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Review

Array lessons from the heart: focus on the genome and transcriptome of cardiomyopathies

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Sanoudou, Despina, Elizabeth Vafiadaki, Demetrios A. Arvanitis, Evangelia Kranias, and Aikaterini Kontrogianni-Konstantopoulos. Array lessons from the heart: focus on the genome and transcriptome of cardiomyopathies. *Physiol Genomics* 21: 131–143, 2005; doi: 10.1152/physiolgenomics.00259.2004.—Our understanding of the cardiovascular system has evolved through the years by extensive studies emphasizing the identification of the molecular and physiological mechanisms involved in its normal function and disease pathogenesis. Major discoveries have been made along the way. However, the majority of this work has focused on specific genes or pathways rather than integrative approaches. In cardiomyopathies alone, over 30 different loci have shown mutations with varying inheritance patterns, yet mostly coding for structural proteins. The emergence of microarrays in the early 1990s paved the way to a new era of cardiovascular research. Microarrays dramatically accelerated the rhythm of discoveries by giving us the ability to simultaneously study thousands of genes in a single experiment. In the field of cardiovascular research, microarrays are having a significant contribution, with the majority of work focusing on end-stage cardiomyopathies that lead to heart failure. Novel molecular mechanisms have been identified, known pathways are seen under new light, disease subgroups begin to emerge, and the effects of various drugs are molecularly dissected. This cross-study data comparison concludes that consistent energy metabolism gene expression changes occur across dilated, hypertrophic, and ischemic cardiomyopathies, while Ca^{2+} homeostasis changes are prominent in the first two cardiomyopathies, and structural gene expression changes accompany mostly the dilated form. Gene expression changes are further correlated to disease genetics. The future of microarrays in the cardiomyopathy field is discussed with an emphasis on optimum experimental design and on applications in diagnosis, prognosis, and drug discovery.

microarrays; heart failure; calcium homeostasis; structural components; metabolism

HUMAN CARDIAC DISEASE remains the principal cause of death and disability in children and adults in developed countries (113). In 1998, an estimated 240,000 deaths were attributed to heart failure within the United States. Unfortunately, this number keeps increasing despite the emphasis placed on prevention programs and new therapeutic agents, rendering heart disease the health epidemic of the 21st century. Thus every year ~4.9 million patients are treated with an estimated cost of $18.8 billion (US dollars) in the United States alone (32, 42, 86).

At the forefront of heart diseases are cardiomyopathies, which are defined as disorders of the myocardium that usually result in inadequate pumping of the heart. They range from lifelong symptomless forms to severe maladies, including arrhythmia, thromboembolism, and progressive heart failure (39, 82). Depending on their phenotypic manifestation and clinical state, cardiomyopathies have been recently classified as stage 1 (latent or potential), stage 2 (early or subclinical), and stage 3 (late or advanced) (41). Stage 1 cardiomyopathies are symptomless, even though a causative factor (e.g., a genetic abnormality or an underlying disease, such as diabetes mellitus or hypertension) is present. In stage 2 forms, heart muscle disease is evident but is accompanied by mild cardiac remodeling and slightly reduced diastolic activity. Stage 3 cardiomyopathies are characterized by extensive structural alterations that result in dilated or nondilated forms, accompanied by systolic and/or diastolic dysfunction that ultimately may precipitate heart failure and cardiac death. Elucidation of the etiologies that lead to the development of cardiac disease will pave the way to early diagnosis, individualized prognosis, and improved treatments.

Patients with heart failure have been traditionally classified into two major groups on the basis of left ventricular dysfunction: cardiomyopathy resulting from ischemic (ischemic cardiomyopathy; ICM) or nonischemic heart disease (4, 22, 39). Ischemic heart disease is the most common underlying cause of heart failure (40–70%). Myocardial ischemia may arise from several etiologies; the most prevalent one is coronary artery disease, which subsequently may lead to myocardial infarction, heart failure, and premature death (1, 28, 34). The pathophysiological manifestations of ICM depend on the extent and severity of coronary artery lesions and include progressive loss of myocytes, neurohormonal activation, and defective systolic contraction (1, 21, 73, 85). In the nonischemic group (25–35%), conditions such as hypertension (~17%), aortic valve pathology (~13%), and hereditary gene defects (~20%) are the main underlying causes that can provoke extensive cardiac remodeling and perturb cardiac function (4, 39, 86, 105). Additionally, viral infection, neoplastic diseases, cytotoxic drugs, inflammation, hyperthyroidism, chronic alcohol abuse, metabolic diseases, systemic disorders, collagen vascular disease, physical agents, and late pregnancy may accelerate the

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functional deterioration of the myocardium and trigger the onset of heart failure (34, 39, 41).

Cardiomyopathies may be monogenic disorders, caused primarily by genetic factors; some forms, however, are complex or polygenic and result from the additive effects of genetic, environmental, and disease-related factors. In line with this notion, development of myocellular hypertrophy and myocardial fibrosis is two times more frequent in diabetic men and five times more common in diabetic women compared with age-matched nondiabetic control subjects (10). Hypertension, another frequent comorbidity of diabetes, may further damage myocardial contractile proteins, alter cellular Ca^{2+} transport, and induce myocardial fibrosis and hypertrophy. The end result of the combinatorial effects of diabetes and hypertension will be a fibrotic, noncompliant myocardium with severe diastolic and systolic dysfunction (10, 82).

Primary cardiomyopathies are also categorized according to anatomical and hemodynamic criteria in four categories: *1*) hypertrophic cardiomyopathy (HCM), *2*) dilated cardiomyopathy (DCM), *3*) restrictive cardiomyopathy (RCM), and *4*) arrythmogenic right ventricular dysplasia (ARVD) (recently reviewed in Ref. 32). This classification system includes both ischemic and nonischemic cardiomyopathies, depending on the degree of morphological adaptation and systolic dysfunction that the affected myocardium has suffered. HCM and DCM are the most prevalent causes of congestive heart failure. According to echocardiographic data from a large population of young adults, the incidence of HCM has been estimated at ~1 in 500 persons, while 40 people in every 100,000 of the population are affected by DCM (29, 69, 82, 113).

Among the variable causes of HCM and DCM, heritable gene mutations have only been recently integrated into the classification system of underlying etiologies of congestive heart failure. Indeed, the recognition that many primary cardiomyopathies are familial disorders has highlighted that heart failure is the result of a complex interplay among genetic factors, molecular modifiers, and environmental stimuli and has considerably shifted the focus of current research toward human molecular genetics (25, 32, 39, 71, 86).

This paper reviews current knowledge on the genetics of cardiomyopathies (DCM, HCM, ICM) and the molecular pathways implicated in their pathogenesis as shown by microarrays. The challenges and potential of microarrays are further discussed in the context of improved clinical care for these patients and drug discovery.

