DILATED CARDIOMYOPATHY

CHARLES J. McKENNA

DOCTOR OF MEDICINE

UNIVERSITY OF EDINBURGH
DECLARATION

The thesis has been composed solely by myself. I identified the probands through retrospective and prospective analysis, set up the Dilated Cardiomyopathy clinic, interviewed all the probands and first-degree relatives, confirmed all echocardiographic measurements, constructed original protocol and datasheets, collected and analysed all the data, and wrote the thesis. It has not been submitted for consideration elsewhere.

Charles J. McKenna
## DILATED CARDIOMYOPATHY

<table>
<thead>
<tr>
<th>Contents</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contributors</td>
<td>5</td>
</tr>
<tr>
<td>Ethics/Grants</td>
<td>6</td>
</tr>
<tr>
<td>Abstract</td>
<td>7-9</td>
</tr>
</tbody>
</table>

## INTRODUCTION

<table>
<thead>
<tr>
<th>General</th>
<th>11-14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment of DCM</td>
<td>15-17</td>
</tr>
<tr>
<td>The autoimmune hypothesis</td>
<td>18-20</td>
</tr>
<tr>
<td>Histological evaluation of biopsy specimens</td>
<td>21</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>22-24</td>
</tr>
<tr>
<td>Objectives</td>
<td>25</td>
</tr>
</tbody>
</table>

## PATIENTS AND METHODS

<table>
<thead>
<tr>
<th>Data gathering</th>
<th>26-33</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>27-28</td>
</tr>
<tr>
<td>First-degree relatives</td>
<td>28</td>
</tr>
<tr>
<td>Histological evaluation of biopsy specimens</td>
<td>29-30</td>
</tr>
<tr>
<td>Case-control study</td>
<td>31-32</td>
</tr>
<tr>
<td>Data analysis</td>
<td>33</td>
</tr>
</tbody>
</table>

## RESULTS

<table>
<thead>
<tr>
<th>General</th>
<th>34-49</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial prevalence</td>
<td>35-37</td>
</tr>
<tr>
<td>HLA distribution</td>
<td>38-40</td>
</tr>
<tr>
<td>Histological evaluation of biopsy specimens</td>
<td>41-42</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>43-44</td>
</tr>
<tr>
<td>Screen-detected DCM</td>
<td>45-48</td>
</tr>
</tbody>
</table>

## DILATED CARDIOMYOPATHY

### Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DISCUSSION</td>
<td>50-73</td>
</tr>
<tr>
<td>Familial prevalence</td>
<td>51-55</td>
</tr>
<tr>
<td>HLA distribution</td>
<td>56-60</td>
</tr>
<tr>
<td>Histological evaluation of biopsy specimens</td>
<td>61-65</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>66-72</td>
</tr>
<tr>
<td>Screen-detected DCM</td>
<td>73</td>
</tr>
<tr>
<td>CONCLUSIONS</td>
<td>74-75</td>
</tr>
<tr>
<td>FUTURE RESEARCH AND CLINICAL IMPLICATIONS</td>
<td>76-78</td>
</tr>
<tr>
<td>APPENDICES I-V</td>
<td>79-84</td>
</tr>
<tr>
<td>PRESENTATIONS AND PUBLICATIONS</td>
<td>85-87</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>88-105</td>
</tr>
</tbody>
</table>
CONTRIBUTORS

*Charles J. McKenna BSc, MB, MRCP*
Cardiology Registrar, Mater Hospital, Dublin, Ireland (1993-96)
- Identified the probands through retrospective and prospective analysis.
- Set up the Dilated Cardiomyopathy clinic.
- Interviewed all the probands and first-degree relatives.
- Confirmed all echocardiographic measurements.
- Performed myocardial biopsies and histological analysis.
- Constructed original protocol and datasheets.
- Collected and analysed all the data.
- Wrote the thesis.

*Mary B. Codd MD, PhD*
Epidemiologist and Biostatistician, Mater Hospital, Dublin, Ireland
- Designed the alcohol case-control study.
- Checked all statistical analysis.
- Reviewed thesis.

*Hugh A. McCann MD, FRCPI*
Consultant Cardiologist, Mater Hospital, Dublin, Ireland
- Confirmed all echocardiographic measurements.
- Reviewed thesis.

*Robert S. Schwartz MD, FACC*
Head of Vascular Biology and Consultant Cardiologist, Mayo Clinic, Rochester, Minnesota, USA
- Confirmed histological analysis of biopsy specimens
- Reviewed thesis

*Declan D. Sugrue MD, FRCPI*
Consultant Cardiologist, Mater Hospital, Dublin, Ireland
- Overviewed entire study as local supervisor and expert in the field.
- Reviewed thesis.
ETHICS

Approval to undertake this work was obtained from the local Ethical Committee (1993). There were no conflicts of interest.

GRANTS

The work was funded in part by two grants from the Mater Misericordiae Hospital Foundation (1993-94). These funds were used solely to pay for HLA-typing.
ABSTRACT

Recent prospective studies in which relatives of patients with dilated cardiomyopathy (DCM) have been screened for the disease using echocardiography have documented a familial prevalence of 20-25%. Those asymptomatic relatives with left ventricular enlargement (LVE) are assumed to have early familial DCM. A serum marker which could identify families at risk of developing dilated cardiomyopathy should be of use in screening for the disease. Alcohol has been implicated as a risk factor for DCM but a causal relationship has not been established.

This study was designed to: (i) compare HLA distribution in familial and non-familial dilated cardiomyopathy; (ii) histologically evaluate myocardial biopsy specimens obtained during diagnostic workup in relatives with echocardiographic LVE; (iii) determine the association between alcohol consumption and DCM, by comparing a cohort of well defined DCM patients with randomly selected, population-based controls.

Methods and Results We report on a group of 100 patients with dilated cardiomyopathy. Two hundred first-degree relatives from 56 of these proband families were screened for dilated cardiomyopathy by echocardiography. The HLA profile of the patients with dilated cardiomyopathy, as well as of the familial and non-familial subgroups, was compared with that of 9000 normal controls. The familial prevalence of dilated cardiomyopathy in this patient group was 'definite' in 14 / 56 (25%) and 'possible' in 25 / 56 (45%). The HLA-DR4 frequency was similar in the 100 patients with dilated cardiomyopathy as
compared with the 9000 controls (39% v 32%; NS). However, the DR4 subtype was significantly more common in the 25 probands with a familial tendency to dilated cardiomyopathy than the 31 probands with non-familial dilated cardiomyopathy (68% v 32%; P < 0.05).

The first 5 relatives demonstrating echocardiographic asymptomatic DCM underwent cardiac catheterization to confirm LVE with normal coronary angiography. Histological evaluation of right ventricular myocardial biopsy specimens obtained included H+E and TUNEL staining, and CPP-32 and HLA-DR expression. The apoptotic index was calculated as the number of positive-staining cells divided by total number of nucleated cells (%) in ten separate high power fields per case. In all cases, mild to moderate myocyte hypertrophy was observed with only mild or no interstitial fibrosis. There was no evidence of inflammation. The apoptotic index ranged from 17-30% (mean=25%). TUNEL positive cells were more prominent in the interstitium than the myocardium and were associated with high levels of CPP-32 immunoreactivity. HLA-DR expression was detected in all cases and was again more pronounced within the interstitium.

Questionnaires were administered detailing average weekly intake of alcohol, total lifetime consumption and alcohol abuse in cases and controls. Significantly more of the 100 patients with DCM than the 211 controls drank greater than the recommended weekly intake of alcohol (40% vs. 24%; P<0.01) and were alcohol abusers according to the CAGE questionnaire (27% vs. 16%; P<0.05). The average total lifetime consumption measured in units of alcohol was also significantly greater in cases than controls (31,200 vs. 7,904; P<0.01).
Conclusions The present finding supports an HLA-linked predisposition to familial dilated cardiomyopathy. The HLA-type DR4 was significantly more common in familial than non-familial cases. More importantly, the DR4 haplotype was associated with two-thirds of the families at risk for dilated cardiomyopathy. This study demonstrates for the first time that asymptomatic relatives, of DCM patients, with echocardiographic LVE have abnormal cellularity. The disease activity does not appear to be inflammatory but is associated with a high apoptotic index. The study also confirms previous suspicion of a causal association between alcohol and DCM, with significantly more patients than controls either abusing alcohol or drinking it in excess of recommended limits.
INTRODUCTION
INTRODUCTION

Specific diseases of the heart muscle were recognised as early as 1933 (1) and the concept of primary myocardial disease was put forward by Mattingly in 1959 (2). It was Harvey in the United States (3) and Goodwin in the United Kingdom (4) who shortly after this called the disease "cardiomyopathy".

Idiopathic dilated cardiomyopathy (DCM) was defined as global ventricular dilatation with impaired systolic function in the absence of a known cause (5). In view of the large amount of ongoing research in the field, the definition has since been expanded to say that DCM "may be familial/genetic, viral and/or autoimmune or alcoholic/toxic" (6). The diagnosis classically excludes patients with known cause for heart muscle disease, e.g. coronary artery disease, hypertension, valvular and congenital heart disease, as well as infective (myocarditis) and metabolic (thyroid disease) causes. The usual method of diagnosing DCM is by echocardiography, to assess ventricular size and function. Echocardiography is also the most useful test for screening and follow-up of patients with DCM. To be certain of the diagnosis one must perform coronary angiography, to exclude coronary artery disease. Endomyocardial biopsy may be performed but is fairly unhelpful as the findings are non-specific.

DCM most commonly presents in young and middle-aged men. Patients are probably asymptomatic for years and tend to present with symptoms of heart failure and less commonly systemic embolism, chest pain, arrhythmias or sudden cardiac death. Some patients die in heart failure but most deaths are sudden and presumed to be secondary to ventricular arrhythmias.
INTRODUCTION

As with other forms of cardiomyopathy, DCM may have a genetic link and be inherited in certain individuals. Triggers thought to be associated with DCM include viral illness and alcohol. The general hypothesis being that such environmental triggers may induce an autoimmune response, through aberrant major histocompatibility antigen expression, in a genetically susceptible individual.

