



## CLINICAL RESEARCH ARTICLE

# Association between genetic variants in the HIF1A-VEGF pathway and left ventricular regional myocardial deformation in patients with hypertrophic cardiomyopathy

Guido E. Pieleś<sup>1,2</sup>, Jaime Alkon<sup>1</sup>, Cedric Manlhiot<sup>1</sup>, Chun-Po Steve Fan<sup>1</sup>, Caroline Kinnear<sup>1</sup>, Leland N. Benson<sup>1</sup>, Seema Mital<sup>1</sup> and Mark K. Friedberg<sup>1</sup>

**BACKGROUND:** Information on genetic etiology of pediatric hypertrophic cardiomyopathy (HCM) rarely aids in risk stratification and prediction of disease onset. Little data exist on the association between genetic modifiers and phenotypic expression of myocardial performance, hampering an individual precision medicine approach.

**METHODS:** Single-nucleotide polymorphism genotyping for six previously established disease risk alleles in the hypoxia-inducible factor-1 $\alpha$ -vascular endothelial growth factor pathway was performed in a pediatric cohort with HCM. Findings were correlated with echocardiographic parameters of systolic and diastolic myocardial deformation measured by two-dimensional (2-D) speckle-tracking strain.

**RESULTS:** Twenty-five children ( $6.1 \pm 4.5$  years; 69% male) with phenotypic and genotypic (60%) HCM were included. Out of six risk alleles tested, one, *VEGF1 963GG*, showed an association with reduced regional systolic and diastolic left ventricular (LV) myocardial deformation. Moreover, LV average and segmental systolic and diastolic strain and strain rate were significantly reduced, as assessed by the standardized difference, in patients harboring the risk allele.

**CONCLUSIONS:** This is the first study to identify an association between a risk allele in the VEGF pathway and regional LV myocardial function, with the *VEGF1 963GG* allele associated with reduced LV systolic and diastolic myocardial performance. While studies are needed to link this information to adverse clinical outcomes, this knowledge may help in risk stratification and patient management in HCM.

*Pediatric Research* (2021) 89:628–635; <https://doi.org/10.1038/s41390-020-0929-z>

**IMPACT:**

- Risk allele in the *VEGF* gene impacts on LV myocardial deformation phenotype in children with HCM.
- LV 2-D strain is significantly reduced in patients with risk allele compared to non-risk allele patients within HCM patient groups.
- Describes that deficiencies in LV myocardial performance in children with HCM are associated with a previously identified risk allele in the angiogenic transcription factor VEGF.
- First study to identify an association between a risk allele in the VEGF pathway and regional LV myocardial deformation measured by 2-D strain in children with HCM.

**INTRODUCTION**

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiomyopathy with significant morbidity and risk of sudden cardiac death.<sup>1</sup> HCM shows a variable genotype–phenotype correlation and penetrance, as well as a remarkable phenotypic heterogeneity.<sup>2</sup> Often, underlying sarcomeric disease-causing mutations do not correlate with the phenotype and genotypic markers that can predict disease severity have yet to be found. This has hampered the use of genetic information in patient risk stratification. In particular, there is little data correlating genetic data with systolic or diastolic myocardial performance in this myocardial disease. While most research has focused on the sarcomeric, disease-producing, genes,

recently, non-synonymous single-nucleotide polymorphisms (SNPs) in modifier genes in the pro-fibrotic and pro-inflammatory pathways have been identified in pediatric patients with HCM and are shown to have an impact on age of presentation, severity of disease, and cardiac diastolic dysfunction that is independent of underlying disease-causing mutation in sarcomeric genes.<sup>3</sup> In particular, allelic variation in these pro-fibrotic and pro-inflammatory genes have been shown to be a predictor of diastolic dysfunction in HCM as measured by two-dimensional (2-D) and tissue Doppler echocardiography.<sup>3</sup> This is of importance as diastolic dysfunction is a hallmark of HCM and a significant cause for HCM-associated morbidity and mortality in adult and pediatric HCM.<sup>1,4</sup> In HCM patients, the grade of diastolic

<sup>1</sup>Department of Pediatrics, Labatt Family Heart Centre, Hospital for Sick Children, University of Toronto, Toronto, ON, Canada and <sup>2</sup>NIHR Cardiovascular Biomedical Research Centre, Bristol Heart Institute, Bristol, UK

Correspondence: Guido E. Pieleś ([guido.pieles@bristol.ac.uk](mailto:guido.pieles@bristol.ac.uk))

Received: 7 June 2019 Revised: 2 February 2020 Accepted: 17 February 2020

Published online: 6 May 2020

dysfunction correlates to exercise capacity<sup>5</sup> and is an outcome predictor.<sup>6,7</sup>

Strain imaging to assess myocardial deformation is a more recent sensitive<sup>8</sup> tool in the assessment of systolic and diastolic myocardial function in HCM.<sup>9</sup> Mean 2-D longitudinal strain is reduced in HCM and it is particularly useful in the assessment of regional wall motion abnormalities and dysfunction, a common feature of HCM.<sup>10</sup> Importantly, as traditional indices of left ventricular (LV) systolic function such as ejection fraction (EF) are typically normal, or even increased, in HCM, myocardial deformation imaging using 2-D strain can detect regional dysfunction in the context of normal EF.<sup>11</sup>

Furthermore, segmental 2-D and 3-D speckle-tracking strain has been shown to correlate with fibrosis in patients with HCM.<sup>11,12</sup> Thus, 2-D systolic and diastolic strain imaging is a promising technology to explore phenotype–genotype correlations in HCM, but its relationship to genotype/modifier genes has not been investigated. Fibrosis in HCM may be mediated through the hypoxia-inducible factor-1 $\alpha$  (HIF1A) and vascular endothelial growth factor (VEGF) pathway, activated by a thickened, hypoperfused, and hypoxic myocardium.<sup>13</sup> This leads to the activation of not only HIF1A, which in turn stimulates angiogenesis,<sup>14</sup> but also endothelial–mesenchymal transition via activation of transforming growth factor  $\beta$ 1 (TGF $\beta$ 1) that can lead to myocardial fibrosis,<sup>15</sup> which is an important predictor of adverse outcome in HCM.<sup>16</sup> In addition, VEGF is an important angiogenic factor and variants that reduce its activity could impair angiogenesis and adversely impact adaptation to myocardial ischemia.

This study investigates the relationship between previously identified allelic variance in the pro-fibrotic and angiogenic gene *VEGF* and hypoxia-response gene *HIF1A* and the level of LV myocardial systolic and diastolic dysfunction as measured by 2-D strain. We hypothesize that functional SNP in the HIF1A-VEGF pathway can lead to reduced myocardial deformation.

## METHODS

In a prospective cohort study, unrelated HCM cases <18 years old were enrolled at the Hospital for Sick Children Toronto (2007–2010). Patients with significant valvar insufficiency, hypertension, congenital heart disease, and metabolic disorders were excluded. The study was approved by the Institutional Review Board of the Hospital for Sick Children and informed consent was obtained from all subjects or parents/legal guardians.

