[Frontiers in Bioscience S2, 653-676, January 1, 2010]

Genetic polymorphisms in dilated cardiomyopathy

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1. ABSTRACT

Dilated cardiomypathies (DCM) are characterized by dilatation and pump dysfunction of the heart. DCM has an incidence of 6/100.000 people a year contributing to a considerable number of cases of heart failure. Although etiology and pathogenesis are known to be multifactorial, they remain mostly unidentified. Recent research identified patients affected with DCM with altered gene products. These alterations can roughly be grouped into causative genes, mostly coding for cytoskeletal proteins. Other genes seem to be activated after the disease onset and are able to influence the clinical course. In this study we systematically analyzed the role of genetic polymorphisms, based on peer-reviewed articles, published in scientific journals. A total of 97 original studies and a selected number of 60 genes, that seem to be related to DCM, have been reviewed.

2. INTRODUCTION

Heart failure is a major condition that affects 700,000 individuals per year in the United States and accounts for annual costs of $10 to $40 billion (1). Similar figures are present in other Western countries. Heart failure is the primary manifestation of dilated cardiomyopathy (DCM), a group of disorders that is characterized by dilation and pump dysfunction of the heart. The etiology and pathogenesis have not yet been fully understood and DCM is widely accepted as a pluricausal or multifactorial disease. This disorder has an incidence of 3.5–8.5/100,000 population per year and a prevalence of approximately 36/100,000 population (1). An important progress in the search of the etiology of cardiomyopathies has been the recognition of hereditary transmission in a subset of DCM patients, which indicates that in these families the disease must be an altered gene product. In this systematic study we analyzed peer-reviewed articles published in scientific medical journals to evaluate the role of genetic polymorphisms in the development of DCM. All studies analyzed in this systematic review have been collected from Pub Med, a service of the National Center for Biotechnology Information (NCBI), which is an online collection of articles published in medical journals for health care purposes.

3. DILATED CARDIOMYOPATHY

According to WHO/ISFC criteria cardiomyopathies are defined as diseases of the myocardium associated with cardiac dysfunction. They are classified as dilated, restrictive, and hypertrophic cardiomyopathy. Arrhythmogenic right ventricular cardiomyopathy has been added as an entity of its own (2).

Dilated cardiomyopathy (DCM) is the most frequent form of cardiomyopathy. The etiology of DCM seems to be idiopathic in about half the patients. Despite recent advances in medical and surgical therapies, DCM remains an important cause of mortality and morbidity and is a leading indication for heart transplantation. The incidence rate is estimated to be 6 /100,000 persons/year (3). Relatively similar results were found in other countries, particularly Western countries. However, the true incidence is probably higher, since all available studies are retrospective. Asymptomatic cases were not identified and not taken into account in these studies. DCM is also associated with high rates of sudden death due to ventricular arrhythmia (mainly ventricular tachycardia (VT) that may occur at any stage. Five-year mortality rates of 30–50% are reported, making early detection and treatment a priority in healthcare.

DCM can be isolated or associated with additional conduction and/or muscular disorders. The majority of patients presents with sporadic DCM. Autosomal recessive, mitochondrial and X-linked DCM have been described (4). In isolated autosomal dominant DCM, null and missense mutations were identified in eight different genes. Thus, mutations in the a-cardiac actin (ACTC), the desmin (DES), the d-sarcoglycan (SGCD) and the metavinculin (VCL) genes are supposed to impair the force transmission from the sarcomere to adjacent sarcomeres and to the extracellular matrix, whereas mutations in the titin (TTN), troponin T (TNNT2), b-myosin heavy chain (MYH7) and a-tropomyosin 1 (TPM1) genes are thought to alter the force production generated by the sarcomere (4). In nonisolated DCM, Lamin A/C (LMNA) gene mutations have been shown responsible for DCM associated with conduction system disease and/or muscular disorders (4). Some of these genes were

previously mapped by a positional cloning strategy in large families (4).

In a genetically heterogeneous disease such as DCM, linkage analysis may be of limited value, mainly because of the high mortality associated with the disease and the incomplete penetrance of mutations leading to this disease. Consequently, one of the key strategies to identify morbid genes is the screening for mutations in candidate genes in large cohorts of DCM patients. The main difficulty in this approach is probably to know if a given molecular variant is a neutral polymorphism or a disease-causing mutation. To assess a causal effect for a DNA variant, one should verify that the variant is only present in affected subjects and absent from a large number of ethnically matched controls, the gene has been found mutated in at least two independent affected subjects, probably preferably familial cases and finally the variant has a significant predicted quantitative or qualitative effect on the encoded protein.

DCM is characterized by ventricular chamber enlargement, due to ventricular dilation with mild or minor hypertrophy, depressed myocardial contractility and increased heart weight (5). Although the heart weight can increase by one fourth, the ventricular wall thickness is generally normal because of the effect of dilation. Both ventricles can be impaired, but sometimes the dysfunction is unilateral (5). Diastolic compliance may be disturbed as well. Subendocardial and transmural scarring can occur even in the absence of thromboembolic obstruction of the coronary arteries. In some cases, interstitial, perivascular and endocardial fibrosis may be prominent. Microscopically, there is evidence of myocyte degeneration, in particular sarcoplasmic degeneration, with ‘‘irregular’’ hypertrophy of myofibers, and the occurrence of systolic contractile dysfunction has been linked to these changes (5).

Although common causes of DCM are viral myocarditis, alcohol toxicity, autoimmune diseases and gene mutations (5), its etiology often remains undetermined and the term idiopathic DCM is used. Of note, a familial origin of the IDCM may be identified in 20% to 25% of cases (3).

3.1. Familial and monogenic forms of dilated cardiomyopathy

The importance of the heredofamilial forms of DCM was under-recognized until prospective studies were performed. Charron *et al.* report that dilated cardiomyopathy was familial in at least 20% of cases (3). This was later confirmed subsequently in different populations (3). If more than one subject in a family is affected with idiopathic DCM, the term familial idiopathic DCM (IDCM) should be used. In familial forms of IDCM, several genetic loci have been identified in rare monogenic forms of the disease. In familial forms of DCM, mutations of genes coding for cytoskeletal proteins related to force transmission, the following genes have been described: dystrophin, cardiac actin, desmin and d-sarcoglycan (5). However, DCM may also be caused by mutations of genes that encode for other sarcomeric proteins in cardiomyocytes that can exhibit sarcoplasmic degeneration (5).

The penetrance of the disease (percentage of subjects who express the disease among carriers of the mutations) is incomplete in most familial studies, and also appears to be influenced by age and sex (6). In particular, some genes implicated in familial DCM (dystrophin, tafazzin, actin, desmin, lamin A/C, d-sarcoglycan, b-myosin heavy chain and troponin T) are also associated with skeletal myopathies, suggesting a common role in pathogenesis. Thus, familial DCM is a very heterogenous disorder, as suggested by the different patterns of inheritance, the different phenotypes and the different genes or loci identified. The autosomal dominant forms are probably the most common ones (7). In complex diseases that do not exhibit a clear pattern of familial aggregation, the candidate gene approach is a strategy widely used to identify susceptibility genes. All genes coding for proteins involved in biochemical or physiological abnormalities of cardiac function are potential candidates for IDC.

Various subtypes of familial DCM have been reported and characterized:

1 DCM with conduction defects and arrhythmias with an autosomal dominant pattern have been associated with one gene, lamin A/C, and one locus 3p22 (3).

2 An autosomal dominant form of DCM with mitral valve prolapse has been associated with a locus on chromosome 10q21–23 (3).

3 An autosomal dominant variety of DCM associated with conduction disorders and myopathy has been linked to chromosome 6q23 (3).

4 An autosomal dominant form of DCM associated with sensorineural hearing loss was described on chromosome 6q23–24 (3).

5 Autosomal recessive forms of the disease have occasionally been reported and no location has been published (for isolated DCM).

6 DCM linked to chromosome X is related to mutations in the dystrophin gene (3).

7 Large deletions of mitochondrial DNA have also been associated with DCM. The causal role of these genetic alterations is not definitely established, because alterations of mitochondrial DNA have also been reported in many other diseases (3).

There have been several studies comparing the clinical characteristics of familial dilated cardiomyopathy with those of sporadic dilated cardiomyopathy, where such sporadic DCM groups could include some familial cases because the term ‘sporadic’ has not been clearly defined. Although their results have not always been consistent, it

has been suggested that familial DCM cases had clinical characteristics such as: younger age at diagnosis, higher ejection fraction, higher frequency of ST-T segment abnormalities on the ECG, and higher frequency of atrioventricular conduction defects than sporadic dilated cardiomyopathy cases (3, 8).

The broad-spectrum of the disease (clinical situations with mild abnormalities or with other cardiac or non-cardiac abnormalities) and the absence of a consensus as to diagnostic criteria prompted the proposal of guidelines with new criteria for the study of familial dilated cardiomyopathy (9). Tsubata *et al.* have reported that for X-linked DCM, two genes have been identified, including tafazzin (G4.5) in cases of the infantile-onset DCM (Barth syndrome), isolated left ventricular noncompaction and dystrophin in later-onset X-linked cardiomyopathy (XLCM) (1, 3).

In the more common autosomal dominant DCM, five loci have been mapped for pure DCM (1q32 [ref. 1], 2q31 [ref. 1], 9q13-q22 [ref. 1], 10q21-q23 [ref. 1], and 15q14 [ref. 1]) and four loci have been mapped in families with DCM and associated with conduction disease (1p1-1q21 [ref. 62], 2q14-q22 [ref. 63], 2q35 [ref. 1], 3p25-p22 [ref. 1], and 6q23 [ref. 1).

DCM is common in patients with Duchenne muscular dystrophy (DMD), but it most frequently occurs late in the disease, being a terminal event in about 10% of patients with DMD (10). In patients with Becker muscular dystrophy (BMD), DCM can manifest earlier and be a prominent clinical feature (1, 4). However, the cardiac genotype to phenotype correlation is not good in patients with DMD or BMD, because some patients with identical mutations in the dystrophin gene may develop a cardiomyopathy while some do not.

4. SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs)

An important requirement in the analysis of genes related to a disease is the identification of genetic markers such as single nucleotide polymorphisms (SNPs) (11). The advantages of SNPs include their abundance in the genome, their relative stability within an organism and the ease with which highly automated analysis systems and simple analytical methods, including PCR-restriction fragment length polymorphism (RFLP), can be developed to genotype individuals for informative SNPs. These advantages make SNPs the markers of choice for mapping and identifying disease-associated genes (12).

SNPs are DNA sequence variations that occur, when a single nucleotide A (adenine), C (cytosine), T (thymine) or G (guanine) in the genome sequence is changed. For example, two sequenced DNA fragments from different individuals, AAGC**C**TA to AAGC**T**TA, contain a difference in a single nucleotide. The second cytosine in the first oligonucleotide sequence is replaced with a thymine. DNA sequencing is used to determine the order of the four bases A (adenine), C (cytosine), T (thymine) or G (guanine), i.e. the base sequence in a DNA molecule. Manual and automatic sequencing of the human genome can be used to identify single DNA variations or SNPs. Genome variations include mutations and polymorphisms that may be distinguished by frequency, in addition to the association with disease. A location in the genome where 94 % of people have an adenine, and the remaining 6% have a thymine, is a polymorphism. If one of the possible sequences is present in less than 1% of the population (99.9% of people have a T and 0.1% have an A), then the DNA variation is called a mutation (11). Thus, SNPs are variations that involve just one nucleotide or base. For a variation to be considered a SNP, it must occur in at least 1% of the population. Any one of the four DNA bases may be substituted for any other: an A instead of a T, a T instead of a C, a G instead of an A, and so on. Theoretically, a SNP could have four possible forms, or alleles, since there are four types of bases in DNA. Almost all common SNPs have only two alleles (11).

Several groups worked to find SNPs and create SNP maps of the human genome. Among these groups were the U.S. Human Genome project and a large group of pharmaceutical companies called the SNP Consortium or TSC Project. SNP data were released at certain intervals and ceased in the autumn of 2002, when the aquisition of genotype and allele frequency data was completed. The two major human gene-based polymorphism databases on the World Wide Web are called: HGVbase and dbSNP. As the results of the Human Genome Project unfold, numerous predictions of its impact have been made. These range from patients having individualized genetic drug profiles to gene therapy reprogramming of dysfunctional organs (11).

