A genome-wide association study identifies two loci associated with heart failure due to dilated cardiomyopathy

Eric Villard1,2, Claire Perret3, Françoise Gary1,2, Carole Proust3, Gilles Dilanian1,2, Christian Hengstenberg4, Volker Ruppert5, Eloïsa Arbustini6, Thomas Wichter7, Marine Germain3, Olivier Dubourg8, Luigi Tavazzi9, Marie-Claude Aumont10, Pascal DeGroote11, Laurent Fauchier12, Jean-Noël Trochu13,14, Pierre Gibelin15, Jean-François Aupetit16, Klaus Stark4, Jeanette Erdmann17, Roland Hetzer18, Angharad M. Roberts19, Paul J.R. Barton20,21, Vera Regitz-Zagrosek22, Cardiogenics Consortium§, Uzma Aslam1,2, Laetitia Duboscq-Bidot1,2, Matthias Meyborg7, Bernhard Maisch5, Hugo Madeira23, Anders Waldenström24, Enrique Galve25, John G. Cleland26, Richard Dorent27, Gerard Roizes28, Tanja Zeller29, Stefan Blankenberg29, Alison H. Goodall30, Stuart Cook19,20, David A. Tregouet3, Laurence Tietj3, Richard Isnard1,2, Michel Komajda1,2, Philippe Charron1,2†, and François Cambien2,31†

1INSERM, U956, Paris, 75013, France; 2University Paris 6 UPMC, U956, AP-HP Hôpital Pitié-Salpêtrière, Paris 75013, France; 3INSERM UMR 937, Paris 6 University (UPMC), Paris 75013, France; 4Klinik und Poliklinik für Innere Medizin II, Universitätsklinikum Regensburg, Franz-Josef-Strauss-Allee 11, Regensburg 93053, Germany; 5Klinik für Innere Medizin-Kardiologie UKGM GmbH Standort Marburg Baldingerstrasse, Marburg 35043, Germany; 6Centre for Inherited Cardiovascular Diseases, Academic Hospital IRCCS Fondazione Policlinico San Matteo, Pavia, Italy; 7Medizinische Klinik und Poliklinik für Innere Medizin C, Kardiologie und Angiologie, Universitätsklinikum Münster, Albert-Schweitzer-Straße 33, Münster 48149, Germany; 8Université de Versailles-Saint Quentin, Hôpital Ambroise Pare, AP-HP, Boulogne 92100, France; 9GVM Hospitals of Care and Research, Cottignola, Italy; 10Service de Cardiologie, Hôpital Bichat, Paris 75018, France; 11Service de Cardiologie, Hôpital Cardiovasculaire, Lille, France; 12Service de Cardiologie, Centre Hospitalier Universitaire Trousseau, Tours 37004, France; 13INSERM UMR915, l’institut du thorax, Nantes, France; 14CHU Nantes, Service de Cardiologie, Nantes F-44000, France; 15Service de Cardiologie, CHU Nice, Nice, France; 16Département de pathologie cardiovasculaire, Hôpital Saint-Joseph-Saint-Luc, Lyon, France; 17Universität zu Lübeck, Medizinische Klinik II, Lübeck 23358, Germany; 18Deutsches Herzzentrum Berlin, Augustenburger Platz 1, Berlin 13353, Germany; 19Medical Research Council Clinical Science Centre, Faculty of Medicine, Imperial College London, Hammersmith Hospital, London, UK; 20Heart Science Centre, National Heart and Lung Institute, Imperial College, Harefield UB9 6JH, UK; 21NIHR Cardiovascular Biomedical Research Unit, Royal Brompton and Harefield NHS Foundation Trust, Sydney Street, London SW3 6NP, UK; 22Institute for Gender in Medicine, Center for Cardiovascular Research, Charité Campus Mitte, Berlin, Germany; 23Faculdade de Medicina de Lisboa, Clínica Universitária de Cardiologia, Portugal; 24Department of Public Health and Clinical Medicine/Medicine, Umeå University, Sweden; 25Servicio de Cardiología, Hospital Vall d’Hebron, Barcelona 08035, Spain; 26Department of Cardiology, University of Hull, UK; 27Service de Cardiologie, CHU Tenon, Paris, France; 28Institut de Génétique Humaine, UPR 1142, CNRS, Montpellier, France; 29Medizinische Klinik und Poliklinik, Johannes-Gutenberg Universität Mainz, Universitätsmedizin Mainz, Mainz, Germany; 30Department of Cardiovascular Sciences, University of Leicester, and Leicester NIHR Biomedical Research Unit in Cardiovascular Disease, Glenfield Hospital, Leicester LE3 9QP, UK; and 31P3S postgenomics platform, Paris, France

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* Corresponding author. Tel: +33 1 4077 9675, Fax: +33 1 4077 9645, Email: eric.villard@upmc.fr
† Present address: Marienhospital Osnabrück, Heart Center Osnabrück-Bad Rothenfelde, Department of Cardiology and Angiology, Bischofstrasse 5, D-49074 Osnabrück, Germany.
‡ A list of participants and their affiliations appears in the Supplementary material online.
† These authors contributed equally to this work.