### Echocardiography

All patients underwent 2-D echocardiography using IE-33 (Philips Ultrasound, Bothell, WA) or Vivid 7 (GE, Horten, Norway) systems. Measurements were made offline by a single investigator (J.A.) who was blinded to subject genotype using commercially available software (Syngo Dynamics, Siemens, Malvern, PA). Echocardiographic data was obtained at last follow-up or last investigation before myectomy or transplantation. Echocardiographic assessment included 2-D measurements of LV dimensions in parasternal long- or short-axis planes, LV fractional shortening (FS), bi-plane Simpson EF, maximal end-diastolic interventricular septal wall thickness (IVSd), and LV posterior wall thickness (LVPWd). Measurements were made offline from digitally stored images using the average from three consecutive cardiac cycles. IVSd and LVPWd z-scores were calculated to adjust for anthropometric differences.

Detailed echocardiographic evaluation of LV systolic and diastolic myocardial deformation was assessed using 2-D speckle-tracking echocardiography. The endocardial border was traced in the apical four-chamber view for longitudinal, and the parasternal short-axis view for circumferential and radial strain. The region of interest was adjusted to wall thickness. Myocardial tracking by the software was verified visually and retraced if

necessary until adequate tracking was achieved. From the deformation curves, the LV lateral and septal wall longitudinal peak systolic and diastolic, radial peak systolic and diastolic, and circumferential peak systolic and diastolic strain (S) and strain rate (SR) were measured in three lateral and three septal, as well as anterior, inferior, and posterior segments to reflect multiplane LV myocardial deformation.

### Genotyping

Blood or saliva was obtained for DNA extraction. Genotyping was performed for SNPs chosen based on previous association studies, functional effects, allele frequency, and prevalence.<sup>17–19</sup> The following SNPs were investigated: *VEGFA* 2578A/C (rs699947) and *VEGFA* 1154A/G (rs1570360) both SNPs in the VEGF promoter, *VEGFA* 634C/G (rs2010963) in the 5'-untranslated region (UTR), and *HIF1A* 145C/T (rs10873142), *HIF1A* 1326C/T (rs2057482), and *HIF1A* 1744C/T (rs11549465) in the 3'-UTR (for primer sequences please see ref. <sup>3</sup>). Functioning alleles (major alleles) in *HIF1A* and loss-of-function alleles (minor alleles) in *VEGFA* were considered risk alleles. These alleles have shown previous associations with coronary artery disease and chronic heart failure.<sup>17,18,20–23</sup> SNP assays were performed using Applied Biosystems Taqman SNP genotyping technology (Assays-by-Design, Burlington, Canada).

Myocardial VEGF expression analysis was performed as described previously<sup>3</sup> on a subset of six samples, where myocardial tissue was available. In brief, myocardial VEGF expression was scaled semi-quantitatively from 1 to 3, where high is 1 and 3 weak.

### Statistics

Patient characteristics (age, sex, and positive family history), genetic variants, and phenotypes were described by summary statistics. Continuous variables were summarized in terms of mean and standard deviation; dichotomous and polytomous variables were summarized in terms of the number and proportion of patients. The association between the SNPs and phenotypes was assessed separately for each echocardiographic parameter using *t* tests at the 5% significance level. We used a dominant model where the presence of at least one risk allele (heterozygous or homozygous) was defined as a risk genotype. Because of the large number of echocardiographic markers and the small number of patients, the reliability of any single significant pairwise association can be limited. However, we hypothesized that if a genotype was associated with a favorable or adverse HCM-related phenotype, we would be able to detect associations across multiple parameters beyond chance alone. We used binomial tests as a means to account for the large number of pairwise comparisons in phenotypes within each genotype. The binomial tests are used to assess if the observed number of “statistically significant” comparisons is significantly larger or smaller than the expected number of false-positive comparisons. A gene is considered associated with HCM phenotype if the *p* value of the binomial test is <5%. Standardized mean difference was calculated as the ratio between between-SNP difference in an echocardiographic parameter and the pooled standard deviation. For intra- and inter-observer variability, we generated scatter plots comparing the original to intra-observers' and inter-observers' assessments. Next, we quantified the intra- and inter-observer variability using the Bland–Altman agreement plot and intraclass correlation (ICC) as described.<sup>24</sup> All statistical analysis was conducted using SAS v9.3.

## RESULTS

Twenty-five patients with phenotypic evidence of HCM defined as increased IVSd with a z-score > 2 were included in the cohort with an average age of 6.1  $\pm$  4.5 (median: 5.8) years at echocardiographic assessment; 19 (69%) were male and 15 (60%) had a positive family history of HCM. Sixty percent of patients harbored

a disease-causing mutation in the following genes: *MYBPC3* ( $n = 8$ ), *MYH7* ( $n = 6$ ), and *PTPN11* ( $n = 1$ ). *PTPN11* in our cohort was associated with LEOPARD syndrome.

Detailed baseline echocardiographic data of our cohort is shown in Table 1; briefly, our HCM cohort had normal LV FS ( $44.8 \pm 13.3\%$ ) and EF ( $67.4 \pm 11.1\%$ ), normal LV LVIDd ( $38.5 \pm 6.9$  mm,  $z = -1.7 \pm 2.3$ ), increased IVSd ( $17.7 \pm 7.7$  mm,  $z = 7.1 \pm 3.5$ ), and upper limit LVPWd ( $9.1 \pm 2.7$ ,  $z = 2.0 \pm 1.8$ ), reflecting values found in a recent large cohort study with pediatric HCM.<sup>25</sup> Impaired LV relaxation was seen by prolongation of isovolumic relaxation time and E-wave deceleration time as described previously.<sup>3</sup>

Table 2 shows the frequency of SNPs for the six alleles considered, which were *VEGF1 947*, *VEGF1 360*, *VEGF1 963*, *HIF1A.101*, *HIF1A.21*, and *HIF1A.51*. Pairwise comparison using these alleles and LV global and regional systolic and diastolic peak strain phenotypes identified the allele *VEGF1 963GG* as a risk allele, present in 13 (52%) of our HCM patients. Table 3 shows a total of

212 pairwise comparisons in LV global and regional systolic and diastolic peak strain phenotypes using *t* tests for each allele. The number of significant *t* tests ranged from 8 to 26 depending on the genotypes. Only two of the genotypes met the binomial probability threshold for statistical significance. For the allele *VEGF1 963*, 26 significant *t* tests (CC/CG vs. GG) were statistically significant ( $p < 0.001$ ), with reduced strain and strain rate values identifying *VEGF1 963GG* as a risk allele for systolic and diastolic mean and segmental strain and strain rate values. The difference of absolute myocardial deformation parameters between non-risk and risk allele (CC/CG vs. GG) in *VEGF1 963* is shown in Table 4. For better illustration, Fig. 1 shows the significant differences between non-risk and risk allele as standardized difference.

