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Increased Frequency of Organ-Specific Cardiac Antibodies in Healthy Relatives of Patients with Dilated Cardiomyopathy: Evidence for Autoimmunity in Polish Families

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Summary

Background and hypothesis: Autoantibodies represent markers of autoimmune involvement and are found with increased frequency in patients and their symptom-free relatives at risk compared with normal controls. Cardiac-specific autoantibodies, detected by immunofluorescence, were found in 20% of symptom-free relatives of patients with dilated cardiomyopathy (DCM) from England and Italy. The role of autoimmunity may vary in DCM patients from Poland due to ethnic differences in genetic susceptibility to autoimmune disease.

Methods: We assessed the frequency of the organ-specific cardiac autoantibodies in 162 symptom-free relatives of DCM patients [85 male, mean (SD) age 27 (18) years] and 80 control subjects from Poland. Familial DCM (> 1 affected member) was present in 4 families, nonfamilial DCM in the remaining 24 pedigrees. We performed antibody screening and noninvasive cardiological assessment in the whole group.

Results: The frequency of cardiac-specific autoantibodies was higher among patients with documented DCM (probands and relatives) (50%) and their symptom-free relatives (38%) than in unrelated normal subjects (10%; p=0.0001). In 24 (86%) of the pedigrees studied, autoantibodies were found in the proband and/or in at least one family member and tended to be more common in familial than in nonfamilial DCM (50 vs. 35%, p=NS). Echocardiographic indices of left ventricul. size and function were similar in relatives with and without de tectable antibodies.

Conclusions: The presence of cardiac-specific autoantibodies in symptom-free relatives of DCM patients provides evidence for autoimmunity in the majority (86%) of our pedigrees, including both familial and nonfamilial forms of DCM.

Key words: autoimmunity, autoantibodies, dilated cardiomyopathy, relatives

Introduction

A feature of organ-specific autoimmune disorders is the presence of detectable organ-specific autoantibodies in patients and their symptom-free relatives at risk.[1] Organ- and disease-specific cardiac autoantibodies were found in a third of patients with dilated cardiomyopathy (DCM) from different countries.[2–5] These autoantibodies were also detected in 20% of symptom-free relatives from England and Italy.[6]

The role of autoimmunity may vary in patients from different countries due to ethnic differences in genetic susceptibility. We assessed cardiological status and frequency of the organ-specific cardiac autoantibodies detected by immunofluorescence (IFL) in symptom-free relatives of DCM patients from Poland.

Methods

Study Groups

Family screening was performed in 28 of 144 consecutive DCM patients;[7] criteria for eligibility have been reported in

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detail.[8] Baseline clinical and diagnostic features of the noneligible patients were similar to those of the index cases.

Probands with DCM: The diagnosis of DCM was based upon World Health Organization (WHO) criteria;[9] all patients had a dilated left ventricle with angiographic left ventricular (LV) ejection fraction ≤ 45% in the absence of any known causes. All patients > 40 years underwent coronary angiography; patients aged ≤ 40 years underwent coronary angiography if there was a history of angina, dyslipoproteinemia, or tobacco abuse. Serum for antibody testing was available in 17 of the 28 probands; 11 had died before the beginning of the study.

Relatives with DCM: Familial disease was defined as the presence of the index case and at least one first-degree relative with DCM diagnosed according to WHO criteria.[9] Of the 28 pedigrees included in the study, 4 were classified as having familial and the remaining 24 as having nonfamilial DCM. In all four familial pedigrees, there was only one first-degree relative with documented DCM. Serum for antibody testing was available in three of the relatives with DCM; one had died before the beginning of the study.

Healthy relatives of the DCM patients: In all, 162 symptom-free relatives (aged 26.5 ± 17.5 years, range 3–84 years, 85 male, 119 first-degree) were studied; of these, 76 were aged ≤ 18 years.There were 47 females. A further 41 first-degree relatives had died and 35 were living, but were unavailable for study. Of the 162 subjects, 34 were from familial and the remaining 128 were from nonfamilial pedigrees. The study was done following approval from the ethics committee at our institution.