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Introduction

Idiopathic dilated cardiomyopathy (DCM) is a form of heart failure (HF) defined by the presence of left ventricular dilatation and left ventricular systolic dysfunction in the absence of an obvious aetiology, such as coronary artery disease (CAD), hypertension, valvular disease, or congenital defect.1–3 Dilated cardiomyopathy is a major cause of systolic HF and the main indication for heart transplantation.1 It affects ≏1/2500 adults and it is more common in men than in women.5

The pathophysiology of DCM is multifactorial with a possible implication of environmental factors and the existence of a strong genetic component attested by a high rate of familial aggregation.3,4 20–35% of DCM cases having an affected first-degree relative.2,3 Mutations in more than 30 genes have been identified in monogenic forms of DCM, most of them encoding proteins of the cytoskeleton or the sarcomere.5 These genes may also carry variants that are over-represented in sporadic cases;7 however, results of candidate gene association studies have been inconsistent8 and the genetic basis of DCM, whether familial or sporadic, remains largely unresolved.

Over the last few years, genome-wide association studies (GWASs) exploiting the power of high density genotyping arrays have led to the discovery of numerous loci implicated in cardiovascular diseases;9,10 however, no GWAS of DCM has been reported so far, probably because the relatively low prevalence of the disease makes the assembly of large clinically homogeneous cohorts of patients difficult.

Here we report the results of a GWAS based on pools of DNA from patients with sporadic DCM and controls from several European populations and the replication of the findings in two independent populations. One of the replicated loci suggests the implication of the B-cell lymphoma 2-associated athanogene 3 (BAG3) in DCM. The subsequent sequencing of all coding regions of the BAG3 gene in patients with familial DCM identified several mutations that strongly affect the predicted protein structure and are likely to cause the disease.

Methods

Subjects

All participants included in the study were of European origin. Detailed description of recruitment and ascertainment in the various studies is given in the Supplementary information. Ethics committees approved the study protocols, and all participants gave written informed consent. All patients had a diagnosis of idiopathic DCM according to conventional criteria,11 characterized by enlarged left ventricle diameter and low ejection fraction (≤45%) in the absence of causal factors, such as CAD. Only apparently sporadic cases without affected relatives were included.

For the GWAS, DCM patients from three European studies (CARDI-GENE, EUROGENE-EHF, and PHRC-DCM)12,13 were assembled and matched with control groups14,15 from the respective populations (see Table 1 and Supplementary information for details).

Constitution of DNA pools

The DNA pools were stratified on study population as well as gender and age when the numbers permitted. We required that at least 25 samples were mixed in a single pool (Supplementary material online, Table S1). Each pool was constituted twice and each pool replicate was analysed on two arrays independently (Supplementary information).
Quantification of allelic signals in DNA pools and statistical analysis

Human 610-quad beadchip arrays were hybridized with DNA pools and scanned on an i-scan instrument (Illumina). After normalization in BeadStudio (Illumina), the data were transferred into the R statistical environment15 for further analyses. Allelic signal intensities within pools were computed from the normalized quantitative signals (x and y) corresponding to the two alleles of each single nucleotide polymorphism (SNP): p(x) = x/(x+y) and p(y) = 1−p(x) (Supplementary methods). Four measurements of x and y were available for each SNP in each pool (two replicates of pools and two arrays/replicate of pool) and were used for statistical modelling. To assess the variation among the four measurements of each pool, we computed the intra-class correlation (IC) of p(x) for each SNP, i.e. the ratio of the between pools variance to the total variance. The median IC was 0.69 with an interquartile range from 0.54 to 0.78.

A mixed linear model (R: LME4) was used to analyse the pooled allelic signals of each SNP, in which p(x) was the dependent variable, disease status, age category, gender, and study population were fixed effects, and the pool identifier was a random effect. In addition, a weight was introduced in the model to account for the number of samples in each pool and the fact that each pool was replicated four times. At the discovery stage, a threshold of P < 5 × 10−7 was adopted for declaring a SNP significant. For in-depth investigation of the genomic context of DCM-associated SNPs, exploration and annotations of loci of interest were carried out using WGAviewer17 and regional association results were plotted with LocusZoom.18

The genomic control inflation factor (λ)19 was computed to see whether after linear adjustment on study population, age, and gender, some population stratification or cryptic relatedness could still bias the distribution of SNP × DCM association statistics. For that purpose, the vector of allelic association P-values from the adjusted linear model was transformed into a vector of 1-df Chi-squared statistics and compared with expected values using the function geantest2 from the package Rgap.

Individual level validation of SNPs associated in the pools—GWAS

For each SNP needing validation, we performed individual level genotyping by TaqMan 5′ nuclease assay technology (Supplementary methods). The association between DCM and individual genotypes was tested using a logistic regression model (R:GLM) assuming an additive allele effect and adjusting on age, gender, and study population as in the analysis of the pools—GWAS data.

BAG3 exonic sequencing in patients with familial dilated cardiomyopathy

To search for mutations in BAG3 in familial DCM, genomic DNA from 168 index patients was sequenced after PCR amplification of the four exons on an ABI 3100 capillary sequencing instrument (Appera). DNAs from other available family members were genotyped for the familial variant by PCR and sequencing. We also sequenced exons 2, 3, and 4 of BAG3 in 364 individuals of European descent without known cardiac disease (Supplementary methods). Moreover, DNA from 95 control subjects of North African origin and 45 control subjects from Turkey were sequenced to check, respectively, for the presence of variants P115S and V468M in non-DCM populations.

