci.* 125 (9), 2087–2093. Internet. doi:10.1242/jcs.087288

Holmström, M., Kivistö, S., Heliö, T., Jurkko, R., Kaartinen, M., Antila, M., et al. (2011). Late gadolinium enhanced cardiovascular magnetic resonance of lamin A/C gene mutation related dilated cardiomyopathy. *J. Cardiovasc Magn. Reson [Internet* 13 (1), 30. doi:10.1186/1532-429X-13-30

Hutchison, C. J. (2011). The role of DNA damage in laminopathy progeroid syndromes. *Biochem. Soc. Trans. [Internet* 39 (6), 1715–1718. doi:10.1042/BST20110700

Izquierdo, I., Rosa, I., Bravo, S. B., Guitián, E., Pérez-Serra, A., Campuzano, O., et al. (2016). Proteomic identification of putative biomarkers for early detection of sudden cardiac death in a family with a LMNA gene mutation causing dilated cardiomyopathy. *J. Proteomics* 148, 75–84. Internet. doi:10.1016/j.jprot.2016.07

Jędrzejowska, M., Potulska-Chromik, A., Gos, M., Gambin, T., Dębek, E., Rosiak, E., et al. (2021). Floppy infant syndrome as a first manifestation of LMNA-related congenital muscular dystrophy. *Eur. J. Paediatr. Neurol.* 32, 115–121. doi:10.1016/j.ejpn.2021.04.005

Komaki, H., Hayashi, Y. K., Tsuburaya, R., Sugie, K., Kato, M., Nagai, T., et al. (2011). Inflammatory changes in infantile-onset lmna-associated myopathy. *Neuromuscul. Disord. [Internet]* 21 (8), 563–568. doi:10.1016/j.nmd.2011.04.010

Koopman, L. P., Rebel, B., Gnanam, D., Menting, M. E., Helbing, W. A., and Boersma, E. (2019). Reference values for two-dimensional myocardial strain echocardiography of the left ventricle in healthy children. *Cardiol. Young* 29 (3), 325–337. doi:10.1017/S1047951118002378

Kumar, S., Androulakis, A. F. A., Sellal, J. M., Maury, P., Gandjbakhch, E., Waintraub, X., et al. (2016). Multicenter experience with catheter ablation for ventricular tachycardia in lamin A/C cardiomyopathy. *Circ. Arrhythm. Electrophysiol.* 9, e004357. doi:10.1161/CIRCEP.116.004357

Lammerding, J., Schulze, P. C., Takahashi, T., Kozlov, S., Sullivan, T., Kamm, R. D., et al. (2004). Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. *J. Clin. Invest.* 113 (3), 370–378. doi:10.1172/JCI19670

Lang, R. M., Bierig, M., Devereux, R. B., Flachskampf, F. A., Foster, E., Pellikka, P. A., et al. (2005). Recommendations for chamber quantification: A report from the American society of echocardiography's guidelines and standards committee and the chamber quantification writing group, developed in conjunction with the European association of echocardiography, a branch of the European society of cardiology. *J. Am. Soc. Echocardiogr.* 18 (12), 1440–1463. doi:10.1016/j.echo.2005.10.005

Lee, C. K., Margossian, R., Sleeper, L. A., Canter, C. E., Chen, S., Tani, L. Y., et al. (2014). Variability of M-mode versus two-dimensional echocardiography measurements in children with dilated cardiomyopathy. *Pediatr. Cardiol.* 35 (4), 658–667. doi:10.1007/s00246-013-0835-9

Lee, T. M., Hsu, D. T., Kantor, P., Towbin, J. A., Ware, S. M., Colan, S. D., et al. (2017). Pediatric cardiomyopathies. *Circ. Res.* 121 (7), 855–873. doi:10.1161/CIRCRESAHA.116.309386

Lipshultz, S. E., Law, Y. M., Asante-Korang, A., Austin, E. D., Dipchand, A. I., Everitt, M. D., et al. (2019). Cardiomyopathy in children: Classification and diagnosis: A scientific statement from the American heart association. *Circulation* 140, 9–68. doi:10.1161/CIR.0000000000000682

Lu, J. T., Muchir, A., Nagy, P. L., and Worman, H. J. (2011). LMNA cardiomyopathy: Cell biology and genetics meet clinical medicine. *Dis. Model Mech. [Internet* 4 (5), 562–568. doi:10.1242/dmm.006346

Maggi, L., Amico, A. D., Pini, A., Sivo, S., Ambrosio, P. D., Sala, S., et al. (2014). LMNA-Associated myopathies: The Italian experience in a large cohort of patients. *Neurology* 83, 1634–1644. doi:10.1212/WNL.0000000000000934

Maggi, L., Carboni, N., and Bernasconi, P. (2016). Skeletal muscle laminopathies: A review of clinical and molecular features. *Cells* 5, 33. doi:10.3390/cells5030033

Marchel, M., Madej-Pilarczyk, A., Steckiewicz, R., Stolarz, P., Peller, M., Tymińska, A., et al. (2021). Predictors of mortality and cardiovascular outcomes in Emery-Dreifuss muscular dystrophy in a long-term follow-up. *Kardiol. Pol.* 79 (12), 1335–1342. doi:10.33963/KP.a2021.0159

Marchel, M., Madej-Pilarczyk, A., Tymí Nska, A., Steckiewicz, R., Ostrowska, E., Wysí Nska, J., et al. (2021). Cardiac arrhythmias in muscular dystrophies associated with emerinopathy and laminopathy: A cohort study. *J. Clin. Med.* 10, 732. doi:10.3390/jcm10040732

Meune, C., Berlo, J. H. V., Anselme, F., Bonne, G., Pinto, Y. M., and Duboc, D. (2006). Primary prevention of sudden death in patients with Lamin A/C gene mutations. *N. Engl. J. Med.* 354 (2), 209–210. doi:10.1056/NEJMc052632

Monagle, P. (2012). Diagnosis and management of deep venous thrombosis and pulmonary embolism in neonates and children. *Semin. Thromb. Hemost.* 38, 683–690. doi:10.1055/s-0032-1326784

Muchir, A., Bonne, G., Kooi, A. J. V. D., Meegen, M. V., Baas, F., Bolhuis, P. A., et al. (2000). Identification of mutations in the gene encoding lamins A/C in autosomal dominant limb girdle muscular dystrophy with atrioventricular conduction disturbances (LGMD1B). *Hum. Mol. Genet.* 9 (9), 1453–1459. doi:10.1093/hmg/9.9.1453

Muscogiuri, G. (2017). Results of late gadolinium enhancement in children affected by dilated cardiomyopathy. *Front. Prediator* 5, 13. doi:10.3389/fped.2017.00013

Ollila, L., Nikus, K., Holmström, M., Jalanko, M., Jurkko, R., Kaartinen, M., et al. (2017). Clinical disease presentation and ECG characteristics of *LMNA* mutation carriers. *Open Hear [Internet]* 4 (1), e000474. doi:10.1136/openhrt-2016-000474

Parent, J. J., Towbin, J. A., and Jefferies, J. L. (2015). Left ventricular noncompaction in a family with lamin A/C gene mutation. *Tex. Hear Inst. J.* 42 (1), 73–76. doi:10.14503/THIJ-13-3843

Pasotti, M., Klersy, C., Pilotto, A., Marziliano, N., Rapezzi, C., Serio, A., et al. (2008). Long-term outcome and risk stratification in dilated cardiolaminopathies. *J. Am. Coll. Cardiol.* 52 (15), 1250–1260. doi:10.1016/j.jacc.2008.06.044

Pasqualin, L. M. A., Reed, U. C., Costa, T. V. M. M., Quedas, E., Albuquerque, M. A. V., Resende, M. B. D., et al. (2014). Congenital muscular dystrophy with dropped head linked to the LMNA gene in a Brazilian cohort. *Pediatr. Neurol. [Internet]* 50 (4), 400–406. doi:10.1016/j.pediatrneurol.2013.11.010

Peretto, G., Di Resta, C., Perversi, J., Forleo, C., Maggi, L., Politano, L., et al. (2019). Cardiac and neuromuscular features of patients with LMNA-related cardiomyopathy. *Ann. Intern Med.* 171 (7), 458–463. doi:10.7326/M18-2768

Pérez-Serra, A., Toro, R., Sarquella-Brugada, G., de Gonzalo-Calvo, D., Cesar, S., Carro, E., et al. (2016). Genetic basis of dilated cardiomyopathy. *Int. J. Cardiol.* 224, 461–472. Internet. doi:10.1016/j.ijcard.2016.09.068

Petillo, R., Ambrosio, P. D., Torella, A., Taglia, A., Picillo, E., Testori, A., et al. (2015). Novel mutations in LMNA A/C gene and associated phenotypes. *Acta Myol.* 34, 116–119.

Pettersen, M. D., Du, W., Skeens, M. E., and Humes, R. A. (2008). Regression equations for calculation of Z scores of cardiac structures in a large cohort of healthy infants, children, and adolescents: An echocardiographic study. *J. Am. Soc. Echocardiogr.* 21 (8), 922–934. doi:10.1016/j.echo.2008.02.006

Priori, S., Blomstrom-Lundqvist, C., Mazzanti, A., Blom, N., Borggrefe, M., Camm, J., et al. 2015 2015 ESC guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: The task force for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death of the European society of cardiology (ESC). Endorsed by: Association for European paediatric and congenital cardiology (AEPC). *Eur. Heart J.*; 36(41): 2793–2867. doi:10.1093/eurheartj/ehv316

Quarta, G., Syrris, P., Ashworth, M., Jenkins, S., Zuborne Alapi, K., Morgan, J., et al. (2012). Mutations in the Lamin A/C gene mimic arrhythmogenic right ventricular cardiomyopathy. *Eur. Heart J.* 33 (9), 1128–1136. doi:10.1093/eurheartj/ehr451

Quijano-roy, S., Mbieleu, B., Bo, C. G., Jeannet, P., Colomer, J., Clarke, N. F., et al. (2008). De novo LMNA mutations cause a new form of congenital muscular dystrophy. *Ann. Neurol.* 64, 177–186. doi:10.1002/ana.21417

Rajdev, A., and Groh, W. (2015). Arrhythmias in the muscular dystrophies. *Card. Electrophysiol. Clin.* 7 (2), 303–308. doi:10.1016/j.ccep.2015.03.011

Rijsingen, I. A. W. V., Arbustini, E., Elliott, P. M., Mogensen, J., Ast, J. F. H., Jenkins, S., et al. (2012). Risk factors for malignant ventricular arrhythmias in lamin A/C mutation carriers A European cohort study. *JAC [Internet]* 59 (5), 493–500. doi:10.1016/j.jacc.2011.08.078

Rijsingen, I. A. W. V., Nannenberg, E. A., Arbustini, E., Elliott, P. M., Mogensen, J., Ast, J. F. H., et al. (2013). Gender-specific differences in major cardiac events and mortality in lamin A/C mutation carriers. *Eur. J. Heart Fail* 15, 376–384. doi:10.1093/eurjhf/hfs191

Steckiewicz, R., Stolarz, P., Świętoń, E., Madej-Pilarczyk, A., Grabowski, M., Marchel, M., et al. (2016). Cardiac pacing in 21 patients with emery-dreifuss muscular dystrophy: A single-centre study with a 39-year follow-up. *Kardiol. Pol.* 74 (6), 576–583. doi:10.5603/KP.a2015.0218

Tan, D., Yang, H., Yuan, Y., Bonnemann, C., Chang, X., Wang, S., et al. (2015). Phenotype-genotype analysis of Chinese patients with early-onset LMNA-related muscular dystrophy. *PLoS One* 10 (6), e0129699. doi:10.1371/journal.pone.0129699

Taylor, M. R. G., Fain, P. R., Sinagra, G., Robinson, M. L., Robertson, A. D., Carniel, E., et al. (2003). Natural history of dilated cardiomyopathy due to lamin A/C gene mutations. *J. Am. Coll. Cardiol. [Internet]* 41 (5), 771–780. doi:10.1016/S0735-1097(02)02954-6

Van Rijsingen, I. A. W., Bakker, A., Azim, D., Hermans-Van Ast, J. F., Van Der Kooi, A. J., Van Tintelen, J. P., et al. (2013). Lamin A/C mutation is independently associated with an increased risk of arterial and venous thromboembolic complications. *Int. J. Cardiol. [Internet]* 168 (1), 472–477. doi:10.1016/j.ijcard.2012.09.118

Wahbi, K., Ben Yaou, R., Gandjbakhch, E., Anselme, F., Gossios, T., Lakdawala, N. K., et al. (2019). Development and validation of a new risk prediction score for life-threatening ventricular tachyarrhythmias in laminopathies. *Circulation* 140, 293–302. doi:10.1161/CIRCULATIONAHA.118.039410

Wang, C. H., Bonnemann, C. G., Rutkowski, A., Sejersen, T., Bellini, J., Battista, V., et al. (2010). Consensus statement on standard of care for congenital muscular dystrophies. *J. Child. Neurol.* 25 (12), 1559–1581. doi:10.1177/0883073810381924

Wang, X., Zabell, A., and Koh, W. (2017). Lamin A/C cardiomyopathies: Current understanding and novel treatment strategies. *Curr. Treat. Options Cardiovasc Med.* 19, 21. doi:10.1007/s11936-017-0520-z

Wolf, C. M., Wang, L., Alcalai, R., Pizard, A., Burgon, P. G., Ahmad, F., et al. (2008). Lamin A/C haploinsufficiency causes dilated cardiomyopathy and apoptosis-triggered cardiac conduction system disease. *J. Mol. Cell Cardiol.* 44 (2), 293–303. doi:10.1016/j.yjmcc.2007.11.008

Worman, H. J., and Bonne, G. (2007). Laminopathies:" a wide spectrum of human diseases. *Exp. Cell Res.* 313 (10), 2121–2133. doi:10.1016/j.yexcr.2007.03.028

Worman, H. J. (2012). Nuclear lamins and laminopathies. *J. Pathology* 226, 316–325. doi:10.1002/path.2999

Yancy, A. C. W., Jessup, M., Bozkurt, B., Butler, J., Jr, D. E. C., Colvin, M. M., et al. 2016 2016 ACC/AHA/HFSA focused update on new pharmacological therapy for heart failure: An update of the 2013 ACCF/AHA guideline for the management of heart failure: A report of the American College of cardiology/American heart association task force on clinical practice guidelines and the heart failure society of America. *J. Card. Fail* [Internet]; 22(9):659–669. doi:10.1016/j.cardfail.2016.07.001

Yancy, C., Jessup, M., Bozkurt, B., Masoudi, F. F. A., Casey, E., Mcmurray, J. J. V., et al. 2013 2013 ACCF/AHA guideline for the management of heart failure: A report of the American College of cardiology foundation/American heart association task force on practice guidelines. *J. Am. Coll. Cardiol.*; 62(16):e147-e239. doi:10.1016/j.jacc.2013.05.019

Supplementary material

TABLE LEGENDS

Table 1. List of *LMNA*-related muscular dystrophy patients, major cardiac events, and rare *LMNA* variants. Abbreviations: L-CMD, *LMNA*-related congenital muscular dystrophy; EDMD, Emery–Dreifuss muscular dystrophy; LGMD1B, Limb–girdle muscular dystrophy 1B; DCM, dilated cardiomyopathy; ICD, implantable cardioverter defibrillator; PM, pacemaker.

Table 2. List of *LMNA*-related muscular dystrophy patients, major and minor cardiac events, and rare *LMNA* mutations. Abbreviations: L-CMD, *LMNA*-related congenital muscular dystrophy; EDMD, Emery–Dreifuss muscular dystrophy; LGMD1B, Limb–girdle muscular dystrophy 1B; M, male; F, female; DCM, dilated cardiomyopathy; ICD, implantable cardioverter defibrillator; PM, pacemaker; AT, atrial tachycardia; AF, atrial fibrillation; NSVT, nonsustained ventricular tachycardia; SVT, sustained ventricular tachycardia; VF, ventricular fibrillation; GLS, global longitudinal strain; N/A, not available; AVB, atrioventricular block; IRBBB, incomplete right bundle branch block.

Table S1. Comparison between the percentage of *LMNA*-related muscular disease published in other series and the percentage found in our series. Abbreviations: EDMD, Emery–Dreifuss muscular dystrophy; L-CMD, *LMNA*-related congenital muscular dystrophy; LGMD1B, Limb–girdle muscular dystrophy type 1B.

Table S2. Statistical analysis of the echocardiographic data. The data in the files show echocardiographic analysis: LV systolic function, LV diastolic function, and RV systolic function. The data in the columns show the compared groups in two times: all patients at enrollment versus final follow-up; non-ICD group versus ICD group at enrollment and at final follow-up; non-DCM group versus DCM group at enrollment and at final follow-up. The *P* value for each group is shown in a separate column. Abbreviations: LVEF, left ventricular ejection fraction; LV, left ventricle; RV, right ventricle; GLS, global longitudinal strain; TAPSE, tricuspid annular plane systolic excursion; ICD, implantable cardiac defibrillator; DCM, dilated cardiomyopathy.

Table S3. Reason for indication for a device and class of device in the cohort of study. Median age was 14.5 years (IQR of 12 to 17 years). Patient number is indicated in the first column. The other columns summarize the type of device, the indication for the device, and the age at device implantation. The younger patient was 2 years old and needed a PM because of an asystole detected in the ILR monitoring device. Abbreviations: ICD, implantable cardiac defibrillator; PM, pacemaker; NSVT, non-sustained ventricular tachycardia.

Table S4. List of *LMNA* variants and related features found in our pediatric cohort. The nucleotide and protein changes, predictions, categorization in the HGMD database, and the score otorged classified as pathogenic or likely pathogenic are presented for each case. Abbreviations: dbSNP, single nucleotide polymorphism database; GnomAD (MAF%), genome aggregation database (minor allele frequency %); ClinVar: clinically relevant variation database; HGMD, the Human Gene Mutation Database; ACMG score, the American College of Medical Genetics and Genomics score; NA, not available.

FIGURE LEGENDS

Figure 1. Figure 1A: Timeline of major and minor events in pediatric patients with lamin-related muscular dystrophy defined on the basis of age. Atrial arrhythmias, asystole/atrial standstill, and malignant arrhythmias preceded structural and cardiac functional abnormalities (DCM criteria or depressed LVEF). Median time values are represented by vertical grey line when required. DCM and LVEF are represented by a dot. Pacemaker are represented by a star. Arrhythmias and ICD are represented by an inverted triangle. Gait loss and NIMV are represented by a square, and death are represented by a cross. When required, minimum to maximum range age is represented in grey box and whiskers. Abbreviations: LVEF, left ventricular ejection fraction. Figure 1B: Timeline of major and minor events in pediatric patients with lamin-related muscular dystrophy defined on the basis of enrollment (time 0 is the time of enrollment represented by a vertical blue dotted line). Median time values are represented by a vertical grey line. DCM and LVEF are represented by a dot, pacemaker is represented by a star, arrhythmias and ICD are represented by an inverted triangle and death is represented by a cross. Abbreviations: LVEF, left ventricular ejection fraction.

Figure 2. Three ECG traces from the ILR. (Figure 2A) Atrial fibrillation (blue arrows) and a ventricular extrasystole (red arrow). (Figure 2B) Ventricular tachycardia, VF, and asystole from a pediatric patient diagnosed with EDMD. (Figure 2C) Sinus pause and asystole in a 3-year-old patient.

Figure 3. Phenotype–genotype graphic correlation. A schematic representation of the *LMNA* gene is shown. Top, major cardiac events, followed by rare pathogenic/likely pathogenic *LMNA* variants identified in our patients with the corresponding amino acid or nucleotide changes. Bottom, structural protein: the coil 1A–1B and coil 2A–2B constituting the α-helicalrod domain. Abbreviations: DCM, dilated cardiomyopathy; ICD, implantable cardioverter defibrillator; PM, pacemaker; NLS, nuclear location signal; Ig, immunoglobulin.

Figure 1

Figure 2

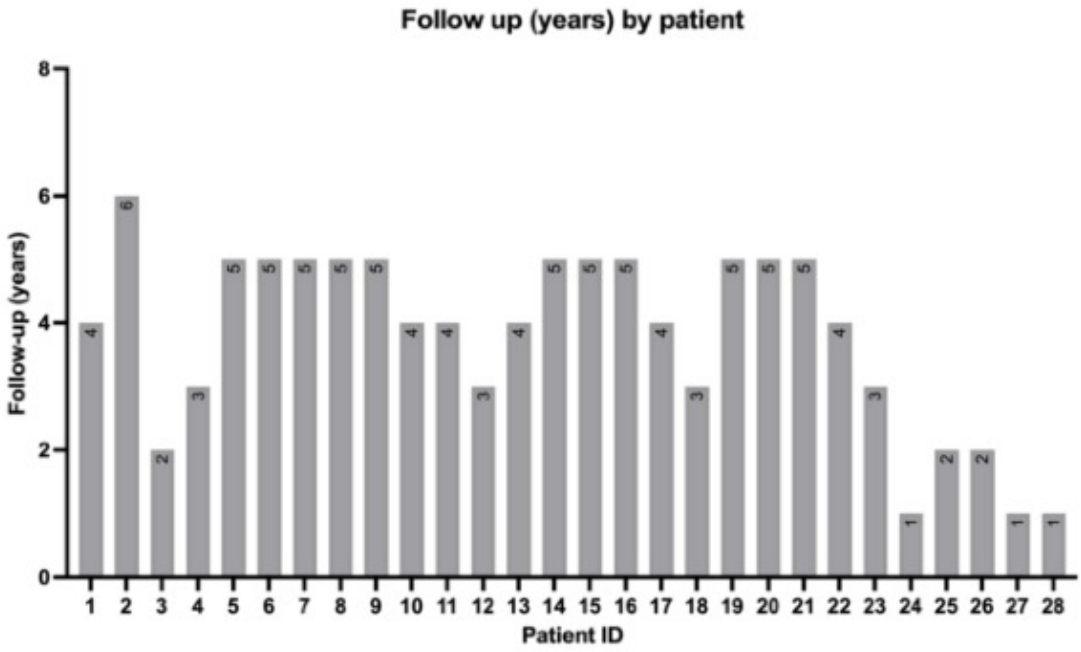


Figure 4. Figure 4A: comparison of values at enrollment and at last follow-up; LVEF values (%). Patients are represented as black dots at enrollment, and triangles represent patients at last follow-up. Red triangles show patients requiring an ICD (gray device represented in the graphic). Cardiomyopathy is represented by a heart. Black lines link the same patient in the two different moments of follow-up. Figure 4B: comparison of values at enrollment and at last follow-up; four-chamber GLS values (%). Patients are represented as black dots at enrollment and triangles at last follow-up. Red triangles show patients requiring an ICD (gray device represented in the graphic). Cardiomyopathy is represented by a heart. Black lines link the same patient in the two different moments of follow-up.

Figure 5. (Figure 5A) Four-chamber GLS values (%) compared with LVEF values (%) for each patient at enrollment. At enrollment, despite normal LVEF (>55%), values below 20.6% are detected (20.6% is the mean normal value published in children). These LVEF values show a decreasing trend at last follow-up (Figure 5B).

Figure S1. Flow chart of the population included in the study.

Figure S2. Time of follow-up (years) represented graphically by patient.

Figure S3. Example of three ECGs from two different patients. (S3A) Sinus rhythm with IRBBB. (S3B) First-degree AVB, wide QRS interval, and repolarization abnormalities. (S3C) Global low QRS voltage and poor R progression in precordial leads in a 4-year-old patient.

Figure S4. Myocardial strain analysis from a pediatric *LMNA* muscular dystrophy patient showing normal ejection fraction with progressive decreasing values of four-chamber GLS from enrollment (S4A) to final control during follow-up (S4B). (S4C) Two-chamber view showing the regional LV deformation represented in colored lines. The basal inferior wall segment (yellow arrow) shows worse deformation, and the mid-anterior septal wall (blue arrow) shows post-systolic deformation (late contraction).

Figure 3

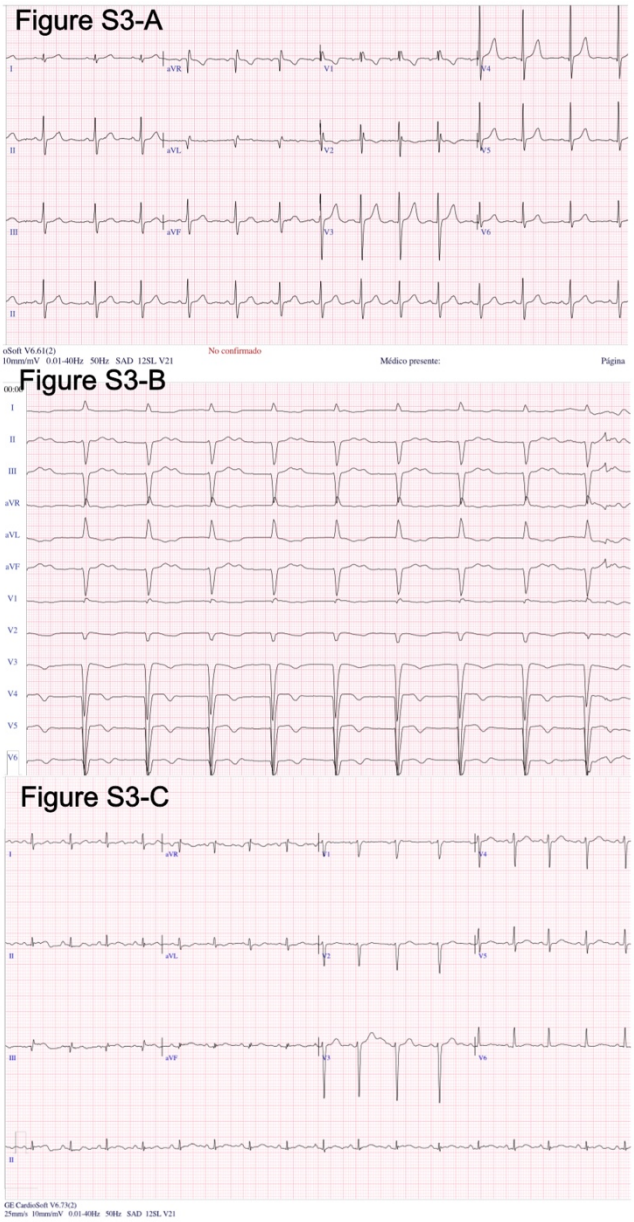


Figure 4

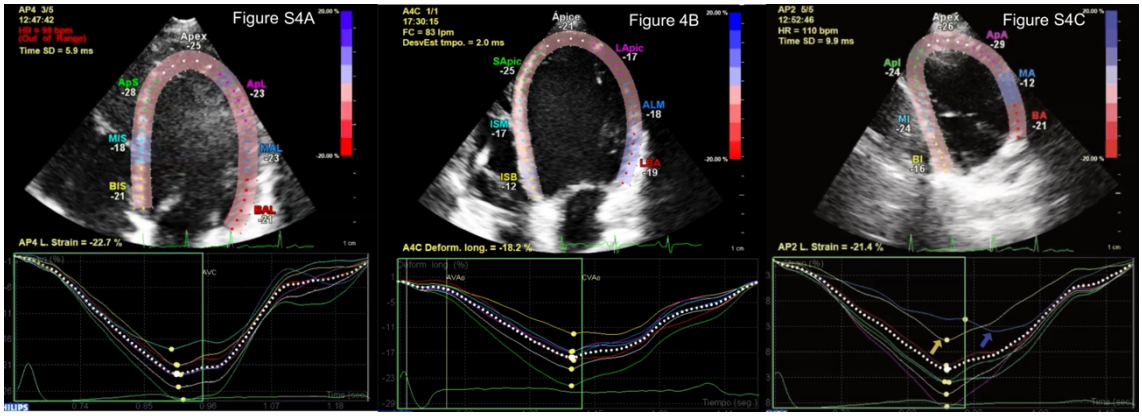


Table 1

Table S1. Cohorts in previous published series

LMNA-related muscular disease	Italy		Chinese	Our cohort
	Benedetti, 2004	Maggi, 2017	Fan, 2019	
EDMD	55.55%	21%	38.1%	46.43%
L-CMD	14.82%	33%	48.8%	39.29%
LGMD1B	29.63%	46%	13.1%	7.14%
Mild weakness	.	.	.	7.14%

Comparison between the percentage of *LMNA*-related muscular disease published in other series and the percentage found in our series. Abbreviations: EDMD, Emery–Dreifuss muscular dystrophy; L-CMD, *LMNA*-related congenital muscular dystrophy; LGMD1B, Limb–girdle muscular dystrophy type 1B.

Table 2

Table S2. Echocardiographic findings in the cohort

	Variable	At Enrollment Median [IQR]	Final follow-up Median [IQR]	*p*
LV systolic function	LVEF (%)	60.75 [56-63.5] n=28	56.75 [51.6-59.75] n=20	**0.04**
	GLS (%)	-21% [-19 ;-22.6] n=28	-17.6% [-16.3;-20.6] n=13	**0.01**
	MAPSE (mm)	11.75 [9.5-12.95] n=28	11 [9.1-12] n=20	0.24
LV diastolic function	Lateral E/E′ ratio	5.8 [5.01-7.22] n= 28	5.9 [5.3-7.9] n=13	0.40
	Septal E/E′ ratio	7.73 [6.37-8.9] n= 28	7.3 [6.42-9.3] n=13	0.88
RV systolic function	TAPSE (mm)	19.9 [15.3-22] n=27	18 [14-19.5] n=13	0.09

Statistical analysis of the echocardiographic data. The data in the files show echocardiographic analysis: LV systolic function, LV diastolic function, and RV systolic function. The data in the columns show the compared groups in two times: all patients at enrollment versus final follow-up; non-ICD group versus ICD group at enrollment and at final follow-up; non-DCM group versus DCM group at enrollment and at final follow-up. The *P* value for each group is shown in a separate column. Abbreviations: LVEF, left ventricular ejection fraction; LV, left ventricle; RV, right ventricle; GLS, global longitudinal strain; TAPSE, tricuspid annular plane systolic excursion; ICD, implantable cardiac defibrillator; DCM, dilated cardiomyopathy.

Table 3

Table S3. Age at device implantation

Patient's number	Device	Indication for device	Age at device implantation *(years)* *Median age: 14.5 (12-17)*
1	ICD	NSVT	12
2	PM	Asystole	2
3	ICD	NSVT	17
5	ICD	NSVT	14
13	ICD	NSVT	18
17	ICD	NSVT	15

Reason for indication for a device and class of device in the cohort of study. Median age was 14.5 years (IQR of 12 to 17 years). Patient number is indicated in the first column. The other columns summarize the type of device, the indication for the device, and the age at device implantation. The younger patient was 2 years old and needed a PM because of an asystole detected in the ILR monitoring device. Abbreviations: ICD, implantable cardiac defibrillator; PM, pacemaker; NSVT, non-sustained ventricular tachycardia.

Table 4

Table S4. LMNA genetic data and classification

Patient	Nucleotide Change	Protein Change	dbSNP	gnomAD (MAF%)	ClinVar (Disease)	HGMD (Disease)	ACMG Score
1	c.745C>T	p.Arg249Trp	rs121912496	NA	LP Lamin-Related Congenital Muscular Dystrophy	CM083718 DM, Muscular Dystrophy	LP
2	c.745C>T	p.Arg249Trp	rs121912496	NA	LP Lamin-Related Congenital Muscular Dystrophy	CM083718 DM, Muscular Dystrophy	LP
3	c.116A>G	p.Asn39Ser	rs57983345	NA	P Charcot-Marie-Tooth, Type 2	CM083713 DM, Muscular Dystrophy	P
4	c.91-93delGAG	p.Glu31del	rs864309525	NA	P Charcot-Marie-Tooth, Type 2	CD156162 DM, Muscular Dystrophy	LP
5	c.1358G>C	p.Arg453Pro	rs267607598	NA	NA	CM083716 DM, Muscular Dystrophy	LP
6	c.745C>T	p.Arg249Trp	rs121912496	NA	LP Lamin-Related Congenital Muscular Dystrophy	CM083718 DM, Muscular Dystrophy	LP
7	c.116A>G	p.Asn39Ser	rs57983345	NA	P Charcot-Marie-Tooth, Type 2	CM083713 DM, Muscular Dystrophy	P
8	c.746G>A	p.Arg249Gln	rs59332535	NA	P Charcot-Marie-Tooth, Type 2	CM1617006 DM, Emery-Dreifuss	P
9	c.91delG	p.Glu31ArgfsTer65	NA	NA	NA	NA	P
10	c.89A>C	p.Gln30Pro	NA	NA	NA	NA	LP
11	c.1487_1488+9del	.	NA	NA	NA	CD1711480 DM, Muscular Dystrophy	P

12	c.1616C>T	p.Ala539Val	NA	NA	NA	NA	LP
13	c.112C>T	p.Leu38Phe	NA	NA	NA	NA	LP
14	c.745C>T	p.Arg249Trp	rs121912496	NA	LP Lamin-Related Congenital Muscular Dystrophy	CM083718 DM, Muscular Dystrophy	LP
15	c.812T>G	p.Leu271Arg	NA	NA	NA	NA	LP
16	c.880_882delCAG	p.Gln294del	NA	NA	NA	NA	LP
17	c.1364G>C	p.Arg455Pro	rs267607597	NA	NA	CM111722 DM, Muscular Dystrophy	LP
18	c.745C>T	p.Arg249Trp	rs121912496	NA	LP Lamin-Related Congenital Muscular Dystrophy	CM083718 DM, Muscular Dystrophy	LP
19	c.810+1G>C	.	rs267607632	NA	P	NA	P
20	c.108G>T	p.Gln36His	NA	NA	NA	NA	LP
21	c.104T>A	p.Leu35Gln	NA	NA	NA	NA	LP
22	c.1357C>T	p.Arg453Trp	rs58932704	NA	P Charcot-Marie-Tooth, Type 2	CM990813 DM, Muscular Dystrophy	LP
23	c.1357C>T	p.Arg453Trp	rs58932704	NA	P Charcot-Marie-Tooth, Type 2	CM990813 DM, Muscular Dystrophy	LP
24	c.91G>A	p.Glu31Lys	rs1228406418	NA	P	CM123360	LP
					Charcot-Marie-Tooth, Type 2	DM, Muscular Dystrophy	
25,26[twins]	c.117T>G	p.Asn39Lys	NA	NA	NA	CM156123 DM, Muscular Dystrophy	LP
27	c.94_96delAAG	p.Lys32del	rs60872029	NA	P Charcot-Marie-Tooth, Type 2	CD033712 DM, Muscular Dystrophy, Emery-Dreifuss	P
28	c.745C>T	p.Arg249Trp	rs121912496	NA	LP Lamin-Related Congenital Muscular Dystrophy	CM083718 DM, Muscular Dystrophy	LP

List of LMNA variants and related features found in our pediatric cohort. The nucleotide and protein changes, predictions, categorization in the HGMD database, and the score otorged classified as pathogenic or likely pathogenic are presented for each case. Abbreviations: dbSNP, single nucleotide polymorphism database; GnomAD (MAF%), genomeaggregationdatabase(minor allele frequency%); ClinVar: clinically relevant variation database; HGMD, the Human Gene Mutation Database; ACMG score, the American College of Medical Genetics and Genomics score; NA, not available.

Supplementary protocol (Protocol S1)

Protocol S1:

Suggested protocol on cardiovascular management in children with *LMNA*-related muscular dystrophy

There is a lack of information on monitoring cardiac involvement in presumably healthy children with *LMNA*-related muscular dystrophy. From our results, it is reasonable to propose the following recommendations for management to detect early cardiac dysfunction and arrhythmias:

- Basal 12-lead ECG: at diagnosis and every 6 months.
- Twenty-four-hour Holter monitoring: at diagnosis and every year.
- ILR monitoring device: due to high incidence of arrhythmias (most asymptomatic) and published studies of SCD, the implantation of a long-term loop recorder should be considered to detect arrhythmias early and prevent malignant arrhythmias, treat them, and prevent sudden death, especially in patients with early-onset skeletal muscular weakness.
- Echocardiography, including strain analysis, at diagnosis and yearly. If arrhythmia or depressed ejection fraction are detected, echocardiography is recommended every 6 months or earlier if needed.
- EPS: perform an EPS in those with suspected arrhythmias that could benefit from cardiac ablation and study those patients at risk of malignant arrhythmias and atrial arrhythmias meanable to provoking cerebral emboli. Brachial venous access should be considered for patients with difficult femoral access.
- An implantable defibrillator should be considered if VT is detected and especially before severe ventricular dysfunction appears. A cardiac resynchronization device should also be considered early.
- CMRI with late gadolinium enhancement imaging is recommended to study the presence and distribution of atrial and myocardial fibrosis that could explain the high incidence of ventricular arrhythmias, cardiac conduction delays, and ventricular dysfunction.
- Consider using NT-proBNP as a cardiac biomarker yearly and when cardiac dysfunction is suspected.
- Nonurgent surgical procedures, such as in other neuromuscular diseases with potential cardiac involvement, and deep cardiac evaluation should be performed before any programmed procedure.

Artículo 2. Resumen estructurado.

LMNA-related muscular dystrophy: identification of variants in alternative genes and personalized clinical translation

Introducción

Las laminopatías son causadas por alteraciones en el gen LMNA que dan lugar a un espectro clínico amplio. Aunque la distrofia muscular puede empezar a edades precoces, la progresión es diferente en cada paciente. En este estudio se investiga la variabilidad fenotípica de las laminopatías mediante el análisis genético de los pacientes diagnosticados de distrofia muscular por LMNA con el fin de identificar otras variantes genéticas raras que expliquen estas diferencias fenotípicas.

Objetivos

Analizar genes conocidos que den lugar a enfermedades musculares para identificar variantes genéticas raras que pueden estar relacionadas con un fenotipo más precoz y/o más severo en pacientes con distrofia muscular por LMNA.

Métodos

Se analizaron 105 genes asociados con enfermedades musculares en 26 pacientes pediátricos con diagnóstico de distrofia muscular por LMNA, y de sus familiares de primer grado, y se correlacionaron con los datos clínicos y con el fenotipo neuromuscular y cardiaco.

Resultados

Todos los pacientes eran portadores de una variante rara en el gen *LMNA.* Los diagnósticos fueron Emery-Dreifuss muscular dystrophy (EDMD, 13 pacientes), LMNA-related congenital muscular dystrophy (L-CMD, 11 pacientes), y Limb-girdle muscular dystrophy 1B (LGMD1B, 2 pacientes). En 9 pacientes se encontraron 10 variantes genéticas raras adicionales en 8 genes diferentes de LMNA: GAA, AGRN, FLNC, LAMP2, NEB, COL6A3, DYSF, CHRND. En la correlación genotipo-fenotipo se encontró que 5 de los 9 pacientes con fenotipos

severos eran portadores de algunas de estas variantes raras detectadas posiblemente patogénicas (3 pacientes con diagnóstico de L-CMD y 2 con EDMD).

Conclusiones

Las laminopatías asociadas a distrofia muscular son un grupo heterogéneo de enfermedades con debut y evolución diferentes, a pesar de presentar, incluso, una misma mutación patogénica en el gen LMNA. El hallazgo de otras variantes como las encontradas en esta cohorte, consideradas posiblemente patogénicas, hace plantearse un estudio específico de si éstas tienen algún papel en la expresividad del fenotipo y en su severidad, por lo que podrían representar un indicador de severidad fenotípica.

Se sugiere que, dada la heterogeneidad clínica, se incluyan otros genes, además del gen LMNA, que estén relacionados con enfermedades neuromusculares y riesgo de arritmias cardíacas. Esto puede ayudar a desenmascarar otras variantes genéticas raras que pueden ser las responsables de los fenotipos severos y expliquen la variabilidad fenotípica que existe.