In detail, between the non-risk alleles (Fig. 2) (*VEGF1 963CC/CG*) and the risk allele (*VEGF1 963GG*), the clinically most important global LV systolic myocardial function values such as mean LV longitudinal peak systolic strain ( $-17.7 \pm 4.8\%$  vs.  $-13.9 \pm 4.0\%$ ),

**Table 1.** Echocardiographic parameters of the LV in all patients investigated (left), patients with the *VEGF1 963CC* or *CG* non-risk alleles (middle) and *VEGF1 963GG* risk allele (right).

Variable	All patients		CC/CG		GG		P value
	N	Stat	N	Stat	N	Stat	
Height (cm)	25	145 ± 22	12	147 ± 19	13	143 ± 25	0.68
Weight (kg)	25	47.3 ± 26.2	12	50.3 ± 29.6	13	44.6 ± 23.5	0.60
BSA (m <sup>2</sup> )	25	1.35 ± 0.45	12	1.39 ± 0.47	13	1.31 ± 0.46	0.67
BPsyst (mmHg)	23	106 ± 14	11	108 ± 13	12	104 ± 16	0.60
BPdias (mmHg)	23	57 ± 12	11	61 ± 13	12	53 ± 11	0.148
MV E (cm/s)	25	95 ± 27	12	103 ± 26	13	87 ± 26	0.154
MV A (cm/s)	25	54.6 ± 16.1	12	53.7 ± 8.5	13	55 ± 21	0.79
E/A	25	1.83 ± 0.61	12	1.95 ± 0.56	13	1.72 ± 0.65	0.35
LVIDd (mm)	25	38.5 ± 6.9	12	40.0 ± 8.1	13	37.1 ± 5.6	0.31
LVIDd-Z	22	-1.7 ± 2.3	12	-1.6 ± 2.9	10	-1.9 ± 1.5	0.78
LVIDs (mm)	25	22.2 ± 5.5	12	22.8 ± 5.7	13	21.7 ± 5.6	0.63
LVPWd (mm)	24	9.1 ± 2.7	12	8.3 ± 2.3	12	10 ± 3	0.149
LVPWd-Z	24	2.0 ± 1.8	12	1.4 ± 1.4	12	2.6 ± 2.0	0.114
IVSd (mm)	25	17.7 ± 7.7	12	16.7 ± 8.7	13	18.7 ± 6.9	0.52
IVSd-Z	24	7.1 ± 3.5	12	6.3 ± 3.9	12	8.0 ± 2.9	0.25
LA diam (mm)	25	32.3 ± 5.7	12	32.8 ± 6.5	13	32 ± 5	0.70
LV FS (%)	25	44.8 ± 13.3	12	48 ± 17	13	42.1 ± 7.6	0.33
LV EF (%)	23	67.4 ± 11.1	11	67.8 ± 7.2	12	67.0 ± 14.1	0.85
LA vol (ml)	25	41.58 ± 28.66	12	46.80 ± 34.68	13	36.76 ± 22.10	0.40
LV mass (g)	24	158.5 ± 136.7	11	180.3 ± 172.8	13	140.1 ± 100.6	0.51
LV wall stress	19	211.3 ± 92.5	8	254.7 ± 77.7	11	179.8 ± 92.6	0.073
LV lat E' (cm/s)	25	11.7 ± 4.8	12	13.3 ± 4.7	13	10.3 ± 4.5	0.116
LV lat A' in (cm/s)	25	7.1 ± 2.8	12	6.6 ± 1.9	13	7.5 ± 3.5	0.46
LV lat S' in (cm/s)	25	9.2 ± 2.9	12	9.3 ± 2.9	13	9.1 ± 3.0	0.87
E/E' lat	25	8.8 ± 3.8	12	8.5 ± 3.8	13	9.2 ± 4.0	0.63
LV med E' in (cm/s)	25	7.7 ± 2.9	12	8.7 ± 2.8	13	6.8 ± 2.7	0.090
LV med A' in (cm/s)	25	5.6 ± 1.7	12	5.6 ± 1.4	13	5.5 ± 1.9	0.83
LV med S' in (cm/s)	25	6.7 ± 1.8	12	7.3 ± 1.3	13	6.1 ± 2.0	0.078
E/E' med	25	11.5 ± 4.0	12	12.3 ± 4.5	13	10.7 ± 3.6	0.35

The echocardiographic parameters are classified as follows: BSA body surface area, BPsyst systolic blood pressure, BPdias diastolic blood pressure, MV E mitral valve pulse wave Doppler E wave, MV A mitral valve pulse wave Doppler A wave, LVIDd left ventricular internal diastolic diameter, LVIDd-Z left ventricular internal diastolic diameter z-score, LVIDs left ventricular internal systolic diameter, LVPWd left ventricular posterior wall diastolic diameter, LVPWd-Z left ventricular posterior wall diastolic diameter z-score, IVSd interventricular septal diastolic wall diastolic diameter, IVSd-Z interventricular septal diastolic wall diastolic diameter z-score, LA diam left atrial diameter, LV FS fractional shortening, LV EF left ventricular ejection fraction, LA vol left atrial volume, LV mass left ventricular mass, LV wall stress left ventricular wall stress, LV lat E' left ventricular lateral pulse wave tissue Doppler E wave, LV lat A' left ventricular lateral pulse wave tissue Doppler A wave, LV lat S' left ventricular lateral pulse wave tissue Doppler S wave, LV med E' left ventricular medial pulse wave tissue Doppler E wave, LV med A' left ventricular medial pulse wave tissue Doppler A wave, LV med S' left ventricular medial pulse wave tissue Doppler S wave.

**Table 2.** The frequency of the genotypes for the six alleles in the 25 patients included in the study.

SNP	SNP A	SNP B	Frequency (%) genotype AA	Frequency (%) genotype AB	Frequency (%) genotype BB	HWE <i>p</i> value
<i>VEGF1 947</i>	A	C	4 (16.0%)	12 (48.0%)	9 (37.5%)	1.00
<i>VEGF1 360</i>	A	G	4 (16.0%)	9 (36.0%)	12 (48.0%)	0.32
<i>VEGF1 963</i>	C	G	0 (0.0%)	12 (48.0%)	<b>13 (52.0%)</b>	0.11
<i>HIF1A.101</i>	C	T	1 (4.0%)	7 (28.0%)	17 (68.0%)	0.80
<i>HIF1A.21</i>	C	T	20 (80.0%)	4 (16.0%)	1 (4.0%)	0.23
<i>HIF1A.51</i>	C	T	19 (76.0%)	5 (20.0%)	1 (4.0%)	0.40

*P* values were calculated using  $\chi^2$  tests to assess Hardy–Weinberg equilibrium. *VEGF1 947* = *VEGFA* (2578A/C) rs699947; *VEGF1 360* = *VEGFA* (1154A/G) rs1570360; *VEGF1 963* = *VEGFA* (634C/G) rs2010963; *HIF1A* (145C/T) rs10873142 (*HIF1A-1*); *HIF1A* (1326C/T) rs2057482 (*HIF1A-2*); *HIF1A* (1744C/T) rs11549465 (*HIF1A-3*). Risk allele is shown in bold. SNP single-nucleotide polymorphism, HWE Hardy–Weinberg equilibrium.

