GNB3 C825T Polymorphism and Myocardial Recovery in Peripartum Cardiomyopathy
Results of the Multicenter Investigations of Pregnancy-Associated Cardiomyopathy Study

Richard Sheppard, MD; Eileen Hsich, MD; Julie Damp, MD; Uri Elkayam, MD;
Angela Kealey, MD; Gautam Ramani, MD; Mark Zucker, MD; Jeffrey D. Alexis, MD;
Benjamin D. Horne, MD; Karen Hanley-Yanez, BS; Jessica Pisarcik, RN, BSN;
Indrani Halder, PhD; James Fett, MD; Dennis M. McNamara, MD; for the IPAC Investigators

Background—Black women are at greater risk for peripartum cardiomyopathy (PPCM). The guanine nucleotide–binding protein β3 subunit (GNB3) has a polymorphism C825T. The GNB3 TT genotype more prevalent in blacks is associated with poorer outcomes. We evaluated GNB3 genotype and myocardial recovery in PPCM.

Methods and Results—A total of 97 women with PPCM were enrolled and genotyped for the GNB3 T/C polymorphism. Left ventricular ejection fraction (LVEF) was assessed by echocardiography at entry, 6 and 12 months postpartum. LVEF over time in subjects with the GNB3 TT genotype was compared with those with the C allele overall and in black and white subsets. The cohort was 30% black, age 30±6, LVEF 0.34±0.10 at entry 31±25 days postpartum. The % GNB3 genotype for TT/CT/CC=23/41/36 and differed markedly by race (blacks=52/38/10 versus whites=10/44/46, P<0.001). In subjects with the TT genotype, LVEF at entry was lower (TT=0.31±0.09; CT+CC=0.35±0.09, P=0.054) and this difference increased at 6 (TT=0.45±0.15; CT+CC=0.53±0.08, P=0.002) and 12 months (TT=0.45±0.15; CT+CC=0.56±0.07, P<0.001). The difference in LVEF at 12 months by genotype was most pronounced in blacks (12 months LVEF for GNB3 TT=0.39±0.16; versus CT+CC=0.53±0.09, P=0.02) but evident in whites (TT=0.50±0.11; CT+CC=0.56±0.06, P=0.04).

Conclusions—The GNB3 TT genotype was associated with lower LVEF at 6 and 12 months in women with PPCM, and this was particularly evident in blacks. Racial differences in the prevalence and impact of GNB3 TT may contribute to poorer outcomes in black women with PPCM. (Circ Heart Fail. 2016;9:e002683. DOI: 10.1161/CIRCHEARTFAILURE.115.002683.)

Key Words: cardiomyopathy ■ heart failure ■ peripartum period ■ polymorphism genetics ■ pregnancy
than in whites. Roughly 50% of black subjects have the GNB3 TT genotype, compared with only 10% of whites. In the genetic substudy of the African-American Heart Failure Trial (AHeFT), blacks with the GNB3 TT genotype had the poorest outcome on placebo and received the greatest benefit from therapy. Despite clear racial differences in outcomes with PPCM, the impact of GNB3 TT genotype on myocardial recovery has not been previously examined.

The Peripartum Cardiomyopathy Network was formed as a 30 center collaborative group to facilitate research in this disorder and recently completed the Investigations of Pregnancy-Associated Cardiomyopathy (IPAC) study. IPAC was a multicenter prospective investigation of the genetic and clinical predictors of outcomes for PPCM patients in North America and reported significantly less myocardial recovery among black women with PPCM when compared with whites. Given the distinct differences in allele frequency for the GNB3 C825T polymorphism between black and white cohorts and the impact of GNB3 TT genotype in black subjects in AHeFT, we examined the relationship of the GNB3 C825T polymorphism between black and white subjects enrolled in IPAC.

Methods

Cohort
Ninety-seven women with newly diagnosed PPCM were enrolled within the first 13 weeks postpartum at 30 centers (Appendix I in the Data Supplement) between December 2009 and September 2012. All women were at least 18 years of age and had no previous history of cardiac disease, an estimated clinical left ventricular ejection fraction (LVEF) of <0.45 at the time of enrollment, and an evaluation consistent with idiopathic nonischemic cardiomyopathy. Women with significant valvular disease, coronary disease (>50% stenosis of a major epicardial vessel or a positive noninvasive study), evidence of ongoing bacterial septicemia (positive blood cultures), ongoing drug or alcohol abuse, history of chemotheraphy or chest radiation with 5 years of enrollment, or a history of a previous cardiomyopathy were excluded.

