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Profile of micro RNAs differentially expressed in hearts from patients with hypertrophic cardiomyopathy and sarcomeric mutations.

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To the Editor:

MicroRNAs (miRNAs) regulate cardiac growth and conduction, playing an important role in cardiac diseases (1). Several miRNAs have been found differentially expressed in cardiac hypertrophic tissue compared with normal tissue, and would contribute to the development of cardiomyocyte hypertrophy (2,3). Hypertrophic cardiomyopathy (HCM) is frequently familial and caused by mutations in sarcomeric genes. To our knowledge no study has reported the miRNA expression profile in HCM tissues with sarcomeric gene mutations. To better define the HCM molecular changes, we defined the expression of well characterized miRNAs in left ventricular (LV) heart tissue from five patients who underwent a cardiac transplant and a normal heart tissue (human left ventricular tissue, Ambion-Applied Biosystems, Foster City, CA), and compared their expression profiles. Two patients were familial HCM cases carriers of *MYH7* mutations (Val822Met and Arg453Cys). Three patients were sporadic cases with LV hypertrophy secondary to heart valve disease.

The study was approved by the Ethical Comittee of Hospital Universitario Central Asturias (HUCA) and all the patients provided written informed consent. Total RNA was isolated (TRIZOL, Invitrogen) and the expression profile of 377 human miRNAs was determined in the normal LV and in a pool of the two tissues with *MYH7* mutations using the Applied Biosystems TaqMan microRNA transcription kit, Megaplex RT human primers pool A, and TaqMan human MicroRNA TLDA plate A. Each sample was analyzed by triplicate and the mean CT value for each miRNA was normalized using the mammalian U6 as the reference gene. A $p<0.05$ in the fold change (HCM-pool vs. the normal LV tissue) was considered as significant for the difference in miRNA expression. The detailed experimental procedure is available upon request to the corresponding author.

Compared to the normal LV, the MCH tissue showed an overall downregulation of miRNAs. However, for most of the miRNAs the difference was not significant, with a CT change between the 2 tissues <4 (data not shown). The expression of 19 miRNAs was significantly different between the 2 tissues (Table 1). These miRNAs were individually assayed in triplicate with real time Taqman miRNA assays (Applied Biosystems) in the LV control and the 5 pathological tissues. A total of 10 miRNAs were underexpressed (miR-1, 133b, 191, 208b, 218, 306, 30b, 374, 454, and 495) or overexpressed (590-5p and 92a) in the 5 pathological tissues. MiR-495 was the only

miRNA that differentiated the hearts with and without sarcomeric mutations. Compared to the normal tissue, miR-495 was underexpressed in the 2 samples with *MYH7* mutations and overexpressed in the 3 samples without sarcomeric mutations. This miRNA has been found deregulated in primary muscular disorders, but not in cardiac diseases. MiRNAs 590-5p and 92a were found overexpressed in all the pathological tissues. None of the two miRNAs had been previously reported as deregulated in cardiac hypertrophy and other heart diseases.

MiR-1 and miR-133 were underexpressed in the hypertrophic tissues and have been previosuly implicated in cardiac development and were significantly downregulated in hearts from patients with idiopathic and ischemic cardiomyopathies (4, 5**)**. MiR-208a and miR-208b are encoded by introns at the *MYH6* and *MYH7* genes, respectively. In mice, the reexpression of myh-7 and miR-208b is a characteristic of cardiac hypertrophy in response to pressure overload. In agreement with a role for these miRNAs in the development of HCM, miR-208a was also overexpressed in the 2 patients with *MYH7* mutations. Interestingly, the miR-208a fold change in one of the HCM patients was the highest among all the miRNAs analysed in our study (Table 1). The downregulation of miR-208b was lower in the 3 patients with cardiac hypertrophy secondary to valve disease, suggesting that the expression changes in this miRNA could differ between hypertrophic hearts with and without sarcomeric gene mutations.