**Table 3.** Assessment of significance of associations between alleles and LV deformation.

Gene	Genotype	Number of <i>t</i> tests	Number of significant <i>t</i> tests	Binomial test <i>p</i> value
<i>VEGF1 947</i>	AA/AC vs. CC	212	9	0.76
<i>VEGF1 360</i>	AA/AG vs. GG	212	9	0.76
<i>VEGF1 963</i>	CC/CG vs. GG	212	26	<0.001
<i>HIF1A.101</i>	CC/CT vs. TT	212	10	1.00
<i>HIF1A.21</i>	CC vs. CT/TT	212	4	0.035
<i>HIF1A.51</i>	CC vs. CT/TT	212	8	0.53

Binomial tests were used to assess if the observed number of “significant” *t* tests is different from its nominal expected 5% level. The genotypes with  $\leq 5$  were combined to ensure adequate sample size in each comparison. While two alleles (*VEGF1 963* and *HIF1A.21*) showed binomial test *p* values of <0.05, only *VEGF1 963* was considered as significantly associated for the following reason: the allele *HIF1A.21* is associated with a lower than expected number of significant associations ( $n = 4$ ), as opposed to *VEGF1 963* ( $n = 26$ ), which is associated with an increased number of significant associations.

mean LV circumferential peak systolic strain, mitral valve level ( $-19.8 \pm 6.2\%$  vs.  $-15.2 \pm 4.1\%$ ) and mean LV radial peak systolic strain, papillary muscle level ( $38.6 \pm 11.5\%$  vs.  $24.9 \pm 18.0\%$ ) were abnormal compared to published pediatric reference data<sup>26</sup> and reduced to below two standardized difference in the risk allele *VEGF1 963GG* (Table 4 and Fig. 1). Myocardial deformation parameters of global LV diastolic function were also significantly reduced for mean LV peak diastolic longitudinal ( $1.6 \pm 0.5\%$  vs.  $1.0 \pm 0.4\%$ ) and mean LV peak diastolic circumferential, mitral level ( $1.6 \pm 0.6\%$  vs.  $0.8 \pm 0.4\%$ ), and papillary muscle level ( $2.0 \pm 0.6\%$  vs.  $1.1 \pm 0.6\%$ ) strain rates.

Besides global LV function impairment, regional LV longitudinal and circumferential strain and strain rates were reduced in multiple segments in the risk allele *VEGF1 963GG* (Fig. 1 and Table 4). Importantly, there was no difference in standard baseline echocardiographic LV function parameters (Table 1), and although indices of diastolic function such as septal pulse wave tissue Doppler (LV med E') and E/E' ratio were abnormal in our HCM cohort, there was no statistical difference between patients harboring the different alleles (Table 1). For the allele *HIF1A.21*, four *t* tests (CC/CT vs. TT) were statistically significant ( $p = 0.035$ ). However, given that the number of statistically significant tests is lower than what would be expected by chance alone, this association was not considered further (Table 3). Intra- and inter-observer variability for myocardial deformation analysis was good,

with intra-observer ICC values between 0.82 and 0.99 and inter-observer ICC values between 0.72 and 0.95, Bland–Altman agreement assessment showed that intra- and inter-observer assessments were within the 95% confidence interval (Supplementary Table 1 and Supplementary Figs. 1 and 2). Semi-quantitative myocardial VEGF expression analysis in a subset of six samples showed weaker expression in the *VEGF1 963GG* risk allele, graded as  $1.83 \pm 0.41$  in non-risk alleles vs.  $2.67 \pm 0.52$  in the risk allele ( $p = 0.01$ ).

### DISCUSSION

HCM is a disease with marked phenotypic regional heterogeneity where myocardial performance may be influenced by hypoxia-related stress signaling pathways. This study investigated if non-synonymous SNPs in the angiogenic gene *VEGF* and the hypoxia-response gene *HIF1A* are associated with altered regional systolic and diastolic myocardial performance of the LV as measured by 2-D strain. In patients with HCM, the risk allele *VEGF1 963G/G* identified in our previous study<sup>3</sup> was associated in the current study with statistically significant reduced mean peak systolic longitudinal, circumferential, and radial strain and strain rate compared to non-risk alleles ( $p < 0.001$  and  $p < 0.05$ ) (Fig. 1). Whereas longitudinal strain reduction in hypertrophied regions has been described in many HCM studies, circumferential strain often remains normal or is indeed increased.<sup>10,27,28</sup> Our study shows that the risk allele *VEGF1 963G/G* has a negative association also with circumferential myocardial dynamics, leading to reduced global (mean) and regional circumferential strain. Longitudinal systolic and diastolic strain impairment in the risk phenotype reflects the hypertrophy in basal and mid part of the septum as shown previously.<sup>11</sup> However, comprehensive assessment of longitudinal, circumferential, and radial strain and strain rates in all LV segments are few, and to the best of our knowledge, no study has previously associated this detailed regional myocardial functional analysis with genes that modify the hypoxia-stress response. In our study, both longitudinal and circumferential strain impairment associated with the risk phenotype were concentrated in the basal and mid portions of the LV, with diastolic circumferential strain impairment in particular at the papillary muscle level.

In pediatric patients with HCM, 2-D deformation imaging has been shown to detect early myocardial abnormalities not only in genotype-positive patients but also in phenotype-negative patients.<sup>29</sup> Together with risk allele stratification as shown in this study, this could evolve into a sensitive tool to detect the impact of non-sarcomeric-modifying genes on disease phenotype and inform decisions on follow-up and management in this patient group. This requires further study.

**Table 4.** Absolute 2-D strain and strain rate values of the LV in all patients (left) investigated, patients with the *VEGF1 963CC* or *CG* non-risk alleles (middle) and patients with *VEGF1 963GG* risk allele (right).