Protocol
The study protocol was approved by the institutional review boards at all participating centers, and informed consent was obtained from all subjects. At the time of enrollment, demographic information including self-designated race, previous clinical evaluation, and current medical therapy were recorded. Women were followed until 1-year postpartum. All hospitalizations and major cardiac events including self-designated race, previous clinical evaluation, and current medical therapy were recorded. Women were followed until 1-year postpartum. All hospitalizations and major cardiac events including self-designated race, previous clinical evaluation, and current medical therapy were recorded.

Left Ventricular Function
All subjects had an echocardiogram to assess LVEF at the time of enrollment, which was repeated at 6 and 12 months postpartum. In addition, women enrolled early (within the 6 weeks postpartum, n=65) had a repeat assessment of left ventricular function at 2 months. Echocardiograms were reviewed by a core laboratory at the University of Pittsburgh for assessment of ventricular volumes and calculation of ejection fraction. The left ventricular (LV) volumes and EF were assessed by biplane Simpson rule using manual tracing of digital images. Left ventricular end-diastolic diameter (LVEDD) was assessed in the parasternal long-axis view. A subset of echocardiograms obtained were not available for assessment by the core laboratory because of format (22 of 310, 7%) and for these studies, the LVEF calculated locally was used.

DNA Isolation and Genotype Assays
Peripheral blood was obtained from subjects at the time of entry and shipped overnight at room temperature to the core laboratory (University of Pittsburgh). DNA was isolated from peripheral blood by leukocyte centrifugation and cell lysis using the PureGene DNA purification kit (Genta Systems, Minneapolis, MN). The GNB protein (G protein) β polypeptide-3 (GNB3) position 825 C/T polymorphism was assessed using a TaqMan SNP Genotyping Assay (Applied Biosystems, Inc, Foster City, CA) with tagged primers (reporter 1 tagged dye 1/4 VIC; reporter 2 tagged dye 1/4 FAM). Context sequence for the GNB3 825 C/T polymorphism was as follows: AGAGCACTACCTGCGCATCACGCCTGTC (VIC) 2 tagged dye (APPLIED BIOSYSTEMS, INC, Foster City, CA) with primers (reporter 1 tagged dye 1/4 VIC; reporter 2 tagged dye 1/4 FAM). Context sequence for the GNB3 825 C/T polymorphism was as follows: AGAGCACTACCTGCGCATCACGCCTGTC (VIC) 2 tagged dye

Statistical and Genetic Analysis
All statistical analyses were performed in SPSS v22. Continuous variables (eg, age and blood pressure) are reported as means and SDs. Student t tests and Fisher exact tests were used to compare subject characteristics by genotype. Non-normally distributed variables (eg, days postpartum, gravid, and para) are summarized as median and 25th and 75th percentiles, and compared by nonparametric Wilcoxon rank sum tests. The impact of GNB3 genotype on LVEF during recovery (defined as LVEF at 12 months) was first examined by 1-way ANOVA with Tukey post hoc tests for pairwise comparisons. Because we had LVEF measured at multiple time points, we performed a repeated measures ANOVA (using the repeated measures under GLM option). LVEF at baseline, 6 and 12 months were entered as repeated measures outcome, genotype (TT versus C allele), and LVEDD were entered as factor and covariate, respectively. In multivariable models, LVEDD and race are significant predictors of LVEF recovery in this cohort. Finally, we used ANCOVA to evaluate the impact of genotype on LVEF over time adjusting for these 2 variables. Because allele and genotype frequencies for this genotype differ significantly between whites and blacks, race-specific analyses were done in white and black subsets. These analyses compared TT genotype carriers to C allele (TC and CC genotype subjects combined) carriers in hierarchical linear regression models. Hierarchical linear regression is a specific analytic approach in which predictors are entered successively in each step. The regression model built at each step is related but separate from the immediately previous and subsequent models. This allows for comparison of related regression models within a single framework. This technique helps to quantify the predictive power of a variable while accounting for other covariates in the model. LVEDD was entered in the first step and genotypes were entered in the second step.

