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 Dilative CM (DCM).

Keywords: Cardiomyopathy; Hypertrophic CM; Dilative CM

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 Dilative 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 Dilative CM (DCM).
Table 1: Classification of Cardiomyopathies (ESC 2008).

| Classification of the Cardiomyopathies |
|---------------------------------------|
| 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                     |

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: myocarditis 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/Dysplasia (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                                                                 |
|--------|---------|-----------|-----------|------------------------------------------------------------------------|
| ABCC9  | 12p12   | AD        | DCM       | ATP-Binding Cassette, Sub-Family C                                      |
| ABLIM1 | 10q25   | AD        | DCM       | Actin binding LIM protein 1                                             |
| ACTC1  | 15q14   | AD        | DCM HCM   | 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                                                            |
| ANKR1D | 10q23–31| AD        | DCM HCM   | BCL2 associate to thanogene                                             |
| APOC3  | 10q26.11| AD        | DCM HCM   | Calretinin 3                                                           |
| CASQ2  | 1p13.1  | AD        | HCM       | Calsequestrin 1                                                         |
| CAV3   | 3p25    | AD        | DCM HCM   | Caveolin 3                                                              |
| COX15  | 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    | 19q13   | AD        | DCM       | Dystrophyn (3685 aa)                                                   |
| DMPK   | 19q13.3 | AD        | DCM       | dystrophya myotonic Protein Kinase                                      |
| DOLK   | 9q34    | AD        | DCM       | Dolico1 Kinase                                                          |
| DSC2   | 18q12   | AD        | DCM       | Desmocollin 2                                                          |
| DSG2   | 18q12   | AD        | DCM       | Desmoglein 2                                                            |
| DSP    | 6q24    | AD        | DCM       | Desmoplakyn                                                             |
| 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       | Fataxin                                                                |
| FHTN   | 9q31-q33| AD        | DCM       | Fukutin                                                                |
| FKRP   | 1q13.32 | AD        | DCM       | Protein correlated to Fukutin                                          |
| GATA1D1| 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       | Junctophilin 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       | Mitochondrial Ribosome Protein                                          |
| MYBPC3 | 11p11   | AD        | DCM HCM   | Myosin ligand Protein C                                                 |
| Gene Symbol | Chromosome Location | Disease | Description |
|-------------|---------------------|---------|-------------|
| MYH6        | 14q1.2           | 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 homeobox 5 |
| PDLM3       | 4q35              | AD DCM | PDZ Lim protein 3 |
| PLN         | 6q22.1            | AD DCM HCM | Phospholamban |
| PKP2        | 12p11             | AD DCM | Plakophilin 2 |
| PRKAG2      | 7q35-q36.36       | AD HCM | Protein 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 tyrosin phosphatase non receptor type |
| RBM20       | 10q25.2           | AD DCM | RNA binding Protein 20 |
| RYR2        | 14q2.1-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 |
| SGGG        | 13q12             | AD DCM | Gamma sarcoglycan |
| SYNE1       | 6q25              | AD DCM | Nexrin 1 |
| SYNE2       | 14q23.2           | AD DCMDCM | Nexrin 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 |
| TMP0        | 12q22             | AD DCM | Tymopheoitin |
| TNNC1       | 3p21.1            | AD DCM HCM | Cardiac Troponin C1 (161 aa) |
| TNN13       | 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 Troponyosin (7 IF) |
| TTD         | 5q31              | AD DCM | Myotilin |
| TTN         | 2q24.3            | XL DCM HCM | Titin (13 IF) |
| TTR         | 18q11             | AD HCM | Transthyretin |
| VCL         | 10q22.1-2q3       | AD DCM HCM | metavinulin |
| MTTLI       | mRNA matrilineal  | DCM | tRNA |
| MTNDI       | mRNA matrilineal  | DCM | NADH dehydrogenase sub. 1 |
| MTATP6      | mRNA matrilineal  | DCM | ATP synthetase 6 |