Se precisan más estudios como el actual con un número mayor de pacientes, ya que por el momento la correlación con la clínica debe llevarse a cabo con cautela.

frontiers | Frontiers in Genetics

TYPE Original Research
PUBLISHED 24 March 2023
DOI 10.3389/fgene.2023.1135438

OPEN ACCESS

EDITED BY
Xiu-An Yang,
Chengde Medical College, China

REVIEWED BY
Vincent Gache,
INSERM U1217 Institut NeuroMyoGène (INMG), France
Jared C. Roach,
Institute for Systems Biology (ISB), United States
Elisa Di Pasquale,
National Research Council (CNR), Italy
Maria Rosaria D'Apice,
Policlinico Tor Vergata, Italy

*CORRESPONDENCE
Oscar Campuzano,
oscar@brugada.org
Georgia Sarquella-Brugada,
georgia@brugada.org

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Human and Medical Genomics, a section of the journal
Frontiers in Genetics

RECEIVED 31 December 2022
ACCEPTED 17 March 2023
PUBLISHED 24 March 2023

CITATION
Cesar S, Coll M, Fiol V, Fernandez-Falgueras A, Cruzalegui J, Iglesias A, Moll I, Perez-Serra A, Martínez-Barrios E, Ferrer-Costa C, Olmo Bd, Puigmulè M, Alcalde M, Lopez L, Pico F, Berrueco R, Brugada J, Zschaeck I, Natera-de Benito D, Carrera-García L, Exposito-Escudero J, Ortez C, Nascimento A, Brugada R, Sarquella-Brugada G and Campuzano O (2023), *LMNA*-related muscular dystrophy: Identification of variants in alternative genes and personalized clinical translation. *Front. Genet.* 14:1135438. doi: 10.3389/fgene.2023.1135438

LMNA-related muscular dystrophy: Identification of variants in alternative genes and personalized clinical translation

Sergi Cesar[1,2,3], Monica Coll[4,5], Victoria Fiol[1,2,3], Anna Fernandez-Falgueras[4,5], Jose Cruzalegui[1,2,3], Anna Iglesias[4,5], Isaac Moll[1,2,3], Alexandra Perez-Serra[4,5], Estefanía Martínez-Barrios[1,2,3], Carles Ferrer-Costa[4,5], Bernat del Olmo[4,5], Marta Puigmulè[4,5,6], Mireia Alcalde[4,5], Laura Lopez[4,5], Ferran Pico[4,5], Rubén Berrueco[7], Josep Brugada[1,2,3,5,8], Irene Zschaeck[1,2,3,9,10], Daniel Natera-de Benito[9,10], Laura Carrera-García[9,10], Jessica Exposito-Escudero[9,10], Carlos Ortez[9,10], Andrés Nascimento[9,10,11], Ramon Brugada[4,5,6,12], Georgia Sarquella-Brugada[1,2,3,6]*† and Oscar Campuzano[4,5,6]*†

[1]Pediatric Arrhythmias, Inherited Cardiac Diseases and Sudden Death Unit, Hospital Sant Joan de Déu, Barcelona, Spain, [2]Arrítmies pediàtriques, Cardiologia Genètica i Mort sobtada, Malalties Cardiovasculars en el Desenvolupament, Institut de Recerca Sant Joan de Déu, Esplugues de Llobregat, Barcelona, Spain, [3]European Reference Network for Rare, Low Prevalence and Complex Diseases of the Heart (ERN GUARD-Heart), Amsterdam, Netherlands, [4]Cardiovascular Genetics Center, University of Girona-IDIBGI, Girona, Spain, [5]Centro de Investigación Biomédica en Red, Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain, [6]Medical Science Department, School of Medicine, Universitat de Girona, Girona, Catalonia, Spain, [7]Pediatric Hematology Service, Hospital Sant Joan de Déu Barcelona, Institut de Recerca Pediàtrica, Hospital Sant Joan de Déu de Barcelona (IRP-HSJD), Universitat de Barcelona, Barcelona, Spain, [8]Arrhythmia Section, Cardiology Service, Hospital Clínic, Barcelona, Spain, [9]Neuromuscular Unit, Department of Neurology, Hospital Sant Joan de Déu, Barcelona, Spain, [10]Investigación Aplicada en Enfermedades Neuromusculares Neurociències Institut de Recerca Sant Joan de Déu, Esplugues de Llobregat, Spain, [11]Instituto Nacional de Investigación Biomédica de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Madrid, Spain, [12]Cardiology Department, Hospital Josep Trueta, Girona, Catalonia, Spain

Background: Laminopathies are caused by rare alterations in *LMNA*, leading to a wide clinical spectrum. Though muscular dystrophy begins at early ages, disease progression is different in each patient. We investigated variability in laminopathy phenotypes by performing a targeted genetic analysis of patients diagnosed with *LMNA*-related muscular dystrophy to identify rare variants in alternative genes, thereby explaining phenotypic differences.

Methods: We analyzed 105 genes associated with muscular diseases by targeted sequencing in 26 pediatric patients of different countries, diagnosed with any *LMNA*-related muscular dystrophy. Family members were also clinically assessed and genetically analyzed.

Results: All patients carried a pathogenic rare variant in *LMNA*. Clinical diagnoses included Emery-Dreifuss muscular dystrophy (EDMD, 13 patients), *LMNA*-related congenital muscular dystrophy (L-CMD, 11 patients), and limb-girdle muscular dystrophy 1B (LGMD1B, 2 patients). In 9 patients, 10 additional rare genetic variants

were identified in 8 genes other than *LMNA*. Genotype-phenotype correlation showed additional deleterious rare variants in five of the nine patients (3 L-CMD and 2 EDMD) with severe phenotypes.

Conclusion: Analysis f known genes related to muscular diseases in close correlation with personalized clinical assessments may help identify additional rare variants of *LMNA* potentially associated with early onset or most severe disease progression.

KEYWORDS

sudden cardiac death, laminopathies, muscular dystrophy, genetics, genetic diagnostic

1 Introduction

Muscular dystrophies caused by deleterious variants in the *LMNA* gene are very rare (<1 per 1,000,000; ORPHA:157973). Cervico-axial and scapuloperoneal weakness, joint contractures, and thoracic lordosis associated with a dystrophic muscle biopsy and variably elevated creatine kinase levels are usually associated with these severe entities (Quijano-Roy et al., 2008). Sudden death (SD) also is common in these patients, mainly due to malignant arrhythmias concomitant with heart alterations (Kumar et al., 2016).

Through alternative splicing, *LMNA* encodes proteins lamin A and C, intermediate filaments that are required during development and cell differentiation and are components of the nuclear envelope (Bonne et al., 2003; Paul and Fulka, 2022). More than 500 rare genetic alterations in *LMNA* have been found to be responsible for a group of diseases called laminopathies (Worman and Bonne, 2007; Dittmer and Misteli, 2011). The most common laminopathies are *LMNA*-related congenital muscular dystrophy (L-CMD) (OMIM: 613205), Emery–Dreifuss muscular dystrophy (EDMD) (OMIM: 181350), limb-girdle muscular dystrophy type 1B (LGMD-1B) (OMIM: 159001), and dilated cardiomyopathy with conduction defects (DCM-CD) (OMIM: 115200). A range of phenotypes have been reported in patients carrying deleterious rare *LMNA* variants (Bertrand et al., 2011; Worman, 2012; Carboni et al., 2013a; Carboni et al., 2013b; Ben Yaou et al., 2021). It has been suggested that different phenotypes can be explained by post-transcriptional modifications of the nuclear envelope/lamina proteins (Maraldi et al., 2011; Zheng et al., 2022). Although *LMNA* is accepted as the main cause of disease, the observed phenotype differences remain poorly understood, mainly at early stages of the disease. Genetic background is suggested to be responsible for these phenotypic differences in disease onset and progression, although no studies investigating the role of additional genetic variants have been reported so far. Understanding genetic background variability can facilitate the early diagnosis of *LMNA*-related muscular dystrophy, which is important for prevention of SD, rehabilitation management, and genetic counseling (Charniot et al., 2003; Choi et al., 2019; Murofushi et al., 2022).

In the present study, we performed a targeted genetic analysis and personalized genotype-phenotype interpretation in families diagnosed with *LMNA*-related muscular dystrophies. We identified rare alterations in genes other than *LMNA* that may help explain the differences in disease onset and phenotype progression.

2 Materials and methods

2.1 Cohort

The study was approved by the Ethics Committee of the Hospital Josep Trueta (Girona, Spain) and Hospital Sant Joan de Déu (Barcelona, Spain), following the Helsinki II declaration. Written informed consent to participate in this study was provided by the participants' legal guardians. Written informed consent was also obtained from all relatives included in the study.

The study enrolled 26 pediatric patients previously diagnosed with any type of *LMNA* muscular dystrophy (2014–2020) and carrying a definite pathogenic rare variant in *LMNA*. We retrospectively collected all available data from each patient's first clinical contact up to their enrollment in our study. Clinical evaluation of index cases included a complete physical examination by a pediatric neurologist, neuromuscular specialist, and pediatric cardiologist. Non-pediatric relatives enrolled in our study also were clinically assessed. Saliva or peripheral blood samples were obtained from each patient as well as all available family members. All individuals were clinically assessed at Hospital Sant Joan de Déu (Barcelona, Catalonia, Spain). The complete pedigree of each family was obtained, including history of neuromuscular and cardiac diseases, syncope, and unexplained deaths.

2.2 Genetic analysis

Genomic DNA was analyzed using next-generation sequencing (NGS). A total of 105 genes involved in neuromuscular diseases and risk of malignant cardiac arrhythmias were analyzed (*ACTA1, AGRN, ANO5, B3GALNT2, B4GAT1, BAG3, BIN1, CAPN3, CAV3, CCND3, CFL2, CHAT, CHKB, CHRNA1, CHRNB1, CHRND, CHRNE, CNTN1, COL6A1, COL6A2, COL6A3, COLQ, DAG1, DES, DMD, DNAJB6, DNM2, DOK7, DPAGT1, DPM1, DPM2, DPM3, DSC2, DSG2, DSP, DYSF, EMD, FHL1, FKRP, FKTN, FLNC, FOS, FXN, GAA, GFPT1, GMPPB, GOSR2, HRAS, ISPD, ITGA7, KBTBD13, KCNQ1, KCNH2, LAMA2, LAMP2, LARGE, LDB3, LMNA, MOK2, MTM1, MUSK, MYF6, MYOT, MYH7, MYBPC3, NEB, NESPRIN2, NUP88, PCNA, PLEC, PKCA, PKP2, POMGNT1, POMGNT2, POMK, POMT1, POMT2, RAPSN, RYR1, RYR2, SCN4A, SCN5A, SEPN1, SGCA, SGCB, SGCD, SGCG, SLC25A4, SREBP, TAZ, TCAP, TMEM5, TMEM43, TMPO,*

TNNT1, TNNI3, TNNT2, TNPO3, TPM2, TPM3, TRIM32, and *TTN*). All gene isoforms described in Ensembl 75 (www.ensembl.org) that have been linked with either a RefSeq code (www.ncbi.nlm.nih.gov/refseq) or CCDS (www.ncbi.nlm.nih.gov/CCDS) were included. Sequence data coordinates were based on UCSC human genome version hg19 (NCBI GRCh37 built). Biotinylated cRNA probe solution was used as a capture probe (Agilent Technologies, Santa Clara, CA, United States). Probes were designed using eArray (Agilent Technologies).

Non-common genetic variants [minor allele frequency (MAF) < 1%] identified throughout NGS analysis were confirmed using Sanger sequencing. Exons and exon–intron boundaries of each gene were amplified (Verities PCR, Applied Biosystems, Austin, TX, United States), and the resulting PCR products were purified (Exosap-IT, Affymetrix Inc., USB Products, Cleveland, OH, United States) and directly sequenced in both directions (Big Dye Terminator v3.1 and 3130XL Genetic Analyzer, both from Applied Biosystems). The Posterior SeqScape Software v2.5 (Life Technologies, Carlsbad, CA, United States) was used to compare results with the reference sequence from hg19. The identified rare variants were contrasted with the Human Gene Mutation Database (www.hgmd.cf.ac.uk/ac/index.php) and Genome Aggregation Database (gnomAD) (www.gnomad.broadinstitute.org). To detect copy number variation (CNV), we looked for significant differences between expected and obtained normalized coverage for a given sample in a region of interest. Several samples were analyzed to corroborate similar levels of coverage between samples. All CNVs were compared with the CNV Control database (www.gwas.biosciencedbc.jp/cgi-bin/cnvdb/cnv_top.cgi), Database of Genomic Variants (www.dgv.tcag.ca/dgv/app/home), DECIPHER (www.decipher.sanger.ac.uk), and gnomAD (www.gnomad.broadinstitute.org). Rare variants that were potentially deleterious and confirmed in the index case were analyzed using the Sanger method in the relatives.

Each rare variant was classified following current recommendations of the American College of Medical Genetics and Genomics (ACMG) (Richards et al., 2015). A vast majority of pathogenic (P) variants are extremely rare (<0.01%). All available data concerning each rare genetic variant was updated until submission time (June, 2022). Variants classified as Variant of Unknown Significance (VUS) in alternative genes were further sub-classified. Variants identified showed no reported MAF or low MAF. Certain association with any neuromuscular disease were considered as VUS with highly suspicious Likely Pathogenic role (VUS-LP); thus, they were included to clarify their potential role in clinical practice. To avoid bias, five investigators independently investigated genetic data concerning each analyzed variant in our study. Finally, all investigators discussed data included in each item of the ACMG and consensus as well as final classification of all rare variants.

3 Results

3.1 Cohort

Our study included 26 pediatric patients (mean age 8.2 years at enrollment; IQR, 4–12.5 years; 53.8% males) of 25 families, with a total of 76 individuals (26 index cases and 50 relatives). Two of the index cases enrolled were female monozygotic twins (index cases 23 and 24). Families were originally from Spain (12), United Kingdom (3), United States (3), Australia (2), Canada (1), France (1), Greece (1), Russia (1), and Argentina (1). No consanguinity occurred in any of families. No potential common ancestor was identified after the family interviews. All index cases accomplished with clinical criteria for LMNA-related muscular disease: 11 Patients (42.3%) were L-CMD, 13 (50%) were EDMD, and 2 (7.7%) presented as LGMD1B. Early-onset skeletal muscle impairment before 2 years of age was detected in 23 of the 26 cases (88.4%) (Figure 1). Clinical assessment was performed in all patients included in our cohort, confirming previous diagnosis for each *LMNA*-related muscular disease.

3.2 Genetic analysis

Our NGS analysis showed an average call rate of 99.25% achieved at 30x coverage. The median coverage per sample was 892 (749–1286). An average of four failed whole exons occurred in each sample, and all these exons were amplified using Sanger sequencing. All rare variants (MAF < 1%) were confirmed also using Sanger sequencing, discarding false positive signal. No CNV were identified in any of genes analyzed, including *LMNA*. Previous karyotype (performed at time of diagnosis, out of our centre) also discarded any large chromosomic alteration in all patients included in our cohort.

3.3 Rare variants in LMNA

All patients included in our study had a previous genetic analysis of the *LMNA* gene. This previous analysis identified rare variants in this gene as potential cause of the disease. Our targeted-gene panel analysis confirmed all previous rare variants in the *LMNA* gene (Figure 2). No additional rare or common variant classified as pathogenic (P) or likely pathogenic (LP) were identified in *LMNA*. As above mentioned, previous genetic analysis identified rare variants in the *LMNA* gene, which were classified according to available data at the moment of genetic analysis was performed. Rare variants in *LMNA* were reclassified following ACMG guidelines and accordingly to current data available (June, 2022). All *LMNA* variants remain classified as P or LP, without any modification in comparison to previous genetic report.

A total of 19 *LMNA* rare variants were identified (17 exonic and 2 intronic). Of exonic rare variants, 4 were delins and 13 missense. All variants were identified in heterozygous state (Figure 2). Eleven rare variants (57.89%) were classified as LP and 7 (36.84%) as definitively P. The most frequent rare variant identified in *LMNA* was p.Arg249Trp (5 patients, 19.23%), as previously reported (Quijano-Roy et al., 2008; Pasqualin et al., 2014; Heller et al., 2017; Ben Yaou et al., 2021; Fan et al., 2021; Jedrzejowska et al., 2021). These 5 patients were diagnosed with L-CMD (4 patients -index case 1, 2, 6, and 26-) and EDMD (1 patient -index case 14-). Other two rare variants were identified two times in different patients each one (p.Asn39Ser, patients 3 and 7; p.Arg453Trp, patients 20 and 21). Both rare variants were also previously

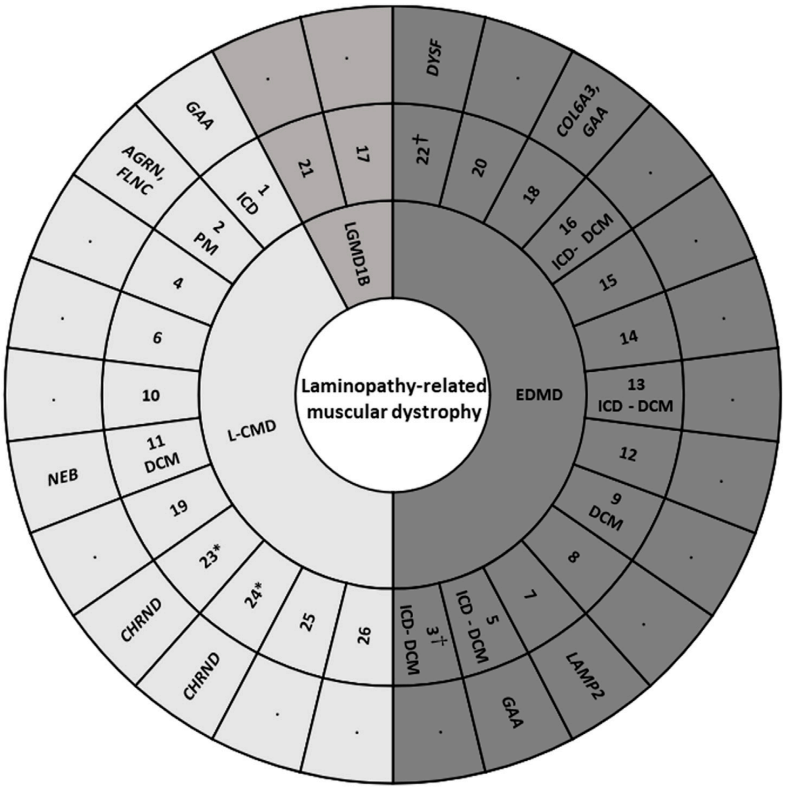


FIGURE 1
Cohort of index cases. DCM, dilated cardiomyopathy. EDMD, Emery-Dreifuss muscular dystrophy. ICD, implantable cardioverter defibrillator. L-CMD, *LMNA*-related congenital muscular dystrophy. LGMD1B, Limb-girdle muscular dystrophy 1B. PM, pacemaker. *, twins. †, sudden death.

reported (Pasqualin et al., 2014; Ben Yaou et al., 2021; Bennett et al., 2021; Fan et al., 2021; Jedrzejowska et al., 2021). A total of 6 cases showed DCM (patient 3, 5, 9, 11, 13, and 16), five of them diagnosed with EDMD and only one with L-CMD (patient 11). Among the patients with ICD (patients 1, 3, 5, 13, and 16), four were diagnosed with EDMD and one with L-CMD (patient 1). Index case number 2, diagnosed with L-CMD and carrying a pacemaker (PM), showed prolonged asystole episodes. Unfortunately, two cases died after inclusion in our study (patient 3 due to rapidly progressive heart failure despite optimal treatment and ICD carrier and 22due to severe respiratory infection) (Tables 1–3) (Figure 1). Finally, focused on intronic *LMNA* rare variant (c.810 + 1G>C) in patient 17, the clinical diagnostic was LGMD1B. The patient was not a carrier of any other rare variant in either the *LMNA* gene or any other gene. In addition, none of relatives showed any clinical symptom (Table 1).

Segregation of genetic variants in families showed that in 24 cases (92.3%), the rare variant in *LMNA* was *de novo* (including intronic variant in case number 17), as widely published (Ben Yaou et al., 2021; Jedrzejowska et al., 2021). Only in two families (index cases 11 and 18), one of parents carried the same deleterious variant in *LMNA*. In both these cases, the parents showed minor muscular impairment (Figure 3).

3.4 Additional rare variants

Eleven rare variants were identified in 8 genes (*AGRN, CHRND, COL6A3, DYSF, FLNC, GAA, LAMP2*, and *NEB*) encoding structural proteins. The *AGRN* gene encodes the protein Agrin, associated with Congenital myasthenic syndrome following an autosomal recessive (AR) pattern of inheritance. The *CHRND* gene encodes the Cholinergic receptor nicotinic delta subunit, mainly related to Myasthenic syndrome following autosomal dominant (AD) or AR pattern of inheritance. The *COL6A3* encodes Collagen type VI alpha 3 chain protein and deleterious variants are mainly associated with muscular dystrophy following both AD and AR patterns of inheritance. The *DYSF* gene encodes the protein Dysferlin, mainly associated with muscular dystrophy following an AR pattern of inheritance. The *FLNC* gene encodes the protein Filamin C, mainly associated wit cardiomyopathies following an AD pattern of inheritance. The *GAA* gene encodes Alpha Glucosidase and deleterious variants in this gene are related to Pompe disease following an AR pattern of inheritance. The *LAMP2*

Gene encodes the Lysosomal associated membrane protein 2, related to Danon disease following a X-linked pattern of inheritance. Finally, the *NEB* gene encodes the protein Nebulin, and pathogenic

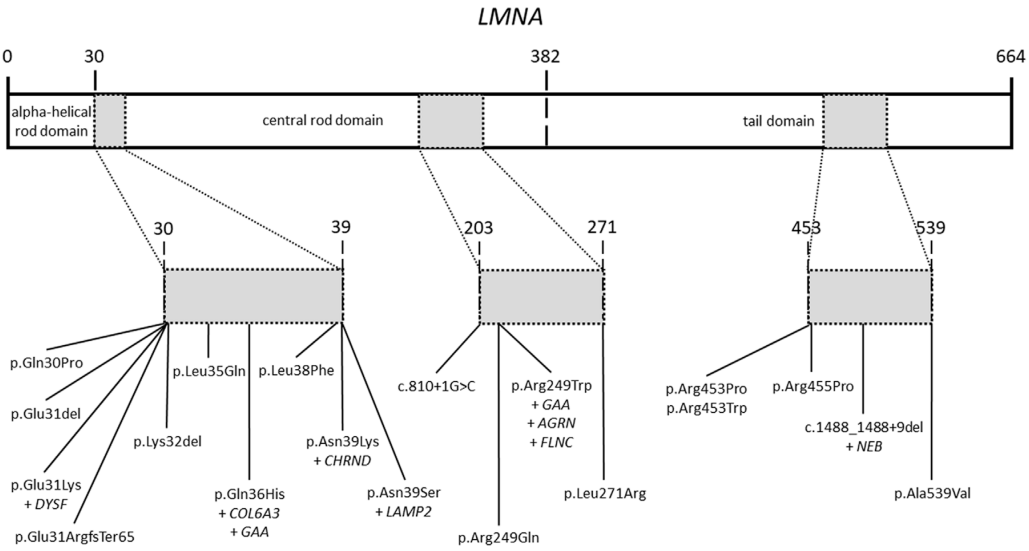


FIGURE 2
Rare variants in the *LMNA* gene. Domains of the *LMNA* gene including rare variants identified in our patients. All rare variants are located in three main zones of the gene.

variants in this gene cause mainly myopathy following an AR pattern of inheritance (Table 2). At this point, it is important to remark that none of these proteins have a close relation to the *LMNA* gene according to consulted protein databases.

These rare variants were identified in 8 families –1, 2, 5, 7, 11, 18, 22, and 23/24- (30.76%). No other rare or common alterations (single variants, delins, or CNV), classified as P, LP, or VUS-LP, were identified in any of additional genes analyzed. All the new 11 rare variants were exonic and in heterozygous state. One variant was non-sense (patient 2), 3 not-in-frame deletions (patients 2, 11, and 18), 2 not-in-frame delins (patients 23/24 -twins-), and 5 missense (patients 1, 5, 7, 18, and 22). All rare variants were classified as LP or VUS-LP following ACMG recommendations. None rare variant was repeated in more than one patient except in twins (patients 23/24) (Tables 1–3) (Figure 1).

Segregation of genetic variants in families showed that none of these rare variants was *de novo*. Curiously, only two variants in *GAA* (p.Arg594His, CM121193-patient 1-and p.Asp645Asn, CM980804-patient 18-) were previously identified and associated with Glycogen Storage Disease (GSD) (Huie et al., 1998; McCready et al., 2007; Liu et al., 2014). None of parents (except families 11 and 18, due to *LMNA*) were previously diagnosed or showed any symptom associated with the genes identified in this study (Figure 3).

3.5 Genotype-phenotype correlation

Previous clinical diagnosis and follow-up showed 9 patients with more severe phenotypes (6 EDMD -patients 3, 5, 9, 13, 16 and 22- and 3 L-CMD -patients 1, 2 and 11-). All nine patients carried a rare missense deleterious variant in *LMNA* except two (patient 9-EDMD-and 11-L-CMD-) whose carried deletions (p.Glu31ArgfsTer65 and c.1488_1488+9del, respectively) (Tables 1–3).

Six patients diagnosed with EDMD (patients 3, 5, 9, 13, 16, and 22) showed different phenotypes: DCM (patient 9) or DCM and carried an ICD (patients 3, 5, 13, and 16). Curiously, patient 22 did not show any risk factor but died suddenly. Patient 3 also died suddenly despite carrying an ICD. Only patient 9 carried a rare variant in *LMNA* (c.91delG) while all other patients carried a missense variant in the same gene. Concerning additional rare variants in other genes, only patient 5 carried a rare missense variant (c.1952G>A) in *GAA*, inherited from their unaffected mother (Figure 1).

Three patients diagnosed with L-CMD (patients 1, 2, and 11) also showed different phenotypes: DCM (patient 11) or carried an ICD (patient 1) or a PM (patient 2) but without DCM. Curiously, patients 1 and 2 (no family relationship) carried the same missense rare variant in *LMNA* (p.Arg249Trp) and patient 11 carried an intronic rare variant (c.1488_1488+9del). Additionally, all patients carried at least one rare variant: patient 1 in *GAA* (p.Arg594His) inherited from their mother and without any symptom to date, patient 2 carried two rare variants, one in *AGRN* and other in *FLNC* (p.Arg740Ter and p.Cys644TrpfsTer27, respectively), both rare variants inherited from their healthy mother, and patient 11 carried a rare variant in *NEB* (p.Met5792_Arp5794delinsIle), inherited from their mother who showed a mild neuromuscular affectation but their brother carried the same variant in *NEB* without any symptom diagnosed to date (Tables 1–3) (Figures 1, 2).

4 Discussion

A cohort of 26 patients diagnosed with *LMNA*-related muscular diseases were analyzed for the increasing number of additional rare

TABLE 1 ***LMNA*-related muscular dystrophy patients, major cardiac end points and rare *LMNA* variants.**

Patient	Sex	Age diagnosis	Phenotype	DCM	Device	*LMNA* variants (LP, P)	Other variants
1	M	11	L-CMD	No	ICD	c.745C > T (p.Arg249Trp)	*GAA*
2	M	2	L-CMD	No	PM	c.745C > T (p.Arg249Trp)	*AGRN, FLNC*
3†	F	16	EDMD	Yes	ICD	c.116A > G (p.Asn39Ser)	None
4	F	9	L-CMD	No	No	c.91_93delGAG (p.Glu31del)	None
5	M	11	EDMD	Yes	ICD	c.1358G > C (p.Arg453Pro)	*GAA*
6	M	9	L-CMD	No	No	c.745C > T (p.Arg249Trp)	None
7	M	3	EDMD	No	No	c.116A > G (p.Asn39Ser)	*LAMP2*
8	F	8	EDMD	No	No	c.746G > A (p.Arg249Gln)	None
9	M	7	EDMD	Yes	No	c.91delG (p.Glu31ArgfsTer65)	None
10	F	2	L-CMD	No	No	c.89A > C (p.Gln30Pro)	None
11	F	3	L-CMD	Yes	No	c.1488_1488+9del	*NEB*
12	M	15	EDMD	No	No	c.1616C > T (p.Ala539Val)	None
13	M	15	EDMD	Yes	ICD	c.112C > T (p.Leu38Phe)	None
14	F	8	EDMD	No	No	c.745C > T (p.Arg249Trp)	None
15	M	13	EDMD	No	No	c.812T > G (p.Leu271Arg)	None
16	M	15	EDMD	Yes	ICD	c.1364G > C (p.Arg455Pro)	None
17	F	11	LGMD1B	No	No	c.810 + 1G > C	None
18	F	3	EDMD	No	No	c.108G > T (p.Gln36His)	*COL6A3, GAA*
19	M	3	L-CMD	No	No	c.104T > A (p.Leu35Gln)	None
20	F	17	EDMD	No	No	c.1357C > T (p.Arg453Trp)	None
21	M	5	LGMD1B	No	No	c.1357C > T (p.Arg453Trp)	None
22†	F	18	EDMD	No	No	c.91G > A (p.Glu31Lys)	*DYSF*
23*,24*	F	5	L-CMD	No	No	c.117T > G (p.Asn39Lys)	*CHRND*
25	M	4	L-CMD	No	No	c.94_96delAAG (p.Lys32del)	None
26	M	4	L-CMD	No	No	c.745C > T (p.Arg249Trp)	None

DCM, dilated cardiomyopathy. EDMD, Emery-Dreifuss muscular dystrophy. F, Female. ICD, implantable cardioverter defibrillator. L-CMD, *LMNA*-related congenital muscular dystrophy. LGMD1B, Limb-girdle muscular dystrophy 1B. LP, Likely Pathogenic. M, Male. P, Pathogenic. PM, pacemaker. *, twins. †, sudden death.

alterations in other genes which may be involved in phenotype differences. We identified that 56% of patients with most severe phenotypes, mainly diagnosed with L-CMD, carried a deleterious rare variant in the *LMNA* gene, but also an additional deleterious rare variant in another gene associated with NMD, and played a potential role in early onset and disease progression.

Only a few cohorts of cases diagnosed with *LMNA*-related muscular diseases have been published to date, all following an autosomal dominant pattern of inheritance, as occurs in our study. In 2007, a cohort including 27 patients (EDMD, 56%; CMD, 15%; LGMD, 30%) (Benedetti et al., 2007). Other cohort was published in 2014, and included 78 cases diagnosed with *LMNA*-related myopathies (EDMD, 21%; L-CMD, 33%; LGMD1B, 46%) (Maggi et al., 2014). In addition, a cohort of 84 patients diagnosed with *LMNA*-related muscular dystrophy were also analyzed (EDMD, 38%; L-CMD, 49%; LGMD1B, 13%) (Fan et al., 2021). Our study shows similar percentages of *LMNA*-related muscular diseases (EDMD, 50%; L-CMD, 42%; LGMD1B, 8%). Recently, the largest cohort including 151 L-CMD patients was also published (Ben Yaou et al., 2021), reinforcing the necessity of anticipatory care of respiratory and cardiac assessment due to rapid progression of symptoms especially in L-CMD. As these are ultra-rare diseases, it is difficult to obtain patients with a definite diagnosis. Various forms of skeletal muscle laminopathies may overlap with each other, creating a phenotypic continuum, as recently reported in a cohort of 15 children with initial symptoms visible during first year of life, included hypotonia, poor head control, or delayed motor development (Jedrzejowska et al., 2021). In addition, involving large number of cases of different ethnic origin, as done in our study for the first time, is crucial to clarify role of genetic background in these ultra-rare diseases in onset as well as progression of disease. Currently, it is widely accepted *LMNA*-related muscular diseases as monogenic entities due to a single P rare variant in the *LMNA* gene. However, due to reported differences in severity of phenotypes,

TABLE 2 Additional genes identified. AD, Autosomic Dominant; AR, Autosomic Recessive; XLD, X-Linked Dominant.

Genes	Location	Protein	Gene ID/ HGNC/MIM	Disease/MIM/Inheritance
GAA	17q25.3	Alpha Glucosidase	2548/4065/606800	Pompe/232300/AR
AGRN	1p36.33	Agrin	375790/329/103320	Congenital myasthenic syndrome-8/615120/AR
FLNC	7q32.1	Filamin C	2318/3756/102565	Cardiomyopathy, familial hypertrophic/617047/AD
				Cardiomyopathy, familial restrictive/617047/AD
				Myopathy, distal/614065/AD
				Myopathy, myofibrillar/609524/AD
LAMP2	Xq24	Lysosomal associated membrane protein 2	3920/6501/309060	Danon/300257/XLD
COL6A3	2q37.3	Collagen type VI alpha 3 chain	1293/2213/120250	Bethlem myopathy/158810/AD, AR
				Dystonia/616411/AR
				Ullrich congenital muscular dystrophy/254090/AD, AR
NEB	2q23.3	Nebulin	4703/7720/161650	Arthrogryposis multiplex congenita/619334/AR
				Nemaline myopathy/256030/AR
DYSF	2p13.2	Dysferlin	8291/3097/603009	Miyoshi muscular dystrophy/254130/AR
				Muscular dystrophy, limb-girdle/253601/AR
				Myopathy, distal, with anterior tibial onset/606768/AR
CHRND	2q37.1	Cholinergic receptor nicotinic delta subunit	1144/1965/100720	Myasthenic syndrome, congenital, slow-channel/616321/AD
				Myasthenic syndrome, congenital, associated with acetylcholine receptor deficiency/616323/AR
				Multiple pterygium syndrome, lethal type/253290/AR
				Myasthenic syndrome, congenital, fast-channel/616322/AR

existence of alternative rare variants as phenotype modifiers is suspected despite not reported to date. Our study aims to solve this gap in *LMNA*-related muscular diseases.

It is widely accepted that early diagnosis of *LMNA*-related muscular diseases is key for appropriate clinical management (Charniot et al., 2003), particularly in L-CMD (Ben Yaou et al., 2021). In addition, clinical familial history and close genotype-phenotype correlation can help clarify the role of genetic variants in onset as well as progression of disease (Cotta et al., 2019; Ben Yaou et al., 2021). Therefore, existence of additional rare genetic modifiers has been suggested as an explanation for clinical phenotype difference observed in families diagnosed with any type of laminopathy (Muntoni et al., 2006; Boudreau et al., 2012; Roncarati et al., 2013) despite no comprehensive genotype-phenotype study. Analysis of variant segregation in families can help unravel the role of the rare variants identified in this study. In summary, we report a targeted genetic analysis and segregation of variants in families, looking for additional rare variants in other genes than *LMNA*, which could explain the phenotypic differences in LMNA-related muscular diseases.

4.1 Genotype-phenotype correlation

All *LMNA* variants were deleterious, which was the main cause of the clinically diagnosed disease. However, different onset as well as disease progression seems to be modified by other variants, in concordance to previously suggested but not exhaustively analyzed to date. The variant p.Arg249Trp was identified in 5 patients (4 L-CMD and 1 EMD2). This variant was previously reported in several patients diagnosed with L-CMD (Ben Yaou et al., 2021). The variant was *de novo* in all reported cases and, in addition to early onset of muscular involvement, patients also showed cardiac involvement and malignant arrhythmias (Quijano-Roy et al., 2008; Komaki et al., 2011; Pasqualin et al., 2014; Ben Yaou et al., 2021; Fan et al., 2021; Jedrzejowska et al., 2021). In view of age-dependent penetrance for heart involvement due to deleterious variants in *LMNA*, a regular cardiological supervision should have been offered (Jedrzejowska et al., 2021), particularly in p.Arg249Trp carriers due to clinical severity (Ben Yaou et al., 2021).

Patients 1 and 2, diagnosed with L-CMD showed most severe phenotype and carried additional potentially deleterious rare variants (patient 1 in *GAA* and patient 2 in *AGRN* and *FLNC*). In patient 1, the *GAA*_p.Arg594His (CM121193) was previously reported and associated with GSD following an autosomal recessive pattern of inheritance (Liu et al., 2014). It was inherited from their asymptomatic mother and none showed any symptom of GSD or Pompe disease due to the heterozygous form. In patient 2, both variants were novel, inherited from their mother and classified as deleterious. The variants in *FLNC* are mainly associated with

TABLE 3 ***LMNA*** **variants and related features found in our pediatric cohort.**

Patient	Gene	Nucleotide change	Protein change	dbSNP	gnomAD (MAF%)	HGMD disease	ClinVar disease	ACMG score	Father	Mother	Other relatives
1	*GAA*	c.1781G>A	p.Arg594His	rs775450536	2/248732 (0.0008%)	CM121193, GSD	P, GSD	LP	-	+	Sister -
	LMNA	c.745C>T	p.Arg249Trp	rs121912496	NA	CM083718, MD	P/LP, MD	LP	-	-	Sister-
2	*AGRN*	c.2218C>T	p.Arg740Ter	NA	NA	NA	NA	LP	-	+	Sister +
	FLNC	c.1932delT	p.Cys644TrpfsTer27	NA	NA	NA	NA	LP	-	+	Sister-
	LMNA	c.745C>T	p.Arg249Trp	rs121912496	NA	CM083718, MD	P/LP, MD	LP	-	-	Sister-
3†	*LMNA*	c.116A>G	p.Asn39Ser	rs57983345	NA	CM083713, MD	P, CMT	P	-	-	NA
4	*LMNA*	c.91_93delGAG	p.Glu31del	rs864309525	NA	CD156162, MD	LP, MD	LP	-	-	NA
5	*GAA*	c.1952G > A	p.Gly651Asp	rs939350425	NA	NA	VUS	LP	-	+	Siblings -
	LMNA	c.1358G > C	p.Arg453Pro	rs267607598	NA	CM083716, MD	NA	LP	-	-	Siblings -
6	*LMNA*	c.745C > T	p.Arg249Trp	rs121912496	NA	CM083718, MD	P/LP, MD	LP	-	-	NA
7	*LAMP2*	c.413A > G	p.Asp138Gly	NA	NA	NA	NA	VUS-LP	-	+	Brother -
	LMNA	c.116A > G	p.Asn39Ser	rs57983345	NA	CM083713, MD	P, CMT	P	-	-	Brother -
8	*LMNA*	c.746G > A	p.Arg249Gln	rs59332535	NA	CM1617006, MD, ED	P, MD	P	-	-	NA
9	*LMNA*	c.91delG	p.Glu31ArgfsTer65	NA	NA	NA	NA	P	-	-	NA
10	*LMNA*	c.89A > C	p.Gln30Pro	NA	NA	NA	NA	LP	-	-	Brother -
11	*LMNA*	c.1487_1488 + 9del		NA	NA	CD1711480, MD	NA	P	-	+	Brother -
	NEB	c.17376_17381del	p.Met5792_Asp5794delinsIle	rs765184893	9/247156 (0.0036%)	NA	NA	VUS-LP	-	+	Brother +
12	*LMNA*	c.1616C > T	p.Ala539Val	NA	NA	NA	NA	LP	-	-	NA
13	*LMNA*	c.112C > T	p.Leu38Phe	NA	NA	NA	NA	LP	-	-	NA
14	*LMNA*	c.745C > T	p.Arg249Trp	rs121912496	NA	CM083718, MD	P/LP, MD	LP	-	-	NA
15	*LMNA*	c.812T > G	p.Leu271Arg	NA	NA	NA	NA	LP	-	-	NA
16	*LMNA*	c.1364G > C	p.Arg455Pro	rs267607597	NA	CM111722, MD	NA	LP	-	-	NA
17	*LMNA*	c.810 + 1G > C		rs267607632	NA	NA	P, MD	P	-	-	NA
18	*COL6A3*	c.8883delA	p.Lys2961AsnfsTer40	NA	NA	NA	NA	LP	+	-	NA
	GAA	c.1933G>A	p.Asp645Asn	rs368438393	2/242414 (0.0008%)	CM980804, GSD	LP, GSD	LP	-	+	NA
	LMNA	c.108G>T	p.Gln36His	NA	NA	NA	NA	LP	+	-	NA

(Continued on following page)

TABLE 3 (*Continued*) ***LMNA*** **variants and related features found in our pediatric cohort.**

Patient	Gene	Nucleotide change	Protein change	dbSNP	gnomAD (MAF%)	HGMD disease	ClinVar disease	ACMG score	Father	Mother	Other relatives
19	*LMNA*	c.104T>A	p.Leu35Gln	NA	NA	NA	NA	LP	-	-	NA
20	*LMNA*	c.1357C>T	p.Arg453Trp	rs58932704	NA	CM990813, MD	P/LP, MD	LP	-	-	NA
21	*LMNA*	c.1357C>T	p.Arg453Trp	rs58932704	NA	CM990813, MD	P/LP, MD	LP	-	-	NA
22†	*DYSF*	c.5546G>A	p.Arg1849Lys	rs786205084	NA	NA	LP, MD	LP	-	+	Brother +
	LMNA	c.91G>A	p.Glu31Lys	rs1228406418	NA	CM123360, MD	P, CMT	LP	-	-	Brother -
23,24 twins	*CHRND*	c.919_920delCCinsAG	p.Pro307Ser	NA	NA	NA	NA	VUS-LP	-	+	Brother +/Twin +
	LMNA	c.117T>G	p.Asn39Lys	NA	NA	CM156123, MD	NA	LP	-	-	Brother -/Twin +
25	*LMNA*	c.94_96delAAG	p.Lys32del	rs60872029	NA	CD033712, MD, ED	P, MD	P	-	-	NA
26	*LMNA*	c.745C>T	p.Arg249Trp	rs121912496	NA	CM083718, MD	P/LP, MD	LP	-	-	NA

dbSNP, single nucleotide polymorphism database. ACMG, score, the American College of Medical Genetics and Genomics score. ClinVar, Clinically relevant Variation database. CMT, Charcot-Marie-Tooth. ED, Emery-Dreifuss. GnomAD (MAF%), Genome Aggregation Database (minor allele frequency %). GSD, Glycogen Storage Disease. HGMD, the Human Gene Mutation Database. LP, Likely Pathogenic. MD, muscular dystrophy. NA, not available. P, Pathogenic. †, sudden death. VUS-LP, variant of unknown significance with highly suspicious likely pathogenic role.

