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Advanced *Ngs* Platforms in Molecular Diagnostics of Hypertrophic and Dilated Cardiomyopathies

Abstract

In the wide scenery of heart pathologies the field of cardiomyopathies is one of those showing a great unreliability. After years of generic classifications the American Heart Association (AHA 2006) first, the European Society of Cardiology (ESC 2008) and just recently the MOGE'S Classification (2013), signed a decided step forward to depict more accurately the different forms of cardiomyopathies. During the last decades, technology improvement realized an important growing of knowledge, about clinical and diagnostics instrumental assessment of the cardiomyopathies. At the same time, the large diffusion of genetic and molecular diagnostic procedures, represent the powerful tool to create a more accurate correlation, between clinical evidences and genetic and molecular damage. Although the important advancements realized, we are still far away to understand why to determinate DNA aberration may correspond a wide range of cardiomyopathies, especially for hypertrophic (HCM) and dilated (DCM) forms, characterized by a different quality and quantity of myocardial damage. The poor alignment between genotypes and phenotypes of these cardiomyopathies is the main reason that makes this topic still obscure and scarcely understood. The newest and most advanced high throughput next generation sequencing (NGS) technologies, could represent, at the moment, the powerful tool able to realize a complete and rapid sequencing of the whole genome (WGS), exome (WES) or transcriptome. This goal is certainly a starting point for each patient affected of cardiomyopathy, making possible to individuate all the genetic mutations, genomic variations and epigenetic keys implicated in the myocardial structural damage. The aim of this work is to review and update the state of the art of the knowledge on Hypertrophic CM (HCM) and Dilatative CM (DCM).

Keywords: Cardiomyopathy; Hypertrophic CM; Dilatative CM

Review Article

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Massimo Scorretti*

Department of Clinical and Molecular Biochemistry, University of Rome, Italy

***Corresponding author:** Massimo Scorretti, Department of Clinical and Molecular Biochemistry, University of Rome "La Sapienza", Molecular Cardiology, Azienda Ospedaliera San Camillo Forlanini, Rome, Italy, Email: mscorretti@hotmail.com

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Abbreviations: AHA: American Heart Association; ESC: European Society of Cardiology; NGS: Next Generation Sequencing; CM: Cardiomyopathy; HCM: Hypertrophic CM; DCM: Dilatative CM; WHO: World Health Organization; WSE: Whole Sequencing Exome; WSG: Whole Sequencing Genome

Introduction

The cardiomyopathies are a large family of heart disease, wherein the main tissue damaged is represented by myocardium. It took many decades and many cognitive efforts, before to arrive to a general cardiomyopathies (CMs) classification, able to catalogue in a reasonable way, the different classes of CMs, emphasizing the causes of the diseases. Just recently, in consequence of the great development of molecular technologies, finally is possible to formulate a classification with a general division among CMs of genetic, non genetic or unknown origin. Following the criteria announced, different classifications have been proposed, including the European Society of Cardiology (ESC) cardiomyopathies classification [1,2], presented in 2008 (Table 1), showing five different forms of CMs, including Hypertrophic CM (HCM) and Dilatative CM (DCM). These two form of CMs, represent the majority of CMs and in both of them are recognized genetic and familial dependence or not.

If today a great step forward, in the field of cardiomyopathies knowledge has been accomplished [2-4], that's due to the capacity of modern technologies to read rapidly and extensively the sequence of DNA and discover the relations among genes and diseases. The high throughput Next Generation Sequencing (NGSs) technologies [5], from the pioneering tools of second generation, to the most advanced of third and fourth generation, represent the key to reveal, maybe completely, the existing relation, among genotypic characteristics and variability of phenotypic expression. Using these powerful tools, we could be able to understand the complicated network of relations existing among gene mutations [6], the role of all type of variants recognized and the epigenetic influence in the etiology and pathophysiology of the cardiomyopathies [7]. The aim of this work is to review and update the state of the art of the knowledge on Hypertrophic CM (HCM) and Dilatative CM (DCM) [8].

Table 1: Classification of Cardiomyopathies (ESC 2008).

Hypertrophic Cardiomyopathy (HCM)	Genetic forms	Unidentified gene defect
		Disease sub type
	Non Genetic forms	Idiopathic
		Disease sub type
Dilated Cardiomyopathy (DCM)	Genetic forms	Unidentified gene defect
		Disease sub type
	Non Genetic forms	Idiopathic
		Disease sub type
Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia (ARVC/D)	Genetic forms	Unidentified gene defect
		Disease sub type
	Non Genetic forms	Idiopathic
		Disease sub type
Restricted Cardiomyopathy (RCM)	Genetic forms	Unidentified gene defect
		Disease sub type
	Non Genetic forms	Idiopathic
		Disease sub type
Unclassified	Genetic forms	Unidentified gene defect
		Disease sub type
	Non Genetic forms	Idiopathic
		Disease sub type

Classification of the Cardiomyopathies

Following the long road of the cardiomyopathies knowledge evolution, as taxonomic entity, there have been different fundamental moments which the modern technologies played a crucial role to partially clarify the etiology, pathophysiology and their clinical expression. For many years the cardiomyopathies represented a group of unrelated pathologies, defined with a long series of non specific nouns, hiding in this way the inability to understand the real cause underlying the pathology.

In 1956, Blankehorne & Gall submitted the international scientific community a classification of the cardiomyopathies, based on the aspecific division of them in two main categories: myocarditys and myocardiosys. One year later, Brigden [1] introduced firstly the concept of "cardiomyopathy" as primitive cardiac disease. In 1959, at NIH was reported and described for the first time a case of hypertrophic cardiomyopathy (HCM). From 1968 at 1995, World Health Organization (WHO) tried, in three different occasions (1968, 1980 e 1995) [4], to describe the myocardium pathologies, reviewing and modifying the previous definitions and classifications of the cardiomyopathies. In every single occasion, WHO's classification didn't reach the objective to create a useful scheme to divide CMs, suitable to catalogue diseases in relation with their causes.