Results

A short description of the populations of patients and controls included at the discovery and replication stages is reported in Table 1, and more details are provided as Supplementary information; all included subjects were of European origin. We used strict criteria11 for patient selection to exclude secondary or familial forms of cardiomyopathy. The discovery GWAS was performed on pools of DNA (pools-GWAS). Overall 26 DNA pools were constituted stratified on study population, disease status, gender, and age (see Supplementary material online, Table S1). To improve the precision of effect estimates when comparing pools of DNA from patients and controls, each pool was duplicated and each duplicate was hybridized to two different genotyping arrays. After filtering out copy number variation markers and SNPs with poor signal intensity, 517 382 SNPs were retained in the analysis.
Table 2  Summary of the results of the discovery phase

<table>
<thead>
<tr>
<th>Retested SNP</th>
<th>Chr</th>
<th>Position</th>
<th>Locus</th>
<th>Pool–GWAS^a</th>
<th>Individual GWAS^b</th>
<th>MAF^c cases/controls</th>
<th>Best proxy^d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OR</td>
<td>95% IC</td>
<td>P-value</td>
<td></td>
</tr>
<tr>
<td>rs16983785</td>
<td>21</td>
<td>29447289</td>
<td>TAK1L</td>
<td>1.97</td>
<td>1.60–2.42</td>
<td>5.4 × 10^-11</td>
<td>0.102/0.059</td>
</tr>
<tr>
<td>rs3728410</td>
<td>13</td>
<td>104824202</td>
<td>Intergenic</td>
<td>1.73</td>
<td>1.45–2.06</td>
<td>3.2 × 10^-10</td>
<td>0.056/0.045</td>
</tr>
<tr>
<td>rs2234962</td>
<td>10</td>
<td>121419623</td>
<td>BAG3</td>
<td>0.64</td>
<td>0.55–0.74</td>
<td>8.8 × 10^-10</td>
<td>0.125/0.208</td>
</tr>
<tr>
<td>rs1991914</td>
<td>4</td>
<td>42600114</td>
<td>OTOPI</td>
<td>0.59</td>
<td>0.49–0.71</td>
<td>1.3 × 10^-8</td>
<td>0.339/0.338</td>
</tr>
<tr>
<td>rs13176432</td>
<td>5</td>
<td>114369723</td>
<td>Intergenic</td>
<td>0.56</td>
<td>0.45–0.68</td>
<td>1.5 × 10^-8</td>
<td>0.047/0.027</td>
</tr>
<tr>
<td>rs10491858</td>
<td>9</td>
<td>1500495</td>
<td>Intergenic</td>
<td>0.68</td>
<td>0.59–0.80</td>
<td>5.8 × 10^-8</td>
<td>0.140/0.111</td>
</tr>
<tr>
<td>rs5970164</td>
<td>X</td>
<td>150849762</td>
<td>MAGEA4</td>
<td>0.61</td>
<td>0.50–0.73</td>
<td>7.2 × 10^-8</td>
<td>0.103/0.167</td>
</tr>
<tr>
<td>rs856003</td>
<td>10</td>
<td>119393553</td>
<td>Intergenic</td>
<td>0.69</td>
<td>0.60–0.79</td>
<td>1.1 × 10^-7</td>
<td>0.195/0.157</td>
</tr>
<tr>
<td>rs10927875</td>
<td>1</td>
<td>16171899</td>
<td>ZBTB17</td>
<td>0.71</td>
<td>0.63–0.81</td>
<td>1.3 × 10^-7</td>
<td>0.269/0.341</td>
</tr>
<tr>
<td>rs11543052</td>
<td>21</td>
<td>46880804</td>
<td>PRMT2</td>
<td>2.18</td>
<td>1.63–2.93</td>
<td>9.7 × 10^-8</td>
<td>0.018/0.006</td>
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<tr>
<td>rs1353456</td>
<td>X</td>
<td>78829377</td>
<td>Intergenic</td>
<td>1.38</td>
<td>1.22–1.57</td>
<td>2.7 × 10^-7</td>
<td>NA</td>
</tr>
<tr>
<td>rs28302070</td>
<td>21</td>
<td>29059787</td>
<td>AF131217.1</td>
<td>1.43</td>
<td>1.24–1.65</td>
<td>3.2 × 10^-7</td>
<td>0.218/0.159</td>
</tr>
<tr>
<td>rs1378796</td>
<td>3</td>
<td>158614482</td>
<td>VEPH1</td>
<td>1.49</td>
<td>1.28–1.75</td>
<td>3.9 × 10^-7</td>
<td>0.099/0.074</td>
</tr>
<tr>
<td>rs2290906</td>
<td>17</td>
<td>73605461</td>
<td>TNRC6C</td>
<td>1.45</td>
<td>1.25–1.67</td>
<td>3.4 × 10^-7</td>
<td>0.164/0.124</td>
</tr>
</tbody>
</table>