The relatives were evaluated by clinical examination with blood pressure measurement, 12-lead electrocardiogram (ECG), M-mode and two-dimensional (2-D) echocardiography, and assessment of sera for cardiac antibodies. Relatives with documented systemic hypertension were not excluded. Systemic hypertension was defined as a resting blood pressure measurement ≥150/90 mmHg on at least two distinct occasions. The ECG was considered to be abnormal according to conventional criteria.[10, 11] Echocardiogram was performed with a Hewlett Packard 77020A machine by a dedicated operator who was blinded to clinical information. Measurements of LV chamber and wall thickness were obtained at papillary muscle level on the 2-D guided M-mode echocardiograms or directly on 2-D echocardiograms. Normal values for LV end-diastolic and end-systolic internal dimensions were calculated according to Henry's method.[12] The regression equation used to calculate predicted LV end-diastolic dimension (LVEDD pred) was: LVEDD pred = 45.3 (body surface area)$^{\gamma_4}$ − 0.03 (age) −7.2 ±12%. Left ventricular enlargement (LVE) was defined as LVEDD>112% (equivalent to >2 SD) of the predicted normal value.[12] Left ventricular enlargement was calculated as the percentage of the predicted normal value, that is: %LVE = LVEDD* 100/LVEDD pred. The specificity of these established cut-off criteria was confirmed in a separate study of 100 symptom-free normal subjects (55 female, mean age 34.3 ± 8.2 years).[8]

Relatives were classified as normal, potentially affected (either LVE or depressed fractional shortening), or suspected of having DCM (LVE and depressed fractional shortening). Relatives suspected of having DCM underwent invasive evaluation, including selective coronary angiography if aged> 40 years.

Controls: Eighty healthy subjects aged 29 ± 9 years, 43 male, unrelated to the DCM patients, represented the normal control group for the immunological study. All had normal clinical and noninvasive examinations (standard 12-lead ECG and 2-D Doppler echocardiography).

Cardiac Antibody Testing

For detection of cardiac antibodies, serum samples were tested by standard indirect IFL at 1/10 dilution on 4 μm-thick, unfixed, fresh frozen cryostat sections of blood group O normal human atrium and skeletal muscle, as previously described.[2, 6] Cardiac antibody titers were measured by doubling dilutions of sera in phosphate-buffered solution. Cardiac antibody patterns were classified as previously reported.[2, 5] Briefly, "organ-specific" antibodies produced a diffuse cytoplasmic staining of atrial tissue, but did not react with skeletal muscle; "cross-reactive 1" antibodies gave a fine striational IFL on cardiac tissue, but stained skeletal muscle fibers only weakly; "cross-reactive 2" antibodies stained both heart and skeletal muscle sections with a broad striational pattern. Absorption studies with relevant tissues had confirmed the organ specificity and cross reactivity of the three antibody types.[2] Two sera were used as standard positive (antibody titer 1/40) and negative controls and titrated in every assay. The intensity of IFL of the positive standard at 1/40 dilution was used as the threshold for positivity. All sera tested at 1/10 dilution were read blindly against these standards. An additional positive control serum was titrated to assess reproducibility.[6] Endpoint titers for this serum were reproducible within one double dilution in all assays.

Data Analysis

Data are reported as mean values ± standard deviation. The two-tailed chi-square test was used to compare frequencies and the two-tailed Student's *t*-test was used for comparison of numerical data. Statistical significance was defined as $p<0.05$.