In 1989 finally, was firstly demonstrated the association between HCM and a locus on chromosome 14 (14q1) [9], where was localized the mutation responsible for HCM, consequently, in 1990 was identified the mutation of the gene MYH7 (B myosin heavy chain), on exon 13 and his association with hypertrophic cardiomyopathy (HCM). Only recently, in 2006 the American Heart Association (AHA) [3], proposed a classification centered on a basic differentiation, between Primary Cardiomyopathies and Secondary Cardiomyopathies.

Primary CMs were sub classified in genetic, non genetic and acquired origin, while in secondary CMs were reported all myocardial diseases where the heart involvement resulted secondary to a systemic disease.

European Society of Cardiology (ESC), in 2008 [2] presented new cardiomyopathies classification including 5 different forms (Table 1):

1) Hypertrophic CMP (HCM),

2) Dilatative CMP (DCM),

3) Arrhythmogenic right ventricular cardiomyopathy/Displasia (ARVC/D)

4) Restricted Cardiomyopathy (RCM) [10]

5) Unclassified

Even in this case, the new classification, in five classes, is sub-articulated around a further division among genetic or non genetic CMs. Last cardiomyopathies classification has been proposed in 2013 [4] and received the endorsement of the World Hearth Federation (WHF). This new scheme, basically take conceptual relevance from the newest evidences emerging with the application of Next generation sequencing (NGS) technologies [11], that documented the relationship between the mutations of almost 100 (Table 2) genes and the cardiomyopathies phenotype.

Table 2: Genes involved in cardiomyopathies.

GENE	CHROM	TRASM (*)	DISEASE	PROTEIN AA
ABCC9	12p12	AD	DCM	ATP-Binding Cassette, Sub-Family C
ABLIM1	10q25	AD	DCM	Actin binding LIM protein 1
ACTC1	15q14	AD	DCM HCM LVNC	Actin alpha cardiac muscle 1
ACTN2	15q14	AD	DCM HCM	Actin Alpha Cardiac muscle 2
AGL	1p21	AD	DCM	Amilo 1,6 glucosydase
ALMS1	2p13	AD	DCM	ALMS1-C
ANO5	11p14.3	AD	DCM	Anoctamine 5
ANKRD1	10q23-.31	AD	DCM HCM	ankyrin
BAG3	10q26.11	AD	DCM HCM	BCL2 associate to athanogene
CALR3	19p13.11	AD	HCM	Calretinin 3
CASQ2	1p13.1	AD	HCM	Calsequestrin 1
CAV3	3p25	AD	DCM HCM	Caveolin 3
COX 15	10q24	AD	DCM	cytochrome c oxidase assembly homolog 15
CRYAB	11q22.3–q23.1	AD	DCM	Alpha B Crystallin
CSRP3	11p15.1	AD	DCM HCM	Protein 3 rich in cysteine e glycine
DES	19q13.4	AD	DCM	Desmin
DMD	Xp21	AD	DCM	Distrophyn (3685 aa)
DMPK	19q13.3	AD	DCM	distrophya myotonic Protein Kinase
DOLK	9q34	AD	DCM	Dolicol Kinase
DSC2	18q12	AD	DCM	Desmocollin 2
DSG2	18q12	AD	DCM	Desmoglein 2
DSP	6q24	AD	DCM	Desmoplakyin
DTNA	18q12	AD	DCM	Dystrobrevin alpha
EMD	Xq28	AD	DCM	Emerin
EYA4	6q23	AD	DCM	Eyes absent 4
FHL1	Xq26	AD	DCM	four and a half LIM domains 1
FHL2	2q12.2	AD	DCM	four and a half LIM domains 2
FXN/FRDA	9q13	AD	HCM	Frataxin
FKTN	9Q31-q33	AD	DCM	Fukutin
FKRP	19q13.32	AD	DCM	Protein correlated to Fukutin
GATAD1	7q21-q22	AD	DCM	GATA zinc finger domain containing 1
GBE	3p12	AD	DCM	Glycogen branching enzyme
GLA	Xq22	XL	HCM	Alpha galactosidase
HFE	6p21	AD	DCM	Protein of emochromatose
ILK	11p15.4	AD	DCM	Integrin linked Kinase
JUP	17q21	AD	ARVC	Junctional Plakoglobin
JPH2	20q12	AD	HCM	Junctophillyn 2
LMNA	1q22	AD	DCM	Lamin A/C
LAMA4	6q21	AD	DCM	Alpha Lamin 4
LAMP2	Xq24	XL	DCM HCM	Prot.2 di membr. ass. a lisosoma
LDB3	10q22	AD	DCM HCM	LIM binding Dominio 3
MRPL3	3q21-q23	AD	DCM	Mithocondrial Ribosome Protein
MYBPC3	11p11	AD	DCM HCM	Myosin ligand Protein C