| MTYY        | mRNA 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 etioloval 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 cardiomyopathy (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, lethal arrhythmias 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, TNNB3, 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 mitochondrial proteins [30]. Familial forms of DCM represent about 20-40% 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, sarcomplasmic 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  |
| MYL2     | <1         | AD          | SARCOMERE  |
| MYL3     | <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          | SARCO. RET. |
| LDB3     | rare       | AD          | Z DISC     |
| VCL      | rare       | AD          | Z DISC     |
| CAV3     | rare       | AD-AR-IU    | PLASMA MEMB. |
| ACTN2    | rare       | AD          | Z DISC     |
| ANKRDI   | rare       | IU          | Z DISC     |
| MYLK2    | rare       | IU          | KINASE     |
| MYOZ2    | rare       | AD          | Z DISC     |
| NEXN     | rare       | AD          | Z DISC     |
| RYR2     | rare       | AD          | SARCO. 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 asymptomatic 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   | Chromosome | 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 thanogene                    |
| CRP3   | 11p15.1    | 15650 | 3     | AD        | DCM | Muscular protein LIM C REACTIVE PROTEIN 3 (223) |
| CSRP3  | 11p15.1    | 28543 | 6     | AD        | DCM | Cystein and Glicin Rich Proteins              |
| DES    | 2q35       | 8363  | 9     | AD        | DCM | Desmin                                        |
| DMD    | Xp21       | 398   | 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 Domnio 3 (727 aa)                |
| MYBPC3 | 11p11      | 21297 | 35    | AD        | DCM | Myosin in 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 senlyn 1 (467 aa)                        |
| PSEN2  | 1q31-q42   | 25922 | 12    | AD        | DCM | Pre senlyn 2 (448 aa)                        |
| SCN5A  | 3p21       | 101617| 28    | AD        | DCM | Channel N voltage dip. Type V alpha (2016 aa) |
| SGCD   | 5q33       | 89746 | 9     | AD        | DCM | Delta sarcoglycan (289 aa)                   |
| SYNE1  | 6q25       | 516118| 147   | AD        | DCM | Nesprin 1 (8997 aa)                          |
| SYNE2  | 14q23.2    | 373485| 116   | AD        | DCM | Nesprin 2 (6885 aa)                          |
| TAZ    | 2q28       | 10212 | 12    | X         | DCM | Tafaxin (292 aa)                             |
| TCAP   | 17q12      | 2369  | 2     | AD        | DCM | Telethonin (167 aa)                          |
| TMP0   | 12q22      |       |       | AD        | DCM | Timopoeotina                                  |
| 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 | Titthin (13 IF)                              |
| VCL    | 10q22.1-q23| 122047| 22    | AD        | DCM | Metavinculin (1134 aa)                       |
| ACTN2  | 15q14      | 78178 | 21    | AD        | DCM | Alpha actin 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    | 1q28       | 2327  | 6     | AD        | DCM | Emerin (254 aa)                              |
| EYA4   | 6q23       | 12912 | 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 Receptor RNA activator (236 aa)      |
| IK     | 5q31.3     | 15423 | 20    | AD        | DCM | IK Cytochine Down regulator HLA II (557 aa)   |
| DNM2   | 19p13.2    | 115436| 22    | AD        | DCM | Dynamin 2 (870 aa)                           |
| SGCb   | 4Q12       | 17708 | 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     | IVS2-1-2a13858g |
| T13213C     | G13980A     |
| G12765A     | Dupl. 15049-15063 |
| C12739G     | IVS23+1:g15131a (I) |
| G12707A     | A15829G (I) |
| G12361A     | Ins. G15919 |
| G12338A     | Del. CGCGT (16189-16194) |
| G12148A     | Del. CT (18566-18567) |
| G11282A     | Del. CT (18773-17774) (I) |
| G11281A     | G20410T     |
| G11271A     | G21059G     |
| G10457A     | Del. CCAGGGA(2376-2382) |
| G9483A      | IVS12+2a:g7308g (I) |
| G9482A      | G7360A      |
| A9483G      | Del. CCAGGGA(2376-2382) |
| C9123T      | 21420 Ins.(21404-21415) Del. (21420-21423) |
| T9094A      | C3277T      |
| C9049T      | Del. CCAGGGA(2376-2382) |
| C8847T      | A5254C      |
| C8848A      | G5614A      |
| C8848T      | G601IA      |
| G8278A      | G601A       |
| G6685C      | G7360A      |
| G6643A      | T7435C      |
| A6491G      | IVS14-2:a10385g (I) |
| G6325A      | Del. TT(10957-10959) |
| C6277A      | Del. T10587 |
| G4508A      | Del. C10618 |
| G1815C      | Del. C10618 |
| G22243A     | C10951T     |
| G21752A     | Del. (10957-10959) |
| T17905G     | Del. GC (11047-11048) |
| G19236T     | G11070C     |
| C19222T     | IVS17+2:11107c (I) |
| G19227A     | Del. A12413 |
| DEL E883    | Y237C       |
| DEL E930    | G623X       |
| S4L         | A3286 del G |
| F247L       | V342D       |

