We describe a simple bioinformatics method for biomarker discovery that is based on the analysis of global transcript levels in a population of inbred mouse strains showing variation for disease-related traits. This method has advantages such as controlled environment and accessibility to heart and plasma tissue in the preclinical selection stage. We illustrate the approach by identifying candidate heart failure (HF) biomarkers by overlaying mouse transcriptome and clinical traits from 91 Hybrid Mouse Diversity Panel (HMDP) inbred strains and human HF transcriptome from the Myocardial Applied Genomics Network (MAGNet) consortium. We found that some of the top differentially expressed genes correlated with known human HF biomarkers, such as galectin-3 and tissue inhibitor of metalloproteinase 1. Using ELISA assays, we investigated one novel candidate, Glycoprotein NMB, in a mouse model of chronic $\beta$-adrenergic stimulation by isoproterenol (ISO) induced HF. We observed significantly lower GPNMB plasma levels in the ISO model compared to the control group ($p$-value $= 0.007$). In addition, we assessed GPNMB plasma levels among 389 HF cases and controls from the METabolic Syndrome In Men (METSIM) study. Lower levels of GPNMB were also observed in patients with HF from the METSIM study compared to non-HF controls ($p$-value $< 0.0001$). In summary, we have identified several candidate biomarkers for HF using the cardiac transcriptome data in a population of mice that may be directly relevant and applicable to human populations.

Heart Failure (HF) is a complex disease characterized by a large number of pathological abnormalities including cardiac overload or injury (Braunwald 2008) and the interplay of environmental and genetic factors. In the last decade, several studies have aimed to identify clinically relevant plasma biomarkers for additional assessment of HF using different approaches. We describe a method to identify biomarker candidates using a systems genetics approach, in which a diverse population of individuals is examined for traits of interest as well as high-throughput molecular phenotypes, such as global transcript levels in relevant tissues. Systems genetics approaches can of course be applied to human populations, but mice offer the advantage of avoiding confounders such as disease heterogeneity and differences in environmental factors (Lusis, Seldin et al. 2016). Among existing inbred strains, genetic and phenotypic diversity is as great as that observed in the human population and unlike humans, mice can be subjected to experimental breeding and tissue collection for detailed phenotyping and transcriptomic analyses (Attie, Churchill et al. 2017). Hundreds of human disease models have been developed in mice and nearly all of these appear to be affected by the genetic background of the mouse (Riordan and Nadeau 2017).

We have developed a systems genetics resource termed the Hybrid Mouse Diversity Panel (HMDP), where the inbred mice were chosen...
for diversity. They have been maintained under a variety of environmental conditions, typed for various clinical traits, and subjected to global transcriptomic profiling of relevant tissues (Lusis, Seldin et al. 2016). We describe a study for one trait previously investigated in the HMDP, heart, failure (HF). We show that the list of genes, whose transcript levels in heart correlate most strongly with HF traits, includes known biomarkers of human HF. We investigate a novel potential HF biomarker, Glycoprotein NMB (GPNMB), in both mice and humans. GPNMB is a type 1 transmembrane protein also known as osteoactivin (Selim 2009) that has been recently involved in inflammation, fibrosis and myocardial remodeling (Järve et al. 2017).

**MATERIALS AND METHODS**

**Analysis of Hybrid Mouse Diversity Panel (HMDP) cardiac transcriptome data**

The differential expression of cardiac transcriptome data from 91 inbred strains of the Heart Failure-HMDP study has been published previously (Wang, Rau et al. 2016). We performed correlation analysis of the change in left ventricular internal dimension (LVIDd) from baseline to week 3 of isoproterenol and cardiac transcript levels at week 3 of isoproterenol.