Compared to other studies on samples from patients with heart failure we analyzed pathological tissues with a recognized sarcomeric mutation that would be the primary cause of the hypertrophy in these patients. Changes in miRNA expression could differ between hypertrophic hearts with sarcomeric mutations and those hearts in which the disease was secondary to another condition that could cause the hypertrophy. The difference between these cases and those without sarcomeric mutations should be replicated in other patients, including cases with mutations at different sarcomeric genes. We also studied failing explanted hearts that represented advanced stages of the disease. We cannot thus exclude that some of the deregulated miRNAs were not representative of the changes at the initial stages of the disease.

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Table 1. Fold change of RQ values compared to the normal left ventricular tissue and p-values (inparentheses) for the 19 miRNAs selected after the TLDA-array screening.

miRNA	**HC** **mean CT**	**H1**[a]	**H9**[a]	**H2**	**H3**	**H5**
1	15	**0.13** (0.003)	**0.13** (0.004)	**0.34** (0.006)	**0.004** (0.002)	**0.15** (0.004)
133a	15	**0.67** (0.04)	**0.23** (0.007)	**0.28** (0.02)	**0.36** (0.006)	**1.00** (0.9)
133b	19	**0.50** (0.05)	**0.30** (0.02)	**0.55** (0.12)	**0.11** (0.002)	**0.04** (0.4)
191	14	**0.37** (0.006)	**0.13** (0.003)	**0.40** (0.01)	**0.84** (0.08)	**0.21** (0.07)
218	22	**0.77** (0.32)	**0.26** (0.02)	**0.62** (0.03)	**0.39** (0.005)	**0.49** (0.05)
30b	16	**0.59** (0.01)	**0.58** (0.03)	**0.48** (0.01)	**0.04** (0.002)	**0.29** **(0.004)**
374	22	**0.46** (0.008)	**0.4** (0.01)	**0.90** (0.93)	**0.19** (0.005)	**0.53** (0.14)
454	25	**0.12** (0.003)	**0.001** (0.05)	**0.19** (0.004)	**0.10** (0.002)	**0.58** (0.04)
495	25	**0.68** (0.05)	**0.42** (0.007)	**1.30** (0.03)	**8.32** (0.29)	**4.40** (0.04)
93	19	**0.79** (0.09)	**0.45** (0.02)	**0.82** (0.28)	**16.51** (0.12)	**1.29** (0.58)
199a-3p	29	**3.48** (0.04)	**1.86** (0.05)	**1.73** (0.07)	**0.12** (0.047)	**1.96** **(**0.40)
590-5p	17	**7.90** (0.003)	**3.33** (0.01)	**10.13** (0.002)	**1.31** (0.05)	**2.62** (0.04)
92a	23	**5.31** (0.006)	**1.65** (0.04)	**5.05** (0.007)	**1.26** (0.25)	**6.68** (0.04)
125a-3p	26	**1.37** (0.06)	**0.003** (0.002)	**0.65** (0.01)	**0.75** (0.03)	**8.26** (0.003)
208a	26	**1.17** (0.82)	**15.40** (0.002)	**7.21** (0.04)	**0.93** (0.87)	**0.47** **(0.008)**
223	17	**1.59** (0.06)	**0.18** (0.006)	**0.22** **(0.004)**	**0.14** **(0.002)**	**0.67** (0.11)
483-5p	23	**1.05** (0.08)	**4.46** (0.007)	**0.04** (0.04)	**0.872** (0.45)	**2.02** (0.10)
451	19	**2.65** (0.01)	**0.07** (<0.001)	**1.33** (0.11)	**0.02** (<0.001)	**1.09** (0.5)
208b	24	**0.79** (0.76)	**0.81** (0.66)	**0.53** (0.05)	**0.06** (0.02)	**0.18** (0.04)

[a] H1 and H9 corresponded to the patients with *MYH7* mutations, while in H2, H3, and H5 the disease was secondary to cardiac valvular disease. The mean CT values for the normal tissue are also indicated.

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