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 new 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 new 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 pyrosequencing 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 gene sequences, suspected to play a crucial role in cardiomyopathy 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 molybdeno 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 grated by frequent 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 | 8365 | 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 Domino 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 | Nesnexin 1 (8997 aa) |
| SYNE2 | 14q23.2 | 373485 | 116 | AD | DCM | Nesprin 2 (6885 aa) |
| TAZ | Xq28 | 10212 | 12 | XL | DCM | Tafaxin (292 aa) |
| TCAP | 1q22 | 1082 | 2 | AD | DCM | Telethonin (167 aa) |
| TMPO | 12q22 | 2369 | | AD | DCM | Timopoietina |
| TNN1 | 3p21.1 | 2980 | 6 | AD | DCM | Cardiac Troponin C1 (161 aa) |
| TNNC1 | 19q13.42 | 6007 | 8 | AD | DCM | Cardiac Troponin C3 (210 aa) |
| TNNT2 | 3q23.1 | 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) |
| Gene       | Chromosome | Position | Dominance | Disease |
|------------|------------|----------|-----------|---------|
| ANKRD1     | 10q23.31   | 9181     | AD        | DCM     |
| ABC9       | 12p12      | 144014   | AD        | DCM     |
| EMD        | Xq28       | 2327     | AD        | DCM     |
| EYA4       | 6q23       | 291523   | AD        | DCM     |
| HBEGF      | 5q23       | 13789    | AD        | DCM     |
| SRA1       | 5q31.3     | 20971    | AD        | DCM     |
| DKM2       | 19p13.2    | 115436   | AD        | DCM     |
| SGCb       | 4Q12       | 17788    | AD        | DCM     |
| RBM20      | 10q25.2    | 195073   | AD        | DCM     |

* 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 new 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 included 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 testifying 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 mutillation 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, indispensible 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.