GENETICS OF HCM

HCM is a complex cardiac disease with unique morphological, functional, and clinical characteristics; the hallmark diagnostic features of HCM are left ventricular (LV) wall hypertrophy that is usually asymmetric, often with particular involvement of the interventricular septum (asymmetric septal hypertrophy), and exaggerated pump function (hypersystolic contraction) accompanied by impaired diastolic relaxation (diastolic dysfunction) (23, 33, 105). Histologically, the hypertrophic myocardium is characterized by pathological myocyte hypertrophy, extensive myofibrillar disarray, and focal or widespread interstitial fibrosis.

HCM is one of the most common inherited monogenic cardiac disorders and is transmitted as an autosomal dominant trait; indeed, a familial cause has been shown in ~50% of affected HCM patients (27, 39, 70). Genetic analyses have causally linked 13 chromosomal loci with the development of HCM. The first 11 of these genes encode protein components of the cardiac sarcomere, whereas mutations in 2 genes that encode nonsarcomeric proteins were recently reported to also cause HCM (Table 1). Notably, a number of different mutations have been reported for most disease genes (Familial Hypertrophic Cardiomyopathy Mutation Database; **http://www.angis.org.au/Databases/Heart/**).

There are two major mechanisms by which mutations in sarcomeric genes could lead to HCM: through haploinsufficiency, in which a dominant gene mutation may inactivate one of the alleles, resulting in a reduced amount of functional protein, or through exertion of a dominant negative effect, in which a dominant mutation may create a transformed protein that impedes normal protein function or has a novel function (95, 105). To date, most hypertrophic cardiomyopathy mutations that have been identified are missense mutations (i.e., single nucleotide substitutions) or minor truncations (i.e., deletions or insertions of single nucleotides) (82, 105). These mutations are highly unlikely to cause haploinsufficiency, via either transcript or protein instability, suggesting that the development of HCM is through a dominant negative effect that the mutant protein exerts within the contractile sarcomere.

GENETICS OF DCM

DCM is a prevalent worldwide disorder characterized by ventricular dilation and contractile dysfunction of the left and/or right ventricles, frequently accompanied by severe cardiac arrhythmias, thromboembolic events, and cardiac conduction abnormalities (15, 16). Although the ventricular and occasionally the atrial walls thicken, most of the heart enlargement results from marked distention of the ventricular chambers. Contrary to the severe histopathological abnormalities that characterize the hypertrophic myocardium, microscopic examination of the dilated myocardium may reveal no substantial myocyte disarray, although occasional areas of subendocardial, focal interstitial, and perivascular fibrosis as well as hypertrophic and atrophic myofibers have been observed (2, 39). Nevertheless, the major clinical manifestation of DCM is the hypocontractile heart due to reduced systolic

Table 1. *HCM-causing gene mutations identified to date*

HCM Gene	Gene Symbol/Locus	No. of Mutations	HCM Cases (%)
β-Myosin heavy chain	*MYH7*/14q12	80	~35%
α-Myosin heavy chain	*MYH6*/14q12	1	<0.5%
Myosin binding protein C	*MYBPC3*/11p11	30	~25%
Regulatory myosin light chain	*MYL2*/12q24.3	8	~1%
Essential myosin light chain	*MYL3*/3p21	2	<1%
Actin	*ACTC*/15q14	5	<5%
Troponin T	*TNNT2*/1q32	14	~15%
Troponin I	*TNNI3*/19q13.4	8	<5%
Troponin C	*TNNC1*/3p21-p14	1	<0.5%
α-Tropomyosin	*TPM1*/15q22	6	~3%
Titin	*TTN*/2q31	1	<0.5%
Cardiac muscle LIM protein	*CLP*/11p15	4	<1%
AMP-activated protein kinase (γ_2-regulatory subunit)	*PRKAG2*/7q36	5	<1%

For detailed review, see Ref. 39. HCM, hypertrophic cardiomyopathy.

and impaired diastolic functions of the injured cardiomyocyte (16, 113).

A diverse array of intrinsic and extrinsic etiologies may lead to the development of DCM, including coronary artery disease, hypertension, thyroid disease, viral infection, and chronic alcohol abuse (32, 105). Although DCM has been traditionally viewed as a sporadic nongenetic disorder, molecular genetic studies have suggested that ~30–40% of DCM-affected individuals have a familial form of the disease (46, 58, 76, 77).

Familial DCM can be transmitted as an autosomal recessive, X-linked, or matrilinear (mitochondrial) trait, but autosomal dominant inheritance is the most common one. To date, ~20 chromosomal loci have been linked to DCM, suggesting that it is a genetically heterogeneous disorder (Table 2). Infantile or childhood-onset forms of DCM have been associated with autosomal recessive (32), X-linked (6, 12, 38, 81, 114), and matrilinear traits (68, 108), whereas adult-onset DCM has been associated with autosomal dominant transmission (32, 46, 48, 77). A wide spectrum of clinical phenotypes frequently accompanies heritable DCM, including early conduction disease, sensorineural hearing loss, and skeletal muscle dystrophies or myopathies (see Ref. 39 and references therein).

DEVELOPMENT OF HCM VS. DCM PHENOTYPE

Mutations in six genes encoding sarcomeric proteins may result in either HCM (Table 1) or DCM (Table 2). This observation raises a question regarding the factors that determine the phenotypic expression of sarcomeric protein gene mutations. It has been shown that HCM- and DCM-causing mutations occur in distinct functional domains of the encoded proteins (32). In line with this notion, HCM-causing mutations are located in regions that are directly involved in force generation ("defective force generation" hypothesis), whereas DCM-causing mutations affect domains that are involved in force transmission from the sarcomere to the extrasarcomeric cytoskeleton ("defective force transmission" hypothesis) (87, 88). Furthermore, whether a mutation will result in HCM or DCM may be determined by the extent of contractile deficit incurred. If myocardial hypertrophy is a compensatory mechanism to contractile malfunction, it is possible that it may be insufficient to salvage severe dysfunction, which may trigger cardiac dilation and myocyte death. Thus it is likely that these two different pathophysiologies may simply reflect gradations of a single pathway. Along these lines, recent findings suggest that mutations in genes encoding sarcomeric and/or cytoskeletal proteins may ultimately result in energy compromise and myofibrillar Ca^{2+} deregulation that lead to development of either HCM or DCM, depending on the severity of contractile dysfunction, by altering downstream signaling cascades that modify gene expression and cardiac function (25, 86, 113). Consequently, dissecting out the molecular mechanisms and cellular events by which specific gene defects trigger distinct disease pathways is imperative. During the past decade, the use of gene expression microarray technology has tremendously advanced our knowledge of genes that participate in intracellular cascades that ultimately lead to myocardial hypertrophy, dilation, and contractile malfunction, providing new targets for individualized therapeutic intervention.