Estimates place the number of genes in the human genome at a surprisingly low 30,000 to 40,000, but with substantial posttranscriptional complexity. The Human Genome Project, at its core, is a vast amount of information about gene location and sequence. The association of certain cytoskeletal gene variants and DCM may also help our understanding of mechanisms and eventually lead to targeted therapeutics (11).

5. SNP's List

5.1. Angiotensin I-converting enzyme (ACE) gene

Angiotensin-converting enzyme (ACE) gene (Gene Bank accession number: NM 000789.2) is one of the most frequently studied genes affecting idiopathic DCM. The ACE gene is localized on chromosome 17q23 and is characterized by a major insertion/deletion polymorphism resulting in 3 genotypes (DD, ID, and II), which affects serum and tissue ACE activity as well as other vasoactive substances. ACE is an ectoenzyme found on the external surface of the endothelial and epithelial cell membranes. It enhances the synthesis of angiotensin-II, which is liberated from angiotensinogen (AGT) by the sequential action of renin and ACE. In adult humans, the effects of angiotensin II are mainly mediated by the AGTR1, a G-protein-coupled receptor expressed by many cell types, in particular cardiomyocytes. Thus, each component of this system may constitute a candidate for DCM. Angiotensin-II promotes

proliferation, migration, and hypertrophy of vascular smooth muscle cells. Moreover, the increased free radical generation by angiotensin-II contributes to endothelial dysfunction (3, 13). A genetic polymorphism in the ACE gene one of the two enzymes of renin-angiotensin system (RAS), a multienzyme, multilocale axis, has been found to have a strong association with higher risk for acute coronary events, sudden cardiac death, vascular restenosis after angioplasty, and idiopathic and hypertrophic cardiomyopathy (14). ACE genotype affects both serum and tissue ACE levels; there is high variability among individuals in ACE concentrations, mainly due to the presence of a genetic polymorphism.

Fifteen studies were retrieved from the literature, but the role of this polymorphism remains controversial. Kucukarabac *et al.* found that ACE gene I/D polymorphism is not associated with idiopathic DCM, although ACE concentration was high in this category of patients (first study, 15). DD genotype is associated with higher concentrations of circulating ACE (16). Kucukarabac *et al.* also reports that plasma ACE concentrations were higher in hypertensive patients with DD genotypes (15). In a second study, Shim *et al.* reported that the ACE gene ID polymorphism is thought to be associated with Kawasaki disease, but not with the development of coronary dilations (13). Cuoco *et al.* reported an association of the D allele of the ACE gene with earlier onset of symptoms in patients with heart failure, due to alcoholic or hypertensive heart disease and decreased survival rate (third study, 17). Mortality was higher in patients older than 50 years with DD genotype. In a fourth study, it was found that stable, treated patients with congestive heart failure (CHF) with the ACE-DD genotype had more restrictive pulmonary changes, a reduced lung diffusing capacity, and poorer exercise tolerance compared with the patients with CHF with the ACE II genotype (18). In addition, the DD genotype was associated with a more tachypneic breathing pattern with a reduced breathing efficiency.

Komajda *et al.* reported that the ACE gene has been the most studied polymorphism with respect to LVH (fifth study, 19). One of the studies cited by Komajda *et al.* found that the D allele was associated with LVH. It included 717 men and 711 women, selected from the population covered by the MONICA (MONItoring of CArdiovascular disease) register of Augsburg, Germany. Another study mentioned by Komajda *et al.* revealed no association between the polymorphism and LVH in the 2439 subjects from the Framingham Heart Study (19). Thus, the exact role of the ACE ID polymorphism remains to be clarified. Candy *et al.* are unanimous with previous studies, where it was demonstrated that a greater percentage of patients with end-stage IDC are homozygous for the deletion polymorphism of the ACE gene compared with a normal population (16). The data of the sixth study also agreed with previous studies, where it was demonstrated that study subjects with idiopathic heart failure and the DD genotype have a greater mortality, than those with the insertion sequence. The main finding of the sixth study is the association between the DD genotype of the ACE gene and a reduced LV systolic performance and an increased LV cavity size in patients with IDC. The insertion–deletion (ID) polymorphism of the ACE gene is a marker linked to differences in plasma and cardiac ACE activity, as well as to an increased mortality in patients with idiopathic heart failure. Vancura *et al.* showed that the patients in endstage DCM do not differ in the frequency of ACE gene alleles compared with the general population (20). The underrepresentation of the ID genotype possibly reflects a protective effect of heterozygosity on the development of the disease, with a limited influence of ID polymorphism on further progression of the disease.

Malik *et al.* reported that the RAS is one of the important factors regulating blood pressure, as well as fluid and electrolyte balance (eighth study, 14). It may have an important role in the pathogenesis of hypertension. The relation between left ventricular mass (LVH) and deletion polymorphism of the ACE gene was evaluated in a white population (19). The main findings of this study, reffered to in a study by Komajda *et al.*, mentioned above, are that the DD genotype was not associated with any increased risk for LVH in women. Male subjects with the DD genotype had LVH more often than those with the II genotype (odds ratio 2.63). The strongest association between LVH and the DD genotype was identified when the blood pressure was normal, thereby implicating the D allele as an independent risk for LVH. The missing relation of ACE gene with hypertension was expected in this study, in accordance with previous results, whereas, the negative association of the genotype with LVH in women was unexpected (19). An explanation might be that the hormonal milieu in women dilutes any predisposing risk of the gene for LVH. In a multicenter case-control study designed to identify any genetic factors predisposing increased risks for myocardial infarction, Furrukh *et al.* reports that in 1300 subjects the DD genotype was seen significantly more frequently in subjects with myocardial infarction than in controls (14). Candy *et al.* cited the detection of an excess of DD genotypes in the subjects with cardiomyopathy compared with controls (16). Keeping in mind the association of DD genotype with atherosclerosis and myocardial infarction, one may not be surprised by these findings. In fact, they support the observations mentioned earlier. In contrast, Sanderson *et al.* could not find an increased frequency of the ACE DD genotype in Chinese patients with end-stage heart failure due to ischemic or dilated cardiomyopathy, compared with controls (ninth study, 21). The discrepancies in the findings between this study, other recent studies and earlier reports are probably due to differences in the criteria for selecting patients and controls and also possibly due to differences in genetic background in the samples examined. Schmidt *et al.* reported that the angiotensin-converting enzyme insertion/deletion polymorphism is not a major risk factor for development of end-stage renal failure and in hemodialysis patients (tenth study, 22). Furthermore, the frequency of hypertension, coronary artery disease, left ventricular hypertrophy and dilated cardiomyopathy were analyzed according to the ACE genotype, but the deletion allele could not be defined as a risk factor in the study's hemodialysis population. Montgomery *et al.* could also not find an association between ACE genotype and either the diagnosis of

idiopathic dilated cardiomyopathy itself or progression of the disease (see below) (eleventh study, 23). An association between the DD genotype of this polymorphism and non-familial dilated cardiomyopathy has been described earlier (16). In contrast, five subsequent studies found a lack of association between ID angiotensin converting enzyme polymorphism and the disease (23). These conflicting results may be the consequence of insufficient sample size and/or bias in recruitment of patients or controls. To avoid these limitations, a national collaboration was established in France which allowed the collection of 433 patients (of European origin) with idiopathic DCM matched with 400 control subjects from the MONICA registry and genetic analyses are currently under way (the Cardigene network). In a further study no association was found between the disease and the ID angiotensin converting enzyme polymorphism or other polymorphisms of the renin–angiotensin–aldosterone system, the b1-adrenoreceptor, tumor necrosis factor alpha, transforming growth factor beta1, nitric oxide synthase 3 and brain natriuretic peptide genes (24). Charron *et al.* citated a genetic polymorphism in intron 16 of the ACE gene, which was strongly related to ACE plasma level (it accounts for nearly half of the plasma level variability) and myocardial concentration (3). The replication of epidemio-genetic studies and the creation of adequate experimental studies will help to definitively establish the pathogenetic role of the permanent increase in ACE expression associated with the deletion polymorphism genotype.

5.2. Angiotensin-II type 1 receptor (AGTR1) gene

The AGTR1 was found selectively downregulated in failing left ventricle from patients with endstage heart failure due to IDC, suggesting that the failing human heart is exposed to increased concentrations of angiotensin-II at the cellular level (24). Genetic variation in the AGTR1 gene might then influence susceptibility to cardiomyopathy or progression of the disease, although this possibility has not yet been examined. The selection of the two polymorphisms investigated in a first study (A-153G and A+39C) was based on the fact that they had been previously suggested to be involved in susceptibility to myocardial infarction and essential hypertension (24). No interactions with daily alcohol use for the various polymorphisms of the renin-angiotensin-aldosterone system were found in a second study (25). These data agree with earlier studies from various countries that showed no associations between either, the ID polymorphism of ACE, or the 1166A/C polymorphism of AGTR1, the LV dimensions and mass at echocardiography. Kajander *et al.* could not identify any genetic susceptibility factors from among the common gene variants of alcohol metabolism and renin-angiotensin-aldosterone system (25).

5.3. Angiotensinogen (AGT) gene

Angiotensinogen (AGT) is the precursor of angiotensin-I. Its concentration is rate limiting for the generation of angiotensin-II. The first study revealed, that a CYP11B2 gene variant, but neither ACE, nor AGT gene variants examined predicted improvement in LVEF measured after initiating medical therapy with furosemide, digoxin and ACE inhibitors in patients of African ancestry with IDC (26). An association between the M235T and T174M polymorphisms of the AGT gene and essential hypertension has been reported in previous reports (24). A possible implication of these two polymorphisms in the susceptibility to IDC was investigated in a welldesigned study, but no association was observed with the disease itself or its severity (24). Thus, there is no association between the T174M and M235T polymorphisms of the AGT gene and DCM.

5.4. Tumor necrosis factor-alpha (TNF-alpha, TNF-a) gene

An increase of TNF-alpha in the heart can cause lethal pump failure (27). An increased expression of TNF-alpha has been observed in the failing human heart (24). The level of TNF-alpha production is, in part, determined by promoter gene polymorphisms. TNF-alpha levels are high in patients with end-stage heart failure, but even higher in patients on left ventricular assisted device (LVAD) support. This increased TNF-alpha production is not associated with the TNF2 polymorphism, but seems to be associated with TNF1. Moreover, it has been shown that patients that received a donor heart with the TNF2 polymorphism had more severe rejection episodes during the first six months after HTx than patients that received a donor heart with TNF1 polymorphism. In HTx the donor TNF-alpha gene seem to play a more important role in severity of acute rejection than that of the patient (27).

Chagas disease, caused by the protozoan Trypanosoma cruzi, is endemic in Latin America, affecting 16-18 million individuals. Up to 30% of the infected individuals develop chronic Chagas disease cardiomyopathy (CCC) 5-30 years after the acute infection. The differential susceptibility towards CCC development is incompletely understood. Familial aggregation of CCC cases in endemic areas indicates that genetic factors may be involved in differential susceptibility to CCC. Genetic susceptibility may play a role in the clinical outcome of Chagas disease and in the differential survival of severe CCC patients. No evidence was found to support an association between TNF2, or TNFa2, alleles and the development of Chagas disease, but it has been shown that patients, positive for TNF2 or TNFa2 alleles, have a shorter survival time compared to those carrying other alleles (28). However, Drigo *et al.* revealed no association between the TNFa microsatellite, the -308 TNF promoter polymorphisms and the development of CCC or progression to more severe forms of cardiomyopathy in Brazilian patients, affected with Chagas disease (28). *(Vide infra)*

Drigo *et al.* also revealed no association between TNF2 or TNFa2 alleles and the development of Chagas disease, but it has been shown that patients positive for TNF2 or TNFa2 alleles have a shorter survival time compared to those carrying other alleles (29).