References

1. Briddon W (1957) Uncommon Myocardial Diseases. Lancet 273(7007): 1179-1184.
2. Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, et al. (2008) Classification of the cardiomyopathies: a position statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J 29(2): 270-276.
3. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, et al. (2006) Contemporary Definitions and Classification of the Cardiomyopathies. An American Heart Association Scientific Statement From the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups and Council on Epidemiology and Prevention. Circulation 113(14): 1807-1816.
4. Arbustini E, Narula N, DeG GW, Reddy KS, Greenberg B, et al. (2013) The MOGE(S) Classification for a Phenotype-Genotype Nomenclature of Cardiomyopathy. J Am Coll Cardiol 62(22): 2046-2072.
5. Hamayak S, Skakian (2014) Cardiomyopathies: Evolution of pathogenesis concepts and potential for new therapies. World J Cardiol 6(6): 478-494.
6. Pascale Richard, Eric Villard, Philippe Charron, Richard Isnard (2006) The Genetic Bases of Cardiomyopathies. Journal of the American College of Cardiology 48(9): A79-A89.
7. Norton N, Li D, Hershberger RE (2012) Next-generation sequencing to identify genetic causes of cardiomyopathies. Curr Opin Cardiol 27(3): 214-220.
8. Meder B, Haas J, Keller A, Heid C, Just S, et al. (2011) Targeted next-generation sequencing for the molecular genetic diagnostics of cardiomyopathies. Circ Cardiovasc Genet 4(2): 110-122.
9. Norton N, Li D, Rieder MJ, Siegfried JD, Rampersaud E, et al. (2011) Genome-wide studies of copy number variation and exome sequencing identify rare variants in BAG3 as cause for dilated cardiomyopathy. Am J Hum Genet 88(3): 273-292.
10. Villard E, Perret C, Gary P, Proust C, Dilanian G, et al. (2011) A genome-wide association study identifies two loci associated with heart failure due to dilated cardiomyopathy. Eur Heart J 32: 1065-1076.
11. Gupta P, Bilinska ZT, Sylvius N, Boudreau E, Veinot JP, et al. (2010) Genetic and ultrastructural studies in dilated cardiomyopathy patients: a large deletion in the lamin A/C gene is associated with cardiomyocyte nuclear envelope disruption. Basic Res Cardioiol 105(3): 365-377.
12. Phelan D, Wilson GR, James PA, Lockhart PJ (2013) The genetics of cardiomyopathy, new technologies and the path to personalised medicine. OA Genetics 1(1): 9.
13. Herman DS, Lam L, Taylor MR, Wang L, Teekakirikul P, et al. (2012) Truncations of Titin Causing Dilated Cardiomyopathy. N Engl J Med 366(7): 619-628.
14. Yanushi Dullewe Wijeyeratne, Elijah R B (2013) Recent Developments in the Genetics of Cardiomyopathies 1(1): 21-29.
15. Tsoutsman T, Bagnall RD, Senscarian C (2008) The impact of multiple gene mutations in determining severity of cardiomyopathy and heart failure. Clin Exp Pharmacol Physiol 35(11): 1349-1357.
16. Baccarelli M, Rienstra E J, Benjamin (2010) Cardiovascular Epigenetics: Basic Concepts and Results From Animal and Human Studies. Circ Cardiovasc Genet 3: 567-573.
17. Geisterfer-Lowrance AA, Kass S, Tanigawa G, Vesberg HP, McKenna W, et al. (1990) A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain gene missense mutation. Cell 62(5): 999-1006.
18. Kamisago M, Sharma SD, DePalma SR, Solomon S, Sharma P, et al. (2000) Mutations in sarcomere protein genes as a cause of dilated cardiomyopathy. N Engl J Med 343(23): 1686-1696.
19. McNally E, Dellefave L (2009) Sarcomere mutations in cardiogenesis and ventricular non compaction. Trends Cardiovasc Med 19(1): 17-21.
20. Mogensen J, Kubo T, Duque M, Uribe W, Shaw A, et al. (2003) Idiopathic restrictive cardiomyopathy is part of the clinical expression of cardiac troponin i mutations. J Clin Invest 111(2): 209-216.
21. Jarcho J, McKenna W, Pare JA, Solomon SD, Holcombe RF, et al. (1989) Mapping a gene for familial hypertrophic cardiomyopathy to chromosome 14q1. J Am Coll Cardiol 2(22): 2046-2072.
22. Maron BJ, Gardin JM, Flack JM, Gidding SS, Kurosaki TT, et al. (1995) Prevalence of hypertrophic cardiomyopathy in a general population of young adults. Echocardiographic analysis of 4111 subjects in the cardia study. Coronary artery risk development in (young) adults. Circulation 92(4): 785-789.
23. Bos JM, Poley RN, Ny M, Tester DJ, Xu X, et al. (2006) Genotype phenotype relationships involving hypertrophic cardiomyopathy-associated mutations in titin, muscle lim protein, and telethonin. Mol Genet Metab 88(1): 78-85.
24. Coats CJ, Elliott PM (2013) Genetic biomarkers in hypertrophic
cardiomyopathy. Biomark Med 7(4): 505-516.

25. Richard P, Chaarron P, Carrier L, Ledieu C, Cheau T, et al. (2003) Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. Circulation 107(17): 2227-2232.

26. Saltzman AJ, Mancini-Di Nardo D, Li C, Chung WK, Ho CY, et al. (2010) Short communication: the cardiac myosin binding protein carg58/terp mutation: a common cause of hypertrophic cardiomyopathy. Circ Res 106(9): 1549-1552.

27. Maron BJ, Haas TS, Goodman JS (2014) Hypertrophic cardiomyopathy: one gene... But many phenotypes. Am J Cardiol 113(10): 1772-1773.