Table 2. *DCM-associated chromosomal loci and disease genes*

DCM-Gene/Symbol	Gene Locus
β-Myosin heavy chain/*MYH7**	14q12
Myosin binding protein C/*MYBPC3**	11p11
Actin/*ACTC**	15q14
Troponin T/*TNNT2**	1q32
α-Tropomyosin/*TPM1**	15q22
Titin/*TTN**	2q31
Desmin/*DES**	2q35
Metavinculin/*VCL**	10q22–q23
Dystrophin/*DMD*‡	Xp21
Tafazzin/*G4.5*‡	Xq28
δ-Sarcoglycan/*SGCD**‡	5q33
Lamin A/C/*LMNA*†‡	1p1–q21
Phospolamban/*PLN**	6q22.1
SERCA2/*ATP2A2**	12q23–q24.1
†	2q14–q22
†	3p22–p25
*	6q12–q16
†‡	6q23
§	6q23–q24
*	9q13–q22
*	9q22–9q31

DCM, dilated cardiomyopathy. *DCM-linked loci only. †DCM loci accompanied by early conduction disease. ‡DCM loci accompanied by skeletal muscle dystrophies. §DCM loci accompanied by sensorineural defects.

MICROARRAYS IN CARDIOMYOPATHIES

Microarrays are glass slides with thousands of cDNAs or oligonucleotides on their surface, representing up to the entire genome of the organism under study. The two main microarray types assess either DNA regional copy number or sequence (DNA arrays) or RNA expression (expression arrays). Both microarray types are used for a broad range and increasing number of applications, with some striking findings to date (Table 3). Expression arrays play a key role in the simultaneous visualization of all transcriptional changes directly or indirectly associated with the disease under study by enabling novel molecular pathways to be uncovered, previously suspected molecular pathways to be better characterized, and interpathway associations to emerge. They are produced at both commercial (Table 4) and academic settings, using a wide range of protocols and approaches. The main steps of a microarray project are summarized in Fig. 1. Experimentally, fluorescently labeled cDNA or cRNA from the samples under study (e.g., patient or control cardiac frozen tissue), enriched with suitable control sequences, is hybridized on the arrays, which are then appropriately washed, stained, scanned, and analyzed (31). Aside from the technical aspect, two areas of paramount importance involve the experimental design and the bioinformatical analysis of the resulting data (thoroughly reviewed in Refs. 92, 123).

An increasing number of such gene expression studies have focused on heart disease, with significant findings and potential. Microarrays capture a global snapshot of the cardiac transcriptome in a single experiment, and thus they are one of the most efficient means for simultaneous identification of all ongoing transcriptional changes in different phases and under different conditions. They put in perspective prior knowledge on individual genes while revealing novel disease-related pathways. Unanswered questions that can now be addressed involve the relative significance of the various molecular aber-

Table 3. *Examples of expression and DNA array applications*

Gene Expression Microarrays			
Understanding normal processes	Understanding disease	Improving health care	DNA Microarrays
Molecular pathways (78)	Molecular pathogenesis (89, 93, 117)	Disease classification (56)	SNP analysis (63, 72, 121)
Differentiation (47)	Disease progression (84)	Diagnosis (45, 57)	Mutation detection (67)
Development (78)	Response to pathogens (74)	Prognosis (e.g., recurrence, metastasis) (53, 62, 120)	Mutation screening (98)
Gene associations/unknown gene characterization (19)	Pathogen characterization (66)	Treatment (40, 119)	Pathogen characterization (50)

Nos. in parentheses refer to reference nos.

rations within and across different heart diseases. Depending on sample availability and wealth of clinical information, microarrays can also shed light on genetic and environmental (e.g., drug) associations with molecular pathway changes during heart disease. Multiple pathological cardiac states have been studied in this manner, with a particular emphasis on cardiomyopathies (DCM, HCM, and ICM) and usually in relation to heart failure. In human cardiomyopathy studies, two microarray platforms have been used to date: "homemade" spotted arrays of cardiac-specific cDNA clones (8, 9, 52) and different generations of Affymetrix whole genome photolithographically synthesized oligonucleotide arrays (**http://www.affymetrix.com**) (107, 110, 122, 124). Of the important findings coming to light, extensive information is unveiled regarding structural [extracellular matrix (ECM), cytoskeletal, and sarcomeric], Ca^{2+} homeostasis-related and energy metabolism pathways. These pathways are likely involved in disease development and progression and form the basis for bridging primary disease stimuli, such as gene mutations, and clinical phenotypes (Fig. 2). Although a considerable overlap is observed in the molecular changes of different cardiomyopathies, there are also distinct cardiomyopathy-specific alterations (Table 5).

STRUCTURAL GENE EXPRESSION CHANGES IN CARDIOMYOPATHIES

Structural genes comprised the largest functional category that was significantly and consistently changed across DCM, HCM, and ICM microarrays studies. This was mostly reflected as overexpression of sarcomeric, cytoskeletal, and ECM genes with decreasing frequency.

The majority of structural gene expression changes were seen in DCM and to a smaller extent in HCM and ICM, respectively. Actin genes were the most commonly changed across the board of different cardiomyopathies, whereas myosin and collagen-related genes were more so in DCM specimens. In particular, collagen type-$1\alpha_1$ and lumican (normally abundant in cardiac collagenous matrices) were the most consistently upregulated genes in DCM (8, 52, 110). Actually, decreased elastin-to-collagen ratio has been suggested as one of the causes of adverse ECM remodeling in heart failure (80).

Table 4. *Examples of commercial sources of microarray companies, software, and public microarray data repositories*