**Analysis of GPNMB transcript level in the human Myocardial Applied Genomics Network (MAGNet) study**

In order to confirm the upregulation of GPNMB in humans during HF, we examined available human cardiac transcriptome data from the MAGNet consortium. The MAGNet consortium has collected and evaluated the cardiac transcriptome using microarrays for 313 subjects at the time of heart transplant or explant (95 individuals with ischemic cardiomyopathy (ICM), 82 with dilated cardiomyopathy (DCM), and 136 non-heart failure (NF) unused donors (Das, Morley et al. 2015, Liu, Morley et al. 2015). RNA-Seq and microarray data have been deposited in the Gene Expression Omnibus (GEO) Database (Accession number GSE57345). Differential gene expression analysis was performed using GEO2R available on the GEO website.

**Mouse Models of Heart Failure**

We assessed circulating GPNMB levels in 2 well-established mouse HF models: The pressure overload by transverse aortic constriction (TAC) and the chronic β-adrenergic stimulation by continuous isoproterenol (ISO)-induced cardiac hypertrophy. For the TAC model, mice were divided into TAC or sham surgery groups. Sham mice received a mid-sternal incision to expose only the transverse aorta. For the ISO model, mice were divided into control and ISO treatment groups. ISO was administered via an intraperitoneal minipump that delivers a continuous infusion of 30 mg/kg/day for 21 days. The ISO dose was determined according to previously published data and our HMDP study (Oudit, Crackower et al. 2003, Berthonneche, Peter et al. 2009, Galindo, Skinner et al. 2009, Wang, Rau et al. 2016). Both HF models were performed in 10-week-old female C57BL/6j mice.

Plasma samples were collected by retro-orbital puncture at the time of euthanasia, which was at 4 weeks after intervention for TAC mice (n = 6) and at 3 weeks after infusion pump implantation for ISO mice (n = 10). Upon conclusion of the experiments, animals were Sacrifice and the hearts were removed. The UCLA Institutional Animal Care and Use Committee (IACUC) approved all animal studies.

**Echocardiography**

Echocardiograms were performed using the Vevo 2100 ultrasound system (VisualSonics, Inc., Toronto, ON, Canada). A parasternal long-axis B-mode image was obtained. The maximal long-axis of the LV was positioned perpendicular to the ultrasound beam. A 90° rotation of the ultrasound probe at the papillary muscle level was performed to obtain a parasternal short-axis view of the LV. A M-mode image to document LV dimensions was captured and saved for analysis using the Vevo 2100 cardiac analysis package. Baseline echocardiograms were performed on all of the mice. In the isoproterenol cohort, final echocardiograms were performed for control and isoproterenol-treated mice at week 3 of the experiment. In the TAC group, final echocardiograms were performed for control and TAC-treated mice at week 4 of the experiment. To ensure adequate sedation while minimizing the effects of inhaled isoflurane on loading conditions, heart rate, cardiac structure and function, we minimized induction and maintenance doses of isoflurane at or below 1.25% and 1%, respectively, while closely monitoring for HR < 475 bpm as a sign of deep sedation and adjusting isoflurane dosage as needed (Wang, Rau et al. 2016).

**Western blot analysis of GPNMB in heart tissues of mice**

Proteins from the heart tissue of ISO treated, TAC mice, and control mice were harvested in buffer (50mM HEPES [pH 7.4], 150mM NaCl, 1% NP-40, 1mM EDTA, 1mM EGTA, 1mM glycophosphate, 2.5mM sodium pyrophosphate 1mNa3VO4, 20mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 μL/g of aprotinin, leupeptin, and pepstatin). Equal amounts of protein were separated on 4–12% Bis-Tris gels (Invitrogen, Carlsbad, CA) using an electrophoretic apparatus (Bio–Rad Laboratories, Hercules, CA) and transferred onto a nitrocellulose blot (Amersham, GE Healthcare). The blot was probed with the indicated primary antibodies using the polyclonal anti-GPNMB (R&D Systems, Minneapolis, MN) and anti-GAPDH (Invitrogen, Carlsbad, CA). Protein signals were detected using HRP conjugated secondary antibodies (Cell Signaling Technologies) and enhanced chemiluminescence (ECL) western blotting detection reagents (Amersham, GE Healthcare).