The Kaplan–Meier method was used to estimate survival free from events (cardiac transplantation or the need for mechanical circulatory support) with days postpartum as the time interval (starting at delivery). Event-free survival of subjects with the GNB3 TT genotype was compared with those with the C allele by the exact log-rank test.

Results

Sample Characteristics
Of the cohort of 97 women, 30% (n=29) were black, 65% were white (n=63), and 5% other (n=5). Seventy percent were New York Heart Association (NYHA) class II–III, and >80% of patients were on angiotensin-converting enzyme (ACE) inhibitor and β-blocker therapy. Baseline LVEF for these patients was 0.34±0.09. For the entire cohort women were enrolled at a median of 24 days postpartum (25th percentile, 75th percentile=11.5, 51 days). Sixty-five women were enrolled early (range, 0–42 days postpartum; median, 14 days), whereas 32 were enrolled late (range, 43–95 days; median, 59).

Genetic Analysis of GNB3 C635T in IPAC
For the GNB3 T/C polymorphism, 22 (23%) subjects were TT genotype, 40 (41%) TC, and 35 (36%) CC. Genotype differed...
A. **Clinical Characteristics by GNB3 Genotype**

<table>
<thead>
<tr>
<th></th>
<th>All (n=97)</th>
<th>TT (n=22)</th>
<th>TC+CC (n=75)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>30±6</td>
<td>28±7</td>
<td>30±6</td>
<td>0.15</td>
</tr>
<tr>
<td>Gravida</td>
<td>2 (1, 4)</td>
<td>2.5 (1, 4)</td>
<td>2 (1, 4)</td>
<td>0.93</td>
</tr>
<tr>
<td>Para</td>
<td>2 (1, 3)</td>
<td>3 (1, 3)</td>
<td>2 (1, 3)</td>
<td>0.38</td>
</tr>
<tr>
<td>Race, % black</td>
<td>30</td>
<td>68</td>
<td>19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Days postpartum</td>
<td>24 (11,51)</td>
<td>34.5 (14,63)</td>
<td>24 (10,49)</td>
<td>0.17</td>
</tr>
<tr>
<td>BMI</td>
<td>29±7</td>
<td>30±7</td>
<td>29±8</td>
<td>0.42</td>
</tr>
<tr>
<td>NYHA class (% I/II/III/IV)</td>
<td>12/45/25/18</td>
<td>18/27/36/18</td>
<td>11/51/21/17</td>
<td>0.22</td>
</tr>
<tr>
<td>HR</td>
<td>86±16</td>
<td>86±15</td>
<td>86±17</td>
<td>0.94</td>
</tr>
<tr>
<td>BP systolic</td>
<td>112±17</td>
<td>115±19</td>
<td>111±17</td>
<td>0.35</td>
</tr>
<tr>
<td>BP diastolic</td>
<td>70±13</td>
<td>73±13</td>
<td>69±13</td>
<td>0.20</td>
</tr>
<tr>
<td>ACE inhibitor (%)</td>
<td>81</td>
<td>91</td>
<td>79</td>
<td>0.16</td>
</tr>
<tr>
<td>Aldosterone receptor antagonist</td>
<td>27</td>
<td>32</td>
<td>25</td>
<td>0.36</td>
</tr>
<tr>
<td>β-Blocker, %</td>
<td>89</td>
<td>96</td>
<td>87</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Comparison of women with the GNB3 TT genotype to those with the GNB3 C allele. For gravidity, para and days postpartum values listed are the mean (with 25th and 75th percentiles). All other values are mean and SD. There was a significantly higher percentage of black women in the subset with the GNB3 TT genotype (68%) than in the subset with the C allele (19%), P<0.001. No other significant differences. ACE indicates angiotensin-converting enzyme; BMI, body mass index; BP, blood pressure; GNB3, guanine nucleotide-binding proteins β-3 subunit; HR, heart rate; and NYHA, New York Heart Association.