MYH6	14q11.2q12	AD	DCM HCM	Myosin Heavy 6 alpha
MYH7	14q12	AD	DCM HCM	Myosin Heavy 7 alpha
MYL2	12q23	AD	HCM	Myosin Light 2 alpha
MYL3	3p	AD	HCM	Myosin Light 3 alpha
MYOZ1	4q26-q27	AD	HCM	Myozenin 1
MYOZ2	4q26-q27	AD	HCM	Myozenin 2
MYPN	10q21.3	AD	DCM HCM	Myopalladin
NEBL	10p12	AD	DCM	nebulette
NEXN	1p31.1	AD	DCM HCM	Nexilin
NKX2-5	5q34	AD	DCM	Nk2 homebox 5
PDLIM3	4q35	AD	DCM	PDZ Lim protein 3
PLN	6q22.1	AD	DCM HCM	Phospholamban
PKP2	12p11	AD	DCM	Plakofillin 2
PRKAG2	7q35-q36.36	AD	HCM	Proteina Kinase activated by AMP
PSEN1	14q24.3	AD	DCM	Presenilin 1
PSEN2	1q31-q42	AD	DCM	Presenilin 2
PTPN	12q24.1-3p25	AD	DCM	Protein tirosyn phosphatase non receptor type
RBM20	10q25.2	AD	DCM	RNA binding Proteina 20
RYR2	1q42.1-q43	AD	HCM	Ryanodine Receptor 2
SCN5A	3p21	AD	DCM	Sodium Channel voltage dip. Tipo V alfa
SDHA	5p15.33	AD	DCM	flavoprotein
SGCD	5q33	AD	DCM	Delta sarcoglycan
SGCB	4Q12	AD	DCM	Beta sarcoglycan
SGCA	17Q12	AD	DCM	Alpha sarcoglycan
SGCG	13Q12	AD	DCM	Gamma sarcoglycan
SYNE1	6q25	AD	DCM	Nexprin 1
SYNE2	14q23.2	AD	DCMDCM	Nexprin 2 (6885 aa)
TAZ	Xq28	AD	DCM	Tafazzin
TCAP	17q12	AD	DCM HCM	telethonin
TCF21	6q23-q24	AD	DCM	Fattore di trascrizione 21 epicardina
TGFB3	14q24	AD	DCM	Fattore di crescita trasformante beta 3
TMEM43	3p25	AD	ARVC	transmembrane protein 43
TMPO	12q22	AD	DCM	Tymophoietin
TNNC1	3p21.1	AD	DCM HCM	Cardiac Troponin C1 (161 aa)
TNNI3	19q13.42	AD	DCM HCM	Cardiac Troponin I3 (210 aa)
TNNT2	1q32.1	AD	DCM HCM	Cardiac Troponin T2
TPM1	15q22.1	AD	DCM HCM	Alpha Tropomyosin (7 IF)
TTID	5q31	AD	DCM	Myotilin
TTN	2q24.3	XL	DCM HCM	Titin (13 IF)
TTR	18q11	AD	HCM	Transthyiretin
VCL	10q22.1-q23	AD	DCM HCM	metavimculin
MTTLI	mDNA	matrilineal	DCM	tRNA
MTNDI	mDNA	matrilineal	DCM	NADH deidhrogenase sub. 1
MTATP6	mDNA	matrilineal	DCM	ATP sinthetase 6
MTTY	mDNA	matrilineal	DCM	tRNA

The new classification has been named "M.O.G.E. (S)" [4], indicating in this way a classification based on genotype-phenotype relation. The MOGES classification [4] is inspired to TNM system of tumors staging and is articulated taking in account five elements, starting from the *morpho-functional aspect (M)* that characterize the phenotypes of HCM, DCM, ARVC and RCM, the organs involvement (O), emphasizing the fact of a single or multiple organs involvement, *genetics or familial inheritance (G)*, that furnish information about the mode of genetics transmission (autosomal dominant, autosomal recessive, X linked dominant or matrilineal).

Finally the *etiological annotation (E)*, that produce indications about causes underlying the pathology development and (S) representing the Heart Failure Stage, described using the ACC/AHA and NYHA classification of heart failure. Most part (50%) of dilated cardiomyophaty (DCM), take origin from myocardial ischemia, hypertension and toxic, or metabolic disease and almost 50% have a more or less recognized genetic origin. Nevertheless, this second fraction of DCM have a surprising heterogeneity, corresponding a similar genotypic aberrations (mutations, different types of variations), different age of presentation of heart failure, lethal arrhythmias and the need of heart transplantation. In few words a wide and global phenotypic diversity.

Epidemiology of HCM and DCM

Dilated and hypertrophic CMs represent two forms more frequently observed, resulting as the major cause of sudden death or heart transplantation [12]. In both CMs there is an important and well documented deep structural damage of the myocardium that shows an architectural disarray of the muscular structure of the heart. Two forms of CMs are characterized by two different anatomical pictures where prevalent hypertrophy (HCM) is or ventricular dilation (HCM). Both forms can evolve in heart failure, letal arrhytmyas and sudden death. In this work we will focus on HCM and DCM genetics, trying to analyze the genetic implications and clarify the several dark sides and the observed incongruity between genotypic structure and phenotypic expression of CMs.

Even through the use of genetic test, resulted that about the 35-40% of people suffering of HCM did not show specific genetic damages, represented by either small or great molecular aberrations [13]. Nevertheless, hypertrophic cardiomyopathy (HCM) is considered as an infrequent inherited heart disease and it has been estimated a prevalence of 1:500 individuals [14] in the general population [15-20]. Different studies, realized during the last two decades, revealed that about 30 genes are implicated in the origin of the disease [9,21-24]. About 65 % of people clinically affected of HCM, show some genetic mutations of genes listed on Table 3 and only 30-40% may be considered sporadic cases, without a familial involvement [24]. Two genes codifying for sarcomeric proteins, as MYH7 and MYBPC3 represent the genetic most "mutated" area, responsible for more than 70% of identified mutations. TNNT2 gene is implicated in almost 10-15% of genetic mutations causing HCM. Others genes, as MYL2, MYL3, ACTC1, TNNI3, TPM1 [25] represent a cluster of genes minorly affected by mutations, but in a variable percentage, still responsible of HCM [26,27]. The extended program of molecular screening, followed during last 10 years using NGS technology, determined the identification of 1400 gene mutations, directly related with HCM [21,23].