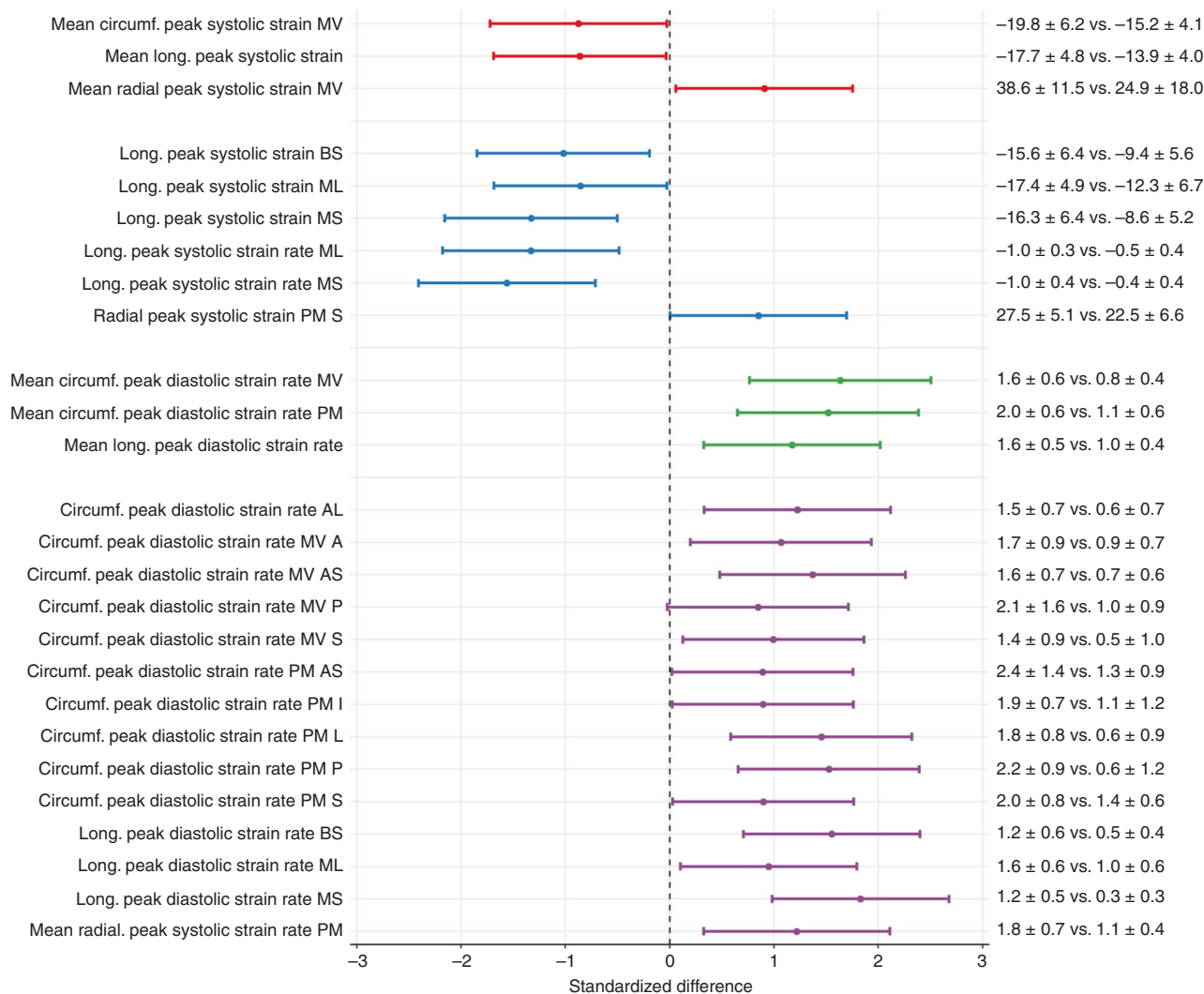
Variable	All patients		CC/CG		GG		P value
	N	Stat	N	Stat	N	Stat	
<b>Mean systolic strain</b>							
Mean longitudinal peak systolic strain	25	-15.8 ± 4.7	12	-17.7 ± 4.8	13	-13.9 ± 4.0	0.044
Mean radial peak systolic strain MV	24	31.8 ± 16.4	12	38.6 ± 11.5	12	24.9 ± 18.0	0.039
Mean circumferential peak systolic strain MV	24	-17.5 ± 5.6	12	-19.8 ± 6.2	12	-15.2 ± 4.1	0.045
Mean radial peak systolic strain rate PM	22	-1.5 ± 0.7	12	-1.8 ± 0.7	10	-1.1 ± 0.5	0.008
<b>Regional systolic strain and strain rate</b>							
Longitudinal peak systolic strain ML	25	-14.8 ± 6.3	12	-17.4 ± 4.9	13	-12.3 ± 6.7	0.042
Longitudinal peak systolic strain MS	25	-12.3 ± 6.9	12	-16.3 ± 6.4	13	-8.6 ± 5.2	0.003
Longitudinal peak systolic strain BS	25	-12.4 ± 6.6	12	-15.6 ± 6.4	13	-9.5 ± 5.6	0.019
Longitudinal peak systolic strain rate ML	24	-0.8 ± 0.4	12	-1.0 ± 0.3	12	-0.5 ± 0.4	0.004
Longitudinal peak systolic strain rate MS	24	-0.7 ± 0.5	12	-1.0 ± 0.4	12	-0.4 ± 0.4	<0.001
Radial peak systolic strain PM S	24	-25.0 ± 6.3	12	-27.6 ± 5.1	12	-22.5 ± 6.6	0.050
<b>Mean diastolic strain rate</b>							
Mean longitudinal peak diastolic strain rate	24	1.3 ± 0.6	12	1.6 ± 0.5	12	1.0 ± 0.4	0.009
Mean circumferential peak diastolic strain rate MV	23	1.2 ± 0.7	12	1.6 ± 0.6	11	0.8 ± 0.4	<0.001
Mean circumferential peak diastolic strain rate PM	23	1.6 ± 0.8	12	2.0 ± 0.6	11	1.1 ± 0.6	0.002
<b>Regional diastolic strain rate</b>							
Longitudinal peak diastolic strain rate ML	24	1.3 ± 0.7	12	1.6 ± 0.6	12	1.0 ± 0.6	0.030
Longitudinal peak diastolic strain rate MS	24	0.8 ± 0.6	12	1.2 ± 0.5	12	0.4 ± 0.3	<0.001
Longitudinal peak diastolic strain rate BS	24	0.9 ± 0.6	12	1.2 ± 0.6	12	0.5 ± 0.4	0.001
Circumferential peak diastolic strain rate MV S	23	1.0 ± 1.0	12	1.5 ± 1.0	11	0.5 ± 0.9	0.027
Circumferential peak diastolic strain rate MV A	23	1.3 ± 0.9	12	1.7 ± 0.9	11	0.9 ± 0.7	0.017
Circumferential peak diastolic strain rate MV P	23	1.5 ± 1.4	12	2.1 ± 1.6	11	1.0 ± 0.9	0.053
Circumferential peak diastolic strain rate MV AS	22	1.1 ± 0.8	11	1.6 ± 0.7	11	0.7 ± 0.6	0.005
Circumferential peak diastolic strain rate AL	22	1.1 ± 0.9	12	1.5 ± 0.7	10	0.6 ± 0.7	0.010
Circumferential peak diastolic strain rate PM S	23	1.7 ± 0.8	12	2.0 ± 0.8	11	1.3 ± 0.6	0.041
Circumferential peak diastolic strain rate PM I	23	1.5 ± 1.1	12	1.9 ± 0.7	11	1.1 ± 1.2	0.052
Circumferential peak diastolic strain rate PM P	23	1.5 ± 1.3	12	2.2 ± 0.9	11	0.7 ± 1.2	0.002
Circumferential peak diastolic strain rate PM L	23	1.3 ± 1.0	12	1.8 ± 0.8	11	0.7 ± 0.9	0.003
Circumferential peak diastolic strain rate PM AS	23	1.9 ± 1.3	12	2.4 ± 1.4	11	1.3 ± 0.9	0.043

All strain values are in %. The deformation parameters are classified as follows: *MV* mitral valve level, *PM* papillary muscle level, *AL* apical lateral, *S* septal, *ML* mid-lateral, *MS* mid-septal, *BS* basal-septal, *AS* antero-septal, *L* lateral, *P* posterior, *I* inferior, *A* anterior.