B. **Clinical Characteristics by GNB3 Genotype**

- **Comparison of 3 genotype classes:**
  - Women homozygous for GNB3 T (black bars), heterozygous women (gray bars), and those homozygous for the C allele (white bars). Lines extending from bars represent SDs. P values represent an ANOVA of an overall difference between the 3 genotype classes. No significant difference at entry (P=0.13) but a significantly lower mean LVEF for GNB3 TT subjects at 6 (P=0.007) and 12 months (P<0.001).
  - Same comparison but combining heterozygous subjects and those homozygous for the C allele into 1 group. Subjects homozygous for GNB3 T (black bars) compared with subjects with the C allele (gray bars). Not statistically significant at entry (P=0.054) but a significantly lower mean LVEF for GNB3 TT subjects at 6 (P=0.002) and 12 months (P<0.0001).

**Figure 1.** Left ventricular ejection fraction (LVEF) over time (entry, 6 and 12 months postpartum) by guanine nucleotide-binding proteins β-3 subunit (GNB3) genotype. **A.** Comparison of 3 genotype classes: women homozygous for GNB3 T (black bars), heterozygous women (gray bars), and those homozygous of the C allele (white bars). Lines extending from bars represent SDs. P values represent an ANOVA of an overall difference between the 3 genotype classes. No significant difference at entry (P=0.13) but a significantly lower mean LVEF for GNB3 TT subjects at 6 (P=0.007) and 12 months (P<0.001). **B.** Same comparison but combining heterozygous subjects and those homozygous for the C allele into 1 group. Subjects homozygous for GNB3 T (black bars) compared with subjects with the C allele (gray bars). Not statistically significant at entry (P=0.054) but a significantly lower mean LVEF for GNB3 TT subjects at 6 (P=0.002) and 12 months (P<0.0001).
and 12 months ($P=0.001$) and from GNB3 CC subjects at 6 ($P=0.02$) and 12 months ($P=0.008$). Tukey post hoc analysis confirmed subjects with the GNB3 TT genotype demonstrated a significantly lower LVEF from CT subjects at 6 ($P=0.009$) and 12 months, ($P<0.0001$) and from CC subjects at 6 ($P=0.02$) and 12 months ($P=0.004$). However, the mean LVEF for TC and CC subjects was similar and did not differ at either 6-month ($P=0.61$) or 12-month ($P=0.22$) follow-up. The association between myocardial recovery (defined as LVEF at 12 months) and GNB3 genotypes persisted after adjustment for race and LVEDD ($F=7.04$, $df=2$, $P=0.002$). Pairwise contrasts showed that TT genotype carriers had significantly lower adjusted LVEF than CT ($P=0.002$) and CC ($P=0.005$) groups. Given their similarities in terms of mean LVEF over time, CC and TC genotype carriers were combined into a single C allele group and compared with GNB3 TT subjects.

The predominant difference in LVEF by genotype was between subjects homozygous for the GNB3 TT allele (GNB3 TT) compared with subjects with the GNB3 C allele. When comparing LVEF over time specifically between subjects with the GNB3 TT genotype versus those with the C allele, subjects enrolled early postpartum had similar mean LVEF regardless of genotype (TT=0.35±0.07; C allele=0.35±0.09, $P=0.98$); however, significant differences were apparent at 2 months (TT=0.38±0.15; C allele=0.44±0.10, $P=0.02$), 6 months (TT=0.45±0.15; C allele=0.53±0.08, $P=0.002$), and 12 months (TT=0.45±0.15; C allele=0.56±0.07, $P<0.0001$). For the entire cohort at the time of entry (combining women enrolled early and late), the mean LVEF for GNB3 TT subjects was slightly lower at entry though this failed to reach significance ($P=0.054$). The differences in LVEF by GNB3 genotype became more pronounced at 6 and 12 months (Figure 1B). These differences persisted when the model was further adjusted for race and baseline LVEDD at 6 months ($P=0.011$) and 12 months ($P=0.00001$). With repeated measures, data assuming 4 time points (entry, 2 months, 6 months, and 12 months) with genotype (TT versus C allele carriers) and LVEDD as predictors, results showed that TT genotype was a significant negative predictor of LVEF recovery over time ($F=15.3$, $P<0.0001$).