Results

Clinical and Diagnostic Features

Patients with DCM: Of the 32 DCM subjects (28 probands and 4 relatives), 21 were in New York Heart Association class III or IV; mean symptom duration was 33.4 ± 31.8 months, mean angiographic LV ejection fraction was 22.8 ± 9.8%. During mean follow-up of 44 months, 11 probands died (4 suddenly, 4 of congestive heart failure, and 3 of stroke), and 4 underwent heart transplantation; 1 relative died of congestive heart failure.

Symptom-free relatives: Clinical features of the 162 symptom-free relatives are shown in Table I. Physical examination

TABLE I Clinical and diagnostic data of 162 symptom-free relatives

Mean age (years)	26.5 ± 17.5
Male sex (%)	85 (52.5)
Children (%)	76 (46.9)
Hypertensive (%)	18 (11.1)
Abnormal ECG (%)	27 (16.6)
2-D echo:	n = 154
LVEDD (mm)	49.3 ± 6.3
LVESD (mm)	32.7 ± 5.7
%FS	33.9 ± 5.1
%LVE	109.7 ± 9.1
Number of potentially affected relatives	66 (43%)

Abbreviations: ECG = 12-lead standard electrocardiogram, 2-D echo = two-dimensional echocardiogram, LVEDD = left ventricular end-diastolic dimension, LVESD = left ventricular end-systolic dimension, %FS = percent fractional shortening, %LVE = percentage left ventricular enlargement.

was normal in all but 18 subjects (11.1%) who were hypertensive. Twelve-lead ECG was abnormal in 27 relatives (16.6%) showing LV hypertrophy in 14 (9%) of cases and conduction disturbances in further 13 (8%) of the relatives (Fig. 1). Similar proportions of relatives with familial and nonfamilial pedigrees had an abnormal ECG (9: 26.5% vs. 18: 14.1%, p=NS).

In 154 (94.1%) relatives, the echocardiographic study was of adequate quality; in the remaining 8, quantitative measurements were not recorded, but LV function was regarded as normal. Of the 154 relatives, 88 (57%) had a normal echocardiographic study and 66 (43%) were classified as potentially affected. The frequency of potentially affected subjects was higher among symptom-free relatives than in our control group of unrelated normals (18%, p=0.0001) and was similar in familial (37.5%) and nonfamilial pedigrees (44.3%, p=NS).

Frequency of cardiac antibodies in the study groups: The frequency of organ-specific antibodies was higher in DCM patients (50%) and their symptom-free relatives (38.3%) than in normals (10%, p=0.0001) (Fig. 2). Antibody titers in the DCM patients and their family members were: 1 in 10 in 45 of the 72 positive sera (62.5%), 1 in 20 in 18 (25%), and 1 in 40 in 9 (12.5%). All positive sera contained autoantibodies of IgG class.

Relatives from familial pedigrees tended to have higher antibody frequency than those from nonfamilial pedigrees (50 vs. 35.2%, p=0.11). In 24 of 28 pedigrees studied (85.7%), the index case and/or at least one first-degree relative were antibody positive. There was at least one first-degree antibody-positive relative in 7 of 9 families whose proband was antibody positive, in 6 of 8 families whose proband was negative, and in 9 of 11 of the pedigrees where antibody status was not known (p=NS). Figure 3 shows antibody status in one representative pedigree with familial DCM.

Table II shows clinical and diagnostic features in relation to cardiac antibody status. Relatives with detectable antibody

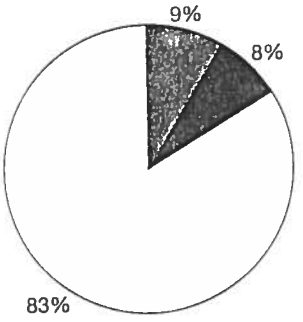

FIG. 1 Electrocardiographic findings in 162 symptom-free relatives. ▩ = Left ventricular hypertrophy, ■ = conduction abnormalities, □ = normal electrocardiogram.

were older than those without (p<0.01); the number of children was higher in the antibody-negative group (p=0.048). All remaining features, including the number of potentially affected relatives, were similar in relatives with and without antibodies.