The role of viral infection in the pathogenesis of DCM has been supported largely by looking at enteroviral serology and RNA persistence in patients with DCM. Higher neutralising antibodies against coxsackievirus B were found in DCM patients than controls, with titres being more elevated in those with recent onset of disease (7). A study of transplanted hearts in patients with end-stage DCM found enteroviral RNA in 6 / 21 (29%), as compared to 1 / 17 (6%) of patients transplanted for ischaemic heart disease (8). This evidence suggested that persistence enteroviral infection may be involved in the pathogenesis of some cases of DCM. The possible aetiological role of enteroviruses was further supported by the detection of enteroviral RNA in myocardial biopsy specimens. In a group of 120 patients with heart muscle disease due to myocarditis or DCM, 41 (34%) demonstrated the presence of myocardial enteroviral RNA (9). Only 16 (13%) of the patients described a history of preceding viral illness, underscoring the underestimation of viral illness obtained through history-taking alone. Interestingly, in this study, the presence of enteroviral RNA within the myocardium at initial diagnosis was independent predictor of outcome over a mean follow-up period of 25 months. It is conceivable that such viral persistence may initiate a secondary, immune-mediated response, leading to an accentuated decline in ventricular function in a susceptible individual.
INTRODUCTION

The relationship between enteroviral persistence and myocardial damage in patients with DCM was strengthened by the finding that enteroviral RNA sequences were present in myocardial biopsy specimens from 4/16 (25%) patients who were positive for antimyosin uptake (a marker of myocardial cell damage), compared to 0/3 patients with negative antimyosin scans (10). Although the majority of patients in study (84%) had positive antimyosin scans, only a minority (25%) also showed evidence of persistence enteroviral infection. Thus, implying that the myocardial damage observed in DCM patients is probably the end result of variety of infectious (viral) and non-infectious (alcohol) agents. The damage caused by these agents may be perpetuated by the production of cardiac autoantibodies in a susceptible individual. Post-viral autoimmune heart disease remains an attractive aetiological concept for the pathogenesis of DCM and myocarditis. However, the interpretation of viral screening is hampered by the high prevalence of the proposed environmental agent in the general population. This problem was addressed by comparing the frequency of antibodies to Coxsackie B in a group of patients with DCM with that of environmentally-matched controls. When these two groups were studied prospectively over the same time period, no difference in the frequency of positive viral serology was observed (11). Hence, it remains true to say that in the vast majority of patients, there is still no definitive evidence of an association between viral infection and DCM (12).
INTRODUCTION

Estimates concerning the epidemiology of DCM are based on the landmark population-based study from Olmsted County (13). Using the unique records linkage system of the Mayo Clinic and the Rochester Epidemiology Project, diagnostic data on the entire population of Olmsted County, Minnesota were accessed. From 1975 to 1984, 45 new cases of DCM were identified. The age- and sex-adjusted incidence and prevalence rates of DCM in the United States were, hence, calculated at 6.0 and 36.5 per 100,000 person-years and 100,000 population, respectively. The frequency in an Irish population is unknown. By extrapolation from US data, there are approximately 2000 cases prevalent in Ireland with about 200 new cases annually. DCM accounts for 50% of the cardiac transplantations performed in Ireland.

Early, retrospective studies on the natural history of DCM indicated median and five year survivals of around 20% and 50% respectively (14,15). However, recent work has demonstrated that the prognosis is more favourable when referral bias is taken into account by comparing hospitalised patients with a population-based cohort (16). Earlier diagnosis and better treatment (ACE inhibitors and beta-blockers, see below) for patients with DCM have contributed to the observed improvement in the prognosis in the last two decades (17,18). The five year survival for patients diagnosed in the 1990's may be as high as 80% (18).
TREATMENT OF DCM

Death in patients with DCM usually occurs as a consequence of ventricular arrhythmia or progressively worsening heart failure. The ultimate goals of pharmacological treatment are, therefore, prevention of lethal arrhythmias and control of left ventricular dysfunction. Routine anti-arrhythmic therapy in patients with DCM has been disappointing. Anti-arrhythmic treatment, guided by electrophysiological study, can improve prognosis in those patients with sustained and inducible ventricular arrhythmias (19,20) and those who present with predominantly right ventricular disease (21). Angiotensin-converting enzyme (ACE) inhibitors, discussed below, may also have a direct electrophysiological anti-arrhythmic effect (22,23).

Digoxin appears to be of symptomatic benefit to patients with non-ischaemic and ischaemic cardiomyopathy, even when their heart failure is clinically mild and they remain in sinus rhythm (24-26). The use of diuretics, for relief of symptoms of heart failure, and warfarin, for prevention of thromboembolism, have little impact on survival or progression of disease. Cardiac transplantation has been shown to improve prognosis with a one-year survival of greater than 80% but is limited to a minority by organ availability (27,28).

β-blockers were first introduced as a treatment for patients with DCM twenty years ago. Metoprolol has beneficial clinical and haemodynamic effects in DCM which are thought to be related to: decrease in myocardial oxygen demand; decrease in circulating catecholamines; up-regulation of β-receptor density; prevention of sudden cardiac death by anti-arrhythmic activity (29-31).
TREATMENT OF DCM

Recent trials with carvedilol, a β-blocker with vasodilator properties related to concomitant α-blockade, show promise in patients with DCM both in terms of symptom relief and reduced mortality (32). A recent meta-analysis of the randomised trails looking at the effect of β-blockade on mortality in patients with heart failure, demonstrated an all-cause mortality benefit, with an impressive overall decrease in the odds of death of 31% and one death prevented for every 35 patients treated (33). This benefit was similar for patients with ischaemic and non-ischaemic cardiomyopathy and there was a trend towards a greater survival benefit with carvedilol compared to β-blockers without α-blocking activity. Similarly, low dose amiodarone may reduce mortality from heart failure and arrhythmia in non-ischaemic dilated cardiomyopathy (34-36).

The degree of left ventricular dysfunction in patients with ischaemic heart disease is known to correlate highly with mortality (37,38). Treatment with ACE inhibitors can reduce the degree of ventricular enlargement and remodelling in the post-myocardial infarction setting (39). ACE inhibitors not only attenuate left ventricular remodelling but also directly inhibit the deleterious effects of neuro-hormonal activation in chronic heart failure (40) and have anti-arrhythmic activity (22,23). Since this earlier work looking at the role of ACE inhibition, many well-constructed, randomised and controlled trials have confirmed their benefit in improving the outlook of patients with left ventricular dysfunction after myocardial infarction (41-47). There is no reason to suppose that ACE inhibitor therapy does not similarly benefit patients with DCM.
TREATMENT OF DCM

A recent randomised study looking at the role of immunosuppressive therapy for patients with myocarditis did not support routine treatment with prednisolone and cyclosporin or azathioprine (48). The patients were, however, identified retrospectively over the preceding two years by the results of endomyocardial biopsy. This trial does not rule out a benefit for selected patients at the time of diagnosis. If a marker could be found to predict those patients at risk, from an inadequate immune response or an autoimmune response, early on in the course of the illness then progressive myocardial damage could theoretically be prevented by timely immunosuppression.

In summary, there are many pharmacological therapeutic strategies of possible symptomatic and survival benefit for patients with DCM. The greatest benefit is achieved for those patients diagnosed early, before the disease and its concomitant ventricular dysfunction are well advanced. Any screening test to identify groups at risk of developing DCM would of course be of use for earlier diagnosis and treatment. By better understanding the aetiology of DCM, one may pin-point such at risk groups and advise them on preventative and follow-up measures.
THE AUTOIMMUNE HYPOTHESIS: HLA-DISTRIBUTION

There are two basic stages in the development of an autoimmune disease. Namely, the creation of the autoantibody followed by tissue damage due to the immune reaction (49). Both long-term humoral and cell-mediated immune responses involve the association of T cells with class II major histocompatibility antigens, i.e. HLA-DR. The expression of these DR antigens is usually limited to the immune system and vascular endothelial cells. When DR expression occurs in tissue where it does not normally exist, subsequent T cell activation leads to an autoimmune reaction. This autoimmune activity can also be detected by the presence of unusually high concentrations of organ-specific autoantibodies in the serum, which can maintain tolerance to the ongoing autoimmune response. Autoimmune disease may be initiated through T cell activation by viruses (discussed above) or other environmental factors, leading to an immune response directed against those self-cells which are aberrantly expressing DR antigen. The linkage of HLA-DR halotypes with various autoimmune disease entities, thus points to a genetic predisposition to respond to such environmental triggers by developing an organ-specific immune response.

The most popular hypothesis regarding the aetiology of DCM is an autoimmune, inherited predisposition initiated by an environmental trigger, e.g. viral illness or alcohol. This theory has been lent support by an increased frequency of HLA-DR4 (50) and the presence of circulating cardiac autoantibodies (51,52) in some patients with DCM. The detection of disease specific cardiac autoantibodies also implies autoimmune involvement in DCM, with anti-α-myosin antibodies have been detected more frequently in subsets of patients than in controls (53).
THE AUTOIMMUNE HYPOTHESIS: HLA-DISTRIBUTION

The majority of DCM patients, however, do not show evidence of autoimmunity in the form of increased frequency of HLA-DR subtypes, enhanced tissue expression of DR antigens or high levels of circulating organ-specific autoantibodies. In these cases, the disease is hypothesized to be non-autoimmune and perhaps solely initiated by an infectious (viral) or toxic (alcohol) agent. Alternatively, serological evidence of autoimmunity may be present only in the early stages of the disease process. In any case, it would seem appropriate to direct any immunotherapy, aimed at slowing or reversing this disease process, at those patients identified as having an ongoing immune reaction to their own myocardium.
THE AUTOIMMUNE HYPOTHESIS: FAMILIAL PREVALENCE

The first prospective studies in which relatives of patients with DCM were screened for the disease by echocardiography were reported recently and gave a familial prevalence of 20-25% (54,55). It is appealing to suggest that the familial tendency for DCM in these patients is linked with a genetic predisposition to develop an autoimmune response to the putative environmental triggers. By screening family members one may potentially identify latent forms of the disease, with actively ongoing autoimmune activity and less severe left ventricular impairment. It is not unreasonable to suspect that such patients may respond better to treatment in the forms of immunotherapy and/or vasodilators and have an improved prognosis secondary to this early detection of the DCM disease state.

The presence of autoantibodies does not adequately distinguish between familial and non-familial DCM (51,52). We postulated that the DR4 subtype might identify families at risk of developing DCM, who would be most likely to benefit from periodic screening with echocardiography and early treatment when left ventricular dysfunction is detected.
EARLY FAMILIAL DCM: HISTOLOGICAL EVALUATION

Prospective studies in which relatives of patients with DCM were screened by echocardiography have consistently demonstrated a familial prevalence of 25%. Screening can potentially identify latent forms of the disease which may respond better to treatment with an improvement in prognosis. Echocardiographic screening of asymptomatic relatives of DCM patients identifies a subset with left ventricular enlargement (LVE) who are assumed to have early familial DCM.