28. Charron P, Carrier L, Dubourg O, Tesson F, DesnoesM, et al. (1997) Penetration of familial hypertrophic cardiomyopathy. Genet Couns 8(2): 107-114.

29. Watkins H, McKenna WJ, Thierfelder L, Suk HJ, Anan R, et al. (1995) Mutations in the genes for cardiac troponin t and alpha-tropomyosin in hypertrophic cardiomyopathy. N Engl J Med 332(16): 1058-1064.

30. Watkins H, Rosenzweig A, Hwang DS, Levi T, McKenna W, et al. (1992) Characteristics and prognostic implications of myosin missense mutations in familial hypertrophic cardiomyopathy. N Engl J Med 326(17): 1108-1114.

31. Charron P, Dubourg O, Desnos M, Bennaceur M, Carrier L, et al. (1998) Clinical features and prognostic implications of familial hypertrophic cardiomyopathy related to the cardiac myosin-binding protein c gene. Circulation 97(22): 2230-2236.

32. Niimura H, Bachinski LL, Sangwatanaroj S, Watkins H, Chudley AE, et al. (1998) Mutations in the gene for cardiac myosin-binding protein c and late-onset familial hypertrophic cardiomyopathy. N Engl J Med 338(18): 1248-1257.

33. Maron BJ, Niimura H, Casey SA, Soper MK, Wright GB, et al. (2001) Development of left ventricular hypertrophy in adults in hypertrophic cardiomyopathy caused by cardiac myosin-binding protein c gene mutations. J Am Coll Cardiol 38(2): 315-321.

34. Michels S, Solomon OJ, Pfefferkon J, Hoedemaekers VM, Koflaid M, et al. (2009) Disease penetrance and risk stratification for sudden cardiac death in asymptomatic hypertrophic cardiomyopathy mutation carriers. Eur Heart J 30(21): 2593-2598.

35. Christians I, Birnie E, van Langen JM, van Spanendonck-Zwartz KY, van Tintelen JP, et al. (2010) The yield of risk stratification for sudden cardiac death in asymptomatic hypertrophic cardiomyopathy using myocardial biopsy in sarcomeric c gene mutation carriers: focus on predictive screening. Eur Heart J 31(7): 842-848.

36. Page SP, Kounas S, Syrris P, Christiansen M, Frank-Hansen R, et al. (2012) Cardiac myosin binding protein-c mutations in families with hypertrophic cardiomyopathy: disease expression in relation to age, gender, and long term outcome. Circ Cardiovasc Genet 5(2): 156-166.

37. Pasquale F, Syrris P, Kaski JP, Mogensen J, McKenna WJ, et al. (2012) Long-term outcomes in hypertrophic cardiomyopathy caused by mutations in the cardiac troponin t gene. Circ Cardiovasc Genet 5(1): 10-17.

38. Girolami F, Ho CY, Sensarian C, Baldi M, Will ML, et al. (2010) Clinical features and outcome of hypertrophic cardiomyopathy associated with triple sarcomere protein gene mutations. J Am Coll Cardiol 55(14): 1444-1453.

39. Ingles J, Doolan A, Chiu C, Seidman J, Seidman C, et al. (2005) Compound and double mutations in patients with hypertrophic cardiomyopathy: implications for genetic testing and counselling. J Med Genet 42(10): e59.

40. Marziliano N, Merlini PA, Vignati G, Orsini F, Motta V, et al. (2012) A case of compound mutations in the mybpc3 gene associated with biventricular hypertrophy and neonatal death. Neonatology 102(4): 254-258.

41. Biagini E, Olivotto I, Iasecone M, Parodi MI, Girolami E, et al. (2014) Significance of sarcomere gene mutations analysis in the end-stage phase of hypertrophic cardiomyopathy. Am J Cardiol 114(5): 769-776.

42. Fujita T, Fujino N, Anan R, Tei C, Kubo T, et al. (2013) Sarcomere gene mutations are associated with increased cardiovascular events in left ventricular hypertrophy: results from multicenter registration in Japan JACC Heart Fail 1(6): 459-466.

43. Li Q, Gruner C, Chan RH, Care M, Siminovich K, et al. (2014) Genotype-positive status in patients with hypertrophic cardiomyopathy is associated with higher rates of heart failure events. Circ Cardiovasc Genet 7(4): 416-422.