Risk alleles in VEGF, including *VEGF 963G/G* are associated with increased IVS thickness.<sup>30</sup> However, the reduced strain values in the risk allele *VEGF 963G/G* cannot be explained by a difference in myocardial thickness of the LV alone as our previous study did not find any associations between IVS thickness and the *VEGF 963G/G* risk allele.<sup>3</sup> Based on the known biology of VEGF-HIF1 signaling, the cause may be impaired angiogenesis and the associated increased fibrosis in the risk allele genotype individuals as shown in our previous study.<sup>3</sup> The findings of our current study suggest that this pro-fibrotic phenotype subsequently results in earlier subclinical myocardial dysfunction of the LV. While knowledge on the pathophysiological mechanisms of the angiogenesis-fibrosis network is still incomplete, recent model animal studies in related disease processes suggest that perturbations in the pro-angiogenetic VEGF pathways are closely linked to fibrosis via TGFβ1,<sup>31</sup> a key player in cardiac fibrosis.<sup>32</sup> While it was out of the scope of this study to provide extensive cell and protein level functional data, we showed that the myocardial VEGF expression is reduced in the *VEGF 963G/G* risk allele. In the animal model, decreased VEGF expression is also associated with myocardial dysfunction in acquired cardiomyopathies.<sup>33</sup> Treatment with VEGF

protein has also been shown to ameliorate LV hypertrophy and decrease myocyte apoptosis in an aortic banding model,<sup>34</sup> and this could be a further mechanism why the loss-of-function VEGF risk allele leads to reduced myocardial deformation. The findings of our study could thus be explained by increased fibrosis and reduced angiogenesis in the loss-of-function risk allele *VEGF 963G/G*. This is of importance as fibrosis is a risk factor for poor outcome in HCM<sup>16,35</sup> and requires further study as we could not correlate our echo and genetic findings with tissue or magnetic resonance imaging (MRI) analysis of fibrosis.

While our study is novel in linking modifier genes to myocardial systolic and diastolic performance in relatively young patients, further longitudinal studies are needed to determine if these genetic variants are associated with progressive myocardial dysfunction and to link these findings to clinical outcomes such as arrhythmia or sudden death in HCM, to date the identified risk allele is known to be associated only to coronary artery disease<sup>36</sup> and hypertrophy.<sup>30</sup> Whereas our previous study showed an association of all risk alleles tested here with diastolic dysfunction, systolic and diastolic myocardial deformation impairment was only associated with the risk allele *VEGF1 963G/G*. Our previous



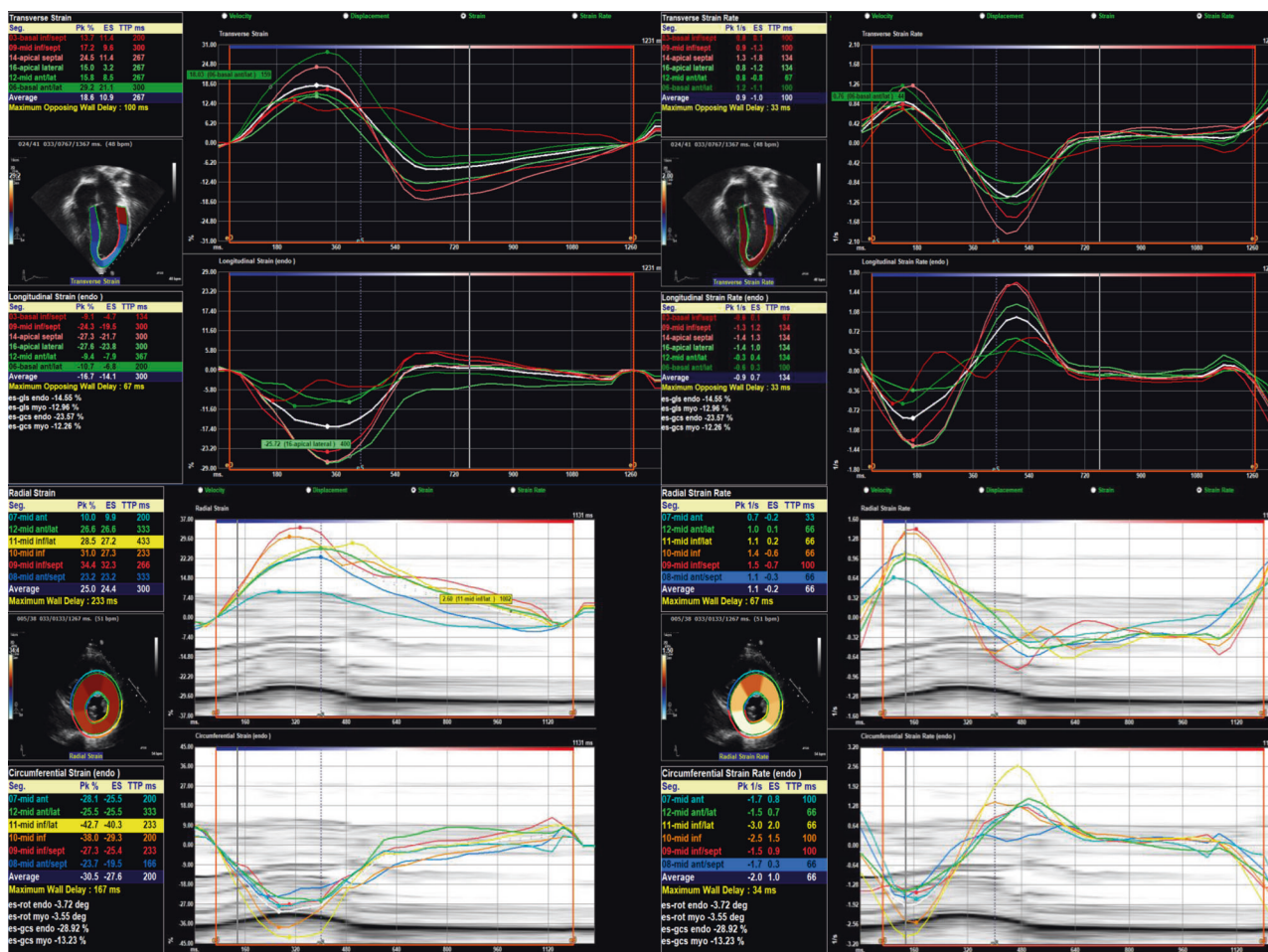
**Fig. 1 Mean and regional LV systolic and diastolic myocardial deformation parameters associated with the risk allele genotype.** Absolute strain and strain rate values for the non-risk alleles *VEGF1* 963CC or CG vs. the risk allele *VEGF1* 963GG are presented on the right. Standardized mean differences and 95% confidence intervals in the phenotypes with significant *t* tests for the risk allele *VEGF1* 963GG are shown. The deformation parameters are classified as follows: red, mean systolic strain; blue, regional systolic strain and strain rate; green, mean diastolic strain and strain rate; purple, regional diastolic strain rate. MV mitral valve level, PM papillary muscle level, PM papillary muscle level, AL apical lateral, S septal, ML mid-lateral, MS mid-septal; BS basal-septal, AS antero-septal, L lateral, P posterior, I inferior, A anterior.