Histological evaluation of explanted hearts of patients with end-stage DCM has revealed myocyte apoptosis (56,57). However, there is little evidence on the presence or role of apoptosis in early (pre-clinical) DCM and some have hypothesized that early DCM may indeed be primarily an interstitial disease. We report the results of myocardial biopsy specimens obtained during diagnostic workup in asymptomatic relatives with echocardiographic LVE.
ALCOHOL CONSUMPTION

By definition the cause of DCM is unknown. Alcohol has been implicated as a risk factor for DCM but a causal relationship has not yet been established. The frequently reported relationship between alcoholism and heart failure has lead to alcohol being postulated as a cause of DCM (14,58). However, many patients with DCM drink alcohol within recommended limits, i.e. are not alcoholics. Nevertheless, alcohol is often cited as the cause of their DCM in a clinical setting.

The aetiological role of alcohol in dilated cardiomyopathy continues to be debated. Twenty years ago Oakley explained that acute myocardial depression by alcohol and improvement of ventricular function after alcohol withdrawal offered no proof of causation (59). In her own words: “Recognizing that a murder has been committed does not necessarily help to identify the criminal.” The evidence linking alcohol consumption with chronic left ventricular failure remains circumstantial and conflicting. Teragaki et al found no correlation between lifetime alcohol intake and cardiac function in men with a history of excessive consumption and congestive heart failure (60). They commented that the amount alcohol required to cause ventricular dysfunction remains unclear. Any stated level above which congestive heart failure is attributed to alcohol abuse is purely arbitrary and, also, does not exclude the possibility of a causative role for alcohol at levels that would be considered socially acceptable in subjects without heart failure.

Marquez et al performed echocardiography on a group of asymptomatic men and women with a diagnosis chronic alcoholism (61). Using the accepted diagnostic criteria, i.e. an end-diastolic diameter more than two standard above the mean and an ejection fraction less than 50%, only 14% of the men and 12% of the women had dilated cardiomyopathy.
ALCOHOL CONSUMPTION

Each patient had reported drinking at least 70 units of alcohol per day in the previous two years. Nevertheless, the vast majority of these patients did not exhibit heart muscle disease.

The concepts of alcoholic heart muscle disease and/or alcohol-induced autoimmune dilated cardiomyopathy are attractive but lacking in definitive proof. The conclusion being, as for the evidence of a viral aetiology, that the vast majority of patients with a diagnosis of DCM are not alcohol abusers. To differentiate a group of patients with DCM on the basis of weekly or total lifetime consumption of alcohol and give them an alternative diagnosis of alcoholic heart muscle disease (AHMD) is a gross simplification. The clinical and pathological findings in patients diagnosed with DCM or AHMD are identical. Only a minority of long-term alcohol abusers, as defined by reported consumption or proven questionnaires, have evidence of left ventricular dysfunction. To make a diagnosis of AHMD in a patient who has dilated cardiomyopathy and is also an alcohol abuser assumes a link for which there is little evidence. The question is why do only minority of alcohol abusers develop left ventricular dysfunction? It would seem logical to assume that this minority is somehow sensitised to its toxic effects and, therefore, represent another subgroup of genetically predisposed DCM patients that have been exposed to an environmental trigger.
ALCOHOL CONSUMPTION

No study to date has compared the alcohol intake of a cohort of DCM patients with that of randomly selected, population-based controls. Two previous groups from France have performed case-control studies of alcohol consumption in DCM (62,63). However, the controls were hospital-based, with associated potential for bias and over-matching for the putative risk factor. This study was designed to determine the association between alcohol consumption and DCM, thus providing important information with regard to the aetiology of DCM and the potential for prevention of this disease.
OBJECTIVES

In summary, the *objectives* of this study were to:

i) determine the frequency of familial DCM in a group of patients with DCM in Ireland, by performing echocardiographic screening on all available asymptomatic first-degree relatives;

ii) determine the distribution of HLA-types in patients with familial and non-familial DCM, and compare this distribution with that of normal controls;

iii) histologically evaluate myocardial biopsy specimens obtained during diagnostic workup in relatives with echocardiographic LVE;

iv) determine the relationship between alcohol consumption and dilated cardiomyopathy, by comparing a cohort of well defined DCM patients with randomly selected, population-based controls.
PATIENTS AND METHODS
DATA GATHERING

CASES
For the purpose of the study, patients with DCM attending the National Cardiac Centre at the Mater Misericordiae Hospital and their first-degree relatives were invited to attend a special clinic. To date 100 probands with DCM have been seen at the clinic. The echocardiographic criteria for the diagnosis of dilated cardiomyopathy were a left ventricular ejection fraction less than 50% and a left ventricular end-diastolic dimension more than two standard deviations above the mean, corrected for the patient's age and body surface area (64) (Appendix 1, page 80).

All the patients had angiographically normal coronary arteries. Patients with a known cause for heart muscle disease e.g. coronary artery disease, hypertension, valvular and congenital heart disease, as well as infective (myocarditis) and metabolic (thyroid disease) causes were excluded. Patients with a history of excessive alcohol consumption were included since they may have a genetic predisposition to develop DCM.

The patients with DCM were contacted and asked to attend for history taking, physical examination and investigation (Appendix 2, page 81). Past medical history was elicited with regards to possible risk factors for development of DCM, e.g. a viral illness in the three months preceding the diagnosis, a peripartum diagnosis, atopy and cigarette smoking.

HLA typing which included class I (HLA-A, B) and class II (HLA-DR) was performed at the National Blood Transfusion Service, using the serological microtoxicity method. The HLA distribution in patients was compared with a reference population of 9000 normal controls.
DATA GATHERING

CASES

Two-dimensional, M-mode and Doppler echocardiographic examinations were performed in the left lateral position by a single experienced operator on all patients. The machine used was a Hewlett Packard Sonos 1500 with a 2.5 MHz transducer. In keeping with convention, end-systolic and end-diastolic cavity dimensions, wall thickness and fractional shortening were measured by M-mode at a level just caudal to the tip of the mitral valve. End-diastole measurements were taken at the R wave using the leading edge method and ejection fractions were calculated using the cubed assumption (64). Echocardiography was performed by a single experienced technician. M-mode left ventricular measurements were taken from paper and checked by CMcK and HMcC to combat inter-observer bias.

FIRST-DEGREE RELATIVES

To date, 200 first-degree from 56 proband families have attended for echocardiography (Appendix 3, page 82). The disease was considered to be familial if at least one first-degree relative was diagnosed as having DCM, as defined above. If a first-degree relative fulfilled only one of the criteria for the diagnosis of DCM, i.e. a left ventricular ejection below 50% or a left ventricular end-diastolic dimension more than two standard above the mean, they were entered in a "possible" category for further follow-up. Inter-observer bias was counteracted as for probands. Also, those relatives demonstrating left ventricular enlargement underwent repeat echocardiography approximately 6 months later to check for intra-patient and intra-observer variability. Any families with a history of premature sudden death (unexplained at an age of less than 50 years within 4 hours of the onset of symptoms) were also included in the "possible" category.
HISTOLOGICAL EVALUATION OF BIOPSY SPECIMENS

The first 5 relatives demonstrating consistent LVE by echocardiography underwent diagnostic workup including right and left cardiac catheterization, and right ventricular myocardial biopsy (x5). Endomyocardial biopsy specimens were formalin-fixed and wax-embedded. Subsequent histological evaluation included haematoxylin and eosin and TUNEL staining, detection of protease CPP-32 immunoreactivity and HLA-DR expression.

DETECTION OF APOPTOTIC CELLS

The apoptotic cells were TUNEL stained using a Boehringer Mannheim In-Situ Cell Death Detection Kit. Briefly, the slides were deparaffinized, transferred to xylene and then rehydrated through a graded series of alcohols. The slides were rinsed in phosphate buffered saline (PBS, 138 mM sodium chloride, 2.7 mM potassium chloride, Sigma chemical) and treated with proteinase K (25μg/ml, Boehringer Mannheim) for 20 minutes at 37°C, before being rinsed again in PBS. The TUNEL reaction mixture, plus 2% normal swine serum (NSS, Vector laboratories), was applied to the slides. They were then coverslipped and incubated in humidity trays at 37°C for 60 minutes and rinsed in PBS. The AP-converter (diluted to 1:5, in 100mM tris-HCL and 150 mM NaCL at a pH of 7.5) was applied to the slides. They were then incubated for a further 30 minutes at 37°C and rinsed again in PBS. Slides were washed in buffer C (100 mM sodium chloride, 100 mM tris-base, 14.85 mM magnesium chloride, pH 9.5, Sigma).
DETECTION OF APOPTOTIC CELLS

Nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolylphosphate p-toluidine salt (Gibco BRL) color development was performed with a maximum development time of 12 minutes. The slides were counterstained with Nuclear Fast Red (Sigma Chemical), dehydrated and coverslipped. The addition of the NSS reduced non-specific labeling and the dilution of the converter-AP raised the specificity of the staining and reduced background staining. The apoptotic index was calculated as the number of positive-staining cells divided by total number of nucleated cells (%) in ten separate high power (x40) fields per case.

DETECTION OF PROTEASE CPP-32 AND HLA-DR EXPRESSION

After deparaffinization and rehydration, the slides were incubated with 5% normal goat serum (Dako) for 10 minutes at room temperature. The primary antibody (mouse monoclonal anti-CPP-32 antibody (Transduction Laboratories) or rabbit anti-HLA-DR antibody (Zymed)) was diluted in 1% normal goat serum and incubated overnight at 4°C in a humidity chamber. Next, the primary antibody was rinsed off and the biotinylated secondary antisera cocktail (goat anti-mouse or anti-rabbit IgG (Dako)) diluted to 1:400 was incubated on the slides for 30 minutes at room temperature. Streptavidin-horseradish peroxidase (Dako) and 1% normal goat serum were applied, before incubation for 30 minutes at room temperature. The slides were color developed in 3-amino-9-ethylcarbazole substrate solution (Sigma) for 15 minutes at room temperature. Counterstaining in haematoxylin was performed for 30 seconds and coverslip applied.
ALCOHOL CASE-CONTROL STUDY

The relationship between alcohol and DCM was evaluated in a case-control study, cases being identified as described above. Population-based controls were selected by RANSAM (65). This is a computer-based system for drawing random samples from the Electoral Register in Ireland. The system is updated each year from the most recent Register and permits a random selection on a National level. RANSAM has been used to select samples for numerous studies and shown to produce estimates which correspond well on variables including age, sex and occupational status. The response to the questionnaire was expected to be of the order of 30% and a ratio of 2:1 controls to cases was deemed necessary for statistical power. Therefore, a sample size of 600 was selected.