Microarray Companies	Public Data Analysis Software	Commercial Data Analysis Software	Public Data Repositories
http://www.affymetrix.com	**http://www.tigr.org/software/**	**http://www.iobion.com/products/products_GENETRAFFIC.html**	**http://genome-ww5.stanford.edu/MicroArray/SMD/**
http://www.codelinkbioarrays.com	**http://rana.lbl.gov/EisenSoftware.htm**	**http://www.insightful.com/products/s-plus_arrayanalyzer/**	**http://www.ncbi.nlm.nih.gov/geo/**
http://www.agilent.com	**http://www-stat.stanford.edu/~tibs/SAM/**	**http://www.sas.com/industry/pharma/mas/**	**http://pga.tigr.org/data_index.shtml**
http://www.mwg-biotech.com/	**http://www.dchip.org/**	**http://www.silicongenetics.com/cgi/SiG.cgi/Products/GeneSpring/index.smf**	**http://microarray.cnmcresearch.org/pgadatatable.asp**
http://www.illumina.com/	**http://www.chip.org/home/resources.cgi**	**http://www.biodiscovery.com/genesight.asp**	**http://cardiogenomics.med.harvard.edu/public-data.html**
http://www.nanogen.com	**http://genome.tugraz.at/Software/**	**http://www.molmine.com/**	**http://www.hugeindex.org/databases/index.html**
http://www.biotechcareercenter.com/Microarray.html	**http://astor.som.jhmi.edu/poe/**	**http://www.biosieve.com/**	**http://www-genome.wi.mit.edu/cgi-bin/cancer/datasets.cgi**
http://www.mergen-ltd.com/	**http://www.bioconductor.org/**	**http://www.genesifter.net/**	**http://162.129.178.60/pga/client/index.php**
http://www.superarray.com/microarrays.php	**http://www.genmapp.org/default.asp**	**http://www.imagingresearch.com/products/ARV.asp**	**http://dbk.ch.umist.ac.uk/StreptoBASE/?page=2**
http://www.arrayit.com/	**http://maexplorer.sourceforge.net/**	**http://www.affymetrix.com/products/software/specific/dmt.affx**	**http://www.ebi.ac.uk/arrayexpress/**
http://www.bdbiosciences.com/clontech/atlas/atlasglass/index.shtml	**http://www.mged.org/Workgroups/MIAME/miame_software.html**	**http://www.mged.org/Workgroups/MIAME/miame_software.html**	**http://www.rzpd.de/gcexpress/query**

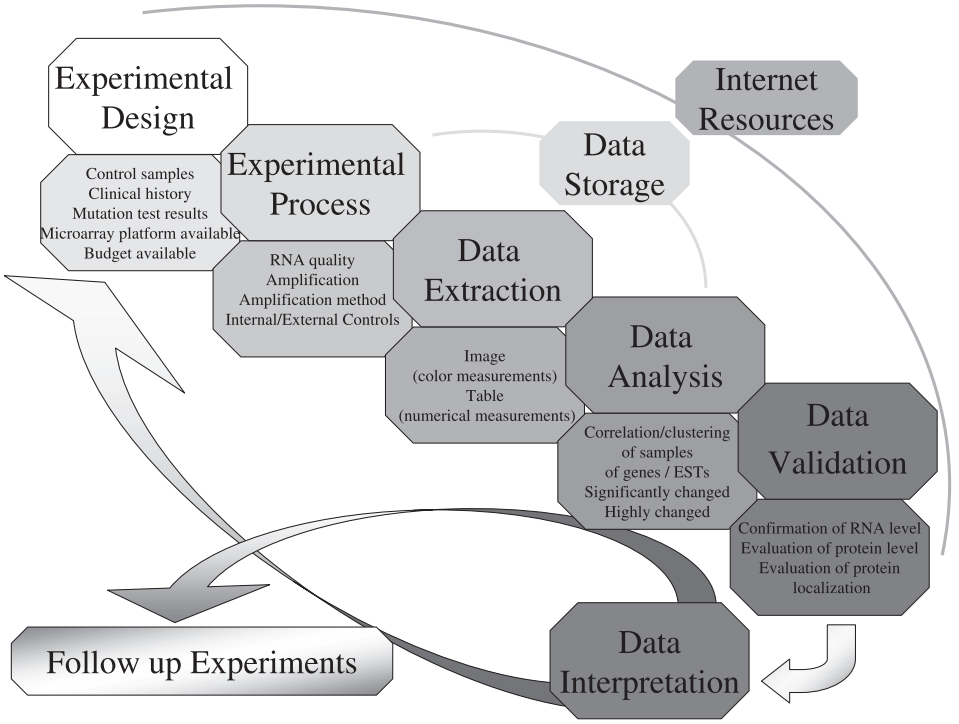

Fig. 1. Diagrammatic representation of the major stages of a microarray project.

Microarray data indicated that this decreased ratio is characteristic of DCM- but not HCM- or ICM-related heart failure, and furthermore pointed to *TGF-β1* and *IGF-I* upregulation as possible triggers of ECM remodeling in DCM (14, 96, 110). Fewer and different collagen genes showed altered expression in HCM, and no such changes were detected in ICM cases (9, 52, 107, 122), further supporting cardiac tissue differences between cardiomyopathies. Overall, changes in ECM are likely to be secondary and associated with fibrosis, impaired contractile function, and cardiac remodeling (36, 94).

The microarray findings of multiple concomitant cytoskeletal and ECM gene expression changes are supported by the known physical association of these two structural components of cardiac muscle via membrane-spanning integrins at sites close to the Z-line, known as costameres (118). Interestingly, various costamere-associated proteins such as integrin-β5, α-actinin, and vinculin were shown to be deregulated in DCM, ICM, and HCM by microarrays (13, 52, 107, 124). Vinculin mutations have also been noted in DCM studies, thus emphasizing the role of costameres in cardiomyopathy development.

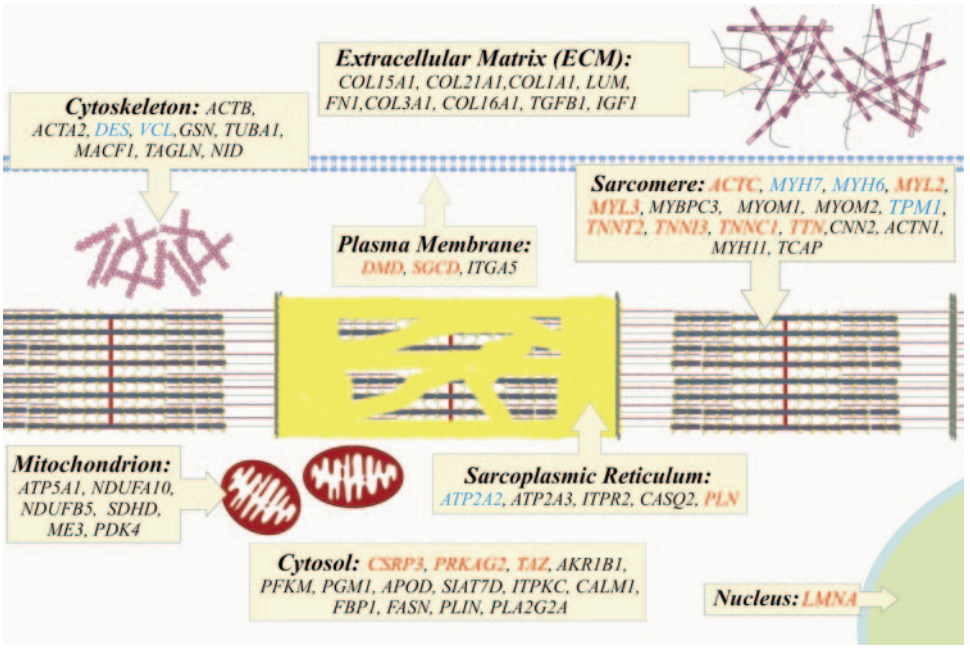

Fig. 2. Major components of the cardiomyocyte associated to human cardiomyopathies; genes with identified mutations are shown in red, genes with altered expression levels in black, and genes exhibiting both in blue.