Discussion

In this study, we detected organ-specific cardiac autoantibodies in 50% of Polish patients with documented DCM, in 38% of their symptom-free relatives from both familial and nonfamilial pedigrees, and in a significantly lower (10%) proportion of normals. Positive autoimmune serology in either proband and/or at least one first-degree relative was present in the majority (86%) of the pedigrees studied. These findings confirm those reported in a distinct cohort of DCM patients and relatives from Western Europe[5] and provide evidence for autoimmune involvement in the pathogenesis of this condition. It is of interest that antibody frequencies in Polish DCM

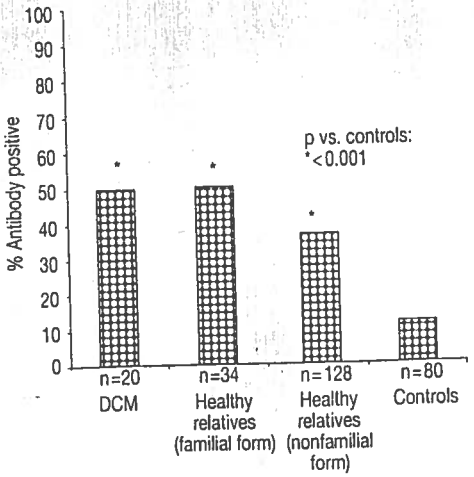

FIG. 2 Frequency of organ-specific cardiac antibodies in familial and nonfamilial pedigrees. DCM = dilated cardiomyopathy.

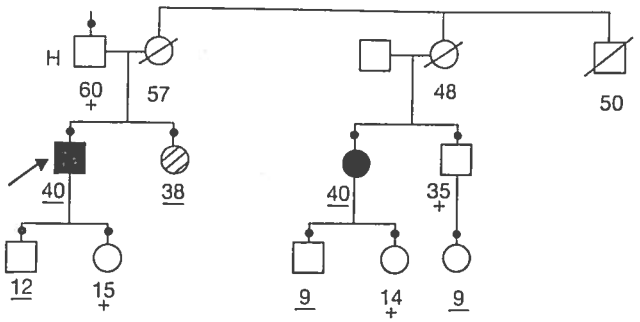

FIG. 3 Antibody status in one representative pedigree with familial dilated cardiomyopathy (DCM). □ = male, ○ = female, open symbols = unaffected subjects, solid symbols = subjects with DCM, shaded symbols = subjects potentially affected (on the basis of an abnormal echocardiogram). Deceased family members are indicated by a slash. The arrow indicates the proband; the minus sign indicates negative cardiac antibody status, and the plus sign shows positive cardiac antibody status. H = hypertensive subject.

TABLE II Clinical and diagnostic features of symptom-free relatives of patients with dilated cardiomyopathy in relation to antibody status

	Cardiac antibody positive (n=62)	Cardiac antibody negative (n=100)	p Value
Number of 1st degree relatives (%)	46 (74.2)	73 (73)	NS
Mean age (years)	31.7 ± 19.3	23.4 ± 15.5	<0.01
Children (%)	23 (36.5)	53 (53)	0.048
Mean age of children	12.8 ± 3.9	12.2 ± 4.2	NS
Male sex (%)	30 (48.4)	55 (55)	NS
Hypertensive (%)	9 (14.5)	9 (9)	NS
2-D echo:	n=56	n=98	
LVEDD (mm)	50.0 ± 5.4	48.9 ± 6.7	NS
LVESD (mm)	33.1 ± 5.1	32.5 ± 5.9	NS
%FS	34.0 ± 4.9	33.7 ± 5.2	NS
%LVE	110.9 ± 9.3	109.1 ± 8.8	NS
Number of potentially affected relatives (%)	27 (48.2)	39 (39.8)	NS

NS = not significant. Other abbreviations as in Table I.

families and normals are higher than those previously reported.[5] This may reflect ethnic differences in genetic susceptibility to autoimmune diseases,[1, 12] as Polish people are more prone to develop autoimmunity to cardiac antigens. Further studies, aimed at assessing potential HLA associations in DCM patients from Poland, should provide new insights.