Patients and controls had a questionnaire filled in regarding their alcohol intake (Appendices 4+5, pages 83-84). The questionnaire detailed the total duration of alcohol intake in years and the average weekly consumption in units. It also accounted for any change or cessation in alcohol intake over the years. From these replies, the total lifetime consumption of alcohol in units was estimated. Reported alcohol consumption was compared with the validated CAGE questionnaire with a high sensitivity, specificity and negative predictive value for excess alcohol intake and alcoholism (66).
ALCOHOL CASE-CONTROL STUDY

The questionnaire was administered to patients at their clinic visit. For practical purposes the controls were posted the same questionnaire as for the DCM patients regarding their alcohol intake, to be completed and returned anonymously in a stamp-addressed envelope. For statistical analysis, patients and controls were divided into three basic categories based on their alcohol consumption:

1 ) those that drank more than the recommended weekly intake,
   i.e. 21 units for men and 14 units for women;

2 ) those that were CAGE positive for alcohol abuse,
   i.e. answered yes to at least two of the four CAGE questions (66);

3 ) average total lifetime consumption in units (ATLC).
DATA ANALYSIS
The multiple objectives of this study necessitated that different types of study design be employed. The HLA-typing in probands and first degree relatives with DCM was a cohort analysis defining the frequency of specific HLA regions in these patients. This could confirm whether certain HLA types were associated with DCM and whether these types were also predictors of DCM in first degree relatives.

To examine the relationship of alcohol to dilated cardiomyopathy required a case-control methodology. One could thus determine whether DCM was associated with excess alcohol consumption from their alcohol intake by questionnaire as compared with controls.

The data was entered from pre-coded datasheets (Appendices 1-5, pages 80-84) onto the mainframe computer for statistical analysis using SAS. Statistics employed include chi square and Mann Whitney U.
RESULTS
GENERAL RESULTS

One hundred proband patients with DCM satisfying the usual exclusions and with angiographically proven coronary arteries attended the special cardiomyopathy clinic. The clinical characteristics of the probands are summarised in Table 1. Their age range (mean) was 19-77 (53.5) years, 74 (74%) were male and 34 (34%) were current smokers. With regards to possible risk factors for DCM, 20 (20%) reported a viral illness within three months of the diagnosis of DCM, 18 (18%) had a personal history of atopy and 2 (2%) were peripartum at the time of the diagnosis.

As detailed below, 56 of the 100 proband families were screened for DCM and 25 of these 56 families demonstrated a familial tendency for the disease. The baseline characteristics of these probands were compared with the 31 probands whose disease was classified as non-familial after screening. Those patients with a familial tendency to the disease showed a stronger male preponderance than the non-familial cases (80% vs. 71%; P=0.72). However, the familial cases were no more likely to report the occurrence of putative risk factors (viral illness, atopy, pregnancy) for the development of DCM (Table 2).
Table 1

Clinical characteristics of DCM probands (n=100)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>54 (19-77) years</td>
</tr>
<tr>
<td>Male</td>
<td>73%</td>
</tr>
<tr>
<td>NYHA</td>
<td>3.2±0.8</td>
</tr>
<tr>
<td>LVEF</td>
<td>32±8%</td>
</tr>
<tr>
<td>LVIDD</td>
<td>68±18 mm</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>90%</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>40%</td>
</tr>
<tr>
<td>Digoxin</td>
<td>75%</td>
</tr>
<tr>
<td>Warfarin</td>
<td>85%</td>
</tr>
<tr>
<td>Nitrate</td>
<td>20%</td>
</tr>
</tbody>
</table>

NYHA=New York Heart Association functional class; LVEF=left ventricular ejection fraction; LVIDD=left ventricular internal diastolic diameter
Table 2

Baseline characteristics for the total DCM patient population compared with familial and non-familial subgroups

<table>
<thead>
<tr>
<th></th>
<th>Total population (n=100)</th>
<th>Familial (n=25)</th>
<th>Non-familial (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>53.5</td>
<td>55</td>
<td>53</td>
</tr>
<tr>
<td>Male</td>
<td>74 (74%)</td>
<td>20 (80%)</td>
<td>22 (71%)</td>
</tr>
<tr>
<td>Smokers</td>
<td>34 (34%)</td>
<td>8 (32%)</td>
<td>11 (35%)</td>
</tr>
<tr>
<td>Viral illness</td>
<td>20 (20%)</td>
<td>4 (16%)</td>
<td>7 (23%)</td>
</tr>
<tr>
<td>Atopy</td>
<td>18 (18%)</td>
<td>4 (16%)</td>
<td>6 (19%)</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>2 (2%)</td>
<td>0 (0%)</td>
<td>1 (3%)</td>
</tr>
</tbody>
</table>
FAMILIAL PREVALENCE

A total of 270 first-degree relatives from 75 of the 100 patient families were potentially available for screening. Twenty-five of the 100 patients either had no family members available for screening or did not wish their relatives to be contacted. So far 200 out of a possible total of 270 (74%) first-degree relatives from 56 out of the 75 (75%) proband families have been screened by echocardiography. Those not screened include relatives still to be contacted, non-attenders and those residing abroad.

Of the 56 families screened, 5 (9%) had a first-degree relative already diagnosed as having DCM and a further 9 (16%) had at least one relative who fulfilled both echocardiographic criteria for DCM, i.e. a left ventricular ejection fraction less than 50% and a left ventricular end-diastolic dimension more than two standard deviations above the mean. Thus, the definite familial occurrence of DCM in this patient group was 14/56 (25%).

In a further 9 families (16%), at least one relative had a possible diagnosis of DCM, i.e. only one of the two echocardiographic criteria was fulfilled. Six families (11%) gave a history of premature sudden death, i.e. unexplained at an age of less than 50 years and within 4 hours of the onset of symptoms, in a first-degree relative and these were recorded as possible cases of DCM. Thus, an additional 15 (9+6)/56 (27%) of this patient group demonstrated a familial tendency to DCM.

Four families had more than one first-degree relative with a definite or possible diagnosis of DCM. Therefore, the familial prevalence of DCM in this patient group was definite in 14/56 proband families (25%) and possible in a total of 25/56 proband families (45%) (Tables 3+4).
Table 3

Familial prevalence of DCM in 56 proband families screened

Definite DCM: already diagnosed 5 (9%)
    both ECHO criteria* 9 (16%)

Possible DCM: one of ECHO criteria 9 (16%)
    sudden death 6 (11%)

Definite familial DCM = 14/56 (25%)
Possible familial DCM = 25/56 (45%)

* LVEF < 50% and LVIDD > 2sd above mean
Table 4

Familial prevalence of DCM in screened first-degree relatives

Number of relatives / proband family available and screened

<table>
<thead>
<tr>
<th></th>
<th>Available</th>
<th>Screened</th>
<th>Familial tendency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1° relatives</td>
<td>270</td>
<td>200 (74%)</td>
<td>22 (11%)</td>
</tr>
<tr>
<td>Proband families</td>
<td>75</td>
<td>56 (75%)</td>
<td>25 (45%)</td>
</tr>
</tbody>
</table>
HLA-DISTRIBUTION

The HLA-types in the 100 probands were compared with a reference population of 9000 normal controls from the National Transfusion Service. All halotypes were checked and significant differences between patients and controls were restricted to the DR4 subtype.

As compared to controls, the total group of patients with DCM showed no significant difference in the frequency of the DR4 halotype (32% vs. 39%; P=0.64). However, when only the DCM probands with a familial tendency were evaluated, the DR4 subtype was significantly more common in comparison with the whole patient population (68% vs. 39%; P<0.05) and controls (68% vs. 32%; P<0.01) (Table 5). Also, within the study group, the proportion of patients with familial DCM and HLA-DR4 (17/25 (68%)) was significantly higher than the proportion of patients with non-familial DCM and HLA-DR4 (10/31 (32%)) (P<0.05).
Table 5

**HLA distribution in DCM patients as compared with controls**

<table>
<thead>
<tr>
<th>DR4 frequency</th>
<th>Controls</th>
<th>DCM probands</th>
<th>DCM probands*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2880 / 9000 (32%)</td>
<td>39 / 100 (39%)</td>
<td>non-familial 10 / 31 (32%)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>familial 17 / 25 (68%)</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

* 56 proband families screened by echocardiography
HISTOLOGICAL EVALUATION OF BIOPSY SPECIMENS

Diagnostic workup in the first 5 relatives included cardiac catheterization and right ventricular biopsy (x5). In all 5 cases, coronary arteries were angiographically normal, as were left and right heart pressures. Histological evaluation of the right ventricular biopsy specimens revealed mild to moderate myocyte hypertrophy with only mild or no interstitial fibrosis. There was no evidence of inflammation (Figure 1a). The apoptotic index ranged from 17-30%, with a mean for the 5 cases of 25% (Table 6). TUNEL positive cells were more prominent in the interstitium than the myocardium (Figure 1b). All five biopsy specimens showed high levels of CPP-32 immunoreactivity (Figure 1c). HLA-DR expression was also detected in all five cases and, as for the evidence of apoptosis, was more prominent in the interstitium than the myocardium (Figure 1d).
Figure 1.
Representative samples of right ventricular biopsy specimens (x40) from the five asymptomatic relative with LVE: (a) stained with H+E, showing mild-to-moderate myocyte hypertrophy, mild-to-no interstitial fibrosis and no evidence of inflammation; (b) TUNEL staining, showing apoptotic activity most marked in the interstitium; (c) CPP-32 immunostaining, showing high levels of activity throughout the biopsy specimen; (d) HLA-DR immunostaining, showing intense expression, also most marked in the interstitium.
Table 6

The apoptotic index calculated as the number of TUNEL positive cells divided by total number of nucleated cells (%) in ten separate high power (x40) fields per case

<table>
<thead>
<tr>
<th>Case</th>
<th>Total nucleated cells</th>
<th>TUNEL positive cells</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54±19</td>
<td>14±4</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>71±13</td>
<td>21±7</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>78±12</td>
<td>11±4</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>66±14</td>
<td>19±8</td>
<td>29</td>
</tr>
<tr>
<td>5</td>
<td>61±10</td>
<td>15±6</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>66±9</td>
<td>16±4</td>
<td>25±6</td>
</tr>
</tbody>
</table>

(Mean ± standard deviation)
ALCOHOL CONSUMPTION

Reported alcohol consumption recorded at interview in the 100 patients with DCM was compared with that of the 211/600 (35%) of randomly selected, population-based controls who returned their anonymous questionnaire by post.

Significantly more of the 100 patients with DCM than the 211 controls drank greater than the recommended weekly intake of alcohol (40% vs. 24%; P<0.01) and were alcohol abusers according to the CAGE questionnaire (27% vs. 16%; P<0.05). The age range in cases and controls was similar but the gender distribution was significantly different. When stratified by gender, the above differences between cases and controls with regards to alcohol consumption no longer reached statistical significance (Table 8). For males, the percentage of cases versus controls drinking alcohol above recommended limits was 40% vs. 30% (P=0.11) and the percentage of alcohol abusers was 33% vs. 22% (P=0.18). For females, the percentage of cases and controls drinking alcohol above recommended limits was 30% vs. 19% (P=0.35) and the percentage of alcohol abusers was 11% vs. 10% (P=0.75).

