On the road from gene to therapy in Inherited Cardiomyopathies

Limongelli Giuseppe[1,2,3], Bossone Edoardo[4], Elliott Perry Mark[2,3], Day Sharlene[5]

[1] Department of Cardiothoracic Sciences, Università della Campania "Luigi Vanvitelli", Monaldi Hospital, AORN Colli, Naples, Italy

[2] Institute of Cardiovascular Science, University College of London, London, UK

[3] European Reference Network - GUARD HEART

[4] Cardiology Division, University of Salerno, Salerno, Italy

[5] Departments of Internal Medicine and Pediatrics, University of Michigan, Ann Arbor, Michigan, USA

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Summary: Almost 30 years after the discovery of the first genetic mutation for HCM, the development of new pharmacological approaches targeting cardiomyopathies and other orphan/rare cardiac disease is becoming closer to reality. Development of targeted therapies is enabled by new insights into the clinical and molecular aspects, and pathogenesis of cardiomyopathies, along with the establishment of large-scale international collaboration and the increasing engagement of the pharmaceutical industry. The road toward "cardiovascular precision medicine" is just beginning, with inherited and rare diseases leading the way in this exciting new era.

Key Points (3–5)

- Cardiomyopathies are a heterogeneous group of myocardial disorders
- Most are related to genetic abnormalities of the structural and functional proteins of the myocyte
- The clinical course recognize 3 phases: preclinical, overt (concealed), and end stage disease
- New insight on pathogenesis and mechanisms underlying inherited cardiomyopathies is opening the hope of present and future therapies

Address for correspondence:

Giuseppe Limongelli, MD, PhD, FESC
Department of Cardiothoracic Sciences,

Università della Campania "Luigi Vanvitelli",

Monaldi Hospital, AORN Colli, Naples, Italy

Email: limongelligiuseppe@libero.it

The century that closed the second millennium was marked by enormous progress in all the life sciences, including medicine. In the 21st Century, we have an unparalled ability to observe, describe,

and define clinical phenomena thanks to the discovery and progressive refinement of new tools able to determine the etiology and pathophysiology of different diseases. With better recognition, many diseases previously thought to be rare have become more commonplace.

The familial nature of cardiomyopathies was first recognized in the mid-20th century (1-5). One of the first families to be reported by Pare et al (5) was the key to the discovery of the genetic substrate of hypertrophic cardiomyopathy when Christine and John Seidman applied–for the first time in cardiology–linkage techniques to discover a mutation (Arg403Glu) in MYH7 on chromosome 14 (6). This landmark paper opened the door to the era of cardiovascular genetics with a cascade of new discoveries about the etiology and pathogenesis of cardiomyopathies. Today, genetic testing is an essential part of diagnosis and management of patients with cardiomyopathies, offering an invaluable tool for risk prediction, cascade genetic testing of at-risk relatives and reproductive testing options (7). Specialized multidisciplinary clinics including cardiologists and genetic counselors are now standard of care (7) and advances in genetic testing technologies have increased the yield and accuracy of genetic testing at ever reducing cost (7,8).

Cardiomyopathies as a group are a heterogeneous group of myocardial disorders in which the heart muscle is structurally and/or functionally abnormal in the absence of any condition that can explain the observed phenotype (9). The estimated combined population prevalence of all cardiomyopathies is at least 3%(10). Most cardiomyopathies are genetic disorders affecting the structural and functional proteins of the cardiomyocyte (9,10,11,12,13,14,15). They can be primary genetic disorders of the myocardium, or be part of multisystem disorders (phenocopies) such as malformation syndromes, neuromuscular disorders, mitochondrial disease, and infiltrative/storage disease (13,14,15). Cardiomyopathies can be acquired–for example following exposure to a toxin or infective agent–but even in this scenario, genetic predisposition plays an important role. The complexity of the different pathways that lead to disease mean that a common classification of cardiomyopathies is still lacking, due to our inability to translate their heterogeneity and complexity in a single nosology. Nevertheless, a common language that encompasses some of the new insights on genotype and phenotype of cardiomyopathies is emerging and impacting on clinical practice.