^aComputed from pooled DNA signals using a linear mixed model adjusting for population, age category, and gender.
^bComputed from individual genotypes using a logistic regression model adjusting for population, age category, and gender.
^cMinor allele frequency estimated from individual genotypes.
^dBest proxy of the retested SNP in the pool–GWAS and its association P-value with DCM. In parentheses are shown the D’/r^2 parameters quantifying the extent of linkage disequilibrium (LD) between the retested SNP and its best proxy. These values were obtained from HAPMAP. The absence of SNPs demonstrating D’ > 0.5 or r^2 > 0.5 with the retested SNP is indicated as NA.
Fourteen SNPs were associated with DCM in the pools–GWAS ($P < 5 \times 10^{-7}$). The values of the genomic control inflation factor ($λ$) computed on the whole set of SNPs and on the 90% least significant SNPs were 1.042 and 0.994, respectively, indicating that stratification was unlikely to inflate the association statistics in an important way; as a consequence, we only present uncorrected statistics. To check the pools–GWAS results, the 14 DCM-associated SNPs identified in the pools–GWAS were genotyped on individual DNA in the discovery samples (Table 2). In the individual-based analysis, two SNPs, rs2234962 on chromosome 10 [odds ratio (95% CI) associated with minor allele = 0.53 (0.44–0.63), $P = 1.1 \times 10^{-13}$] and rs10927875 on chromosome 1 [OR = 0.71 (0.62–0.81), $P = 3.6 \times 10^{-7}$], remained significantly associated with DCM. A third SNP, rs16983785 on chromosome 21 [OR = 1.79 (1.41–2.27), $P = 7.4 \times 10^{-7}$] was just above the pre-specified threshold (Supplementary material online, Figure S1). These three SNPs were tested in two independent replication studies (Table 1). The pools–GWAS associations were replicated in the two replication studies combined for rs10927875 [OR = 0.76 (0.70–0.84), $P = 3.6 \times 10^{-7}$] and rs2234962 [OR = 0.66 (0.53–0.82), $P = 9.5 \times 10^{-7}$]; while for rs16983785, replication was not achieved [OR = 1.25 (0.98–1.61), $P = 0.076$]. In the data set combining the discovery and replication samples, rs10927875 and rs2234962 were associated with DCM with respective $P$-values of $9.5 \times 10^{-7}$ and $4.0 \times 10^{-12}$. The ORs and 95% CIs for each study group and for men and women separately in the discovery and replication phases are reported in Figure 1 and more detailed information is provided in Supplementary material online, Table S2. This low frequency may be due to chance as the MAF of rs2234962 in German men and women was 0.214 and 0.218, respectively, in a large population-based sample of 3149 individuals from the Gutenberg Heart Study.\(^{24}\)

**Figure 1** Allelic odds ratios for associations with dilated cardiomyopathy of the three identified lead SNPs in the discovery and replication samples. Meta-analysis summary effects: rs2234962 (BAG3) OR: 0.66 (95% CI 0.53–0.82), rs10927875 (ZBTB17) OR: 0.76 (0.70–0.84), rs16983785 (TAK1L) OR: 1.53 (1.25–1.89). The values used to draw the Forest plots are derived from Supplementary material online, Table S2. Replication samples are prefixed by ‘REP.’ The black squares are centred at the corresponding odds ratio and their surface is proportional to the sample size, the 95% confidence intervals are shown. For convenience, in this representation, statistics are shown separately for men and women; however, the global statistics for the discovery and replication samples reported in the Results section are adjusted on population, gender, and age as indicated in Table 2. The association of rs2234962 with DCM was replicated in men ($P = 0.0016$) but not in women ($P = 0.59$). This difference is largely the consequence of the low minor allele frequency (MAF) of this SNP in women included in the German replication control sample (0.138 compared with >0.188 in the other groups, Supplementary material online, Table S2). This low frequency may be due to chance as the MAF of rs2234962 in German men and women was 0.14 compared with 0.188 in the other groups, Supplementary material online, Table S2.

**Further exploration of the rs10927875 association signal on chromosome 1**

rs10927875 is not the best marker of dilated cardiomyopathy risk in the region

rs10927875 is located in an intron of ZBTB17 (zinc finger and BTB domain containing-17) also frequently referred to as MIZ-1) on chromosome 1p36.2–p36.1. The locus of interest is located in a genomic region, covering ~210 kb, that spans several other genes—SPEN (spen homolog, transcriptional regulator), HSPB7 (heat shock 27 kDa protein family, member 7), CLCNKA (chloride channel Ka), and CLCNKB (chloride channel Kb)—and exhibits strong linkage disequilibrium (LD) (Figure 2). Recently, Stark et al.\(^{20}\) reported an association between a SNP in HSPB7 (rs1739843) and DCM, in a study conducted in the group of German patients used for replication in the present study. Because DNA pooling introduces some error that may affect the
estimates of relative effects of linked SNPs, we additionally individually genotyped four SNPs in the region (Supplementary material online, Table S3): rs1763601 (a proxy of rs1739843), rs945417 (a proxy of rs1805152), rs945425 (a SNP located in the proximal promoter region of \textit{CLCNKA} which may generate an alternative splice site), and rs1048261 (a SNP located in the 3\textquotesingle UTR of \textit{HSPB7}). These SNPs are presented in their genomic context in Supplementary material online, Figure S2. Three of the four additional SNPs exhibited stronger associations with DCM than the lead SNP identified in the pools–GWAS (rs1763601: \(P = 3.4 \times 10^{-9}\); rs945417: \(1.4 \times 10^{-9}\); rs945425: \(4.2 \times 10^{-7}\); and rs1048261: \(7.9 \times 10^{-9}\) when compared with \(P = 1.3 \times 10^{-7}\) for rs10927875) and, for all these SNPs, the minor allele was associated with a reduced risk of DCM.