A short list of three genes mutations [28], represent the more consistent nucleus of inherited HCM. The mutation of MYBPC3 (30-40%), MYH7 (20-30%), TNNT2 (10-15%) [29] represent together almost 70-85% of mutations responsible of HCM (Table 3). The familial DCM is a heart disease inherited as monogene disorder. The prevalence of DCM is estimated around 1:2500-3000, with an incidence of 7/100.000/year [28] and a way of inheritance mainly dominant autosomal (85%), in minor part recessive autosomal, X linked and rarely matrilineal [30]. A great number of gene mutations, occurred in DCM are related with an involvement of sarcomeric proteins [31], cytoskeletal proteins, z disk proteins, nuclear and mithocondrial proteins [30]. Familial forms of DCM represent about 20-48% of all forms of DCM, showing an incomplete penetrance and different degrees of expressivity [31]. Almost 20% of familial forms of DCM are due to mutations of the TTN gene [29], determining an important alteration of the titin structure. In conclusion, has been well documented that same genetic mutations, observed in the same families groups, can arise distinct forms of cardiomyopathies [32], as HCM and DCM, with different profiles of clinical expression, documented by the variations of onset age of heart failure symptoms and the presence of lethal arrhythmias as well.

The Genetic Alterations in HCM and DCM

The growing role of molecular diagnostics, during last two decades, realized a substantial improving of knowledge about genetic mechanism, implicated in cardiomyopathies determinism. HCM and DCM represent, actually, two major form of CMs wherein has been possible to demonstrate the dependence from genes mutations (Table 3 & 4). Although many genes mutations have been well correlated with HCM and DCM phenotypes [32], by the other hand there are many controversial situations, where is not possible to reach a rapid and easy correlation among two phenotypes described above and a gene mutation [24]. Furthermore, in many occasions has been possible to observe, a phenotypes overlapping, represented by different gene mutations able to reproduce a similar phenotype. In many cases, related to a familial disease recurrence, there are not evidences supporting the genetic damage, remaining hence largely unresolved. Actually are known a large number of genes strictly related with the different typology of CM.

At least 100 and more genes and more than 1500 their mutations have been genetically identified, characterized and tried to associate either to HCM or DCM [33]. The hypertrophic form of CM, is the cardiomyopathy mainly related with genetic alterations (Table 3) [12,15-17,25,34-43]. In the great pool of genes investigated, the major attentions are reserved to mutations of 15 genes described as "determinants" to generate HCM phenotypes [1-15] of (Table 3) [44-48]. Actually more than 400 mutations represent the genetic base of HCM. An important number of these mutations involve the proteins network representing the myocyte architecture. Sarcomeric proteins, zeta disc proteins, sarcoplasmic reticular proteins, enzymes ecc. [21] are differently involved in genetic aberrations registered in HCM [49]. As affirmed above, 400 mutations have

been characterized as reflecting the alterations of MYH7 gene (more than 200 mutations) and MYBPC3 gene (150 mutations) (Table 5). Sarcomeric proteins are primarily damaged in their structural part (globular head of beta myosin heavy chain) [21]. About 96% of mutations described in MYH7 are classified as missense, resulting in a non-disruption of the reading frame. Conversely the 150 mutations observed on MYBPC3, in 70 % of cases are classified as non sense mutations (stop codon, deletion, insertion, and translocation), resulting in disruption of the reading frame and generating a null allele.

Table 3: Genes Involved in HCM.

Gene	Prevalence	Inheritance	Proteins
MYH7	30-35	AD	SARCOMERE
MYBPC3	25-30	AD	SARCOMERE
TNNT2	15-20	AD	SARCOMERE
TPM1	<5	AD	SARCOMERE
TNNI3	5	AD	SARCOMERE
MYL 2	<1	AD	SARCOMERE
MYL 3	<1	AD	SARCOMERE
ACTC	<1	AD	SARCOMERE
TTN	<1	AD	SARCOMERE
PRKAG2	<1	AD	KINASE
TNNC1	<1	AD	SARCOMERE
TNNI3	<1	AD	SARCOMERE
MYH6	<1	AD	SARCOMERE
TCAP	<1	AR, AD, IU	Z DISC
CSRP3	<2	AD	Z DISC
BAG3	rare	AD	Z DISC
TTR	rare	AD	TRANSP. PROT.
PLN	rare	AD	SARCOP. RET.
LDB3	rare	AD	Z DISC
VCL	rare	AD	Z DISC
CAV3	rare	AD-AR-IU	PLASMA MEMB.
ACTN2	rare	AD	Z DISC
ANKRD1	rare	IU	Z DISC
MYLK2	rare	IU	KINASE
MYOZ2	rare	AD	Z DISC
NEXN	rare	AD	Z DISC
RYR2	rare	AD	SARCOP. RET.
CASQ2	rare	AD	SARCOMERE
GLA	rare	XL	LYSOSOME
JPH2	rare	AD	SARCOMERE
LAMP2	rare	XL	LYSOSOME
MYPN	rare	AD	SARCOMERE

In the last years, many others genes have been included in the HCM determinism, arriving to describe about 1000 variants, including not only sarcomeric proteins, but also zeta disc, plasma membrane and sarcoplasmic proteins (Tables 3 & 5). In about 5% of cases, have been signaled multiple mutations [50], occurring in MYH7 and MYBPC3 genes. Multiple genes mutations have been found in four families, wherein people were affected of symptomatic HCM testifying the role of private mutations of certain type on gene mutation. Recent guidelines for HCM diagnosis, recommend genetic test including five genes: MYH7, MYBPC3, TNNT2, TNNI3 and TPM1 (Table 3).