The average total lifetime consumption (ATLC) measured in units of alcohol was also significantly greater in cases than controls (31,200 vs. 7,904; P<0.01). This difference remained significant after gender stratification. The ATLC in male cases was 51,126 as compared to 10,311 in male controls (P<0.01). Female ATLC was 14,262 in cases and 6,241 in controls (P<0.05).

The rates of alcohol consumption above recommended weekly limits were comparable for patients with familial (10/45; 40%) and non-familial (13/31; 42%) DCM. All the familial cases whose alcohol intake was above recommended limits were also alcohol abusers according to the CAGE questionnaire, making alcohol abuse non-significantly more common in this subgroup of patients (40% vs. 23%; P=0.26) (Table 9).
Table 7

Prevalence of alcohol abuse in DCM cases v Population-based controls

<table>
<thead>
<tr>
<th></th>
<th>Probands (n=100)</th>
<th>Controls (n=211)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>54</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>19-77</td>
<td>18-82</td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol consumption</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; weekly limit</td>
<td>40 (40%)</td>
<td>50 (24%)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Cage + ve</td>
<td>27 (27%)</td>
<td>33 (16%)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>ATLC</td>
<td>31,200</td>
<td>7,904</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

ATLC=average total lifetime consumption (units)
Table 8
Prevalence of alcohol abuse in DCM cases vs Population-based controls, stratified by gender

<table>
<thead>
<tr>
<th></th>
<th>Probands (n=100)</th>
<th>Controls (n=211)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; weekly limit</td>
<td>73 (73%)</td>
<td>86 (41%)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Cage + ve</td>
<td>32/73 (40%)</td>
<td>26/86 (30%)</td>
<td>0.18</td>
</tr>
<tr>
<td>ATLC</td>
<td>51,126</td>
<td>10,311</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; weekly limit</td>
<td>27 (27%)</td>
<td>125 (59%)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Cage + ve</td>
<td>8/27 (30%)</td>
<td>24/125 (19%)</td>
<td>0.35</td>
</tr>
<tr>
<td>ATLC</td>
<td>14,262</td>
<td>6,241</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

ATLC=average total lifetime consumption (units)
Table 9

Prevalence of alcohol abuse in familial v non-familial DCM

<table>
<thead>
<tr>
<th></th>
<th>Familial (n=25)</th>
<th>Non-familial (n=31)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; weekly limit</td>
<td>10 (40%)</td>
<td>13 (42%)</td>
<td>NS</td>
</tr>
<tr>
<td>Cage + ve</td>
<td>10 (40%)</td>
<td>7 (23%)</td>
<td>NS</td>
</tr>
</tbody>
</table>
SCREEN-DETECTED DCM

With regards to the 22 asymptomatic first-degree relatives with left ventricular enlargement on echocardiographic screening, 18 (82%) were male and the average age was 40 (range 17-60) years, i.e. younger than the proband population but with a similar male preponderance. The ECG was abnormal in only 4 (18%) of these cases, with two patients in atrial fibrillation and two showing non-specific ST changes.

As was shown for the probands with familial DCM, the screen-detected relatives were significantly more likely than controls (64% vs. 32%; P<0.01) and the total patient group (64% vs. 39%; P<0.05) to have the HLA-DR4 subtype. Compared to population-based controls, they were non-significantly more likely to be alcohol abusers according to the CAGE questionnaire (27% vs. 16%; P=0.27).
DISCUSSION
FAMILIAL PREVALENCE

In this study of patients with DCM, 25% of cases were definitely familial in nature and a total of 45% showed a familial tendency. Somewhat more surprising is the 11% frequency of premature sudden death. The majority of patients with "idiopathic" life-threatening ventricular arrhythmias would appear to have abnormal myocardium on biopsy (67) and these findings may indicate an early form of DCM which is too subtle to be detected by usual cardiac investigations. These results are potentially an underestimate of the prevalence of familial DCM as not all of the relatives were screened.

It seems likely that left ventricular enlargement, as measured by echocardiography, in asymptomatic first-degree relatives of probands with DCM is an early marker of the disease. A recent report from the Framingham Heart Study demonstrated that an increase in echocardiographic left ventricular dimension in subjects without a history of myocardial infarction or heart failure is a risk factor for subsequent congestive heart failure (68). The risk-factor-adjusted hazard ratios for congestive heart failure were 1.47 and 1.43 per increment of one standard deviation in left ventricular end-diastolic dimension. The study, involving 4744 men and women with a mean age of 50, identified left ventricular dimensions as the most important echocardiographic predictor of congestive heart failure. Thus, it appears that overt ventricular systolic dysfunction is preceded by an increase in cavity dimensions, with early and asymptomatic left ventricular dilatation being a subclinical compensatory response to this dysfunction. The predictive value of left ventricular enlargement in relatives of patients with DCM would be expected to be much higher than that estimated from this large, community-based sample.
FAMILIAL PREVALENCE

One of the earliest discourses on "the non-coronary cardiomyopathies" by Brigden in 1957 (69) found "strong evidence for familial heart disease" in 7/50 (14%) of cases of isolated heart muscle disease. Numerous studies dating from the 1980's have re-looked at the familial incidence of DCM. The initial work on familial DCM was retrospective and involved only symptomatic or previously diagnosed first-degree relatives. The disease was considered familial if at least one first-degree relative was diagnosed as having DCM and the percentage familial incidence calculated as that of the total number of probands with one or more affected relatives. The familial incidence calculated in these studies was generally low, varying between 2-9% (14,70-74).

In 1992 Michels et al reported a prospective study from the United States, in which 315 first-degree relatives of 59 patients with DCM were screened by echocardiography (54). The relatives were diagnosed as having DCM if their left ventricular ejection fraction was less than 50% and their left ventricular internal dimension in diastolic was greater than 56 mm. By these criteria 18 relatives from 12 families were shown to have DCM, giving a familial incidence of 12/59 (20%). The majority of the relatives with DCM were asymptomatic, only 3 having been previously diagnosed. A study by Zachara et al in 1993 retrospectively calculated, from previously diagnosed first-degree relatives, a familial incidence of 14 cases from 105 probands (13%) with DCM (75). They prospectively screened 109 asymptomatic relatives of these 14 cases and identified 23 (21%) potential cases of DCM based on the same echocardiographic criteria as above. A prospective study in the United Kingdom found a familial tendency in 10 of 40 (25%) families of DCM patients screened (55).
FAMILIAL PREVALENCE

Pedigree analysis of the potential carriers of DCM in the latter two studies revealed a pattern of inheritance consistent with autosomal dominant with incomplete penetrance (55,75). It is difficult to comment on possible modes of inheritance in the present study as only first-degree relatives were screened. However, those families with more than one affected member showed a pattern in keeping with an autosomal dominant pattern, as previously noted (76.77). However, others have recorded a recessive or more commonly mixed dominant and recessive modes of inheritance in familial DCM (54,71,74,78,79). A large family with X-linked inheritance of DCM has also been described (80). All this tends to suggest that multiple genetic causes may be responsible for disease transmission and lead to a final common phenotype in familial DCM.

There have been two separate reports of inherited dilated cardiomyopathy associated with deletion and mutation of mitochondria (81,82). Such mutations lead to impaired oxidative phosphorylation, limit the cellular energy available to cardiac muscle during exercise and, thus, cause myocardial degeneration (83). Further to this, characteristic electron-microscopic changes have been demonstrated in myocardial mitochondria from patients with familial cardiomyopathy (84). Mutation in the muscle promoter region of the dystrophin gene has also been linked to an X-linked form of familial DCM (85).
FAMILIAL PREVALENCE

The definite familial prevalence of DCM in this study is similar to those reported from the USA (86) and the UK (55), showing a 25% occurrence of familial DCM (Table 10). Obviously, some relatives with a normal echo at one-off screening may later develop DCM. Only an ongoing prospective study screening all available first-degree relatives, on a regular basis, would determine the true prevalence of familial DCM. When sufficient asymptomatic, screen-detected relatives with DCM are identified, randomised trials of ACE inhibition and/or immunotherapy to slow disease progression should be initiated.
Table 10

**Familial DCM: prospective screening studies (USA, UK, IRL)**

<table>
<thead>
<tr>
<th></th>
<th>Probands</th>
<th>Relatives</th>
<th>Familial DCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goerrs  1995 (86)</td>
<td>95</td>
<td>457</td>
<td>23 families (24%)</td>
</tr>
<tr>
<td>Keeling 1995 (55)</td>
<td>40</td>
<td>236</td>
<td>10 families (25%)</td>
</tr>
<tr>
<td>Present study</td>
<td>56</td>
<td>200</td>
<td>14 families (25%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>191</strong></td>
<td><strong>893</strong></td>
<td><strong>47 (25%)</strong></td>
</tr>
</tbody>
</table>
HLA-DISTRIBUTION

In this study of patients with DCM, the HLA-type DR4 was significantly more common in patients with familial DCM than in those with non-familial DCM. More importantly, HLA-DR4 was associated with over two-thirds (68%) of the families at risk from DCM. DR4 has been associated with other autoimmune diseases. The present finding supports an HLA-linked predisposition to the development of familial DCM. Families of DCM patients with the DR4 subtype are at particular risk of developing the disease and should probably be screened regularly by echocardiography, as well as counselled regarding potential lifestyle and pharmacological interventions which might alter the natural history of the disease. It is of interest to note that patients undergoing heart transplantation have less cellular rejection and increased long-term survival if they are HLA-DR matched with the donor heart (87). This matching process may be of particular importance in patients with familial DCM being considered for transplantation.

The commonest mechanism cited with regard to the aetiology of DCM, which relates to the diseases' apparent familial predisposition, is an autoimmune (HLA-linked) predisposition initiated by an environmental trigger, e.g. viral illness or alcohol. There are already many well known associations between diseases with autoimmune features and the human leucocyte histocompatibility (HLA) antigens. HLA links have been made with diseases of presumed viral-immune origin, e.g. rheumatoid arthritis and lupus erythematosis. The major histocompatibility complex (MHC) plays an important role in presenting antigens such as a virus to the immune system. Increased expression of the MHC antigens occurs in tissue undergoing autoimmune destruction.
HLA-DISTRIBUTION

Anderson et al in 1984 were the first to report a HLA link in patients with DCM (50). They found that the HLA types DR4 and B27 were commoner in patients than controls. The haplotype frequency of B27 was 14.5% in patients with DCM as compared with 3.3% in controls (P=0.001) and that of DR4 was 54% in DCM and 32% in controls (P=0.02). The HLA-DR4 association with DCM has since been confirmed by many other studies (88-92) and refuted by one (93). The latter was a retrospective look at a particular subset of patients who underwent cardiac transplantation.