For patients, the burden of cardiomyopathies lies in the development of heart failure and sudden cardiac death. The latter is particularly relevant in children and young adults (7, 16) where physical exercise can be a trigger. However, the physical and psychological benefit of sport activity in daily life should be balanced case-by-case with the potential risk of cardiac arrest (17,18). In the last 20 years, the use of implantable cardioverter defibrillators (ICD) have transformed primary and secondary prevention, although risk stratification for primary prevention and complications related to ICD implantation still represent challenges. Much work on risk prediction focuses on the search for new biomarkers such as high resolution imaging with tissue characterization. Efforts to improve the

risk/benefit of ICD implantation focus on new technologies such as subcutaneous ICD leads (S-ICD). The role of genotype in risk stratification for sudden cardiac death is still unclear, but molecular autopsy (proband genetic testing performed on postmortem DNA) can be a valuable tool for clarification of the cause of death and for allowing appropriate screening and risk stratification of family members (7,16).

Recent practice guidelines have highlighted the important contribution of inherited cardiomyopathies to the burden of heart failure (19). Dilated cardiomyopathy and advanced stage-HCM and arrhythmogenic cardiomyopathy (AC) represent important causes of heart failure with reduced ejection fraction (HFREF), while RCM and restrictive HCM represent extremes of heart failure with preserved ejection fraction (HFPEF) (20,21,22). Moreover, increased recognition of infiltrative disease (i.e. amyloidosis) is revealing common and potentially treatable causes of HFPEF in specific subgroups and ethnic populations (23,24). The future development of heart failure services will lie in closer collaboration between HF and cardiomyopathy specialists and multidisciplinary teams. Progress will also come from large scale collaborations, registries and national electronic health records which hopefully will provide the power to appreciate cumulative disease burden, define accurate risk estimates for adverse events, and determine how genotype impacts disease (9).

The mechanisms by which gene mutations lead to protein and cell dysfunction and clinical disease is an area of active investigation. Phenotypic characterization of preclinical sarcomere gene mutation carriers has yielded insights into the earliest biomechanical defects that link pathogenic variants to cardiac remodeling and dysfunction (25,26). For example, hyperdynamic ventricular contraction and diastolic dysfunction are the earliest identified biomechanical defect in human HCM, while systolic dysfunction is the first sign of pathophysiology in DCM (25,26). Sarcomeric protein gene mutations can result in either phenotype, but functional studies have shown that disease may relate directly to their impact on different functional domains and protein-protein interactions (25,26). Another recent discovery is that titin truncating variants (TTNtv), yielding titin haploinsufficiency, represent the most common cause of familial DCM (25-28). Experimental data seem to support the hypothesis that titin is critical for sarcomere assembly and content and that mutations lead to an abnormal and inadequate stress response (for example during increased haemodynamic load in pregnancy) (25-28). Considerable progress has also been made in understanding AC caused by genes encoding proteins of the cardiac desmosomes, which lead to disruption of inter-myocyte connections and alteration of intracellular signal transduction. Wnt/beta catenin and Hippo signaling pathways have been implicated in disease pathogenesis, as well known regulators of adipogenesis, fibrogenesis and apoptosis, the main cellular mechanisms underpinning the disease phenotype.

Therapies in cardiomyopathies vary according to the disease stage. Some treatments are essentially palliative but in DCM, ACE inhibitors and beta blockers delay progression and improve prognosis.