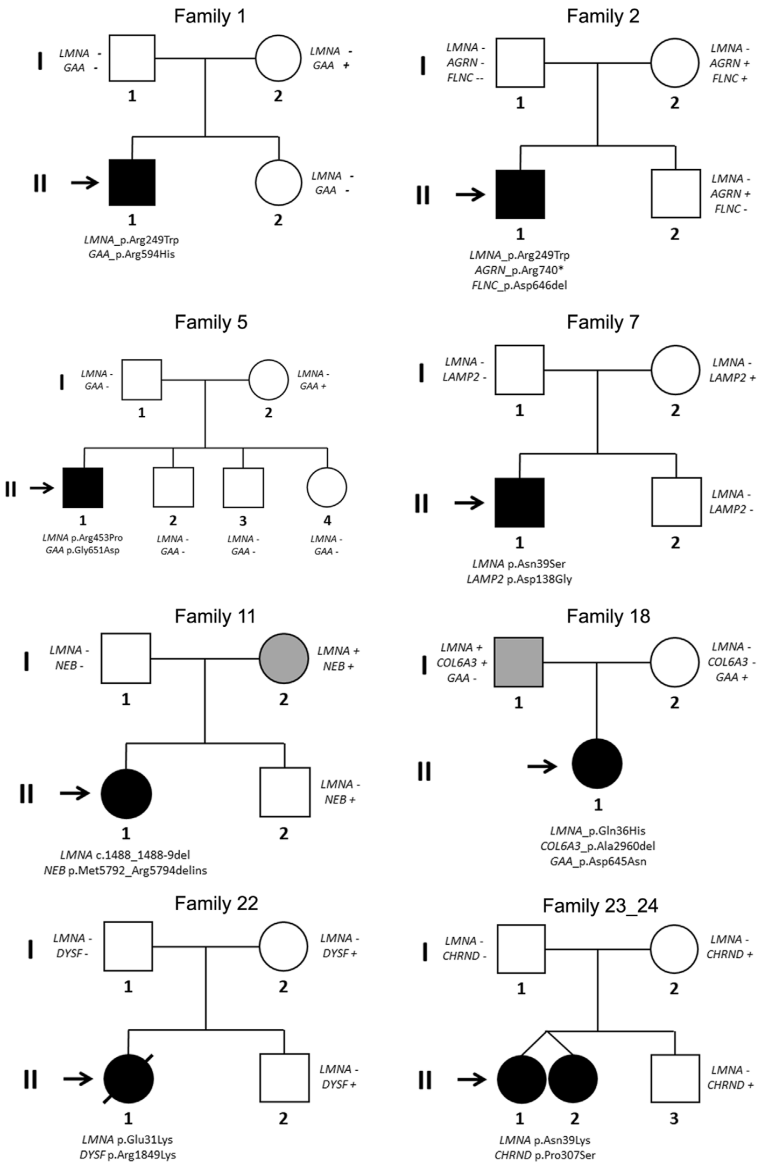


FIGURE 3
Pedigrees of families 1, 2, 5, 7, 11, 18, 22, and 23/24 (twins). Generations are indicated in the left side. Each individual of direct family linage is identified with a number. Clinically affected patients are shows in black, clinically unaffected patients are show in white, and mild phenotype is indicated in grey color. Slash indicates deceased. Index case is indicated with an arrow. Sign plus indicates carrier of the genetic variant. Minus sign indicates not carrier of the genetic variant.

Hypertrophic cardiomyopathy (not observed in patient 2 or the mother) and variants in *AGRN* are mainly associated with congenital myasthenic syndrome following an autosomal recessive pattern of inheritance. Therefore, neither mother or sister showed any symptom of muscular weakness as both are carriers of the same heterozygotic variant. Therefore, the presence of any deleterious rare variant may explain the most severe muscular weakness and malignant arrhythmias observed in patients 1 and 2, in comparison to patients 6 and 26 (both showing same diagnosis and carrying the same *LMNA* variant but without aggressive phenotype). However, further molecular studies should be performed to unravel the pathophysiological mechanism

involved in these potential phenotype modifications. Curiously, patient 14, diagnosed with EDMD and carrying the same variant p.Arg249Trp, showed a different phenotype possibly due to an unidentified alteration, reinforcing the targeted genetic analysis not only limited to the *LMNA* gene in patients diagnosed with *LMNA*-related muscular diseases. In addition, in p.Arg249, other deleterious variants were identified in patient 8, who was diagnosed with EDMD. This variant (p.Arg249Gln) was also previously reported in 3 infants showing slow muscular degeneration and slight arrhythmias (Bonne et al., 2000; Fan et al., 2021; Jedrzejowska et al., 2021), similar to this patient.

Patient 5, who showed a severe phenotype of EDMD, with DCM and implanted ICD due to malignant arrhythmias, carried two rare variants (*LMNA*_p.Arg453Pro and *GAA*_p.Gly651Asp). Both variants were novel. The *GAA* variant was inherited from the asymptomatic mother and no symptoms of GSD/Pompe disease in any carrier were observed due to widely-accepted recessive pattern of inheritance in this gene. Curiously, in the same aminoacid p.Arg453, another deleterious rare variant was previously reported as *de novo* in several cases (p.Arg453Trp) (Bonne et al., 2000; Fan et al., 2021; Jedrzejowska et al., 2021). In these reported cases, the diagnosis was EDMD concomitant with arrhythmias and slow muscular degeneration. Only in one case, the diagnosis was LGMD1B. In our cohort, two cases carried this rare variant *LMNA*_p.Arg453Trp, patient 20 diagnosed with EDMD and patient 21 diagnosed with LGMD1B (both showing slow muscular degeneration, without any arrhythmia or cardiac alteration).

Patient 7, diagnosed with EDMD but without any cardiac alteration, carried the deleterious *LMNA*_p.Asn39Ser variant. She carried an additional rare variant in the *LAMP2* gene, also identified in her mother. Rare variants in this gene are associated with Danon disease, following an X-linked pattern of inheritance. However, the mother did not show any symptom/phenotype related to Danon disease. The rare variant in *LMNA* was previously published in 5 patients, of which 3 were diagnosed with L-CMD and slow muscular weakness progression, and 2 with EDMD and no cardiac affectation (Pasqualin et al., 2014; Fan et al., 2021). In our cohort, patient 3 also carried the same variant but with a clinical diagnosis of EDMD. Despite no additional deleterious variant identified in any of all analyzed genes, DCM and malignant arrhythmias were documented. Unfortunately, this patient died due to rapidly progressive heart failure despite optimal treatment and being an ICD carrier. It suggests the targeted genetic analysis looking for other new genes not currently associated with any muscular diseases. Curiously, in the same aminoacid *LMNA*_p.Asn39, twins included in our cohort (patients 23/24) carried the deleterious variant (p.Asn39Lys) responsible of L-CMD diagnosed. This variant was recently reported in one case of L-CMD with slow muscular weakness progression and no cardiac affectation (Fan et al., 2021) and in two cases showing hypotonia, waddling gait and normal heart (Jedrzejowska et al., 2021), in concordance to our twins.

Patient 11, diagnosed with concomitant L-CMD and DCM, carried a *de novo* and novel deletion *LMNA*_c.1488_1488+9del. This variant was inherited from her mother who showed a minor muscular impairment. No history of any muscular disease was documented in previous generations. Patient 11 also carried an additional deletion in *NEB*, a gene associated with myopathies following an autosomal recessive pattern of inheritance. Both their mother and brother carried this *NEB* variant in heterozygosis form and, as expected, showing no symptom to date. In our cohort, three more *de novo* deletions in *LMNA* were identified in patients 4, 9, and 25, where two were diagnosed with L-CMD (patients 4 and 25) and showed slow muscular weakness progression with no cardiac affectation, while patient 9 was diagnosed with EDMD and DCM. In concordance, both deleterious variants were previously reported in L-CMD patients showing phenotypes similar to our patients (Fan et al., 2021).

Patient 18, diagnosed with EDMD, showed slow muscular weakness progression and no cardiac affectation. The patient carried *LMNA*_p.Gln36His, inherited from the father who showed a minor muscular impairment. No history of any muscular disease was documented in previous generations. This variant was never reported, to the best of our knowledge. This patient also carried the deleterious variant *GAA*_p.Asp645Asn, previously identified and associated with GSD (CM980804) (McCready et al., 2007). This *GAA* variant was inherited during heterozygosis from the healthy mother, not showing any symptom of GSD or Pompe disease. In addition, this patient also carried a deleterious indel in *COL6A3*. This variant has not been reported so far, and the gene is associated with dystonia and muscular dystrophy. The variant was inherited from the father who showed a minor muscular impairment. As mentioned above, no history of any muscular disease was documented in previous generations.

Patient 22, diagnosed with EDMD, showed slow muscular weakness progression and no cardiac affectation. The *LMNA*_p.Glu31Lys variant was *de novo*. Despite no aggressive phenotype, the patient died at 10 years old due to severe respiratory infection. This variant has been recently reported in one patient diagnosed with L-CMD, slow muscular weakness progression, and no cardiac affectation (Fan et al., 2021). This patient also carried a deleterious variant in *DYSF* (p.Arg1849Lys), the gene associated with an autosomal recessive muscular dystrophy. This variant was inherited from the asymptomatic mother, as also observed in the brother who also carried the same *DYSF* variant. No history of any muscular disease was documented in previous generations. Finally, patients 13 and 16, both diagnosed with EDMD, DCM and with an ICD, carried only one deleterious variant in *LMNA* (p.Leu38Phe and p.Arg455Pro, respectively). Both rare variants are novel and *de novo*, after segregation of both variants in relatives.

In conclusion, laminopathies associated with muscular disorders are a group of heterogeneous conditions with different onset and development. We suggest that a targeted genetic diagnosis including *LMNA* as well as other genes related to muscular diseases may help to unravel additional potential rare variants that could be associated with more severe phenotypes. However, translation into clinical practice should be performed with caution due to further studies in large cohorts are necessary to clarify role additional variants.

5 Limitations

The study had a few limitations. First was the reduced cohort. Due to the rarity of the disease worldwide, it is difficult get enough number of families to obtained a conclusive result in a genotype-phenotype correlation. Therefore, despite reduced number of patients, our cohort of 26 patients is the largest reported so far, other than the 84 patients reported by Fan et al. (2021). Other limitation is the

potential pathophysiological role of additional genetic alterations located in other genes not included in our NGS custom-panel and that could be implicated in phenotype modification. A potential future approach is to perform whole exome sequencing and/or whole genome sequencing to identify new alteration in any region of the genome. Our study includes a comprehensive genotype-phenotype correlation in relatives, at our point of view the main fact in genetic interpretation and clinical translation of genetic variants identified. However, both *in vivo* and *in vitro* studies should be also performed to clarify the pathophysiological mechanism associated with the progressive disable phenotype associated with the disease. Therefore, classification of rare variants should be done following ACMG recommendations and should be periodically reanalyzed, particularly if classified as having an ambiguous role. A periodic update of previous classification may help to clarify role of rare variants, helping to clinicians to obtain genetic diagnosis and, if appropriate, adopt preventive measures.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the ethics committee of Hospital Sant Joan de Déu and followed the World Medical Association Declaration of Helsinki. Written informed consent was obtained both from parents of all patients and from all relatives included in the study. Written informed consent to participate in this study was provided by the participants and legal guardian/next of kin.

Author contributions

GS-B, SC, OC, and RB developed the concept and prepared the manuscript. VF, MC, AF-F, AI, AP-S, EM-B, IM, JC, CF-C, BO, MP, LL, MA, FP, RB, CO, DA, LC-G, JE-E, IZ, AN, and JB acquired, pre-processed, and analyzed the data. GS-B, SC, OC, JB, and RB supervised the study. All authors contributed to manuscript revision, read and approved the submitted version.

Funding

This work was also supported by Obra Social. La Caixa Foundation (LCF/PR/GN16/50290001, and LCF/PR/GN19/50320002) and Instituto de Salud Carlos III, Fondo Investigación Sanitaria-FIS-(PI21/00094). Both CIBERCV and CIBERER are an initiative of the ISCIII (Instituto de Salud Carlos III), Spanish Ministry of Economy and Competitiveness.

Acknowledgments

This long-term work could not have been possible without the unconditional support of Fundación Andrés Marcio–Niños contra la Laminopatía to who we are deeply thankful. Finally, we would like to truly thank all our patients and their families, especially to Carlota who inspired us to become better scientist and doctors.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Ben Yaou, R., Yun, P., Dabaj, I., Norato, G., Donkervoort, S., Xiong, H., et al. (2021). International retrospective natural history study of LMNA-related congenital muscular dystrophy. *Brain Commun.* 3 (3), fcab075. doi:10.1093/braincomms/fcab075

Benedetti, S., Menditto, I., Degano, M., Rodolico, C., Merlini, L., D'Amico, A., et al. (2007). Phenotypic clustering of lamin A/C mutations in neuromuscular patients. *Neurology* 69 (12), 1285–1292. doi:10.1212/01.wnl.0000261254.87181.80

Bennett, R., Campbell, T., Eslick, A., Pudipeddi, A., Hing, A., Yeates, L., et al. (2021). Ventricular tachycardia in a patient with dilated cardiomyopathy caused by a novel mutation of lamin A/C gene: Insights from features on electroanatomic mapping, catheter ablation and tissue pathology. *Heart Lung Circ.* 30 (2), 310–317. doi:10.1016/j.hlc.2020.08.024

Bertrand, A. T., Chikhaoui, K., Yaou, R. B., and Bonne, G. (2011). Clinical and genetic heterogeneity in laminopathies. *Biochem. Soc. Trans.* 39 (6), 1687–1692. doi:10.1042/BST20110670

Bonne, G., Mercuri, E., Muchir, A., Urtizberea, A., Becane, H. M., Recan, D., et al. (2000). Clinical and molecular genetic spectrum of autosomal dominant Emery-Dreifuss muscular dystrophy due to mutations of the lamin A/C gene. *Ann. Neurol.* 48 (2), 170–180. doi:10.1002/1531-8249(200008)48:2<170::aid-ana6>3.0.co;2-j

Bonne, G., Yaou, R. B., Beroud, C., Boriani, G., Brown, S., de Visser, M., et al. (2003). 108th ENMC international workshop, 3rd workshop of the MYO-CLUSTER project: EUROMEN, 7th international emery-dreifuss muscular dystrophy (EDMD) workshop, 13-15 september 2002, naarden, The Netherlands. *Neuromuscul. Disord.* 13 (6), 508–515. doi:10.1016/s0960-8966(03)00063-4

Boudreau, E., Labib, S., Bertrand, A. T., Decostre, V., Bolongo, P. M., Sylvius, N., et al. (2012). Lamin A/C mutants disturb sumo1 localization and sumoylation *in vitro* and *in vivo*. *PLoS One* 7 (9), e45918. doi:10.1371/journal.pone.0045918

Carboni, N., Mateddu, A., Marrosu, G., Cocco, E., and Marrosu, M. G. (2013a). Genetic and clinical characteristics of skeletal and cardiac muscle in patients with lamin A/C gene mutations. *Muscle Nerve* 48 (2), 161–170. doi:10.1002/mus.23827

Carboni, N., Politano, L., Floris, M., Mateddu, A., Solla, E., Olla, S., et al. (2013b). Overlapping syndromes in laminopathies: A meta-analysis of the reported literature. *Acta Myol.* 32 (1), 7–17.

Charniot, J. C., Pascal, C., Bouchier, C., Sebillon, P., Salama, J., Duboscq-Bidot, L., et al. (2003). Functional consequences of an LMNA mutation associated with a new cardiac and non-cardiac phenotype. *Hum. Mutat.* 21 (5), 473–481. doi:10.1002/humu.10170

Choi, S. A., Cho, A., Kim, S. Y., Kim, W. J., Shim, Y. K., Lee, J. S., et al. (2019). Importance of early diagnosis in LMNA-related muscular dystrophy for cardiac surveillance. *Muscle Nerve* 60 (6), 668–672. doi:10.1002/mus.26700

Cotta, A., Paim, J. F., Carvalho, E., Valicek, J., da Cunha Junior, A. L., Navarro, M. M., et al. (2019). LMNA-related muscular dystrophy with clinical intrafamilial variability. *J. Mol. Neurosci.* 69 (4), 623–627. doi:10.1007/s12031-019-01390-0

Dittmer, T. A., and Misteli, T. (2011). The lamin protein family. *Genome Biol.* 12 (5), 222. doi:10.1186/gb-2011-12-5-222

Fan, Y., Tan, D., Song, D., Zhang, X., Chang, X., Wang, Z., et al. (2021). Clinical spectrum and genetic variations of LMNA-related muscular dystrophies in a large cohort of Chinese patients. *J. Med. Genet.* 58 (5), 326–333. doi:10.1136/jmedgenet-2019-106671

Heller, F., Dabaj, I., Mah, J. K., Bergounioux, J., Essid, A., Bonnemann, C. G., et al. (2017). Cardiac manifestations of congenital LMNA-related muscular dystrophy in children: Three case reports and recommendations for care. *Cardiol. Young* 27 (6), 1076–1082. doi:10.1017/S1047951116002079

Huie, M. L., Tsujino, S., Sklower Brooks, S., Engel, A., Elias, E., Bonthron, D. T., et al. (1998). Glycogen storage disease type II: Identification of four novel missense mutations (D645N, G648S, R672W, R672Q) and two insertions/deletions in the acid alpha-glucosidase locus of patients of differing phenotype. *Biochem. Biophys. Res. Commun.* 244 (3), 921–927. doi:10.1006/bbrc.1998.8255

Jedrzejowska, M., Potulska-Chromik, A., Gos, M., Gambin, T., Debek, E., Rosiak, E., et al. (2021). Floppy infant syndrome as a first manifestation of LMNA-related congenital muscular dystrophy. *Eur. J. Paediatr. Neurol.* 32, 115–121. doi:10.1016/j.ejpn.2021.04.005

Komaki, H., Hayashi, Y. K., Tsuburaya, R., Sugie, K., Kato, M., Nagai, T., et al. (2011). Inflammatory changes in infantile-onset LMNA-associated myopathy. *Neuromuscul. Disord.* 21 (8), 563–568. doi:10.1016/j.nmd.2011.04.010

Kumar, S., Baldinger, S. H., Gandjbakhch, E., Maury, P., Sellal, J. M., Androulakis, A. F., et al. (2016). Long-term arrhythmic and nonarrhythmic outcomes of lamin A/C mutation carriers. *J. Am. Coll. Cardiol.* 68 (21), 2299–2307. doi:10.1016/j.jacc.2016.08.058

Liu, X., Wang, Z., Jin, W., Lv, H., Zhang, W., Que, C., et al. (2014). Clinical and GAA gene mutation analysis in mainland Chinese patients with late-onset Pompe disease: Identifying c.2238G > C as the most common mutation. *BMC Med. Genet.* 15, 141. doi:10.1186/s12881-014-0141-2

Maggi, L., D'Amico, A., Pini, A., Sivo, S., Pane, M., Ricci, G., et al. (2014). LMNA-Associated myopathies: The Italian experience in a large cohort of patients. *Neurology* 83 (18), 1634–1644. doi:10.1212/WNL.0000000000000934

Maraldi, N. M., Capanni, C., Cenni, V., Fini, M., and Lattanzi, G. (2011). Laminopathies and lamin-associated signaling pathways. *J. Cell Biochem.* 112 (4), 979–992. doi:10.1002/jcb.22992

McCready, M. E., Carson, N. L., Chakraborty, P., Clarke, J. T., Callahan, J. W., Skomorowski, M. A., et al. (2007). Development of a clinical assay for detection of GAA mutations and characterization of the GAA mutation spectrum in a Canadian cohort of individuals with glycogen storage disease, type II. *Mol. Genet. Metab.* 92 (4), 325–335. doi:10.1016/j.ymgme.2007.07.006

Muntoni, F., Bonne, G., Goldfarb, L. G., Mercuri, E., Piercy, R. J., Burke, M., et al. (2006). Disease severity in dominant Emery Dreifuss is increased by mutations in both emerin and desmin proteins. *Brain* 129, 1260–1268. doi:10.1093/brain/awl062

Murofushi, Y., Hayakawa, I., Abe, Y., Nakao, H., Ono, H., and Kubota, M. (2022). The most severe form of LMNA-associated congenital muscular dystrophy. *Brain Dev.* 44, 650–654. doi:10.1016/j.braindev.2022.05.006

Pasqualin, L. M., Reed, U. C., Costa, T. V., Quedas, E., Albuquerque, M. A., Resende, M. B., et al. (2014). Congenital muscular dystrophy with dropped head linked to the LMNA gene in a Brazilian cohort. *Pediatr. Neurol.* 50 (4), 400–406. doi:10.1016/j.pediatrneurol.2013.11.010

Paul, J. C., and Fulka, H. (2022). Nuclear lamins: Key proteins for embryonic development. *Biol. (Basel)* 11 (2), 198. doi:10.3390/biology11020198

Quijano-Roy, S., Mbieleu, B., Bonnemann, C. G., Jeannet, P. Y., Colomer, J., Clarke, N. F., et al. (2008). De novo LMNA mutations cause a new form of congenital muscular dystrophy. *Ann. Neurol.* 64 (2), 177–186. doi:10.1002/ana.21417

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., et al. (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. *Genet. Med.* 17 (5), 405–424. doi:10.1038/gim.2015.30

Roncarati, R., Viviani Anselmi, C., Krawitz, P., Lattanzi, G., von Kodolitsch, Y., Perrot, A., et al. (2013). Doubly heterozygous LMNA and TTN mutations revealed by exome sequencing in a severe form of dilated cardiomyopathy. *Eur. J. Hum. Genet.* 21 (10), 1105–1111. doi:10.1038/ejhg.2013.16

Worman, H. J., and Bonne, G. (2007). Laminopathies": A wide spectrum of human diseases. *Exp. Cell Res.* 313 (10), 2121–2133. doi:10.1016/j.yexcr.2007.03.028

Worman, H. J. (2012). Nuclear lamins and laminopathies. *J. Pathol.* 226 (2), 316–325. doi:10.1002/path.2999

Zheng, M., Jin, G., and Zhou, Z. (2022). Post-translational modification of lamins: Mechanisms and functions. *Front. Cell Dev. Biol.* 10, 864191. doi:10.3389/fcell.2022.864191

Otros resultados no publicados.

Artículo 3. Resumen estructurado.

Cardiovascular involvement in pediatric Myotonic Muscular Dystrophy type-1.

Introducción

La distrofia miotónica tipo 1 es la distrofia muscular más prevalente en adultos y, en muchos casos, el diagnóstico se establece ya en edad pediátrica. En adultos existe un riesgo alto de muerte súbita por arritmias potencialmente letales y el riesgo está bien definido, aunque la afectación cardiovascular en pediatría está infraestimada y no existen guías clínicas específicas o recomendaciones.

Objetivos

Descripción del estado cardiovascular y la identificación de predictores potenciales de arritmias y muerte súbita en una cohorte pediátrica con distrofia miotónica tipo 1.

Métodos

El estudio se basa en datos retrospectivos, desde 1998 hasta 2021, de una cohorte pediátrica con distrofia miotónica tipo 1, añadiendo una intervención prospectiva para valorar el estado cardiovascular mediante ecocardiografía, electrocardiograma, Holter cardiaco 24h y, según el caso, estudio electrofisiológico. Los pacientes se clasificaron según si la distrofia miotónica fue congénita (primer mes de vida), infantil (desde el mes de vida hasta loa 10 años) o juvenil (de 11 a 20 años).

Resultados

Se reclutó datos clínicos pertenecientes a 40 pacientes de <18 años al diagnóstico de distrofia miotónica tipo 1 (40% sexo femenino). El seguimiento medio de la intervención prospectiva fue de 3.12 años y la distribución según subgrupos fue de un 30% forma congénita, 47.5% forma infantil y 22.5% forma juvenil; 1 paciente tuvo criterios limítrofes para la forma congénita/infantil. Sólo

falleció un paciente en época neonatal debido a la prematuridad y a presentar debilidad muscular importante por una forma congénita de la DM1. En cuanto a los datos ecocardiográficos, no hubo diagnóstico de miocardiopatía ni insuficiencia cardíaca. Tan sólo 4 pacientes tuvieron hallazgos menores (hipertrabeculación apical del ventrículo izquierdo, con fracción de eyección conservada). Durante el seguimiento se destaca el hallazgo de bloqueo AV de primer grado (7 pacientes mediante ECG y 5 pacientes mediante Holter ECG 24h). No hubo otros grados de bloqueo AV más avanzados excepto bloqueo AV de segundo grado tipo 1 en horas de descanso nocturno. Se encontraron también otros hallazgos ECG: bloqueo incompleto de rama derecha (8 pacientes), bloqueo completo de rama derecha (1 paciente), desviación del eje de QRS (7 pacientes), bradicardia sinusal (7 pacientes), hipovoltaje del QRS (16 pacientes), elevación del punto J (9 pacientes), QS en V1 (4 pacientes). La suma de algunos de los hallazgos ECG descritos fueron encontradas en 18 pacientes. Se detectaron arritmias ventriculares en 8 pacientes, uno de ellos con alta carga de extrasístoles ventriculares y duplas ventriculares asociado a bloqueo AV de primer grado, sin precisar colocación de marcapasos ni desfibrilador después de realizar un estudio electrofisiológico. Ningún paciente tuvo síntomas relacionados con los hallazgos ecocardiográficos o electrocardiográficos ni tampoco hubo criterios de implante de marcapasos o desfibrilador.

Los datos clínicos y cardiovasculares no se pudieron correlacionar con el número de expansión de CTG, debido a que las copias fueron contabilizadas tan sólo en algunos pacientes.

Conclusiones

En la cohorte estudiada de pacientes pediátricos con Distrofia miotónica tipo se hallaron alteraciones electrocardiográficas, especialmente bloqueo AV de primer grado y arritmias ventriculares, que podrían ser el sustrato potencial para que en la etapa adulta presenten arritmias malignas o muerte súbita. No hubo pacientes que requirieran estimulación con marcapasos ni criterios para implante de desfibrilador. Un seguimiento exhaustivo a nivel cardiológico en un equipo multidisciplinar es clave para la detección de arritmias y determinar el riesgo que presentan estos pacientes durante la infancia, aunque se necesitan más estudios

con mayor número de pacientes para determinar si estos hallazgos son la base de futuras complicaciones cardiovasculares en la etapa adulta joven.

Cardiovascular involvement in pediatric Myotonic Muscular Dystrophy type 1.

Sergi Cesar, Jose Cruzalegui, Andres Nascimento, Carlos Ignacio Ortez, Daniel Natera, Laura Carrera, Jessica Exposito, Josep Brugada, Georgia Sarquella-Brugada

Abstract

Myotonic muscular dystrophy type 1 is the most prevalent neuromuscular dystrophy in adults, and, in most cases, diagnosis is stablished during childhood. Adults are at high risk of sudden death because of life-threatening arrhythmias and risk are well-defined, but cardiovascular involvement is underestimated in pediatric population and there is no specific cardiovascular guidelines or recommendations. This study focus on cardiovascular involvement in a cohort of pediatric DM1 patients and to investigate potential predictors of arrhythmias and sudden death.

Introduction

Myotonic muscular dystrophy type 1 (DM1) is the most prevalent inherited neuromuscular disease in adult population with an estimated prevalence of 1:8000 (1,2). Inherited pattern is characterized by autosomal-dominant expansion of CTG trinucleotide repeat on chromosome 19q13.3 and anticipation in consecutive generations associates earlier onset and increased severity. (3,4)

The main manifestations in this multisystemic disease are the neuromuscular symptoms and includes progressive muscle weakness and myotonia, but other manifestations can be expressed (ocular, gastrointestinal, central nervous, respiratory, endocrine, urinary and cardiac).

The classification during the pediatric age is based on age of onset and clinical severity: congenital (cDM1), infantile (iDM1) and juvenile (jDM1) forms. (5)

Mortality rate during neonatal period in cDM1 is very high and can reach up to 40%, mainly due to respiratory disease secondary to severe global weakness. (6,7)

Cardiac manifestations are very frequent in adults diagnosed with DM1. Heart failure, progressive conduction defects and ventricular arrhythmias can be related with sudden cardiac death (SCD), that is estimated at 0.5%/year in adult population.(5) There is a lack of information about cardiovascular involvement in children diagnosed with DM1 and

there are no published specific pediatric guidelines. Last published recommendations from the HRS expert consensus for cardiovascular evaluation are provided for both pediatric and adult DM1 patients, but specific studies are needed evaluating the risk of arrhythmias and SCD and to stablish predictors in pediatric population. (2,8)

Objective

The aim of this study is to describe the cardiovascular profile and to find predictors of arrhythmias and sudden death of a cohort with genetically confirmed diagnosis of DM1, based on retrospective data and a prospective intervention during 5 years with echocardiography, electrocardiogram and 24h-holter monitoring.

Methods

A retrospective study with a prospective intervention in pediatric patients diagnosed with DM1 was conducted in our national referral center for pediatric neuromuscular disease. The patients were classified in congenital form (from birth to 1 month of age), infantile form (iDM1, from 1 month to 10 years of age) and juvenile form (jDM1, from 11 years to 20 years of age). According to the age and severity, medical data, family history, 12-lead ECG, transthoracic echocardiography, 24h ECG Holter monitoring and electrophysiological study were performed. Data was reviewed retrospectively from 1998 to 2021, and the prospective data was performed until the transition to adult hospital, the end of study period (maximum 6 years of follow-up in the prospective period) or the end due to mortality.

The study was approved by the Ethics Committee of the Hospital Sant Joan de Déu (Barcelona, Spain), following the Helsinki II declaration.

Cardiac Imaging

Echocardiogram data were collected following the standard assessment of cardiac dimensions and function. Normal left ventricular systolic function was defined as left ventricular ejection fraction >55%. (9,10)

Electrocardiographic analysis

12-lead ECG analysis was evaluated from retrospective data and from the last ECGs during the interventional prospective period. Definitions of normality were based on published data. (11,12) Arrhythmias and SDNN values were assessed from 24h-holter ECG monitoring.

Statistics

Normally distributed data are presented as mean values [± standard deviation (SD)] and non-normally distributed data as a median [interquartile range (IQR)]. Categorical variables are presented as number and percentages.

Results

Clinical data

A total of 40 patients <18 years of age (40% female, 60% male, mean age 11 (±5.52), from 1998 to 2021, were included in the retrospective analysis. The interventional prospective follow-up had a mean of 3.12 years (±1.9). Table 1 shows the clinical and demographic characteristics of the cohort. From the overall cohort, the distribution of the different forms of DM1 were as follows: 12 patients (30%) were cDM1 form, 19 patients (47.5%) were iDM1, 1 patient met borderline criteria c/iDM1 and 9 were jDM1 form (22.5%). During the follow-up, only two patients experimented cardiovascular symptoms (dizziness) with no relation with cardiovascular involvement. In our cohort, none of the patients included died suddenly. Only one patient died due to the global severity of the disease in a cDM1 form in association to the extreme prematurity and with the largest CTG expansion (2333) of the cohort.

Echocardiographic data

All alive patients showed preserved LV ejection fraction in the retrospective and prospective analysis. Four patients showed LV apical hypertrabeculation, one of them were a cDM1 patient with moderate trabeculation with criteria for LV non compaction with preserved ejection fraction. Echocardiographic analysis, including functional parameters and myocardial deformation, did not show abnormalities in pediatric age during the follow-up. Other findings were bicuspid aortic valve in 1 patient, with no

stenosis and no valvular insufficiency. No other cardiomyopathies and no other congenital heart disease were found in the pediatric cohort.

Electrocardiographic data

Electrocardiographic features are described in the table 3. At last assessed ECG, 75% of patients had different abnormalities in the ECG: 7 patients showed 1st degree AV block (17.5%). No complete AV block were found. Incomplete right branch bundle block was a frequent finding (8 patients, 20%), and only one patient had criteria for complete RBBB. Other findings were: QRS axis deviation (left -3 patients-, right -3 patients-, superior -3 patients), sinus bradycardia in 7 patients (17.5%), low QRS voltage (V1, V6 and/or global QRS low voltage in 16 patients -40%-), elevated J point in V2 and/or V3 in 9 patients (22.5%), QS wave in V1 in 4 patients (10%). An accumulation of different ECG abnormalities in the same patient were frequent (18 patients, 45%).

Table 4 summarizes data about 24h-Holter ECG monitoring. At least, 37 patients (92.5%) had a 24h-Holter ECG monitoring during follow-up and about 72.9% had a second 24h-Holter ECG monitoring during follow-up. Data are represented in the table 4. One patient had isolated sinus pause during night rest, 5 patients showed 1st degree AV block non previously described in the 12-lead ECG (12.5%), 2 patients had type I 2nd degree AV block during night rest (5%), 2 sinus bradycardia, and 8 patients (20%) had ventricular extrasystoles (1 of them -patient 19- ventricular bigeminy and ventricular couplets and 1st degree AV block that required an EPS with no ICD or PM criteria). No other patients met criteria for PM/ICD implantation neither EPS during follow-up.

Other systemic involvement

In the table 5 are represented the main systemic involvement and other findings in this cohort. Cognitive delay, language delay and global developmental delay were frequent (52.5%). Attention-deficit/hyperactivity disorder (ADHD) was diagnosed in 7 patients. Autism Spectrum Disorder was diagnosed in 2 patients.

About respiratory involvement, all patients were functionally evaluated and 12 met criteria for non-invasive ambulatory ventilation (NIV) during follow-up (5 cDM1, 10 iDM1 and 1jDM1). In some cases, NIV treatment was discontinued because of inadaptability.

Three patients with cDM1 form had dysphagia but with no percutaneous endoscopic gastrostomy was required.

Considering tendon retractions, Achilles tendon retraction was a frequent finding (6 patients, 2 cDM1 and 3 iDM1).

Ophthalmological evaluation was performed in all patients as routine evaluation, found cataract in 3 male patients (2 with cDM1 form and 1 with iDM1 form).

Genetics

CTG expansion were pathological in all cases, but only numerically calculated or available in 57.5% of the cohort. The 4 patients with apical LV hypertrabeculation showed 1250, 400 and 1000 CTG repeat length but only 1 of them with moderate apical trabeculation showed abnormalities in the ECG (low QRS voltage in V1 and V6 leads and deep R wave in V3 lead).

Inheritance was maternal in 26 cases (65%) and 14 was paternal (35%).

Discussion

Myotonic muscular dystrophy is classically related with arrhythmias and cardiomyopathy during adulthood. (13) Cardiac involvement may present as asymptomatic ECG abnormalities and symptomatic arrhythmias due to sinus-node dysfunction, heart block, atrial and ventricular arrhythmias. (2,13) In addition to arrhythmias and heart failure, progressive respiratory failure is a mechanism of death. (2,14) There is a lack about evidence and recommendations in pediatric population. Recently, Brunet et al published a retrospective study of 65 cDM1 patients highlighting that 3 deaths were defined as sudden death but no major ECG abnormalities or arrythmias and no classic risk ECG criteria were detected in these patients. (7)

The pathophysiology of DM1 relies on a toxic RNA gain of function and CTG repeats are retained in the nuclei and cause alternative splicing deregulation of a subset of pre-mRNAs. This mechanism of abnormal splicing on SCN5A gene has been identified as a contributor of arrhythmias in this population. (2) Moreover, previous studies have demonstrated that the larger CTG amplification size the higher prevalence of cardiac manifestations, but arrhythmias are not only exclusive in patients with larger CTG

repeats. (2,15,16) We cannot evaluate the real impact of CTG expansion on cardiac involvement or ECG features in our cohort, due to the small size and the lack of data about exact expansion since in the oldest genetic evaluations CTG repeats were not systematically calculated.

Cardiomyopathy

The onset of cardiomyopathy during childhood is extremely rare. Previous published series did not identify any patient with cardiomyopathy. (7,17) We found mild apical LV hypertrabeculation in 3 patients and 1 one moderate hypertrabeculation with borderline criteria for non-compaction cardiomyopathy with preserved ejection fraction. Echocardiographic analysis and myocardial deformation did not show abnormalities during follow-up. Probably, those patients with structural cardiomyopathy or echocardiographic abnormalities will be present during adulthood. Other findings were patent foramen ovale (2 patients) and bicuspid aortic valve (1 patient). We did not find any other cardiac findings like in previous published series: patent ductus arteriosus, aortic root dilatation, ventricular septal defect, mitral valve prolapse and hypertrophic cardiomyopathy. (7)

ECG findings, conduction defects and arrhythmias

Previous pediatric series described more cardiac arrhythmias in cDM1. Ho et al. published that only 1 cDM1 patient required an intervention because of atrial fibrillation during pediatric age. (17) Other series did not document supraventricular or ventricular arrhythmias during childhood. (7) Last published expert consensus describes that atrial arrhythmias and ventricular tachycardia, sometimes exercise-induced, can be present in pediatric patients after 10 years of age but no complete AV block have been described.(2,18)

In our cohort, 75% of patients had different ECG findings classically considered minor or clinically in general population but that become in future predictors for SCD in adulthood.

The most frequent conduction defect found in our cohort was asymptomatic 1st degree AV block (12 patients, 30%) diagnosed with 12-lead ECG and 24h-Holter ECG monitoring, that is congruent with a recent pediatric series of cDM1 but, interestingly, we detected

this conduction defect in both cDM1 (3 patients) and iDM1 (9 patients) forms. (7) Other series did not find this rate about conduction defects, or it was considered as a minor finding with no numerical estimation within the cohort. (17,19) Early onset conduction defects during childhood could be in relation with more severe phenotype during adulthood, but further studies including natural history from infancy are needed.

Within the other ECG findings described in our cohort, low QRS voltage have been described in other cohorts suggesting that could be related with more severity and mortality in cDM1 form. (7) This global low QRS voltage have been related to an increased risk of mortality in other diseases such as amyloidosis or in patients with apparently no cardiovascular disease. (20,21)

QRS axis deviation could be related to chest deformities and respiratory treatment with NIV, but it should be assessed in further studies in a largest cohort.

Ventricular arrhythmias were found in 8 asymptomatic patients, 1 of them with frequent ventricular ectopy (ventricular bigeminy, ventricular couplets) associated to 1st degree AV block that required an EPS but with no criteria for ICD implantation.

There is no pediatric data about the electrophysiological data related to an increased risk for complete AV block and sudden death. An increased risk of complete AV block and sudden death have been described in adult population with an HV interval > 70ms and a prolongation of HV interval of 1.2ms/year when serial EPS were done. (2,22,23)

Sudden death

Predictors of SCD in adults have been published (PR ≥ 240ms, QRS ≥ 120, QTc ≥ 450ms), as well other arrhythmias like complete AV block, asystole, and ventricular arrhythmias. (13,24). There is no information about the risk of SCD in patients with mild-to-moderate AV conduction impairment (PR 200-240ms and QRS 100-120ms). (2) These last values are, frequently, found in pediatric patients as we described in our cohort. Sudden death was no present in our cohort during pediatric age. Death in patient 28 was related with the severity of the disease in a cDM1 form in association with extreme prematurity, sepsis, and respiratory complications. Pediatric published series highlighted a rate of 6.1% of unexplained deaths within cDM1 patients, and 1 patient with symptomatic AV conduction disease. (7)We did not find mortality in relation with cardiac involvement in

our cohort, but these previous series suggest that these patients, probably cDM1 and iDM1 forms, are a higher risk of SCD during childhood.