44. Olivotto I, Girolami F, Ackerman MJ, Nistri S, Bos JM et al. (2008) MyosinA light chain protein gene mutation screening and outcome of patients with hypertrophic cardiomyopathy. Mayo Clin Proc 83(6): 630-638.

45. Colan SD, Lipschultz SE, Lowe AM, Sleeper LA, Messere J, et al. (2007) Epidemiology and cause-specific outcome of hypertrophic cardiomyopathy in children: findings from the pediatric cardiomyopathy registry. Circulation 115(6): 773-781.

46. Raperzzi C, Arbustini E, Ciafora AL, Chairon P, Gimeno-Blanes J, et al. (2013) Diagnostic work-up in cardiomyopathies: bridging the gap between clinical phenotypes and final diagnosis. A position statement from the esc/working group on myocardial and pericardial diseases. Eur Heart J 34(19): 1448-1458.

47. Chairon P, Araad M, Arbustini E, Basso C, Bilinska Z, et al. (2010) Genetic counselling and testing in cardiomyopathies: a position statement of the European society of cardiology working group on myocardial and pericardial diseases. Eur Heart J 31(22): 2715-2726.

48. Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, et al. (2011) Hrs/ehrA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the heart rhythm society (hrs) and the european heart rhythm association (ehrA). Heart Rhythm 8(8): 1308-1339.

49. Elliott PM, Anastasakis A, Borger MA, Borggrefe M, Cecchi F, et al. (2014) 2014 esc guidelines on diagnosis and management of hypertrophic cardiomyopathy: the task force for the diagnosis and management of hypertrophic cardiomyopathy of the european society of cardiology (esc). Eur Heart J 35(59): 273-2779.

50. Chairon P, Heron D, Garghiu ML, Feingold J, Oury JF, et al. (2004) Diagnosis and management of hypertrophic cardiomyopathy of the european society of cardiology (esc)/eur heart rhythm association (ehra). Heart Rhythm 8(8): 1308-1339.

51. Mardis ER (2008) Next-generation DNA sequencing methods. Annu Rev Genomics Hum Genet 9: 387-402.

52. Voelkerding KV, Dames SA, Durtschi JD (2009) Next-generation sequencing: from basic research to diagnostics. Clin Chem 55(4): 641-658.

53. Sikkema-Raddatz B, Johansson LG, de Boer EN, Almomani R, Boven
LG, et al. (2013) Targeted next-generation sequencing can replace sanger sequencing in clinical diagnostics. Hum Mutat 34(7): 1035-1042.

54. Manovanov L, Cohey AJ, Scott CE, Kozarewa I, Turner EH, et al. (2010) Target-enrichment strategies for next-generation sequencing. Nat Methods 7(2): 111-118.

55. Glenn TC (2011) Field guide to next-generation DNA sequencers. Mol Ecol Resour 11(5): 759-769.

56. Frommolt P, Abdallah AT, Altmuller J, Motamney S, Thiele H, et al. (2012) Assessing the enrichment performance in targeted resequencing experiments. Hum Mutat 33(4): 635-641.

57. Lopes LR, Zekavati A, Syrris P, Hubank M, Gambartolomei C, et al. (2013) Genetic complexity in hypertrophic cardiomyopathy revealed by high-throughput sequencing. J Med Genet 50(4): 228-239.

58. Norton LN, LiD, Hersberger RE (2012) Next-generation sequencing to identify genetic causes of cardiomyopathies. Curr Opin Cardiol 27(3): 214-220.

59. Rehm HL, Balle SJ, Bayrak-Toydemir P, Berg JS, Brown KK, et al. (2013) Acng clinical laboratory standards for next-generation sequencing, Genet Med 15(9): 733-747.

60. Meder B, Haas J, Keller A, Heil C, Just S, et al. (2011) Targeted next-generation sequencing for the molecular genetic diagnostics of cardiomyopathies. Circ Cardiovasc Genet 4(2): 110-122.

61. Spudich JA (2014) Hypertrophic and Dilated Cardiomyopathy: Four Decades of Basic Research on Muscle Lead to Potential Therapeutic Approaches to These Devastating Genetic Diseases. Biophys J 106(6): 1236-1249.