In subset analysis by race, black women with the GNB3 TT genotype had a significantly lower mean LVEF at entry compared with C allele carriers (0.28±0.09 versus 0.35±0.08; $P=0.04$), and this difference increased to 14 EF units at 12 months (12 months LVEF TT=0.39±0.16; C allele=0.53±0.09, $P=0.02$, Figure 2A). In white women, the mean LVEF for GNB3 TT subjects was similar to C allele carriers at entry (GNB3 TT=0.37±0.04; C allele=36±0.10, $P=0.85$), but significantly lower at 12 months (0.50±0.11 versus 0.56±0.06; $P=0.04$, Figure 2B). In hierarchical regression models adjusted for baseline LVEDD, presence of the TT genotype was associated with 6.2% lower LVEF recovery in blacks (β [SE]=−6.2 (2.8), $P=0.03$) and the genotype explained only 10% of variation in this race. In whites, the TT genotype corresponded to a 9.7% lower LVEF recovery (β [SE]=−9.7 (4.4), $P=0.04$) and explained 53% of variation.

Overall, 71% of women recovered to a final LVEF of ≥0.50. Recovery to this degree was significantly less likely overall in GNB3 TT subjects than in those with the GNB3 C allele (TT=48% versus C allele=78%, $P=0.01$). Subset analysis by race revealed similar trends in both white women (final LVEF ≥0.50 for GNB3 TT=50% versus C allele=81%, $P=0.12$) and black women (TT=43% versus C allele=77%, $P=0.12$). In addition, analysis of NYHA functional class by GNB3 genotype revealed no significant difference at study entry (Table, $P=0.22$) but a significant difference at 12-month follow-up with higher NYHA class in GNB3 TT subjects (% NYHA class I/II/III/IV at 12 months GNB3 TT subject=35/41/18/6 versus the C allele=79/17/2/2, $P=0.004$).

Event-Free Survival

Among the 97 women in IPAC, there were few events. Two women died and 4 received LVADs before 1-year postpartum. Of the subjects who received LVADs, 2 women subsequently died and 1 was transplanted. No women recovered on LVAD and the remaining LVAD subject remained on support at 1 year. Analysis of event-free survival revealed that the overall
Discussion

Although genetic predispositions have been increasingly recognized as a risk factor for development of PPCM,16,20 the role genomic background plays in racial differences in myocardial recovery remains largely unexplored. In this study from IPAC, women with PPCM with the GNB3 TT genotype demonstrated a significantly lower LVEF at 6 and 12 months postpartum. Although this genotype clearly affects both whites and blacks, the greater impact and higher frequency of the GNB3 TT homogygote among blacks seems to contribute to the lower LVEF evident in black women with PPCM at 12 months postpartum.

This common GNB3 polymorphism, although functionally silent, is in 100% linkage disequilibrium with a splicing variant of exon 9 (GNB3s) and the T allele is always coinherited with the truncated subunit. This truncated subunit enhances α-adrenergic activation,21,22 and the GNB3 825T allele, which is much more prevalent in blacks,17 has been associated with low-renin hypertension.15 In IPAC, GNB3 TT subjects did not demonstrate significant systolic or diastolic hypertension, although an association with hypertension may have been masked by the use of both ACE inhibitors and β-blockers in the majority of subjects.

The effect of GNB3 genotype on outcomes in blacks with chronic heart failure has been examined in a genetic substudy of AHeFT.18 Subjects with the GNB3 TT genotype on placebo in AHeFT demonstrated poorer hospitalization-free survival than subjects with the C allele. This was reversed by treatment with a fixed dose combination of isosorbide dinitrate and hydralazine, suggesting that the GNB3 genotype influences the impact of pharmacotherapy in black with chronic heart failure. Of note, although β-blockers and ACE inhibitors were widely used in IPAC, fixed dose combination of isosorbide dinitrate and hydralazine received limited use. Whether the use of fixed dose combination of isosorbide dinitrate and hydralazine could have improved myocardial recovery in IPAC subjects with the GNB3 TT genotype remains to be determined.