Table 4: Genes involved in DCM.

Gene	Crom	Bases	Exons	Trasm (*)	DIS	Proteins
ACTC1	15q14	7631	7	AD	DCM	Actin alpha cardiac 1
BAG3	10q26.11	26450	4	AD	DCM	BCL2 associate to athanogene
CRP3	11p15.1	156560	3	AD	DCM	Muscular protein LIM C REACTIVE PROTEIN 3 (223)
CSRP3	11p15.1	28543	6	AD	DCM	Cystein and Glycin Rich Proteins 3
DES	2q35	8363	9	AD	DCM	Desmin
DMD	Xp21	2241933	79	AD	DCM	Dystrophin (3685 aa)
DSP	6p24	45143	24	AD	DCM	Desmoplakin (2871 aa)
FKRP	19q13.32	30943	3nc 1pc	AD	DCM	Protein correlated to Fukutine
LMNA	1q22	57517	12	AD	DCM	Lamin A/C
LDB3	10q22.2-q23.3	67620	16	AD	DCM	LIM legante Dominio 3 (727 aa)
MYBPC3	11p11	21297	35	AD	DCM	Myosin link C Protein
MYH7	14q12	22981	40	AD	DCM	Heavy chain 7 Beta Myosin
MYOZ1	10q22.1	10104	6	AD	DCM	Myozenin 1 (299 aa)
PLN	6q22.31	12452	2	AD	DCM	Phospholamban (52 aa)
PSEN1	14q24.3	87257	14	AD	DCM	Pre senilyn 1 (467 aa)
PSEN2	1q31-q42	25922	12	AD	DCM	Pre senilyn 2 (448 aa)
SCN5A	3p21	101617	28	AD	DCM	Channel N voltage dip. Type V alpha (2016 aa)
SGCD	5q33	897446	9	AD	DCM	Delta sarcoglycan (289 aa)
SYNE1	6q25	516118	147	AD	DCM	Nexprin 1 (8997 aa)
SYNE2	14q23.2	373485	116	AD	DCM	Nexprin 2 (6885 aa)
TAZ	Xq28	10212	12	XL	DCM	Tafaxin (292 aa)
TCAP	17q12	2369	2	AD	DCM	Telethonin (167 aa)
TMPO	12q22			AD	DCM	Timopoietina
TNNC1	3p21.1	2980	6	AD	DCM	Cardiac Troponin C1 (161 aa)
TNNI3	19q13.42	6007	8	AD	DCM	Cardiac Troponin I3 (210 aa)
TNNT2	1q32.1	18755	18	AD	DCM	Cardiac Troponin T2
TPM1	15q22.1	29284	15	AD	DCM	Alpha Trophomyosin (7 IF)
TTN	2q24.3	304814	367	AD	DCM	Tithin (13 IF)
VCL	10q22.1-q23	122047	22	AD	DCM	Metavimculin (1134 aa)
ACTN2	15q14	78178	21	AD	DCM	Alpha actinin 2 (894 aa)
ANKRD1	10q23-.31	9181	9	AD	DCM	Ankirin (319 aa)
ABCC9	12p12	144014	38	AD	DCM	ATP binding cassette sub family C member 9 (1545 aa)
EMD	Xq28	2327	6	AD	DCM	Emerin (254 aa)
EYA4	6q23	291523	19	AD	DCM	Eyes absent homolog 4 (639 aa)
HBEGF	5q23	13789	6	AD	DCM	Heparin Bindin Epidermal Growth Factor(208 aa)
SRA1	5q31.3	20971	5	AD	DCM	Steroid Recptor RNA activator (236 aa)
IK	5q31.3	15423	20	AD	DCM	IK Cytochine Down regulator HLA II (557 aa)
DNM 2	19p13.2	115436	22	AD	DCM	Dynamin 2 (870 aa)
SGCB	4Q12	17788	6	AD	DCM	BETA sarcoglycan (318 aa)
RBM20	10q25.2	195073	14	AD	DCM	RNA ligand Protein 20 (1227)

(*)AD: Autosomal Dominant; AR: Autosomal Recessive; XL: X Linked.

Table 5: MYH7 and MYB3 mutations in HCM.

MYH7	MYB3
C13267G	IVS21-2a13858g
T13213C	G13980A
G12765A	Dupl. 15049-15063
C12739G	IVS23+1:g15131a (I)
C12707A	A15829G (I)
G12361A	Ins. G15919
G12338A	A16088G
G12307T	Del. CGCGT (16189-16193)
G12148A	Del. CGCGT (16190-16194)
G12147T	C16154T
G12138A	Del. C16212
G11306T	G17721A
G11282A	IVS26 Del. Gt (17773-17774) (I)
G11281A	Del. CT (18566-18567)
G11271A	G20410T
G10457A	G21034A
G9494A	Del. G21059
A9483G	G21524A
C9123T	21420 Ins.(21404-21415) Del. (21420-21423)
T9094A	C2377T
C9049T	Del. CCAGGGA(2376-2382)
C8847T	A5254C
C8848A	IV57+5:g5828a (I)
C8848T	G6011A
G8278A	G6014A
76685C	IVS12+2a:g7308g (I)
G6643A	G7360A
A6491G	T7435C
G6325A	IVS14-2:a10385g (I)
C6277A	Del. TT(10957-10959)
G4508A	Del. T10587
G18153C	Del. C10618
G22243A	C10951T
G21752A	Del. (10957-10959)
T17905G	Del. GC (11047-11048)
G19236T	G11070C
C19222T	IVS17+2:111073c (I)
G19227A	Del. A12413
DEL. E883	Y237C
DEL. E930	G263X
S4L	A328fs del G
F247L	V342D
R453C	Q 404 fs del C
A583V	R495V
R663H	G531R
R723G	G532 fs del G
R787C	E542Q
M822V	A627N
R870H	R726C
K1459N	R733H
	V771M
	M844 fs ins GA
	R891 fs ins G
	Q998E
	R1022S
	R1138H

