Involvement of BAG3 and HSPB7 loci in various etiologies of systolic heart failure: Results of a European collaboration assembling more than 2000 patients

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Involvement of *BAG3* and *HSPB7* loci in various etiologies of systolic heart failure:

Results of a European collaboration assembling more than 2,000 patients

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Heart failure (HF), a major public health burden affecting 2% of industrialized populations, is a syndrome resulting from structural or functional myocardial impairment leading to inadequate cardiac output to meet the body's metabolic demands (1). Half of HF patients present systolic dysfunction (systolic-HF), also called reduced ejection fraction (HF-REF), a disease related to various causes including idiopathic Dilated Cardiomyopathy (DCM) and Coronary Artery Diseases (CAD) (ischemic-HF). HF is usually a multifactorial disease but the genetic variants contributing to its susceptibility or its severity may be different according to its underlying causes (2) and their identification is only beginning.

A previous genome wide association study (GWAS) (3) identified two single nucleotide polymorphisms (SNPs) associated with DCM: rs2234962 within *BAG3* gene (B-cell lymphoma 2-associated athanogene 3) and rs10927875 within a locus encompassing five genes including *HSPB7* (heat shock 27 kDa protein family, member 7) (further called *HSPB7* locus). Other studies also reported associations between polymorphisms in the *HSPB7* locus and HF (4-6), two of them assessing the implication of the locus in the subgroup of ischemic-HF patients (4, 5), but none addressed the role of *BAG3* in ischemic-HF nor the potential impact of *HSPB7* and *BAG3* loci on HF severity. We hypothesized that these two loci could be involved in susceptibility to syst-HF due to CAD (ischemic-HF) and/or in severity of syst-HF due to CAD or DCM.

All participants were of European origin, had a diagnosis of systolic-HF (Left Ventricle Ejection Fraction, LVEF, < 45%) and gave written informed consent. The study complies with the principles of the Declaration of Helsinki and was approved by local ethics committees. In total, 1160 ischemic-HF patients and 1612 controls and 1141 DCM patients were studied. Genotyping of rs2234962-*BAG3* and rs10927875-*HSPB7* was performed with TaqMan technology. Disease severity was assessed in patients with Ischemic-HF or DCM as

following: LVEF, Left Ventricle End Diastolic Diameter (LVEDD), NYHA dyspnea at inclusion and Age at diagnosis.

We analyzed the data following this summarized methodology. After quality controls and Hardy-Weinberg equilibrium testing in controls (p<0.05), the association between Ischemic-HF and individual genotypes was tested using a logistic regression model adjusting on age, gender, and study population in the R statistical environment (7). We assumed an additive mode of inheritance and decided to adjust the p-value threshold following Bonferroni correction for two independent tests (p-value <0.025 considered significant). Severity assessment in patients was performed via linear regression analyses adjusted for age, sex, and origin depending on the SNP genotype exposure.

Considering the allelic odds ratios for rs10927875-*HSPB7* and rs2234962-*BAG3* associations with DCM (0.71 and 0.54 respectively) (3) and the p-value threshold of 0.025, our study had a 95% and 99% power to detect similar associations.

We observed that rs10927875- *HSPB7* is significantly associated with Ischemic-HF (Table 1), with a MAF lower in patients than in controls (0.29 *vs.* 0.33, p=0.0017, odds ratio 0.96 [0.94-0.98]), which is consistent with a protective effect of the T minor variant against Ischemic-HF. No significant association was detected between rs2234962-*BAG3* and Ischemic-HF (Table 1), even though a trend was detected.

Regarding severity, rs2234962-*BAG3* was significantly associated with LVEDD both in DCM (adjusted-p: 0.0056, Table 2, N=1076 patients) and ischemic-HF patients (adjusted-p: 0.01, N=359 patients). In the ischemic-HF population, the major allele T of rs2234962-*BAG3* was associated with enlarged LVEDD (TT: 64.0 ±8.7 mm; TC: 62.7 ±8.0 mm and CC: 61.8 ±9.8 mm) and thus with a greater severity of the disease. Results are less clear in DCM

patients although the same trend could be observed between major homozygous and heterozygous alleles (TT: 70.9 ±9.6 mm and TC: 69.1 ±9.2 mm respectively).

No other association could be revealed between the two SNPs and any of the other severity criteria.

The protective effect of the T minor allele of rs10927875-*HSPB7* against ischemic-HF is consistent with previous findings in DCM patients (3). Matkovich and Cappola also described SNPs in *HSPB7* locus, rs1739840 and rs1739843 respectively, which exhibited a lower MAF in Ischemic-HF cases than in controls (0.4126 *vs.* 0.4735 and 0.367 *vs* 0.436 respectively) (5, 6).