Table 5. *Summary of major molecular pathway changes in human cardiomyopathies*

	Sarcomeric	Cytoskeletal	Extracellular Matrix	Ca^{2+} Homeostasis	Apoptosis	Energy Metabolism	Refs.
DCM	↑ ↑ ↑ ↑	↑ ↑ ↑ ↓	↑ ↑ ↑ ↑	↓ ↓	↑ ↓	↑ ↑ ↓ ↓	(8, 52, 107, 110, 122, 124)
HCM	↑ ↑	↑ ↑ ↑	↑ ↓	↓	↓	↓ ↓	(9, 52)
ICM	↑ ↑	↑ ↑	↑	~	↑	↑ ↑	(107, 122)

Arrows indicate overexpression (↑) or underexpression (↓). Their no. reflects the proportion of genes changed compared with other functional categories in that disease group.

In addition, DCM-related mutations have also been detected in the desmin gene. However, desmin overexpression was solely and consistently observed in the HCM cases analyzed, favoring a differential role of this gene in the two conditions (DCM and HCM). In addition to its primary structural role, desmin can interfere with mitochondrial proliferation, localization, and respiration, which are indeed observed altered by microarray studies (see ENERGY METABOLISM) (20, 79).

Of particular interest was the novel implication of gelsolin and the plakin family genes in cardiomyopathies (9, 122, 124). These genes are involved in cytoskeletal and tissue integrity, and their overexpression may represent an additional step in the cardiac remodeling process during cardiomyopathies. Furthermore, gelsolin can interfere with L-type Ca^{2+} channel activation and may contribute to the cardiomyopathic Ca^{2+} homeostasis aberrations (see CA^{2+} SIGNALING).

Among the thousands of genes analyzed by microarrays, only a few discrepancies (upregulated in some studies and downregulated in others) were noted in structural genes such as calponin, β-actin, and striated muscle LIM protein-1, possibly reflecting interindividual variation of molecular response to the disease (52, 107, 122, 124).

Microarray findings of disease-specific structural gene expression changes reveal putative mechanisms of cardiac remodeling and muscle integrity rescue, and they begin to uncover molecular pathways specific to each cardiomyopathy form with significant potential in better understanding their pathophysiology. These observations in combination with previously identified mutations in structural genes (Tables 1 and 2) (5, 88) propose a pivotal and possibly primary role of structural abnormalities in the development and/or progression of cardiomyopathies. It is, however, important to note that microarray studies do not permit differentiation between cause and effect. Hence, some of the observed structural changes may be downstream consequences of primary disease-causing defects, potentially aggravating disease severity, whereas others may be compensatory mechanisms triggered in an effort to rescue muscle fibers. Further work, particularly on animal models, could help clarify the order of cellular events during cardiomyopathy progression and perhaps enable the selection of more effective therapeutic targets.

CA^{2+} SIGNALING

Structural gene expression changes such as gelsolin upregulation can affect Ca^{2+} homeostasis. Although the majority of identified cardiomyopathy mutations involve structural genes, the known mutations in phospholamban (49, 102) and potentially other Ca^{2+} regulators when present could also impact Ca^{2+} transport and signaling. A concomitant deregulation of the two functional categories has been described before in microarray studies of skeletal myopathies and dystrophies, where structural gene mutations led to significant structural and Ca^{2+} homeostasis-related gene expression changes (24, 90, 100).

In physiological situations, muscle excitation-contraction is tightly regulated by Ca^{2+} cycling via channels and pumps (predominantly L-type channels and sarcoplasmic reticulum Ca^{2+}-ATPases) between the cytosol, where it activates the myofilaments, and the sarcoplasmic reticulum. However, in end-stage cardiomyopathies leading to heart failure, systolic peak Ca^{2+} is reduced, diastolic Ca^{2+} levels are increased, and diastolic Ca^{2+} decay is prolonged. An understanding of the molecular pathways involved in Ca^{2+} homeostasis disruption and potential molecular differences between different forms or stages of cardiomyopathies and heart failure could significantly expand and improve current pharmacological options (91).

Microarrays revealed significant changes in Ca^{2+}-signaling gene expression in DCM and HCM but not in ICM studies. In support of previous observations, the most consistent finding involved the ATPase, Ca^{2+}-transporting, cardiac muscle, slow twitch-2 (also called sarcoplasmic reticulum Ca^{2+}-ATPase-2; *ATP2A2* or *SERCA2*), which was downregulated in almost all DCM and HCM studies (8, 9, 52, 124). ATP2A2 is a phospholamban-regulated sarcoplasmic reticulum ion pump responsible for actively transporting Ca^{2+} from the cytosol into the sarcoplasmic reticulum lumen, resulting in muscle relaxation (65). Reduced ATP2A2 pump activity can result in prolonged muscle stiffness and impaired relaxation. Through microarray findings and additional studies, it was concluded that, despite regional variation of *ATP2A2* expression in failing hearts, this enzyme was consistently depressed partly due to its altered regulation (37). In addition to *ATP2A2*, Ca^{2+} homeostasis-related gene expression changes differed between diseases. Inositol 1,4,5-trisphosphate 3-kinase C (*ITPKC*), a Ca^{2+} homeostasis modulator via phosphorylation of inositol 1,4,5-trisphosphate to inositol 1,3,4,5-tetrakisphosphate, as well as ATPase, Ca^{2+} transporting, ubiquitous (*ATP2A3*), and inositol 1,4,5-trisphosphate receptor, type 2 (*ITPR2*), was underexpressed in DCM (8, 52). Although generally unaltered in humans, calsequestrin (a sarcoplasmic reticulum Ca^{2+} storage protein) was the only overexpressed Ca^{2+} signaling-related gene, altered specifically in DCM patients (52). In mice, its overexpression is associated with hypertrophy and heart failure, probably reflecting interspecies variations in molecular pathogenesis, highlighting the importance of careful cross-species data extrapolation (61, 101). Interestingly, one of the largest and more uniform microarray studies of human DCM specimens displayed the largest number of Ca^{2+} signaling-related gene expression changes, suggesting that these changes are disease specific and/or of moderate magnitude, so therefore

harder to detect (8). Overall, Ca^{2+} homeostasis appears significantly impaired in DCM at the Ca^{2+} transport, signaling, and storage levels. Although some of these gene expression changes may be secondary events, they could contribute toward the final phenotype.