The impact of genotype on cardiac remodeling and LVEF in patients with chronic heart failure was examined in AHeFT and several small studies. Of note, genotypes related to the renin–angiotensin aldosterone system,23 nitric oxide synthesis,24 and the adrenergic receptors25 have been explored as potentially modulating remodeling and the impact of therapy. In contrast to its impact on event-free survival, GNB3 TT genotype did not impact remodeling in AHeFT.18 In addition to the work in AHeFT, the GNB3 T allele has been associated with and in increase in arrhythmic events in chronic heart failure.26 Modulation of GNB3 has been associated with reverse remodeling after cardiac resynchronization therapy.16 However, no previous analysis has evaluated the impact of GNB3 on myocardial recovery in recent onset cardiomyopathy, and it is possible that factors influencing left ventricular recovery in PPCM are distinct from those affecting remodeling in more chronic left ventricular dysfunction.

The impact of the GNB3 T allele on myocardial recovery in IPAC was evident by 2 months postpartum and may involve a response to injury, which reflects differences in vascular tone and increased afterload. Despite previous work linking GNB3 T to the increase risk of hypertension, blood pressure was not significantly different by genotype in IPAC. Black subjects with the GNB3 TT genotype had a greater heart rate response to the cold pressor test of sympathetic activation,27 suggesting the GNB3 polymorphism may influence vascular tone through alterations of autonomic function. Metabolic stress testing revealed an association between the GNB3 T allele and poorer peak oxygen consumption and altered heart rate variability, further suggesting that the T allele may be associated with autonomic modulation and cardiovascular stress response.28

This study is limited by the small number of patients which diminishes the power to firmly establish a genetic association between GNB3 T allele and LV recovery in patients with PPCM. This is particularly evident in subset analysis by race, given the small number of black women per genotype class. Therapy with ACE inhibitors and β-blockers was used at all centers, but was not standardized or optimized by protocol, and variation in treatment by site may have contributed to the heterogeneity of outcomes. The association of GNB3 TT with less myocardial recovery may reflect its higher prevalence in blacks and its role as a nonspecific marker of African genomic heritage, rather than a true functional impact of the truncated subunit itself. However, the strong impact of the GNB3 TT genotype within the black subset itself argues that its impact is not just as a marker of race.

Conclusions

This is the first study to establish a relationship between difference in genetic background and LV remodeling and recovery in women with PPCM. Black women are more predisposed to PPCM and less likely to recover. GNB3 TT genotype, more prevalent in black women, was associated with lower LVEF at 6 and 12 months postpartum. Women presenting with PPCM with this specific genotype may be at higher risk for chronic cardiomyopathy. The relationship between this specific genotype, adrenergic signaling, and the potential for genotype-targeted therapy requires further investigation.

Disclosures

None.

References

The ability to predict recovery of left ventricular function after a patient develops cardiomyopathy remains a significant challenge to clinicians. Clinical predictors have been examined, including patient factors, serological factors, and factors on cardiac imaging such as echocardiographic features. These have been explored in patients with both ischemic and nonischemic cardiomyopathy. There are also some studies that have subsequently examined genetic factors that might help predict recovery of left ventricular function as well. This is the first study to explore genetic factors that might help predict left ventricular recovery in women with peripartum cardiomyopathy (PPCM). The particular gene explored (guanine nucleotide–binding proteins β-3 subunit CR282T) is associated with hypertension and also with black background, both of which are associated with the development of PPCM. The ability to predict recovery of left ventricular function in patients with PPCM remains clinically relevant, and the role of patient genetic profile might also help further our understanding of the physiology underlying the development of PPCM. Patient genotype may potentially help clinicians better individualize the patient discussions about the ramifications of the cardiomyopathy and also help potentially tailor medical therapy individually to women with PPCM.
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APPENDIX:
IPAC Investigators