Systemic involvement: extracardiac features

Cognitive delay and global developmental delay are frequent features in DM1 population, especially in those congenital and infantile forms. Motor symptoms and delayed milestones are frequent and respiratory evaluation is mandatory for investigate the necessity of ventilation. Other frequent extracardiac features are gastrointestinal (fecal incontinence, constipation, dysphagia), urinary incontinence and cataracts. Behavior and learning difficulties are very frequent and in some cases are related with attention-deficit hyperactivity disorder (ADHD). In our series 7 patients met criteria for ADHD (1 cDM1, 3 iDM1 and 3 jDM1). Neuropsychological evaluation is needed to evaluate cognitive strengths and weaknesses, including global intellectual ability, executive functions, social cognition, visuomotor and visuospatial ability, language abilities, daytime sleepiness and learning disabilities. (17,25)

Follow-up during childhood

As published in previous recommendations (24) and in the last expert consensus (2), coordinated care of patients with DM1 is needed to assess neurological, cardiac, ophthalmologic, endocrine, gastrointestinal, and other specialties according to phenotypic manifestations. About cardiac evaluation, ECG, Holter ECG monitoring and cardiac imaging is needed during follow-up even in the absence of cardiac symptoms. Close monitoring for arrhythmic complications should be perform when there is a treatment with a sodium channel blocker because of the increasing loss of function of the cardiac sodium channel. EPS is indicated when there are symptoms of bradycardia with mild-to-moderate conduction disorder in the ECG and/or symptoms suggestive of ventricular tachycardia, to assess the risk of AV block, sustained arrhythmias and sudden cardiac death.

Conclusions

In our cohort, children with DM1 had high yield of ECG cardiac abnormalities, especially 1st AV block and ventricular arrhythmias, that could be potential basis on future life-

threatening arrhythmias and sudden cardiac death in adulthood. In our series, death was present in only 1 patient in relation with prematurity with a severe congenital phenotype DM1 and no patients required a PM or an ICD. Comprehensive cardiac follow-up from the diagnosis or suspected diagnosis, in a multidisciplinary team, is key to assess patients with DM1. Further studies are needed to clarify the role of the ECG abnormalities found in children and the probable relation to higher risk in adulthood.

References

1. Wahbi K, Babuty D, Probst V, Wissocque L, Labombarda F, Porcher R, et al. Incidence and predictors of sudden death, major conduction defects and sustained ventricular tachyarrhythmias in 1388 patients with myotonic dystrophy type 1. Eur Heart J. 2017;38(10):751–8.

2. Groh WJ, Bhakta D, Tomaselli GF, Aleong RG, Teixeira RA, Amato A, et al. 2022 HRS expert consensus statement on evaluation and management of arrhythmic risk in neuromuscular disorders. Heart Rhythm. 2022;19(10):e61–120.

3. Šabovič M, Medica I, Logar N, Mandić E, Zidar J, Peterlin B. Relation of CTG expansion and clinical variables to electrocardiogram conduction abnormalities and sudden death in patients with myotonic dystrophy. Neuromuscul Disord. 2003;13(10):822–6.

4. Lau JK, Sy RW, Corbett A, Kritharides L. Myotonic dystrophy and the heart: A systematic review of evaluation and management. Int J Cardiol. 2015;184:600–8.

5. Dogan C, De Antonio M, Hamroun D, Varet H, Fabbro M, Rougier F, et al. Gender as a modifying factor influencing myotonic dystrophy type 1 phenotype severity and mortality: A nationwide multiple databases cross-sectional observational study. PLoS One. 2016;11(2):30148264.

6. Pelargonio G, Dello Russo A, Sanna T, De Martino G, Bellocci F. Myotonic dystrophy and the heart. Heart. 2002;88:665–70.

7. Brunet Garcia L, Hajra A, Field E, Wacher J, Walsh H, Norrish G, et al. Cardiac Manifestations of Myotonic Dystrophy in a Pediatric Cohort. Front Pediatr. 2022;10:910660.

8. Zeppenfeld K, Tfelt-Hansen J, De Riva M, Winkel BG, Behr ER, Blom NA, et al. 2022 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. Eur Heart J. 2022;43(40):3997–4126.

9. Lopez L, Colan SD, Frommelt PC, Ensing GJ, Kendall K, Younoszai AK, et al. Recommendations for Quantification Methods During the Performance of a

Pediatric Echocardiogram: A Report From the Pediatric Measurements Writing Group of the American Society of Echocardiography Pediatric and Congenital Heart Disease Council. J Am Soc Echocardiogr. 2010;23(5):465–95.

10. Lopez L, Colan S, Stylianou M, Granger S, Trachtenberg F, Frommelt P, et al. Relationship of Echocardiographic Z Scores Adjusted for Body Surface Area to Age, Sex, Race, and Ethnicity: The Pediatric Heart Network Normal Echocardiogram Database. Circ Cardiovasc Imaging. 2017;10(11):e006979.

11. Rijnbeek PR, Van Herpen G, Bots ML, Man S, Verweij N, Hofman A, et al. Normal values of the electrocardiogram for ages 16-90 years. J Electrocardiol. 2014;47(6):914–21.

12. Rijnbeek PR, Witsenburg M, Schrama E, Hess J, Kors JA. New normal limits for the paediatric electrocardiogram. Eur Heart J. 2001;22(8):702–11.

13. Petri H, Vissing J, Witting N, Bundgaard H, Kober L. Cardiac manifestations of myotonic dystrophy type 1. In J Cardiol. 2012;160(2):82–8.

14. Wahbi K, Babuty D, Probst V, Wissocque L, Labombarda F, Porcher R, et al. Incidence and predictors of sudden death, major conduction defects and sustained ventricular tachyarrhythmias in 1388 patients with myotonic dystrophy type 1. Eur Heart J. 2017;38(10):751–8.

15. Chong-Nguyen C, Wahbi K, Algalarrondo V, Bécane HM, Radvanyi-Hoffman H, Arnaud P, et al. Association between Mutation Size and Cardiac Involvement in Myotonic Dystrophy Type 1: An Analysis of the DM1-Heart Registry. Circ Cardiovasc Genet. 2017;10(3).

16. Groh WJ, Lowe MR, Zipes DP. Severity of cardiac conduction involvement and arrhythmias in myotonic dystrophy type 1 correlates with age and CTG repeat length. J Cardiovasc Electrophysiol. 2002;13(5):444–8.

17. Ho G, Carey KA, Cardamone M, Farrar MA. Myotonic dystrophy type 1: Clinical manifestations in children and adolescents. Arch Dis Child. 2019;104(1):48–52.

18. Bassez G, Lazarus A, Desguerre I, Varin J, Laforêt P, Bécane HM, et al. Severe cardiac arrhythmias in young patients with myotonic dystrophy type 1. Neurology. 2004;63(10):1939-41.

19. Sharma A, Singh S, Mishra SK. Cardiac abnormalities in congenital and childhood Myotonic muscular dystrophy type 1. Neuropediatrics. 2017;48(1):42–4.

20. Usoro AO, Bradford N, Shah AJ, Soliman EZ. Risk of mortality in individuals with low QRS voltage and free of cardiovascular disease. Am J Cardiol. 2014;113(9):1514–7.

21. Mussinelli R, Salinaro F, Alogna A, Boldrini M, Raimondi A, Musca F, et al. Diagnostic and prognostic value of low QRS voltages in cardiac AL amyloidosis. Ann Noninvasive Electrocardiol. 2013;18(3):271–80.

22. Wahbi K, Meune C, Porcher R, Bécane HM, Lazarus A, Laforêt P, et al. Electrophysiological Study With Prophylactic Pacing and Survival in Adults With Myotonic Dystrophy and Conduction System Disease. JAMA;307(12):1292-301.

23. Lallemand B, Clementy N, Bernard-Brunet A, Pierre B, Corcia P, Fauchier L, et al. The evolution of infrahissian conduction time in myotonic dystrophy patients: Clinical implications. Heart. 2012;98(4):291–6.

24. Feingold B, Mahle WT, Auerbach S, Clemens P, Domenighetti AA, Jefferies JL, et al. Management of cardiac involvement associated with neuromuscular diseases: A scientific statement from the American Heart Association. Circulation. 2017;136(13):e200–e231.

25. Johnson NE, Aldana EZ, Angeard N, Ashizawa T, Berggren KN, Marini-Bettolo C, et al. Consensus-based care recommendations for congenital and childhood-onset myotonic dystrophy type 1. Neurol Clin Pract. 2019;9(5):443–54.

Table 1. Clinical and demographic data															
Patient ID	DM1 form	Age at genetic diagnosis (years)	Age at first symptoms (months or years)	Death	CTG expansion	Inheritance	NIV	Gender	Age (inclusion)	Follow-up (years)	CV Symptoms	PM/ICD	Cardiomyopathy	Echo-cardiographic analysis	Cataract
1	iDM1	NA	12yo	N	NA	maternal	Y	M	18	3	N	N	N	Normal	N
2	cDM1	NA	<1month	N	1250	maternal	N	M	6	5	N	N	Moderate apical LV hypertrabeculation	Normal	N
3	cDM1	NA	<1month	N	1667	paternal (exitus, arrhythmia)	N	M	5	5	N	N	N	Normal	Y
4	jDM1	5	Asymptomatic	N	135	paternal	N	F	7	3	N	N	N	Normal	N
5	jDM1	9	Asymptomatic	N	123	paternal	N	F	12	6	N	N	N	Normal	N
6	iDM1	5	NA	N	NA	paternal	N	M	20	2	N	N	N	Normal	Y
7	iDM1	10	NA	N	NA	paternal	N	M	17	1	N	N	N	Normal	N
8	jDM1	NA	12yo	N	NA	paternal	N	F	16	2	Dizziness (18yo)	N	N	Normal	N
9	jDM1	NA	Asymptomatic	N	NA	paternal	N	F	17	1	N	N	N	Normal	N
10	iDM1	NA	3yo (motor symptoms)	N	NA	maternal	Y	M	11	3	N	N	N	Normal	N
11	cDM1	NA	<1month	N	NA	maternal	Y	M	10	5	N	N	Mild apical LV hypertrabeculation	Normal	N
12	cDM1	11	<1month	N	NA	maternal	Y	M	17	1	N	N	N	Normal	N
13	cDM1	1	<1month	N	1000	maternal	Y	M	11	6	N	N	N	Normal	N
14	iDM1	1	NA	N	97	maternal	Y	F	7	4	N	N	N	Normal	N
15	iDM1	NA	NA	N	NA	maternal	N	F	18	1	N	N	N	Normal	N
16	iDM1	NA	NA	N	NA	maternal	Y	F	10	5	N	N	N	Normal	N
17	iDM1	12	NA	N	333	paternal	N	M	16	4	N	N	N	Normal	N
18	iDM1	NA	NA	N	NA	maternal	Y	M	12	5	N	N	N	Normal	N
19	iDM1	NA	8yo	N	NA	maternal	N	M	17	4	N	N	N	Normal	N
20	cDM1	NA	<1month	N	NA	maternal	N	F	19	1	N	N	N	Normal	N
21	iDM1	NA	NA	N	300	maternal	N	F	11	6	N	N	N	Normal	N

22	iDM1	NA	NA	N	600	maternal	Y	F	13	6	N	N	N	Normal	N
23	iDM1	3	6yo	N	667	maternal	Y	M	7	6	N	N	N	Normal	N
24	iDM1	NA	NA	N	NA	paternal	Y	M	16	3	N	N	N	Normal	N
25	cDM1	NA	<1month	N	NA	maternal	N	M	14	1	N	N	N	Normal	Y
26	jDM1	NA	Asymptomatic	N	600	paternal	N	M	11	3	N	N	N	Normal	N
27	jDM1	15	13yo	N	500	paternal	N	F	16	3	N	N	N	Normal	N
28	cDM1	At birth	<1month	Y	2333	maternal	NA	F	0.1	NA exitus	Y (prematurity, weakness, respiratory insufficiency)	NA	N	Normal	N
29	cDM1	At birth	<1month	N	400	maternal	N	M	4	1	N	N	Mild apical LV hypertrabeculation	Normal	N
30	i/jDM1	Prenatal	Asymptomatic	N	115	maternal	N	F	3	1	N	N	N	Normal	N
31	iDM1	5	5yo	N	1600	maternal (exitus)	N	F	8	6	N	N	N	Normal	N
32	cDM1	At birth	<1month	N	2000	maternal	Y	M	2	1	N	N	N	Normal	N
33	cDM1	Prenatal	<1month	N	1733	maternal	Y	M	2	1	N	N	N	Normal	N
34	jDM1	14	14yo	N	600	paternal	N	F	14	3	N	N	N	Normal	N
35	iDM1	1.5	1.5yo	N	1300	maternal	Y	M	5	6	N	N	N	Normal	N
36	iDM1	8	8yo	N	1000	maternal	Y	M	14	2	N	N	Mild apical LV hypertrabeculation	Normal	N
37	cDM1	3 months	<1month	N	1333	maternal	N	M	0.1	2	N	N	N	Normal	N
38	iDM1	7	5yo	N	NA	maternal	N	F	11	1	N	N	N	Normal	N
39	iDM1	13	asymptomatic	N	333	paternal	N	M	13	2	Dizziness (15yo)	N	N	Normal	N
40	jDM1	12	16	N	500	paternal	Y	M	11	1	N	N	N	Normal	N

cDM1: congenital myotonic muscular dystrophy type 1. iDM1: infantile myotonic muscular dystrophy type 1. jDM1: juvenile myotonic muscular dystrophy type 1.
CV: cardiovascular; PM: pacemaker; ICD: implantable cardiac defibrillator.
NA: not available/not applicable; Y: Yes; N: No; M: male; F: female. LV: left ventricle.

Table 2. Echocardiographic findings			
Patient ID	**DM1 form**	**Structural findings**	**Other findings**
2	cDM1	Moderate apical LV hypertrabeculation. Preserved ejection fraction.	N
9	jDM1	N	Bicuspid Aortic Valve
11	iDM1	Mild apical LV hypertrabeculation	N
27	jDM1	N	Patent foramen ovale
29	cDM1	Mild apical LV hypertrabeculation	N
34	jDM1	N	Patent foramen ovale
36	iDM1	Mild apical LV hypertrabeculation	N

cDM1: congenital myotonic muscular dystrophy type 1. iDM1: infantile myotonic muscular dystrophy type 1. jDM1: juvenile myotonic muscular dystrophy type 1.
N: No; LV: left ventricle

Table 3. Electrocardiographic features

Patient ID	DM1 form	Rhythm	Heart rate (bpm)	ECG analysis					
				PR interval (ms)	QRS interval (ms)	QRS axis (°)	cQT (ms, Bazett)	Repolarization	Global ECG abnormalities
1	iDM1	Sinus	60	200	90	-29	401	Early repolarization	1st degree AV block, elevated J point in V2, low QRS voltage in V1
2	cDM1	Sinus	130	160	80	-150	390	N	Low QRS voltage in V1 and V6, deep R wave in V2-V3
3	cDM1	Sinus	110	160	80	-155	416	Asymmetric T wave	IRBBB, global Low QRS voltage
4	jDM1	Sinus	80	120	40	60	412	N	NA
5	jDM1	Sinus/low atrial	80	110	60	20	420	N	NA
6	iDM1	Sinus	75	190	80	-8	353	N	Elevated J point in V2, 1st degree AV block
7	iDM1	Sinus	70	180	80	90	360	N	Sinus bradycardia, elevated J point in V2, 1st degree AV block, Low QRS voltage in V1
8	jDM1	Sinus	90	120	60	85	424	N	NA
9	jDM1	Sinus	60	140	60	60	404	N	Sinus bradycardia
10	iDM1	Sinus/low atrial	50	140	60	-10	378	N	Sinus bradycardia
11	cDM1	Sinus	105	140	80	80	435	N	Elevated J point in V2, deep R wave in V2 and V3
12	cDM1	Sinus	90	160	40	90	360	N	NA
13	cDM1	Sinus/low atrial	120	120	80	90	420	N	NA
14	iDM1	Sinus	75	150	80	68	410	Early repolarization	Global Low QRS voltage, QS in V1, 1st degree AV block
15	iDM1	Sinus	60	204	90	60	420	N	QS in V1, Global low QRS voltage, sinus bradycardia, 1st degree AV block
16	iDM1	Sinus	100	140	90	100	400	N	NA
17	iDM1	Sinus	60	180	88	53	380	N	Elevated J point in V2 and V3
18	iDM1	Sinus	90	140	80	20	440	N	Deep R wave in V2
19	iDM1	Sinus	72	206	90	69	405	N	Elevated J point in V2 and V3, Low voltage in V1, 1st degree AV block
20	cDM1	Sinus	53	186	92	-43	424	N	QS in V1, Global low QRS voltage, sinus bradycardia
21	iDM1	Sinus	83	130	80	87	430	N	IRBBB

22	iDM1	Sinus	40	160	100	180	400	N	IRBBB, Sinus bradycardia, low voltage in V1
23	iDM1	Sinus	68	140	100	60	400	N	IRBBB
24	iDM1	Sinus/low atrial	71	152	110	76	432	N	Elevated J point in V2 and V3
25	cDM1	Sinus	100	149	82	70	422	N	QS wave in V1
26	jDM1	Sinus	65	146	84	80	401	N	NA
27	jDM1	Sinus	73	176	114	6	450	N	QRS fragmentation, Low QRS voltage in V1
28	cDM1	Sinus	NA	NA	NA	NA	NA	NA	NA (exitus)
29	cDM1	Sinus	131	126	78	78	451	N	NA
30	i/jDM1	Sinus	87	100	68	81	406	N	Short PR interval
31	iDM1	Sinus	90	160	84	95	450	N	Borderline PR interval, Low QRS voltage in V1
32	cDM1	Sinus	127	134	70	85	441	N	IRBBB, Low QRS voltage in V1 and V2
33	cDM1	Sinus	140	116	78	106	440	N	IRBBB, low QRS voltage in V1 and V6, hypervoltage R and S waves in V4
34	jDM1	Sinus	71	128	90	81	430	N	NA
35	iDM1	Sinus	92	132	84	74	403	N	Low QRS voltage in V1 and V6
36	iDM1	Sinus	85	166	98	83	433	Early repolarization	J Point elevation in V2
37	cDM1	Sinus	87	136	100	-100	438	N	CRBBB, intraventricular conduction delay
38	iDM1	Sinus	82	168	94	63	432	N	IRBBB, Low QRS voltage V1 and V2
39	iDM1	Sinus	44	230	104	87	374	N	Sinus bradycardia, IRBBB, 1st degree AV block
40	jDM1	Sinus	81	140	108	23	440	N	Short PR interval, elevated J point in V2 and V3

cDM1: congenital myotonic muscular dystrophy type 1. iDM1: infantile myotonic muscular dystrophy type 1. jDM1: juvenile myotonic muscular dystrophy type 1.
NA: not available/not applicable; Y: Yes; N: No
IRBBB: incomplete right bundle branch block; CRBBB: complete right bundle branch block

Table 4. 24h-Holter ECG monitoring features							
Patient ID	DM1 form	First 24h-Holter ECG monitoring			Last 24h-Holter ECG monitoring		
		Age (years)	Ventricular arrhythmia	Atria and AV conduction	Age (years)	Ventricular arrhythmia	Atrial and AV conduction
1	iDM1	18	N	Type I 2nd degree AV Block	21	N	N
2	cDM1	8	N	N	13	N	N
3	cDM1	5	N	1st degree AV Block	10	Ventricular extrasystoles	1st degree AV block
4	jDM1	8	N	N	11	N	N
5	jDM1	11	N	N	17	N	N
6	iDM1	21	N	N	23	Ventricular extrasystoles	N
7	iDM1	16	N	Type I 2nd degree AV Block	NA	NA	NA
8	jDM1	15	N	N	17	N	N
9	jDM1	17	N	N	NA	NA	NA
10	iDM1	14	N	N	17	N	1st degree AV block
11	cDM1	10	N	N	15	N	1st degree AV block
12	cDM1	17	N	N	NA	NA	NA
13	cDM1	12	N	N	18	N	N
14	iDM1	8	N	N	12	N	N
15	iDM1	18	N	1st degree AV Block	NA	NA	NA
16	iDM1	10	N	N	15	N	N
17	iDM1	16	N	Type I 2nd degree AV Block	20	N	N
18	iDM1	12	N	N	17	Ventricular extrasystoles	N
19	iDM1	17	Ventricular bigeminy	1st degree AV block	21	Ventricular couplets	N
20	cDM1	19	N	1st degree AV block	NA	NA	NA
21	iDM1	11	N	N	17	N	1st degree AV block
22	iDM1	14	N	Sinus bradycardia	20	N	Sinus bradycardia
23	iDM1	7	N	N	13	N	N

24	iDM1	18	N	N	21	N	N
25	cDM1	14	N	N	NA	NA	NA
26	jDM1	11	N	N	14	N	N
27	jDM1	16	N	Sinus pause	19	N	N
28	cDM1	NA	NA	NA	NA	NA	NA
29	cDM1	NA	NA	NA	NA	NA	NA
30	i/jDM1	3	N	N	NA	NA	NA
31	iDM1	8	N	N	15	N	N
32	cDM1	2	N	N	3	N	N
33	cDM1	2	N	N	NA	NA	NA
34	jDM1	15	N	N	18	N	N
35	iDM1	5	N	N	12	Ventricular extrasystoles	Atrial extrasystoles
36	iDM1	14	N	N	16	N	N
37	cDM1	NA	NA	NA	NA	NA	NA
38	iDM1	12	N	N	NA	NA	NA
39	iDM1	15	N	1st degree AV block	17	N	1st degree AV block, sinus bradycardia
40	jDM1	13	N	Atrial extrasystoles	NA	NA	NA

NA: not available/not applicable; Y: Yes; N: No
cDM1: congenital myotonic muscular dystrophy type 1. iDM1: infantile myotonic muscular dystrophy type 1. jDM1: juvenile myotonic muscular dystrophy type 1.

Table 5. Other systemic involvement							
Patient ID	DM1 form	Age at genetic diagnosis (years)	Age at first symptoms (months or years)	Exitus	NIV	Cataracts	Other involvement
1	iDM1	NA	12yo	N	Y	N	Cognitive delay, Mild Achilles tendon retraction
2	cDM1	NA	<1month	N	N	N	Cognitive delay, Bilateral cryptorchidism
3	cDM1	NA	<1month	N	N	Y	Global developmental delay, Inguinal hernia, Cryptorchidism, Bilateral Achilles tendon retraction
4	jDM1	5	Asymptomatic	N	N	N	N
5	jDM1	9	Asymptomatic	N	N	N	Mild sleep apnea-hypopnea syndrome
6	iDM1	5	NA	N	N	Y	Cognitive delay
7	iDM1	10	NA	N	N	N	Cognitive delay
8	jDM1	NA	12yo	N	N	N	ADHD
9	jDM1	NA	Asymptomatic	N	N	N	Bicuspid Aortic Valve
10	iDM1	NA	3yo (motor symptoms)	N	Y	N	Global developmental delay, Achilles tendon retraction
11	cDM1	NA	<1month	N	Y	N	Global developmental delay, Achilles tendon retraction
12	cDM1	11	<1month	N	Y	N	Global developmental delay, Inguinal hernia
13	cDM1	1	<1month	N	Y	N	Global developmental delay, Achilles tendon retraction
14	iDM1	1	NA	N	Y	N	Cognitive delay, Scoliosis
15	iDM1	NA	NA	N	N	N	Cognitive delay, scoliosis, elbow retraction, Achilles retraction
16	iDM1	NA	NA	N	Y	N	Cognitive delay
17	iDM1	12	NA	N	N	N	ADHD
18	iDM1	NA	NA	N	Y	N	Cognitive delay, Scoliosis
19	iDM1	NA	8yo	N	N	N	Cognitive delay, Scoliosis
20	cDM1	NA	<1month	N	N	N	ADHD
21	iDM1	NA	NA	N	N	N	Dysphagia
22	iDM1	NA	NA	N	Y	N	ADHD
23	iDM1	3	6yo	N	Y	N	Autism Spectrum disorder
24	iDM1	NA	NA	N	Y	N	Cognitive delay

25	cDM1	NA	<1month	N	N	Y	Global developmental delay, Autism Spectrum Disorder, Encopresis
26	jDM1	NA	Asymptomatic	N	N	N	ADHD
27	jDM1	15	13yo	N	N	N	Patent foramen ovale, ADHD
28	cDM1	At birth	<1month	Y	NA	N	Extreme prematurity
29	cDM1	At birth	<1month	N	N	N	NA
30	i/jDM1	Prenatal	Asymptomatic	N	N	N	NA
31	iDM1	5	5yo	N	N	N	Cognitive delay, Thyroglossal duct cyst, Von Willebrand disease, Scoliosis
32	cDM1	At birth	<1month	N	Y	N	Cognitive delay, dysphagia
33	cDM1	Prenatal	<1month	N	Y	N	Diaphragmatic paralysis
34	jDM1	14	14yo	N	N	N	Patent foramen ovale
35	iDM1	1.5yo	1.5yo	N	Y	N	Cognitive delay, Celiac disease
36	iDM1	8	8yo	N	Y	N	Cognitive delay
37	cDM1	3m	<1month	N	N	N	Global developmental delay, dysphagia
38	iDM1	7yo	5yo	N	N	N	Cognitive delay
39	iDM1	13	Asymptomatic	N	N	N	ADHD
40	jDM1	12	16	N	Y	N	NA

cDM1: congenital myotonic muscular dystrophy type 1. iDM1: infantile myotonic muscular dystrophy type 1. jDM1: juvenile myotonic muscular dystrophy type 1.
NA: not available/not applicable; Y: Yes; N: No
ADHD: attention-deficit/hyperactivity disorder. NIV: non-invasive ventilation.

Otros resultados no publicados.

Artículo 4. Resumen estructurado. Resultados no publicados.

Cardiac characterization of pediatric Duchenne muscular Dystrophy: finding predictors of arrhythmias and sudden death

Introducción

La distrofia muscular de Duchenne (DMD) es la miopatía más frecuente en niños. Es una enfermedad muscular progresiva ya presente en etapas precoces de la infancia. Presenta complicaciones musculoesqueléticas, respiratorias y cardíacas ya incluso durante la etapa de la infancia y adolescencia. La miocardiopatía dilatada con diferentes grados de insuficiencia cardiaca puede manifestarse también en la infancia. Las arritmias y muerte súbita también han sido descritas en esta población. Globalmente, la afectación cardiovascular en pediatría está infraestudiada, a pesar de existir guías internacionales sobre el tipo de seguimiento que debe realizarse por cada una de las disciplinas.

Objetivos

Descripción del estado cardiovascular y la identificación de predictores potenciales de arritmias y muerte súbita en una cohorte pediátrica con distrofia muscular de Duchenne.

Métodos

El estudio se basa en datos retrospectivos de una cohorte pediátrica diagnosticada genéticamente de Distrofia muscular de Duchenne, añadiendo una intervención prospectiva para valorar el estado cardiovascular, al inicio y al final del seguimiento, mediante ecocardiografía con parámetros básicos y avanzados incluyendo *strain* miocárdico, RM cardiaca con contraste según práctica habitual, electrocardiograma, Holter cardiaco 24h, y, si precisa según guías de práctica clínica habitual, estudio electrofisiológico.

Resultados

Se reclutó datos clínicos pertenecientes a 40 pacientes de <18 años con diagnóstico genéticamente confirmado de DMD. El seguimiento medio fue de 4.11 años (±1.24). Los fenotipos fueron la mayoría de DMD (90%), siendo los restantes fenotipos intermedios DMB/D (3 pacientes) y 1 caso de distrofia muscular de Becker. La mayoría de los pacientes no tuvieron síntomas cardiovasculares, tan sólo 4 pacientes con palpitaciones y no hubo pacientes con otros síntomas como síncope, a pesar de haber diferentes grados de severidad de insuficiencia cardiaca crónica. Sólo falleció un paciente durante el seguimiento, debido a insuficiencia cardiaca rápidamente progresiva pasando de leve a terminal en unos 18 meses sin detectar arritmias en este período. No hubo casos de muerte súbita en esta cohorte durante el seguimiento.

De los datos electrocardiográficos y de arritmias, todos los pacientes tenían alteraciones del ECG al inicio del seguimiento, típicas de la enfermedad. Siete pacientes (17.5%) tuvieron arritmias auriculares y/o ventriculares, pero éstas fueron aisladas y no se correlacionaron con fibrosis en las RM cardiacas (sólo 1 de los casos en el que había combinación de extrasístoles auriculares aisladas y ventriculares aisladas). Tan sólo un paciente tuvo una taquicardia auricular ablacionada y en otro caso, sin fibrosis y sin datos de insuficiencia cardiaca, mostró fibrilación auricular que también tuvo que ser ablacionada dada la refractariedad al tratamiento farmacológico.

En cuanto a los datos ecocardiográficos, el 42.5% de los pacientes tuvo criterios de miocardiopatía dilatada con diferentes grados de disfunción ventricular. De forma significativa ($p<0.0001$), la fracción de eyección del ventrículo izquierdo (LVEF o FEVI) y el *strain* miocárdico del ventrículo izquierdo (GLS apical 4 cámaras) se vieron reducidos comparando los valores al inicio y al final de la intervención de seguimiento. Además, algunos de los pacientes ya tenían el GLS apical 4 cámaras alterado al inicio del seguimiento a pesar de mantener valores conservados de FEVI. En este grupo se hallaba el paciente que falleció por insuficiencia cardiaca rápidamente progresiva.

El 55% tuvo una RM cardiaca con contraste durante el seguimiento, realizada con una media de 14.9 años (SD±2.12). Se detectó que las regiones más frecuentemente afectadas por fibrosis fueron las basales inferior, inferolateral y anterolateral, y la medio-ventricular inferolateral, anterolateral y apical lateral. El

paciente 9 fue el que tenía extrasístoles auriculares y ventriculares aisladas y tenía fibrosis en la RM cardiaca, no encontrando fibrosis en el resto de pacientes con las arritmias descritas.

En cuanto a la genética, el tipo de mutaciones en el gen DMD más frecuentemente detectadas en nuestra cohorte fueron las deleciones de exones (13/40, deleciones tipo *frame-shift* (12/40) y las mutaciones puntuales tipo *nonsense* (9/40), concordante con la literatura. El paciente 9 que tenía fibrosis miocárdica y las extrasístoles auriculares y ventriculares tenía una deleción tipo *frame-shift* en el exón 7. En cuanto al tipo de mutaciones de los pacientes que ya tenían un valor bajo de GLS apical 4 cámaras al inicio del seguimiento, con FEVI preservada, se encontraron: deleción *frame-shift* de los exones 3-11 (2 pacientes, hermanos), mutación *nonsense* del exón 8 (1 paciente), deleción *inframe* de los exones 22-44 (1 paciente), deleción de los exones 49-50 (1 paciente), deleción *frame-shift* del exón 44 (1 paciente) y mutación *nonsense* del exón 51 (1 paciente).

Correlacionando la fibrosis y el tipo de mutación, el tipo de mutaciones más frecuentemente relacionadas con fibrosis en la cohorte estudiada fueron las deleciones tipo *frame-shift* (5 pacientes), las mutaciones *nonsense* (3 pacientes), la deleción de exones (2 pacientes) y 1 paciente con deleción *inframe.*

Conclusiones

Durante el tiempo de seguimiento en la cohorte pediátrica estudiada con DMD no se han encontrado ni arritmias potencialmente malignas ni muerte súbita, por lo que no se ha podido correlacionar ningún predictor para poder establecer medidas preventivas. Estos hallazgos no son sorprendentes, pues en otras publicaciones se ha reportado que, en edad pediátrica, la tasa de arritmias potencialmente letales y la muerte súbita son eventos raros, especialmente con la mejoría del tratamiento global del paciente neuromuscular ya en fases iniciales de la enfermedad.

Se ha podido demostrar que la función cardíaca se afecta rápidamente ya durante la adolescencia, e incluso algunos pacientes ya demostraban tener al inicio del seguimiento valores bajos de GLS apical 4 cámaras a pesar de mantener una FEVI preservada. Estos pacientes con valores precozmente bajos de GLS apical 4 cámaras estarían a mayor riesgo durante la adolescencia y sería

una oportunidad poder implementar medidas de mejora del seguimiento para poder optimizar el tratamiento farmacológico.

Se propone enfocar el seguimiento en detectar de forma precoz alteraciones de la función cardíaca, en la fibrosis y en los pacientes que manifiesten algún síntoma cardiovascular incluso aquellos que puedan ser atípicos. Esto permitirá detectar a los pacientes que puedan tener más eventos inesperados de arritmias potencialmente letales o muerte súbita, como pueden ser los pacientes con disfunción cardíaca moderada a severa y los pacientes con fibrosis transmural.

Una evaluación exhaustiva por un cardiólogo pediátrico especialista en miocardiopatías y análisis ecocardiográfico y que esté en un equipo multidisciplinar del paciente neuromuscular es clave para establecer protocolo de manejo específicos en este tipo de pacientes tan vulnerables y que presentan síntomas atípicos o que están asintomáticos a pesar de estar en fases avanzadas de insuficiencia cardiaca.

Cardiac characterization of pediatric Duchenne muscular Dystrophy: finding predictors of arrhythmias and sudden death

Sergi Cesar, Jose Cruzalegui, Flavio Zuccarino, Alejandro Pérez-Casares, Andres Nascimento, Carlos Ignacio Ortez, Daniel Natera, Laura Carrera, Jessica Exposito, Josep Brugada, Georgia Sarquella-Brugada

Abstract

Duchenne muscular dystrophy (DMD) is the most frequent myopathy in children. Progressive muscular weakness is present in early childhood, with the subsequent musculoskeletal, respiratory and cardiac complications. Cardiovascular complications are a leading cause of morbidity and mortality. The signs and symptom of heart failure in DMD children are frequently subtle and overlooked. Dilated cardiomyopathy related to DMD is the most frequent cardiac phenotype, with different heart failure degrees over time and can be studied by basic and advanced echocardiography and cardiac deformation with myocardial strain that can be affected earlier than other parameters. Life threatening arrhythmia and sudden death are described in DMD population, probably underestimated and often are unpredictable events. Myocardial fibrosis can be detected early using Gadolinium in the magnetic resonance imaging and could help to detect those cases that can manifest heart failure during childhood.

The aim of this study was to describe cardiovascular profile of a cohort of 40 patients diagnosed with DMD, following them about 4 years and comparing demographic, clinical, imaging and electrocardiographic data at enrollment and at final follow-up in order to find predictors of arrhythmias and sudden death.

No life-threatening arrhythmias and no sudden death were detected in our cohort during the follow-up. One patient died because of end-stage heart failure, showing fibrosis and low values of GLS at enrollment. Imaging data showed overall worsening of left ventricular function over time, and in some cases 4-chamber global longitudinal strain was low at enrollment despite preserved left ventricular ejection fraction. According to other published papers about DMD in children, we propose focusing on imaging tests to detect earlier cardiac dysfunction that would allow optimize medical treatment since, according with

previous literature, transmural fibrosis and moderate-to-severe cardiac dysfunction are the most frequent findings related with life-threatening arrhythmias and sudden death.

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder, with an incidence 1:3500-6300 male births.(1–3) A mutation in DMD gene is the responsible of marked reduction or absence in the dystrophin.(4) DMD, as well other dystrophinopathies, are characterized by variable degrees of skeletal and cardiac muscle impairment. (2) Cellular damage is present in both skeletal and cardiac muscles because of a lack of structural support of the myocyte and sarcolemmal membrane and in the T-tubular membranes of myocytes, leading to progressive replacement by fibrofatty tissue. (5)

Loss of ambulatory capacity, decline of respiratory and cardiac functions are the most important features of DMD patients. The onset of muscle weakness is early around 4-7 years old, and the progression is characterized by non-ambulatory phase around 13 years old. (6,7) Respiratory care has changed the prognosis and prolonged survival with the use, when applied, of non-invasive home ventilators. (7)

Dilated cardiomyopathy (DCM), arrhythmias, and congestive heart failure (HF) are the most important life-threatening conditions in DMD.(1,2,7–9) DMD-DCM is characterized by thinner left ventricle wall and progressive left ventricular (LV) dilatation, because of the progressive loose of myocytes.(1,9) Apoptosis and fibrotic substitution and scarring that proceeds from the epicardium to the endocardium are typically found behind the posterior and mitral valve apparatus.(1) Heart failure remains asymptomatic for many years because oxygen consumptions are severely diminished by muscle weakness. Mortality rate for DMD patients with cardiomyopathy is worse than Becker muscular dystrophy. (7,10)

As published in the current guidelines for cardiovascular evaluation, early during infancy, is recommended to detect early onset and progression of the DCM. (7) Serum biomarkers can be useful to characterize HF and to assess function status

in adult and pediatric patients.(11) Troponin are known to be associated to the extension of myocardial damage but there are conflicting results about the diagnostic and prognostic implications in these patients.(11)

Echocardiography is recommended to evaluate dimensions and function. LV dysfunction is defined by left ventricular ejection fraction (LVEF) < 55% and a fractional shortening <28%. Diastolic function can be assessed by transmitral flow (increased mitral A-wave velocity and lower E/A ratio), lateral TDI (lower E wave velocities).(12,13) Speckle tracking myocardial deformation, showing low global longitudinal strain (GLS) values, can be useful to evaluate subclinical LV dysfunction before the onset of LVEF reduction.(14) Inferolateral and antero-lateral mid-basal segments are the regions with lower values of strain. (15) Circumferential global myocardial strain has been detected more impaired in DMD. (16) Speckle tracking analysis is limited by the poor image quality of these patients (13,17), but myocardial strain could be useful to detect impairment despite normal or mildly altered ejection fraction using a non-invasive method with no contrast and taking a key role during the pediatric age. (15)

RV function is often preserved probably because the reduced overload and the respiratory improving management.(1,18)

Cardiac magnetic resonance imaging (cMRI) is assuming and increasingly important role, to analyze cardiac dimensions, function and myocardial fibrosis. cMRI allows a non-invasive myocardial tissue characterization by late Gadolinium enhancement (LGE) and T1 mapping techniques using non-ionizing radiations.(1) The presence of transmural LGE pattern, often located at the inferolateral wall, is recognized as independent predictor of adverse cardiac events in DMD patients.(1,8,19,20) Subepicardial LGE in inferolateral free LV wall is common in DMD patients, and other frequent affected segments are inferior an anterolateral segments.(18) LGE can detect focal macroscopic fibrosis while T1 mapping technique pre and post-contrast is able to quantify diffuse myocardial fibrosis and extracellular volume expansion. T1 mapping has been demonstrated to identify early myocardial fibrosis in absence of LGE, but there are several limitations depending of the type of sequence, heart rate, vendors, and the inability to discriminate diffuse fibrosis from inflammation or fat infiltration. (21)

Arrhythmias are described in DMD population. The most frequent findings in the ECG are right axis deviation, deep and narrow Q waves in inferolateral leads,

conduction defects, sinus tachycardia, short PR interval, and high voltage R wave in right precordial leads, right bundle branch block and inverted T waves. Left bundle branch block was associated with cardiac events and mortality.(22) The incidence of supraventricular and ventricular tachycardia is about 6% and 2% respectively (1), but, as published recently, the lower LVEF the most frequent ventricular arrhythmias: NSVT prevalence of 0%, 7% and 40% with LVEF >55%, 35-55% and <35% respectively. (22) Standard antiarrhythmic medications and device management are considered in DMD population, but ICD should be individualized according general and neuromuscular status. (7,8,22)

Sudden death showed a very low rate in the pediatric population in contrast to adult, as described in Cardiomyopathy Registry. (8,22–24) Consequently, it is unclear when ICD implantation is most beneficial considering the low rate of sudden arrhythmic death in pediatric age. (8)

Objective

The aim of this study is to describe the cardiovascular profile and to find predictors of arrhythmias and sudden death of a cohort with genetically confirmed diagnosis of DMD, based on retrospective data and a prospective intervention during 5 years with echocardiography, electrocardiogram and 24h-holter monitoring.

Methods

A retrospective study with a prospective intervention in pediatric patients diagnosed with DMD was conducted in our national referral center for pediatric neuromuscular disease. The patients were classified in DMD, BMD of intermediate phenotype. According to the age and severity, medical data, family history, 12-lead ECG, transthoracic echocardiography, 24h ECG Holter monitoring and electrophysiological study were performed. Data was reviewed retrospectively and the prospective data was performed until the transition to adult hospital, the end of study period (maximum 5 years of follow-up in the prospective period) or the end due to mortality.

The study was approved by the Ethics Committee of the Hospital Sant Joan de Déu (Barcelona, Spain), following the Helsinki II declaration.