Following on from their original retrospective study linking HLA-DR4 with DCM, Anderson’s group validated the association in a new set of patients identified prospectively and in a meta-analysis of five studies comparing the frequency of HLA-DR antigens in DCM patients (94). They found a DR4 frequency of 20/41 (49%) in patients versus 11/53 (21%) in controls (P<0.005). They relative risk for DCM with the DR4 subtype was 2.8, with the presence of this haplotype estimated as attributable to one-third of all DCM cases. The meta-analysis also indicated a significant increase in the frequency of HLA-DR4 in patients with DCM, the overall odds ratio for having a DR4 allele being 2.06. These results supported the belief that predisposing genetic factors linked to immunoregulation, in conjunction with one of the putative environmental triggers, may lead to the development of DCM, at least in a subset of patients. The present study adds considerable weight to this hypothesis by linking the DR4 association, as a surrogate for autoimmune aetiology, specifically with the subset of DCM patients with a familial tendency to the disease.
HLA-DISTRIBUTION

Normal adult cardiac myocytes express only low levels of MHC class I and no class II antigens. Human myocarditis is, however, associated with abnormal myocardial expression of both class I and II MHC antigens (95). This supports a specific immune-mediated cardiomyopathy in these patients. HLA-DR expression has also been demonstrated in myocardial biopsy specimens from patients with DCM (96). Eighty-three of 342 patients (24%) in this study had HLA-DR expression on the vascular endothelium. The relative odds of myocardial ischaemia in the biopsy specimens, as measured under electron microscopy, by the preferential loss of actin over myosin, in DR positive as compared to DR negative samples were 7.8 (P=0.01). Thus, there appears to be association between HLA-DR expression and ongoing myocardial ischaemia in patients with DCM, the location of DR positivity on the vascular endothelium suggesting that this ischaemia is secondary to microvascular injury. Thus, exposure to an infectious (viral) or toxic (alcohol) agent may lead to altered HLA-DR expression in myocardial vascular endothelium. These DR positive cells then, potentially, initiate an autoimmune response with resulting microvascular injury and myocardial ischaemia, in a genetically susceptible individual.

The search for humoral markers of autoimmunity in patients with DCM has been ongoing for the last 20 years. The function of T-cells is to recognise antigens as foreign, in conjunction with the MHC. Natural killer (NK) leucocytes are known to be important in host immune surveillance and mediate resistance to viral infections. NK cell deficiency has been shown to be a disease marker for DCM and this subgroup of patients with DCM was distinguished from others by an increase in specific HLA halotypes (97).
HLA-DISTRIBUTION

An increase in T-helper/T-suppressor cell ratios has been variably demonstrated in DCM (98) and is a typical feature of autoimmune disease. Nearly half of patients with DCM have evidence for a chronic autoimmune process by immunohistochemical analysis of their cardiac biopsies (99).

Circulating organ-specific cardiac autoantibodies have been demonstrated in some patients with DCM and been more frequently detected in those patients with recent onset of disease (51,52). This also suggests a cell-mediated autoimmune mechanism. The reason for the presence of serological markers in only some patients with DCM is postulated to be due to either different aetiologies or a reduction in antibody level with disease progression.

The evidence suggests that DCM can be associated with the host's immune response and the genetic factors that control this response. It is conceivable that familial DCM is the subgroup of patients with the disease with, an inherited, defect in their immune response. This defect may then manifest itself as an autoantibody directed against the myocardium following an environmental stimulus, e.g. a viral illness. This theory is lent support by correlations between specific T-cell gene alleles (100) and HLA-types (101) and the presence of autoantibodies in familial cardiomyopathy. Organ-specific cardiac antibodies have also been detected in symptom-free relatives of patients with DCM (52). Once again implying that the body's autoimmune response, under the control of specific MHC genes manifested by HLA types, is important in the aetiology of familial DCM.
HLA-DISTRIBUTION

Halotype screening may help to elucidate if a genetic predisposition can be identified in certain family members and, hence, point to a possible pathogenesis for familial DCM. Hence, those family members with specific MHC genes could be counselled, advised and followed-up with regard to the possibility of development of DCM. The presence of circulating autoantibodies does not adequately distinguish between familial and non-familial DCM (52,102).

If family members at risk from DCM could be identified, early treatment in the form of ACE inhibition and/or immunotherapy could be tested in a randomised, controlled with the end-points of ventricular function and mortality (103). The search for a marker to predict families at risk of developing DCM has so far been in vain. Perhaps HLA-typing could act as a serum marker to identify families most likely to benefit from periodic screening with echocardiography and early treatment when left ventricular dysfunction is detected.

As mentioned already, some relatives with a normal echo at one-off screening may later develop DCM. Only an ongoing prospective study screening all available first-degree relatives, on a regular basis, would determine the true prevalence of familial DCM and the usefulness of markers such HLA-typing in identifying those families at greatest risk. When sufficient asymptomatic, screen-detected relatives with DCM are identified, randomised trials of ACE inhibition and/or immunotherapy to slow disease progression should be initiated.
HISTOLOGICAL EVALUATION OF BIOPSY SPECIMENS

Asymptomatic relatives with LVE have active disease which does not appear to be inflammatory in nature but is associated with a high apoptotic index. Apoptosis, as measured by TUNEL staining and CPP-32 activity, was more prominent in the interstitium than the myocardium. The early disease activity was associated with abnormal HLA-DR expression, again more pronounced within the interstitium. Little or no fibrosis was evident in the biopsy specimens.

Active myocardial damage without inflammation has previously been described in symptomatic DCM patients undergoing cardiac transplantation (104). Apoptosis has been demonstrated in end-stage, ischaemic and non-ischaemic heart, failure. In this regard, it may be merely a non-specific marker of slow myocyte loss. More than one method of showing apoptosis is now required to obtain strong evidence of apoptosis in any human study (105). Narula et al showed evidence of DNA fragmentation and DNA laddering in explanted hearts of four patients with idiopathic dilated cardiomyopathy. Only rare apoptosis was observed in the hearts of four persons who suffered a non-cardiac, sudden death (57). In contrast to Narula et al, who detected apoptosis mainly in myocytes, we found apoptosis to be more frequent in the interstitium. It is possible that early DCM is primarily an interstitial disease, with alteration of the myocardial interstitium being a primary event in the transition from compensated to symptomatic heart failure (106).
Apoptosis has also been shown in 6 of 8 patients with arrhythmogenic right ventricular, by TUNEL staining and detection of protease CPP-32. The latter protease is important for the induction of apoptosis in mammalian cells (107). Apoptosis was prominent in areas with viable myocardium and less frequent in areas replaced by fat and fibrous tissue (108). Thus, suggesting that the loss of myocardium through apoptosis may be a primary process, preceding fibrosis in the absence of an inflammatory response. High levels CPP-32 immunoreactivity were present in right ventricular myocytes of the six patients. The protease was undetectable or barely detectable in four control subjects.

Various stimuli may produce TUNEL positive cells within the myocardium. Myocardial stretch can initiate apoptosis and subsequent impairment of muscle performance, which can be prevented by the addition of nitric oxide (NO) releasing drugs (109). The countervailing balance between vasoactive substances appears to control cell growth and cell death (110). Thus, by inhibiting vasoconstrictor effects, the homeostatic balance shifts in favor of NO which may protect the myocardium from overstretching and apoptotic cell death.

Activation of apoptosis may also be inhibited by expression of the proto-oncogene bcl2. Olivetti et al examined the hearts of 36 patients with end-stage heart failure who underwent transplantation. They observed a significant increase in apoptosis within the myocardium by TUNEL staining and DNA gel electrophoresis. The percentage of myocytes that labeled positively for bcl2 was
HISTOLOGICAL EVALUATION OF BIOPSY SPECIMENS

1.8 times higher in patients with cardiac failure, as compared to normal hearts (56). This suggests that compensatory mechanisms may be initiated in the failing heart to limit apoptotic cell death and maintain myocardial function.

The presence of p53 in ventricular myocyte cell culture has been shown to cause a significant increase in transcription of the bax gene, which opposes bcl2 and, hence, promotes apoptosis. The expression of bcl-2 in these cultured myocytes prevented p53-induced apoptosis. This implies that bcl-2 is an antiapoptotic agent which may have clinical potential in the overloaded heart (111).

The cytoplasmic region of the Fas protein is thought to transduce the intracellular signals required to initiate apoptosis. Patients with the inherited autoimmune disorder, Canale-Smith syndrome, have mutations in the Fas gene (112). Such mutations may lead to susceptibility to environmental agents in autoimmune disorders, for example, viral infection which can activate and induce programmed cell death in T cells by way of antigen-receptor signaling involving major histocompatibility complex (MHC) class II antigens (113,114).

MHC class II antigens are expressed mainly on antigen presenting cells of the immune system and increased expression is present in tissue undergoing autoimmune injury. Normal myocardium has no detectable levels of MHC class (including HLA-DR) antigens. Such induction has been demonstrated in the myocardium and microvascular endothelium of patients with active myocarditis,
HISTOLOGICAL EVALUATION OF BIOPSY SPECIMENS

with abnormal MHC class I and II antigen expression present in 11/13 patients and 1/8 controls (88).

The autoimmune hypothesis regarding the etiology of familial DCM is supported by the increased frequency of HLA-DR4 and the presence of cardiac autoantibodies in some patients with DCM (50-54,88,90,94,95). We have shown that the DR4 subtype is significantly more common in familial cases, when compared to non-familial cases or controls. Thus, there appears to be an HLA linked predisposition to the development of familial DCM. The expression of HLA-DR antigen within the myocardium of all the cases in the present study adds support to the autoimmune hypothesis for familial DCM.