In HCM, calmodulin underexpression was the only other Ca^{2+} homeostasis-related change along with *ATP2A2*. Calmodulin acts as a Ca^{2+} sensor that modulates ion channels and activates other Ca^{2+}-dependent signaling molecules. L-type Ca^{2+} channels, the main portal for Ca^{2+} entry into cardiac myocytes, belong to those regulated by calmodulin (125). Impaired L-type Ca^{2+} channel function has been implicated in the genesis of arrhythmias during cardiac hypertrophy, which would be consistent with calmodulin downregulation (3).

All microarray studies involved end-stage cardiomyopathy cases presenting with heart failure, and therefore the consistent *ATP2A2* downregulation in DCM and HCM was not unexpected. Importantly, distinct Ca^{2+}-related molecular mechanisms are involved in these two cardiomyopathy forms that seem to converge as disease progresses to heart failure. In DCM, more Ca^{2+} homeostasis-related changes were noted, involving primarily intracellular signaling and transport mechanisms. In HCM, the limited changes observed involved predominantly Ca^{2+} transport-related genes. Finally, ICM cases had no significant Ca^{2+} homeostasis-related gene expression changes, and despite the clinical phenotype of heart failure, *ATP2A2* levels were normal, unlike DCM and HCM cases. Overall, alterations in gene expression of Ca^{2+} handling proteins would cause impaired removal of cytosolic Ca^{2+}, reduced loading of cardiac sarcoplasmic reticulum Ca^{2+} stores, and defective sarcoplasmic reticulum Ca^{2+} release, resulting in diminished peak and prolonged decay of Ca^{2+} transients. This would affect excitation-contraction coupling, leading to depressed myocardial contractility and slow relaxation of the failing heart.

ENERGY METABOLISM

It is noteworthy that intracellular Ca^{2+} levels interfere with energy metabolism in addition to other cellular functions. For example, elevated extramitochondrial Ca^{2+} levels activate key oxidative metabolism enzymes and therefore stimulate energy production (75). Meantime, energy availability is a prerequisite for efficient Ca^{2+} homeostasis, such as Ca^{2+} pumping via ATP2A2 (43).

Through microarrays, energy metabolism was revealed to be impaired across all three forms of cardiomyopathy (DCM, HCM, and ICM). One of the most consistent observations was the upregulation of mitochondrial genes, primarily in DCM but also in HCM and ICM (52, 122, 124). Examples include NADH dehydrogenases, ATP synthases, succinate dehydrogenase, malic enzyme-3 ($NADP^+$ dependent), and methylene tetrahydrofolate dehydrogenase (NAD^+ dependent) (8, 107, 110, 122, 124). Certain glucose metabolism steps were occasionally downregulated (e.g., phosphofructokinase, phosphoglucomutase-1) in DCM, whereas others were upregulated (e.g., fructose-1,6-bisphosphatase) (52, 110), with similar variation observed in lipid metabolism-related genes (e.g., perilipin, fatty acid synthase, phospholipase A2, group IIA) (107, 110, 124). Overall energy metabolism genes were predominantly underexpressed in DCM and HCM but overexpressed in ICM. Changes in energy metabolism would alter the availability of high-energy phosphates required for the increased work demands of the overloaded failing myocardium. Functional consequences of energy alterations could include changes in free energy of ATP hydrolysis and in phosphorylation potential to levels that would affect the Ca^{2+} pump and cross-bridge cycling, therefore causing impaired contractility and relaxation of the heart.

Inconsistent findings were only reported for aldose reductase and apolipoprotein D in DCM (8, 107, 110, 122). In HCM, there was some evidence of glucose metabolism downregulation (52). For the most part, different genes from each pathway were implicated in each study. The variable expression levels of different metabolic genes may reflect the secondary involvement of these pathways in cardiomyopathies, perhaps in relation to the stage of the disease. Importantly, many heart disease drugs also have an effect on energy metabolism that may vary with dose and duration of treatment (18, 51, 103, 115). Inevitably, all patients recruited in cardiomyopathy/heart failure microarray projects were taking varying combinations of these drugs. Studying the gene expression changes induced by these drugs in biopsies from other unaffected tissues of the same individuals could help in understanding the significance of the observed molecular effects in cardiac tissue.

Consistent metabolism-associated gene expression changes across studies, unrelated to energy production, include downregulation of sialyltransferase in DCM and HCM cases (8, 52) and upregulation of dioxin-inducible cytochrome *P*450 (or cytochrome *P*450, family 1, subfamily A, polypeptide 1-CYP1A1) in DCM and ICM cardiac specimens (107, 110). Cytochrome *P*450 monoxygenases play a key role in drug metabolism, specifically inactivation and toxicity. In the cardiac milieu, cytochrome *P*450 monoxygenases, including CYP1A1, have been reported preferentially upregulated in the right ventricles of DCM patients and healthy individuals, and they are postulated to play a significant role in pharmacotherapy (112). Although many factors influence the expression levels of the cytochrome *P*450 family of genes (e.g., hypoxia downregulates them), the LV upregulation of *CYP1A1* observed by microarrays in DCM and ICM cases could be induced by the specific drug combinations administered to the patients that, among other drugs, consistently included amiodarone and captoril (35, 107, 110). Current data are too limited to permit conclusions on drug and *CYP1A1* gene expression associations. Future work could clarify whether such associations exist, and whether combinations of certain drugs reduce their individual potency by triggering cytochrome *P*450 overexpression and thus expediting their metabolic inactivation. This example of *CYP1A1* upregulation demonstrates the powerful role microarrays could play in future evaluations of drug efficacy.

OTHER PATHWAYS

Several other pathways displayed significantly changed expression in the microarray studies of DCM, HCM, and ICM. Apoptosis appeared upregulated by microarrays primarily in DCM but also in ICM and HCM, with several apoptosis and anti-apoptosis genes over- or underexpressed, respectively, in accordance with previous evidence (55, 59). Altered expression was observed for antioxidant protein-2 (*AOP2*), modulator of apoptosis-1 (*MAP-1*), pleiomorphic adenoma gene-like-1

(*PLAGL1*), and α_1-antichymotrypsin among other genes. Metallothionein L1, a stress-inducible protein shown to participate in cardiac apoptosis (60) (observed in both DCM and ICM), and c-Fos, a transcription factor occasionally implicated in apoptosis (observed in DCM and HCM), were significantly and interchangeably over- or underexpressed in different studies, suggesting a secondary and perhaps disease stage-dependent cellular event (8, 107, 110). Although cross-species comparisons must be made with caution, the above findings are consistent with the notion of a variable role of the apoptotic pathway at different disease stages; the microarray study of four cardiac hypertrophy mouse models revealed overexpression of apoptosis genes only in the most severely affected models (7).