Cardiac Imaging

Echocardiogram data were collected following the standard assessment of cardiac dimensions and function. Normal left ventricular systolic function was defined as left ventricular ejection fraction >55%.

Electrocardiographic analysis

12-lead ECG analysis was evaluated from retrospective data and from the last ECGs during the interventional prospective period. Definitions of normality were based on published data. Arrhythmias and standard values were assessed from 24h-holter ECG monitoring.

Statistics

Normally distributed data are presented as mean values [± standard deviation (SD)] and non-normally distributed data as a median [interquartile range (IQR)]. Categorical variables are presented as number and percentages. Data analysis and graphics were performed using GraphPad Prism version 10.0.0 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com.

Results

Clinical data

A total of 40 male patients <18 years of age genetically diagnosed with a pathogenic mutation in DMD were included in the retrospective analysis. The interventional prospective follow-up had a mean of 4.11 years (±1.24). Table 1 shows the clinical and demographic characteristics of the cohort. From the overall cohort, most patients were diagnosed with DMD (90%). The other patients included were 3 intermediate phenotypes (B/DMD) and 1 patient with BMD phenotype. During the follow-up, cardiovascular symptoms were reported only in 4 patients (palpitations) but no patients were reported syncope. Despite different severity of heart failure, patients had no related symptoms. In our cohort, none of the patients included died suddenly. Only one patient died during the follow-up due to the rapid progression of cardiac disease despite of medical treatment. In this case, severe cardiac dysfunction and cardiac failure were stablished in 18 months and no arrhythmias were detected during this terminal heart disease.

All patients received enalapril after 10 years old following the international recommendations. Medical treatment of heart failure was started when LVEF was under 50% and was a combination of Enalapril or Sacubitril/Valsartan, diuretics (Eplerenone or Spironolactone and/or Furosemide), beta-blockers (Carvedilol or Bisoprolol). About corticotherapy, all patients received standard doses of prednisone or deflazacort from 4 to 6 years of age in almost all cases, following the international recommendations.

Arrhythmias and ECG data

Table 3 showed the ECG findings from 12-lead ECG and 24h-Holter monitoring. All patients had ECG abnormalities at enrollment, regardless of age and before finding criteria of cardiomyopathy. The most frequent alterations were sinus tachycardia, short PR interval, high voltage criteria in V2-V4 leads and Q waves (inferior leads and left chest leads). Seven patients (17.5%) showed atrial or ventricular arrhythmias. Isolated ventricular extrasystoles were detected in two patients, in combination to atrial extrasystoles. Atrial extrasystoles were detected in 5 patients and in 1 patient symptomatic atrial tachycardia that required an ablation. One patient, with no cardiac dysfunction and no fibrosis in the MRI, showed atrial fibrillation that was refractory to antiarrhythmic treatment and required ablation. LGE was not detected in the other patients with arrhythmias.

Echocardiographic data

Around 42.5% of patients find criteria for dilated cardiomyopathy with different severity of cardiac dysfunction. Table 2 resumes echocardiographic analysis comparing echocardiographic values at enrollment and at last control. We compared LVEF and 4 chamber myocardial strain (4 chamber GLS) during follow-up. Graphic 1 represents the migration of GLS values under the value considered as <p5 during follow-up. Some of these depressed GLS values have preserved LVEF (around 55-57%). The died patient (patient 19) had a low GLS value of -15.7% despite preserved LVEF (56.5%) at enrollment. The other patients with similar values were patient 3 (55%, -12.8), patient 14 (35.6%, -16.1), patient 20 (55%, -15.9), patient 22 (60.7%, -15.7), patient 29 (64.3%, -16.4) and patient 35 (-57.1%, -12.2). Except patients 29 and 35 (7 and 4 years old at enrollment), the other patients (3, 14, 19, 20, 22) had cardiomyopathy criteria but no arrhythmias

(isolated ventricular extrasystole in patient 3). Statistical analysis with paired t test showed significant lower LVEF and worst GLS values at last control, comparing with enrollment values ($p < 0.0001$).

We did not find significant differences between the other functional echocardiographic analysis during follow-up, including diastolic functional analysis.

cMRI and correlation with arrhythmias

Only 22 patients (55%), with a mean age at cMRI of 14.9 years (SD±2.12), were evaluated with cMRI with LGE because of age or distance problems from their referral county. LGE was detected in 11 patients (50%). Figure 1 showed the 16 myocardial segments and the most frequent segments with fibrosis (≥3 patients) are represented in purple basal inferior, inferolateral and anterolateral, mid-ventricular inferolateral and anterolateral and apical lateral).

One patient, with no cardiac dysfunction and no fibrosis in the MRI, showed atrial fibrillation that was refractory to antiarrhythmic treatment and required ablation. The other patients with arrhythmias (isolated atrial and ventricular arrhythmias, atrial tachycardia and atrial fibrillation) had no fibrosis except patient 9 with isolated atrial and ventricular extrasystoles.

Sudden death

No sudden death was present in the cohort. Patient 19 died at 16 years old due to refractory medical treatment for chronic heart failure. No arrhythmias were detected during follow-up.

Phenotype-genotype correlation

Table 1 showed genetic data and cardiac outcomes (cardiomyopathy, arrhythmia, death). Those patients with worst GLS values at enrollment had the following mutations in DMD gene: frameshift deletion exons 3-11 (2 patients, brothers), non-sense mutation exon 8 (1 patient), in-frame deletion exons 22-44 (1 patient), deletion exons 49-50 (1 patient), and frameshift deletion exon 44 (1 patients) and nonsense mutation in exon 51 (1 patient). The patient with myocardial fibrosis and arrhythmias had a frameshift deletion in exon 7. Most patients with fibrosis

had no arrhythmias during follow-up (10/11 patients, 90% of patients with fibrosis) with a mean age at final follow-up of 17 years (SD±2.25).

Figure 2 shows the different mutations by type and by exon. Graphic 3 shows the different mutations (A) and the relation with LGE in the cMRI in those patients that can be complete this imaging test. The most frequent mutation type were exon deletions (13/40), following by frameshift deletion (12/40) and nonsense mutations (9/40). Inframe deletions (4) and duplication (2) were the less frequent in our cohort. The most frequent mutation type related with LGE in cMRI in our cohort were frameshift deletions (5), following by nonsense mutations (3), exon deletions (2) and inframe deletion (1).

Discussion

Cardiovascular complications are a leading cause of disease-related morbidity and mortality among DMD population.(7) As the myocardial disease progresses, clinical heart failure develops over time and life-threatening arrhythmias can appear.(7) The prevention of cardiomyopathy stands as one of the most challenging clinical research issues in children with DMD, so is key to stablish a proactive strategy of early diagnosis and treatment to maximize duration and quality of life.

Classically, routine pediatric cardiology examinations, first and foremost echocardiography, failed to determine when and to what level the cardiomyopathy will occur. Using both conventional and advanced echocardiographic analysis could improve the detection of cardiomyopathy before standard features will be present in the DMD heart. (1) As we observed in our cohort, both LVEF and 4-chamber GLS showed a progressive and statistically significant worsening over time, but 4-chamber GLS showed, in some individuals, decreased values regardless the short age. These features justify a very specific follow-up involving a pediatric cardiologist specialist in cardiomyopathies and neuromuscular diseases.

Children with DMD, the myocardial strain can be decreased before the onset of related-DMD cardiomyopathy, as we detected in some patients of our cohort over time.(15) The existence of altered LV strain despite a normal or mildly altered LVEF could be represent as a future target to drug trials for cardiomyopathy

prevention and to do strategical medical intervention to treat early the inevitable evolution to heart failure in few years.

The poor echo window in this population, especially those >15 years of age, difficult the reproducibility and precision of overall strain (longitudinal, radial and circumferential).(15) In our cohort, 4-chamber myocardial strain offered the possibility to compare values over time. Despite we added other views to analyze radial and circumferential strain, they were suboptimal, and the strain values offer poor reproducibility for most segments. This could be solved doing myocardial strain with MRI, but this increases the invasiveness of the technique. (15) Patients with early affectation of myocardial strain could be at future higher risk of cardiac dysfunction despite initial preserved LVEF values. These patients could be benefit with a medical intervention protocol such as earlier cMRI, closer monitoring biomarkers and preventive pharmacologic treatment.

About LGE in the cMRI, the presence of transmural LGE has been related with adverse cardiac events. (20) Thus, LGE progression are directly related to continuous decline in LV systolic function. (20) About the distribution of LGE, the subepicardium of the LV lateral free wall is most frequently the first described onset, and additional septal or transmural LGE in the LV lateral free wall mostly occurs in advanced stages.(20)

In our cohort, the LV myocardial segments most frequently affected with fibrosis were inferior, inferolateral and anterolateral segments (basal and mid-ventricular segments), according with other published papers. (18) The quantification of the LGE and the follow-up of this LGE over time could be a useful tool to identify patients at risk and to optimize medical treatment. Because of the low rate of cMRI achieved in our cohort, we cannot stablish conclusions about de cardiac involvement, but we did not detect life-threatening arrhythmias during the follow-up despite the presence of LGE in 50% (11 patients) of overall cMRI.

The most common changes in dystrophin are intragenic deletions (about 65% of dystrophin mutations), and around 20-35% have point mutations. (2,4) Some studies revealed that deletions in exons 50 and/or 51 showed less LGE, while gene duplications showed more LGE, but concluding that did not find association between genotype and severity of cardiomyopathy.(20) Other published papers found early onset cardiomyopathy for deletions affection amino-terminal domain (exons 2 to 9) and a later onset for deletions removing part of the central rod

domain and hinge 3 (exons 50 and/or 51).(25) There is controversial data about this genotype-phenotype correlation, probably because of genetic modifiers that determine cardiac disease severity in unrelated DMD patients with the same age and DMD mutation.(20) Our data about the genotype-phenotype correlation revealed that the earliest lower values in 4-chamber GLS included 2 patients (brothers) with a frameshift deletion in exons 3-11, 1 patient with nonsense mutation in exon 8 (1 patient), and the other described patients have other locations including exons 49, 50 and 51. About fibrosis, the most frequent mutation type related with LGE in cMRI in our cohort was frameshift deletion (5), following by nonsense mutations (3), exon deletions (2) and inframe deletion (1). Probably, a comprehensive long-term follow-up describing the cardiac involvement and severity is needed to describe this genotype-phenotype correlation.

All patients were under oral corticotherapy and with different combinations of pharmacological treatment, most of them started earlier or at the time of DMD diagnosis and before the onset of decreased cardiac function. So, the role and the effect of these therapies will remain unclear. Recently, a published paper suggested that circumferential strain improves after the combination of angiotensin-converting enzyme inhibitors (ACEi) or angiotensine receptor blockers (ARB) with eplerenone. (26) This finding could support the idea that DMD patients can benefit for early medical treatment with more than one drug, probably before 10 years of age, but more trials are needed to confirm this in initial phases of the disease with normal and mid-range reduction of LVEF.

Patients with severe ventricular dysfunction in DMD dilated cardiomyopathy, the therapy indicated is ACEi +/- betablockers: the combination has been proved to be superior in the prevention of major cardiac events and in long-term survival. (1,27). Other drugs can be added: spironolactone or eplerenone, especially in symptomatic patients and despite treatment with ACEi and betablockers, to reduce mortality and heart failure hospitalization. (1,28,29)

Probably, other strategies to find life-threatening arrhythmias are needed to explore. Some studies suggested again that 24h-Holter monitoring could be not useful to detect significant findings for those DMD patients with LVEF >35% specially if there were no symptoms. Then, 24h-Holter monitoring could be not useful to detect significant findings for those DMD patients with LVEF >35%

specially if there were no symptoms. (24) In our experience, no significant arrhythmias and no sudden arrhythmic death were detected during follow-up. The patient with end-stage heart failure that finally dead did not show any significant arrhythmia during follow-up. In our experience, during the pediatric age, other strategies than 24-hour Holter should be adopted to monitoring cardiac dysfunction because of the low rate of fatal arrhythmias and mortality in DMD. Probably, the rate of arrhythmias in pediatric patients with BMD could be higher than DMD patients, as suggested in some publications, due to the higher mobility, but the rate of arrhythmias in both diagnosis is lower than expected. (24,30) The development of arrhythmias in patients with DMD increases with the decrease of cardiac function to moderato-to-severe LVEF values, something that happens most frequently in the third decade of life. (31) Advanced age is the other factor that is correlated with arrhythmia events. (31) Considering these features in DMD population, probably it may be reasonable to monitor closer for arrhythmias in patients with DMD with moderate-to-sever cardiac dysfunction, potential symptoms such as palpitations, and advanced age. Accordingly with our findings and the conclusions from other published papers about pediatric DMD, these recommendations can play a key role in the transition to adult stage and the young adult with DMD.

Limitations

This study has some limitations. Echocardiographic data is incomplete because of poor cardiac echo window and myocardial strain analysis were consistent only in 4-chamber views. There is published data about global longitudinal, radial and circumferential strain, but the reproducibility of myocardial strain analysis in this population is extremely difficult comparing with other cardiomyopathies. About the quality of the imaging, cMRI is the preferred imaging test to analyze cardiac function and deformation. In our case, cMRI was not available in all patients because of distance from referral county and data is missed, and the age for cMRI evaluation differs between the patients and this is an important limitation to interpretate the data and to make decisions. Moreover, advanced cardiac imaging team evaluating these special population is needed to increase the analysis quality and to decrease the time inverted in the test that, very frequently, sedation and/or general anesthesia is needed to complete all the views. Biomarkers are

not systematically collected in this study, but troponins and NT-proBNP are the routine biomarkers that we collect in this neuromuscular disease and other cardiomyopathies.

Conclusions

The aim of this study was to describe the cardiovascular profile of a cohort of DMD patients and to find predictors of arrhythmias and sudden death during an intervention of 5 years. We did not find life-threatening arrhythmias and no sudden death in our cohort, and we cannot correlate the collected data. These findings are in relation with other publications, since sudden death and life-threatening arrhythmias have a very low rate during the pediatric age. We demonstrate that cardiac function decreases rapidly during adolescence and some patients had low GLS values at enrollment despite preserved LVEF. These patients with low GLS values at early stage of the disease are at higher risk during adolescence, and it could be an opportunity to improve the follow-up and to optimize pharmacological treatment. We propose focusing on analyze and detect early cardiac dysfunction, on fibrosis detection and on the symptomatic patients (even when the symptoms are not typical for heart failure and arrhythmias i.e. chest discomfort, dizziness, and gastrointestinal symptoms).

A comprehensive evaluation involving a cardiologist with experience in cardiomyopathies and echocardiographic analysis is key to describe cardiac involvement and to stablish specific management protocols in these vulnerable patients that, often, present heart failure-related atypical symptoms or incredibly asymptomatic even in advanced stages of heart failure.

Table 1. Clinical and demographic data												
Patient ID	**Phenotype**	**Genetics**	**Neuromuscular onset (months)**	**Corticoid therapy (years)**	**Loose of gait (years)**	**Scoliosis**	**Achyles retraction**	**NIV**	**Cardiomyopathy**	**Arrhythmia**	**Ablation**	**Death**
1	DMD	Nonsense Exon 7 c.583C>T; p.Arg195*	36	6	15	N	N	N	N	N	N	N
2	DMD	Deletion exons 46-48	NA	6	8	Y	Y	N	Y	N	N	N
3	DMD	Deletion exons 49-50	NA	NA	NA	Y	Y	N	Y	Y (ventricular extrasístole)	N	N
4	DMD	Deletion exons 45-50	18	6	7	N	Y	N	N	Y (ventricular extrasístole)	N	N
5	DMD	Nonsense Exon 26 c.3578T>A; p.Leu1193*	18	12	11	Y (surgery)	Y (surgery)	Y	N	N	N	N
6	DMD	Nonsense mutation (NA)	NA	6	8	N	Y (surgery)	Y	Y	N	N	N
7	DMD	Nonsense Exon 65 c.9380C>G;p.Ser3127*	NA	5	14	N	Y (surgery)	N	Y	Y (ventricular extrasístole)	N	N
8	B/DMD	Inframe Deletion exons 45-49	84	NA	NA	NA	Y	NA	N	N	N	N
9	DMD	Frameshift deletion exón 7	72	6	NA	N	Y	Y	Y	Y (atrial and ventricular extrasístole)	N	N
10	DMD	Deletion exons 45-52	14	5	NA	N	N	N	N	N	N	N
11	DMD	Inframe deletion exons 3-25	48	6	14	N	Y (surgery)	N	Y	N	N	N
12	DMD	Nonsense exon 40 c.5611A>T p.Lys1871*	48	5	15	N	Y	Y	Y	N	N	N

13	DMD	Nonsense exon 43 c.6286C>T p.Gln2096*	60	6	NA	Y	Y (surgery)	N	N	Y (atrial tachycardia)	Y	N
14	DMD	Frameshift deletion exon 44	NA	NA	8	Y	Y (surgery)	Y	Y	N	N	N
15	DMD	Deletion exon 19	NA	NA	12	N	Y (surgery)	N	N	N	N	N
16	B/DMD	Deletion exon 18-41	NA	7	NA	Y	Y	N	Y	N	N	N
17	BMD	Deletion exons 3-11	60	11	NA	N	Y (surgery)	N	N	N	N	N
18	DMD	Duplication exons 8-29 and 42-44	18	6	11	Y	Y	N	Y	Y (atrial and ventricular extrasistole)	N	N
19	DMD	Frameshift deletion exons 3-11	72	6	NA	N	N	Y	Y	N	N	Y (heart failure)
20	DMD	Frameshift deletion exons 3-11	48	NA	NA	N	N	N	Y	N	N	N
21	DMD	Frameshift deletion exon 39 c.5517_5521delAGATG p.Asp1840fs	12	10	13	Y	Y	N	Y	N	N	N
22	DMD	Nonsense mutation exon 8 c.693C>A; p.Tyr231Ter	72	6	10	N	Y (surgery)	N	Y	N	N	N
23	DMD	Frameshift deletion exons 48-54	18	6	16	Y	Y (surgery)	N	Y	N	N	N
24	DMD	Frameshift deletion exon 21 c.2773insG; p.Val925Glyfs*13	16	7	13	N	Y (surgery)	N	N	Y (atrial fibrillation)	Y	N

25	DMD	Deletion exon 51	36	8	NA	N	Y (surgery)	N	N	N	N	N
26	DMD	Frameshift mutation exon 46 c.6651_6652del p.Asp2210Phefs*3	36	6	NA	N	N	N	N	N	N	N
27	B/DMD	Deletion exons 45-47	18	7	NA	Y	N	N	N	N	N	N
28	DMD	Deletion exons 8-17	48	5	NA	N	Y (surgery)	N	N	N	N	N
29	DMD	Nonsense mutation exon 51 c.7436 G>A p.Trp2479*	36	5	11	N	Y (surgery)	N	N	N	N	N
30	DMD	Duplication exons 3-7	48	6	NA	Y	Y	N	N	N	N	N
31	DMD	Frameshift deletion exons 8-12	18	6	NA	N	Y	N	N	N	N	N
32	DMD	Deletion exons 10-44	18	8	NA	Y	Y	N	N	N	N	N
33	DMD	Frameshift deletion exon 45	36	6	13	Y	Y	Y	N	N	N	N
34	DMD	Inframe deletion exons 48-55	36	7	NA	N	Y	N	N	N	N	N
35	DMD	Inframe deletion exons 22-44	36	7	12	N	Y	N	Y	N	N	N
36	DMD	Nonsense mutation exon 74 c.10245C>A p.Tyr3475*	48	10	NA	N	Y	N	N	N	N	N
37	DMD	Deletion exons 8-9	48	7	15	Y	Y (surgery)	N	N	N	N	N

38	DMD	Deletion exons 48-52	36	6	14	N	Y (surgery)	N	N	N	N	N
39	DMD	Frameshift deletion exons 46-50	24	6	12	N	Y (surgery)	N	Y	N	N	N
40	DMD	Frameshift deletion exons 8-34	36	6	NA	N	Y (surgery)	N	N	N	N	N

DMD: Duchenne muscular dystrophy. B/DMD: intermediate phenotype
NIV: non invasive ventilation; PM: pacemaker; ICD: implantable cardiac defibrillator.
NA: not available/not applicable; Y: Yes; N: No

Table 2. Echocardiographic and MRI data																		
Patient ID	Phenotype	Genetics	Follow-up years (age at enrollment-final)	LV dimensions (mm)		LA/Ao ratio		LVEF (%)		FS (%)		E/E' ratio		LV Myocardial strain (%)		Cardiac index (mL/m^2/min)		cMRI
				I	F	I	F	I	F	I	F	I	F	I	F	I	F	Fibrosis (segments), Age at MRI
1	DMD	Nonsense Exon 7 c.583C>T; p.Arg195*	5 (14-19yo)	48.9/32.3	51.9/34.3	1.25	1.3	62.6	59.3	33.9	30.5	8	NA	-23.1	-20.2	3527	NA	Fibrosis: Y (5,6,11,12,16) Age: 14yo
2	DMD	Deletion exons 46-48	4 (13,17yo)	39.9/27.7	45/33	1	1.08	58.6	52	30.6	26	7.2	6.69	-25.9	-15.6	2246	1790	Fibrosis: N Age: 13
3	DMD	Deletion exons 49-50	4 (17-21yo)	61.6/50.8	67.5/55.2	1.3	1.16	35.6	36.6	17.5	18.2	6.4	4.78	-12.8	.8.8	3455	1239	Fibrosis: N Age: 17
4	DMD	Deletion exons 45-50	6 (11-17yo)	39.1/20.5	47/30.4	1.1	0.8	79.5	64.8	47.6	35.4	6.5	6.08	-22.7	-14.6	2819	1972	Fibrosis: N Age: 11
5	DMD	Nonsense Exon 26 c.3578T>A; p.Leu1193*	5 (12-17yo)	36/22.9	36.2/24.8	1.16	0.68	67.1	60.4	36.4	31.5	6.08	7	-18.5	-13.8	2742	2442	Fibrosis: N Age: 17
6	DMD	Nonsense mutation (NA)	2 (14-16yo)	40.3/27.2	37.4/27.5	1.16	1.27	61.4	52.5	32.5	26.5	4.83	NA	-20.9	-12.6	2512	NA	Fibrosis: N Age: 14
7	DMD	Nonsense Exon 65 c.9380C>G;p. Ser3127*	5 (13-18yo)	35.4/23.1	36.6/25.3	1.05	1	65	59.2	34.7	28.5	4.71	5.6	-21	-17.1	2870	NA	Fibrosis: N Age: 18
8	B/DMD	Inframe Deletion exons 45-49	1.5 (14-15yo)	52.3/33.1	55.9/32.8	1.37	1.17	66	71.7	36.7	41,4	6.32	6.6	-24.2	-25	2812	1322	Fibrosis: N Age: 14
9	DMD	Frameshift deletion exón 7	6 (15-21yo)	49/36.1	58.3/40.1	1.64	1.27	51.5	49.3	26.3	NA	5.84	9	-23.1	-13.9	2365	NA	Fibrosis: Y (1,2,3,4,6,9) Age: 15

10	DMD	Deletion exons 45-52	6 (5-11yo)	38.8/26	41.9/29.4	1.33	0.82	62.2	57.4	33	29.8	4.68	4.8	-23.1	-22-1	3729	4372	NA
11	DMD	Inframe deletion exons 3-25	6 (11-17yo)	41.6/27.3	50.4/39	1.35	1.11	63.8	47.5	34.4	22.6	8	9.4	-23.7	-12.3	4259	2860	Fibrosis: Y (4,5,6,11,12,16) Age: 11
12	DMD	Nonsense exon 40 c.5611A>T p.Lys1871*	2 (18-yo)	48.7/37	49/37	1.24	1.33	47.3	47	23.8	23	6	8.3	-21.3	-17.1	3268	2905	Fibrosis: Y (4,5,6,8,9,10, 11,12,14,15,16) Age: 18
13	DMD	Nonsense exon 43 c.6286C>T p.Gln2096*	6 (10-16yo)	37.6/22.4	38.3/23.7	1.42	1,12	71.9	69	40.4	38.18	5.5	4.9	-25.4	-22.6	3138	2423	Fibrosis: N Age: 16
14	DMD	Frameshift deletion exon 44	4 (13-17yo)	43.4/30.8	51.2/37.4	1	1.1	55	52.2	29	26.9	5	11.2	-16.1	-12.2	3339	NA	Fibrosis: Y (1,5,6,7,11,12,13,16) Age: 13
15	DMD	Deletion exon 19	2 (17-19yo)	53.4/41.5	66.5/12.1	1,52	1.5	44.6	31,6	22.3	15.3	6.6	9.2	-18.1	-8.8	3816	NA	Fibrosis: Y (all segments) Age: 17
16	B/DMD	Deletion exon 18-41	4 (13-17yo)	45.3/33.5	57.1/42	1,09	NA	51.2	51.3	26	26.6	6.3	5.1	-23	-21.1	3492	3134	Fibrosis: Y (3,4,5,6,9,10,11,12,15,16) Age: 17
17	BMD	Deletion exons 3-11	5 (12-17yo)	42/23.2	45.3/24.5	1.2	1.06	76.5	77.4	44.8	45.9	4.7	5.1	-22.2	-20.6	4610	3896	Fibrosis: N Age: 12
18	DMD	Duplication exons 8-29 and 42-44	3 (11-14yo)	41.4725	38.5/28.2	1.06	1.19	66.2	51	39.7	25.5	5.2	4.7	-19.2	-14.8	NA	3131	NA
19	DMD	Frameshift deletion exons 3-11	4 (12-16yo)	49.7/35	72.4/58.3	1.37	1.82	56.5	38	29.6	19.5	6.5	7.3	-15.7	-9.9	3899	2075	Fibrosis: Y (4,5,10,11) Age: 16
20	DMD	Frameshift deletion exons 3-11	3 (8-11yo)	46.7/33.3	50.1/26.3	1.43	1.49	55.2	45.8	28.6	22.9	6.7	6.4	-15.9	-14.9	3552	2580	NA
21	DMD	Frameshift deletion exon 39 c.5517_5521delAGATG p.Asp1840fs	5 (9-14yo)	40.3/26.3	47.4/39.2	1.12	0.96	64.4	36.1	34.7	17.3	5.9	9.1	-22.1	1-8.7	4656	2756	Fibrosis: Y (4,5,9,10,13,15,16) Age: 14

22	DMD	Nonsense mutation exon 8 c.693C>A; p.Tyr231Ter	2 (14-16yo)	46.7/31.6	44.8/34.5	0.93	1.07	60.7	46.5	32.4	23.1	5.8	5.5	-15.7	-15.6	3037	3234	NA
23	DMD	Frameshift deletion exons 48-54	5 (12-17yo)	40.5/26.6	48.2/37	0.93	0.97	65,1	46.7	35.3	28.1	5.5	6.2	-16.9	-13.6	6281	2613	Fibrosis: Y (4,5,6,10,11,12) Age: 16
24	DMD	Frameshift deletion exon 21 c.2773insG; p.Val925Glyfs *13	5 (10-15yo)	40.8/27.2	39.9/24.3	1.37	1	64.1	68.2	34.5	36	6.6	6.1	-22.5	-22.7	2459	2713	Fibrosis: N Age: 14
25	DMD	Deletion exon 51	5 (7-12yo)	40.8/26.2	46.2/32.7	1.17	1.16	65.8	56	35.7	39.2	4.88	6.8	-22	18.9	3028	2000	NA
26	DMD	Frameshift mutation exon 46 c.6651_6652del p.Asp2210Phefs*3	5 (6-11yo)	37.3/24.6	40.9/25.2	1.44	1.25	63.7	69	34	38.3	7.4	4.2	-25	-22.1	3163	3253	NA
27	B/DMD	Deletion exons 45-47	3 (7-10yo)	33/19	32.7/19.9	1.3	1.03	76.6	70.6	44	38.9	6.1	5	-23.8	-20.7	3355	2342	NA
28	DMD	Deletion exons 8-17	3 (8-11yo)	45.8/28.5	44.8/29.7	1.42	1.46	68.1	62.9	37.9	33.9	5.5	6.6	-22.9	-21.7	5122	2952	NA
29	DMD	Nonsense mutation exon 51 c.7436 G>A p.Trp2479*	5 (7-12yo)	41/26.8	49.7/32.6	1.24	1.44	64.3	63.2	34.6	34.4	5.6	9.1	-16.5	-15.3	4362	3993	NA
30	DMD	Duplication exons 3-7	3 (7-11yo)	35.2/22.8	41.1/27.3	1.11	1.01	65.5	63	35.2	33.7	NA	5.2	-23.7	-16.8	3288	3587	NA
31	DMD	Frameshift deletion exons 8-12	4 (6-10yo)	37.2/20.4	42.3/27.7	1.45	1.25	77.3	62.4	45.2	33.3	4.4	5.1	-20.4	-16.6	3868	3554	NA
32	DMD	Deletion exons 10-44	5 (6-11yo)	32.7/18.4	38.3/24.2	1.24	1.15	76.3	67.4	43.8	36.8	9.9	4.6	-24	-24.2	3605	2586	NA
33	DMD	Frameshift deletion exon 45	3 (11-14yo)	37.4/22.5	46.3/28.8	0.97	1	71.2	68.1	39.8	37.9	NA	7.53	-22.1	15.6	NA	2508	NA

34	DMD	Inframe deletion exons 48-55	5 (4-9yo)	37.6/23.7	43.2/27.1	1.24	1.74	67.6	67.5	36.9	37.3	6.3	4.9	-20.3	-21.5	NA	3283	NA
35	DMD	Inframe deletion exons 22-44	2 (12-14yo)	44.6/31.3	44.7/32.6	1.03	1.03	57.1	53.1	29.8	27.2	5.5	6.9	-12.2	-10	2392	1750	NA
36	DMD	Nonsense mutation exon 74 c.10245C>A p.Tyr3475*	4 (9-13yo)	43.6/26.3	42.5/27.7	1.48	1.27	72.8	64.6	39.5	35	4.7	6.6	-22.5	-19.2	3945	4220	Fibrosis: Y (5,12) Age: 17
37	DMD	Deletion exons 8-9	4 (13-17yo)	40.7/21.2	48.3/27.4	1.07	1.18	66.9	74.	36.2	43.3	4.4	5.3	-22.2	-19.2	3389	2292	NA
38	DMD	Deletion exons 48-52	4 (11-15yo)	36.7/27.2	40.8/27.2	1.02	1.06	71.1	62.5	39.6	33.3	5.6	5	-22.8	-20.8	5575	2557	NA
39	DMD	Frameshift deletion exons 46-50	4 (8-12yo)	40.1/28	42/31	1.07	1	58.1	51.6	30.2	26.1	5.1	5.2	-22.1	-15.3	3850	3809	NA
40	DMD	Frameshift deletion exons 8-34	4 (11-15yo)	37.7/23.6	40.2/25.3	1.27	1.39	68	67.3	37.2	36.9	9.5	8.4	-17.2	-17.4	3343	3898	Fibrosis: N Age: 15

DMD: Duchenne muscular dystrophy. B/DMD: intermediate phenotype
I: first control; F: last control
Ao: aorta; LA: left atria; LV: left ventricle; LVEF: left ventricular ejection fraction; SF: shortening fraction; cMRI: cardiac magnetic resonance imaging
NA: not available/not applicable; Y: Yes; N: No

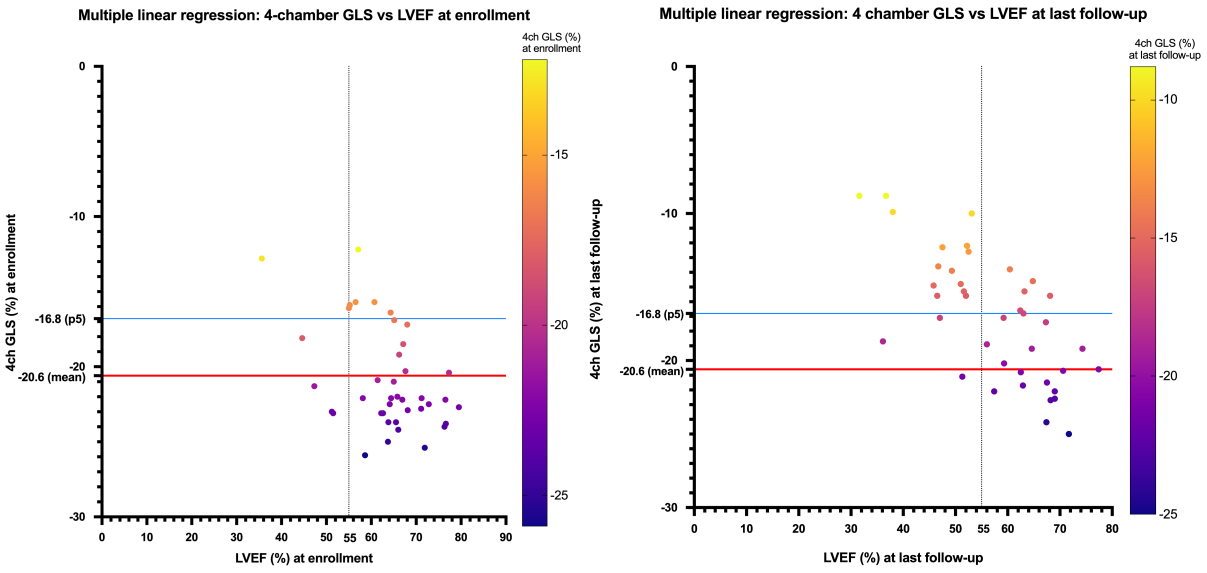
Table 3. Electrocardiographic features									
Patient ID	Phenotype	Rhythm (I/F)	Heart rate (bpm) (I/F)	ECG and 24h-Holter analysis					
				PR interval (ms) (I/F)	QRS interval (ms) (I/F)	QRS axis (°) (I/F)	cQT (ms, Bazett) (I/F)	Q waves (I/F)	Global ECG and 24h-Holter abnormalities (I/F)
1	DMD	Sinus/Sinus	79/92	118/105	92/ 99	84/43	435/444	V4,V5,V6 / V5,V6	R and S waves with high voltage V1, V2, V3 in first and final ECG
2	DMD	Sinus/Sinus	86/84	126/126	78/86	78/59	390/399	V6 / V6	R wave with high voltage I, II, V1, V2, V3 / Last ECG with high R voltage I, II and ST elevation in II
3	DMD	Sinus/Sinus	61/66	100/122	82/88	67/63	400/394	V5,V6 / V5, V6	Global low voltage in first and final ECG Isolated ventricular extrasystoles
4	DMD	Sinus/Sinus	114/79	134/134	92/90	28/62	432/401	III, V6 / V6	R wave with high voltage in I and poor R wave progression / ST elevation in V2 Isolated ventricular extrasystoles
5	DMD	Sinus/Sinus	107/93	102/94	78/86	57/19	421/405	V5, V6 / V6	R wave high voltage V2 and ST elevation in V2, V3 / R wave high voltage in I, V1, V2 and ST elevation in V2
6	DMD	Sinus/Sinus	94/76	110/110	76/80	50/71	427/414	III, aVF,V5,V6 / NA	R wave with high voltage in I / R wave with high voltage in II
7	DMD	Sinus/Sinus	97/130	116/120	90/84	83/72	439/462	V4,V5,V6,II,III,aVF/ V4,V5,V6,II,III,aVF	R wave high voltage in II, III, avF, V2, V3, V4 / R wave high voltage in II, III, avF, V2, V3, V4 Isolated ventricular extrasystoles
8	B/DMD	Sinus/Sinus	54/68	110/116	94/94	78/76	422/416	V4,V5,V6,II,III,aVF/ V4,V5,V6,II,III,aVF	R wave high voltage in II, III, V1, V2, V3, Negative T waves in V4-V6 and III, aVF/ R wave high voltage in II, III, V1, V2, V3, Negative T waves in V4-V6 and III, aVF/
9	DMD	Sinus/Sinus	90/103	105/105	95/138	10/-85	435/468	NA/NA	ST elevation in V1,V2, V3 and R wave high voltage in V3, V4 / Wide QRS, RBBB Isolated atrial and ventricular extrasystoles
10	DMD	Sinus/Sinus	101/100	130/138	100/106	75/94	448/466	NA / II,III,aVF, V6	R and S waves high voltage V1,V2,V3 / R and S waves high voltage V1,V2.
11	DMD	Sinus/Sinus	78/92	110/130	96/98	71/-14	435/435	II, aVF, V4,V5,V6 / V5,V6	R wave high voltage V1,V2,V3 / R wave high voltage in I, V1, V2

12	DMD	Sinus/Sinus	74/66	106/110	88/86	75/91	455/392	V5,V6 / II, III, aVF, V5,V6	R wave high voltage in V2 / R wave high voltage in II, III
13	DMD	Sinus/Sinus	87/103	116/110	94/90	65/80	406/460	III,aVF, V4,V5,V6 / II, III, aVF, V5,V6	R wave high voltage in V1, V2 and ST elevation in V2,V3 / R wave high voltage in II, III, V2, V4, V5 Atrial tachycardia
14	DMD	Sinus/Sinus	83/75	114/114	82/92	95/81	434/410	NA/NA	Low voltage T wave in V4-V6 / Low voltage T wave in V4-V6
15	DMD	Sinus/Sinus	77/82	122/134	96/94	79/19	427/448	NA/NA	Low voltage T wave in V4-V6 / Low voltage T wave in V4-V6
16	B/DMD	Sinus/Sinus	89/96	106/112	92/100	7/71	418/434	NA/NA	R wave high voltage in V2 and V5,V6 / R wave high voltage in V3, V4 and RBBB
17	BMD	Sinus/Sinus	102/95	134/124	110/118	58/70	456/455	II,III, V5,V6 / V5,V6	R wave high voltage in V1, V2, V3, V4 / R wave high voltage in II, V1, V2, V3, V4 and RBBB
18	DMD	Sinus/Sinus	89/93	122/132	88/88	65/0	452/457	NA/NA	ST elevation in V2, V3 / Global low voltage, IRBBB, ST elevation in V3 Atrial and ventricular extrasystoles.
19	DMD	Sinus/Sinus	95/88	108/110	86/92	49/63	457/459	V5, V6 / II, III, aVF, V5,V6	R wave high voltage in V1,V2, V3, V4 / R wave high voltage I, V2
20	DMD	Sinus/Sinus	79/81	110/114	84/86	74/69	421/427	II, V4, V5, V6 / II, V4,V5,V6	R wave high voltage in V1, V2, V3 / R wave high voltage in V1, V2, low voltage T wave in V4-V6.
21	DMD	Sinus/Sinus	70/122	106/130	88/82	83/87	425/447	II, III, aVF, V4-V6 / II,III,aVF, V4-V6	R wave high voltage in II, V1, V2, V3 /R wave high voltage in V2, V3, low voltage T wave in II, aVF, V6
22	DMD	Sinus/Sinus	84/74	110/114	86/78	76/75	399/419	V5,V6 / V5,V6	R wave high voltage in V2, ST elevation in V2 / ST elevation in V2
23	DMD	Sinus/Sinus	90/83	112/110	84/82	10/58	447/437	V5, V6 / V6	R wave high voltage in V1,V2 / R wave high voltage in V1,V2, low voltage in T wave in V6
24	DMD	Sinus/Sinus	98/88	114/100	84/78	69/69	441/447	V4-V6 / III, V4-V6	R wave high voltage in V1-V2 / R wave high voltage in V2 Atrial fibrillation
25	DMD	Sinus/Sinus	102/99	106/120	82/88	79/48	427/428	II,III,aVF, V4-V6 / II,III,aVF, V4-V6	R wave high voltage in II, III, aVF, V1-V3 / R wave high voltage in I, II, and low voltage T wave in V4-V6 and III, aVF
26	DMD	Sinus/Sinus	86/91	90/102	76/78	76/74	399/400	V4-V6 / III	R wave high voltage in V2, V3 / T wave low voltage in V5-V6
27	B/DMD	Sinus/Sinus	87/82	104/114	80/84	76/63	418/420	II,III,aVF,V4-V6 / NA	R wave high voltage in II, III, V3, V4 / NA
28	DMD	Sinus/Sinus	99/97	82/86	88/84	68/74	444/429	II,III,aVF,V4-V6 / II,III,aVF,V4-V6	R wave high voltage in V1-V3 / R wave high voltage in V1-V2
29	DMD	Sinus/Sinus	87/92	120/118	82/82	76/52	394/420	NA / NA	R wave high voltage in II, V2 / R wave high voltage in V2, ST elevation in V2
30	DMD	Sinus/Sinus	94/103	140/138	92/90	83/79	412/416	II,III,aVF, V4-V6 / NA	R wave high voltage in II, V1, V2 / R wave high voltage in II, V2, ST elevation in V3
31	DMD	Sinus/Sinus	94/96	108/110	90/86	77/69	425/439	II,III,aVF, V4-V6 / V4-V6	R wave high voltage in II, V1-V3 / R wave high voltage in V2, deep S wave in V2
32	DMD	Sinus/Sinus	82/71	120/118	88/90	76/76	427/419	II,III,aVF,V4-V6 / II,III,aVF,V4-V6	R wave high voltage in II, III, V1-V3 / R wave high voltage in II, V1-V3, ST elevation in V3-V4