This study shows for the first time that asymptomatic relatives of DCM patients who demonstrate LVE on echocardiographic screening have ongoing disease activity. This was evident by mild to moderate myocyte hypertrophy, with little or no fibrosis and no inflammation in the biopsy samples examined. Apoptotic activity, as detected by TUNEL activity and CPP-32 immunoreactivity, and abnormal HLA-DR expression were present in all cases. Interestingly, these findings were more prominent within the interstitium than the myocardial cells. Anti-apoptotic therapy may be beneficial at this pre-clinical stage of the disease process.
HISTOLOGICAL EVALUATION OF BIOPSY SPECIMENS

LIMITATIONS

The histological analysis was performed on a small number of right ventricular biopsy specimens and the interesting observations described must therefore be viewed as provocative but preliminary. As the previous work cited in the discussion has shown little or no apoptosis or HLA-DR expression in normal myocardium, we accepted this as historical control evidence rather than analysing biopsy specimens obtained from apparently normal myocardium.
ALCOHOL CONSUMPTION

This is the first reported case-control study to compare the alcohol intake of a group of DCM patients with that of randomly selected, population-based controls. Both consumption of alcohol above recommended weekly limits (40% vs. 24%; P<0.01) and alcohol abuse as defined by the CAGE questionnaire (27% vs. 16%; P<0.05) were significantly more common in patients with DCM than controls. Those patients with familial DCM were non-significantly more likely than non-familial probands to be alcohol abusers according to the CAGE questionnaire (40% vs. 23%; P=0.26). These results support the evidence for alcohol consumption as an aetiological agent in a minority (20-40%) of patients with dilated cardiomyopathy. The CAGE questionnaires has a higher predictive value for identifying alcohol abuse than self-reporting or standard biochemical markers and it identified 16% of the controls as being alcoholics. Obviously, not all of these subjects develop dilated cardiomyopathy. The definition of alcoholic heart muscle disease, hence, over simplifies the relationship between alcohol and heart failure. To develop alcohol-related dilated cardiomyopathy one must presumably either be unusually sensitive to its toxic effect and/or develop an immune response which perpetuates the initial myocardial insult.

Two previous French studies have also demonstrated a greater alcohol consumption in patients with DCM compared to controls. However, the controls were patients attending hospital with either surgical complaints (62) or coronary disease (63). They were, therefore, neither randomly selected nor population-based, and 50% of them were defined as heavy alcohol consumers. Also, the numbers of patients and controls in the present case-control study achieved a higher statistical power than either of the above.
ALCOHOL CONSUMPTION

Assessing alcohol intake, particularly in alcoholics, is notoriously difficult. The well known CAGE questionnaire gives a sensitivity of 81-91% and specificity of 77-89% for discriminating between alcoholic and non-alcoholic patients (66). The negative predictive value of the CAGE questionnaire in the detection of excessive drinking and alcoholism is 98%. This compares favourably to the negative predictive value of the biochemical markers gamma-GT and MCV which are 86% and 84% respectively (115). The most important requirement of a useful screening test for alcoholism is that it should detect the majority of cases. In this regard the CAGE questionnaire performs better than biochemical markers of alcohol intake and will identify nine out of ten alcoholics.

Chronic alcoholism is associated with symptomatic left ventricular dysfunction in up to one-third of cases (116), with up to two-thirds of asymptomatic chronic alcoholics demonstrating significant cardiac abnormalities on echocardiography (117). Large doses of alcohol are, however, required to demonstrate impaired ventricular function in chronic alcoholics with no preceding symptoms of cardiac disease (118). Reversibility of this association has also been demonstrated, with improvement in ventricular function being observed in those who reduce their alcohol intake or abstain (58,119,120). Although, abstinence does not guarantee a clinical improvement and myocardial damage may continue to progress.

Alcohol is negatively inotropic, arrhythmogenic and can cause histological change in the myocardium in the short term (121). This toxic effect appears to be dose-related (116) and causative toxins include both ethanol and acetaldehyde (122,123). Acetaldehyde is a metabolite of ethanol and inhibits myocardial protein synthesis (123).
Both ethanol and acetaldehyde inhibit the association of actin and myosin, thus reducing the contractility of human muscle, in vitro (124). The mechanism of this inhibition seems to be related both to the binding and uptake of calcium by the sarcoplasmic reticulum (125) and ATPase activity (126). Another possible mechanism by which alcohol may cause dilated cardiomyopathy is through sympathetic overstimulation, with accompanying tachycardia and hypertension (116,127). Although none of the patients in the present gave a history of hypertension, one cannot fully exclude the possibility of "burnt-out" hypertensive heart disease.

The evidence that acute administration of alcohol impairs cardiac function does not allow the leap of faith to causation in chronic heart failure. There is still no definitive proof that excess alcohol intake leads to congestive heart failure. This would require the demonstration of a consistent and progressive damaging effect of alcohol on myocardial function (128). The evidence linking chronic alcohol abuse with cardiomyopathy remains, for the most part, conflicting and circumstantial. The term alcoholic heart muscle disease (AHMD) has been coined to include those patients with dilated cardiomyopathy who also consume an arbitrarily set chronic excess amount of alcohol. Their alcohol intake is the only clinical distinction between these patients and those with a diagnosis of DCM. There is no proof that excess alcohol is the cause of ventricular dysfunction in those patients labeled with a diagnosis of AHMD. Similarly, there is no proof that alcohol is not involved in the pathogenesis of DCM in those patients whose intake is not defined as excessive.
ALCOHOL CONSUMPTION

A myocardial biopsy study of 38 patients with dilated cardiomyopathy found increased myocardial enzyme activity in the 18 (47%) defined as heavy drinkers (reported total lifetime consumption greater than 25,000 units) compared with the rest of the group (129). However, there were no significant differences in ejection fraction between the two groups. They concluded that, although a threshold dose of alcohol for heart muscle damage cannot be determined, the results are “supportive” of an alcoholic aetiology in patients with dilated cardiomyopathy. The lack of correlation between ejection fraction and alcohol intake or enzyme activity does, however, somewhat negate this conclusion. By the same definitions, 7/21 (33%) of heavy drinkers with dilated cardiomyopathy had circulating antibodies against cardiac protein-acetaldehyde adducts compared with 2/64 (3%) controls (130). The controls were somewhat of a mixed bag of patients: 32 with cardiac failure and an alcohol intake of less than 21 units/week, 22 with dilated cardiomyopathy and undefined alcohol intake, and 10 with coronary disease. The result suggests that a minority of heavy drinkers with dilated cardiomyopathy develop immunogenic cardiac antibodies, which may not only act as a marker of disease activity but also participate in ongoing heart muscle damage.

Obrador et al used the heart-to-lung ratio of monoclonal antimyosin antibodies as a marker of myocardial damage in 117 patients with DCM being considered for transplantation (131). In this study, 29 (25%) of the patients had a reported total lifetime alcohol consumption of greater than 36,000 units and were defined as having “alcoholic dilated cardiomyopathy”. This subgroup showed a significantly less frequent and less intense antibody uptake than the rest of the patients with dilated cardiomyopathy.
ALCOHOL CONSUMPTION

Once more, total alcohol intake did not correlate with the echocardiographic degree of left ventricular dilatation. However, antibody uptake was significantly higher in present than previous drinkers and abstention was associated with an increase in ejection fraction. The results suggest that continued alcohol intake causes chronic left ventricular impairment which is potentially reversible upon alcohol withdrawal. The antibody response, as for protein-acetaldehyde adducts, may be a marker of disease activity and/or involved in ongoing immune-mediated myocardial damage.

Defining patients with dilated cardiomyopathy as having an alcoholic aetiology on the basis of a cut-off total lifetime intake, whether it be 25,000 or 36,000 units, is convenient for the purposes of a study but an over-simplification. The above two studies do indicate that chronic alcohol abuse can cause ongoing myocardial damage which may be of an immune nature. The lack of a dose-response between total alcohol intake and ejection fraction, however, suggests that variable sensitivity to its toxic effects, rather than simply lifetime consumption, determines which alcohol abusers will develop dilated cardiomyopathy. In contrast to patients with dilated cardiomyopathy who abuse alcohol, subjects attending hospital primarily for treatment of alcoholism have demonstrated a negative correlation between ejection fraction and alcohol consumption (116). This correlation being strongest for total lifetime alcohol intake. Nonetheless, as discussed by the authors of the latter study, individual susceptibility to myocardial damage through alcohol abuse clearly varies greatly, with no exact duration of abuse or amount consumed predicting the development of symptomatic heart failure (132).
ALCOHOL CONSUMPTION

It is conceivable that even those patients with a low alcohol intake may have a genetically predisposed greater sensitivity to its toxic effects (133,134). An autoimmune process initiated by myocardial necrosis secondary to the toxic effects of alcohol may be responsible for the continuation of the disease process, even after long-term abstinence, at least in familial DCM. Alcohol abuse was no more or less common in familial cases of DCM. Nonetheless it would seem prudent to advise not only patients but also their first-degree relatives to at least maintain their alcohol intake within the present recommended weekly limits. It is, however, important to note that 60% of patients either drank alcohol within the recommended limits or abstained. This supports the hypothesis that alcohol is only one of many possible triggers for the development of DCM in a susceptible individual.
ALCOHOL CONSUMPTION

LIMITATIONS OF CASE-CONTROL STUDY

This retrospective determination of alcohol intake was by questionnaire with potential for recall bias and a reticence to report consumption accurately. Patients were interviewed and controls returned answers anonymously. This is defended on the basis of pragmatism and cost. No retest data were generated and no independent verification of survey responses was sought. Reporting of alcohol intake is not necessarily any more accurate the second time around. Independent verification would raise ethical considerations and, also, not necessarily be more accurate. It may be that interviewing patients resulted in a more honest appraisal of alcohol consumption. On the other hand, anonymous reporting may enhance honesty with regards to reporting alcohol intake. While the majority of patients were male, a larger proportion of the controls were female. When stratified by gender, only the differences between cases and controls with regards to average total lifetime consumption of alcohol remained statistically significant. The study was not powered for subgroup gender analysis. Population-based controls were selected in favour of other sources of control selection to avoid the risk of over-matching for the putative risk factor. Also, the more equal numbers of both sexes in the control group provides a population-based estimate of alcohol use and abuse. It is interesting to note that the percentage of the population estimated to be consuming alcohol above recommended “safe” levels in this Irish study is similar to The Health of the Nation from the United Kingdom (135). In the latter report, 28% of men and 11% of women exceeded 21 and 14 units a week respectively, as compared to 30% of men and 19% of women in the present study.
SCREEN-DETECTED DCM

As for the probands, the asymptomatic screen-detected relatives with echocardiographic DCM were significantly more likely than controls to have the DR4 haplotype (64% vs. 32%; P<0.05). They were also non-significantly more likely to be alcohol abusers according to the CAGE questionnaire (27% vs. 16%; P=0.27). This suggests that familial DCM is an autoimmune disease with alcohol being one of the environmental triggers in a susceptible individual. It is, however, well known that children born to alcoholics are at increased risk for alcohol abuse themselves. Therefore, it is possible that part of the familial trend witnessed in DCM is due to an inherited susceptibility to abuse alcohol. The increased DR4 frequency in these patients does, nonetheless, point to an autoimmune predisposition to familial DCM. As with the probands with DCM, the majority of the effected relatives (73%) were not alcohol abusers. Thus, while some of the screen-detected cases may possibly be the result of familial alcohol abuse alone, the rest either have an inherited sensitivity to the toxic effects of alcohol or have experienced some other myocardial insult initiating left ventricular dysfunction.
CONCLUSIONS

The familial prevalence of DCM in this patient group was a 'definite' 25% and a 'possible' 45%. This is the first study to show that familial DCM is linked to the DR4 haplotype, implying an autoimmune predisposition in this subgroup of patients. The HLA-type DR4 was significantly more common in familial dilated cardiomyopathy, as compared with non-familial cases and controls, and was associated with over two-thirds of the families at risk for the disease.