To get better insight into the haplotypic structure of the region and its impact on the association of the locus with DCM, haplotypic relative risks were computed.\(^2^3\) As reported in Supplementary material online, Table S4, the five genotyped SNPs determine six common haplotypes. The two major haplotypes CTGCT (0.508 and 0.603 in controls and DCM cases, respectively) and TGCTA (0.301 and 0.230) differ at the five sites (Yin/yang configuration) and are likely to represent ancestral alleles, the four less common haplotypes resulting from recombination between them. In comparison with CTGCT, TGCTA was associated with a reduced risk of DCM [OR = 0.64 (0.53–0.74), \(P = 3.2 \times 10^{-9}\)].

\textbf{HSPB7 is a possible candidate at the locus but is devoid of coding variant} 
Among the genes located in the region, \textit{HSPB7}, which encodes the \textquoteleft cardiovascular heat shock protein\textquoteright\(^2^2\) and exhibits cardiac-specific expression, is a possible candidate for an association with DCM. However, none of the DCM-associated SNPs or tight proxies identified through systematic sequencing by Matkovich et al.\(^2^3\) or genotyped in the present study affects the coding sequence of \textit{HSPB7}. Moreover, we sequenced the \textit{HSPB7} exons in 168 independent index cases diagnosed with familial DCM but were unable to identify any coding variant (data not shown).

\textbf{Possible cis-eQTLs for HSPB7 and CLCNKA} 
Given the lack of coding variant in \textit{HSPB7}, we envisaged that sequence variations in the \textit{HSPB7} region might be related to the DCM risk through an effect on gene expression. To investigate this possibility, we took advantage of genome-wide expression data generated using RNA from circulating monocytes in the Gutenberg Heart Study\(^2^4\) and from circulating monocytes and in

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{association_plot.png}
\caption{Association plot of SNPs with dilated cardiomyopathy (DCM) around the lead SNP rs10927875, based on pools–GWAS results. Upper panel: pairwise \(r^2\) (derived from HAPMAP) between the lead SNP and the SNPs in its flanking regions are shown together with the association \(P\)-value with DCM of each SNP. Annotations of SNPs: none (circle), non-synonymous (triangle), synonymous or UTR (square), transcription factor binding site consensus (asterisk). Lower panel: genes encompassing the associated region. Linkage disequilibrium may explain the presence of several DCM-associated SNPs in the region (see also Supplementary material online, Table S3), especially in the sequences of \textit{HSPB7} (rs1763601, pools–GWAS: \(P < 1.0 \times 10^{-8}\), this SNP being a perfect proxy of rs1739843, the DCM-associated SNP identified by Stark et al.\(^2^0\) according to HapMap release 22) and \textit{CLCNKA} (rs1805152, pools–GWAS: \(P = 5.6 \times 10^{-9}\)).}
\end{figure}
vitro-derived macrophages in the Cardiogenics study. In both data sets, \( HSPB7 \) expression was not detectable in monocytes. Conversely, in the Cardiogenics resource, the gene was weakly expressed in macrophages and several SNPs were associated in cis with its expression, the strongest signal was observed with rs945425 \( (P = 6.1 \times 10^{-57}) \). The same SNPs were also, but less strongly, associated with the expression of the nearby \( CLCNKA \) gene \( (P = 2.2 \times 10^{-26} \text{ with rs945425}) \) (see Supplementary material online, Table S5). These results might indicate a role of \( HSPB7 \) or \( CLCNKA \) genes through mediation of expression in macrophages.

On the other hand, Cappola et al. recently showed that the rs1739843 \( HSPB7 \) SNP, which is in strong LD with rs945425, had no effect on \( HSPB7 \) mRNA expression or splicing in left ventricular myocardium tissue. The authors further suggested that the variant responsible for modulating the risk of heart failure was more likely to be a non-synonymous polymorphism in the nearby \( CLCNKA \) gene, which altered the activity of the encoded chloride channel. However, the mechanism linking this variant to heart failure remains speculative since this channel is not expressed in the heart. The association of SNPs with \( CLCNKA \) and \( HSPB7 \) gene expression found in macrophages but not in the myocardium tissue suggests that these eQTL may be cell specific or may depend on specific activation mechanisms. However, the associations observed in macrophages must be interpreted with caution because the \( HSPB7 \) probe present on the Illumina array targets a polymorphic sequence and the two \( CLCNKA \) probes may also target polymorphic sequences (see legend of Supplementary material online, Table S5 for more details). The strong LD reflected by the Yin/yang haplotypic structure in the region could thus account for the observed co-association of the signals generated by the polymorphic probes and several SNPs. Functional studies will be needed to assess whether the \( HSPB7 \) and \( CLCNKA \) eQTLs in circulating macrophages may have any relevance for DCM. The other genes in the region (\( SPEN \), \( ZBTB17 \), \( CLCNKB \)) were either not detected in monocytes or macrophages or their expression was not or only weakly influenced by the DCM-associated SNPs in macrophages (Supplementary material online, Table S5). Importantly, given the extensive LD in the region, it cannot be excluded that variant(s) located at a distance of the initially identified markers may implicate unforeseen genetic regulatory mechanisms.