33	DMD	Sinus/Sinus	98/113	120/138	88/94	51/-6	428/466	V5,V6 / NA	R wave high voltage in V1, V2 / Global low voltage
34	DMD	Sinus/Sinus	145/89	106/98	76/82	54/50	441/401	II,III,aVF,V4-V6 / II,III,aVF	R wave high voltage in V1-V2 / NA
35	DMD	Sinus/Sinus	99/91	124/126	82/82	49/45	464/452	III / III	R wave high voltage in I, Deep S wave in V1,V2 / R wave high voltage in I, low voltage T wave V4-V6
36	DMD	Sinus/Sinus	97/91	156/144	92/92	74/75	429/410	II,III,aVF,V4-V6 / II,III,aVF, V5-V6	R wave high voltage in II, V2 / R wave high voltage in II, Deep S wave in V2, ST elevation in V2
37	DMD	Sinus/Sinus	81/97	118/114	84/86	64/35	429/419	V5,V6 / II,III,aVF, V5-V6	R wave high voltage in V2, ST elevation in V2 / ST elevation in V2, IRBBB
38	DMD	Sinus/Sinus	92/98	138/130	94/76	15/16	440/431	NA / NA	IRBBB, R wave high voltage in V3,V4 / R wave high voltage in V2, Deep S wave in V2, ST elevation in V2
39	DMD	Sinus/Sinus	96/90	116/114	96/90	48/36	470/465	V6 / NA	R wave high voltage in I, II, V2-V4, Deep S wave in V2,V3, ST elevation in V3 / R wave high voltage in I, II, V2, Deep S wave in V2, ST elevation in V2, low voltage T wave in V5-V6 and III
40	DMD	Sinus/Sinus	92/88	112/114	92/98	69/72	422/396	II,III,AVF,V6 / NA	R wave high voltage in V1-V3, deep S wave in V2,V3 / R wave high voltage in II, V1-V3, ST elevation in V2

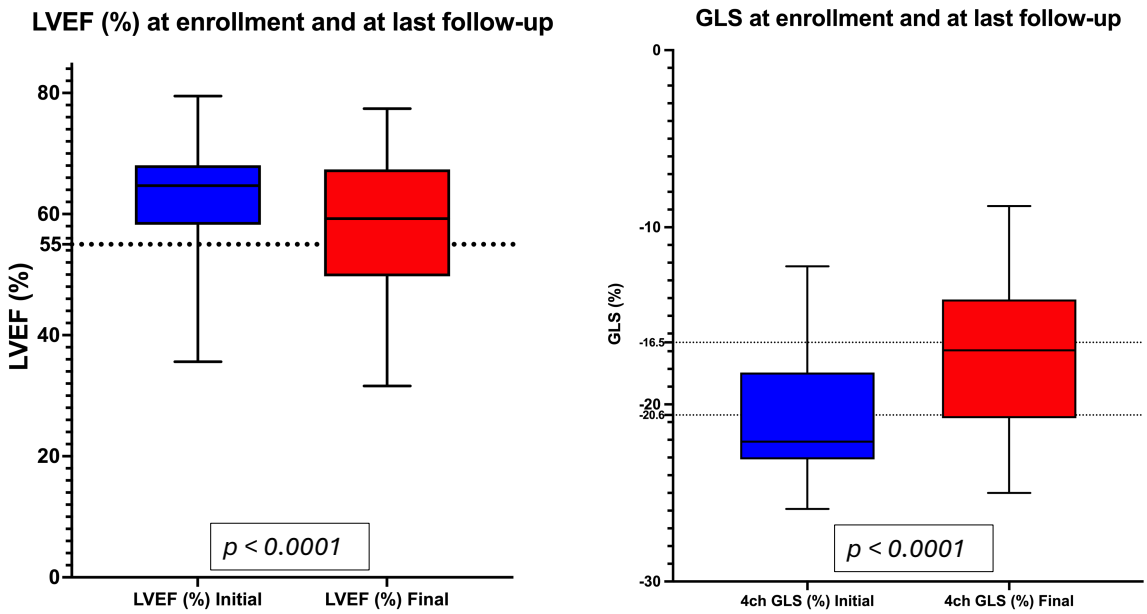
DMD: Duchenne muscular dystrophy. B/DMD: intermediate phenotype
I: first control; F: last control.
RBBB: right bundle branch block; IRBBB: incomplete bundle branch block
NA: not available/not applicable; Y: Yes; N: No

Graphic 1. Relationship between LVEF and 4-chamber GLS values for every patient over time



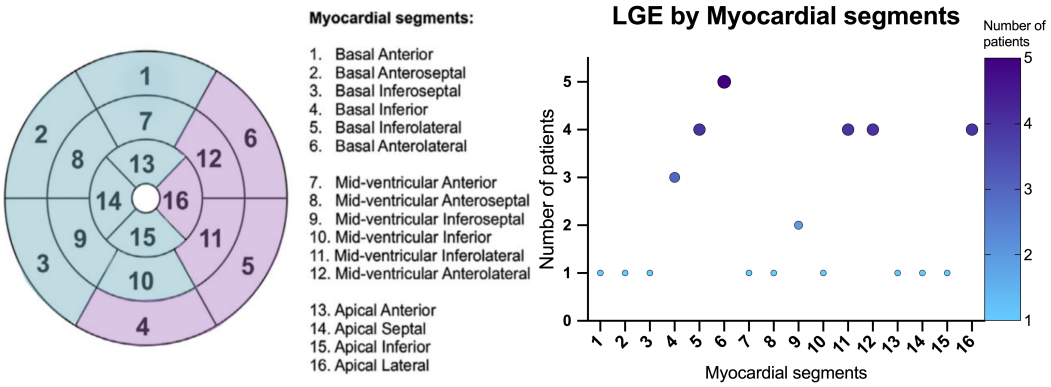
Left graphic represents the relationship between 4-chamber GLS and LVEF values at enrollment. Right graphic represents the relationship between 4-chamber GLS and LVEF values final follow-up. Note that patients have worsening values of LVEF and GLS over time. At enrollment, 4-chamber GLS is depressed in some patients despite relatively well preserved LVEF.
LVEF: left ventricular ejection fraction. 4-chamber GLS: 4-chamber global longitudinal strain.

Graphic 2. LVEF and 4-chamber GLS values over time during follow-up.



Left graphic represents a Box-plot with LVEF values at enrollment (blue) and final follow-up (red) with a statistically significant reduction of median values over time (p < 0.0001). Right graphic represents a Box-plot with 4-chamber GLS values at enrollment (blue) and final follow-up (red) with a statistically significant reduction of deformation (more positive values) over time (p < 0.0001). LVEF: left ventricular ejection fraction (%). 4-chamber GLS: 4-chamber global longitudinal strain.

Figure 1. Myocardial segments and late gadolinium enhancement (fibrosis)



Blue areas represent < 3 patients with fibrosis in these segments. Purple areas represent ≥ 3 patients with fibrosis in these segments: basal inferior, basal inferolateral, basal anterolateral, mid-ventricular inferolateral, mid-ventricular anterolateral and apical lateral. LGE: Late Gadolinium Enhancement

Figure 2. Type of mutations and affected exons in DMD gene.

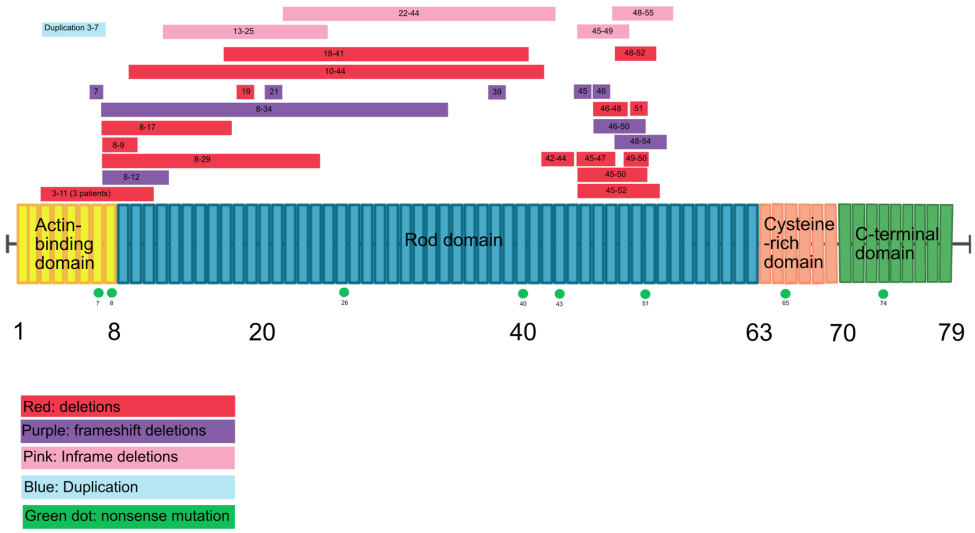
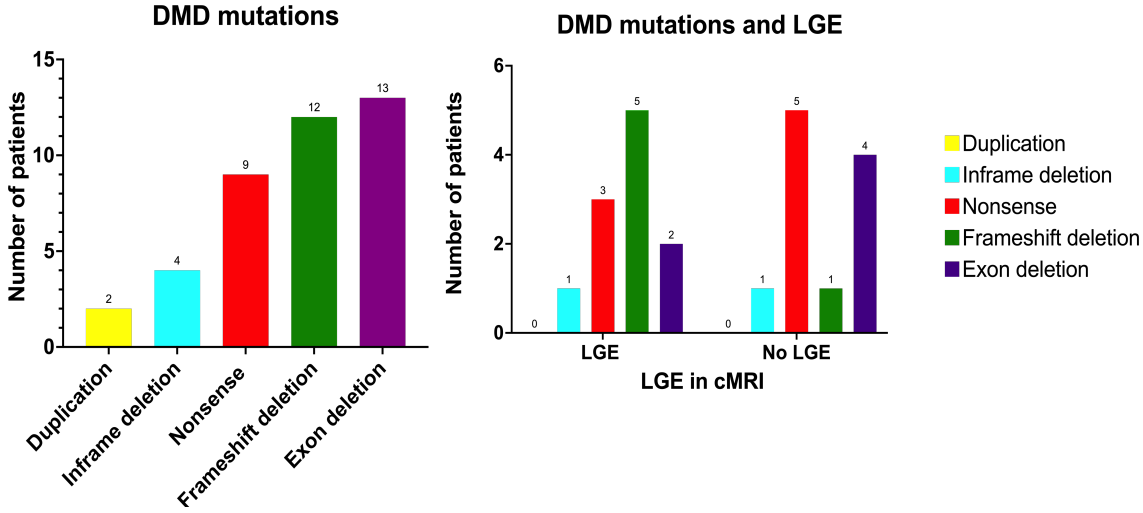


Figure 2 shows the different type of mutations represented by exons. In yellow, the actin-binding domain, in blue the Rod-domain, in orange the Cysteine-rich domain and in green the C-terminal domain. The color legends shows the classification of the different mutations. Two hotspot regions of high prevalence has been detected: deletions in exons 3-20 and exons 45-55.

Graphic 3. DMD mutations and its relationship with LGE in the cMRI



Graphic 3 shows the different type of DMD gene mutations in the cohort. Note that de most frequent mutation type in the cohort were exon deletions and frameshift deletions. LGE was found most frequently in patients with frameshift deletion and nonsense mutations.

DMD: Duchenne muscular dystrophy. LGE: late Gadolinium enhancement. cMRI: cardiac magnetic resonance imaging.

References

1. Adorisio R, Mencarelli E, Cantarutti N, Calvieri C, Amato L, Cicenia M, et al. Duchenne dilated cardiomyopathy: Cardiac management from prevention to advanced cardiovascular therapies. J Clin Med. 2020;9(10):3186.

2. Nascimento Osorio A, Medina Cantillo J, Camacho Salas A, Madruga Garrido M, Vilchez Padilla JJ. Consensus on the diagnosis, treatment and follow-up of patients with Duchenne muscular dystrophy. Neurologia (Engl Ed). 2019;34(7):469–81.

3. Mendell JR, Shilling C, Leslie ND, Flanigan KM, Al-Dahhak R, Gastier-Foster J, et al. Evidence-based path to newborn screening for Duchenne muscular dystrophy. Ann Neurol. 2012;71(3):304–13.

4. Muntoni F, Torelli S, Ferlini A. Dystrophin and mutations: one gene, several proteins, multiple phenotypes. Lancet Neurol. 2003;2:731–40.

5. Campbell KP. Three muscular dystrophies: loss of cytoskeleton-extracellular matrix linkage. Cell. 1995;80(5):675-9.

6. Birnkrant DJ, Bushby K, Bann CM, Apkon SD, Blackwell A, Brumbaugh D, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. Lancet Neurol. 2018;17(3):211–2.

7. Birnkrant DJ, Bushby K, Bann CM, Alman BA, Apkon SD, Blackwell A, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: respiratory, cardiac, bone health, and orthopaedic management. Lancet Neurol. 2018;17(4):347–61.

8. Groh WJ, Bhakta D, Tomaselli GF, Aleong RG, Teixeira RA, Amato A, et al. 2022 HRS expert consensus statement on evaluation and management of arrhythmic risk in neuromuscular disorders. Heart Rhythm. 2022;19(10):e61–120.

9. Finsterer J, Cripe L. Treatment of dystrophin cardiomyopathies. Nat Rev Cardiol. 2014;11(3):168–79

10. McNally EM, Kaltman JR, Woodrow Benson D, Canter CE, Cripe LH, Duan D, et al. Contemporary cardiac issues in Duchenne muscular dystrophy. Circulation. 2015;131(18):1590–8.

11. Castro-Gago M, Gómez-Lado C, Eirís-Puñal J. Cardiac troponin I for accurate evaluation of cardiac status in myopathic patients. Brain Dev. 2009;31(2):184–184.

12. Soslow JH, Xu M, Slaughter JC, Stanley M, Crum K, Markham LW, et al. Evaluation of Echocardiographic Measures of Left Ventricular Function in Patients with Duchenne Muscular Dystrophy: Assessment of Reproducibility and Comparison to Cardiac Magnetic Resonance Imaging. J Am Soc Echocardiogr. 2016;29(10):983–91.

13. Spurney CF, McCaffrey FM, Cnaan A, Morgenroth LP, Ghelani SJ, Gordish-Dressman H, et al. Feasibility and Reproducibility of Echocardiographic Measures in Children with Muscular Dystrophies. J Am Soc Echocardiogr. 2015;20010(8):1–10.

14. Levy PT, Machefsky A, Sanchez AA, Patel MD, Rogal S, Fowler S, et al. Reference Ranges of Left Ventricular Strain Measures by Two-Dimensional Speckle-Tracking Echocardiography in Children: A Systematic Review and Meta-Analysis. J Am Soc Echocardiogr. 2016;29(3):209-225.e6.

15. Amedro P, Vincenti M, De La Villeon G, Lavastre K, Barrea C, Guillaumont S, et al. Speckle-Tracking Echocardiography in Children With Duchenne

Muscular Dystrophy: A Prospective Multicenter Controlled Cross-Sectional Study. J Am Soc Echocardiogr. 2019;32(3):412–22.

16. Siegel B, Olivieri L, Gordish-Dressman H, Spurney CF. Myocardial Strain Using Cardiac MR Feature Tracking and Speckle Tracking Echocardiography in Duchenne Muscular Dystrophy Patients. Pediatr Cardiol. 2018;39(3):478–83.

17. Earl CC, Soslow JH, Markham LW, Goergen CJ. Myocardial strain imaging in Duchenne muscular dystrophy. Front Cardiovasc Med. 2022;9:1031205.

18. Bilchick KC, Salerno M, Plitt D, Dori Y, Crawford TO, Drachman D, et al. Prevalence and distribution of regional scar in dysfunctional myocardial segments in Duchenne muscular dystrophy. J Cardiovasc Magn Reson. 2011;13(1):20.

19. Mavrogeni S, Markousis-mavrogenis G, Papavasiliou A. Cardiac involvement in Duchenne and Becker muscular dystrophy. World J Cardiol. 2015;7(7):1–14.

20. Florian A, Ludwig A, Engelen M, Waltenberger J, Rösch S, Sechtem U, et al. Left ventricular systolic function and the pattern of late-gadolinium-enhancement independently and additively predict adverse cardiac events in muscular dystrophy patients. J Cardiovasc Magn Reson. 2014;16(1):81.

21. Olivieri LJ, Kellman P, McCarter RJ, Cross RR, Hansen MS, Spurney CF. Native T1 values identify myocardial changes and stratify disease severity in patients with Duchenne muscular dystrophy. Journal of Cardiovascular Magnetic Resonance. 2016;18(1):72.

22. Hakimi M, Burnham T, Ramsay J, Cheung JW, Goyal NA, Jefferies JL, et al. Electrophysiologic and Cardiovascular Manifestations of Duchenne and Becker Muscular Dystrophies. Heart Rhythm. 2024;S1547-4271(24)02992-0.

23. Pahl E, Sleeper LA, Canter CE, Hsu DT, Lu M, Webber SA, et al. Incidence of and risk factors for sudden cardiac death in children with dilated cardiomyopathy: A report from the pediatric cardiomyopathy registry. J Am Coll Cardiol. 2012;59(6):607–15.

24. Villa CR, Czosek RJ, Ahmed H, Khoury PR, Anderson JB, Knilans TK, et al. Ambulatory monitoring and arrhythmic outcomes in pediatric and adolescent patients with Duchenne muscular dystrophy. J Am Heart Assoc. 2016;5(1):e002620.

25. Kaspar RW, Allen HD, Ray WC, Alvarez CE, Kissel JT, Pestronk A, et al. Analysis of dystrophin deletion mutations predicts age of cardiomyopathy onset in Becker muscular dystrophy. Circ Cardiovasc Genet. 2009;2(6):544–51.

26. Raman S V., Hor KN, Mazur W, Halnon NJ, Kissel JT, He X, et al. Eplerenone for early cardiomyopathy in Duchenne muscular dystrophy: a randomised, double-blind, placebo-controlled trial. Lancet Neurol. 2015;14(2):153–61.

27. Ogata H, Ishikawa Y, Ishikawa Y, Minami R. Beneficial effects of beta-blockers and angiotensin-converting enzyme inhibitors in Duchenne muscular dystrophy. J Cardiol. 2009;53(1):72–8.

28. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, et al. 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure: a report of the American College of Cardiology / American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society. Circulation. 2017;136(6):e137-e161.

29. McDonagh TA, Metra M, Adamo M, Baumbach A, Böhm M, Burri H, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J. 2021;42(36):3599-3726.

30. Bourke J, Tynan M, Stevenson H, Bremner L, Gonzalez-Fernandez O, McDiarmid AK. Arrhythmias and cardiac MRI associations in patients with established cardiac dystrophinopathy. Open Heart. 2024;11(1):3002590.

31. Chiang DY, Allen HD, Kim JJ, Valdes SO, Wang Y, Pignatelli RH, et al. Relation of Cardiac Dysfunction to Rhythm Abnormalities in Patients with Duchenne or Becker Muscular Dystrophies. Am J Cardiol. 2016;117(8):1349–54.

8. Discusión

Las enfermedades neuromusculares de las que se ha completado la revisión y seguimiento en los artículos presentados tienen origen ya en etapas precoces de la vida. En algunos de estos casos, puede haber manifestaciones clínicas en época fetal (pocos movimientos fetales) o en los primeros meses de la vida, como pueden ser las distrofias musculares congénitas por laminopatía (fenotipo L-CMD) y la forma congénita de la Distrofia muscular tipo 1 o Steinert (cDM1). De forma general, se puede resumir la presentación fenotípica habitual en la figura 14.

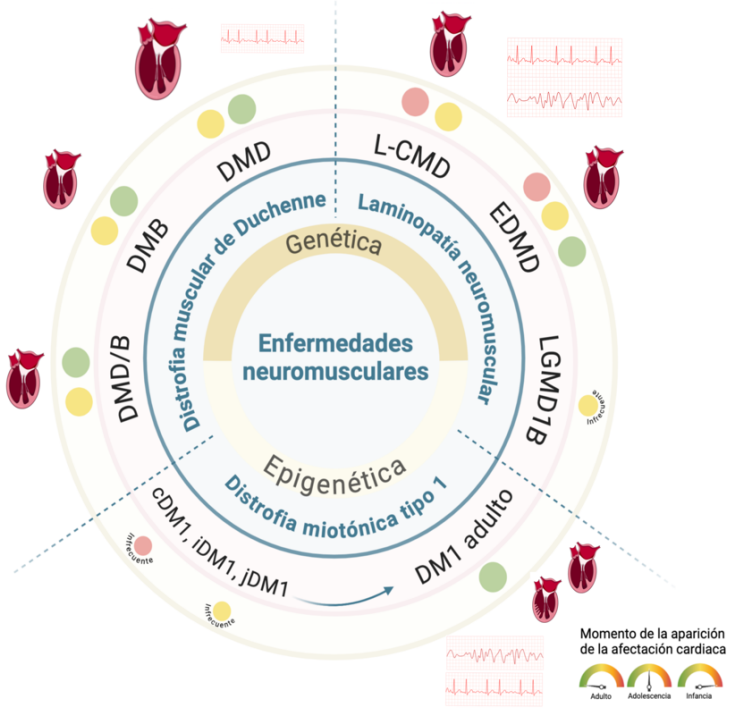


Figura 14 (creación propia). En esta figura se representan las enfermedades neuromusculares estudiadas, destacando le influencia que tiene la genética y epigenética en la expresión fenotípica de cada una. Se destaca también el momento de aparición de complicaciones cardiovasculares (arritmias, miocardiopatía dilatada) que, de forma más frecuente, suelen aparecer en cada una de ellas siendo en rojo la infancia, amarillo la adolescencia y en verde en la etapa adulta. La genética, incluyendo otros genes modificadores de fenotipo, y la epigenética son factores que pueden ser fundamentales en la expresión fenotípica tanto en la forma de expresividad como en la severidad. En el caso de DMD, la miocardiopatía dilatada e insuficiencia cardíaca ha sido la complicación cardiovascular más frecuente. En el caso de las laminopatías, las formas de L-CMD y EDMD muy relacionadas con arritmias potencialmente malignas y miocardiopatía dilatada e insuficiencia cardiaca. En el caso de DM1 se han detectado alteraciones electrocardiográficas y ecocardiográficas menores en la infancia y adolescencia que pueden sentar la base de las arritmias potencialmente letales y miocardiopatía que se manifiestan en la etapa adulta. + DMD: Distrofia muscula de Duchenne. DMB: distrofia muscular de Becker. DMD/B: fenotipo intermedio. DM1: distrofia miotónica tipo 1 o de Steinert. cDM1: forma congénita de distrofia miotónica tipo 1. iDM1: forma infantil de la distrofia miotónica tipo 1. jDM1: forma juvenil de la distrofia miotónica tipo 1. L-CMD: distrofia muscular congénita por laminopatía. EDMD: distrofia muscular de Emery-Dreifuss. LGMD1B: distrofia muscular de cinturas tipo 1B.

Hay que tener en cuenta que algunos casos congénitos o de inicio precoz en los primeros años de la vida van a tener una debilidad muscular muy importante que va a empeorar el pronóstico global. En otros casos, la manifestación cardiovascular va a ser potencialmente letal, motivo por el que debemos tener la responsabilidad de estudiar indicadores de aquellas situaciones que puedan comprometer la vida del paciente de forma brusca e inesperada, como pueden ser las arritmias y la muerte súbita.

A continuación, se resume la discusión por cada una de las enfermedades estudiadas en la presente tesis doctoral.

8.1. Laminopatías neuromusculares

Las laminopatías son un grupo de enfermedades cuya base genética se encuentra en una mutación patogénica en el gen LMNA, que codifica para unas proteínas nucleares llamadas laminas. Las laminas son proteínas nucleares de filamento intermedio que tienen como función, entre otras, la modulación de la organización de la cromatina, la modulación de la expresión génica y la estabilidad genómica. El gen LMNA codifica para las laminas tipo A (AD10, C, y C2) vía *splicing* alternativo, y se expresan en todas las células somáticas diferenciadas. (3,17,18,24) Las hipótesis que se manejan y que explicarían que los miocitos y miocardiocitos sean más sensibles a alteraciones en la lamina tipo A son el daño en la membrana nuclear celular que daría lugar a la muerte celular por estrés mecánico (por su papel clave en la interacción con el citoesqueleto) y la interrupción de vías de señalización (MAPK, ERK, JNK, p38_ y mTOR) debido al papel de las lamina tipo A en la expresión génica e interacción con reguladores transcripcionales (c-Fos, ERK1/2, y SREBP1). (18,97,98) La patogénesis de la miocardiopatía también podría estar involucrado el efecto negativo de la haploinsuficiencia de la lamina A/C. (99)

En cuanto al fenotipo, la distrofia muscular por LMNA puede dar lugar a diferentes fenotipos, lo que complica su estudio ya desde el período neonatal, danto lugar a discrepancias en el manejo desde el punto de vista cardiológico. (25,32,33,100–102)

En la cohorte presentada de pacientes pediátricos con distrofia muscular por laminopatía se han podido observar eventos cardíacos mayores (taquicardia

ventricular, fibrilación ventricular), miocardiopatía e insuficiencia cardiaca, ictus y, en 2 casos muerte.

A pesar de que todos estos pacientes tuvieron un estudio electrofisiológico normal, el implante del holter subcutáneo en estos pacientes permitió discriminar arritmias malignas (taquicardia ventricular, fibrilación ventricular) en 5 casos (17% de los pacientes incluidos), además de otros casos de asistolia, pausas sinusales, fibrilación auricular y arritmias supraventriculares. Estos 5 pacientes recibieron el implante de un desfibrilador como prevención de la muerte súbita. Además, cabe destacar que los pacientes con eventos cardíacos mayores y arritmias fueron los que debutaron con sintomatología neuromuscular antes de los 2 años de edad. Esto, sumado a la vulnerabilidad de estos pacientes, la poca o nula deambulación en muchos casos, así como la atipicidad de los síntomas cardiovasculares, apoyan el uso de herramientas que ayuden a discriminar y estratificar mejor el riesgo cardiovascular para poder tomar actitudes terapéuticas o preventivas dentro de un período aún asintomático de la enfermedad cardíaca y así prevenir arritmias potencialmente malignas que desenlacen en una muerte súbita. Con esto se demuestra que, al igual que en recomendaciones de pacientes neuromusculares en población adulta (EDMD y LGMD1B) en las que se indicaría implantar dispositivos de monitorización ECG, con el seguimiento de esta cohorte de pacientes pediátricos con laminopatía neuromuscular este tipo de dispositivos tiene un papel muy relevante. (33,38,103)

Aunque pueda verse limitada la indicación del implante de dispositivos de control del ritmo cardíaco, como puede ser un DAI, en pacientes con distrofia muscular en edad pediátrica (por cuestiones anatómicas, de descargas inapropiadas y otras complicaciones) en centros expertos en enfermedades neuromusculares, con un equipo multidisciplinar de manejo integral, los dispositivos pueden ser implantados y programados de manera segura. Existen publicaciones al respecto del implante de DAI en enfermedades neuromusculares, abogando por la individualización de la indicación en cada caso.(57) En la cohorte de pacientes estudiada, de los 5 pacientes a los que se implantó un desfibrilador, todos tenían ya unos valores bajos del *strain* miocárdico del corte de 4 cámaras, a pesar de tener valores preservados de FEVI, lo que el *strain* miocárdico podría

corresponder a un predictor de mal pronóstico cardiológico ya en edad pediátrica.

Durante el seguimiento de estos 5 desfibriladores, hubo dos choques apropiados y ningún choque inapropiado durante el tiempo de seguimiento. Ha de valorarse también, en el momento del implante del dispositivo, la expectativa de vida y el estado global del paciente. Los resultados de esta tesis han permitido detectar arritmias potencialmente letales en edades muy precoces que hubieran podido limitar la expectativa de vida del paciente, ya que a nivel neuromuscular no existía un deterioro lo suficientemente severo como para desencadenar en un evento de muerte.

En cuanto a los fenotipos, los que presentaron peor fenotipo cardíaco fueron los que tuvieron un debut neuromuscular más precoz, antes de los 2 años, independientemente del fenotipo neuromuscular final (23/28 pacientes incluidos). Esto se ve apoyado con la serie retrospectiva publicada recientemente, en pacientes con diagnóstico de L-CMD.(25) El debut precoz comentado, independientemente del fenotipo neuromuscular descrito o sospechado, es muy relevante ya que los criterios diagnósticos para estratificar los diferentes fenotipos de laminopatías neuromusculares han ido cambiando en los últimos años y esto dificulta el análisis global de los resultados de las publicaciones. En la cohorte de pacientes del estudio, los eventos cardiovasculares se centraron en los pacientes con L-CMD y EDMD. Aunque los pacientes con LGMD1B pueden manifestar miocardiopatía dilatada, arritmias y muerte súbita, puede que este riesgo se vea desplazado más adelante a la etapa adulta, tal y como se sugiere en las diferentes publicaciones.(6,35,36,57) También los pacientes con fenotipos atípicos y leves podrían manifestar miocardiopatía dilatada, aunque tampoco ninguno de los dos pacientes incluidos ha manifestado miocardiopatía ni trastornos ECG en la monitorización, al menos en edad pediátrica. (33,97) En cuanto a los pacientes con EDMD de la cohorte, aquellos con un debut neuromuscular muy precoz, tuvieron más riesgo de miocardiopatía dilatada al cabo de los años, mientras que las arritmias potencialmente malignas estuvieron más presentes en el fenotipo L-CMD. Esto concuerda con estudios previos reportados. (33,34,49)

En la figura 15 se muestra un resumen de la historia natural de este grupo de pacientes con manifestación precoz de la enfermedad neuromuscular y los

momentos clave que, probablemente, si se actúa de modo multidisciplinar y con un alto índice de sospecha, puedan vitarse la mortalidad por arritmias y muerte súbita. A pesar de los avances en la tecnología que permiten detectar situaciones de forma muy precoz, queda aún trabajo por hacer en cuanto al manejo de los fármacos para insuficiencia cardiaca y arritmias en población pediátrica.

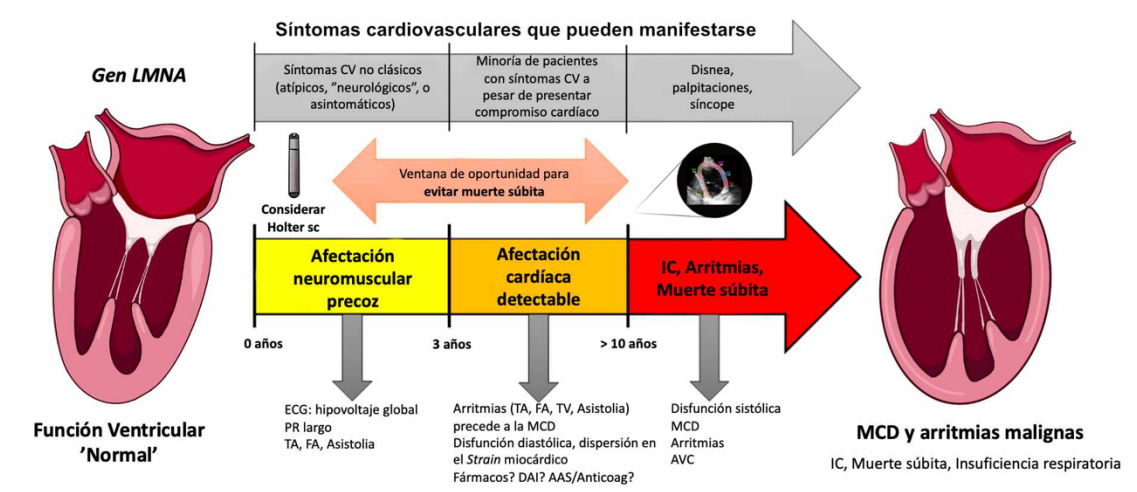


Figura 15 (creación propia). Resumen de la historia natural de los pacientes con laminopatía neuromuscular, con las manifestaciones más frecuentemente reportadas desde el punto de vista sintomático y cardiovascular. Se introduce el elemento de Holter subcutáneo como dispositivo a considerar implantar en las formas de manifestación precoz de la enfermedad neuromuscular y el análisis de *strain* miocárdico como indicador de mal pronóstico cardíaco precoz.

De los dos pacientes que fallecieron durante el seguimiento de la cohorte pediátrica, las causas fueron infección respiratoria en uno de los casos, a los 10 años de edad y no presentaba afectación cardíaca hasta ese momento, y en el otro caso por insuficiencia cardíaca congestiva terminal a los 18 años. En este último caso, se asociaron, además, arritmias auriculares (silencio auricular, fibrilación auricular) y ventriculares potencialmente malignas (taquicardia ventricular, fibrilación ventricular) y refractarias al tratamiento médico propuesto y a la optimización del algoritmo del DAI. Ambos casos se trataban de fenotipos precoces con manifestación clínica neuromuscular antes de los 2 años de edad y eran no ambulantes y requerían soporte ventilatorio no invasivo en domicilio de forma intermitente.

En cuanto a la genética, han sido identificadas 20 variantes en el gen LMNA (18 exónicas y 2 intrónicas), de las que 12 fueron *de novo.* La mutación más prevalente identificada fue p.Arg249Trp (21.4%) y, a pesar de tener la misma

mutación, hubo expresividad variable, pues los fenotipos encontrados en los pacientes portadores fueron 4 L-CMD (de éstos, 1 portador de marcapasos y otro de DAI), 1 EDMD y 1 manifestación con debilidad muscular leve. Esta mutación se relacionó más con las arritmias que con miocardiopatía dilatada, pues no se encontró ningún caso, al menos en edad pediátrica.

Tanto la miocardiopatía dilatada como la presencia de DAI indicado por arritmias tuvo una localización muy variada en el gen LMNA. La figura 16 representa esta correlación y, la cohorte de seguimiento, la miocardiopatía dilatada se relacionó más con mutaciones en el exón 1 y 7 (ninguno de los pacientes del exón 4), mientras que las DAI por arritmias tuvo una distribución heterogénea entre todos los exones. Este dato puede tener relevancia para el seguimiento, al menos, durante la edad pediátrica. A pesar de esto, pacientes con la misma mutación expresan fenotipos variables, con penetrancia también variable confiriendo diferentes grados de severidad de la enfermedad y, por ende, con diferentes velocidades de progresión de la enfermedad global.

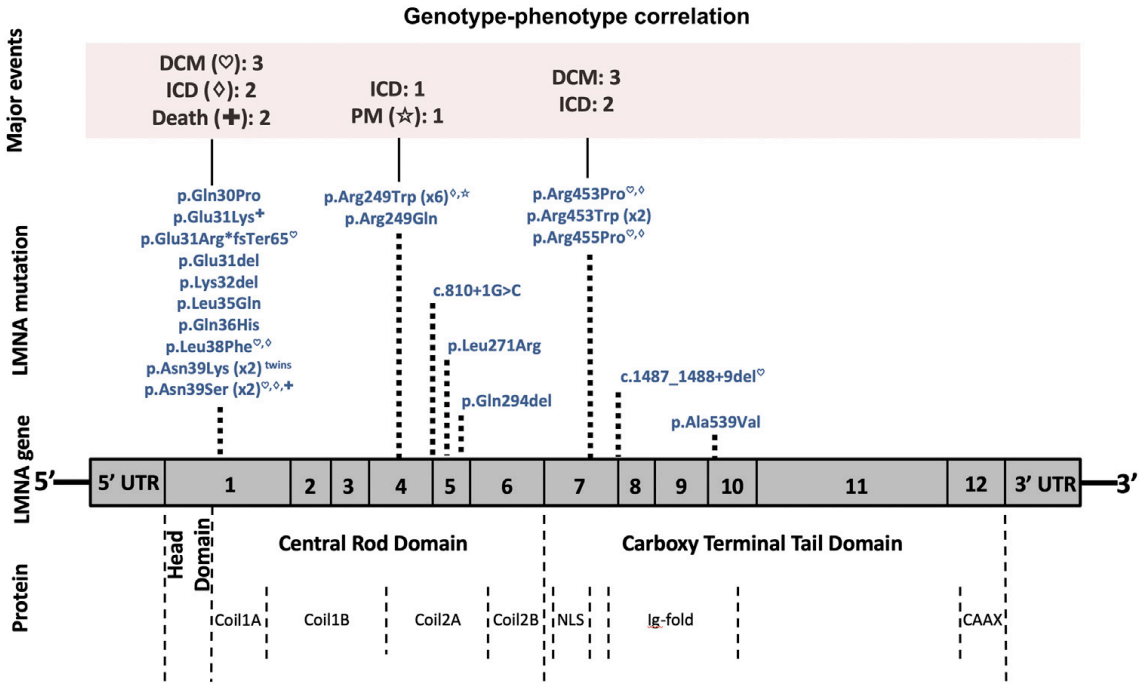
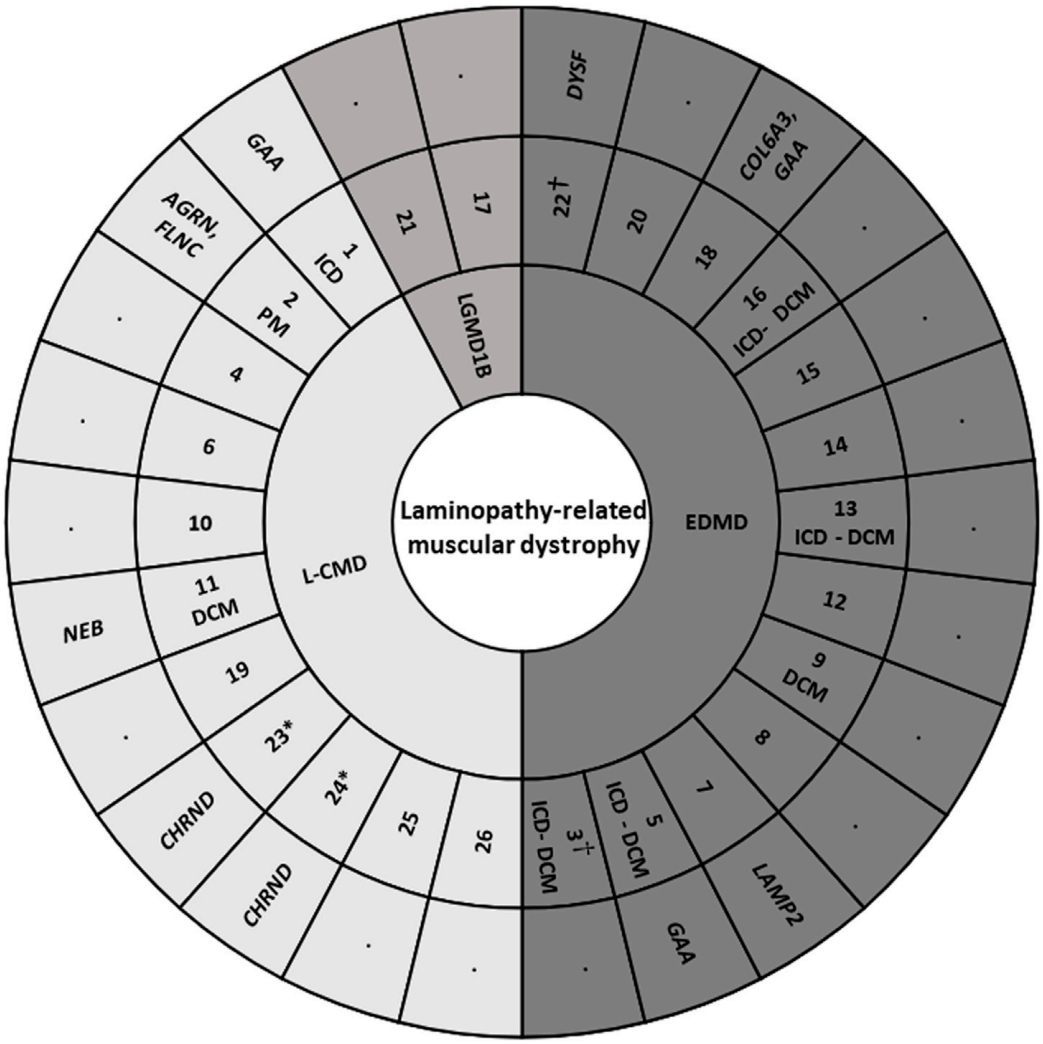


Figura 16 (publicada en el artículo 1 de esta tesis, Cesar et al). Correlación fenotipo-genotipo de la cohorte pediátrica con laminopatía neuromuscular. *DCM: miocardiopatía dilatada. ICD: desfibrilador automático implantable. Death: muerte. PM: marcapasos.*

Siguiendo en la línea de las características fenotípicas observadas, a parte de la expresividad variable incluso con la misma mutación patogénica, cabe destacar un debut y progresión diferentes entre los pacientes incluso con el mismo fenotipo neuromuscular. Esto genera la hipótesis de que existen otros modificadores del fenotipo y que ha justificado encontrar otros predictores que empeoren el pronóstico cardíaco.(31) Además, durante el desarrollo de la tesis y después de la publicación de los artículos que se presentan, dos pacientes pediátricas de la cohorte, gemelas monocigóticas, han tenido una progresión rápidamente progresiva desde el punto de vista neuromuscular y cardíaco, con debut de arritmias auriculares e insuficiencia cardíaca rápidamente progresivas y refractarias al tratamiento médico, aunque con diferencias en la velocidad de progresión de la debilidad muscular y de las complicaciones cardiovasculares entre ellas. Todo esto justifica un análisis profundo de los modificadores del fenotipo, como pueden ser factores genéticos, epigenéticos y ambientales. En esta línea, en el artículo 2 se describen factores genéticos encontrados en la cohorte estudiada. Para entender que pueda haber otros factores genéticos, la miocardiopatía dilatada, que es la miocardiopatía más frecuente, se caracteriza por una dilatación ventricular y disfunción sistólica, con adelgazamiento de las paredes ventriculares y fibrosis miocárdica. A nivel genético, se ha relacionado con más de 60 genes y se puede detectar en aproximadamente el 60% de los casos, principalmente mediante herencia autosómica. (104,105) Entre los genes que se hallan más frecuentemente en la miocardiopatía dilatada, alrededor del 30-35% de los casos corresponde a una mutación en el gen TTN, seguido de LMNA en el 10-15% de los casos. También se han descrito otros genes implicados como los que codifican a canales iónicos o proteínas asociadas (SCN5A, KCNQ1 y ABCC9), factores de transcripción (*TBX5, TBX20, NKX2-5, GATA4, GATA5, GATA 6, EYA4,* and *FOXD4*), y proteínas sarcoméricas (*ACTC1, MYBPC3, MYH6, MYH7, TNNC1, TNNI3, TNNT2, TPM1)*, entre otras.(104–107) Cuando existe un fenotipo de miocardiopatía dilatada con trastorno de la conducción, se ha podido relacionar al gen LMNA en hasta el 8% de los casos. (42,105) Esta particularidad del defecto de la conducción le confiere un peor pronóstico y mayor riesgo de muerte súbita, algo demostrado también en modelos murinos. (108,109)

La ampliación del estudio genético en la cohorte de pacientes con laminopatía neuromuscular surgió de, precisamente, la necesidad de dar explicación a las diferencias de la forma y severidad de la expresión fenotípica, tal y como también se ha sugerido en otras publicaciones.(110–112) Así, en el artículo 2 se detalla la ampliación del estudio genético a 105 genes más relacionados de alguna manera con enfermedades neuromusculares, miocardiopatía y arritmias. Se pudieron analizar 26 pacientes (casos índice) de 25 familias diferentes y un total de 50 familiares. La mayoría de los pacientes incluidos (23/26) tuvieron un debut de síntomas neuromusculares antes de los 2 años. Gran parte de los casos fueron variantes raras en el gen LMNA *de novo* (24/26 casos, 92.3%), y en tan sólo 2 familias se encontró la misma variante en alguno de los padres (siendo en este caso una expresión fenotípica muscular muy leve).