Multiple transcription, translation, and protein modification-related genes were significantly overexpressed in DCM and HCM, consistent with a highly active cellular phase of the cardiac muscle, as it deteriorates with disease progression, responds to medications, and compensates for the accumulating molecular aberrations (8, 9, 52, 110, 124). The most consistent gene expression changes in this functional category were elongation factor-2 (*EF2*) and runt-related transcription factor-2 (*RUNX2* or *OSF2*). The overexpression of calponin, observed in two independent studies of DCM and ICM, led to the hypothesis of an ongoing cardiac muscle dedifferentiation process (107, 110). Finally, two genes, namely atrial natriuretic peptide (*ANP*) and brain natriuretic peptide (*BNP*), have been observed to consistently change across multiple microarray studies, usually with a high-fold change, and are often referred to as biomarkers of heart failure [8.5-fold (52), 4.2-fold (110), 7.2-fold (107)]. In hypertrophic mouse models, the microarray detected *ANP* upregulation with increasing degrees of disease severity; this was in fact the only consistent finding between them (7). ANP and BNP act mainly as cardiac hormones, produced primarily by the atrium and ventricle, respectively. They participate in the regulation of blood pressure and body fluid homeostasis and modify growth and development of cardiovascular tissues and bone. *ANP* is an embryonic cardiac gene, frequently overexpressed in pressure and volume overload, ischemic damage, and other naturally occurring events that stimulate cardiac hypertrophy (30). BNP is thought to act as a cardiomyocyte-derived antifibrotic factor, with a role in ventricular remodeling (109).

Overall, the considerable overlap in mutated genes, gene expression changes, and molecular pathways affected in HCM and DCM supports previous hypotheses of specific pathogenesis pathways (Table 6). Furthermore, the greater number of gene expression changes in DCM compared with HCM is consistent with moderate contractile dysfunction favoring cardiac hypertrophy, while severe dysfunction leads to cardiac dilation.

CHALLENGES

Gene expression microarrays have been widely used since they were first developed for a broad range of different applications and conditions. Their sensitivity, specificity, and reproducibility have been established, yet variations exist between different microarray systems (54). Although conceptually simple, microarrays present many technical and analytical challenges that need to be carefully considered and addressed when planning, performing, interpreting, and comparing cardiomyopathy studies. Thus appreciation of the system's limitations can maximize the extraction of information from previous studies, explain interstudy discrepancies, and expand the capabilities of future studies.

Table 6. *Representative genes from pathways significantly affected in end-stage cardiomyopathies*

Genes	DCM	HCM	ICM
Structural			
Tropomyosin 1 (alpha)/*TPM1*	+	+	
Actin, beta/*ACTB*	+	+	+
Actin, alpha/*ACTC*	+	+	+
Myomesin 1 (skelemin)/*MYOM1*	+		+
Calponin 2/*CNN2*	+		+
Gelsolin/*GSN*	+	+	+
Actinin, alpha 1/*ACTN1*	+		+
Collagen, type XV, alpha 1/*COL15A1*	+		+
Myosin, heavy polypeptide 6/*MYH6*	+		
Myosin, heavy polypeptide 7/*MYH7*	+		
Collagen, type XXI, alpha 1/*COL21A1*	+		
Collagen, type I, alpha 1/*COL1A1*	+		
Lumican/*LUM*	+		
Fibronectin 1/*FN1*	+		
Tubulin, alpha 1/*TUBA1*	+		
Microtubule-actin crosslinking factor 1/*MACF1*	+		
Integrin, beta 5/*ITGB5*	+		
Vinculin/*VCL*		+	
Desmin/*DES*		+	
Titin-cap (telethonin)/*TCAP*		+	
Collagen, type III, alpha 1/*COL3A1*		+	
Collagen, type XVI, alpha 1/*COL16A1*			+
Transgelin/*TAGLN*			+
Nidogen (enactin)/*NID*			+
Ca^{2+} homeostasis			
ATPase, Ca^{++} transporting, cardiac muscle, slow twitch 2/*ATP2A2*	+		+
ATPase, Ca^{++} transporting/*ATP2A3*	+		
Inositol 1,4,5-trisphosphate 3-kinase C/*ITPKC*	+		
Inositol 1,4,5-triphosphate receptor, type 2/*ITPR2*	+		
Calsequestrin 2, cardiac/*CASQ2*	+		
Calmodulin 1 (phosphorylase kinase, delta)/*CALM1*		+	
Metabolism			
ATP synthase, H^+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1/*ATP5A1*	+		+
Aldo-keto reductase family 1, member B1 (aldose reductase)/*AKR1B1*	+		+
Phosphofructokinase/*PFKM*	+	+	
Phosphoglucomutase 1/*PGM1*	+	+	
Apolipoprotein D/*APOD*	+		+
Sialyltransferase 7D/*SIA77D*	+	+	
Dioxin inducible cytochrome P450/*CYP1A1*	+		+
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10/*NDUFA10*	+		
NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5/*NDUFB5*	+		
Succinate dehydrogenase complex, subunit D/*SDHD*	+		
Malic enzyme 3, NADP(+)-dependent/*ME3*	+		
Fructose-1,6-bisphosphatase 1/*FBP1*	+		
Fatty acid synthase/*FASN*	+		
Perilipin/*PLIN*	+		
Phospholipase A2, group IIA/*PLA2G2A*	+		
Others			
Transforming growth factor, beta 1/*TGFB1*	+		
Insulin-like growth factor 1 (somatomedin C)/*IGF1*	+		

ICM, ischemic cardiomyopathy.

A good microarray experimental design is the first critical step of the process, aiming to address the scientific questions at hand with maximum efficiency and power while considering the constraints of the methodology and the available material. Important factors include the number and uniformity of patient samples (when low, it is difficult or even impossible to reach statistical significance) as well as the number and type of control samples, since "normal" is often a relative term. In cardiomyopathies, this becomes a significant challenge, given the inherent difficulty in obtaining affected and, even more so, normal cardiac tissue, and has been leading to compromises in that respect. The age, gender, and ethnicity of individuals included in the study need also be considered. Additional levels of complexity are introduced by the multifactorial disease etiology of cardiomyopathies. Knowledge of the clinical history leading to full-blown cardiomyopathy and/or the causative mutations would permit focused studies on better-matched patient populations and therefore clearer, highly specific results. It was particularly interesting to witness the power of microarray analysis in identifying two of eight cases of DCM that proved to be familial and alcoholic cardiomyopathies, respectively (110). The anatomical origin of a tissue sample (e.g., left or right ventricle) (107), the duration and severity of the disease, and the types, combinations, and length of time that medications were administered, before tissue sampling, can also impact gene expression. However, sample availability is again a decisive factor. It is therefore imperative that the above parameters are carefully considered in all cases, at least at the data analysis and interpretation stages.