No association was observed between DCM and the alpha-238 and alpha-308 (G/A) polymorphisms of the TNF-alpha gene in another study (30). These data agree with earlier studies that showed no associations between

the TNF-alpha/-308 (G/A) polymorphism and DCM (24, 30). Ito *et al.* showed that the TNF-alpha-308 A allele was more frequent in Japanese patients with idiopathic DCM, suggesting that a variability of TNF-alpha polymorphisms in patients with DCM have different ethnic origins (31). Alikasifoglu *et al.* suggested that there is no association between the alpha-238 and alpha-308 (G/A) polymorphisms of the TNF-alpha gene and DCM in Turkish patients, at least (30). Tiret *et al.* selected the G-308A polymorphism, because it had been shown to affect transcriptional activity (24). This study also revealed no association between the G-308A polymorphism and DCM. Ito *et al.* stated that the frequency of TNFA2 in the control group was 3.0 % and this value was similar to a prior report for Japanese patients (31). The frequency of the TNFa2 allele was high in Japanese patients with idiopathic DCM compared to the normal controls. TNF-alpha-308 position polymorphisms were, however, not associated with clinical characteristics, hemodynamic variables, or clinical courses of patients affected with DCM. Serum TNF-alpha levels of patients with idiopathic DCM were found to be higher than those of the controls. These data indicate that the TNFa2 allele has the potential to produce high levels of TNF-alpha under stimulated conditions. This is the first report, which revealed an association between TNF-alpha polymorphism and idiopathic DCM. This data seems to be controversial, but there may be two reasons to explain the difference between the previous report and the present study. The etiology of DCM cardiomyopathy may be diverse among races or countries, similar to Chagas disease (31). The second reason may be related to differences in the underlying diseases of the study groups. The present study included only patients with idiopathic dilated cardiomyopathy. The previous study contained patients with congestive heart failure caused by miscellaneous etiologies, such as ischemic heart disease, cardiomyopathy and other diseases. At least in the Japanese population, the TNFA2 allele seems to be associated with the pathogenesis of idiopathic dilated cardiomyopathy.

5.5. Interleukin-4 (IL-4) gene

Interleukin-4 (IL-4) is a cytokine of the T-helper 2- cell (Th2) sub-type, which is mainly produced by activated T cells and mast cells. Bijlsma *et al.* found that IL-4 production within the donor's heart and by the donor's cells is important for reducing the incidence of rejection episodes (32). In transplantation Th2 cells are believed to induce graft tolerance. Previous studies revealed that patients with a relatively high frequency of IL-4 producing helper T lymphocytes (HTL) before heart transplantation (HTX) had no or less rejection episodes compared with patients with a low frequency of IL-4 producing HTL. This study showed that the incidence of rejection was significantly lower in patients that received a donor heart with the T-positive genotype compared with patients that received a heart from a T-negative donor. Patients, who had the T-negative genotype and received a heart from a T-positive donor, suffered significantly less from rejection than T-negative patients that received a T-negative donor heart. This was not significant in the T-positive patient group.

5.6. Interleukin-10 gene

Various cytokines play important roles in the pathogenesis of congestive heart failure. IL-10 has anti-inflammatory actions and its production is, in part, induced by TNF-alpha in an autoregulatory feedback manner (31). Ito *et al.* revealed no association between IL-10 -1082 polymorphism and the presence or severity of idiopathic DCM (31). The frequency of the IL-10 A/A genotype was very low. This illustrates the point that studies with very large patient numbers may be necessary to assess the impact of gene polymorphisms, which occur at low frequency.

Cytokine release by T-lymphocytes and macrophages in the microenvironment of a transplanted graft is of critical importance in acute rejection of the graft. This rejection is believed to be induced through pro-inflammatory Th1 cytokines, whereas Th2 cytokines are involved in the induction of transplant tolerance (33). An important Th2 cytokine is interleukin (IL)-10. Bijlsma *et al.* included 70 patients who underwent heart transplantation as treatment for end-stage heart disease in their study (33). Sixty cardiac donors served as healthy controls, 35 patients were suffering from ischaemic heart disease (IHD), and 29 from DCM. There were no differences in allele- and genotype distribution between patients, donors, rejectors and nonrejectors. These data suggest that SNP genotypes and IL10.G microsatellite alleles have no influence on the development of heart failure and graft rejection after heart transplantation.

5.7. Phospholamban (PLN) gene

Phospholamban (PLN) is a protein that regulates the sarcoplasmatic reticulum Ca2+ pump and controls the size of the sarcoplasmatic reticulum Ca2+ store during diastole (34).

Abnormal calcium homeostasis is a prototypical mechanism for contractile dysfunction in failing cardiomyocytes. Depressed calcium cycling in experimental and human heart failure reflects, at least in part, impaired calcium sequestration by the smooth reticulum (34). It has been shown that increases in the relative levels of PLN to Ca2+-ATPase in failing hearts and resulting inhibition of Ca2+ sequestration during diastole, impairs contractility. The results of this study indicated that the g.203A4C genetic variant in the human PLN promoter might contribute to depress contractility and accelerate functional deterioration in heart failure (34). No mutation was found in the coding exon of phospholamban (PLN) gene in a further study (35). The mutation frequency in PLN appears to be very low in the population screened in this study (no mutation). Estimates from previous reports evaluate the frequency of familial dilated cardiomyopathy causing mutations in PLN (one mutation) to 5%. These data indicate that mutations in phospholamban gene are rare in familial subtype of DCM (35).

5.8. Heat Schock Protein A1-like and A1B genes

Polymorphisms in the MHC-associated stressprotein genes HSPA1L and HSPA1B have been associated with (auto-) immune disorders, although these

associations seem to be due to linkage dysequilibria with other HLA markers (36). Since the HSPA1B 1267 A->G polymorphism remains silent and studies examining expression of HSPA1A and HSPA1B mRNA after stimulation by lipopolysaccharide did not show an association with HSPA1B 1267 A->G polymorphism, other polymorphic sites in linkage with HSPA1B 1267 A->G were postulated to be responsible for the above mentioned association with autoimmune and inflammatory diseases. The pathogenetic mechanism responsible has not yet been identified. Further study of HSPA1B/HSPA1L genes in addition to neighboring markers (TNF, HLA-DR,-B, and C4) may identify ancestral haplotypes associated with inflammatory DCM, which may help in defining potential sites for MHC associated susceptibility genes (36).

5.9. Bone morphogenetic protein-10 (BMP10) gene

Bone morphogenetic protein-10 (BMP10) interacts with a protein called titin-cap (Tcap). BMP10 is localized on the cell surface and at the stretch-sensing Z disc of cardiomyocytes. Nakano *et al.* reported an association of the variant of the human BMP10 gene, Thr326Ile, with susceptibility to hypertensive DCM (37). The variant BMP10 showed decreased binding to Tcap and increased extracellular secretion of BMP 10. BMP10 is a member of the TGF-beta family. About 5% of hypertensive patients eventually developed systolic dysfunction, despite having blood pressure similar to others, who did not develop it, suggesting that genetic factors might play pivotal roles in the transition from compensated hypertrophy to heart failure (37). It has been shown that BMP10 possessed prohypertrophic activity and was upregulated in hypertensive cardiac hypertrophy

5.10. Titin/connectin gene

Matsumoto *et al.* reported in an earlier study several mutations in titin/connectin gene found in patients with hypertrophic cardiomyopathy or DCM (38). A hypertrophic cardiomyopathy-associated titin/connectin mutation (Arg740Leu) was found to increase the binding to actinin, while other DCM-associated titin/connectin mutations (Ala743Val and Val54Met) decreased the binding to actinin and Tcap/telethonin, respectively. Since the N2-B region expresses only in the heart, it was speculated that functional alterations due to the mutations cause cardiomyopathies. Matsumoto *et al.* showed in another study a novel TTN mutation found in the is2 region of titin/connectin and functional alterations due to the N2-B mutations in binding to FHL2 (38). These findings indicate that N2-B region mutations may cause cardiomyopathy through dysregulation of recruitment of metabolic enzymes. Further studies, including mutational analysis of FHL2 as a candidate disease gene for hypertrophic cardiomyopathy and dilated cardiomyopathy, are needed to clarify this issue.

5.11. Human leukocyte antigen (HLA) gene

The immune system is strictly related to human leukocyte antigen (HLA). Components of the major histocompatibility complex may serve as markers for the propensity to develop immune-mediated myocardial damage. HLA class II genes, especially highly polymorphic HLA-DQ genes, play an important role in the activation of immune responses and thus control the predisposition for or protect from idiopathic DCM. Development of autoimmune inflammatory damage occurs only in patients with a predisposing genetic background. Lin *et al.* reported that HLA-G could be a genetic factor in the development of IDC (first study, 39). Both the -14 bp/-14 bp genotype and the 14 bp deletion allele could be a susceptibility marker, whereas both the +14 bp/+14 bp genotype and the 14 bp insertion allele could be associated with protection to developing idiopathic dilated cardiomyopathy. Because the conclusions were based on the analysis of small number of patients, other studies with larger number of patients will be required. Further Investigation of other polymorphisms will be required. In a second study, Liu *et al.* suggested that HLA-DQA1 0501 and DQB1 0303 were related to the genetic susceptibility to IDC while DQA1 0201, DQB1 0502 and DQB1 0504 alleles conferred protection from IDC (40). It has been shown that HLA-DQ allele polymorphisms may serve as genetic markers for IDC and be involved in the regulation of immune specific response to auto- or exterior anti-myocardium antibody. Similar results were found in a third and fourth study (41, 42). Liu *et al.* suggested that HLA-DQA1 0501 and DQB1 0303 are related to the genetic susceptibility to IDC while DQA1 0201 allele confers protection from IDC. They found that HLA-DQA1 0501 is associated to the genetic susceptibility to IDC while DQA1 0201 allele confers protection from IDC (42). The data of the fifth study indicate that polymorphism of HLA-DR and –DQ molecules, as well as beta-cardiac myosin, do not influence the susceptibility to different clinical forms of Chagas disease or the progression to severe Chagas cardiomyopathy (36). HLA typing of class I and class II antigens have been carried out in Chagas disease. The results turned out quite variable, due to technical problems, low number of samples, and lack of adequately matched controls. CCC patients typically migrated to bigger towns for specialized care in cardiology units. The majority of studies have compared cardiomyopathy outpatients with normal controls, who do not come from endemic areas. Ethnic variability is also a ponderable factor in Latin American countries where these studies have been carried out. The association of IDC with HLA-DRB1 1401 in nonfamilial Japanese patients was confirmed in the sixth study (5).

5.12. Non-HLA gene block consisting of the NFKBIL, ATP6V1G2, BAT1, MICB, and MICA genes within the MHC class III - class I boundary region

Hepatitis C virus (HCV) infects various extrahepatic tissues and causes clinical manifestations that do not originate from hepatopathy (43). The extrahepatic manifestation includes cardiomyopathy. DCM and hypertrophic cardiomyopathy (HCM) are two major clinical phenotypes of the hepatitis C virus (HCV)-associated cardiomyopathy. DCM and HCM can be found in 5.7 and 6.6%, respectively, of random patients with positive HCV antibody (43). Shichi *et al.* showed that the non-HLA gene block consisting of the NFKBIL, ATP6V1G2, BAT1, MICB, and MICA genes within the MHC class III - class I boundary region was strongly associated with the susceptibility to HCV-DCM. No

significant association between the MHC genomic region and HCV-HCM was found. This observation suggests that HCV-DCM and HCV-HCM have at least two distinct pathogenic mechanisms in relation to the MHC-meditated immune response, although the patients that developed DCM/HCM with HCV infection consisted of a small number of cases. The conclusions were based on the analysis of a small number of patients. Thus, further studies with a greater number of patients are necessary. The difference in the MHC-related disease susceptibility for HCV-associated cardiomyopathy strongly suggests that the development of HCV-DCM and HCV-HCM is under the control of different pathogenic mechanisms.

5.13. Alpha2C-adrenoceptor gene

The 2C-Adrenoceptor deletion may be a novel, strong and independent predictor of reduced event rates in DCM patients treated according to guidelines (99% ACEI, 76% b-blockers).

DCM patients with the deletion variant Del322–325 in the a2C-adrenoceptor showed significantly decreased event rates. Genetic variation in the a2C-adrenoceptor gene (a2CDel322–325) is independently associated with survival and absence of events in patients with severe heart failure due to dilated DCM. Regitz-Zagrosek *et al.* suggest that the a2C-adrenoceptor gene (a2CDel322–325) polymorphism is an important and independent genetic factor that determines survival in patients with advanced DCM (44). Caucasian patients with the a2C322–325 deletion have a functional advantage in the presence of an equally impaired left ventricular function in comparison with the 'wild-types'. Further studies are needed to analyse the effect of this polymorphism on the clinical course of patients with DCM and its implications for therapy.