La siguiente figura 17 (publicada en el artículo 2 de la tesis) muestra la correlación de los hallazgos genéticos con el fenotipo cardíaco y neuromuscular:



Ninguna de las proteínas de las que son responsables los genes que se han encontrado tras el análisis, participan de forma cercana con el gen LMNA. En total, el 30.76% de los pacientes tuvieron un hallazgo de una variante rara en alguno de estos genes: AGRN, CHRND, COL6A3, DYSF, FLNC, GAA, LAMP2, and NEB). Todos fueron exónicos, en estado heterozigoto y ninguno fue *de novo.* De estas variantes raras encontradas, las únicas publicadas previamente, y relacionadas con trastorno del depósito de Glucógeno, fueron las del gen GAA en dos pacientes diferentes (p.Arg594His, p.Asp645Asn).(113–115) Es importante destacar que, a excepción de los 2 casos en que los padres tenían manifestaciones leves musculares por mutación en LMNA, ninguno tenía otros síntomas. El hallazgo de variantes raras como las halladas en GAA (paciente 1), AGRN y FLNC (paciente 2) podrían conferir una severidad especial al fenotipo neuromuscular y cardiaco por LMNA, pues los dos pacientes (6 y 26) que tienen la misma mutación en LMNA que los pacientes 1 y 2 (c.745C>T, p.Arg249Trp) no han expresado la misma severidad fenotípica, aun tener también el mismo diagnóstico fenotípico L-CMD, y no son portadores de estas variantes raras en GAA, AGRN ni FLNC.

En la cohorte, otros pacientes con la misma mutación en p.Arg249Trp (paciente 14) han presentado otro fenotipo neuromuscular (EDMD). Esto sugiere que la ampliación del análisis genético tenga un sentido para dar explicación a estas diferencias de la expresión fenotípica.

El paciente 5, que tenía una mutación en LMNA pArg453Pro, tenía un fenotipo EDMD con afectación cardíaca severa (miocardiopatía dilatada y arritmias que justificaron el implante de un DAI), tuvo un hallazgo de una mutación rara en GAA p.Gly641Asp. Se desconoce el grado de participación como modificador del fenotipo porque en este caso la enfermedad de depósito de glucógeno se manifiesta de forma recesiva y la madre, que era portadora, no tenía síntomas relacionados con la enfermedad. En este caso desconocemos el papel que pueda tener ya que falta literatura al respecto del comportamiento fenotípico de este cambio de aminoácido en el gen LMNA.

Los pacientes 20 y 21 tenían también una mutación en el mismo aminoácido p.Arg453: en estos dos casos la mutación fue en p.Arg453Trp pero tenían diferente diagnóstico fenotípico (EDMD y LGMD1B) y la afectación muscular era

lentamente progresiva sin afectación cardíaca. Esta mutación ha sido descrita en fenotipos de EDMD con arritmias y debilidad muscular lentamente progresiva. (34,116,117) En estos dos casos no se hallaron otros genes en la ampliación del estudio genético.

La mutación en p.Asn39Ser fue responsable del fenotipo EDMD del paciente 7 y del paciente 3. Mientras que la literatura publicada (34,118) concuerda con la no afectación cardíaca del paciente 7 (sí hay diferentes fenotipos publicados: EDMD, L-CMD), el paciente 3 tuvo un fenotipo EDMD con una afectación muscular y cardíaca severas (arritmias malignas, portadora de DAI y, finalmente, fue el paciente que falleció con 18 años de causa cardíaca). El estudio genético ampliado no mostró ningún hallazgo en este paciente 3 mientras que en el paciente 7 se halló una variante rara en LAMP2 de la cual era portadora la madre y no tenía fenotipo ni síntomas.

En el caso de las pacientes gemelas monocigóticas, portadoras de la misma mutación en LMNA p.Asn39Lys, con fenotipo L-CMD y, a pesar que inicialmente no tuvieron fenotipo cardíaco, en el seguimiento clínico han presentado una evolución rápidamente progresiva tanto a nivel neuromuscular como también a nivel cardíaco, presentando arritmias (taquicardia auricular de difícil control, fibrilación auricular en la gemela 1 y taquicardia auricular y ventricular e ictus en la gemela 2) e insuficiencia cardiaca, ambas refractarias de comportamiento agresivo y refractarias al tratamiento médico. Esto, una vez más, difiere de los casos publicados con la misma mutación en LMNA, en los que se describe una evolución muscular lentamente progresiva y sin afectación cardíaca. (34,116) En este caso se ha ampliado el estudio genético a posteriori (ver artículo en Anexos Martínez-Olorón et al) y se ha encontrado, a parte de la variante posiblemente benigna en CHRND p.Pro307Ser, dos variantes de comportamiento ambiguo en AGRN p.Pro325Arg y en DMD p.Gln206Leu. La variante AGRN fue heredada del padre (asintomático) y la variante DMD y CHRND de la madre (asintomática). A pesar de la discrepancia en la velocidad del empeoramiento de la enfermedad neuromuscular y cardíaca en ambas gemelas, el resultado final fue el mismo, éxitus debido a complicaciones cardiológicas (arritmias e insuficiencia cardiaca rápidamente progresiva y refractaria al tratamiento médico). Dada la relevancia clínica de estos dos casos, se representa la evolución clínica en la figura 18.

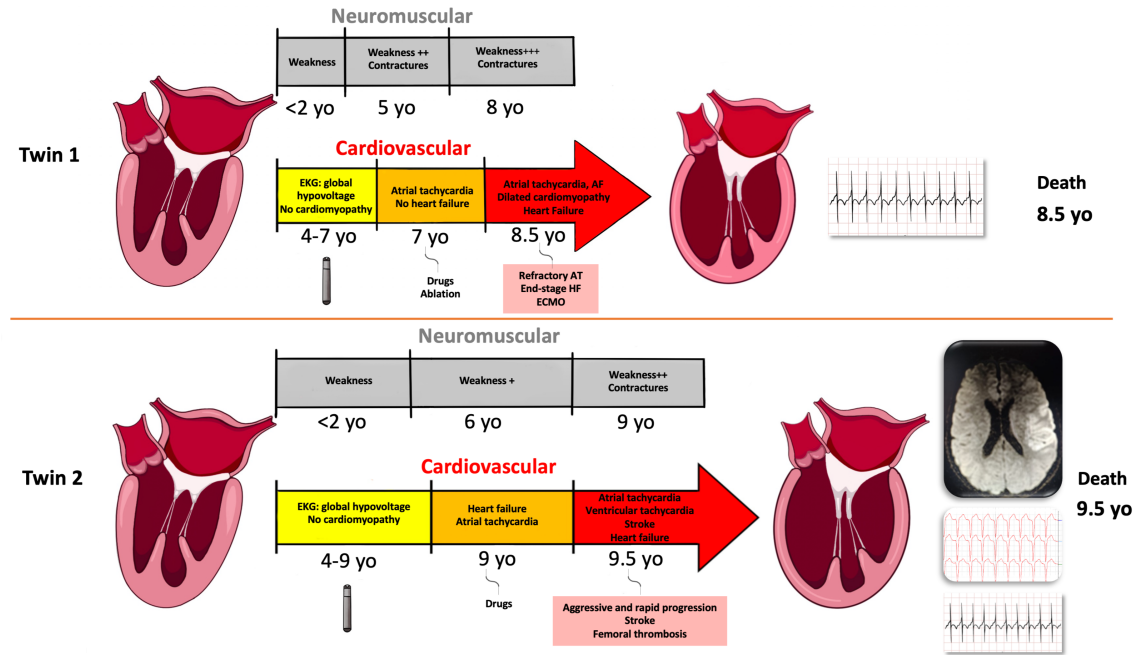


Figura 18 (creación propia). Representación clínica de las pacientes gemelas monocigóticas con mutación en LMNA y fenotipo de laminopatía neuromuscular tipo L-CMD. Ambas eran portadoras de holter subcutáneo que permitió identificar las arritmias para optimizar el tratamiento clínico. El fenotipo de la gemela 1 fue más precoz y severo a nivel de debilidad muscular y el debut cardíaco también fue más precoz presentando taquicardia auricular y, finalmente, una miocardiopatía dilatada con insuficiencia cardíaca rápidamente progresiva.

En el paciente 11, con fenotipo neuromuscular L-CMD y miocardiopatía dilatada como expresión cardíaca, se encontró una deleción en el gen NEB (relacionado con miopatías autosómico recesivas), aunque el hermano y la madre eran portadores y no tenían síntomas. Los otros pacientes con deleciones en el gen LMNA (4, 9 y 25) tuvieron una expresión fenotípica similar a lo reportado en la literatura, con afectación muscular lentamente progresiva, sin afectación cardíaca o expresión de miocardiopatía dilatada. (34)

En el paciente 18 con fenotipo de EDMD, se encontraron dos variantes raras más: GAA p.Asp645Asn heredada por parte materna, sin síntomas al respecto y en heterocigosis, y COL6A3 p.Lys2961AsnfsTer40 por parte paterna). En este caso, la mutación en LMNA, no reportada previamente, en p.Gln36His fue heredada por rama paterna que también tenía una debilidad muscular leve, al igual que el hijo, que tampoco tenía afectación cardíaca. En este caso no había más familiares clínicamente afectos, pero se desconoce el papel que pueda jugar la variante COL6A3 en la expresión de la debilidad muscular.

El paciente 22, con fenotipo EDMD presentó una afectación muscular lentamente progresiva y sin afectación cardíaca. En este caso el paciente tenía una mutación *de novo*, LMNA p.Glu31Lys, previamente publicada en un caso con fenotipo L-CMD con las mismas características fenotípicas.(34) En este paciente 22, se encontró además una variante en DYSF p.Arg1849Lys, asociado a distrofia muscular recesiva y heredado por parte materna. Ni la madre ni el hermano, también portador, tenían síntomas al respecto.

En los pacientes 13 y 16, a pesar de tener un fenotipo de EDMD, tener miocardiopatía dilatada y ser portadores de DAI, no se encontró ninguna variante más en el estudio genético ampliado. Estos dos pacientes tenían mutaciones *de novo* en el gen LMNA (p.Leu38Phe y p.Arg455Pro, respectivamente).

En resumen, en algunos de estos pacientes hay discrepancias genotipo-fenotipo teniendo en cuenta lo que existe ya publicado y surgen dudas sobre el papel de DYSF del paciente 18, el rol del gen CHNRD, AGRN y DMD (recientemente ampliado el estudio genético, ver Anexos) en las gemelas con L-CMD fallecidas de causa cardíaca (a los 8.5 y 9.5 años de edad), el papel de COL6A3 en el caso del paciente 18, si existe algún modificador genético más en el paciente 3 que también fue éxitus por causa cardíaca, si el paciente 5 tiene una expresión fenotípica concordante a la mutación en LMNA o existe algún papel del hallazgo de la variante en GAA encontrada y si, finalmente, la expresión fenotípica neuromuscular diferente en los paciente 20 y 21 corresponde o no a algún modificador genético no encontrado en este análisis genético. Así, estos hallazgos permiten abrir otras líneas de investigación para poder estudiar qué sucede fisiopatológicamente para dar explicación a las diferencias fenotípicas y si estos hallazgos genéticos pueden tener una participación en la modificación del fenotipo. Comprender esto es de vital importancia para este grupo de pacientes tan vulnerables y con una expresión fenotípica agresiva en algunos de estos casos.

8.2. Distrofia miotónica de Steinert

La Distrofia miotónica de Steinert, o DM1, es la enfermedad neuromuscular más frecuente en la etapa adulta, cuyo patrón hereditario es autosómico dominante y corresponde a una expansión de trinucleótidos de CTG en el cromosoma 19q13.1. Existe un fenómeno de anticipación en las generaciones sucesivas y esto lleva a expresar un fenotipo más precoz y severo.(57,60,61,72)

En el artículo 3, cuyos resultados no han sido publicados, se describe una revisión retrospectiva de la cohorte de DM1. Es relevante destacar que, en la edad pediátrica, existen diferentes grados de severidad que han motivado una clasificación basada en el momento de aparición de los síntomas: la forma congénita, la infantil y la juvenil (cDM1, iDM1 y jDM1, respectivamente). (119) Se representan gráficamente tanto las formas como la evolución habitual en la figura 19.

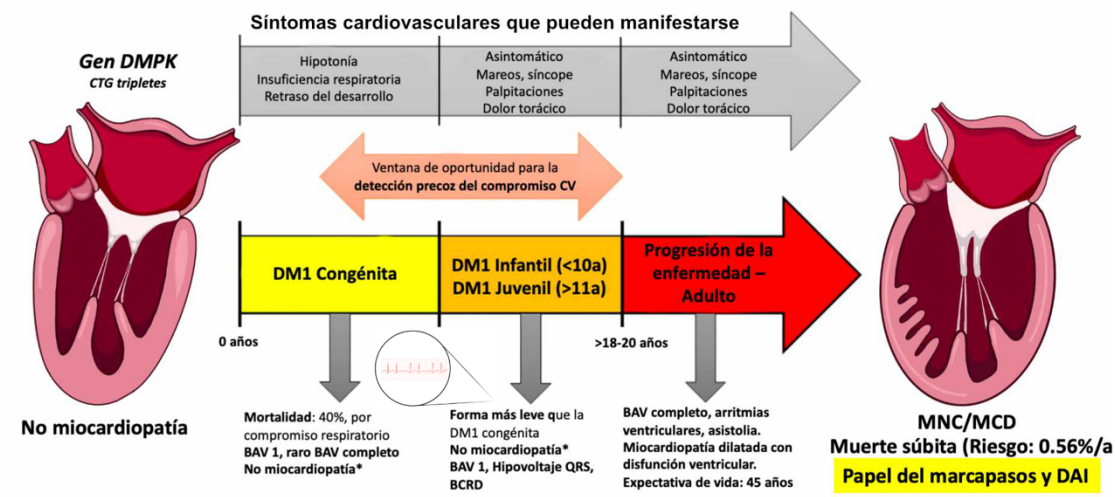


Figura 19 (creación propia). Historia natural según lo que hay descrito y los principales hallazgos que se pueden encontrar en función del fenotipo: cDM1, iDM1, jDM1 y del adulto. Se introduce el indicador de alteraciones electrocardiográficas como hallazgos principales de la cohorte en formas de cDM1, iDM1 y jDM1 después del estudio de la cohorte.
cDM1: Distrofia miotónica tipo 1 congénita; iDM1: Distrofia miotónica tipo 1 infantil; jDM1: Distrofia miotónica tipo 1 juvenil; MNC: miocardiopatía no compactada. MCD: miocardiopatía dilatada. DAI: desfibrilador automático implantable. BAV: bloqueo auriculo-ventricular. BCRD: bloqueo completo de rama derecha.
(*) No suele haber BAV completo ni miocardiopatía en las formas cDM1 y iDM1, aunque está descrito en publicaciones previas.

A pesar de haberse descrito predictores de muerte súbita en población adulta, sigue sin haber información en población pediátrica, a pesar de que ésta ha sido reportada previamente en la forma cDM1 y sin hallarse alteraciones clásicas de

riesgo en el electrocardiograma ni haberse podido registrar arritmias (manifestación sistémica más precoz y más severa). (64)

A pesar de que en la literatura están descritos hallazgos como ductus arterioso persistente, dilatación del arco aórtico, defectos del septo interventricular, prolapso de la válvula mitral y miocardiopatía hipertrófica(64), en la cohorte descrita de 40 pacientes en el artículo 3, no se han encontrado alteraciones cardiacas estructurales mayores. En el análisis ecocardiográfico no se halló ninguna alteración significativa funcional, y en el análisis estructural tan sólo 1 válvula aórtica bicúspide, hipertrabeculación leve apical del ventrículo izquierdo en 3 pacientes y en sólo 1 pacientes se hallaron criterios limítrofes para no compactación del ventrículo izquierdo, pero con función conservada. Estos hallazgos, a pesar de no ser significativos, pueden sentar la base para futuras investigaciones y seguimientos a largo plazo hasta la edad adulta, para identificar si la hipertrabeculación apical del ventrículo izquierdo pueda ser un predictor precoz para aquellos pacientes que puedan presentar miocardiopatía relacionada con la DM1 en la etapa adulta.

En cuanto a la descripción ECG y de arritmias, no hay estudios específicos sobre su incidencia en el grupo de cDM1, aunque sí están descritas arritmias supraventriculares y ventriculares durante la infancia. (57,64,120,121)

Cabe destacar que los hallazgos ECG, en la cohorte pediátrica descrita en el artículo 3, no son potencialmente malignos en la población general, aunque podrían establecer un predictor de muerte súbita o arritmias potencialmente letales en la etapa adulta. Esto se basa en que un 30% de la cohorte se pudo observar ya un bloqueo AV de primer grado (3 pacientes del grupo con cDM1 y 9 pacientes del grupo iDM1). Se desconoce el grado de progresión del bloqueo AV a otros grados más avanzados o si estos pacientes consiguen alcanzar un PR mayor a 240, que sería uno de los predictores, junto al QRS y QTc, para muerte súbita en adultos.(74,122,123) Tampoco hay predictores de bloqueo AV completo y muerte súbita en estudios electrofisiológicos en población pediátrica con DM1, aunque sí en adultos, determinando un intervalo HV >70ms o bien una progresión de la prolongación de este intervalo >1.2ms/año. (57,124,125) Esto también podría abrir un campo de investigación para prevenir la muerte súbita del adulto conociendo los intervalos HV en población pediátrica con DM1 y que ya tenga alteraciones leves-moderadas ECG en el PR y QRS (PR 200-240ms,

QRS 100-120ms) antes de la transición al equipo multidisciplinar del adulto, como se ha detectado en la cohorte descrita. La muerte súbita descrita en edad pediátrica corresponde a los grupos cDM1 (hasta 6.1% de muertes inexplicadas) y iDM1, aunque en nuestra cohorte no se ha detectado muerte súbita relacionada con ningún grupo. (64)

Para poder correlacionar la genética con los hallazgos cardiovasculares, independientemente de cuáles sean, se necesitaría reanalizar y precisar más en los tripletes de CTG, ya que estos son responsables de causar disregulación en el *splicing* en un subgrupo de *mRNA.* Justamente este es el mecanismo que, sobre el gen SCN5A, puede contribuir a las arritmias descritas en esta población, como ya ha sido publicado previamente.(57) Lo que sí se ha relacionado previamente en población adulta que a mayor tamaño de amplificación de CTG, mayor prevalencia de manifestaciones cardiacas.(57,58,126) Por tanto, este análisis del número de repeticiones de CTG junto con otros indicadores clínicos como los hallazgos ECG podrían sentar las bases para determinar qué pacientes son están más a riesgo de presentar arritmias potencialmente letales y/o muerte súbita.

8.3. Distrofia muscular de Duchenne

En el artículo 4, resultados no publicados, se describe el seguimiento cardiovascular de una cohorte de 4 pacientes con diagnóstico genéticamente confirmado de distrofinopatía, la mayoría con DMD. Las complicaciones cardiovasculares siguen siendo, hoy en día, la causa principal de morbilidad y mortalidad en pacientes con DMD.(78,80) En la figura 20 se representa la historia natural de las complicaciones cardíacas en DMD.

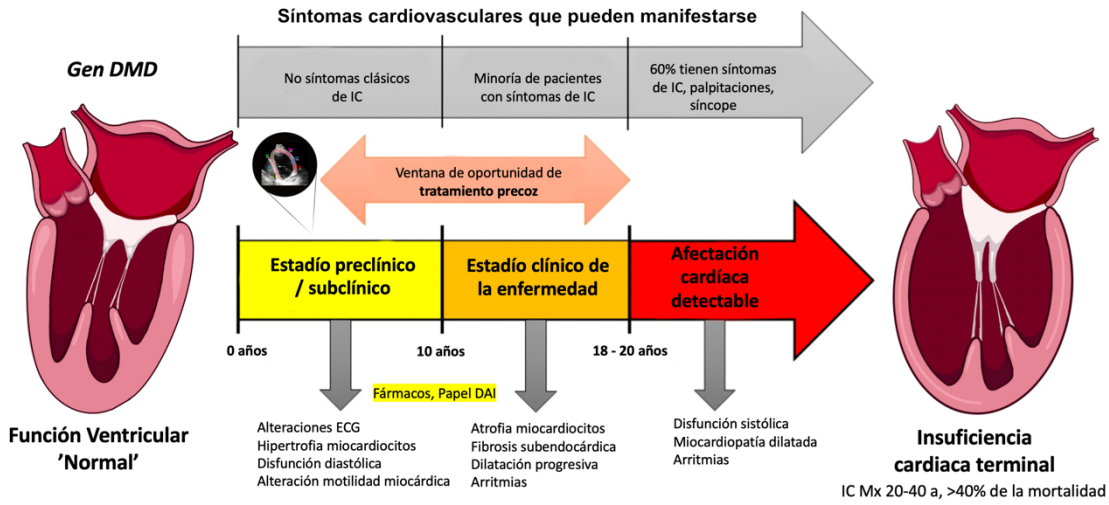


Figura 20 (creación propia). Etapas de las complicaciones cardíacas en DMD. En la etapa preclínica o subclínica antes de los 10 años existen ya alteraciones en el ECG y también las alteraciones de la deformación cardíaca detectada en la cohorte (*Strain* miocárdico), introduciendo este elemento en la etapa preclínica/subclínica. Las arritmias e insuficiencia cardíacas empiezan a aparecer en el estadío clínico de la enfermedad, durante la adolescencia, siendo habitualmente la etapa del adulto joven la que va relacionada a la mortalidad cardiovascular.
IC: insuficiencia cardiaca; Mx: mortalidad; DAI: desfibrilador automático implantable; ECG: electrocardiograma.

Entre las complicaciones cardiovasculares que ensombrecen el pronóstico se hallan la miocardiopatía dilatada (MCD), arritmias e insuficiencia cardíaca. (57,76,78,80,127)

La MCD de la DMD tiene como base celular la pérdida de miocitos, que se traducen a nivel ventricular en un adelgazamiento de la pared y dilatación del ventrículo izquierdo. Característicamente, la apoptosis celular se traduce en fibrosis miocárdica que va desde el epicardio hasta el endocardio y de localización preferente a nivel posterior, algo que es muy frecuente en la MCD y que también se ha encontrado en la cohorte de seguimiento. (80,127)

La MCD suele ser asintomática en este grupo de pacientes, tal y como también se ha encontrado en nuestra cohorte de pacientes pediátricos, por lo que dificulta el seguimiento y la detección de complicaciones. La base de este razonamiento suele ser que la pérdida de deambulación por la debilidad muscular hace que el consumo de oxígeno baje de forma muy importante. (78,128) Es por este motivo que el seguimiento cardiovascular debe empezarse en etapas precoces de la DMD. (78)

En la cohorte descrita en el artículo 4, el análisis ecocardiográfico básico y avanzado, con *strain* miocárdico, ha sido clave para determinar alteración de la deformación cardíaca incluso antes de que los valores de FEVI se deprimieran tal y como se ha visto en algunos de los pacientes de la cohorte estudiada. Este comportamiento del *strain* miocárdico ha sido ya sugerido en la literatura. (129)

El comportamiento de la función cardíaca global a lo largo de todo el seguimiento en edad pediátrica fue hacia un deterioro lento y progresivo en la mayoría de los casos, con significancia estadística. Esto justifica el uso de herramientas de análisis ecocardiográfico básico y avanzado, con *strain* miocárdico, para detectar aquellos pacientes que puedan estar más a riesgo de presentar un deterioro en la adolescencia y poder poner medidas de soporte clínico y/o tratamiento médico. Esto supone un hándicap ya que, a medida que avanzan y se modernizan las herramientas de detección, los protocolos de actuación no lo hacen con la misma velocidad, especialmente en edad pediátrica, lo que supone un reto para el clínico. La decisión de iniciar un tratamiento farmacológico en una etapa pre-sintomática de la DMD, incluso a pesar de tener indicadores precoces de afectación cardíaca en una enfermedad que no tiene un tratamiento curativo y que se conoce que la evolución será hacia la insuficiencia cardiaca, es, por el momento, motivo de debate y controversia. Ya hay artículos donde se demuestra una mejoría del *strain* miocárdico circunferencial tras la combinación de IECA o ARA. (130)

Hay que destacar también que, precisamente por la obesidad en muchos casos, la ventana ecográfica del paciente con DMD es deficiente por lo que el análisis ecocardiográfico se hace más dificultoso, limitando en muchas ocasiones el análisis avanzado con *strain* miocárdico, algo que se ha podido comprobar en la cohorte estudiada y que se ha limitado al análisis del GLS apical 4 cámaras: en este caso se compararon los valores de GLS apical 4 cámaras al inicio y final de seguimiento, detectando que algunos pacientes ya tenían valores de GLS apical 4 cámaras deprimidos a pesar de que el valor de FEVI estaba preservado (paciente 3, 14, 20, 22, 20 y 35), pero sin arritmias. A pesar de que sólo se ha podido analizar este valor del GLS apical 4 cámaras, hay otros reportes en la literatura que han podido demostrar también que el *strain* miocárdico se altera también en el estudio longitudinal en 2 y 3 cámaras, radial y circunferencial y que, además, es un valor pronóstico de mortalidad tal y como recientemente se

ha publicado. (129,131) Para solucionar esto, se podría analizar el *strain* miocárdico con resonancia magnética cardíaca (RMc), aunque esto implicaría la utilización de software específico para disminuir el artefacto de la respiración en el caso que se pueda realizar sin anestesia general. (129) Las ventajas de la RMc es la utilización de contraste para el estudio del realce tardío de Gadolinio, aunque otras técnicas como el T1 *mapping* también se están utilizando. La presencia de fibrosis suele empezar en localización subepicárdica en la pared lateral del ventrículo izquierdo, siendo la septal y transmural una manifestación más avanzada del daño miocárdico y, esta última, relacionada directamente con eventos cardíacos adversos. (132)En la cohorte estudiada con DMD, de los pacientes en los que se ha podido analizar la RMc, se pudo detectar fibrosis en 11 pacientes (50% de los pacientes con RM cardiaca): la localización de la fibrosis fue en segmentos inferiores, inferolaterales y anterolaterales (basales y medio ventriculares), concordante con la literatura. (133) A pesar de detectar esta fibrosis no se encontró relación directa con la presencia de arritmias potencialmente malignas a pesar de que la edad media de los pacientes con RMc era de 14.8 años (DE±2.12). La cuantificación de esta fibrosis podría ayudar a establecer la velocidad de progresión del daño miocárdico en edad pediátrica y así poder proponer más indicadores precoces de progresión rápida de la miocardiopatía. Identificar esto es vital y necesario, aunque nuevamente faltarían protocolos de actuación y las decisiones clínicas se relegan a criterio del cardiólogo pediátrico. A pesar de que la RMc puede ser de ayuda, existen discrepancias sobre la correlación con las arritmias, como ha pasado con el paciente número 24 de la cohorte (fibrilación auricular sin datos de miocardiopatía ni fibrosis). En contraposición, el paciente número 9 con arritmias auriculares y ventriculares, y el paciente número 13, con taquicardia auricular, ambos con fibrosis miocárdica en el estudio con RMc. Los pacientes que más se relacionaron con fibrosis en la RMc fueron las deleciones tipo *frame-shift,* seguidas de las *nonsense*, deleción de exones y deleciones *inframe*, en este orden.

En cuanto al tratamiento, es el de la insuficiencia cardíaca por miocardiopatía dilatada, que se inicia cuando hay disfunción ventricular significativa: suelen ser IECA con o sin betabloqueantes y diuréticos (estos últimos, especialmente si hay

síntomas, para reducir la mortalidad y el ingreso por insuficiencia cardiaca). (80,134,135)

A pesar de que las troponinas pueden estar asociadas a la extensión del daño miocárdico, hay resultados controvertidos en cuanto al diagnóstico y pronóstico al respecto. (131,136) Además, los valores de NT-proBNP pueden ser normales a pesar de tener una insuficiencia cardíaca establecida, ya que la movilidad y el ejercicio de los pacientes con DMD se ven muy reducidos, y tampoco serviría para discriminar a los pacientes con miocardiopatía dilatada tal y como ha sido publicado previamente. (137) A pesar de esto, en una publicación reciente se ha asociado los niveles de NT-proBNP con la mortalidad, aunque la frecuencia para obtener niveles de este biomarcador no queda claro. (131) En nuestra cohorte el seguimiento de biomarcadores no era un objetivo principal, además de haber sido un parámetro de obtención errática, por lo que no se ha analizado. No obstante, los biomarcadores pueden ayudar en el paciente inestable, sintomático, como marcador de respuesta terapéutica o para comparar los cambios de los valores en el tiempo de seguimiento. (131) Existe ya un campo en la transcriptómica y proteómica que puede ser también un campo de investigación en el grupo de las enfermedades neuromusculares en la edad pediátrica, y que pueden ayudar a detectar indicadores de riesgo cardiovascular.(138,139)

En cuanto a la genética y qué mutaciones son más de riesgo cardíaco en edad pediátrica, no hay consenso al respecto. De forma general, las variantes patogénicas en las que están dentro del marco de lectura (deleciones/duplicaciones *in-frame*) se correlacionarían con un fenotipo neuromuscular más leve y las que hay una alteración por fuera del marco de lectura (deleciones/duplicaciones *out-of-frame*) se relacionan con un fenotipo neuromuscular más severo, aunque no en todos los casos se cumple esto. A pesar de esto, sigue siendo un desafío predecir la severidad individual de cada caso.(140) En la figura 21 se representan las mutaciones de la cohorte estudiada, coincidiendo los *hotspot* de deleciones en la región de los exones 3-20 y en los exones 45-55. (141)

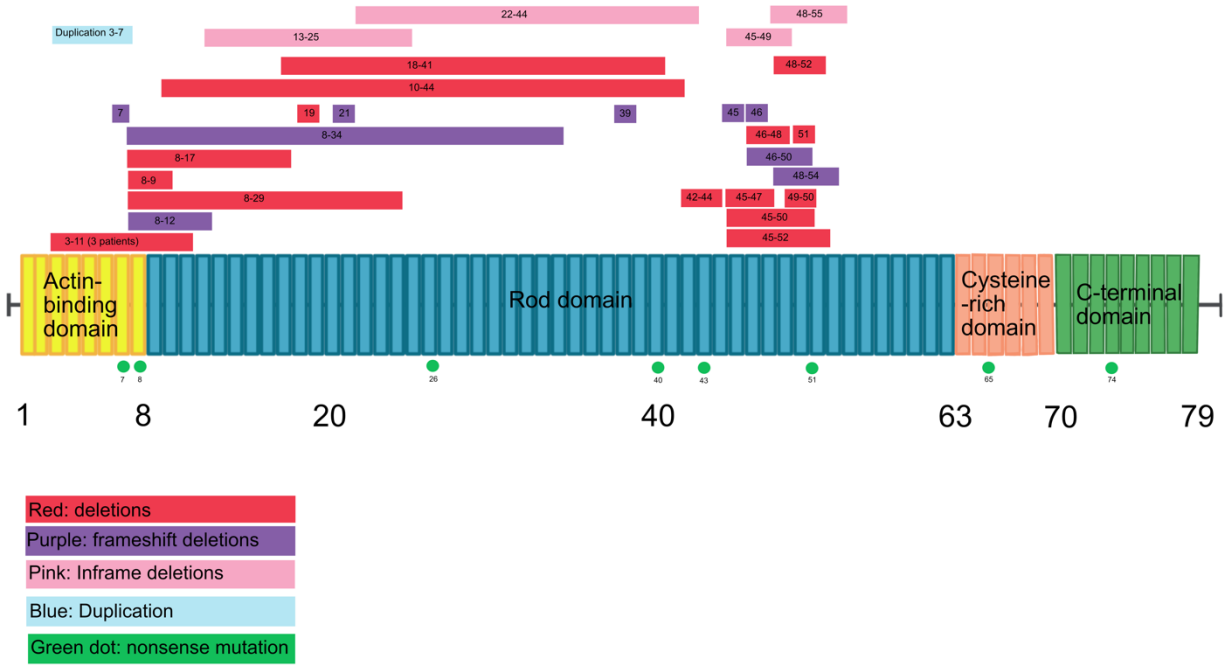


Figura 21 (creación propia). Representación gráfica del gen DMD con la distribución de los 79 exones. En amarillo la *Actin-binding domain,* en azul la *Rod-domain,* en naranja la *Cysteine-rich domain* y en verde la *C-terminal domain*. Los tipos de mutaciones de la cohorte de DMD estudiada están representadas en los colores de la leyenda. Se destacan los *hotspot* de las deleciones en los exones 3-20 y en la región de la *rod domain* de los exones 45-55. DMD: distrofia muscular de Duchenne.

La miocardiopatía de inicio precoz parece haberse relacionado con deleciones del dominio proximal N-terminal (exones del 2 al 9), aunque existe variabilidad fenotípica, probablemente por la existencia de modificadores genéticos del fenotipo, que hace que no se pueda concluir una relación genotipo-fenotipo. (87,89,90,132,141,142) En este sentido, en la cohorte estudiada, dos de los pacientes con valores bajos de GLS apical 4 cámaras y FEVI preservada al inicio del estudio tenían una mutación tipo *frame-shift* en los exones 3-11, otro con una mutación *nonsense* en el exón 8 y el resto de pacientes tenían una localización en los exones 49, 50 y 51. Por tanto, en cuanto a la distribución exónica de los pacientes con *strain* miocárdico precozmente alterado, ha sido heterogénea y se han encontrado en ambos *hotspot* de prevalencia de las mutaciones del gen DMD (exones en región proximal N-terminal y exones 49, 50 y 51).

En nuestra cohorte no hubo muerte súbita ni tampoco se detectaron arritmias potencialmente malignas, por lo que no he ha podido correlacionar este aspecto con los resultados obtenidos del estudio ecocardiográfico y del seguimiento del ritmo cardíaco durante el período de seguimiento. Esto sugiere que se deberían tomar otras estrategias diferentes a las habituales, como es el Holter cardiaco 24h, ya que la mayoría de los pacientes se encuentran asintomáticos y el rendimiento de esta prueba en DMD parece insuficiente. Una estrategia sería la monitorización con Holter cardiaco implantable para el seguimiento estrecho de aquellos pacientes sintomáticos o con algún indicador de peor pronóstico en la edad pediátrica, como pueden ser aquellos con GLS apical 4 cámaras alterado a edades precoces, tener una FEVI <35% u otros datos como una prolongación del intervalo *T peak-T end* del electrocardiograma tal y como se ha sugerido algunas publicaciones. (143–145)

8.4. Limitaciones

El seguimiento de las cohortes de pacientes con enfermedades neuromusculares tiene algunas limitaciones. La Unidad de Arritmias y la Unidad de Neuromuscular son referentes nacionales en el hospital Sant Joan de Déu, pero también se reciben pacientes internacionales, ya que se trata de enfermedades raras. Esto hace que sean derivados los pacientes con fenotipos más severos, con el consecuente sesgo, además de perder datos de seguimiento de pruebas de imagen, especialmente en los pacientes internacionales o aquellos que viven lejos del hospital.

En cuanto a la ecocardiografía, la adquisición de imágenes ecocardiográficas de calidad en pacientes neuromusculares, como es el caso, es muy difícil. Esto hace que en el análisis se reduzcan el número de estructuras o de datos que se pueden obtener, incluyendo el *strain* miocárdico. Esto se solucionaría, en parte, con la realización de RMc, aunque para evitar una anestesia general se precisa que el paciente colabore con las apneas y la inmovilidad durante la prueba y, a menudo, no es posible y se deben utilizar herramientas avanzadas que ayuden al análisis en respiración espontanea. Esto es un reto para unidades de tratamiento integral del paciente neuromuscular con riesgo cardiovascular, especialmente en población pediátrica.

Los biomarcadores no han sido obtenidos de forma homogénea en la cohorte de pacientes y, a pesar de no ser un objetivo del estudio de la cohorte, no se puede establecer una correlación de los valores de troponinas ni de NT-proBNP con el grado de severidad del fenotipo cardíaco. En este sentido, probablemente la proteómica y la transcriptómica puedan ofrecer una respuesta a muchas de las dudas que hay al respecto del riesgo cardiovascular de estos pacientes.

