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Original article

Clinical and mutational spectrum in a cohort of 105 unrelated patients with dilated cardiomyopathy

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ABSTRACT

Dilated Cardiomyopathy (DCM) is one of the leading causes of heart failure with high morbidity and mortality. More than 30 genes have been reported to cause DCM. To provide new insights into the pathophysiology of dilated cardiomyopathy, a mutational screening on 4 DCM-causing genes (*MYH7, TNNT2, TNNI3* and *LMNA)* was performed in a cohort of 105 unrelated DCM (64 familial cases and 41 sporadic cases) using a High Resolution Melting (HRM)/sequencing strategy. Screening of a highly conserved arginine/serine (RS)-rich region in exon 9 of *RBM20* was also performed. Nineteen different mutations were identified in 20 index patients (19%), including 10 novels. These included 8 *LMNA* variants in 9 (8.6%) probands, 5 *TNNT2* variants in 5 probands (4.8%), 4 *MYH7* variants in 3 probands (3.8%), 1 *TNNI3* variant in 1 proband (0.9%), and 1 *RBM20* variant in 1 proband (0.9%). One proband was double-heterozygous. *LMNA* mutations represent the most prevalent genetic DCM cause. Most patients carrying *LMNA* mutations exhibit conduction system defects and/or cardiac arrhythmias. Our study also showed than prevalence of mutations affecting *TNNI3* or the (RS)-rich region of *RBM20* is lower than 1%. The discovery of novel DCM mutations is crucial for clinical management of patients and their families because pre-symptomatic diagnosis is possible and precocious intervention could prevent or ameliorate the prognosis.

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1. Introduction

Dilated cardiomyopathy (DCM), primarily characterized by left ventricular dilatation and impaired systolic function, is one of the leading causes of heart failure with high morbidity and mortality [1]. The prevalence is 1/2500 individuals. About 20–48% of DCM individuals had familial forms of the disease [1]. Disease-causing genes in familial DCM predominantly follow autosomal dominant inheritance patterns. The penetrance and presentation of familial DCM are highly variable concerning the functional impairment, the extent of cardiac involvement and the presence of other clinical manifestations. Over the past decade, private mutations were scattered among more than 30 genes encoding essentially sarcomeric, cytoskeletal, and nuclear proteins [2]. Available molecular

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data suggested that the three most prevalent genes involved in DCM are lamin A/C (*LMNA*, OMIM#: 150330), β-myosin heavy chain (*MYH7*, OMIM +160760), and cardiac troponin T (*TNNT2*, OMIM *191045) [2]. However, except an important mutational screening performed on a cohort of more than three hundred probands with familial or idiopathic dilated cardiomyopathy, no other cohort with a significant number (>100) of DCM Caucasian patients was investigated for mutations in these prevalent DCM genes [3–6].

To provide new insights into the pathophysiology of dilated cardiomyopathy, a mutational screening on these prevalent DCM-causing genes was performed in a cohort of 105 unrelated DCM index probands (64 familial cases and 41 sporadic cases) using a High Resolution Melting (HRM)/sequencing strategy which was previously reported as an highly sensitive and high-throughput method to allow identification of mutations in the coding sequences of prevalent DCM genes [7,8]. Additional *TNNI3* screening (OMIM +191044) was also performed as mutations in this gene were recently reported in autosomal dominant DCM [9]. Finally, as a recent study suggested that mutations affecting a highly conserved arginine/serine (RS)-rich region in exon 9 of *RBM20* could accounted for 3% of all DCM cases, we also undertook to study this exon in order to confirm its prevalence [10].

2. Methods

2.1. Subjects

The study included 105 unrelated index patients. Most of them were recruited from a general cardiology hospital. All probands underwent a comprehensive assessment including a clinical examination, ECG, echocardiogram and for most of the cases MRI, angiography and coronarography. High blood pressure, coronary disease, chronic excess of alcohol consumption and a systemic disease were excluded prior to genetic analysis. The clinical diagnostic criteria were established according to international criteria and included a left ventricular enlargement with systolic dysfunction after the exclusion of other detectable causes of DCM [11]. Patients were enrolled in this study after obtaining informed consent form. Using pedigree data, patients were classified as familial cases when at least two first-degree patients in the same family were affected. Cases were considered sporadic when no evidence of familial disease was observed or when no relative could be clinically evaluated. Family screening was proposed to families only when a putative pathogenic mutation was identified in the proband.