5.14. Beta1-adrenoceptor gene

The beta1-adrenergic receptor (b1-AR) is a G protein-coupled receptor expressed in the heart and other tissues, acting as a receptor for catecholamines. Coding and promoter polymorphisms of this receptor have been identified in the general population (3). The variants beta(1)Ser49, beta(1)Arg389,and alpha(2c)Del322-325 were found in negative association with the susceptibility to risk factors for chronic heart failure due to DCM. However, the alpha(2c)Del322-325 variant might be protective (45). Wenzel *et al.* reported that the –2146T>C polymorphism existed in strong linkage disequilibrium with the Ser49Gly mutation in the N-terminus of the receptor, in the probands studied (46). The -2146C homozygotes were found in patients only. Genotype frequencies differed significantly between the controls and the patients affected with idiopathic DCM. The possible involvement of genetic variants in other cardiac diseases related to the b1-adrenoceptor remains to be clarified. An additional study (47) showed that the Arg389 allele frequency in the Japanese population is similar to that of a Scandinavian group, but a little lower than that in the French group. The beta1-AR gene is not responsible for DCM. No relationship with the left ventricular function of DCM was found. Finally, the Gly389 allele suppressed the occurrence of VT in patients with DCM. The involvement of the Arg389Gly b1-adrenoreceptor gene polymorphism in heart failure was also assessed in a group of 297 patients in a further study (3). The variant was associated with significant differences in exercise capacity. Furthermore, the Gly389 b1-adrenoreceptor variant has been reported, with an increased incidence in the Afro–American population as compared to non-African.

5.15. Beta2-adrenoceptor gene

Beta1- and beta2-adrenergic receptors are G protein-coupled receptors for the catecholamines, epinephrine and norepinephrine. Beta2-adrenoceptors (AR) play an important role in the regulation of vascular and bronchial smooth muscle tone (48). They also exist in the human heart and contribute to the regulation of heart rate and contractility (48). The results of the first study do not support the hypothesis of a higher abundance of the Thr164Ile-beta2AR variant in HTX-patients (48). Leineweber *et al.* mentioned a study of 259 patients with chronic heart failure (CHF) due to ischemic or DCM and found that those patients harboring the heterozygous status for Thr164Ile-beta2AR, exhibited a rapid progression to death or heart transplantation (HTX).

5.16. Beta myosin heavy chain gene

A mutation analysis of four genes involved in familial DCM in a population of idiopathic DCM was carried out in 96 independent patients (54 familial and 42 sporadic) in the first study (35). Seven mutations in MYH7, one in TNNT2 and none in PLN or in the VCL cardio-specific exon were found. In HCM patients, more than 80 different disease mutations have been identified to date in MYH7 (35). Together with the very low occurrence of myosin heavy chain mutation or polymorphism in the general population, as observed by systematic MYH7 screening and with an almost complete association of nonconservative MYH7 mutations with cardiac diseases, such as hypertrophic cardiomyopathy (HCM) or DCM, these genetically based observations strongly support a role in disease for the seven newly identified mutations in this study (35). These findings confirm the genetic heterogeneity of FDCM, with a prominent role of MYH7 in DCM with a 10% mutation frequency in familial forms and delayed onset of the disease in the studied population and a low occurrence of PLN and VCL mutation, a 2% frequency of TNNT2 mutation (R141W is associated with high penetrance and early onset) (35).

Genetic susceptibility may play a role in the clinical outcome of Chagas disease and in the differential survival of severe Chagas disease patients. Chronic Chagas disease cardiomyopathy (CCC) is the most important clinical outcome of infection by the parasite Trypanosoma cruzi. One-third of CCC patients develop heart failure due to end-stage dilated cardiomyopathy, and their survival is reduced by 50% compared to patients with other cardiomyopathies. Fae *et al.* suggested that polymorphism of HLA-DR and –DQ molecules, as well as beta-cardiac myosin, do not influence the susceptibility to different clinical forms of Chagas disease or the progression to severe Chagas cardiomyopathy (36). Male sex was

identified as a risk factor for progression to the more severe forms of cardiomyopathy (relative risk = 8.75).

5.17. Myosin binding protein-C (MyBP-C) gene

Myosin binding protein-C (MyBP-C) is one of the sarcomeric proteins. Mutations in the MyBP-C gene, on chromosome 11, are a frequent cause of HCM (49). Konno *et al*. detected an Arg820Gln missense mutation in the MyBP-C gene in 8 probands (7 in HCM, 1 in DCM) (49). This sequence variant was found in clinically affected patients and was absent in 100 normal controls. Konno *et al.* suggest that the Arg820Gln missense mutation in the MyBP-C gene may be associated with disease. Elderly carriers with the Arg820Gln missense mutation may show LV systolic dysfunction and dilation. The Arg820Gln missense mutation in the MyBP-C gene is associated with variable clinical features, and the clinical expression of this mutation is often delayed until middle age. Elderly patients with Arg820Gln mutation may show "burned-out" phase HCM and patients with this mutation may be included among those diagnosed as having DCM. Screening of patients with DCM, as well as HCM, for this mutation is of significant importance, because patients with this mutation may be diagnosed clinically as having DCM.

5.18. Cardiac troponin I (TNNI3) gene

The main function of the troponin complex is to regulate muscle contraction and relaxation. This regulation is mediated via conformational changes of the I, T, and C complexes, which are induced by variation of intracellular calcium ion concentration. Researchers, investigating the N-terminus of cTnI, have identified important interaction sites with both cTnC and cTnT (50). TNNI3 is the first recessive disease gene identified in DCM. Functional studies suggested that mutated cTnI and cTnT interaction is impaired. Murphy *et al.* suggested that this impairment leads to diminished myocardial contractility and disease (50). The mutation in this gene could cause disease because this genetic variant was not found in 150 controls. TNNI3 mutations in DCM are rare but these data suggest that other recessive disease genes might be identified by use of molecular genetic strategies suitable for identification of homozygous sequence variations.

5.19. Cardiac troponin T (TNNT2) gene

Troponin T is a regulatory protein of the striated muscle. Mutations in the troponin T gene are the most frequent (51). A mutation analysis of four genes involved in familial DCM in a population of idiopathic DCM was carried out. Seven mutations in MYH7, one in TNNT2, and none in PLN or in the VCL cardio-specific exon were found. MYH7 appears as the most frequently mutated gene in this FDCM population and mutation carriers present a delayed onset, in contrast to TNNT2. These findings confirm the genetic heterogeneity of familial DCM with a 2% frequency of TNNT2 mutation (R141W is associated with high penetrance and early onset), a low occurrence of PLN and VCL mutation, a prominent role of MYH7 in DCM with a 10% mutation frequency in familial forms and delayed onset of the disease in the studied population (35).

5.20. Alpha-cardiac actin gene

ACTC is one of six actin genes in humans, none of which have thus far been implicated in human disease. In cardiac myocytes, cardiac actin is the main component of the thin filament of the sarcomere (52). One end of the polarized actin filament forms cross-bridges with myosin, and the other end is immobilized, attached to a Z band or an intercalated disc (52). Thus, actin transmits force between adjacent sarcomeres and neighboring myocytes to effect coordinated contraction of the heart. The sarcomeric protein, actin, plays a central, dual role in cardiac myocytes, generating contractile force by interacting with myosin and also transmitting force within and between cells. Two missense mutations in the cardiac actin gene (ACTC), postulated to impair force transmission, have been associated with familial DCM. Monserrat *et al.* reported that HCM, DCM or left ventricular non-compaction (LVNC) and restrictive cardiomyopathy may appear as overlapping entities (53). The E101K mutation in the alpha-cardiac actin gene (ACTC) should be considered in the genetic diagnosis of LVNC, apical HCM, and septal defects. The data of another study indicates that the cardiac actin gene seldom appears to be involved in DCM. Several populations were subsequently screened for mutations in this gene and no other mutations were found: 44 probands recorded in the USA, 30 Japanese patients affected with FDCM and 106 Japanese sporadic cases, 11 patients belonging to eight families and 46 sporadic cases of mostly black African origin and 43 probands of familial forms and 43 sporadic cases of European origin were also analized. In addition, to avoid the identification of polymorphisms in biased or selected subgroups of patients, other independent studies with larger number of patients will be required (3, 4, 54).

Karkkainen *et al.* revealed no association between the ACTC variants and DCM or HCM in subjects from Eastern Finland and confirmed the earlier results that the ACTC gene does not play an important role in the genetics of DCM or HCM (55). Tesson *et al.* suggested that cardiac actin and desmin gene mutations are unlikely to cause DCM in the European population studied (54). Olson *et al*. found that mutations in ACTC cause either HCM or DCM, depending on the functional domain of actin that is affected (56). Based upon these data, it appears that actin defects, that alter force generation, lead to progressive, maladaptive cardiac hypertrophy, while defects, that impair force transmission, lead to congestive heart failure. Sylvius *et al.* reported about a study, where all six exons of the cardiac actin gene in a relatively large DCM population (4). Olson *et al.* identified two mutations (G867A and A1014G) in two DCM families of German or Swedish–Norwegian ancestry (52). These mutations were not found by Sylvius *et al.* (4). It is likely that genetic differences between ethnic groups influence the results. This is supported by the fact that the Japanese population (patients and normal controls) used have three polymorphisms in exon 6, whereas none was detected in 435 control subjects in the study by Olson *et al* (52). It is unclear how the mutant actin leads to DCM. Further studies on the mechanism of cardiac dilation and hypertrophy are needed to clarify the pathogenesis of DCM and develop direct treatments for the disease. Previous

studies support the hypothesis that relatively subtle molecular defects in force transmitting proteins, like actin, lead to myocyte dysfunction and heart failure. Firstly, missense mutations throughout the actin gene in Drosophila result in abnormal structure and function of flight muscle (52). Secondly, transgenic expression of a noncardiac actin in cardiac actin–deficient mice causes heart enlargement and dysfunction, resembling human IDC. Thirdly, missense mutations in dystrophin have been identified in X-linked dilated cardiomyopathy. In mice, heterozygous disruption of ACTC is not associated with heart abnormalities. Therefore, the missense mutations in ACTC are likely to lead to altered actin function rather than loss of function (52).

5.21. Alstroem syndrome1 gene

Alstroem syndrome (ALMS1; MIM] 203800) is a recessively inherited disorder with a complex and variable clinical spectrum (57). Patients develop progressive cone-rod dystrophy leading to blindness, sensorineural hearing loss (SNHL), hyperinsulinemia, and obesity in early childhood. Type 2 diabetes mellitus (T2DM) is observed in nearly all patients before the second decade. Dilated cardiomyopathy (DCM) occurs in approximately 70% of patients during infancy or adolescence. A large cohort of patients with Alstroem syndrome was screened for mutations in the ALMS1 gene. Marshall *et al.* detected 79 disease-causing variants, of which 55 are novel mutations (57). 66 SNPs were also found. A significant association was found between alterations in exon 8 and absent, mild or delayed renal disease (P50.0007). This article might support the understanding of the molecular mechanisms of ALMS1 and provides the basis for further investigation of how alternative splicing of ALMS1 contributes to the severity of the disease.

5.22. Endothelin-1, endothelin-A (ETA) and endothelin-B (ETB) receptor genes

Plasma endothelin-1 (ET-1) levels and endothelin-A (ETA) receptor densities are increased in patients with DCM (58). Several genetic polymorphisms in the genes encoding the endothelin system have been reported (58). A common genetic polymorphism in the ETA receptor gene has been more frequently found in French patients with DCM, than in controls (58). Herrmann *et al.* (58) found that patients carrying the T allele of the ETA receptor gene polymorphism H323H show significantly worse cumulative survival compared to non-carriers. The data strongly suggest a role of a genetic variation in the ETA receptor on survival in DCM patients. This might have important consequences for the identification of high-risk individuals and also indicate that ETA antagonists might be beneficial for dilated cardiomyopathy patients. Further studies are necessary to evaluate the potentially functional impact of the ETA H323H polymorphism on receptor expression or structure.

5.23. Ryanodine receptor 2 gene

Mutations in the cardiac ryanodine receptor (RYR2) gene have been shown to cause arrhythmogenic right ventricular cardiomyopathy (ARVC). RYR2 is one of the largest human genes (105 exons) encoding an mRNA of about 15 kb. Mutations in this gene have been associated with catecholaminergic polymorphic ventricular tachycardia, CPVT (59) and ARVC. Milting *et al.* could not find any of the published RYR2 mutations linked to the development of ARVC. Polymorphisms in the RYR2-gene are associated with ARVC in a subgroup of patients. Other molecular principles might be responsible for the development of ARVC like those involving desmosomal proteins (59).