Differently to HCM genetics, the dilated cardiomyopathy shows a less connection with genetic damage, resulting in many cases the idiopathic origin and only in 30% a familiar relation. As already evidenced above, the familial way of inheritance may change from the autosomal dominant form, to autosomal recessive and x linked recessive as well [51]. In few cases, even mitochondrial inheritance patterns and matrilineal transmission have been described [30]. A relevant number of genes were studied and sequenced during last year's and about 40 at the moment seems to be related with DCM [50,52,53]. TTN gene, for example, is one of the largest genes of our genome that seems to have a relevant role in DCM [29,31]. TTN gene (379 exons), indeed, shows a large number of rare variants, that in 25% of familial cases can result causative of DCM [54].

Lamin A/C gene, [51,55] is normally related with heart conduction system diseases but recently is suspected to be linked to DCM since about 5-8 familial cases of familial DCM are considered to be depending by alterations of lamin protein. Two news mutations of LMNA, described as R349L and R190W, have been correlated with DCM. Nevertheless LMNA gene, received further investigation and it revealed an interesting spreading of number variants, showing in 11 of them, important signs of pathogenicity. To complete the picture of LMNA gene mutations [55] and their correlations with pathological phenotypes, recently has been determined the mutation R189W, very close to the common mutation R190W, representing an "hot spot" region at exon 3. The "hot spot" DNA regions represent a very sensitive area, with high susceptibility to mutation, due to his intrinsic instability, where is very frequent to find mutations. Many others genes correlated with sarcomeric and not sarcomeric proteins, are actually related with DCM determinism.

Phospholamban (PLN), Ankyrin (ANKRD1), Nesprin (SYNE1), Emerin (EMD), Dystrophin (DMD), BCL-2 Athanogene (BAG3) [53], RBM20 [52] and many others, represent a large family genes, involved a different title in DCM. Investigators keep on continuing in active study strategy, in the attempt to understand the still obscure and unexplained relation genotype-phenotype.

In addition to the known mutations, that play a role in DCM, is fundamental to recognize the importance of "private

mutations", when the mutation is the only described in a single family and found in different members of the family. Although the study of news mutations is always active, actually a great relevance is attributed to the rare variants as crucial keys for disease determination. The genetic analysis of families affected of DCM often revealed a "private" stage of the abnormalities observed, with the impossibility to extend their significance a role of general law [52,53,56]. Furthermore, there is a general consensus to refer the phenotypic diversity, among individuals with common genetic aberrations, as an epigenetic inheritance. Furthermore, there is a general consensus to refer the phenotypic diversity, among individuals with common genetic aberrations, as an epigenetic inheritance [52,53]. In few words, as a consequence of a "force" acting in the direction to modify and conditioning the gene expression, even in absence of real genes mutations. As already affirmed above, often different mutations of the same gene may reveal two different phenotypes, DCM or HCM, consolidating the hypothesis of a final common pathway of expression, for a great number of familial cardiomyopathies.

Next generation sequencing technologies

It was nearly ten years since when, on 2005, new high throughput technologies and their applications were available for molecular and clinical diagnostics [57-65]. The new frontiers of sequencing, simultaneously, a great number of genes at reasonably low costs, changed suddenly the picture of the diagnostics for many diseases, since long time under molecular investigation. The pyrosequencig and sequencing by synthesis, were the more promising technologies that finally realized the purpose to determine, in a short time and a low cost, the entire unknown genes sequence, suspected to play a crucial role in cardiomyophaty etiology, as well in oncology and other diseases [58-61]. However the limiting factors of NGS applications were substantially represented by the amplification and the long time of sequencing, on the other hand the need to manage a huge amount of digital data, furnished by the sequencing process of the genes studied.

The high throughput NGS technologies, were built to produce a large amount of data that needed of a continuous process of verification, analyses and alignment [8,59-62]. The need to use powerful digital tools, as dedicated hardware and software able to manage and analyze many terabytes of digital data, represented a weak point that moved the research toward new technologies able to give a major support to NGS applications of first and second generation [8]. Following the way of a guided evolution, the NGS technologies in their development are grown, passing through different steps, as NGS of third generation and reaching, recently the fourth generation level of evolution. The main objective of this high level is to solve a group of problems related to the fine analysis of certain DNA zone, including the characterization of the genetic variants such as single nucleotide polymorphism (SNPs).

To analyze the global contest of genetic individual profile, through whole sequencing exome (WSE) and whole sequencing genome (WSG) [8,66], looking for further evidences, as structural variants and copy number variations (CNV) [67], is fundamental to try clarify correlation between an insufficient explained genotype structure and the presence of a cardiomyopathy phenotype. We need further evidences, about the role of genetic and epigenetic influences and abnormalities, to create a solid base that make possible a linear correlation between genetic damage and a catalogue of thousands of molecular alterations and their hypothetic diseases type. More recent NGS techniques known as NGS of third and fourth generation, are already in use, even though only for research purpose [68-72]. One of important steps forward, made through the aid of NGS technologies is represented by the possibility to avoid the amplification phase of DNA, reducing sequencing errors and biases. The single molecule sequencing technique (SMS) [70], represents the core of NGS platform of third generation and has been introduced in 2008.