Disease modifiying treatments have not been identified for HCM or AC but there has been a recent surge of clinical trials testing new therapies for cardiomyopathies. One such therapy being tested for HCM is mavacampten (MYK-461, Myokardia, San Francisco, CA, USA), a small-molecule allosteric myosin inhibitor that restores contractile balance in HCM hearts by decreasing adenosine triphosphatase activity of the cardiac myosin heavy chain (29,30). A phase 2a study has recently been completed to evaluate the efficacy, safety and tolerability of mavacamten in subjects with symptomatic HCM and LVOT obstruction (NCT02842242); a large Phase 2/3 study is due to start in 2018. This trial is supported by data from preclinical studies showing that in an HCM mouse model, mavacampten administered in an early stage prevented disease development (LVH, myocyte disarray and fibrosis) and down regulated both hypertrophic and pro-fibrotic gene expression (29,30).
Phenotype prevention or reverse remodeling is the ultimate goal of pharmacologic therapy. Different therapies have been, or are being tested in HCM. Diltiazem has shown some promise in sarcomere gene mutations carriers in a small clinical trial (31) while no benefit was apparent with N-acetylcysteine, atorvastatin, or ranolazine analogs (30, 32-35). Valsartan is currently under investigation in the VANISH trial (36). This trial was based on encouraging findings from a preclinical trial in which inhibiting TGFbeta by neutralizing antibodies or with losartan prevented phenotypic development in mice carrying a MYH7 mutation (37). Gene-targeted therapies are also on the horizon, enabled by recent advances in gene-editing technology. In a recent study, genetic engineering using CRISPR–Cas9 technique corrected a heterozygous MYBPC3 mutation in human preimplantation embryos (38, 39). However, the applicability of this technique to clinical practice is uncertain, in light of concerns about off-target effects and the current availability of pre-implantation genetic diagnosis which allows for implantation of genetically unaffected embryos.

Other innovative breakthroughs have emerged in the field of rare multisystem diseases, such as chaperone therapy (migalastat) for Fabry disease and antisense oligonucleotides (eteplirsen) for Duchenne muscular dystrophy, both recently approved in clinical practice (30). New developments are underway in Rasopathies, where short-term therapy with mTOR inhibitors (everolimus) has recently been used to prevent disease complications in patients with a severe form of HCM (40) and in Lamin A/C disease, where a p38α inhibitor. is under investigation in a small phase 2 study trial of Lamin A/C DCM (NTC02351856) (41).

In conclusion, almost 30 years after the discovery of the first genetic mutation for HCM the development of new pharmacological approaches targeting cardiomyopathies and other orphan/rare cardiac disease is closer to reality. Development of targeted therapies is enabled by new insights into

clinical phenotypes and molecular pathogenesis, along with the establishment of large-scale international collaboration and engagement of the pharmaceutical industry. The road toward cardiovascular precision medicine is just beginning, with inherited and rare diseases leading the way.

REFERENCES

(1) Evans W. Familial Cardiomegaly. Br Heart J. 1949 Jan; 11(1): 68–82;

(2) DE MATTEIS F, OZZANO T. [Familial idiopathic cardiomegaly].Minerva Med. 1954 May 30;45(43):1549-55;

(3) HOLLMAN A, GOODWIN JF, TEARE D, RENWICK JW.A family with obstructive cardiomyopathy (asymmetrical hypertrophy).Br Heart J. 1960 Sep;22:449-56.

(4) TEARE D. Asymmetrical hypertrophy of the heart in young adults.Br Heart J. 1958 Jan;20(1):1-8.

(5) PARE JA, FRASER RG, PIROZYNSKI WJ, SHANKS JA, STUBINGTON D.Hereditary cardiovascular dysplasia. A form of familial cardiomyopathy.Am J Med. 1961 Jul;31:37-62.

(6) Geisterfer-Lowrance AA, Kass S, Tanigawa G, Vosberg HP, McKenna W, Seidman CE, Seidman JG. A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain gene missense mutation. Cell. 1990;62:999 –1006.

(7) Ingles JJ, Bagnall RD, Semsarian C.Genetic Testing for Cardiomyopathies in Clinical Practice. HFC 2018 in press.

(8) D'Argenio V, Frisso G, Precone V, Boccia A, Fienga A, Pacileo G, Limongelli G, Paolella G, Calabrò R, Salvatore F. DNA sequence capture and next-generation sequencing for the molecular diagnosis of genetic cardiomyopathies. J Mol Diagn. 2014 Jan;16(1):32-44.

