Figures and figure supplements

A deleterious gene-by-environment interaction imposed by calcium channel blockers in Marfan syndrome

Figure 1. Effect of amlodipine in wild-type (WT) and Marfan mice. (A) Echocardiography data showing mean (±2 SEM) growth in the aortic root and ascending aorta from 2 to 4 months of age. Number of mice per group (male/female) = WT placebo 11 (5/6), WT amlodipine 10 (6/4), Marfan placebo 14 (7/7), Marfan amlodipine 11 (6/5). Mean (±2 SEM) weight per group (in grams) at 4 months = 27.4 ± 2.4 g (WT placebo), 27.6 ± 2.8 g (WT amlodipine), 28.1 ± 3.0 g (Marfan placebo), 27.9 ± 2.8 g (Marfan amlodipine). (B) Survival curve from 2 to 5 months of age. Number of mice per group (male/female) = WT placebo 11 (5/6), WT amlodipine 10 (6/4), Marfan placebo 14 (7/7), Marfan amlodipine 19 (9/10). (C) Representative VVG staining (upper panel) and Masson’s trichrome staining (lower panel) of the proximal ascending aorta in 5-month-old male mice. Scale bar: 40 μm. (D) Mean (±2 SEM) aortic wall architecture score of the proximal ascending aorta in 5-month-old mice. Number of mice per group = 4 (2 male, 2 female). Scale: 1 (normal) to 5 (extensive damage). Plac, placebo; Aml, amlodipine.

DOI: 10.7554/eLife.08648.003
Figure 1—figure supplement 1. Effect of amlodipine in wild-type (WT) and Marfan mice. (S1) Mean (±2 SEM) systolic and diastolic blood pressure, and heart rate, in 3-month-old mice. Number of mice per group = 8 (4 male; 4 female). (S2) Representative latex-injected images showing ascending aortic size (distance between arrowheads) in 5-month-old male mice. Scale bar: 2 mm. (S3) Representative VVG staining (upper panel) and Masson’s trichrome staining (lower panel) of the descending thoracic aorta in 5-month-old male mice. Scale bar: 40 μm. (S4) Mean (±2 SEM) aortic wall architecture score of the descending thoracic aorta in 5-month-old mice. Number of mice per group = 4 (2 male, 2 female). Scale: 1 (normal) to 5 (extensive damage). (S5) Mean (±2 SEM) aortic root and ascending aortic growth from 2 to 4 months of age in mice treated with 3 mg/kg/day amlodipine. Number of mice per group (male/female) = WT placebo 5 (3/2), WT amlodipine 5 (2/3), Marfan placebo 10 (6/4), Marfan amlodipine 8 (4/4). Plac, placebo; Los, losartan; Aml, amlodipine.

DOI: 10.7554/eLife.08648.004
Figure 2. Effect of verapamil in wild-type (WT) and Marfan mice. (A) Mean (±2 SEM) growth in the aortic root and ascending aorta from 2 to 6 months of age. Number of mice per group (male/female) = WT placebo 8 (4/4), WT verapamil 10 (4/6), Marfan placebo 9 (5/4), Marfan verapamil 11 (6/5). Mean (±2 SEM) weight per group (in grams) at 6 months = 30.3 ± 2.6 g (WT placebo), 30.6 ± 2.2 g (WT verapamil), 31.3 ± 3.3 g (Marfan placebo), 31.5 ± 3.2 g (Marfan verapamil). (B) Representative VVG staining (upper panel) and Masson’s trichrome staining (lower panel) of the proximal ascending aorta in 6-month-old male mice. Scale bar: 40 μm. (C) Mean (±2 SEM) aortic wall architecture score of the proximal ascending aorta in 6-month-old mice. Number of mice per group = 4 (2 male, 2 female). Scale: 1 (normal) to 5 (extensive damage). Plac, placebo; Ver, verapamil.

DOI: 10.7554/eLife.08648.005
Figure 2—figure supplement 1. Effect of verapamil in wild-type (WT) and Marfan mice. (S1) Representative latex-injected images showing aortic size (distance between arrowheads) in 6-month-old male mice. Scale bar: 2 mm. (S2) Representative VVG staining (upper panel) and Masson’s trichrome staining (lower panel) of the descending thoracic aorta in 6-month-old male mice. Scale bar: 40 μm. (S3) Mean (±2 SEM) aortic wall architecture score of the descending thoracic aorta in 6-month-old mice. Number of mice per group = 4 (2 male, 2 female). Scale: 1 (normal) to 5 (extensive damage). Plac, placebo; Ver, verapamil.

DOI: 10.7554/eLife.08648.006
Figure 3. CCB effect is ERK1/2- and AT1R-dependent in wild-type (WT) and Marfan mice. (A) Western blot analysis of the aortic root and ascending aorta in 5-month-old mice. Number of mice per group = 4 (2 male, 2 female). (B) Mean (±2 SEM) ascending aortic growth from 2 to 4 months of age. Number of mice per group (male/female) = WT placebo 9 (5/4), WT amlodipine 8 (4/4), WT amlodipine + RDEA119 7 (3/4), Marfan placebo 9 (4/5), Marfan amlodipine 10 (6/4), Marfan amlodipine + RDEA119 11 (6/5). (C) Survival curve from 2 to 4 months of age. (D) Western blot analysis of the aortic root and ascending aorta.
Figure 3. Continued

aorta in 4-month-old mice. Number of mice per group = 4 (2 male, 2 female). (E) Mean (±2 SEM) ascending aortic growth from 2 to 4 months of age. Number of mice per group (male/female) = WT placebo 11 (6/5), WT amlodipine 11 (6/5), WT amlodipine + losartan 8 (4/4), Marfan placebo 7 (4/3), Marfan amlodipine 6 (3/3), Marfan amlodipine + losartan 9 (4/5). (F) Western blot analysis of the aortic root and ascending aorta in 4-month-old mice. Number of mice per group = 3 (2 male, 1 female; or 1 male, 2 female). Plac, placebo; Aml, amlodipine; RDEA, RDEA119; Los, losartan; Geno, genotype; Treat, treatment; I/A, interaction.