5.24. Apolipoprotein E (ApoE) gene

It has been shown that ApoE alleles are associated with both cardiovascular and Alzheimer's diseases (60). Among alleles, the epsilon4 has been found to be associated with higher plasma cholesterol levels and is related to the risk of lipid disorder and coronary heart disease (60). Apolipoprotein E (ApoE) is a major component of low-density and high-density lipoproteins. Three common alleles – epsilon2, epsilon3, and epsilon4 – encode for the three main isoforms – ApoE epsilon2, ApoE epsilon3, and ApoE epsilon4 – circulating in the bloodstream. The ApoE epsilon4 allele is a significant risk factor for coronary heart disease (60) suggested an association of Apolipoprotein E (ApoE) polymorphism with a severe form of DCM.

5.25. Sodium channel 5A gene

Mutations in the SCN5A gene coding for the a-subunit of the cardiac Na+ ion channel cause long QT syndrome, Brugada syndrome, idiopathic ventricular fibrillation, sick sinus node syndrome, progressive conduction disease, DCM and atrial stillstand. The identification of gene carriers is clinically important, particularly in sudden infant and adult death syndromes. SCN5A (MIM 600163) is the gene coding for the a-subunit of the cardiac depolarizing Na+ ion channel Nav1.5 responsible for phase 0 of the cardiac action potential (AP) (61). Hofman-Bang *et al.* developed a specific and sensitive multiplex CE-SSCP analysis for high efficiency mutation analysis of SCN5A (61).

5.26. Cluster of Diferentiation 45 gene

Thude *et al.* suggested that the CD45 77C-G polymorphism is not associated with the susceptibility to idiopathic DCM in a German population (62). The group reported about an investigation of the linkage of different immune function genes including the CD45 gene in a large multi-generation Italian family (63 members) affected from DCM. No evidence for genetic linkage to idiopathic DCM has been found for all the loci analysed. Further studies are needed to clarify the association between CD45 77-G polymorphism and idiopathic DCM.

5.27. Trypanosoma cruzi mini-exon (ME) gene

Trypanosoma cruzi is classified into two major groups named T. cruzi I and T. cruzi II. T. cruzi I is mainly observed in wild mammals and more adapted to marsupials and sylvan triatomines. It is only occasionally isolated from humans, whereas T. cruzi II is apparently more associated with primates and is usually found in human infections. Until now all parasites that have been isolated from seropositive individuals in Brazil belong to T. cruzi II (63).

The ME gene is presented in the nuclear genome of all Kinetoplastida. Ruiz-Sanchez *et al.* analyzed 16 stocks isolated from human cases and four isolated from triatomines from diverse geographical origins (Mexico and Guatemala) (63). Of 16 human cases, four were acute cases, six indeterminates and six chronic chagasic cardiophatic patients with diagnosis of dilated cardiomyopathy. All the Mexican and Guatemalan isolates regardless their host or vector origin generated a 350 bp amplification product, consequently all of them belong to T. cruzi I in spite of their broad geographic distribution, since stocks were isolated from individuals living in Northwest of Mexico, the Pacific Coast, the Central part of Mexico and the Gulf of Mexico Coast, including Guatemala. It has been reported in earlier studies that Mexican stocks from eight states out of 31 in Mexico belonged to T. cruzi I (63). Ruiz-Sanchez *et al.* confirmed and extended previous findings. Furthermore Ruiz-Sanchez *et al.* reported that T. cruzi I may play a major role in human infection in Mexico and Guatemala. These results contrast with the situation reported in Brazil, where parasites belonging to T. cruzi II are preferentially associated with human infection, while T. cruzi I are associated with the sylvatic cycle of the parasite (65). However, the results of Ruiz-Sanchez *et al.* are in agreement with another article where 74% of Venezuelan isolates from acute chagasic patients were typed as T. cruzi I (63). These observations suggest that T. cruzi I predominates in human and sylvatic cycle, at least in Mexico and Guatemala.

5.28. Myotrophin gene

To define the characteristics of each transcript and its pathophysiological significance, transcripts of myotrophin were examined in spontaneously hypertensive rat (SHR) heart during progression of hypertrophy. The myotrophin gene is a single copy gene, consisting of 4 exons separated by 3 introns. The myotrophin gene has been mapped and shown to be a novel gene localized in human chromosome 7q-33. G. Adhikary *et al.* found that this protein may be a common link initiating different types of cardiac hypertrophy (64).

5.29. Vinculin (VCL) gene

Vinculin and its isoform metavinculin are protein components of intercalated discs, structures that anchor thin filaments and transmit contractile force between cardiac myocytes. Vinculin is located on chromosome 10q22.1-q23 (65). The smaller isoform, metavinculin, is ubiquitously expressed. Metavinculin, containing an additional 68 amino acids, is expressed exclusively in cardiac and smooth muscle (65). In cardiac myocytes, vinculin and metavinculin colocalize to intercalated discs and costameres (65). Thus, vinculin and metavinculin are located at principle sites of contractile force transmission. Human studies have suggested a potential relationship between metavinculin and vinculin expression, intercalated disc abnormalities and DCM (65). Olson *et al.* examined the possible heritable dysfunction of metavinculin in the pathogenesis of DCM. They suggest that metavinculin plays an important role in the structural integrity and function of the heart (65). They also showed that inherited dysfunction of this protein is associated with altered actin filament organization *in vitro*, disrupted intercalated disc structure *in situ* and DCM. These results agree with the hypothesis that defective contractile force transmission leads to DCM. These findings establish vinculin as a DCM gene.

5.30. Metavinculin (meta-VCL) gene

Metavinculin, an isoform of the vinculin, is one of the membrane-associated proteins located in the intercalated discs and costameres and has a role in force transmission and in anchoring of thin filaments. A mutation analysis of four genes involved in FDCM in a population of idiopathic DCM was performed in the first study (35). These findings confirm the genetic heterogeneity of familial DCM with a low occurrence of PLN and metavinculin mutation, a 2% frequency of TNNT2 mutation (R141W is associated with high penetrance and early onset) and a prominent role of MYH7 in DCM with a 10% mutation frequency in familial forms and delayed onset of the disease in the studied population (35). The mutation frequency in metavinculin cardio-specific exon 19 appears to be very low in the population screened in the present study (no mutation). Estimates from previous reports evaluate the frequency of familial DCM causing mutations in VCL exon 19 (two mutations) to 3% (65). In another study no disease-associated mutations were found in the metavinculin-specific exon of the vinculin gene or the desmin gene (7). These data indicate that mutations in the metavinculin gene are rare in familial DCM.

5.31. Lamin A/C gene

Lamin contributes to the structural integrity of the nuclear envelope and provides mechanical support for the nucleus. Missense mutations of the lamin gene may alter interactions with some cytoplasmic proteins, particularly intermediate filaments of cytoskeleton (e.g. cytoskeleton), but this has not yet been demonstrated. Lamin A and C are components of the nuclear envelope and are located in the lamina, a multimeric structure associated with the nucleoplasmic surfangiotensin converting enzyme of the inner nuclear membrane (3). This gene is also responsible for two skeletal myopathies: Emery-Dreifuss and limb-girdle muscular dystrophy (3). LMNA mutations have been associated with familial or sporadic DCM, with or without conduction system disease. The main findings of Hermida-Prieto *et al.* were the description of one novel and one recurrent mutation in the lamin A/C gene associated with severe forms of familial DC and the identification of isolated LVNC in a young carrier of the R190W lamin A/C mutation (66). Hermida-Prieto *et al.* found that the novel R349L mutatuion may contribute (but not definitely) to the disease. The R190W mutation has been associated with severe forms of familial DCM with conduction system disease (66). The R190W mutation may contribute (but not definitely) to isolated LVNC in the patients studied. Arbustini *et al.* reported that LMNA gene mutations accounted for 33% of the cases of DCM with AVB, all of which were familial autosomal dominant DCMs (67). Increased sCPK levels in DCM patients without AVB were not useful in predicting LMNA mutations. Some data were published in a further study. The lamin A/C gene was

found associated with the autosomal dominant form of DCM associated with a particular phenotype. Charron *et al.* reports of a screening of 11 families with DCM for mutations in the lamin A/C gene (3). A mutation in the lamin A/C gene was found in five of the families. There were five missense mutations, four in the alpha-helical rod domain of the lamin A/C gene and one in the lamin C tail domain. The results of Speckman *et al.* indicated that missense mutations in the lamin A/C gene cosegregate with familial partial lipodystrophy. However, it is not clear how the alterations described, lead to adipocyte apoptosis or initiate loss of fat at puberty (68). Further studies are necessary to clarify why different alterations within lamin A/C are responsible for three clinically distinct diseases.

5.32. Coxsackievirus B-adenovirus receptor (CAR) gene

Coxsackie B viruses (genus, Enterovirus; family, Picornaviridae) are involved in the pathogenesis of aseptic meningitis, encephalitis, pleurodynia and myocarditis. They are implicated in the pathogenesis of DCM. Coxsackie B viruses consist of six serotypes (1–6), classified within the enterovirus genus of the family Picornaviridae. Coxsackie B viruses are the etiological agents of a wide spectrum of human diseases, including mild respiratory infection, aseptic meningitis, and fatal myocarditis. Outbreaks of coxsackie B virus infection occur annually throughout the world (69). Infection of newborns and infants by these viruses can induce paralysis, aseptic meningitis, and febrile illnesses that can be fatal, while infections in adults are mostly asymptomatic (69). The RFLP assay for coxsackie B virus developed by Patel *et al.* (69) has many advantages over other procedures used for the purpose. The assay is very simple and sensitive. In addition, all the techniques used in this assay were very simple and can be done in any laboratory with high accuracy. Moreover, by using single restriction enzyme all six subtypes of coxsackie B viruses were clearly differentiated. The RT-PCR-based RFLP assay developed for the 5'-UTR is a useful approach to differentiate coxsackie B virus clinical isolates into their subtypes and is a valuable supplement to enterovirus identification for diagnostic and epidemiological studies. Bowels *et al.* have reported that mutations in CAR are unlikely to cause myocarditis or DCM, although it remains possible that mutations in CAR or interacting proteins, controlling viral uptake and processing, result in disease in some patients. Among this cohort of patients with acquired, familial or idiopathic myocarditis or DCM, no mutations were found that could account for changes in virus susceptibility or CAR function (70). Bowels *et al.* reported about the increased expression of CAR in the myocardium of patients with DCM by comparison with normal hearts.

5.33. Adenosine monophosphate deaminase-1 (AMPD1) gene

The human adenosine monophosphate deaminase-1 (AMPD1) gene is located in the region p13-p211 of chromosome 1 and contains 16 exons. AMPD1 is an enzyme that catalyzes the deamination of AMP to inosine monphosphate as a part of purine catabolism. A C-T transition at nucleotide 34 (codon 12 in exon 2) results in a nonsense mutation, predicting a severely truncated protein that looses its catalytic activity (71). The mutant AMPD1 cannot catalyze the deamination of AMP inosine monphosphate; thus, AMP turns into adenosine. The loss of the catalytic activity of the mutant AMPD1 increases the adenosine production in skeletal muscle (71). Adenosine in turn is able to attenuate the expression of TNF-a. This suggests a TNF-a-related mechanism being responsible for a better clinical outcome, observed in patients with CHF who have a mutant AMPD1 allele. Gastmann *et al.* confirmed the result of a better survival associated with the mutant AMPD1 allele. This is in agreement with earlier results (71).

5.34. Cypher/Z-band alternatively spliced PDZ-motif protein gene

Cypher/ZASP appears to be an ideal candidate for the cardiomyopathy causative gene, because Cypher/ZASP encodes a Z-disc associated protein. Myocardial function is associated with the regulation or activation of cell signal kinases such as protein kinase C (PKC). For example, modification of myocardial proteins by PKC plays a key role in the regulation of contractility and the growth of cardiomyocytes, whereas alterations in the expression, activity, or localization of PKC are associated with cardiac hypertrophy and failure (72). A gene for the PDZ and LIM domain-containing cytoskeletal protein, Cypher/ZASP, was identified in mouse (Cypher) and human (ZASP) (72). It was demonstrated by two assays that the D626N mutation of Cypher/ZASP increased the affinity of the LIM domain for protein kinase C. These findings suggest a novel biochemical mechanism of the pathogenesis of DCM. In addition, these observations imply that the cardiac dysfunction might be associated not only with the alteration in each sarcomeric interaction, but also with the altered recruitment of molecules participating in intracellular signaling.