**Further exploration of the rs2234962 association signal on chromosome 10**

rs2234962 is a non-synonymous SNP (c.T757C, p.C151R) located within the coding sequence of \( BAG3 \) on chromosome 10q25.2–q26.2 (Figures 3 and 5). Our results show that the minor allele of this variant is associated with a reduced risk of DCM. The cysteine to arginine substitution at Position 151 of the \( BAG3 \) protein is predicted to be probably damaging by the Polyphen tool. As rs2234962 could tag functional SNPs absent from the genotyping array used in our study, we examined the LD of this SNP with other variants in the region and observed that only two SNPs in HapMap release 22 were in tight LD \( (r^2 > 0.80) \) with the lead

![Figure 3](image-url)
SNP and both were intronic with no evidence that they could be functional.

Several variants on BAG3 may affect dilated cardiomyopathy risk

BAG3 presents several non-synonymous SNPs that are referenced in the HapMap database. To complement our analysis of the locus, we selected two of these SNPs, rs3858340 (c.C1526T, p.P407L) and rs35434411 (c.G518A, p.R71Q), on the basis of a MAF > 5% in the HAPMAP CEU population, and individually genotyped them in the discovery cohorts. The rs35434411 was relatively rare and it was not associated with DCM status (MAF 0.032 and 0.029 in patients and controls, respectively). Conversely, rs3858340 was significantly associated with DCM (P = 3.6 × 10⁻³, MAF of 0.12 and 0.081 in patients and controls, respectively). Multiple logistic regression analysis indicated that both rs2234962 and rs3858340 were independently associated with DCM (P = 3.1 × 10⁻¹² and P = 3.6 × 10⁻³, respectively). In the discovery cohort, these two SNPs were in complete LD (D' = −1) defining three haplotypes, C757-C1526 (R-P), T757-C1526 (C-P), and T757-T1526 (C-L). According to the Forest plots in Figure 4, the presence of an arginine (R) at Position 151 and a proline (P) at Position 407 of BAG3 is associated with a reduced risk of DCM.

Finally, simultaneously testing the associations of HSPB7 and BAG3 individual genotypes with DCM in the discovery samples showed that the effect of both SNPs was additive (rs945417: P < 2.5 × 10⁻⁸, rs2234962: P < 5.6 × 10⁻¹³), with no evidence of interaction (P = 0.7).

Rare mutations in BAG3 are present in patients with familial forms of dilated cardiomyopathy

Given the apparent involvement of non-synonymous BAG3 SNPs in sporadic DCM, we investigated whether mutations in BAG3 might contribute to monogenic forms of DCM. The plausibility of such implication was reinforced by a previously reported linkage between markers in the chromosome 10q25–26 region, including the BAG3 locus, with familial DCM.28 The four BAG3 exons and intron–exon boundaries were sequenced in 168 independent index cases from DCM families of mainly European origin. A total of 19 molecular variants were detected (Figure 5 and Supplementary material online, Table S6); among them, six were known SNPs referenced in dbSNP, four were present in a control group of 347 healthy individuals of European descent in which they were tested, and three were unlikely to be deleterious (see legend of Supplementary material online, Table S6). The six remaining variants include four truncating and two missense mutations (Supplementary material online, Figure S3). Two of the BAG3 mutations were observed in relatively large families (six and five proven mutation carriers, respectively) and the four others were observed in small families (one to two mutation carriers in each of these families) (Supplementary material online, Figure S4). The presence of DCM was observed in 13/18 mutation carriers (72%) and possible DCM in 3 (LV dilatation and mild LVEF < 60% in 3, congestive heart failure in 2). Two mutation carriers had a normal cardiac examination (a 7-year-old male and a 33-year-old female). Overall, the predicted severity of the mutations, their pattern of distribution in affected families, and their absence in a sample of 347 healthy individuals suggested that they may be disease causing.

Phenotypically, DCM patients carrying a mutation showed isolated DCM, without associated conduction defect or skeletal myopathy and a normal serum creatine kinase level in all but one patient (Supplementary material online, Table S7). Four patients had heart transplantation and five relatives, whose DNA was not available, died prematurely from cardiac cause with a previous diagnosis of DCM.

Our GWAS and familial results implicating BAG3 in DCM may constitute an example of a gene harbouring common susceptibility variants implicated in sporadic forms of the disease and rare mutations responsible for monogenic forms.