DOI: 10.7554/eLife.08648.007

Figure 3—figure supplement 1. CCB effect is ERK1/2- and AT1R-dependent in wild-type (WT) and Marfan mice. (S1) Representative latex images, VVG and trichrome staining in 4-month-old male mice. Latex scale bar: 2 mm. VVG and trichrome scale bar: 40 μm. (S2) Mean (±2 SEM) aortic architecture score in 4-month-old mice. Number of mice per group = 4 (2 male, 2 female). Plac, placebo; Aml, amlodipine; RDEA, RDEA119.

DOI: 10.7554/eLife.08648.008
Figure 4. PKC activation in placebo- and CCB-treated wild-type (WT) and Marfan mice. (A) Western blot analysis of the aortic root and proximal ascending aorta in 4-month-old mice. Number of mice per group = 4 (2 male, 2 female). (B) Western blot analysis of the aortic root and proximal ascending aorta in 4-month-old mice. Number of mice per group = 3 (2 male, 1 female; or 1 male, 2 female). (C) Mean (±2 SEM) ascending aortic growth from 2 to 4 months of age. Number of mice per group (male/female) = WT placebo 8 (4/4), WT amlodipine 9 (4/5), WT amlodipine + enzastaurin 8 (3/5), Marfan placebo 3 (1/2). Figure 4. continued on next page.
Figure 4. Continued

12 (7/5), Marfan amlodipine 8 (5/3), Marfan amlodipine + enzastaurin 8 (5/3). (D) Mean (±2 SEM) aortic root growth from 2 to 4 months of age. Number of mice per group (male/female) = WT placebo 8 (4/4), WT enzastaurin 6 (3/3), Marfan placebo 12 (7/5), Marfan enzastaurin 8 (4/4). (E) Western blot analysis of the aortic root and ascending aorta in 4-month-old mice. Number of mice per group = 4 (2 male, 2 female). Plac, placebo; NAb, neutralizing antibody; Los, losartan; Aml, amlodipine; Enz, enzastaurin; Geno, genotype; Treat, treatment; I/A, interaction.

DOI: 10.7554/eLife.08648.009

Figure 4—figure supplement 1. PKC activation in placebo- and CCB-treated wild-type (WT) and Marfan mice. (S1) Representative latex-injected images of 4-month-old male mice. Scale bar: 2 mm. (S2) Representative VVG staining (upper panel) and Masson’s trichrome staining (lower panel) of the proximal ascending aorta in 4-month-old male mice. Scale bar: 40 μm. (S3) Mean (±2 SEM) aortic architecture score of the aortic root and proximal ascending aorta in 4-month-old mice. Number of mice per group = 4 (2 male, 2 female). Scale: 1 (normal) to 5 (extensive damage). Plac, placebo; Aml, amlodipine; Enz, enzastaurin.

DOI: 10.7554/eLife.08648.010
Figure 5. Effect of hydralazine in wild-type (WT) and Marfan mice. (A) Mean (±2 SEM) aortic root growth from 2 to 6 months of age. Number of mice per group (male/female) = WT placebo 9 (4/5), WT hydralazine 12 (7/5), Marfan placebo 15 (6/9), Marfan hydralazine 12 (6/6). Mean (±2 SEM) weight per group (in grams) at 6 months = 31.4 ± 2.4 g (WT placebo), 31.2 ± 3.1 g (WT hydralazine), 31.0 ± 2.7 g (Marfan placebo), 30.4 ± 3.5 g (Marfan hydralazine). (B) Western blot analysis of the aortic root in 6-month-old mice. Number of mice per group = 4 (2 male, 2 female). (C) Diagram illustrating key nodal points in Marfan mouse aortic disease pathogenesis. Drugs shown in red ameliorate aneurysm progression, while manipulations shown in blue exacerbate it. Plac, placebo; Hyd, hydralazine; Geno, genotype; Treat, treatment; I/A, interaction.

DOI: 10.7554/eLife.08648.011
Figure 5—figure supplement 1. Effect of hydralazine in wild-type (WT) and Marfan mice. (S1) Mean (±2 SEM) systolic and diastolic blood pressure, and heart rate, in 3-month-old mice. Number of mice per group = 8 (4 male, 4 female). (S2) Representative parasternal long-axis in vivo echocardiography images of 6-month-old male mice. (S3) Representative VVG staining (upper panel) and Masson’s trichrome staining (lower panel) of the aortic root in 6-month-old mice. (S4) Mean (±2 SEM) aortic wall architecture score of the aortic root in 6-month-old mice. Number of mice per group = 4 (2 male, 2 female). Scale: 1 (normal) to 5 (extensive damage). (S5) Western blot analysis of the aortic root in 4-month-old mice. Number of mice per group = 4 (2 male, 2 female). Plac, placebo; Hyd, hydralazine; Aml, amlodipine; RDEA, RDEA119; Geno, genotype; Treat, treatment. DOI: 10.7554/eLife.08648.012