(9) Masarone D, Kaski JP, Pacileo G, Elliott PM, Bossone E, Day SM, Limongelli G. Epidemiology and Clinical Aspects of Genetic Cardiomyopathies. HFC 2018 in press.

(10) McKenna WJ, Maron BJ, Thiene G. Classification, epidemiology, and global burden of cardiomyopathies. Circ Res 2017;121:722–730.

(11) Garfinkel AC, Seidman JG, Seidman CE. Genetic Pathogenesis of Hypertrophic and Dilated Cardiomyopathy. HFC 2018, in press.
(12) Basso C, Corrado D, Thiene G. Diagnostic criteria, genetics, and molecular basis of ARVC. Heart Failure Clinics 2018, in press.
(13) Dhandapany PS, Razzaque MA, Muthusami U, Kunnoth S, Edwards JJ, Mulero-Navarro S, Riess I, Pardo S, Sheng J, Rani DS, Rani B, Govindaraj P, Flex E, Yokota T, Furutani M, Nishizawa T, Nakanishi T, Robbins J, Limongelli G, Hajjar RJ, Lebeche D, Bahl A, Khullar M, Rathinavel A, Sadler KC, Tartaglia M, Matsuoka R, Thangaraj K, Gelb BD. RAF1 mutations in childhood-onset dilated cardiomyopathy. Nature genetics 46 (6), 635-639 282014.
(14) Sweet ME, Mestroni L, Taylor MRG. Genetic infiltrative cardiomyopathies. HFC 2018 in press.
(15) Calcagni G, Digilio MC, Adorisio R, Martinelli S, Grutter G, Baban A, Versacci P, Drago F, Gelb B, Tartaglia M. Clinical presentation and natural history of hypertrophic cardiomyopathy in Rasopathies. HFC 2018, in press.

(16) Bagnall RD, Weintraub RG, Ingles J, Duflou J, Yeates L, Lam L, Davis AM, Thompson T, Connell V, Wallace J, Naylor C, Crawford J, Love DR, Hallam L, White J, Lawrence C, Lynch M, Morgan N, James P, du Sart D, Puranik R, Langlois N, Vohra J, Winship I, Atherton J, McGaughran J, Skinner JR, Semsarian C. A Prospective Study of Sudden Cardiac Death among Children and Young Adults.N Engl J Med. 2016 Jun 23;374(25):2441-52. doi: 10.1056/NEJMoa1510687.
(17) Atteya G, Lampert R. Controversies Surrounding Exercise in Genetic Cardiomyopathies. Heart Failure Clinics 2018, in press.
(18) Saberi S, Wheeler M, Bragg-Gresham J, Hornsby W, Agarwal PP, Attili A, Concannon M, Dries AM, Shmargad Y, Salisbury H, Kumar S, Herrera JJ, Myers J, Helms AS, Ashley EA, Day SM. Effect of Moderate-Intensity Exercise Training on Peak Oxygen Consumption in Patients With Hypertrophic Cardiomyopathy: A Randomized Clinical Trial. JAMA. 2017 Apr 4;317(13):1349-1357.
(19) Ezekowitz JA, O'Meara E, McDonald M, Abrams H, Chan M, Ducharme A, Giannetti N, Grzeslo A, Hamilton P, Heckman G, Howlett J, Koshman SL, Lepage S, McKelvie R, MoeG, Rajda M, Swiggum E, Virani S, Zieroth S, Secondary Panel Members, Al-Hesayen A, Cohen-Solal A, D'Astous M, De S, Estrella-Holder E, Fremes S, Green L, Haddad H, Harkness K, Hernandez AF,Kouz S, LeBlanc M-H, Masoudi FA, Ross HJ, Roussin A, Sussex B, 2017 Comprehensive Update of theCanadian Cardiovascular Society Guidelines for the Management of Heart Failure, Canadian Journal of Cardiology (2017), doi: 10.1016/j.cjca.2017.08.022.
(20) Pinto YM, Elliott PM, Arbustini E, Adler Y, Anastasakis A, Böhm M, Duboc D, Gimeno J, de Groote P, Imazio M, Heymans S, Klingel K, Komajda M, Limongelli G, Linhart A, Mogensen J,