Finding an association between ischemic-HF and *HSPB7* locus but not with *BAG3* locus suggests that the genetic background of multifactorial systolic-HF is different according to its underlying causes. This is meaningful since the pathophysiological pathways of these two diseases are different, DCM resulting from a primary myocardial defect whereas ischemic-HF is due to ischemia and necrosis. Since *HSPB7* locus appears involved in both causes, this may reflect a pivotal role of gene(s) in the locus as a poorly adaptive process to various triggers. The restricted association of *BAG3* locus with DCM suggests a more specific role in the context of a primary myocardial defect.

rs10927875-*HSPB7* locates in an intron of *ZBTB17* on chromosome 1p36, but the locus encompasses several other genes—*SPEN*, *HSPB7*, *CLCNKA* and CLCNKB—exhibiting strong linkage disequilibrium. *HSBP7* is considered as a strong candidate because it exhibits cardiac-specific expression and some of its variants have been independently associated with advanced HF, systolic-HF (8) or with DCM (4-6). A recent study found that *HSPB7* is early expressed in embryonic heart and have a major role in heart embryogenesis (9).

BAG3 is mainly expressed in striated muscle and is involved in anti-apoptosis and anti-proteotoxicity pathways (10). rs2234962-*BAG3,* a non-synonymous SNP (c.T757C, p.C151R) predicted as probably damaging by Polyphen, is associated in our study with LVEDD in both ischemic-HF and CMD populations. The number of T alleles appears related with enlarged LVEDD in ischemic-HF patients but this was less clear for DCM patients, possibly due to the very low T homozygous DCM patients (9 out of the 1141). A less efficient *BAG3* activity may favour HF severity but the hypothesis is speculative.

Out of the two loci previously associated with DCM, we observed that *HSPB7* locus was also associated with ischemic-HF whereas *BAG3* locus was not. Conversely, *BAG3* locus was associated with LVEDD in both DCM and syst-HF patients. Our results suggest investigating further the role of these two loci in the pathophysiology of HF of various causes.

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CONFLICT OF INTEREST

The authors report no relationships that could be construed as a conflict of interest

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Table 1: Genotypic repartition of cases (ischemic-HF), controls and allelic comparison test assuming an additive allele effect

SNP (genotypes)	Population	Patients Genotype	MAF	Controls Genotypes	MAF	p-adj*	OR [CI 95%]
rs10927875-*HSPB7* (TT/TC/CC)	Germany	18/133/147	0.28	32/127/115	0.35	0.06	0.95 [0.90-1.00]
	France	28/153/173	0.29	84/310/312	0.34	0.17	0.97 [0.94-1.01]
	UK	33/221/235	0.29	47/271/256	0.32	0.30	0.98 [0.96-1.01]
	All	79/507/555	0.29	163/708/683	0.33	$1.7E^{-3}$	0.96 [0.94-0.98]
rs2234962-*BAG3* (CC/TC/TT)	Germany	12/92/192	0.20	12/90/171	0.21	0.75	0.99 [0.93-1.05]
	France	13/103/241	0.18	32/232/433	0.21	0.13	0.96 [0.92-1.00]
	UK	13/151/323	0.18	18/199/346	0.21	0.43	0.99[0.95-1.02]
	All	38/346/756	0.18	62/521/960	0.21	0.07	0.97 [0.94-1.00]

This table gives the genotypic repartition for the three SNPs in the corresponding population. The deducted Minor Allele Frequency (MAF) is also given. *p-adj, p-value adjusted for age, gender and population. OR calculation was done on the row genotypes data thus no adjustment was performed.

Table 2: Association with severity sub-phenotypes for French, German and Italian DCM patients.

Population		France (N=749)	Germany (N=278)	Italy (N=114)	Total (N=1141)
SNP	Severity criteria	p-adj*	p-adj*	p-adj*	p-adj*
rs10927875-HSPB7	LVEF (N=1004)	0.14	0.24	0.05	0.87
	LVEDD (1076)	0.47	0.82	0.79	0.72
	NYHA (738)	0.79	0.02	0.35	0.50
	Age at diagnosis (1141)	0.66	0.07	0.95	0.2
rs2234962-BAG3	LVEF (1004)	0.13	0.40	0.10	0.21
	LVEDD (1076)	$4.1E^{-3}$	0.37	0.78	**$5.6E^{-3}$**
	NYHA (738)	0.59	0.09	0.02	0.34
	Age at diagnosis (1141)	0.57	0.74	0.27	0.99

*The phenotypic information was not always available for the 1141 DCM cases. The exact number of tested individuals is given for each sub-phenotype. *p-adj, p-value of the test adjusted on age, gender and population.*