analysis concentrated on parameters of global diastolic dysfunction and did not investigate myocardial deformation. While VEGF protein serum levels were recently shown to be associated with the morphological and functional HCM phenotype,<sup>37</sup> it is yet unknown to what extent an unfavorable VEGF modifier genotype as described here can influence myocardial mechanics and our results are hence not contradictory. Future molecular functional studies will have to better quantify the effect of a loss-of-function VEGF allele on myocardial angiogenic capacity and its subsequent effect on systolic and diastolic dysfunction.

#### Limitations

We did not separately investigate endo-, mid-, and epicardial strain—although recent studies have identified myocardial layer-specific deformation patterns with preservation of endocardial deformation in patients with HCM,<sup>38</sup> and modifier genes as investigated here might show a myocardial layer-specific interaction. Myocardial deformation imaging has been used in several pediatric studies to provide detailed description of myocardial function in pediatric cardiomyopathies, such as

HCM,<sup>29</sup> arrhythmogenic right ventricular cardiomyopathy,<sup>39</sup> dilated cardiomyopathy,<sup>40</sup> and left ventricular non-compaction cardiomyopathy<sup>41</sup> with good intra- and inter-observer variability, and this corresponds to the satisfactory reliability data of our study. The role of strain imaging in pediatric HCM as an outcome predictor has, however, not been sufficiently explored, and at present our findings have therefore limited predictive value. While we have included VEGF expression data, a more detailed molecular functional characterization of the risk modifier allele might help explain the cause for the observed reduction in myocardial deformation, but this was beyond the scope of this study. It would have been informative to quantify myocardial extra-cellular volume and fibrosis burden using cardiac MRI, but this information was unavailable in our cohort also due to ethical concerns (e.g., general anesthesia for cardiac MRI). Additional studies are required to investigate this aspect, as well as correlation with tissue samples, although availability of tissues is limited in the pediatric population. Further studies to investigate the association of our findings with clinical outcomes, such as ventricular tachycardia and sudden death, are needed.



**Fig. 2 Representative strain and strain rate curves.** Longitudinal (top panels) and radial and circumferential strain (lower panels) showing regional differences in strain and strain rate values.

**CONCLUSION**

This is the first study to investigate the association between modifier gene risk alleles and LV myocardial deformation in children with HCM. It shows that early subclinical deficiencies in myocardial performance in children with HCM are associated with a previously identified risk allele in the angiogenic transcription factor VEGF. Our results describe new modifiers of myocardial function in HCM. This knowledge can potentially aid in patient risk stratification and prediction for myocardial dysfunction in patients with HCM.

**ACKNOWLEDGEMENTS**

This study was supported by the NIHR Biomedical Research Center at the University Hospitals Bristol NHS Foundation Trust and the University of Bristol. The views expressed in this publication are those of the author(s) and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health. At the time of the study, GEP held a National Institute for Health Research (NIHR) UK Academic Clinical Lectureship and a Labatt Family Heart Center Toronto/CA scholarship. The study was approved by the Institutional Review Board of the Hospital for Sick Children and informed consent was obtained from all subjects or parents/legal guardians

**AUTHOR CONTRIBUTIONS**

G.E.P. analyzed the data, designed, wrote, edited, and finalized the manuscript. J.A. designed the study, collected the study data, and reviewed the manuscript. C.M. performed the statistical analysis and reviewed the manuscript. C.-P.S.F. and C.M. performed the statistical analysis and reviewed the manuscript. C.K. collected the study data and reviewed the manuscript. S.M. designed the study, supervised data

collection and analysis, and reviewed the manuscript. L.N.B. designed the study, supervised study data collection, and reviewed the manuscript. M.K.F. designed the study, supervised data collection, analyzed the data, wrote, edited, and reviewed the manuscript.

**ADDITIONAL INFORMATION**

The online version of this article (<https://doi.org/10.1038/s41390-020-0929-z>) contains supplementary material, which is available to authorized users.

**Competing interests:** The authors declare no competing interests.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**REFERENCES**

1. Authors/Task Force members. et al. 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy: the Task Force for the Diagnosis and Management of Hypertrophic Cardiomyopathy of the European Society of Cardiology (ESC). *Eur. Heart J.* **35**, 2733–2779 (2014).
2. Watkins, H., Ashrafian, H. & Redwood, C. Inherited cardiomyopathies. *N. Engl. J. Med.* **364**, 1643–1656 (2011).
3. Alkon, J. et al. Genetic variations in hypoxia response genes influence hypertrophic cardiomyopathy phenotype. *Pediatr. Res.* **72**, 583–592 (2012).
4. McMahon, C. J. et al. Characterization of left ventricular diastolic function by tissue Doppler imaging and clinical status in children with hypertrophic cardiomyopathy. *Circulation* **109**, 1756–1762 (2004).
5. Ha, J. W. et al. Tissue Doppler-derived indices predict exercise capacity in patients with apical hypertrophic cardiomyopathy. *Chest* **128**, 3428–3433 (2005).