62. Maron BJ, Maron MS, Semsarian C (2012) Genetics of Hypertrophic Cardiomyopathy After 20 Years. J Am Coll Cardiol 60(8): 705-715.

63. Sanoudou D, Vafiadis E, Arvanitis DA, Kranias E, Kontogiani-Konstantopoulos A (2005) Array lessons from the heart: focus on the Genome and Transcriptome of cardiomyopathies. Physiol Genomics 21(2): 131-143.

64. Giesiewicz Artur and Jablacka Anna (2012) Role of Genetic Factors in Dilated Cardiomyopathy. Extract from Cardiomyopathies - From Basic Research to Clinical Management.

65. Keller D, Carrier L, Schwartz K (2002) Genetics of familial cardiomyopathies and arrhythmias. Swiss Med Wkly 132(29-30): 401-407.

66. Hedley PL, Haundrup O, Andersen PS, Aalid FH, Jensen M, et al. (2011) The KCNE gene in hypertrophic cardiomyopathy: a candidate gene study. J Negat Results Biomed 10: 12.

67. Voelkerding KV, Dames S, Durtschi JD (2010) Next generation sequencing for clinical Diagnostics principles and application to targeted resequencing for hypertrophic cardiomyopathy. J Mol Diagn 12(5): 539-551.

68. Richard P, Charpon P, Carrier L, Ledeuil C, Chev T, et al. (2003) Hypertrophic cardiomyopathy. Circulation 107(17): 2227-2232.

69. García-Castro M, Coto E, Reguero JR, Berruzeta JR, Alvarez V, et al. (2009) Mutations in sarcomeric genes MYH7, MYBPC3, TNN2, TNNI3, and TPM1 in patients with hypertrophic cardiomyopathy. Rev Esp Cardiol 62(10): 48-56.

70. Li Z, Huang J, Zhao J, Chen C, Wang H, et al. (2014) Rapid molecular genetic diagnosis of hypertrophic cardiomyopathy by semiconductor sequencing. J Transl Med 12: 173.

71. Curilla K, Benesova L, Penicka M, Minarik M, Zemanek D, et al. (2012) Spectrum of clinical manifestations of mutations in genes responsible for hypertrophic cardiomyopathy. Acta Cardiol 67(1): 23-29.

72. Geer C, Perrot A, Oszczel C, Binner P, Counsell D, et al. (2003) Mutations in Human muscle LIM protein gene in families with hypertrophic cardiomyopathy. Circulation 107(10): 1390-1395.

73. Watkins H (2003) Genetic clues to disease pathways in hypertrophic and dilated cardiomyopathies. Circulation 107(10): 1344-1346.

74. Teekakirijul P, Kelly MA, Rehm HL, Lakdawala NK, Funke BH (2013) Inherited cardiomyopathies: molecular genetics and clinical genetic testing in the postgenomic era. J Mol Diagn 15(2): 158-170.

75. Ku CS, Roukes DH (2013) From Next Generation Sequencing to nanopore sequencing technology: paving the way to personalized genomic medicine. Expert Rev Med Devices 10(1): 1-6.

76. Liang L, Wang Q, Agren H, Tu Y (2014) Computational studies of DNA sequencing with solid state nanopores: key issue and future prospects. Front Chem 2: 5.

77. Srinivasan S, Batra J (2014) Four generations of sequencing: is ready for the clinical yet? Next generation sequencing and applications 1(107).

78. Mignardi M, Nilsson M (2014) Fourth generation sequencing in the cell and the clinic. Genome Med 6(4): 31.

79. Steinbock LJ, Radenovic A (2015) The emergence of nanopores in next generation sequencing. Nanotechnology 26(7): 074003.

80. Haas J, Katus HA, Meder B (2011) Next-generation sequencing entering the clinical arena. Mol Cell Probes 25(5-6): 206-211.

81. Zaragoza MV, Fass J, Diegoli M, Lin D, Arbustini E (2010) Mitochondrial DNA variant discovery and evaluation in human cardiomyopathies through next generation sequencing. Plos One 5(8): e12295.

82. McNally EM, Golbus JR, Puckebrwitz MJ (2013) Genetic mutations and mechanism in dilated cardiomyopathy. J Clin Investigation 123(1):19-26.