Discussion

We report the results of the first GWAS conducted in patients with HF due to DCM. For the discovery phase of this study, we used a DNA-pooling approach and identified 14 SNPs at different loci showing some evidence of association with DCM. Three of these associations were confirmed by individual genotyping and two of them were replicated in independent DCM patients and controls.
The first DCM-associated SNP is located in a region exhibiting strong LD that encompasses the HSPB7 and the CLCNKA gene, two potential candidate genes. Recently, Matkovich et al. \textsuperscript{23} identified several SNPs in the HSPB7 sequence in patients with sporadic cardiomyopathy. According to the HapMap data, \textsuperscript{29} some of these polymorphisms are in strong LD with the SNPs investigated in the present study, suggesting that the variants reported in both studies correspond to the same Yin/yang haplotypic structure. Yin/yang haplotypes are commonly observed in the human genome. \textsuperscript{30} Such configuration has already been reported for the CLCNKA and CLCNKB genes \textsuperscript{31} and it is likely that the rs10927887 exonic CLCNKA SNP recently suggested to be responsible for modulating the risk of heart failure \textsuperscript{26} also belongs to this yin/yang haplotype, given its strong LD with the rs1739843 intronic HSPB7 SNP. Although functional experiments demonstrated a 50% loss of function of the CLCNKA chloride channel associated with rs10927887, \textsuperscript{26} linking this mechanism with risk of heart failure remains speculative. On the other hand, the association of several CMD-associated SNPs with HSPB7 gene expression in macrophages suggests that HSPB7 might be the culprit gene. HSPB7 is also called cardiovascular HSP as a consequence of its selective expression in cardiovascular tissues. \textsuperscript{22} and it belongs to the small HSP (sHSP) family, \textsuperscript{32} whose members bind denatured proteins. The physiological response of muscle fibres to stress involves sHSPs and HSPs of high molecular mass, such as HSP70. An example is provided by HSPB5 (\(\alpha\)B-crystallin); a dominant mutation in this gene causes a severe form of desmin-related cardiomyopathy characterized by impaired autophagy and accumulation of misfolded proteins. \textsuperscript{33} In humans, genetic variants in HSPB7 have been reported to be associated with advanced heart failure and systolic dysfunction of unspecific origin. \textsuperscript{23,34}

The second DCM-associated SNP affects the sequence of the protein encoded by BAG3. Even if suggestive evidence supporting a role for the C151R and P407L common variants has been

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**Figure 5** Variants in BAG3 found in index patients with familial dilated cardiomyopathy (DCM). (A) Genomic structure of the BAG3 gene. The four exons are presented as boxes (white for UTR, grey for coding). Upper horizontal lines indicate sequenced regions. (B) BAG3 transcript with all missense and frame shift variants positions identified in familial DCM cases indicated. The variants are classified as likely disease causing (red), possibly disease causing (black), or probably neutral (green) as explained in the Results section. Indicates SNPs associated with sporadic DCM in the GWAS. All likely and possibly disease-causing variants were found each in a single independent individual at the heterozygous state. The electrophoregrams represent the heterozygous mutated (upper) and homozygous wild-type (lower) sequences are shown for each DCM mutation. The arrows indicate the modified nucleotides and the sequenced strand orientation. (C) Schematic representation of the BAG3 protein with referenced domain signature according to UniprotKB database \textsuperscript{38} and dark grey boxes. The consequences of the DNA variants in (B) are shown as result predicted amino acid changes with the same colour code. (D) The ClustalW multiple alignments of orthologous BAG3 sequences from different species restricted to the immediate vicinity of each missense variant (red boxes) with MAF > 5% is shown. Interspecies conservation is indicated as blue boxes (dark blue: identical; light blue: similar; white: not conserved amino acid).
presented in this report, extensive work is required to better characterize the exact contribution of all common BAG3 SNPs on the disease. BAG3 is a member of a conserved family of cytoprotective co-chaperone proteins containing a conserved domain able to interact with HSC70/HSP70 and sHSPs proteins. BAG3 is involved in numerous activities including macro-autophagic protein degradation in aging cells. BAG3 is mainly expressed in striated muscle and colocalizes with Z-disc (a sarcomeric protein assembly essential for actin anchoring in striated muscles). Following normal muscle development, Bag3-deficient mice present a progressive myopathy with Z-disc disruption and develop a fulminating myopathy characterized by non-inflammatory myofibrillar degeneration with apoptotic features. Autophagic degradation appears essential for maintaining Z-disc integrity and muscle contractility and Bag3 plays an essential role in this process which also involves small Hsps, such as HspB8.

In humans, a mutation in BAG3 (P209L), which affects a conserved I-P-V motif of the BAG3 protein known to play an important role in the interaction of BAG3 with sHSP, causes severe dominant childhood muscular dystrophy with cardiomyopathy. In the present study, six mutations that affect the BAG domain of BAG3, either by substituting highly conserved amino acids or by deleting the whole domain, were identified. Surprisingly, none of the carriers of the mutations presented with myofibrillar disorders as reported for the P209L mutation, suggesting that different mutations in the BAG3 gene could result in different phenotypes. In Bag3 knock-out mice, fulminant myopathy and cardiomyopathy develop in homozygous --/-- but not in heterozygous animals, while in Drosophila, the Bag3 ortholog starvin deletion is associated with locomotion decline and myofibre sarcomeric disorganization even in heterozygous animal. Further experiments are clearly needed to better understand the pathophysiology of DCM caused by BAG3 mutations.

Chaperone-assisted degradation or selective autophagy appears to be an essential mechanism for the preservation of cellular structures in striated muscles that is evolutionarily conserved from flies to human and implicates Bag3, Hsc70, and small Hsps. As far as we know, no mention of interaction between Bag3 and HspB7 has been made in the literature, but the possible implication of these two proteins in human DCM may point to a common important pathophysiological pathway implicating an inappropriate handling of degraded proteins within striated myocytes. Alternatively, in the absence of identification of the functional variant(s) in the region with strong LD around HSPB7, an effect on the DCM risk that is independent of HSPB7 cannot be ruled out, an alternative candidate being CLCNKA.