5.35. B-sarcoglycan (SGCB) and d-sarcoglycan (SGCD) genes

Hypothesizing that DCM is a disease of the cytoskeleton and sarcolemma, SGCB and SGCD genes may play a role in the pathogenesis of DCM. Both genes have a muscular restricted expression profile, due to a molecular link between extracellular matrix and sarcolemal cytoskeletal proteins in the myocytes. Mutational data linking mutations in these genes with genetically inherited muscular disorders, such as DCM and/or myopathy. The b-sarcoglycan (SGCB) and d-sarcoglycan (SGCD) genes are strong candidates for a morbid role in DCM for several reasons. These genes are highly expressed in cardiac and skeletal muscle. They encode proteins involved in the cytoarchitecture of the cardiac cell as they are components of the dystrophin associated sarcoglycan complex that forms a structural link between the F-actin cytoskeleton and the extracellular matrix. Moreover, the SGCB and SGCD genes have been implicated in limb-girdle muscular dystrophy (LGMD2E and LGMD2F, respectively) and both disorders have been found associated with DCM (4). D-Sarcoglycan is one of the four proteins (a, b, g, and d) in the sarcoglycan complex, which in turn forms a part of the dystrophin-associated glycoprotein complex. This complex is located in the transmembrane region and its function is to form a link between the intracellular and extracellular

matrix. (Leiden muscular dystrophy pages, d-sarcoglycan, http://www.dmd.nl/sgcd_home.html). It has been suggested that the d-sarcoglycan gene is unlikely to cause DCM in patients from eastern Finland (7). Only two DCM associated mutations in this gene have been previously reported. Tsubata *et al.* found two mutations in the d-sarcoglycan gene (1). The Ser151Ala mutation was detected in three family members of one family and a 3-bp deletion in position 238 (del Lys238) in two sporadic cases without signs of skeletal muscle disease. The Ser151Ala and del Lys238 mutations caused a relatively severe form of DCM characterized by sudden cardiac death and heart failure at young age and a need for HTX (4). The carriers of the Arg71Thr mutation have a relatively mild, late-onset disease and a good response to medication (7). Therefore the phenotype of the subjects carrying this mutation seems to be less severe than that in patients described by Tsubata *et al.* (1). Sylvius *et al.* estimated the prevalence of SGCD gene mutations responsible for DCM at less than 1.5% (4). This underlines the fact that the SGCD gene is only marginally implicated in the disease. This is in agreement with previous results obtained by mutation screening of other candidate genes such as DES, ACTC or TNNT, MYH7, and TPM1, which are also rarely mutated in DCM as none were over a 10% mutation frequency (4). Sylvius *et al.* found that the most frequently implicated gene in DCM appears to be the LMNA gene, since 10 different mutations responsible for DCM associated with conduction and/or muscular disorder have been reported (4). However, these clinically non-isolated forms of DCM represent only 10% of all familial DCM (4). As no major gene or locus have been identified in DCM and given the fact that morbid mutation identification concerns only a minor percentage of familial cases of DCM, it may be speculated that a large number of morbid genes remains to be identified.

The delta-sarcoglycan gene was also demonstrated to be responsible for dilated cardiomyopathy (3). In one family with autosomal dominant mode of inheritance, a Ser151Ala mutation was found in three patients with isolated DCM at a young age (1).Tsubata *et al.* suggested that d-sarcoglycan is a disease-causing gene responsible for familial and idiopathic DCM and lend support to the “final common pathway” hypothesis that DCM is a cytoskeletalopathy (1). The fact that mutations in d-sarcoglycan and dystrophin, as well as mutations in G4.5, can also result in skeletal myopathy, suggests that patients with DCM should be carefully evaluated for skeletal muscle weakness and that neurologists caring for patients with skeletal myopathies should be cognizant of the potential for associated cardiomyopathies in their patients.

5.36. Multidrug Resistance Protein 5 (MRP5/ABCC5) gene

The multidrug resistance protein 5 (MRP5/ABCC5) has been described as cellular export pump for cyclic nucleotides. The multidrug resistance protein 5 (MRP5/ABCC5) represents the first molecular biologically identified ATP-dependent export pump for cyclic nucleotides with cGMP as a high-affinity substrate and cAMP as a low affinity substrate. 5MRP5 expression in human heart is important, because several features of cGMP, as a second messenger of nitric oxide (NO), have emerged in heart, not only in the regulation of the vascular smooth muscle tone, but also in the regulation of cardiac contractility (74). Besides the export of cyclic nucleotides, MRP5 as organic anion export pump may have a protective function against potential toxic compounds that can be pumped from the endothelial cells back into blood (74). Variations in the MRP5 expression in the cardiovascular endothelium, as well as in the cardiomyocytes, may therefore influence the concentration of these compounds in the heart tissue. Results from earlier studies revealed a possible association with 20 of a total of 95 identified SNPs within the MRP5 gene. None of the 20 SNPs found in the MRP5 gene and promoter region altered the expression. The expression of MRP5 in cardiac and cardiovascular myocytes as well as endothelial cells indicates the presence of ATP-dependent cGMP export as potential novel component and pharmacological target in the regulation of cardiac tissue cGMP levels. Moreover, Dazert *et al.* found an increased expression in patients with ICM (74).

5.37. Polyadenylate-binding protein 2 (PABP2) gene

Weakness of distal limb muscle groups is a clinical hallmark of MPDs, even though somepatients may also experience weakness of proximal muscles. The molecular basis of most distal myopathy (MPD) is still unknown. The first genetic locus for autosomal dominant (AD) MPD was discovered in an Australian family, in which affected individuals developed selective weakness of foot and toe extensors, followed by progressive weakness of finger extensors and neck muscles. Distal myopathy (MPD) linked to chromosome 14q11-q13 (MPD1) is rare.

MPDs are a genetically heterogeneous group of muscle disorders. The coding sequence of PABP2 (the polyadenylate-binding protein 2 gene) was evaluated by Hedera *et al.* (75). They reported, that the described family is only the second known kindred with a chromosome 14–linked MPD in whom the linkage has been unequivocally established. There were no signs of involvement of hand or finger extensors and neck muscles, seen in the original family with MPD1. The degree and frequency of proximal weakness seem to be more prominent than in other patients with MPD1. Haplotype analysis suggests that the gene causing MPD1 is located between polymorphic microsatellite markers D14S283 and D14S1034 on chromosome 14q11-q13. The MPD1 locus contains PABP2. A small expansion of the polyalanine tract (GCG) is a cause of oculopharyngeal muscular dystrophy. They further reported exclusion of the PABP2 gene in their family with MPD1 (75). Hedera *et al.* also did not detect a coding change in this gene, thus, excluded it as the cause of MPD1. Another important candidate gene within the MPD1 locus is MYH7 (heavy chain cardiac beta-myosin). Mutations in this gene cause familial HCM, and these patients do not have signs of clinical myopathy (75). Similarly, mutations in a giant skeletal muscleprotein, titin, localized on chromosome 2q31, cause a dilated form of cardiomyopathy, and these patients do not have clinical signs of MPD (75). The history of idiopathic cardiomyopathy in some affected individuals may supply an important indication for candidate genes (75).

5.38. Genes encoding the four major components of the heart calcineurin pathway, PPP3CA, PPP3CB, GATA4, NFATC4

Despite the investigation of a large number of polymorphisms, only one among the four non-synonymous polymorphisms, NFATC4/G160A, was associated with the investigated phenotype. A Gly/Ala substitution at position 160 of the NFATC4 protein (G160A) was associated with left ventricular mass and wall thickness. The other polymorphisms identified by the gene screen were not associated with cardiac phenotypes. The data suggest that this polymorphism could be involved in the genetic susceptibility to develop human cardiac hypertrophy. Furthermore, the data indicate that compared to noncarriers, carriers of the NFATC4/A160 allele have lower mean LVM and WT in the LOVE Study and are less frequently observed in patients with DCM than in controls in the CARDIGENE study (24, 76). This may suggest that the A160 allele protects against cardiac hypertrophy.

5.39. Aldosterone synthase (CYP11B2) gene

Aldosterone, whose production is regulated primarily by the renin–angiotensin system, may have indirect effects on cardiac structure and function through its role in blood pressure regulation (24). The CYP11B2 T-344C polymorphism, which is associated with plasma aldosterone levels, has been shown, in a small study, to strongly affect left ventricular size and mass in young adults free of clinical heart disease (77). Takai *et al.* reported that the TC+CC genotype in the CYP11B2 was significantly associated with larger LV volume in DCM. The genotype distribution in DCM was not statistically different from that in controls, suggesting that this polymorphism cannot represent a susceptibility gene to DCM. The prevalence of the CC genotype in Europeans is twice of that found in Japanese, suggesting that ethnic differences may exist regarding this genotype (24, 77). Tiret *et al.* reported that T-344C polymorphism was not associated with severity of DCM (24). This discrepancy may be due to either the difference of methods used for the evaluation of the disease severity or ethnic differences. The LV volume was measured in this study with LVG in order to minimize the methodological bias, whereas Tiret *et al.* analyzed the combined data obtained by LVG, radionucleotide angiogram or echocardiogram(24). Tiago *et al.* found that a CYP11B2 gene variant, but neither ACE, nor AGT gene variants examined predicted improvement in LVEF measured after initiating medical therapy with furosemide, digoxin and ACE inhibitors in patients of African ancestry with IDC (26). The CARDIGENE study is the largest study conducted so far on this topic, including more than 400 patients with DCM (24). In control subjects, there was no difference of allele frequencies among the three MONICA regions despite their geographical distance (northern, eastern and southern France), making it an unlikely risk of stratification of the population for the polymorphisms considered. Tiret *et al.* have reported that several reasons might explain the negative findings. The first one is that the investigated genes, despite being strong candidates a priori, do not play any significant role in the pathogenesis of DCM (24). A second reason might be that the polymorphisms selected in each gene were not appropriate and that there exist other unmeasured polymorphisms of these genes, whose effect on disease could not be detected through linkage disequilibrium with the polymorphisms studied. However, this explanation is rather unlikely, given the strong linkage disequilibrium generally observed within candidate genes. A third explanation might be related to the heterogeneity of patients with respect to progression of the disease. In actuality, the sample included both new and old patients, some of them being followed up for more than 10 years. If the same genetic factor contributed to both, the susceptibility to disease and its mortality, then mixing new cases and long-term survivors might mask the genetic effect. This question would have to be clarified in longitudinal studies. The populations in these studies were remarkably different, and the earlier work involved relatively young men and women free of coronary artery disease. Kajander *et al.* also reported a large negative study on the role of aldosterone synthase -344C/T polymorphism to LV structure and function (25).

5.40. Dystrophin gene

Dystrophin is a large (427 kDa) cytoskeletal protein that localizes to the inner angiotensin-converting enzyme of the plasma membrane or sarcolemma (3). Through the amino-terminal actin-binding domain, dystrophin is related with F-actin and the sarcomere. The carboxy-terminal domain is associated with a large transmembrane complex of glycoproteins, termed the dystrophin associated glycoprotein complex (DAG). In this manner, dystrophin is believed to play a critical role in establishing connections between the internal cytoskeleton and/or the sarcomeric structure and the external basement membrane. The absence of dystrophin leads to a disruption of the DAG complex, a loss of integrity of the plasmalemma and fiber necrosis. Although no skeletal muscle involvement is evident (in contrast with Duchenne or Becker muscular dystrophy, where the dystrophin gene is also involved), plasma creatine kinase levels are usually high. DNA alterations involved in the dystrophin gene are located in the muscular promotor-first, muscular exon-first intron regions. These alterations consist of deletions or of a point mutation in the splice consensus site of the first intron. They result in absence of the protein in the cardiac muscle, whereas dystrophin expression is preserved or slightly reduced in skeletal muscle; in exons 2–7, 9, 45–49, 48–49, 49–51, where other genetic alterations have been identified (deletions, duplication and missense mutations). As dystrophin mutations may cause clinical or subclinical skeletal myopathy, it is possible that the muscle fatigue, seen chronically in many patients with DCM, could be due to primary skeletal muscle disease and not due primarily to chronic heart failure (3). Examining the dystrophin gene in DCM is this group's first application of Mendel leaping. More work is necessary to evaluate the general utility of this approach for selecting candidate genes for complex disease. Point mutations seem to be associated with sporadic DCM without clinical evidence of skeletal myopathy (3). Charron *et al.* have reported that the first gene responsible for DCM was identified in 1993, the dystrophin gene, as responsible for X-linked DCM. The

genetically affected individuals usually develop a severe form of DCM at adolescence or in young adulthood.