Our group of relatives was young, children accounting for half of the relatives. We found that antibody-positive relatives were older than antibody-negative relatives. Conversely, positive antibody status was associated with younger age in the previously reported Anglo-Italian series.[5] We recently reported that positive cardiac autoantibody status in Polish DCM patients was associated with a long latency period and insidious onset of symptoms.[13] Thus, the lack of association between antibody and younger age in our study could indicate that the autoimmune disease process in Polish patients has a more subtle, slow progression and delayed onset. This would indicate clinical and genetic heterogeneity in DCM as in other organ-specific autoimmune diseases.[14]

Mild abnormalities of LV function were found in 43% of our symptom-free relatives. Echocardiography has limitations in terms of reproducibility, in particular when assessing mild abnormalities. However, the frequency of LV enlargement was higher among the relatives compared with that seen in our unrelated normal subjects. This is consistent with two previous reports from different centers[5, 15] and raises the issue that the echocardiographic abnormalities, at least in some relatives, may represent latent forms of DCM and/or early disease changes.

In our study, antibody status was not associated with echocardiographic abnormalities. Conversely, in a previous report on DCM family members from Western Europe, in which the same immunofluorescent technique was used, the antibodies were associated with greater end-diastolic echocardiographic dimension and lower fractional shortening, suggesting that they might represent early markers of disease.[5] Since half of our relatives were children, and echocardiographic assessment of mild LV enlargement is likely to be more difficult in children than in adult normal subjects because of age-related cardiac growth phenomena, this may explain the lack of correlation between antibody status and echocardiographic findings in the present study. Extended longitudinal observation and better understanding of both clinical and immunologic phenotype (e.g., cell-mediated immune markers) and genotype (HLA and other predisposing genes) is necessary to clarify which of the relatives at present classified as potentially affected will develop DCM. Follow-up is also needed to establish the role of the antibodies detected by IFL as predictive markers for relatives at risk.

In DCM, additional humoral markers, besides those found by IFL, include antibodies to mitochondrial antigens (M7 and adenine nucleotide translocator), β-1 receptor, α- and β-myosin heavy chain.[16–19] In a previous report, autoantibodies against myosin, branched-chain keto acid dehydrogenase, and the adenine nucleotide translocator were studied[16] and found both in familial and nonfamilial genealogies. It would be interesting to elucidate whether relatives classified as negative for one antibody marker are positive for another, and to assess predictive value for each identified antibody specificity.

Although cardiac autoantibodies are markers of autoimmune involvement, this does not imply that they are directly pathogenic. At present there is no conclusive evidence for antibody-mediated damage in DCM patients. None of the antibody markers identified so far in patients[2, 5, 16–19] has been shown to be able to produce the disease phenotype following inoculation of high-titer antibody in susceptible animal models. In a well-characterized mouse model of autoimmune heart disease, high-titer antibody from autoimmune animals was also not capable of inducing disease in syngeneic normal recipients, whereas the disease could be

transferred by T cells.[20, 21] Thus, it is likely that also human DCM is a T cell-mediated disease.

Conclusion

Our study confirms that symptom-free relatives of Polish DCM patients from both familial and nonfamilial pedigrees have increased frequency of organ- and disease-specific cardiac autoantibodies compared with normal control subjects. This is consistent with autoimmune involvement, as shown in other series from Western Europe[5] and the USA.[15] Prospective studies are needed to assess whether DCM in Poland has slower progression and delayed onset. Antibody-positive relatives with or without echocardiographic abnormalities may be at risk and should be followed up, although the predictive value of the antibody for the development of DCM remains to be established.

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