2.2. Molecular analysis

Genomic DNA was extracted from whole blood using a WIZARD Genomic DNA Purification kit (Promega, Madison, WI) according to the manufacturer's instructions. All coding exons and intronic junctions of *MYH7, LMNA, TNNT2* and *TNNI3* genes were amplified and scanned using an HRM/sequencing strategy as previously reported [7,8]. Exon 9 of *RBM20* was partially amplified (amplification conditions available under request) using Light-Cycler® 480 High Resolution Melting Master kit according to the manufacturer's instructions (Roche Applied Science, Meylan, Fra) and further scanned by HRM analysis.

PCR products showing divergent HRM profiles were purified by using MultiScreen-PCR Plates (Millipore, Bedford, MA) and directly sequenced on both strands using the BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Forster City, CA). Sequencing products were purified with Montage SEQ96 Sequencing Reaction Clean Up kit (Millipore) and applied onto an ABI 3730 automatic sequencer (Applied Biosystems).

Identified nucleotidic changes were considered as possibly disease-causing if they predicted a change in a conserved amino acid (conversation between chimp, mouse, rat, dog, cow and pig peptidic sequences) and were absent in 200 healthy ethnic matched controls. Nucleotide changes considered as likely disease-causing either met the above criteria with segregation of the disease in multiple affected individuals within a family or led to a premature truncation (frameshift, splice mutations, nonsense), or had previously been reported as causative of DCM. The pathogenicity of the novel missense mutations was also evaluated with *in silico* studies based on the use on 2 different softwares (PolyPhen-2, and SIFT).

3. Results

3.1. Global study population

Molecular screening was performed on a cohort constituted of 105 unrelated index probands who were recruited with an original clinical diagnosis of DCM. The mean onset age of this cohort was 37.3 ± 17.1 years. The presence of the disease was classified as familial in 64 cases (61.9%), and sporadic in 41 (38.1%) cases (Table 1). Mean age of onset was significantly different between sporadic and familial cases (41.5 ± 16.9 versus 30.7 ± 14.6 years; $p = 0.0026$). No statistical difference for onset age, left ventricular end diastolic diameter (LVEDD) and ejection fraction (EF) could be observed in patients with versus without identified mutations (Table 1).

Based on the nature of the mutation, segregation studies and experimental data previously reported, 19 mutations could be classified as likely or possibly disease-causing mutations: 10 are novel and 9 have been previously reported (Table 2). Novel missense identified mutations affected highly conserved amino-acids among mammals and were observed neither in 200 healthy ethnic matched controls nor in the 1000 genomes database. Except TNNI3-p.Ala116Gly, *in silico* studies based on the use of 2 different softwares (PolyPhen-2 and SIFT) suggested that these new missense variants could be deleterious. Among the 9 known mutations, 7 were already associated with DCM cases but 2 (TNNT2-p.Arg278Cys, TNNT2-p.Ala28Val) were previously associated only with HCM cases (Table 2). Unfortunately, left ventricular

Table 1
Clinical features at diagnosis of DCM cohort.

Presentation	*n* (%)	Age at onset (years)	LVEDD[a] (mm)	EF[b] (%)	Mutation positive cases	Mutation negative cases
Familial	64 (61.9%)	41.5 ± 16.9	62.9 ± 9.3	32.3 ± 9.4	15 (23.4%)	49 (76.6%)
Sporadic	41 (38.1%)	30.7 ± 14.6	65.1 ± 11.9	30.5 ± 12	5 (12.2%)	36 (87.8%)
Presentation	***n* (%)**	**Age at onset (years)**	**LVEDD[a] (mm)**	**EF[b] (%)**	**Familial**	**Sporadic**
Mutation positive cases	20 (19%)	37.1 ± 19.2	61.6 ± 9.2	34.9 ± 9.3	15 (75%)	5 (25%)
Mutation negative cases	85 (81%)	37.4 ± 16.7	64.2 ± 10.6	31 ± 10.6	49 (57.6%)	36 (42.3%)

[a] LVEDD: Left Ventricular End Diastolic Diameter.
[b] EF: Ejection fraction.

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