5.41. Gene for platelet-activating factor acetylhydrolase (PAF-AH)

It was found that a variant allele (279Phe allele) of the gene for platelet-activating factor acetylhydrolase (PAF-AH), which has a reduced enzymatic activity as compared with the normal 279Val allele, is significantly associated with nonfamilial IDC in a Japanese population (78). Because PAF-AH is related to both inflammation and superoxide-induced tissue damage, this result might support the immune-related and oxygen stress-related etiologies of IDC. This association should be confirmed in other patients and control panels. The reported association of the PAF-AH polymorphism with nonfamilial IDC (78) was examined in 106 nonfamilial IDC patients (78). In contrast, no evidence was found to support the reported association, suggesting that the contribution of the PAF-AH variant in the susceptibility to nonfamilial IDC (if there is indeed any such contribution) may not be large enough to be confirmed by the analysis of 106 nonfamilial IDC patients.

5.42. Transforming growth factor (TGF)-b1 gene

Transforming growth factor–b1 (TGF-b1), a regulatory cytokine produced by many cell types, has been studied in relation to the pathogenesis of coronary artery disease. Both anti-atherogenic and pro-atherogenic activities of TGF-b1 have been reported (79). Transforming growth factor–b1 inhibits the proliferation of many cells, including smooth muscle cells, endothelial cells and epithelial cells.It could therefore inhibit development of atherosclerosis. Furthermore, TGF-b1 has chemoattractant activities, enhances cell adhesion, and stimulates intracellular matrix deposition (79). The involvement of TGF-b1 in cardiomyopathy is less extensively studied. Elevated TGF-b1 gene expression was measured in ventricular biopsies from hypertrophic and dilated hearts, whereas others found decreased TGF-b1 plasma levels in patients with DCM (79). The contradictory findings in patients with ischemic heart disease (IHD) as well as in patients with CMP could result from different biologic activities of TGF-b1 during various stages of both, disease processes or to intraindividual variations in TGF-b1 protein production. Analysis of TGF-b1 polymorphisms showed that the presence of the Arg25 allele is associated with increased blood pressure and with the development of graft vascular disease after cardiac transplantation, whereas the Pro25 allele was associated with myocardial infarction (79). A recent study, however, could not confirm a relation between these TGF-b1 polymorphisms and coronary artery disease. Holweg *et al.* reported an association of the Leu10-Pro (codon 10) polymorphism in the TGF-b1 gene with end-stage heart failure caused by DCM. Although other cytokines are involved, these observations suggest that TGF-b1 is implicated in the pathophysiology of DCM (79). The difference in TGF-b1 gene polymorphism distribution in the patient group with DCM needs further investigation. This group is of interest, because a difference may exist between patients with hereditary DCM and patients with cardiomyopathy caused by toxic agents or viral infection.

5.43. G4.5 (Tafazzin, TAZ) gene

Mutations have been described in the gene G4.5 in patients with classic Barth syndrome (BTHS) as well as in patients with infantile DCM and isolated left ventricular noncompaction LVNC (80). Ichida *et al.* have reported the identification of novel mutations in G4.5 in patients with isolated LVNC (80). In patients with LVNC associated with CHD no mutations were found in G4.5. Instead, a mutation in the calcium-binding EF-hand domain of a-dystrobrevin was identified in one family. Several important points are becoming apparent with regard to G4.5 mutations. Many affected individuals develop severe infantile disease and succumb. The gene defect usually differs among families; however, there seems to be no obvious genotype: phenotype correlations that allow the differentiation of clinical course to be predicted. The cardiac phenotypes that occur as a result of G4.5 mutations may vary significantly. The cardiac manifestations include DCM, endocardial fibroelastosis, LVNC and HCM. In addition, this phenotype can differ among family members and change over time, possibly in response to therapy. Finally, the systemic manifestations of BTHS are equally unpredictable. In some children, sudden death occurs. It is likely that modifier genes are involved in determining the phenotype and clinical severity.

Ichida *et al.* suggest that studies of patients with myocardial disorders having prominent systolic dysfunction should include evaluation of members of the cytoskeleton-sarcolemma complex, as well as G4.5, as candidate genes (80).

5.44. HS426 (nebulette) gene

Abnormalities in genes for cytoskeletal proteins related to Z-disc function have been reported to cause idiopathic DCM. Therefore, the genomic organization of the gene for nebulette, a novel actin-binding Z-disc protein, may be an interesting investigational field. Arimura *et al.* found that the Asn654Lys variation was associated with nonfamilial idiopathic DCM only in the homozygous state and no increase in frequency of heterozygotes was observed in the patients (78). Because the Asn654Lys variant of nebulette is the polymorphism present in the healthy population, the functional difference between the alleles of nebulette may not be so large as to cause any disease phenotypes in the heterozygous state. Further investigation into the functional alteration related to the Asn654Lys polymorphism will be required to demonstrate whether the polymorphism itself is related to the disease or whether it is merely a genetic marker linked to the susceptibility gene to idiopathic DCM.

5.45. Human cardiotrophin-1 gene (CTF1)

CT-1 (CTF1) belongs to the IL-6 family of cytokines and can stimulate cardiac myocyte growth *in vitro*, suggesting that the gp130-signaling pathway may play a role in cardiac hypertrophy. Erdmann *et al.* found genetic variants in the coding and promoter region of CT-1, which might modify gene function and gene expression (81).

Because of the low prevalence of this promoter polymorphism, further investigation is ongoing with larger

patient and control groups to achieve a more precise estimate of the relative risk.

5.46. Desmin gene

Desmin is the chief intermediate filament of skeletal and cardiac muscle (8). It functions as a cytoskeletal protein linking Z bands to the plasma membrane and nuclear membrane and it maintains the structural and functional integrity of the myofibrils. Desmin-related myopathy is a familial disorder of skeletal muscle characterized by intracytoplasmic accumulation of desmin-reactive deposits in muscle cells and is often accompanied by cardiac involvement, such as conduction blocks and/or cardiomyopathy. The myopathy is partly caused by mutations of the desmin gene. Nine disease-causing mutations have been identified; eight in the rod domain and one in the carboxy-terminal domain. All those in the rod domain were associated with skeletal muscle involvement while those in the carboxy-terminal domain were not always associated. Miyamoto *et al* reported that the mutation (Ile451Met) located in the carboxy-terminal domain caused familial DCM without clinically evident skeletal muscle abnormalities (8). The desmin gene was identified as responsible for the autosomal dominant inherited form of DCM. The desmin gene was also identified as responsible for restrictive cardiomyopathy in several families. Miyamoto *et al.* showed that in a relatively large Japanese population affected with DCM three out of 265 patients (1.1%) had the missense mutation (Ile451Met), previously reported as disease-causing (8). Another study in Europe showed that no mutation was detected in the population of 41 probands of DCM families and 22 sporadic cases (54). In conclusion this pathogenetic mechanism of DCM is rare. Another characteristic of cardiac involvement due to the mutations of the desmin gene is conduction blocks. Seven mutations in the rod domain led to conduction blocks. Neither of the patients had any conduction blocks, similar to the cases due to the Ile451Met mutation reported by Miyamoto *et al.* (8). As mentioned above the desmin gene is probably uncommon in DCM, since only one proband among 40 had a mutation in the study above and no mutations were found in 41 probands of FDCM and 22 sporadic cases (54). The same group indicated that desmin and cardiac actin gene mutations are unlikely to cause DCM in the European population studied.

Karkkainen *et al.* showed that the desmin and d-sarcoglycan genes are unlikely to cause DCM in patients from eastern Finland (7).

5.47. Endothelial nitric oxide synthase (NOS3) gene

Tiret *et al.* studied eight candidate genes: the endothelial nitric oxide synthase (NOS3), the angiotensin I-converting enzyme (ACE), the angiotensin-II type 1 receptor (AGTR1), the angiotensinogen (AGT), the aldosterone synthase (CYP11B2), the tumor necrosis factor-alpha (TNF), the transforming growth factor beta1 (TGFB1) and the brain natriuretic peptide (BNP) genes (24). 433 patients with IDC and 401 controls were included. The controls were randomly sampled from the French population surveys carried out in Lille (northern France), Strasbourg (eastern France) and Toulouse (southern France) within the framework of the WHO MONICA project (24). Enhanced basal production of nitric oxide has been reported in patients with heart failure while increased activity of inducible nitric oxide synthase has been found in cardiac tissue from patients with DCM (24). The CARDIGENE study is a large case-control study designed to investigate genetic factors involved in IDC. Tiret *et al.* could not find any association between the polymorphisms and the risk or the severity of IDC.

5.48. Brain Natriuretic Peptide (BNP) gene

Brain natriuretic peptide is mainly produced by the ventricles and its secretion has been found increased in the failing heart in proportion to the severity of left-ventricular dysfunction (24). Its expression has been observed primarily in myocytes in the interstitial fibrous area in DCM (24). Plasma levels of BNP are raised in patients with heart failure, left-ventricular dysfunction, DCM and have a prognostic role in mortality of patients with congestive heart failure (24). The investigation of these eight candidate genes was based on pathophysiological considerations, previously reported in published articles. Tiret *et al.* could not find any association between the polymorphisms and the risk or the severity of idiopathic DCM.

5.49. Skeletal muscle alpha-actin gene (ACTA1)

Muscle contraction results from the force generated between the thin filament protein actin and the thick filament protein myosin, which causes the thick and thin muscle filaments to slide past each other (82). There are skeletal muscle, cardiac muscle, smooth muscle and non-muscle isoforms of both actin and myosin. Inherited diseases in humans have been associated with defects in cardiac actin (DCM and HCM), cardiac myosin (HCM) and non-muscle myosin (deafness) (82). It has been reported that mutations in the human skeletal muscle alpha-actin gene (ACTA1) are associated with two different muscle diseases, 'congenital myopathy with excess of thin myofilaments' (actin myopathy) and nemaline myopathy (82). Both diseases are characterized by structural abnormalities of the muscle fibres and variable degrees of muscle weakness. 15 different missense mutations resulting in 14 different amino acid changes were detected. Three genes mutated in several types of nemaline myopathy were found: TPM3 (encoding alpha-tropomyosin slow) in both dominant and recessive nemaline myopathy in which the nemaline bodies are restricted to slow, type I muscle fibres; NEB (encoding nebulin) in typical non- or slowly progressive congenital nemaline myopathy and ACTA1 (82). Mutations in ACTA1 can also cause additional phenotypes. Nowak *et al.* identified a spectrum of phenotypes associated with mutations in ACTA1. These are 'congenital myopathy with excess of thin myofilaments' with or without intranuclear nemaline bodies (n=3 patients), severe nemaline myopathy (n=11) and mild nemaline myopathy (n=4). 7 of 11 patients with ACTA1 mutations and severe nemaline myopathy died within the first year of life, 2 are alive at 3 and 10 years (82).

5.50. Bradykinin B2 receptor gene

Bradykinin plays an important role in the cardiovascular system, affecting blood pressure regulation, cell proliferation, and matrix synthesis by fibroblasts. Bradykinin receptors belong to a family of seven transmembrane receptors and are classified into two types, B1 and B2. Most of the actions of bradykinin are mediated by the B2 receptor (12). In the initial mutation screening study three promoter mutations, one in a patient with HCM (−704C/T) and two in patients with DCM (-412G/C and -78C/T) were found. The coding mutation T21M was identified in only one patient with hypertension. All patients had no obvious family history for the diseases. The -412C/G variant was also found in one anonymous blood donor whose phenotype could not be evaluated, but the T21M mutation was not found in the extended population. Because of the low frequency of the potentially functional variants in the promoter region and in the coding region (<1%) large populations will be necessary for epidemiological studies (12). Further study is needed to evaluate whether the identified variants are of clinical significance.