University of Pittsburgh Medical Center (10) Dennis M McNamara, MD; James D. Fett, MD; Jessica Pisarcik, RN, BSN; Charles McTiernan, PhD; Karen Hanley-Yanez, BS; John Gorcsan III, MD; Erik Schelbert, MD. Intermountain Medical Center (8) Rami Alharethi, MD; Kismet Rasmusson, CRNP; Kim Brunisholz; Amy Butler, BS, CCRP; Deborah Budge; A.G. Kfoury, MD; Benjamin Horne, PhD; Joe Tuinei; Heather Brown. Vanderbilt University (7) Julie Damp, MD; Allen J. Naftilan, MD; Jill Russell, RN, MSN; Darla Freehardt, LPN, BS, CCRP. Cleveland Clinic (7) Eileen Hsich, MD; Cynthia Oblak, CCRC. Washington University, St. Louis (6) Greg Ewald, MD; Donna Whitehead, RN; Jean Flanagan, RN; Anne Platts. University of Southern California (6) Uri Elkayam, MD; Jorge Caro, MPH; Stephanie Mullin, RN. Brigham and Women’s Hospital (5) Michael M. Givertz, MD; M. Susan Anello, RN, BS. University of Kentucky (5) Navin Rajagopalan, MD; David Booth, MD; Tiffany Sandlin, RN; Wendy Wijesiri, RN. Mayo Clinic (4) Leslie T. Cooper, MD; Lori A. Blauwet, MD; Joann Brunner, RN; Mary Phelps; Ruth Kempf. Louisiana State University (4) Kalgi Modi, MD; Tracy Norwood. University of Illinois (4) Joan Briller, MD; Decebal Sorin Griza, MD. Duke University (4) G. Michael Felker, MD; Robb Kociol, MD; Patricia Adams, RN. Wake Forest (4) Gretchen Wells, MD; Vinay Thohan, MD; Deborah Wesley-Farrington, RN, BSN, CCRC, CCA; Sandra Soots, RN, CCRC. Jewish General (3) Richard Sheppard, MD; Caroline Michel, MD; Nathalie Lapointe, RN, PhD; Heather Nathaniel. University of Calgary (3) Angela Kealey, MD. Massachusetts General (2) Marc Semigran, MD; Maureen Daher, RN. Penn State Milton S. Hershey Medical Center (2) John Boehmer, MD; David Silber, MD; Eric Popjes, MD; Patricia Frey, RN; Todd Nicklas, RN. University of Rochester (2) Jeffrey Alexis, MD; Lori Caufield, RN, BSN, CCRC. Georgia Health Sciences University (2) John W. Thornton III, MD; Mindy Gentry, MD; Vincent J.B. Robinson, MBBS; Gyanendra K. Sharma, MD; Joan Holloway, BS; Maria Powell, LPN, CCRC. University of Texas, Southwestern (2) David Markham, MD; Mark Drazner, MD; Lynn Fernandez, RN. Newark Beth Israel Medical Center (2) Mark Zucker, MD; David A. Baran, MD; Martin L. Gimovsky, MD; Natalia Hochbaum, MD; Bharati Patel, RN,
CCRC; Laura Adams, RN, BSN. University of Maryland (2) Gautam Ramani, MD; Stephen Gottlieb, MD; Shawn Robinson, MD; Stacy Fisher, MD; Joanne Marshall, BSN, MS. Columbia University (2) Jennifer Haythe, MD; Donna Mancini, MD; Rachel Bijou, MD; Maryjane Farr, MD; Marybeth Marks, Henry Arango. Baylor College (2) Biykem Bozkurt, MD, PhD, FACC, FAHA; Mariana Bolos. Thomas Jefferson (1) Paul Mather, MD; Sharon Rubin, MD; Raphael Bonita, MD; Susan Eberwine, RN. Stony Brook University Medical Center (1) Hal Skopicki, MD, PhD; Kathleen Stergiopoulos, MD; Ellen McCathy-Santoro, MD; Jennifer Intravaia, RN, CCRCII; Elizabeth Maas. Morristown Hospital (0) Jordan Safirstein, MD; Audrey Kleet, RN, MS, ACNP-BC, CCRN, CCTC; Nancy Martinez, RN; Christine Corpoin, RN; Donna Hesari, RN. University of Miami (0) Sandra Chaparro, MD; Laura J. Hudson, MA, MPH, CCRC. Harper University Hospital (0) Jalal K. Ghali, MD; Zora Injic, RN, BSc, MSA. Johns Hopkins Hospital (0) Ilan S. Wittstein, MD.