The majority of human microarray cardiomyopathy studies have used Affymetrix arrays (GeneChips), with a few exceptions of "home-spotted" arrays (8, 9, 52). The variety of microarray platforms (e.g., **http://biotech.deep13.com/Research/Microarray.html**) and the range of protocols used to prepare arrays (including probe sequences representing each gene, accuracy of database used to design probe sequences, length of probe, no. of probes used per gene, glass surface coating, method of probe placement on slide, etc.) are a considerable source of interstudy discrepancies that have been directly addressed by individual researchers and consortiums (the Association of Biomolecular Resource Facilities; **http://www.abrf.org/**) (54, 111). Although utilization of different approaches is key for scientific progress and could increase our detection power, it is important to realize the limits it sets and the need for careful validation. In addition, variations can also be observed between array generations within the same platform (83). The four cardiomyopathy studies using Affymetrix microarrays worked with three different whole human genome array generations (107, 110, 122, 124).

Importantly, the bioinformatical analysis of raw microarray data is one of the most critical steps in a microarray study. Together with the general interindividual variability and microarray platform differences, bioinformatical analysis is one of the most common sources of discrepancy between studies and is likely to account for some of the discrepancies between human cardiomyopathy studies. Key decisions regard the normalization and analytical approaches and the thresholds for determining significance and high-fold change (92, 104). Hundreds of commercial and publicly available software have been developed (Table 4), but a consensus on minimal requirements for analysis and raw data publication are only beginning to emerge (17). Proposals are now being made for standardizing microarray analysis (11). Although the analytic method depends partially on the nature of the study and the scientific questions addressed, the choice of thresholds is a little more arbitrary and requires confirmation by additional methods. Confirmation and follow-up of microarray findings is critical, given the large scale of the analysis and the imperfect concordance between RNA and protein expression levels. Commonly used approaches are RT-PCR, Northern and Western blotting, and immunohistochemistry (26, 100). The vast majority of human DCM/HCM/ICM studies reviewed here used at least one validation approach to confirm microarray findings. These included real-time RT-PCR (8, 52, 107, 110, 124) and Northern and Western blotting (122). Microarray gene expression measurements were predominantly in agreement with the findings of other methods, even when protein expression of the same genes was assessed. Interestingly, most discrepancies were observed in genes with nonsignificant and relatively low-fold expression changes between affected and control cardiac tissue. Furthermore, increasing the stringency in microarray data analysis led to increased concordance between microarrays and real-time RT-PCR measurements (52). These results jointly demonstrate the power of microarray technology and emphasize the vital role of thorough and stringent data analysis.

In summary, careful experimental design, accurate experimental techniques, detailed bioinformatical analysis, and confirmation of results form the basis for a solid microarray project. Cardiomyopathy microarray-based research could particularly benefit from *1*) studies of increased sample size (patient and control specimens), *2*) more detailed clinical and histological characterization of the specimens, and *3*) the establishment of minimum-application technical, analytical, and validation standards best suited to the idiosyncrasy of cardiomyopathies.

FUTURE PROSPECTS

Microarrays are already playing a key role in deciphering cardiomyopathy-related molecular pathways, but their potential is even more promising. As previously demonstrated in the context of cancer and skeletal myopathies (44, 99, 106), microarrays could contribute in the improvement of disease classification and prognosis by identifying gene expression profiles characteristic of disease subgroups. Hierarchical clustering enabled the identification of two cases with an initial diagnosis of DCM that were later shown to be patients with alcoholic and familial cardiomyopathies, respectively (110). In a similar fashion, the association of characteristic global gene expression profiles to specific cardiomyopathy subgroups, phase, or disease progression will be possible to facilitate diagnosis and improve prognosis. Diagnosis can further benefit from the development of diagnostic DNA microarrays that will contain all known cardiomyopathy or cardiomyopathy subtype-specific mutations. Low-density microarrays testing for known HCM mutations have already been developed (116). Furthermore, diagnostic microarrays could be used presymptomatically in those cases where individuals could benefit from preventative measures or medications, and after all bioethical considerations have been addressed.

In the pharmaceutical arena, microarrays can be used for a range of applications in combating cardiomyopathy, including *1*) therapeutic target identification, primarily by deciphering the molecular pathways of disease pathogenesis; *2*) drug selection, by assessing the drug effect on global gene expression in in vitro and in vivo models; *3*) optimization of current and novel drugs by "dissecting" their molecular mechanism of action; and *4*) evaluation of drug doses and side effects.

The need for novel effective drugs against cardiomyopathies can be greatly served by utilizing microarrays in the context of pharmacogenetics (the use of genetic analysis to predict drug responses, efficacy, and toxicity). With the extensive worldwide effort on single nucleotide polymorphism (SNP) mapping, SNP microarrays are rapidly evolving and should soon start being applied in the cardiomyopathy field, in parallel with gene expression arrays. SNP arrays will make it increasingly feasible to *1*) define diagnostic markers, *2*) identify individuals who will respond to therapies by determining which genomes predispose to optimal metabolic breakdown of specific drugs, *3*) associate different genomes with specific drug side effects, and *4*) facilitate and expedite clinical trials (particularly phase IIA) by segmentation of responders and nonresponders on genetic grounds. Such a segmentation scheme would enrich subsequent clinical trials, with patients more likely to respond to the tested drug, and consequently it would increase the statistical significance of clinical findings and the specificity of the drug under testing.

It is noteworthy that the overwhelming data output of microarray experiments leaves an excess of biological information from each project unutilized. Most cardiomyopathy studies have focused only on a small number of significantly changed genes. Meantime, sample number limitations sometimes render results statistically weak or even inconclusive. The advent of new and more powerful bioinformatical approaches, together with increasing numbers of cardiomyopathy microarray studies and publicly available datasets (Table 4), will gradually enable the performance of large-scale meta-analysis studies, where results from many different studies can be reanalyzed to increase statistical significance and/or answer novel scientific questions (97). The concept of meta-analysis has already been successful in increasing the power of genetic association studies on susceptibility to common diseases (64). Meta-analysis enabled the identification of disease-related gene variants, which were easy to miss in average-size studies due to their moderate effect on disease risk.

In conclusion, the genomic analysis of cardiomyopathy patients has led to identification of mutations in numerous genes, yet the number of mutations documented per gene is limited and the disease etiology remains uncertain. However, the majority of genes code for proteins with similar functional roles, and as a whole, they form the basis toward understanding this complex disease. The studies on the transcriptome of cardiomyopathy patients by microarrays have taken the molecular characterization of the disease to a new level. The identified similarities between functional categories affected in different cardiomyopathies support hypotheses of common pathogenetic mechanisms behind DCM and HCM. The large amount of unutilized data, together with follow-up work, can lead to the establishment of new disease biomarkers as well as an understanding of current drug efficacy. Importantly, it is becoming increasingly apparent that the power of microarray-defined molecular players may directly impact diagnostic, prognostic, and disease classification potential in providing the means to a new era of therapeutics.

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