5.51. Myocyte enhancer factor 2 (MEF2A) gene

MEF2A is a gene of one of four members of a family of transcription enhancer factors commonly known as the Myocyte Enhancer Factor 2 (MEF2) family. The gene for the human MEF2A (Genebank accession number X68505) has been localized to 15q26 by *in situ* hybridization and by mapping in somatic cell hybrids. The polymorphic (CAG)n repeat begins at position 1672 in the cDNA sequence of the MEF2A gene and codes for a polyglutamine tract of variable length. Co-dominant inheritance of this polymorphism was observed in three families of three or more generations. Bachinski *et al.* found that MEF2A is not associated with DCM (83). The identification of a polymorphic repeat in this gene and the development of a PCR-based assay make this locus directly amenable to linkage analysis and provide an easy tool to evaluate families with a linked disease for the expansion of the normally polymorphic (CAG)n repeat.

5.52. Chromosome 10 linkage in familial DCM

In 1995, the discovery of gene locations for FDCM in families with pure FDCM was reported. Bowels *et al.* reported about evidence for linkage to 9q13-q22 in three families (84). Genetic heterogeneity exists in pure FDCM,) and also about the linkage to 1q32 in one family. Although these genes have not been identified, a number of candidate genes have been described, all based on the various potential pathophysiologic mechanisms of DCM. Bowles *et al.* have reported the discovery of the third locus for pure autosomal dominant FDCM to chromosome 10q21-q23 (84). The search for the disease causing gene and the responsible mutation(s) is ongoing. The region of interest on 10q21-q23 contains a number of candidate genes, including muscle membrane proteins (i.e., vinculin, metavinculin, actin, ankyrin, laminin, energy-producing proteins (ATP synthase) and proteins responsible for energy transport (perforin) (84). Identification of new FDCM-causing genes and the responsible mutations will enable to verify the speculation, that cytoskeletal protein abnormalities underlie the resultant ventricular dilation and dysfunction characteristic of DCM. Once the genes and their mutations are identified, it is possible that pre-symptomatic diagnosis and improved therapeutic options based on the underlying cause of disease can be developed, resulting in better long term care and survival for patients with DCM and their family members who are at risk.

5.53. Chromosome X linkage in infantile cardiomyopathy

For X linked DCM, two genes have been identified, including tafazzin (G4.5) in cases of the infantile-onset DCM (Barth syndrome) and isolated LVNC and dystrophin in later-onset X-linked cardiomyopathy (XLCM) (85). The familial X linked cardiomyopathies so far reported tend to have infantile or childhood onset of symptoms (85). The best delineated clinically is Barth syndrome, which features X-linked cardiomyopathy with variable skeletal myopathy, short stature and neutropenia. It has not been possible to delineate genetically the more severe congenital form of cardiomyopathy reported in this study from Barth syndrome. If the family described in this study represents a severe form of Barth syndrome, at least some of the variable expression between families might represent allelic rather than locus heterogeneity (85).

5.54. Locus on chromosome 6q12-q16 for autosomal dominant DCM

Haplotype reconstruction showed that all affected subjects, as well as all individuals with unknown status, shared a common haplotype on chromosome 6, between markers D6S1627 and D6S1716 .The candidate interval corresponds to a 16.4-cM region localized on chromosome 6q12-16. The disease interval on chromosome 6q12-16 contains known genes encoding collagen IXa-1 polypeptide (COL9A1 [MIM 120210]), myosin VI (MYO6 [MIM 600970]), vascular endothelial growth factor (VEGF [MIM 192240]), malic enzyme cytoplasmic (ME1 [MIM 154250]) and several other genes encoding anonymously expressed sequence tags. In addition, the genes encoding cardiac phospholamban (PLN [MIM 600133]) and laminin-a4 (LAMA4 [MIM 600133]), located near the disease interval, could also be considered as candidate genes. The entire coding sequence and promotor region of PLN (Z99496) and ME1 (NM002395) genes were screened for mutation by PCR and SSCP. Sylvius *et al.* could not identify any genetic susceptibility to DCM (86). The screening of the remaining candidate genes within the 6q12-16 region is in progress in this laboratory.

5.55. Tafazzin (TAZ) gene in Irish wolfhounds

DCM is a common disease in humans and dogs. Large-breed dogs and especially Irish wolfhounds belong to the frequently affected breeds. Male Irish wolfhounds show a significantly higher prevalence of DCM than females. X chromosome markers for linkage with DCM as well as a human candidate gene on the X chromosome have been investigated. Some dog breeds, especially large-breed dogs such as Doberman Pinschers, Newfoundlands, Boxers, Great Danes and Irish wolfhounds show a high prevalence of DCM. Therefore, a genetic cause seems to be likely in these breeds (73). In Irish wolfhounds, the onset of

the disease occurs between 3 and 7 years. Mutations in the TAZ gene are known to cause Barth syndrome, endocardial fibroelastosis and infantile DCM (OMIM 302060, 305300 and 300069). The role of the TAZ gene is still unknown. It is supposed to function as an acyltransferase in the remodelling of cardiolipin in the inner mitochondrial membrane (73). No relation between the TAZ gene and the development of DCM was, however, found.

5.56. A-dystrobrevin

The a-dystrobrevin gene is alternatively spliced, resulting in multiple isoforms of dystrobrevin (a, b, g), with different tissue distributions, of which only a-dystrobrevin is expressed in the heart (80). A-Dystrobrevin is a member of the DAPC, which is composed of 3 subcomplexes: the dystroglycan complex, the sarcoglycan complex and the cytoplasmic complex, which includes the syntrophins and dystrobrevins (80). The DAPC, which is located at the sarcolemma, connects the cysteine-rich and C-terminal domains of dystrophin with b-dystroglycan and the cytoplasmic complex, respectively. B-Dystroglycan is a transmembrane protein that binds to the laminin-binding protein a-dystroglycan in the extracellular matrix (80). At the N-terminus, dystrophin binds to actin. These interactions effectively link the extracellular matrix to the dystrophin-based cytoskeleton of the muscle fiber at the C-terminus and to the contractile apparatus at the N-terminus. Furthermore, a-dystrobrevin links the DAPC to the signaling protein neuronal nitric oxide synthase (nNOS) (80). Disruption of these links results in severe muscle wasting or cardiac muscle pathology. For example, dystrophin mutations cause Duchenne muscular dystrophy (80) or X-linked DCM. Ichida *et al.* reported the identification of novel mutations in G4.5 in patients with isolated LVNC (80). In patients with LVNC associated with CHD no mutations were found in G4.5. Instead, a mutation in the calciumbinding EF-hand domain of a-dystrobrevin was identified in 1 family. The a-dystrobrevin mutation described in this study results in a phenotype of dilated HCM with deep trabeculations, associated with congenital heart disease, consistent with the criteria for LVNC. Further study is needed to clarify this issue.

5.57. Manganese superoxide dismutase gene (SOD2)

The SOD2 gene encodes for manganese superoxide dismutase (MnSOD). MnSOD is an antioxidant enzyme localized in mitochondria to protect cells from oxidative damages and preferentially expressed in the heart, brain, kidney, and liver (5). Expression of MnSOD is induced by inflammatory cytokines such as IL1 and TNF that are increased in sera of patients with IDC or myocarditis (5) and overexpression of MnSOD promotes survival of cells damaged by these cytokines (5). Because the heart is rich in mitochondria as compared with the other organs, mitochondrial disorders frequently involve the cardiac tissue. It also has been suggested that MnSOD is involved in the pathogenic process of ischemic heart disease and adriamycin-induced cardiomyopathy, both of which often show the IDC-like phenotype (5). Hiroi *et al.* confirmed the association of IDC with HLA-DRB1 1401 in nonfamilial Japanese patients and showed that the Val allele of the SOD2 gene, especially in the homozygous state, was associated with nonfamilial IDC in Japanese (5). They also showed that there was a difference in the mitochondrial processing efficiency of MnSOD (SOD2) leader signal depending on the Ala/Val polymorphism.

5.58. Mitochondrial DNA abnormalities

The mtDNA is a double-stranded, circular DNA molecule. Because of the lack of histones, a repair system, and an exposure to oxygen-free radicals the mutation rate in mtDNA is more than 10 times higher as in nDNA. In addition, mtDNA has no introns, so that a random mutation will usually strike a coding DNA sequence. The high mutation rate of the mtDNA causes a prevalent mtDNA variation rate in the human population and is a common cause for mitochondrial disease. Mitochondrial DNA mutations have the potential to affect OXPHOS and in turn cellular death, but the mechanisms whereby these mutations cause disease are unknown. The maternal inheritance and high mutation rate of the mtDNA mean that mtDNA alterations are common in human populations and that these alterations make it difficult to define disease related mtDNA mutations (2). Sequence alterations that have not yet been described before could be detected in both, patients and controls, respectively. These sequence alterations leading to a replacement of amino acid residues may be normal polymorphisms and therefore no cause for the disease. A number of pathogenic mtDNA mutations identified in patients with cardiomyopathy reside in tRNA genes of the mtDNA. These mutations have been shown to negatively affect mitochondrial protein synthesis and specific respiratory enzyme activities (2). Two tRNA mutations were found in patients with DCM (c.10475T>C tRNAArg and c.15924A>G tRNAThr) (2). The c.15924A>G tRNAThr mutation is connected with respiratory enzyme deficiency, mitochondrial myopathy and cardiomyopathy. The tRNAArg—c.10458T>C mutation is described as a known sequence polymorphism. In the D-loop region several sequence alterations could be detected. The c.16189T>C variant that is associated with susceptibility to DCM could be detected in the Caucasian cohort in 15.6% of patients with DCM and in 9.7% of the control subjects. A number of 17.2% of the c.16189T>C variant in white Europeans with DCM versus 8.8% in controls have also been detected (2). A significant difference could be found in new mtDNA mutations in protein coding genes of patients with DCM. Some of the mutations were found to be potentially pathogenic to DCM, because they substituted evolutionarily conserved residues, which might alter the function of the respective protein subunits. Taken all data together this study showed that mutations altering the function of the enzyme subunits of the respiratory chain could be responsible for the pathogenesis of DCM (2).

5.59. Alcohol and acetaldehyde dehydrogenases (ADHs and ALDHs) genes

Kajander *et al.* reported that the genes that code for alcohol and acetaldehyde dehydrogenases (ADHs and ALDHs) also contain common functional polymorphisms (25). ADH2 has two metabolically faster alleles, 2 and 3, and the ADH2:2 allele has been associated with alcoholic liver cirrhosis. A comparable faster allelic variant of ADH3

(allele 1) causes a 3- to 4-fold increase in ethanol oxidation rate. ALDH2 has an inactive allelic isoform ALDH2:2, which is rare in Caucasians but common in Asians and results in acetaldehyde accumulation and marked acute effects on LV function (25).

5.60. Cytochrome P-450 2E1 (CYP2E1)

The gene that codes for the major component of microsomal ethanol oxidation system, cytochrome P-450 2E1 (CYP2E1), also contains several polymorphic sites, of which the PstI and RsaI polymorphisms have been associated with alcoholic liver disease (25). DraI and MspI polymorphisms of CYP2E1 are of unknown functional significance, but DraI polymorphism was linked with the prevalence of alcoholism in one study (25).

6. FINAL CONSIDERATIONS

DCM is a primary myocardial disease that causes considerable morbidity and mortality. Although this cardiomyopathy is clinically heterogeneous, genetic factors play an important role in its etiology and pathogenesis. To clarify the genetic background of idiopathic DCM, the association between gene polymorphisms and this disease has been investigated for various genes. The genetic contributions to heart failure can be broadly grouped into causative and modifier genes. Numerous components of the cytoskeletal system have been implicated as genes that cause DCM. In contrast, modifier genes become active after the disease is present and thus influence clinical course. The identification of genetic risk factors is important for better understanding the pathogenesis of DCM. Up to now, several chromosomal loci and disease genes have been identified. But the data suggests that nearly all of the disease genes account for a relatively small proportion of all cases of DCM. As no major gene or loci have been identified in DCM, it may be speculated that a large number of morbid genes remains to be identified.

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Key Words: Heart, Dilated Cardiomyopthy, Etiology, DNA, Single Nucleotide Polymorphisms, Review

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