The strong point of single molecule sequencing technology, include an higher throughput, longer read lengths and direct RNA sequencing. The availability of longer read lengths creates the conditions to enable the direct detection of haplotypes and the discover of rare variants. Furthermore, the use of a different way (laser or pH measurement) to ensure a correct lecture of the nucleotides sequence, of genes studied have simplified and shortened the entire process of sequencing. The NGS platform of fourth generation [70], started with his applications around 2011 and is linked to the nanopore technology. Nanopore sequencing [68,69,72] is an apparently easy way to sequence a gene, or a larger part of DNA structure, as the whole exome or whole genome. This technology is based on the concept that a single DNA molecule can be sequenced, without amplification, when passing through a tiny nanopore channel, in a biological nanopore or on a solid-state support as graphene, silicon nitride or silicon oxide and aluminium nitride or molibdeno disulfide materials [72].

The main objective of this technology is to simplify the complexity of sequencing methodologies, in the attempt to short the time of sequencing and limiting the errors. The nanopore platform, in his proposal, try to include the five elements that make winning a technology: cheaper instruments, lower cost for determination, longer reads, faster speed and transportability, nevertheless this technology is graved by frequents errors, during sequencing, probably due to the pore diameter and his geometry and the charge of the pore surface as well [72]. The advent of NGS platforms of third and fourth generation, represented an evident advantage, creating the bases to disclose the unknown area of knowledge represented by the study of genetic variants (SNPs) [8]. The study of polymorphism, including novel and rare variants and variants of unknown clinical significance (VCS) [70], copy number variations (CNVs) [67], structural variants and the genes fusion, is an important chapter that is waiting to be written.

All these aberrations and their complexes network of interactions still represent a wall for the comprehension of the CMs determinism. NGS advanced platforms using a single molecule sequencing, hence are considered important factors need to fill the existing diagnostics gap represented by the NGS platforms of second generation, unsuitable to perform whole genome sequencing (WAS), whole exome sequencing (WES) and RNA sequencing, useful for transcriptome analysis [68] (Table 6 & 7).

Table 6: Advanced NGS of third and fourth generation. Strategic and potential utility in molecular and clinical diagnostics

Single Point Mutations
Large genomic Alterations
Insertions, deletions, Translocations
Common Variants (SNPs)
Copy Number Variations (CNV)
Copy number alterations
Variants of unknown clinical significance
WES
WGS
Transcriptome Analysis

Table 7: Genes involved in DCM.

GENE	CROM	BASES	EXONS	TRASM (*)	DIS	PROTEINA AA
ACTC1	15q14	7631	7	AD	DCM	Actin alpha cardiac 1
BAG3	10q26.11	26450	4	AD	DCM	BCL2 associate to athanogene
CRP3	11p15.1	156560	3	AD	DCM	Muscular protein LIM C REACTIVE PROTEIN 3 (223)
CSRP3	11p15.1	28543	6	AD	DCM	Cystein and Glycin Rich Proteins 3
DES	2q35	8363	9	AD	DCM	Desmin
DMD	Xp21	2241933	79	AD	DCM	Dystrophin (3685 aa)
DSP	6p24	45143	24	AD	DCM	Desmoplakin (2871 aa)
FKRP	19q13.32	30943	3nc 1pc	AD	DCM	Protein correlated to Fukutine
LMNA	1q22	57517	12	AD	DCM	Lamin A/C
LDB3	10q22.2-q23.3	67620	16	AD	DCM	LIM legante Dominio 3 (727 aa)
MYBPC3	11p11	21297	35	AD	DCM	Myosin link C Protein
MYH7	14q12	22981	40	AD	DCM	Heavy chain 7 Beta Myosin
MYOZ1	10q22.1	10104	6	AD	DCM	Myozenin 1 (299 aa)
PLN	6q22.31	12452	2	AD	DCM	Phospholamban (52 aa)
PSEN1	14q24.3	87257	14	AD	DCM	Pre senilyn 1 (467 aa)
PSEN2	1q31-q42	25922	12	AD	DCM	Pre senilyn 2 (448 aa)
SCN5A	3p21	101617	28	AD	DCM	Channel N voltage dip. Type V alpha (2016 aa)
SGCD	5q33	897446	9	AD	DCM	Delta sarcoglycan (289 aa)
SYNE1	6q25	516118	147	AD	DCM	Nexprin 1 (8997 aa)
SYNE2	14q23.2	373485	116	AD	DCM	Nexprin 2 (6885 aa)
TAZ	Xq28	10212	12	XL	DCM	Tafaxin (292 aa)
TCAP	17q12	2369	2	AD	DCM	Telethonin (167 aa)
TMPO	12q22			AD	DCM	Timopoietina
TNNC1	3p21.1	2980	6	AD	DCM	Cardiac Troponin C1 (161 aa)
TNNI3	19q13.42	6007	8	AD	DCM	Cardiac Troponin I3 (210 aa)
TNNT2	1q32.1	18755	18	AD	DCM	Cardiac Troponin T2
TPM1	15q22.1	29284	15	AD	DCM	Alpha Trophomyosin (7 IF)
TTN	2q24.3	304814	367	AD	DCM	Tithin (13 IF)
VCL	10q22.1-q23	122047	22	AD	DCM	Metavimculin (1134 aa)
ACTN2	15q14	78178	21	AD	DCM	Alpha actinin 2 (894 aa)