También hay limitaciones en cuanto al papel patogénico que puedan tener otras variantes genéticas que no hayan podido explorarse en el panel NGS personalizado en el caso de las laminopatías neuromusculares y, también, en el resto de enfermedades neuromusculares que muestren una expresividad y severidad fenotípica variables incluso teniendo la misma mutación. Esto debe servir como punto de referencia para futuras investigaciones en las enfermedades neuromusculares, como podría ser la investigación funcional de las variantes raras obtenidas en la cohorte de laminopatías o, también, secuenciación de un exoma o del genoma completo. Además, cada vez más, deben reevaluarse a lo largo del tiempo las variantes ambiguas o de significado incierto, ya que pueden ser objeto de reclasificación y pueden dar respuesta a incógnitas de la correlación genotipo-fenotipo de algunos pacientes.

9. Conclusiones

9.1. En las enfermedades neuromusculares, a pesar de existir insuficiencia cardíaca y arritmias, es frecuente que no se presente ningún síntoma cardiovascular o que los síntomas sean atípicos, lo que dificulta el manejo y seguimiento de los pacientes.

9.2. En las enfermedades neuromusculares, la expresión variable del fenotipo y la diferencia en la severidad de las manifestaciones cardíacas, incluso con una misma mutación patogénica, sugieren que existen modificadores genéticos que podrían representar potenciales indicadores de letalidad y posibles dianas terapéuticas.

9.3. El subgrupo de pacientes pediátricos con enfermedad neuromuscular que presentaron más accidentes cerebrovasculares fueron las laminopatías neuromusculares que mostraron sintomatología neuromuscular precoz antes de los 2 años de edad.

9.4. En las laminopatías neuromusculares es frecuente encontrar precozmente, antes de los 10 años de edad, insuficiencia cardíaca por miocardiopatía dilatada y arritmias potencialmente malignas relacionadas con la muerte súbita.

9.5. La monitorización electrocardiográfica con dispositivos implantables (holter subcutáneo) es útil y ayuda a identificar arritmias potencialmente letales y, por tanto, a tomar decisiones clínicas para prevenir la muerte súbita en la población pediátrica con laminopatías neuromusculares, especialmente las que debutan con clínica neuromuscular de forma precoz antes de los 2 años de edad.

9.6. El estudio electrofisiológico realizado de forma sistemática en pacientes con laminopatía no mostró indicadores predictores de arritmias potencialmente malignas.

9.7. En población pediátrica con laminopatías neuromusculares y distrofia muscular de Duchenne, el análisis ecocardiográfico básico y avanzado con *strain* miocárdico es útil para identificar alteraciones de la deformación cardíaca de forma más precoz que valores clásicos como la fracción de eyección del ventrículo izquierdo, relacionándose con un peor pronóstico

cardíaco tener un *strain* miocárdico del ventrículo izquierdo precozmente alterado en el caso de las laminopatías neuromusculares.

9.8. En la distrofia miotónica tipo 1 (enfermedad de Steinert), las arritmias potencialmente malignas y la muerte súbita son muy poco frecuentes durante la edad pediátrica, aunque hay hallazgos electrocardiográficos precoces que pueden ser motivo de investigación en la etapa adulta para poder comprobar si son indicadores precoces de un peor pronóstico cardiovascular.

9.9. En la distrofia muscular de Duchenne, las arritmias potencialmente malignas y la muerte súbita son muy poco frecuentes durante la edad pediátrica, aunque la miocardiopatía dilatada y la insuficiencia cardiaca son progresivas en la adolescencia y la función cardíaca se deprime de forma significativa.

10. Bibliografía

1. Mah JK, Korngut L, Fiest KM, Dykeman J, Day LJ, Pringsheim T, et al. A Systematic Review and Meta-analysis on the Epidemiology of the Muscular Dystrophies. Can J Neurol Sci. 2016;43(01):163–77.
2. Finsterer J, Stöllberger C. Heart Disease in Disorders of Muscle, Neuromuscular Transmission, and the Nerves. Korean Circ J. 2016;117–34.
3. Bonne G, Quijano-Roy S. Emery-Dreifuss muscular dystrophy, laminopathies, and other nuclear envelopathies. Handb Clin Neurol. 2013;113:1367–76.
4. Groh WJ. Arrhythmias in the muscular dystrophies. Heart Rhythm. 2012;9(11):1890–5.
5. Rajdev A, Groh WJ. Arrhythmias in the muscular dystrophies. Card Electrophysiol Clin. 2015;7(2):303–8.
6. Finsterer J, Stöllberger C, Maeztu C. Sudden cardiac death in neuromuscular disorders. Int J Cardiol. 2015;203:508–15.
7. Priori S, Blomstrom-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, Camm J, et al. 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: The Task Force for the Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death of the European Society of Cardiology (ESC). Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). Eur Heart J. 2015;36(41):2793–867.
8. Palladino A, D'Ambrosio P, Papa AA, Petillo R, Orsini C, Scutifero M, et al. Management of cardiac involvement in muscular dystrophies: paediatric versus adult forms. Acta Myol. 2016;35(3):128–34.
9. Ryan TD, Taylor MD, Mazur W, Cripe LH, Pratt J, King EC, et al. Abnormal circumferential strain is present in young Duchenne muscular dystrophy patients. Pediatr Cardiol. 2013;34(5):1159–65.

10. Mertens L, Ganame J, Claus P, Goemans N, Thijs D, Eyskens B, et al. Early Regional Myocardial Dysfunction in Young Patients with Duchenne Muscular Dystrophy. J Am Soc Echocardiogr. 2008;21(9):1049–54.
11. Judge DP, Kass D a, Thompson WR, Wagner KR. Pathophysiology and therapy of cardiac dysfunction in Duchenne muscular dystrophy. Am J Cardiovasc Drugs. 2011;11(5):287–94.
12. Mavrogeni S, Markousis-Mavrogenis G, Papavasiliou A, Kolovou G. Cardiac involvement in Duchenne and Becker muscular dystrophy. World J Cardiol. 2015;7(7):410-4.
13. Abe Y. Myocardial fibrosis of the left ventricular posterior wall can be a target for early detection of cardiac involvement in patients with Duchenne muscular dystrophy. J Cardiol. 2015;66(3):203–4.
14. Quijano-roy S, Mbieleu B, Bönnemann CG, Jeannet PY, Colomer J, Clarke NF, et al. De novo LMNA Mutations cause a new form of congenital muscular dystrophy. Ann Neurol. 2008;64(2):177–86.
15. Dubowitz V. 68th ENMC International Workshop (5th international workshop) on Congenital Muscular Dystrophy, 9-11 April 1999, Naarden, The Netherlands. Neuromuscul Disord. 1999;9(6-7):446–54.
16. Bonne G, Yaou RB, Béroud C, Boriani G, Brown S, de Visser M, et al. 108th ENMC International Workshop, 3rd Workshop of the MYO-CLUSTER project: EUROMEN, 7th International Emery-Dreifuss Muscular Dystrophy (EDMD) Workshop, 13–15 September 2002, Naarden, The Netherlands. Neuromuscul Disord. 2003;13(6):508–15.
17. Aebi U, Cohn J, Buhle L, Gerace L. The nuclear lamina is a meshwork of intermediate-type filaments. Nature. 1986;323(6088):560–4.
18. Worman HJ, Bonne G. “Laminopathies”: a wide spectrum of human diseases. Exp Cell Res. 2007;313(10):2121–33.
19. Lammerding J, Schulze PC, Takahashi T, Kozlov S, Sullivan T, Kamm RD, et al. Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. J Clin Invest. 2004;113(3):370–8.
20. Dechat T, Shimi T, Adam SA, Rusinol AE, Andres DA, Spielmann HP, et al. Alterations in mitosis and cell cycle progression caused by a mutant lamin A

known to accelerate human aging. Proc Natl Acad Sci U S A. 2007;104(12):4955–60.

21. Andrés V, González JM. Role of A-type lamins in signaling, transcription, and chromatin organization. J Cell Biol. 2009;187(7):945–57.
22. Dauer WT, Worman HJ. The Nuclear Envelope as a Signaling Node in Development and Disease. Dev Cell. 2009;17(5):626–38.
23. Gonzalez-Suarez I, Redwood AB, Perkins SM, Vermolen B, Lichtensztejin D, Grotsky DA, et al. Novel roles for A-type lamins in telomere biology and the DNA damage response pathway. EMBO J. 2009;28(16):2414–27.
24. Hutchison CJ. The role of DNA damage in laminopathy progeroid syndromes. Biochem Soc Trans. 2011;39(6):1715–8.
25. Ben Yaou R, Yun P, Dabaj I, Norato G, Donkervoort S, Xiong H, et al. International retrospective natural history study of LMNA-related congenital muscular dystrophy. Brain Commun. 2021;3(3):fcab075.
26. Benedetti S, Menditto I, Degano M, Rodolico C, Merlini L, D'Amico A, et al. Phenotypic clustering of lamin A/C mutations in neuromuscular patients. Neurology. 2007;69(12):1285–92.
27. Bertrand AT, Chikhaoui K, Yaou RB, Bonne G. Clinical and genetic heterogeneity in laminopathies. Biochem Soc Trans. 2011;39(6):1687–92.
28. Wahbi K, Ben Yaou R, Gandjbakhch E, Anselme F, Gossios T, Lakdawala NK, et al. Development and Validation of a New Risk Prediction Score for Life- Threatening Ventricular Tachyarrhythmias in Laminopathies. Circulation. 2019;140(4):293–302.
29. Crasto S, My I, Di Pasquale E. The Broad Spectrum of LMNA Cardiac Diseases: From Molecular Mechanisms to Clinical Phenotype. Front Physiol. 2020;11:761.
30. Chatzifrangkeskou M, Le Dour C, Muchir A. Modulation of cytoskeleton in cardiomyopathy caused by mutations in LMNA gene. Am J Physiol Cell Physiol. 2023;324(6):C1223-C1235.
31. Granger B, Gueneau L, Drouin-Garraud V, Pedergnana V, Gagnon F, Ben Yaou R, et al. Modifier locus of the skeletal muscle involvement in Emery-Dreifuss muscular dystrophy. Hum Genet. 2011;129(2):149–59.

32. Carboni N, Politano L, Floris M, Mateddu A, Solla E, Olla S, et al. Overlapping syndromes in laminopathies: a meta-analysis of the reported literature. 2013;31(1):7–17.
33. Maggi L, D'Amico A, Pini A, Sivo S, Pane M, Ricci G, et al. LMNA-associated myopathies: the Italian experience in a large cohort of patients. Neurology. 2014;83:1634–44.
34. Fan Y, Tan D, Song D, Zhang X, Chang X, Wang Z, et al. Clinical spectrum and genetic variations of LMNA -related muscular dystrophies in a large cohort of Chinese patients. J Med Genet. 2021;58(5):326-333.
35. Muchir A, Bonne G, van der Kooi AJ, van Meegen M, Baas F, Bolhuis PA, et al. Identification of mutations in the gene encoding lamins A/C in autosomal dominant limb girdle muscular dystrophy with atrioventricular conduction disturbances (LGMD1B). Hum Mol Genet. 2000;9(9):1453–9.
36. Lu JT, Muchir A, Nagy PL, Worman HJ. LMNA cardiomyopathy: cell biology and genetics meet clinical medicine. Dis Model Mech. 2011;4(5):562–8.
37. Groh WJ, Bhakta D, Tomaselli GF, Aleong RG, Teixeira RA, Amato A, et al. 2022 HRS expert consensus statement on evaluation and management of arrhythmic risk in neuromuscular disorders. Heart Rhythm. 2022;19(10):e61-e120.
38. Heller F, Dabaj I, Mah JK, Bergounioux J, Essid A, Bönnemann CG, et al. Cardiac manifestations of congenital LMNA-related muscular dystrophy in children: three case reports and recommendations for care. Cardiol Young. 2017;27(6):1076–82.
39. Tan D, Yang H, Yuan Y, Bonnemann C, Chang X, Wang S, et al. Phenotype-genotype analysis of Chinese patients with early-onset LMNA-related muscular dystrophy. PLoS One. 2015;10(6):e0129699.
40. Finsterer J, Stöllberger C, Blazek G. Neuromuscular implications in left ventricular hypertrabeculation/noncompaction. 2006;110(3):288–300.
41. Finsterer J, Ramaciotti C, Wang CH, Wahbi K, Rosenthal D, Duboc D, et al. Cardiac findings in congenital muscular dystrophies. Pediatrics. 2010;126(3):538–45.
42. Quarta G, Syrris P, Ashworth M, Jenkins S, Zuborne Alapi K, Morgan J, et al. Mutations in the Lamin A/C gene mimic arrhythmogenic right ventricular cardiomyopathy. Eur Heart J. 2012;33(9):1128–36.

43. Cattin ME, Muchir A, Bonne G. “State-of-the-heart” of cardiac laminopathies. Curr Opin Cardiol. 2013;28(3):297–304.
44. Hasselberg NE, Edvardsen T, Petri H, Berge KE, Leren TP, Bundgaard H, et al. Risk prediction of ventricular arrhythmias and myocardial function in Lamin A/C mutation positive subjects. Europace. 2014;16(4):563–71.
45. Alastalo TP, West G, Li SP, Keinänen A, Helenius M, Tyni T, et al. LMNA mutation c.917T>G (p.L306R) leads to deleterious hyper-assembly of Lamin A/C and associates with severe right ventricular cardiomyopathy and premature aging. Hum Mutat. 2015;36(7):694–703.
46. Dobrzynska A, Gonzalo S, Shanahan C, Askjaer P. The nuclear lamina in health and disease. Nucleus. 2016;7(3):233–48.
47. Muscogiuri G, Ciliberti P, Mastrodicasa D, Chinali M, Rinelli G, Santangelo TP, et al. Results of Late Gadolinium Enhancement in Children Affected by Dilated Cardiomyopathy. Front Pediatr. 2017;5:13.
48. Wang X, Zabell A, Koh W, Tang VHW. Lamin A/C Cardiomyopathies: Current Understanding and Novel Treatment Strategies. Curr Treat Options Cardiovasc Med. 2017;19(3):21.
49. Marchel M, Madej-Pilarczyk A, Tymińska A, Steckiewicz R, Ostrowska E, Wysińska J, et al. Cardiac Arrhythmias in Muscular Dystrophies Associated with Emerinopathy and Laminopathy: A Cohort Study. J Clin Med. 2021;10(4):732.
50. Steckiewicz R, Stolarz P, Świętoń E, Madej-Pilarczyk A, Grabowski M, Marchel M, et al. Cardiac pacing in 21 patients with Emery-Dreifuss muscular dystrophy: A single-centre study with a 39-year follow-up. Kardiol Pol. 2016;74(6):576–83.
51. Haugaa KH, Hasselberg NE, Edvardsen T. Mechanical dispersion by strain echocardiography: a predictor of ventricular arrhythmias in subjects with lamin A/C mutations. JACC Cardiovasc Imaging. 2015;8(1):104–6.
52. den Boer SL, du Marchie Sarvaas GJ, Klitsie LM, van Iperen GG, Tanke RB, Helbing WA, et al. Distribution of strain patterns in children with dilated cardiomyopathy. Echocardiography. 2017;34(6):881-887.
53. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Drazner, MH, et al. 2013 ACCF/AHA Guideline for the management of heart failure: a report of the

American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2013;62(16):e147-239.

54. Yancy ACW, Jessup M, Bozkurt B, Butler J, Jr DEC, Colvin MM, et al. 2016 ACC/AHA/HFSA Focused Update on New Pharmacological Therapy for Heart Failure: An Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure. J Card Fail. 2016;22(9):659–69.

55. Bozkurt B, Colvin M, Cook J, Cooper LT, Deswal A, Fonarow GC, et al. Current Diagnostic and Treatment Strategies for Specific Dilated Cardiomyopathies: A Scientific Statement from the American Heart Association. Circulation. 2016;134(23):e579-e646.

56. Atalaia A, Ben Yaou R, Wahbi K, De Sandre-Giovannoli A, Vigouroux C, Bonne G. Laminopathies' Treatments Systematic Review: A Contribution Towards a 'Treatabolome'. J Neuromuscul Dis. 2021;8(3):419–39.

57. Groh WJ, Bhakta D, Tomaselli GF, Aleong RG, Teixeira RA, Amato A, et al. 2022 HRS expert consensus statement on evaluation and management of arrhythmic risk in neuromuscular disorders. Heart Rhythm. 2022 Oct 1;19(10):e61–120.

58. Chong-Nguyen C, Wahbi K, Algalarrondo V, Bécane HM, Radvanyi-Hoffman H, Arnaud P, et al. Association between Mutation Size and Cardiac Involvement in Myotonic Dystrophy Type 1: An Analysis of the DM1-Heart Registry. Circ Cardiovasc Genet. 2017;10(3):e001526.

59. Freyermuth F, Rau F, Kokunai Y, Linke T, Sellier C, Nakamori M, et al. Splicing misregulation of SCN5A contributes to cardiac-conduction delay and heart arrhythmia in myotonic dystrophy. Nat Commun. 2016;7:11067.

60. Sabovic M, Medica I, Logar N, Mandić E, Zidar J, Peterlin B. Relation of CTG expansion and clinical variables to electrocardiogram conduction abnormalities and sudden death in patients with myotonic dystrophy. Neuromuscul Disord. 2003;13(10):822–6.

61. Lau JK, Sy RW, Corbett A, Kritharides L. Myotonic dystrophy and the heart: A systematic review of evaluation and management. Int J Cardiol. 2015;184:600-608.

62. Thornton CA. Myotonic dystrophy. Neurol Clin. 2014;32(3):705-19, viii.

63. Pearson CE, Edamura KN, Cleary JD. Repeat instability: mechanisms of dynamic mutations. Nat Rev Genet. 2005;6(10):729–42.
64. Brunet Garcia L, Hajra A, Field E, Wacher J, Walsh H, Norrish G, et al. Cardiac Manifestations of Myotonic Dystrophy in a Pediatric Cohort. Front Pediatr. 2022;10:910660.
65. Harper PS. Congenital myotonic dystrophy in Britain I. Clinical aspects. Arch Dis Child. 1975;50(7):505-13.
66. Martorell L, Cobo AM, Baiget M, Naudó M, Poza JJ, Parra J. Prenatal diagnosis in myotonic dystrophy type 1. Thirteen years of experience: implications for reproductive counselling in DM1 families. Prenat Diagn. 2007 Jan;27(1):68–72.
67. Breton R, Mathieu J. Usefulness of clinical and electrocardiographic data for predicting adverse cardiac events in patients with myotonic dystrophy. Can J Cardiol. 2009;25(2):e23-7.
68. Russo V, Capolongo A, Bottino R, Carbone A, Palladino A, Liccardo B, et al. Echocardiographic Features of Cardiac Involvement in Myotonic Dystrophy 1: Prevalence and Prognostic Value. J Clin Med. 2023;12(5):1947.
69. Leali M, Aimo A, Ricci G, Torri F, Todiere G, Vergaro G, et al. Cardiac magnetic resonance findings and prognosis in type 1 myotonic dystrophy. J Cardiovasc Med (Hagerstown). 2023;24(6):340–7.
70. Wahbi K, Ederhy S, Bécane HM, Meune C, Béhin A, Stojkovic T, et al. Impaired myocardial deformation detected by speckle-tracking echocardiography in patients with myotonic dystrophy type 1. Int J Cardiol. 2011;152(3):375–6.
71. Izzo M, Battistini J, Provenzano C, Martelli F, Cardinali B, Falcone G. Molecular Therapies for Myotonic Dystrophy Type 1: From Small Drugs to Gene Editing. Int J Mol Sci. 2022;23(9):4622.
72. Wahbi K, Babuty D, Probst V, Wissocque L, Labombarda F, Porcher R, et al. Incidence and predictors of sudden death, major conduction defects and sustained ventricular tachyarrhythmias in 1388 patients with myotonic dystrophy type 1. Eur Heart J. 2017;38(10):751–8.
73. Groh WJ, Groh MR, Saha C, Kincaid JC, Simmons Z, Ciafaloni E, et al. Electrocardiographic Abnormalities and Sudden Death in Myotonic Dystrophy Type 1. N Engl J Med. 2008;358(25):2688-97.

74. Petri H, Vissing J, Witting N, Bundgaard H, Køber L. Cardiac manifestations of myotonic dystrophy type 1. In J Cardiol. 212;160(2):82–8.
75. Douglas AGL, Wood MJA. Splicing therapy for neuromuscular disease. Mol Cell Neurosci. 2013;56:169–85.
76. Nascimento Osorio A, Medina Cantillo J, Camacho Salas A, Madruga Garrido M, Vilchez Padilla JJ. Consensus on the diagnosis, treatment and follow-up of patients with Duchenne muscular dystrophy. Neurologia (Eng Ed). 2019;34(7):469–81.
77. Bladen CL, Salgado D, Monges S, Foncuberta ME, Kekou K, Kosma K, et al. The TREAT-NMD DMD global database: analysis of more than 7,000 Duchenne muscular dystrophy mutations. Hum Mutat. 2015;36(4):395–402.
78. Birnkrant DJ, Bushby K, Bann CM, Alman BA, Apkon SD, Blackwell A, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: respiratory, cardiac, bone health, and orthopaedic management. Lancet Neurol. 2018;17(4):347–61.
79. Birnkrant DJ, Bushby K, Bann CM, Apkon SD, Blackwell A, Brumbaugh D, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. Lancet Neurol. 2018;17(3):211–2.
80. Adorisio R, Mencarelli E, Cantarutti N, Calvieri C, Amato L, Cicenia M, et al. Duchenne dilated cardiomyopathy: Cardiac management from prevention to advanced cardiovascular therapies. J Clin Med. 2020;9(19):3186.
81. D'Amario D, Amodeo A, Adorisio R, Tiziano FD, Leone AM, Perri G, et al. A current approach to heart failure in Duchenne muscular dystrophy. Heart. 2017;103(22):1770–9.
82. Cho MJ, Lee JW, Lee J, Shin YB. Evaluation of Early Left Ventricular Dysfunction in Patients with Duchenne Muscular Dystrophy Using Two-Dimensional Speckle Tracking Echocardiography and Tissue Doppler Imaging. Pediatr Cardiol. 2018;39(8):1614–9.
83. Oreto L, Vita GL, Mandraffino G, Carerj S, Calabrò MP, Manganaro R, et al. Impaired myocardial strain in early stage of Duchenne muscular dystrophy: its relation with age and motor performance. Acta Myol. 2020;39(4):191–9.

84. Earl CC, Soslow JH, Markham LW, Goergen CJ. Myocardial strain imaging in Duchenne muscular dystrophy. Front Cardiovasc Med. 2022;9(1031205).
85. Silva MC, Magalhães TA, Meira ZM, Rassi CH, Andrade AC, Gutierrez PS, et al. Myocardial fibrosis progression in Duchenne and Becker muscular dystrophy: A randomized clinical trial. JAMA Cardiol. 2017;2(2):190–9.
86. Lim KRQ, Nguyen Q, Yokota T. Genotype-phenotype correlations in Duchenne and Becker muscular dystrophy patients from the Canadian Neuromuscular Disease Registry. J Pers Med. 2020;10(4):241.
87. Tandon A, Jefferies JL, Villa CR, Hor KN, Wong BL, Ware SM, et al. Dystrophin genotype–cardiac phenotype correlations in Duchenne and Becker Muscular Dystrophies using cardiac magnetic resonance imaging. Am J Cardiol. 2015;115(7):967–71.
88. Kaspar RW, Allen HD, Ray WC, Alvarez CE, Kissel JT, Pestronk A, et al. Analysis of dystrophin deletion mutations predicts age of cardiomyopathy onset in Becker muscular dystrophy. Circ Cardiovasc Genet. 2009;2(6):544–51.
89. Jefferies JL, Eidem BW, Belmont JW, Craigen WJ, Ware SM, Fernbach SD, et al. Genetic predictors and remodeling of dilated cardiomyopathy in muscular dystrophy. Circulation. 2005;112(18):2799–804.
90. Magri F, Govoni A, D'Angelo MG, Del Bo R, Ghezzi S, Sandra G, et al. Genotype and phenotype characterization in a large dystrophinopathic cohort with extended follow-up. J Neurol. 2011;258(9):1610–23.
91. Cirino RHD, Scola RH, Ducci RDP, Wermelinger ACC, Kay CSK, Lorenzoni PJ, et al. Predictors of early left ventricular systolic dysfunction in Duchenne muscular dystrophy patients. Muscle Nerve. 2018;58(1):84–9.
92. Poyatos-García J, Martí P, Liquori A, Muelas N, Pitarch I, Martinez-Dolz L, et al. Dystrophinopathy Phenotypes and Modifying Factors in DMD Exon 45–55 Deletion. Ann Neurol. 2022;92(5):793–806.
93. Pahl E, Sleeper LA, Canter CE, Hsu DT, Lu M, Webber SA, et al. Incidence of and risk factors for sudden cardiac death in children with dilated cardiomyopathy: A report from the pediatric cardiomyopathy registry. J Am Coll Cardiol. 2012;59(6):607–15.

94. Dimas VV, Denfield SW, Friedman RA, Cannon BC, Kim JJ, Smith EO, et al. Frequency of Cardiac Death in Children With Idiopathic Dilated Cardiomyopathy. Am J Cardiol. 2009;104(11):1574–7.
95. Wittlieb-Weber CA, Knecht KR, Villa CR, Cunningham C, Conway J, Bock MJ, et al. Risk Factors for Cardiac and Non-cardiac Causes of Death in Males with Duchenne Muscular Dystrophy. Pediatr Cardiol. 2020;41(4):764–71.
96. Zeppenfeld K, Tfelt-Hansen J, de Riva M, Winkel BG, Behr ER, Blom NA, et al. 2022 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. Eur Heart J. 2022;43(40):3997–4126.
97. Maggi L, Carboni N, Bernasconi P. Skeletal Muscle Laminopathies: A Review of Clinical and Molecular Features. Cells. 2016;5(3):33.
98. Davidson PM, Lammerding J. Broken nuclei--lamins, nuclear mechanics, and disease. Trends Cell Biol. 2014;24(4):247–56.
99. Wolf CM, Wang L, Alcalai R, Pizard A, Burgon PG, Ahmad F, et al. Lamin A/C haploinsufficiency causes dilated cardiomyopathy and apoptosis-triggered cardiac conduction system disease. J Mol Cell Cardiol. 2008;44(2):293–303.
100. Malashicheva A, Perepelina K. Diversity of Nuclear Lamin A/C Action as a Key to Tissue-Specific Regulation of Cellular Identity in Health and Disease. Front Cell Dev Biol. 2021;9:761469.
101. Bonne G, Di Barletta MR, Varnous S, Bécane HM, Hammouda EH, Merlini L, et al. Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. Nat Genet. 1999;21(3):285–8.
102. Worman HJ. Nuclear lamins and laminopathies. J Pathol. 2012;226(2):316-25.
103. Taylor MRG, Fain PR, Sinagra G, Robinson ML, Robertson AD, Carniel E, et al. Natural history of dilated cardiomyopathy due to lamin A/C gene mutations. J Am Coll Cardiol. 2003;41(5):771–80.
104. Garcia-Pavia P, Cobo-Marcos M, Guzzo-Merello G, Gomez-Bueno M, Bornstein B, Lara-Pezzi E, et al. Genetics in dilated cardiomyopathy. Biomark Med. 2013;7(4):517–33.
105. Pérez-Serra A, Toro R, Sarquella-Brugada G, de Gonzalo-Calvo D, Cesar S, Carro E, et al. Genetic basis of dilated cardiomyopathy. Int J Cardiol. 2016;224:461–72.

106. Hinson JT, Chopra A, Nafissi N, Polacheck WJ, Craig C, Swist S, et al. Titin Mutations in iPS cells Define Sarcomere Insufficiency as a Cause of Dilated Cardiomyopathy. Science. 2015;349(6251):982–6.

107. Haas J, Frese KS, Peil B, Kloos W, Keller A, Nietsch R, et al. Atlas of the clinical genetics of human dilated cardiomyopathy. Eur Heart J. 2015;36(18):1123-35a.

108. Nikolova V, Leimena C, McMahon AC, Tan JC, Chandar S, Jogia D, et al. Defects in nuclear structure and function promote dilated cardiomyopathy in lamin A/C–deficient mice. J Clin Invest. 2004;113(3):357–69.

109. Frock RL, Chen SC, Da DF, Frett E, Lau C, Brown C, et al. Cardiomyocyte-specific expression of lamin A improves cardiac function in Lmna-/-mice. PLoS One. 2012;7(8):e42918.

110. Roncarati R, Viviani Anselmi C, Krawitz P, Lattanzi G, von Kodolitsch Y, Perrot A, et al. Doubly heterozygous LMNA and TTN mutations revealed by exome sequencing in a severe form of dilated cardiomyopathy. Eur J Hum Genet. 2013;21(10):1105–11.

111. Boudreau É, Labib S, Bertrand AT, Decostre V, Bolongo PM, Sylvius N, et al. Lamin A/C mutants disturb sumo1 localization and sumoylation in vitro and in vivo. PLoS One. 2012;7(9):e45918.

112. Muntoni F, Bonne G, Goldfarb LG, Mercuri E, Piercy RJ, Burke M, et al. Disease severity in dominant Emery Dreifuss is increased by mutations in both emerin and desmin proteins. Brain. 2006;129(Pt5):1260–8.

113. Huie ML, Tsujino S, Brooks SS, Engel A, Elias E, Bonthron DT, et al. Glycogen Storage Disease Type II: identification of four novel missense mutations (D645N, G648S, R672W, R672Q) and two insertions/deletions in the acid α-glucosidase locus of patients of differing phenotype. Biochem Biophys Res Commun. 1998;244(3):921–7.

114. Liu X, Wang Z, Jin W, Lv H, Zhang W, Que C, et al. Clinical and GAA gene mutation analysis in mainland Chinese patients with late-onset Pompe disease: Identifying c.2238G>C as the most common mutation. BMC Med Genet. 2014;15:141.

115. McCready ME, Carson NL, Chakraborty P, Clarke JTR, Callahan JW, Skomorowski MA, et al. Development of a clinical assay for detection of GAA mutations and characterization of the GAA mutation spectrum in a Canadian cohort of

individuals with glycogen storage disease, type II. Mol Genet Metab. 2007;92(4):325–35.

116. Jędrzejowska M, Potulska-Chromik A, Gos M, Gambin T, Dębek E, Rosiak E, et al. Floppy infant syndrome as a first manifestation of LMNA-related congenital muscular dystrophy. Eur J Paediatr Neurol. 2021;32:115–21.

117. Bonne G, Mercuri E, Muchir A, Urtizberea A, Bécane HM, Recan D, et al. Clinical and molecular genetic spectrum of autosomal dominant Emery-Dreifuss muscular dystrophy due to mutations of the lamin A/C gene. Ann Neurol. 2000;48(2):170–80.

118. Pasqualin LMA, Reed UC, Costa TV, Quedas E, Albuquerque MA, Resende MB, et al. Congenital muscular dystrophy with dropped head linked to the LMNA gene in a Brazilian cohort. Pediatr Neurol. 2014;50(4):400–6.

119. Dogan C, De Antonio M, Hamroun D, Varet H, Fabbro M, Rougier F, et al. Gender as a modifying factor influencing Myotonic Dystrophy type 1 phenotype severity and mortality: a nationwide multiple databases cross-sectional observational study. PLoS One. 2016;11(2):e0148264.

120. Ho G, Carey KA, Cardamone M, Farrar MA. Myotonic dystrophy type 1: Clinical manifestations in children and adolescents. Arch Dis Child. 2019;104(1):48–52.

121. Bassez G, Lazarus A, Desguerre I, Varin J, Laforêt P, Bécane HM, et al. Severe cardiac arrhythmias in young patients with Myotonic Dystrophy type 1. Neurology. 2004;63(10):1939-41.

122. Mussinelli R, Salinaro F, Alogna A, Boldrini M, Raimondi A, Musca F, et al. Diagnostic and prognostic value of low QRS voltages in cardiac AL amyloidosis. Ann Noninvasive Electrocardiol. 2013;18(3):271–80.

123. Feingold B, Mahle WT, Auerbach S, Clemens P, Domenighetti AA, Jefferies JL, et al. Management of cardiac involvement associated with neuromuscular diseases: a scientific statement from the American Heart Association. Circulation. 2017;136(13):e200–31.

124. Wahbi K, Meune C, Porcher R, Bécane HM, Lazarus A, Laforêt P, et al. Electrophysiological study with prophylactic pacing and survival in adults with Myotonic Dystrophy and Conduction System Disease. JAMA. 2012;307(12):1292-301.

125. Lallemand B, Clementy N, Bernard-Brunet A, Pierre B, Corcia P, Fauchier L, et al. The evolution of infrahissian conduction time in myotonic dystrophy patients: clinical implications. Heart. 2012;98(4):291–6.
126. Groh WJ, Lowe MR, Zipes DP. Severity of cardiac conduction involvement and arrhythmias in myotonic dystrophy type 1 correlates with age and CTG repeat length. J Cardiovasc Electrophysiol. 2002;13(5):444–8.
127. Finsterer J, Cripe L. Treatment of dystrophin cardiomyopathies. Nature. 2014;11(3):168–79.
128. McNally EM, Kaltman JR, Benson DW, Canter CE, Cripe LH, Duan D, et al. Contemporary cardiac issues in Duchenne muscular dystrophy. Circulation. 2015;131(18):1590–8.
129. Amedro P, Vincenti M, De La Villeon G, Lavastre K, Barrea C, Guillaumont S, et al. Speckle-Tracking Echocardiography in Children With Duchenne Muscular Dystrophy: A Prospective Multicenter Controlled Cross-Sectional Study. J Am Soc Echocardiogr. 2019;32(3):412–22.
130. Raman SV, Hor KN, Mazur W, Halnon NJ, Kissel JT, He X, et al. Eplerenone for early cardiomyopathy in Duchenne muscular dystrophy: a randomised, double-blind, placebo-controlled trial. Lancet Neurol. 2015;14(2):153–61.
131. Soslow JH, Xu M, Slaughter JC, Crum K, Kaslow JA, George-Durrett K, et al. Cardiovascular Measures of All-Cause Mortality in Duchenne Muscular Dystrophy. Circ Heart Fail. 2023;16(8):e010040.
132. Florian A, Ludwig A, Engelen M, Waltenberger J, Rösch S, Sechtem U, et al. Left ventricular systolic function and the pattern of late-gadolinium-enhancement independently and additively predict adverse cardiac events in muscular dystrophy patients. J Cardiovasc Magn Reson. 2014;16(1):81.
133. Bilchick KC, Salerno M, Plitt D, Dori Y, Crawford TO, Drachman D, et al. Prevalence and distribution of regional scar in dysfunctional myocardial segments in Duchenne muscular dystrophy. J Cardiovasc Magn Reson. 2011;13(1):20.
134. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Colvin MM, et al. 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure: a report of the American College of

Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. Circulation. 2017;136(6):e137-e161.

135. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J. 2021;42(36):3599–726.
136. Castro-Gago M, Gómez-Lado C, Eirís-Puñal J. Cardiac troponin I for accurate evaluation of cardiac status in myopathic patients. Brain Dev. 2009;31(2):184.
137. Schade van Westrum S, Dekker L, de Haan R, Endert E, Ginjaar I, de Visser M, et al. Brain natriuretic peptide is not predictive of dilated cardiomyopathy in Becker and Duchenne muscular dystrophy patients and carriers. BMC Neurol. 2013;13:88.
138. Toro R, Blasco-Turrión S, Morales-Ponce FJ, Gonzalez P, Martínez-Camblor P, López-Granados A, et al. Plasma microRNAs as biomarkers for Lamin A/C-related dilated cardiomyopathy. J Mol Med (Berl). 2018;96(8):845–56.
139. Alonso-Villa E, Bonet F, Hernandez-Torres F, Campuzano Ó, Sarquella-Brugada G, Quezada-Feijoo M, et al. The Role of MicroRNAs in Dilated Cardiomyopathy: New Insights for an Old Entity. Vol. 23, International Journal of Molecular Sciences. MDPI; 2022.
140. Bürger O, Humbel A, Ivanovski I, Baumer A, Rauch A. Further evidence for an attenuated phenotype of in-frame DMD deletions affecting the central rod domain of dystrophin around exon 48. Am J Med Genet A. 2024:e63842.
141. Lim KRQ, Nguyen Q, Yokota T. Genotype-phenotype correlations in Duchenne and Becker muscular dystrophy patients from the Canadian Neuromuscular Disease Registry. J Pers Med. 2020;10(4):241.
142. Kaspar RW, Allen HD, Ray WC, Alvarez CE, Kissel JT, Pestronk A, et al. Analysis of dystrophin deletion mutations predicts age of cardiomyopathy onset in Becker muscular dystrophy. Circ Cardiovasc Genet. 2009;2(6):544–51.
143. Villa CR, Czosek RJ, Ahmed H, Khoury PR, Anderson JB, Knilans TK, et al. Ambulatory monitoring and arrhythmic outcomes in pediatric and adolescent patients with Duchenne muscular dystrophy. J Am Heart Assoc. 2016;5(1):e002620.

144. Chiang DY, Allen HD, Kim JJ, Valdes SO, Wang Y, Pignatelli RH, et al. Relation of Cardiac Dysfunction to Rhythm Abnormalities in Patients with Duchenne or Becker Muscular Dystrophies. Am J Cardiol. 2016;117(8):1349–54.
145. Baskan S, Karaca Ozer P, Orta H, Ozbingol D, Yavuz ML, Ayduk Govdeli E, et al. Prognostic Value of Tpeak–Tend Interval in Early Diagnosis of Duchenne Muscular Dystrophy Cardiomyopathy. Diagnostics (Basel). 2023;13(14):2381.
146. Charron P, Arbustini E, Bonne G. What Should the Cardiologist know about Lamin Disease? Arrhythm Electrophysioil Rev. 2012;1(1):22-28.
147. Davies KE, Nowak KJ. Molecular mechanisms of muscular dystrophies: Old and new players. Nat Rev Mol Cell Biol. 2006;7(19):762–73.

11. Anexos

Debido a la falta de consistencia de las manifestaciones clínicas en las enfermedades neuromusculares, el manejo de forma individualizada de estos pacientes es clave durante el seguimiento en edad pediátrica y debe ser llevado por un equipo multidisciplinar que incluya un cardiólogo pediátrico experto en arritmias y análisis ecocardiográfico avanzado.
En este sentido y fruto del intenso trabajo, se detallan otros artículos y colaboraciones, en investigación clínica y ciencia básica en enfermedades neuromusculares. Aunque no forman parte de esta tesis doctoral, gracias a ésta se han creado grupos de trabajo y colaboraciones de las que los pacientes se ven beneficiados directa o indirectamente.

- Revisión: Neuromuscular diseases with hypertrophic cardiomyopathy. **Cesar S**. Glob Cardiol Sci Pract. 2018;(3):27.

- Artículo original: Paredes-Fuentes AJ, **Cesar S**, Montero R, Latre C, Genovès J, Martorell L, et al. Plasma Idebenone monitoring in Friedreich's Ataxia patients during al long-term follow-up. Biomed Pharmacother. 2021;143:112143.

- Artículo original: Natera-de Benito D, Ortez C, Jou C, Jimenez-Mallebrera C, Codina A, Carrera-García L, Expósito-Escudero J, **Cesar S**, et al. The phenotype and genotype of congenital myopathies based on a large pediatric Cohort. Pediatr Neurol. 2021;115:50-65.

- Artículo original: Congenital LMNA-related muscular dystrophy in paediatrics: cardiac management in monozygotic twins. Martínez Olorón P, Alegría I, **Cesar S**, Del Olmo B, Martínez-Barrios E, Carrera-García L, et al. Int J Mol Sci. 2024;25(11):5836.

- Colaboración en el análisis ecocardiográfico de ratones con laminopatía en el Centro de Salud Carlos III de Madrid, Unidad de terapia Génica, a

cargo del Dr. Ignacio Pérez de Castro, del cual han sido presentados los siguientes pósters y comunicaciones orales a congresos:

- Evolution of CRISPR Tools for the Treatment of LMNA-associated congenital muscular dystrophy. Congreso: 2024 Intermediate Filaments Gordon Research Conference. Formato: póster.
- Targeted genetic Intervention with Cas9 and a mutation-specific single guide RNA to rescue survival and cardiac deficits in LMNA-related congenital muscular dystrophy. Congreso: 30th annual meeting of the ESGCT. Formato: póster.
- Heterogeneous responses to the application of different gene therapy strategies on an LMNA-R249W mouse of LMNA-related congenital muscular dystrophy. Congreso: 4th International Meeting on Laminopathies. 9-12 May 2023. Formato: comunicación oral.
- Study of the therapeutic potential of the Compound Arry-371797 in congenital dystrophy associated to LMNA (L-CMD). Congreso: 4th International Meeting on Laminopathies. 9-12 May 2023. Formato: póster.