This study shows for the first time that asymptomatic relatives of DCM patients who demonstrate LVE on echocardiographic screening have ongoing disease activity. This was evident by mild to moderate myocyte hypertrophy, with little or no fibrosis and no inflammation in the biopsy samples examined. Apoptotic activity, as detected by TUNEL activity and CPP-32 immunoreactivity, and abnormal HLA-DR expression were present in all cases. Interestingly, these findings were more prominent within the interstitium than the myocardial cells.

This is also the first case-control study to compare alcohol consumption in a group of DCM patients with that of randomly selected, population-based controls. Significantly more DCM patients than controls either abuse alcohol or drink it in excess of recommended limits. Excess alcohol appears to be causally linked to both familial and non-familial DCM. It is, however, important to note that 60% of patients either drank alcohol within recommended limits or abstained. This supports the hypothesis that alcohol is only one of many potential triggers for the development of DCM in a susceptible individual.
FUTURE RESEARCH AND CLINICAL IMPLICATIONS
FUTURE RESEARCH AND CLINICAL IMPLICATIONS

The special DCM clinic will continue to recruit patients with DCM and screen as many of their first-degree relatives as possible, in an attempt to obtain a true estimate of the familial frequency of the disease. Those first-degree relatives already "cleared" of having DCM by one-off echocardiography will be recalled on a regular basis, to better estimate how frequently the disease develops at some time in family members. In this way, more screen-detected asymptomatic first-degree relatives will be detected. They will have the diagnosis of DCM confirmed by excluding the conditions already mentioned and performing angiography to confirm normal coronary arteries.

The screen-detected patients will be HLA-typed to see if their halotype distribution corresponds with the original proband diagnosed in that family. Viral serology will be performed looking for evidence of recent infection (Coxsackie, Echo) which may have initiated an autoimmune process. Serology will also screen for cardiac autoantibodies (organ-specific, anti-β, myosin) and specific T-cell subsets (CD 3,4+8), as further evidence indicating an autoimmune process in the early stages of DCM in asymptomatic patients.

The screen-detected DCM cases will undergo exercise testing and VO2 Max to look for early signs of effort limitation. ECG and holter rhythm monitoring will identify any conduction disturbances or arrhythmias in the early stages of the disease. At the time of catheterisation the screen-detected patients will have a right ventricular biopsy performed. Their myocardium can then be examined for evidence of an autoimmune reaction early in the course of DCM. This detailed clinical and serological assessment of the asymptomatic screen-detected patients will give us a better understanding of the exact nature of autoimmune dysfunction that is present early in the course of familial DCM.
FUTURE RESEARCH AND CLINICAL IMPLICATIONS

The available evidence suggests that the initial immune response is mainly humoral and, if the environmental trigger is not eradicated effectively, then a cellular autoimmune response ensues which is ongoing and leads to further myocardial damage. It is conceivable that immunotherapy at the early humoral phase may help the host to eradicate the environmental pathogen, prevent a cellular response and stop disease progression. Anti-apoptotic therapy, e.g. bcl-2, may also be beneficial at this pre-clinical stage. Certainly initiation of ACE inhibitor therapy should slow disease progression by inhibiting further ventricular remodelling.

When enough cases of early DCM are identified, trials assessing the effectiveness of the above treatment modalities in altering the natural history of DCM should be organised. There is a realistic hope that, by detecting and treating the disease early, one may continue to improve the prognosis.
APPENDICES
APPENDIX I

Normal Adult Echocardiographic Measurements (reference 62)

<table>
<thead>
<tr>
<th>Number</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>13-54</td>
<td>26</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.45-2.22</td>
<td>1.8</td>
</tr>
<tr>
<td>LVIDD-flat (mm)</td>
<td>37-56</td>
<td>47</td>
</tr>
<tr>
<td>LVIDD/m² (mm)</td>
<td>21-32</td>
<td>26</td>
</tr>
</tbody>
</table>

LVIDD = left ventricular internal diastolic diameter
APPENDIX II

Proband datasheet
Name: 
Address: 
Hospital No.: 

<table>
<thead>
<tr>
<th>Item</th>
<th>Column</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study No.</td>
<td></td>
<td>1-3</td>
</tr>
<tr>
<td>D.O.B. (dd/mm/yy)</td>
<td></td>
<td>4-9</td>
</tr>
<tr>
<td>Gender (1 = m, 2 = f)</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Date of last follow-up (dd, mm, yy)</td>
<td></td>
<td>11-16</td>
</tr>
<tr>
<td>Year of diagnosis</td>
<td></td>
<td>17-18</td>
</tr>
</tbody>
</table>

**Risk Profile. (1=y, 2=n)**

- Viral Illness {< 3/12 before}     | 19
- Peripartum {at diagnosis}        | 20
- Atopy                           | 21
- Cigarettes (no./d.) at diagnosis | 22-23
- at follow-up                    | 24-25

**ECHO: {at follow-up}**

- LVIDD (mm)                      | 26-27 |
- LVIDS                           | 28-29 |
- LVEF% (1=normal)                | 30-31 |
- LVPWD                           | 32-33 |
- LVPWS                           | 34-35 |
- IVSD                            | 36-37 |
- IVSS                            | 38-39 |
- E/A reversal (1=y, 2=n)         | 40
- mitral regurg.                  | 41
- {1 = no, 2 = mild, 3 = mod, 4 = severe} | 42-43 |
- est. RVSP (mmHg)                |      |
APPENDIX III

Datasheet for First Degree Relatives

Name:
Address:

<table>
<thead>
<tr>
<th>Item</th>
<th>Column</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study No.</td>
<td></td>
<td>1-3</td>
</tr>
<tr>
<td>Study No. of proband</td>
<td></td>
<td>4-6</td>
</tr>
<tr>
<td>Relation to proband</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>(1=sibling,2=parent,3=child)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D.O.B. (dd/mm/yy)</td>
<td></td>
<td>8-13</td>
</tr>
<tr>
<td>Gender (1=m,2=f)</td>
<td></td>
<td>14</td>
</tr>
</tbody>
</table>

ECHO:
- LVIDD (mm)
- LVIDS
- LVEF% (1=normal)

Diagnosis of DCM
(1=y,2=n,3=possible)

Yes: EF < 50% and LVIDD > 56 mm
Possible: EF < 50 or LVIDD > 56 mm

If yes to be entered into proband study and be HLA typed.
APPENDIX IV

Alcohol questionnaire for patients with DCM

Name: 
Address: 

<table>
<thead>
<tr>
<th>Item</th>
<th>Column</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study No.</td>
<td></td>
<td>1-3</td>
</tr>
<tr>
<td>D.O.B. (dd/mm/yy)</td>
<td></td>
<td>4-9</td>
</tr>
<tr>
<td>Gender (1=m,2=f)</td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

Alcohol History

<table>
<thead>
<tr>
<th>Item</th>
<th>Column</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life-long non-drinker? (1=y,2=n)</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Age when started</td>
<td></td>
<td>12-13</td>
</tr>
<tr>
<td>Ex-drinker? (1=y,2=n)</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Age when stopped</td>
<td></td>
<td>15-16</td>
</tr>
<tr>
<td>Total duration of drinking (yrs)</td>
<td></td>
<td>17-18</td>
</tr>
<tr>
<td>Change in intake anytime?</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>Year of change</td>
<td></td>
<td>20-21</td>
</tr>
<tr>
<td>Ave. weekly intake presently (units)</td>
<td></td>
<td>22-24</td>
</tr>
<tr>
<td>Ave. weekly intake past (ex-drinkers)</td>
<td></td>
<td>25-27</td>
</tr>
</tbody>
</table>

CAGE Questionnaire (1=y,2=n)

<table>
<thead>
<tr>
<th>Item</th>
<th>Column</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you ever felt you should cut down on your drinking?</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>Have people annoyed you by criticising your drinking?</td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>Have you ever felt bad or guilty about your drinking?</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Have you ever had a drink first thing in the morning to steady your nerves or get rid of a hangover?</td>
<td></td>
<td>31</td>
</tr>
</tbody>
</table>
APPENDIX V

Alcohol questionnaire for randomly selected controls

1. Age (years) = ___

2. Sex (m/f) = ___

3. Are you a life-long abstainer from alcohol (non-drinker)? (y/n): ___

If your answer to Qu.3 is yes, you may leave the rest of the form blank.

4. If not, roughly what age were you when you started to drink?: ___

5. Are you an ex-drinker? (y/n): ___

6. If so, at what age did you stop drinking? (years): ___

7. Did you change your alcohol consumption at anytime? (y/n): ___

8. If so, what age were you at that time? (years): ___

In Qu. 9 + 10, you are asked to express your average weekly alcohol intake in units. A unit of alcohol is equivalent to half a pint of beer, one measure of spirit or a glass of wine. Thus, if you drink an average of 10 pints of beer a week that is equal to 20 units of alcohol etc..

9. What is your present average weekly alcohol intake, in units? ___

10. If you are an ex-drinker or changed your alcohol intake at sometime, what was your average weekly intake in the past, in units? ___

11. Have you ever felt that you should cut down on your drinking? (y/n): ___

12. Have people ever annoyed you by criticising your drinking? (y/n): ___

13. Have you ever felt bad or guilty about your drinking? (y/n): ___

14. Have you ever had a drink first thing in the morning to steady your nerves or get rid of a hangover? (y/n): ___
PRESENTATIONS AND PUBLICATIONS
1. Idiopathic dilated cardiomyopathy: familial prevalence and HLA distribution.
McKenna, Codd, McCann, Sugrue.
Heart 1997;77:549-552.

2. Alcohol consumption and idiopathic dilated cardiomyopathy: a case-control study.
McKenna, Codd, McCann, Sugrue.

3. Histopathologic changes in asymptomatic relatives with early familial dilated cardiomyopathy.
McKenna, Sugrue, Kwon, Edwards, Holmes, Schwartz.
In progress.
PUBLICATIONS
ABSTRACTS


REFERENCES
REFERENCES