83. Dellesewe L, McNally EM (2010) The genetic of dilated cardiomyopathy. Curr Opin Cardiol 25(3): 198-204.

84. Chami N, Tadros R, Lemarbre F, Lo KS, Beaudoin M, et al. (2014) Nonsense mutation in BAG3 are associated with early on dilated cardiomyopathy in French Canadians. Can J Cardiol 30(12): 1655-1661.

85. Baccarelli A, Rienstra M, Benjamin EJ (2010) Cardiovascular epigenetics: basic concepts and results from animal and human studies. Circ Cardiovasc Genet 3(6): 567-573.

86. Egger G, Liang G, Aparicio A, Jones PA (2004) Epigenetics in human disease and prospects for epigenetic therapy. Nature 429(6990): 457-463.

87. Yang J, Xu WW, Hu SJ (2015) Heart Failure: advanced development in genetics and epigenetics. BioMed Res Int 352734.

88. Haas J, Frese KS, Park YJ, Keller A, Vogel B, et al. (2013) Alterations in Genetics and epigenetics. BioMed Research Int 352734.

89. Ramachandran G, Qureshi SF, Anantapur V, Nallari P (2011) Interplay of histone acetylation and transcription factors in cardiac
hypertrophy. Int J Biol Med res 2(2): 581-588.

90. Bird A (2007) Perceptions of epigenetics. Nature 447(7143): 396-398.

91. Clarke SL, McKale R, Davis MS, Ramanjulu S (2012) Biogenesis of mammalian mirna. In: Baron N. MicroRNA as Tools in Biopharmaceutical Production. Dordrecht: Springer.

92. Jinek M, Doudna JA (2009) A three-dimensional view of the molecular machinery of RNA interference. Nature 457(7228): 405-412.

93. Forman JJ, Coller HA (2010) The code within the code: microRNAs target coding regions. Cell Cycle 9(8): 1533-1541.

94. Meola N, Gennarino VA, Banfi S (2009) Micrornas and genetic diseases. Pathogenetics 2(1): 7.

95. Oliveira-Carvalho V, da Silva MM, Guimaraes GV, Bacal F, Bocchi EA (2013) Micrornas: new players in heart failure. Mol Biol Rep 40(3): 2663-2670.

96. Small EM, Frost RJ, Olson EN (2010) MicroRNAs add a new dimension to cardiovascular disease. Circulation 121(8): 1022-1032.

97. Millat G, Chanavat V, Rousson R (2014) Evaluation of a new NGS method based on a custom ampliseq library and ion torrent pgm sequencing for the fast detection of genetic variations in cardiomyopathies. Clin Chim Acta 433: 266-271.

98. Hershberger RE, Lindenfeld J, Mestroni L, Seidman CE, Taylor MR, et al. Genetic evaluation of cardiomyopathy heart failure society of America practice guideline. J Card Fail 15(2): 81-97.

99. Gupta P, Bilinska ZT, Sylvius N, Boudreau E, Veinot JP, et al. (2010) Genetic and ultrastructural studies in dilated cardiomyopathy patients: a large deletion in the lamin a/c gene is associated with cardiomyocyte nuclear envelope disruption. Basic Res Cardiol 105(3): 365-377.

100. Caleshu C, Day S, Rehm HL, Baxter S (2010) Use and interpretation of genetic tests in cardiovascular genetics. Heart 96(20): 1669-1675.

101. Van El CG, Cornel MC, Borris P, Hastings RJ, Fellmann F, et al. (2013) Whole-genome sequencing in health care. Recommendations of the european society of human genetics. Eur J Hum Genet 21(6): 580-584.

102. Norton N, Robertson PD, Rieder MJ, Zuchner S, Rampersaud E, et al. (2012) Evaluating pathogenicity of rare variants from dilated cardiomyopathy in the exome era. Circ Cardiovasc Genet 5(2): 167-174.

103. Ho CY, Charron P, Richard P, Girolami F, Van Spaendonck-Zwarts KY, et al. (2015) Genetic Advances in sarcomeric cardiomyopathies: state of the art. Cardiovasc Res 105(4): 397-408.