**Study limitations**

Even when a very strict methodology is applied, estimates of allelic effects based on DNA pools are affected by variation in the quantity and concentration of individual DNAs composing the pools and by quantification errors. The reduced specificity of pools—GWAS compared with individual-based GWAS may be compensated by individual-based genotyping of the most significant hits in the same samples. As shown in Table 2, associations observed in the pools—GWAS were more frequently reproduced by individual genotyping when several SNPs in LD at the same locus were associated with the disease. The observation made at the ZBTB17/HSPB7/CLCNKA locus is interesting in this regard, because the strongest hits were not the same in the pools—GWAS and in the individual genotyping. As a consequence of errors inherent to the DNA pooling approach, pools—GWAS also exhibit a reduced sensitivity, implying that our study may have missed some associations that would have been identified by an individual-based GWAS. Nevertheless, this approach is efficient and it allowed us to replicate the only locus identified so far for sporadic DCM and to identify another locus whose implication in this disease was unsuspected.

Among the six mutations detected in family index cases, 5 were observed in C151C homozygous individuals while one was observed in a C151R heterozygous individual. Resequencing of BAG3 in all sporadic DCM cases will be needed to assess whether rare causal mutations may occur preferentially in association with the C151 allele and contribute to the association observed between the C151R SNP and sporadic DCM.

**Conclusion**

This pools—GWAS identified two loci associated with sporadic DCM. The most likely candidate gene at the chromosome 10 locus is BAG3, whereas HSPB7 and CLCNKA are two potential candidates at the chromosome 1 locus. Beyond DCM, it will be of interest to investigate the implication of the these loci in other forms of heart failure and muscle dysfunction, as well as in the age-related changes that affect the heart and muscle physiology.

**Supplementary material**

Supplementary material is available at European Heart Journal online.

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Heart failure due to dilated cardiomyopathy


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**Left bundle branch block during pregnancy as a sign of imminent peripartum cardiomyopathy**

_Saida Labidi, Denise Hilfiker-Kleiner*, and Gunnar Klein_

Departments of Cardiology and Angiology, Medizinische Hochschule Hannover, Medical School Hannover, Carl-Neuberg Str. 1, 30625 Hannover, Germany

*Corresponding author. Tel: +49 511 532 2531, Fax: +49 511 532 3263, Email: hilfiker.denise@mh-hannover.de*

We describe the development of peripartum cardiomyopathy (PPCM) in a 32-year-old woman. Because of a known cardiomyopathy of her mother, she obtained five cardiologic examinations from the gestational age of weeks 16–38 with ECG and echocardiograms. Except that the ECG showed a complete left bundle branch block (LBBB), the patient experienced no cardiac disorder throughout her pregnancy (Panels A and B, Supplementary material online). After spontaneous and uncomplicated vaginal delivery of an eutrophic daughter, she was discharged from hospital in a healthy condition. Six months later, she had another follow-up ECG and echocardiogram where she did not report any dyspnoea or oedema but the LBBB persisted and she displayed a left ventricular enlargement and reduced ejection fraction (EF) of 40% (Panel B). A week later, she presented with an EF of 30% and persistent LBBB (Panel B), symptoms of dyspnoea and dry cough, and was admitted to hospital. After exclusion of common causes for heart failure, i.e. no evidence of ischaemia and late enhancement by MRI and no evidence of significant valvular heart disease by echo, no evidence of myocarditis and amyloidosis by right ventricular biopsy, PPCM was diagnosed. The patient, still nursing at that time, was ablactated with the prolactin blocker bromocriptine (2.5 mg/day for 10 days, Supplementary material online) and heart failure medication (ACE-inhibitors, diuretics, beta-blockers) was initiated. Since an anti-angiogenic 16 kDa fragment generated from prolactin seems to be a driving factor in PPCM (see Hilfiker-Kleiner et al., *Cell* 2007;128:589–600), blocking prolactin by bromocriptine may promote recovery in PPCM patients, a notion supported by a small, randomized clinical trial in PPCM patients (Silwa et al., *Circulation* 2010;121:1165–1173). Indeed, the patient showed rapid improvement (Panel B). Follow-up visits up to 12 months postpartum showed normalized cardiac dimension and function and LBBB had disappeared (Panels B and C). Our case report reveals that LBBB in a pregnant woman should be taken seriously and may be the first and only sign of developing PPCM.

**Panel A.** ECG of the patient at 8 months of pregnancy, recorded at 50 mm/s and 10 mm/mV, showing complete left bundle branch block during uneventful pregnancy.

**Panel B.** Table displaying heart rate (HR), PQ, QRS, ejection fraction (EF), and left ventricular end-diastolic diameter (LVEDD) throughout pregnancy (Prg, blue), onset of PPCM 6 months postpartum (PP, red), and recovery starting 8 months postpartum monitored to 12 months postpartum (PP, black). Asterisks indicate abnormal QRS, EF, LVEDD, and HR.

**Panel C.** Normal ECG of the patient 8 months postpartum, recorded at 50 mm/s and 10 mm/mV.

**Supplementary material**
Supplementary material is available at *European Heart Journal* online.

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