6. Kitaoka, H. et al. Tissue doppler imaging and plasma BNP levels to assess the prognosis in patients with hypertrophic cardiomyopathy. *J. Am. Soc. Echocardiogr.* **24**, 1020–1025 (2011).
7. Biagini, E. et al. Prognostic implications of the Doppler restrictive filling pattern in hypertrophic cardiomyopathy. *Am. J. Cardiol.* **104**, 1727–1731 (2009).
8. Kansal, M. M. et al. Usefulness of two-dimensional and speckle tracking echocardiography in “Gray Zone” left ventricular hypertrophy to differentiate professional football player’s heart from hypertrophic cardiomyopathy. *Am. J. Cardiol.* **108**, 1322–1326 (2011).
9. Garceau, P. et al. Evaluation of left ventricular relaxation and filling pressures in obstructive hypertrophic cardiomyopathy: comparison between invasive hemodynamics and two-dimensional speckle tracking. *Echocardiography* **29**, 934–942 (2012).
10. Carasso, S. et al. Systolic myocardial mechanics in hypertrophic cardiomyopathy: novel concepts and implications for clinical status. *J. Am. Soc. Echocardiogr.* **21**, 675–683 (2008).
11. Urbano-Moral, J. A. et al. Investigation of global and regional myocardial mechanics with 3-dimensional speckle tracking echocardiography and relations to hypertrophy and fibrosis in hypertrophic cardiomyopathy. *Circ. Cardiovasc. Imaging* **7**, 11–19 (2014).
12. Saito, M. et al. Clinical significance of global two-dimensional strain as a surrogate parameter of myocardial fibrosis and cardiac events in patients with hypertrophic cardiomyopathy. *Eur. Heart J. Cardiovasc. Imaging* **13**, 617–623 (2012).
13. Johansson, B. et al. Myocardial capillary supply is limited in hypertrophic cardiomyopathy: a morphological analysis. *Int. J. Cardiol.* **126**, 252–257 (2008).
14. Rey, S. & Semenza, G. L. Hypoxia-inducible factor-1-dependent mechanisms of vascularization and vascular remodelling. *Cardiovasc. Res.* **86**, 236–242 (2010).
15. Zeisberg, E. M. et al. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat. Med.* **13**, 952–961 (2007).
16. Bruder, O. et al. Myocardial scar visualized by cardiovascular magnetic resonance imaging predicts major adverse events in patients with hypertrophic cardiomyopathy. *J. Am. Coll. Cardiol.* **56**, 875–887 (2010).
17. Zheng, Z. L. et al. Genetic polymorphisms of hypoxia-inducible factor-1 alpha and cardiovascular disease in hemodialysis patients. *Nephron Clin. Pract.* **113**, c104–c111 (2009).
18. van der Meer, P. et al. The VEGF +405 CC promoter polymorphism is associated with an impaired prognosis in patients with chronic heart failure: a MERIT-HF substudy. *J. Card. Fail.* **11**, 279–284 (2005).
19. Hsieh, Y. Y., Wang, J. P. & Lin, C. S. Four novel single nucleotide polymorphisms within the promoter region of p53 gene and their associations with uterine leiomyoma. *Mol. Reprod. Dev.* **74**, 815–820 (2007).
20. Buraczynska, M. et al. Association of the VEGF gene polymorphism with diabetic retinopathy in type 2 diabetes patients. *Nephrol. Dial. Transpl.* **22**, 827–832 (2007).
21. Hlatky, M. A. et al. Polymorphisms in hypoxia inducible factor 1 and the initial clinical presentation of coronary disease. *Am. Heart J.* **154**, 1035–1042 (2007).
22. Breunis, W. B. et al. Vascular endothelial growth factor gene haplotypes in Kawasaki disease. *Arthritis Rheum.* **54**, 1588–1594 (2006).
23. Howell, W. M. et al. VEGF polymorphisms and severity of atherosclerosis. *J. Med. Genet.* **42**, 485–490 (2005).
24. Shrout, P. E. & Fleiss, J. L. Intraclass correlations: uses in assessing rater reliability. *Psychol. Bull.* **86**, 420–428 (1979).
25. Norrish, G. et al. Yield of clinical screening for hypertrophic cardiomyopathy in child first-degree relatives. *Circulation* **140**, 184–192 (2019).
26. Marcus, K. A. et al. Reference values for myocardial two-dimensional strain echocardiography in a healthy pediatric and young adult cohort. *J. Am. Soc. Echocardiogr.* **24**, 625–636 (2011).
27. Yang, H. et al. Hypertrophy pattern and regional myocardial mechanics are related in septal and apical hypertrophic cardiomyopathy. *J. Am. Soc. Echocardiogr.* **23**, 1081–1089 (2010).
28. Serri, K. et al. Global and regional myocardial function quantification by two-dimensional strain: application in hypertrophic cardiomyopathy. *J. Am. Coll. Cardiol.* **47**, 1175–1181 (2006).
29. Forsey, J. et al. Early changes in apical rotation in genotype positive children with hypertrophic cardiomyopathy mutations without hypertrophic changes on two-dimensional imaging. *J. Am. Soc. Echocardiogr.* **27**, 215–221 (2014).
30. Lacchini, R. et al. Effect of genetic polymorphisms of vascular endothelial growth factor on left ventricular hypertrophy in patients with systemic hypertension. *Am. J. Cardiol.* **113**, 491–496 (2014).
31. Kariya, T. et al. TGF-beta1-VEGF-A pathway induces neoangiogenesis with peritoneal fibrosis in patients undergoing peritoneal dialysis. *Am. J. Physiol. Ren. Physiol.* **314**, F167–F180 (2018).
32. Leask, A. Potential therapeutic targets for cardiac fibrosis: TGFbeta, angiotensin, endothelin, CCN2, and PDGF, partners in fibroblast activation. *Circ. Res.* **106**, 1675–1680 (2010).
33. Han, B. et al. Decreased cardiac expression of vascular endothelial growth factor and redox imbalance in murine diabetic cardiomyopathy. *Am. J. Physiol. Heart Circ. Physiol.* **297**, H829–H835 (2009).
34. Friehs, I. et al. Vascular endothelial growth factor prevents apoptosis and preserves contractile function in hypertrophied infant heart. *Circulation* **114**(Suppl.), I290–I295 (2006).
35. O’Hanlon, R. et al. Prognostic significance of myocardial fibrosis in hypertrophic cardiomyopathy. *J. Am. Coll. Cardiol.* **56**, 867–874 (2010).
36. Ma, W. Q. et al. Association of genetic polymorphisms in vascular endothelial growth factor with susceptibility to coronary artery disease: a meta-analysis. *BMC Med. Genet.* **19**, 108 (2018).
37. Pudil, R. et al. Vascular endothelial growth factor is associated with the morphologic and functional parameters in patients with hypertrophic cardiomyopathy. *Biomed. Res. Int.* **2015**, 762950 (2015).
38. Okada, K. et al. Myocardial shortening in 3 orthogonal directions and its transmural variation in patients with nonobstructive hypertrophic cardiomyopathy. *Circ. J.* **79**, 2471–2479 (2015).
39. Pieleś, G. E. et al. Association of echocardiographic parameters of right ventricular remodeling and myocardial performance with modified task force criteria in adolescents with arrhythmogenic right ventricular cardiomyopathy. *Circ. Cardiovasc. Imaging* **12**, e007693 (2019).
40. Forsha, D. et al. Patterns of mechanical inefficiency in pediatric dilated cardiomyopathy and their relation to left ventricular function and clinical outcomes. *J. Am. Soc. Echocardiogr.* **29**, 226–236 (2016).
41. Sabatino, J. et al. Left ventricular twist mechanics to identify left ventricular noncompaction in childhood. *Circ. Cardiovasc. Imaging* **12**, e007805 (2019).