ANKRD1	10q23-.31	9181	9	AD	DCM	Ankirin (319 aa)
ABCC9	12p12	144014	38	AD	DCM	ATP binding cassette sub family C member 9 (1545 aa)
EMD	Xq28	2327	6	AD	DCM	Emerin (254 aa)
EYA4	6q23	291523	19	AD	DCM	Eyes absent homolog 4 (639 aa)
HBEGF	5q23	13789	6	AD	DCM	Heparin Bindin Epidermal Growth Factor(208 aa)
SRA1	5q31.3	20971	5	AD	DCM	Steroid Recptor RNA activator (236 aa)
IK	5q31.3	15423	20	AD	DCM	IK cytochine Down regulator HLA II (557 aa)
DNM 2	19p13.2	115436	22	AD	DCM	Dynamin 2 (870 aa)
SGCB	4Q12	17788	6	AD	DCM	BETA sarcoglycan (318 aa)
RBM20	10q25.2	195073	14	AD	DCM	RNA ligand Protein 20 (1227)

(*) AD: Autosomal Dominant; AR: Autosomal Recessive; XL: X Linked; Matrilineal: Maternal Transmission

Role of Variants and Epigenetic Influence on Phenotypic Variability

As extensively underlined above, many questions about correlations among genetics alterations and phenotype expression are still without answers. Even though the NGS most advanced technologies will be able to discover news mutations using WGS, WES and transcriptome analysis [73], there is a real perception that epigenetic factors play a fundamental role in phenotype variability of cardiomyopathies. Meanwhile specific attention is reserved to the role played by DNA variants (common variants, rare variants and unknown clinical significance variants) and their capacity to influence and modulate the expression of a nearby mutated gene. The different types of variants identified, can be include in the "family" of genetic modifiers, having a role of modulator, eliciting either down regulation or up regulation of the gene expression. The influence exercised, by locally acting variants (SNP), on a mutated gene, either close or remote from it, has been well documented in the case of Long QT syndrome [8], where a SNPs located in UTR (untranslated region) of KNC1 gene, is able to modify the expression of allele mutating the global amount of protein produced by the normal and mutated allele [54].

Summarizing, common SNPs are normally silent but when they are located close to a strategic area as a UTR of a gene mutated may become relevant influencing directly the gene expression [8]. To find a correct and exhaustive explanation for those cases which initially were associated with HCM and subsequently after years have been reclassified in DCM, in many occasion has been invoked the epigenetic involvement [74-85]. Reasoning about epigenetic factors, recent investigations highlighted the role of DNA methylation in cardiomyopathies determination. There are growing and consolidate evidences testimony a strong correlation among an aberrant CpG islands (CGIs) methylation and the phenotypic modulation of cardiomyopathies [77]. Recent studies conducted in patients affected of heart failure, focusing LY75 (lymphocyte antigen 75), ADORA2A (adenosin receptor A2) genes and their level of methylation [77], revealed a reduced expression of proteins related genes, in heart myocardial cells, potentially responsible of heart failure. It is reasonable the idea that an increased DNA mutilation of CGIs area, nearby LY75 gene and the promoter area [77], play a key role favoring the emersion of disease in DCM patients. Talking about epigenetic factors, very important attention have to be reserved to genetic and clinical significance of micro RNA (miRNA) [8,81-83]. Micro RNA and their role in cardiomyopathies have been well investigated during last 10 years [82,83]. A crucial role seems to be related to the capacity of miRNA to influence and modulate the genes expression acting as post transcriptional factors able either to silence or upregulate the gene [82].

In 2007, was first discovered the relation among the increase of miR-21, miR-29h, miR-129, miR-210, miR-211, miR-212, miR-423, the reduction of miR-30, miR182, miR526 and a picture of heart failure [8,85]. Furthermore, has been evidenced as the over expression of miR-208a is correlated with an increased expression of gene MYH7 and hypertrophy, in patient documenting heart failure. Many others miRNA have been correlated with the gene expression modulation, inducing phenotype variability [8,82]. Recently, investigators are studying the role of circulating miRNA and their potential use as biomarker, useful for diagnostic and therapeutic purpose. It has been demonstrated a close relation among circulating BNP (brain natriuretic peptide) and plasma circulating miR-93 and miR-106b [85], in patients affected of heart failure. The plasma levels of those miRNA could be responsible of the BNP modulation in patients undergone to HF pharmacological treatment [84].

Conclusion

The study of cardiomyopathies, unfortunately, demonstrated a nature complicated and a multi factorials and multigenic determinism. The picture frame, where actually DCM and HCM are positioned, is certainly more defined, even if lights and shadows make the CMs landscape still uncertain and confuse. The important level of heterogeneity shown by CMs, still represent a concrete obstacle to the full etiology comprehension. Therefore the NGS revolution, accomplished in ten years, represents the path to follow to wide and improve the knowledge of genes structure, their damages and the complicate network frame, connecting the gene expression, epigenetic factors,

environmental factors and the role played by DNA, coding or non coding, variants. Actually NGS technologies are providing, routinely, the identification of genes involved in CMs, operating sequencing of a selected panel of targeted genes. It will result fundamental the use of NGS most advanced applications, defined of third and fourth generation, as for example the promising nanopore technologies, indispensables tools to realize a fine investigation of the whole exome, whole genome and the transcriptome, in the attempt to isolate all gene mutations, variations a their relationship [8,66].

A further problem to solve is represented by the huge amount of digital data furnished by the high throughput applications that will need of appropriate digital tools to manage correctly and rapidly genetics information coming from sequencing procedures [8]. The future scenery of diagnostics, therapy and prevention of cardiomyopathies is all concentrated in the evolution of the ultra modern technologies of DNA sequencing and their applications, as useful tools to realize genetic testing. It is important emphasize the relevance of genetic testing, even though their nature still result to be probabilistic rather than deterministic. Accordingly with the recent guidelines (2011 - HRS/EHRA) genetic testing should be effected by members of families [8,86-103] whom CMs are suspected in consequence of disease acquired by other family members. The goal to reach in the next future of CMs will be to use genetic testing not only for diseases prevention or diagnostics purposes, but above all to drive a correct therapeutic regimen in personalized protocols.

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