Moon J, Pieper PG, Seferovic PM, Schueler S, Zamorano JL, Caforio AL, Charron P. Proposal for a revised definition of dilated cardiomyopathy, hypokinetic non-dilated cardiomyopathy, and its implications for clinical practice: a position statement of the ESC working group on myocardial and pericardial diseases. Eur Heart J. 2016 Jun 14;37(23):1850-8. doi: 10.1093/eurheartj/ehv727.

(21) Biagini E, Olivotto I, Iascone M, Parodi MI, Girolami F, Frisso G, Autore C, Limongelli G, Cecconi M, Maron BJ, Maron MS, Rosmini S, Formisano F, Musumeci B, Cecchi F, Iacovoni A, Haas TS, Bacchi Reggiani ML, Ferrazzi P, Salvatore F, Spirito P, Rapezzi C. Significance of sarcomere gene mutations analysis in the end-stage phase of hypertrophic cardiomyopathy. Am J Cardiol. 2014 Sep 1;114(5):769-76.

(22) Limongelli G, Masarone D, Frisso G, Iacomino M, Ferrara I, Rea A, Gravino R, Bossone E, Salvatore F, Calabro R, Elliott P, Pacileo G. Clinical and genetic characterization of patients with hypertrophic cardiomyopathy and right atrial enlargement. J Cardiovasc Med (Hagerstown). 2017 Apr;18(4):249-254.

(23) González-López E, Gallego-Delgado M, Guzzo-Merello G, de Haro-Del Moral FJ, Cobo-Marcos M, Robles C, Bornstein B, Salas C, Lara-Pezzi E, Alonso-Pulpon L, Garcia-Pavia P. Wild-type transthyretin amyloidosis as a cause of heart failure with preserved ejection fraction. Eur Heart J. 2015 Oct 7;36(38):2585-94.

(24) Dungu JN, Papadopoulou SA, Wykes K, Mahmood I, Marshall J, Valencia O, Fontana M, Whelan CJ, Gillmore JD, Hawkins PN, Anderson LJ.Afro-Caribbean Heart Failure in the United Kingdom: Cause, Outcomes, and ATTR V122I Cardiac Amyloidosis.Circ Heart Fail. 2016 Sep;9(9).

25) Garfinkel AC, Seidman JG, Seidman, CE. Genetic Pathogenesis of Hypertrophic and Dilated Cardiomyopathy. Heart failure Clinics 2018, in press.

26) Lynn, ML, Lehman SJ, Tardiff, J. Biophysical Derangements in Genetic Cardiomyopathies. Heart failure Clinics 2018, in press.

27) Hinson JT, Chopra A, Nafissi N, et al. Titin mutations in iPS cells define sarcomere insufficiency as a cause of dilated cardiomyopathy. Science (80-). 2015;349(6251):982-986. doi:10.1126/science.aaa5458.

28) Schafer S, de Marvao A, Adami E, et al. Titin-truncating variants affect heart function in disease cohorts and the general population. Nat Genet. 2016;49(1):46-53. doi:10.1038/ng.3719.

29) Green, E.M., Wakimoto, H., Anderson, R.L., Evanchik, M.J., Gorham, J.M., Harrison, B.C., Henze, M., Kawas, R., Oslob, J.D., Rodriguez, H.M. and Song, Y., 2016. A small-molecule inhibitor of sarcomere contractility suppresses hypertrophic cardiomyopathy in mice. Science, 351(6273), pp.617-621.

30) Maurizi N, Ammirati E, Coppini R, Morrone A, Olivotto I. Clinical and Molecular Aspects of Cardiomyopathies: Emerging Therapies and Clinical Trials. Heart failure Clinics 2018, in press.

31) Ho CY, Lakdawala NK, Cirino AL, Lipshultz SE, Sparks E, Abbasi SA, Kwong RY, Antman EM, Semsarian C, Gonzalez A, Lopez B, Diez J, Orav EJ, Colan SD, Seidman CE. Diltiazem treatment for pre-clinical hypertrophic cardiomyopathy sarcomere mutation carriers: a pilot randomized trial to modify disease expression. JACC Heart Fail 2015;3:180–188.

32) Lombardi R, Rodriguez G, Chen SN, Ripplinger CM, Li W, Chen J, Willerson JT, Betocchi S, Wickline SA, Efimov IR, Marian AJ. Resolution of established cardiac hypertrophy and fibrosis and prevention of systolic dysfunction in a transgenic rabbit model of human cardiomyopathy through thiol-sensitive mechanisms. Circulation 2009;119:1398–1407.

33) Senthil V, Chen SN, Tsybouleva N, Halder T, Nagueh SF, Willerson JT, Roberts R, Marian AJ. Prevention of cardiac hypertrophy by atorvastatin in a transgenic rabbit model of human hypertrophic cardiomyopathy. Circ Res 2005;97:285–292.

34) Nagueh SF, Lombardi R, Tan Y,Wang J, Willerson JT, Marian AJ. Atorvastatin and cardiac hypertrophy and function in hypertrophic cardiomyopathy: a pilot study. Eur J Clin Invest 2010;40:976–983.

35) Olivotto I, Camici PG, Merlini PA et al. 'Efficacy of ranolazine in patients with symptomatic hypertrophic cardiomyopathy: the "Restyle-HCM" randomised, double-blind, placebo-controlled study'- 2017 Circ Heart Fail, in press.

36) Ho CY, McMurray JJV, Cirino AL, Colan SD, Day SM, Desai AS, Lipshultz SE, MacRae CA, Shi L, Solomon SD, Orav EJ, Braunwald E; VANISH trial investigators and executive committee.The Design of the Valsartan for Attenuating Disease Evolution in Early Sarcomeric Hypertrophic Cardiomyopathy (VANISH) Trial.Am Heart J. 2017 May;187:145-155. doi: 10.1016/j.ahj.2017.02.008.

37) Teekakirikul P1, Eminaga S, Toka O, Alcalai R, Wang L, Wakimoto H, Nayor M, Konno T, Gorham JM, Wolf CM, Kim JB, Schmitt JP, Molkentin JD, Norris RA, Tager AM, Hoffman SR, Markwald RR, Seidman CE, Seidman JG.Cardiac fibrosis in mice with hypertrophic cardiomyopathy is mediated by non-myocyte proliferation and requires Tgf-β.J Clin Invest. 2010 Oct;120(10):3520-9. doi: 10.1172/JCI42028.

38) Ma, H., Marti-Gutierrez, N., Park, S.W., Wu, J., Lee, Y., Suzuki, K., Koski, A., Ji, D., Hayama, T., Ahmed, R. and Darby, H.,. Correction of a pathogenic gene mutation in human embryos. Nature, 2017;548(7668), pp.413-419

39) Ohiri JC, McNally EM. Gene editing and gene-based therapeutics for cardiomyopathies. Heart failure Clinics 2018, in press.

40) Giulio Calcagni, Rachele Adorisio, Simone Martinelli, Giorgia Grutter, Anwar Baban, Paolo Versacci, Maria Cristina Digilio, Fabrizio Drago, Bruce D Gelb, Marco Tartaglia, Bruno Marino. Clinical presentation and natural history of hypertrophic cardiomyopathy in RASopathies. In press.

41) Captur G, Arbustini E, Bonne G, Syrris P, Mills K, Wahbi K, Mohiddin SA, McKenna WJ, Pettit S, Ho CY, Muchir A. Lamin and the heart. Heart. 2017 Nov 25:heartjnl-2017.