**Cross-sectional study of the METabolic Syndrome In Men (METSIM) cohort**

The METSIM study is comprised of 10,197 Finnish men recruited between age 45 to 74 years (mean ± SD = 58 ± 7 years) by random sampling from the population register of Kuopio, Eastern Finland. The METSIM study and its methods have been described in detail elsewhere (Stancakova, Javorsky et al. 2009, Laasko, Kuusisto et al. 2017). The METSIM HF cases were identified by screening medical records for HF diagnostic codes and by querying the Finnish medication reimbursement database for HF medications. A total of 119 subjects with HF were identified and 270 control subjects with no previous diagnosis of HF or...
G3 Genes | Genomes | Genomics | Genetica
Volume 8 | November 2018 | Biomarkers for Heart Failure | 3501

current clinical or biochemical indication of cardiovascular diseases or other chronic disease including chronic kidney disease and end stage renal disease patients were determined to be controls. The study was approved by the Ethics Committee of the University of Eastern Finland and Kuopio University Hospital.

**GPNMB measurements in mice and humans**

Plasma GPNMB levels in mice and human samples were assayed using commercial enzyme-linked immunosorbent assay kits (R&D systems, Minneapolis, MN) (Catalogue numbers: DY2330 and DY2550, respectively).

**Statistical analysis**

The t-test statistic was used to examine differences between HF and control plasma GPNMB protein levels in mice. The p-value threshold of < 0.05 was considered statistically significant. Clinical characteristics of HF cases and non-HF controls were compared using t-tests for continuous variables and Fisher’s exact tests for categorical variables. A Spearman rank correlation test was used to assess the correlation between GPNMB and proBNP. The associations between GPNMB and HF were investigated by univariate and multivariate logistic regression models with the SPSS statistical software package.

**Data availability**

The HMDP cardiac transcriptome data are available at the Gene Expression Omnibus (GEO) online database by the accession GSE48760 (Wang, Rau et al. 2016). The complete correlation data of cardiac transcripts with ISO-induced left ventricular dilation is presented in Supplemental Table 1. Supplemental table 2 includes unidentifiable clinical data of all METSIM study. All the statistical analyses were performed with the SPSS statistical software package.

**RESULTS**

**Selection of GPNMB as a candidate biomarker for HF**

The list of transcripts that were most perturbed in terms of fold change by isoproterenol, including GPNMB (FC = 1.6, p-value = 1.92x10^-12) are shown in Table 1. In addition, we ranked transcripts by the magnitude of correlation with left ventricular dilation, a clinical trait we used as a surrogate marker of adverse cardiac remodeling (Table 2). Of interest, the top correlated transcripts with left ventricular dilation corresponded to genes involved in collagen synthesis and degradation (Col5a1, Luther, Thodeti et al. 2012), Col5a1 (Roulet, Ruggiero et al. 2007), Fbn1 (Fedak, de Sa et al. 2003), remodeling of the heart and arterial calcification (Dtr, Spp1 (Peacock, Huk et al. 2011), Enpp1 (Pillai, Li et al. 2017)), extracellular matrix synthesis and degradation (Ctsk (Hua, Xu et al. 2013), Sparc (Bradshaw 2009, Toba, de Castro Bras et al. 2016) and Mfaps5 (Vaittinen, Kolehmainen et al. 2015)).

We examined cardiac expression of Npph, Timp1 and Lgals3, which are transcripts encoding three well-known heart failure plasma biomarkers brain natriuretic peptide (BNP), tissue inhibitor of metalloproteinase 1 (TIMP1), and galectin-3 (de Boer, Voors et al. 2009, Goldbergova, Parenica et al. 2012, Ho, Liu et al. 2012). Timp1 and Lgals3 increased by 3.5- to 4 fold with isoproterenol treatment (Table 1). Both transcripts were also positively correlated with isoproterenol-induced left ventricular dilation (Table 2; Timp1: r = 0.25, p-value = 0.02; Lgals3: r = 0.29, p-value = 0.006). Interestingly, although Nppb level was positively correlated with left ventricular dilation (r = 0.26, p-value = 0.01), Nppb level was not significant altered by isoproterenol.

Next, we performed differential gene expression analysis of microarray-based transcriptome data deposited in the Gene Expression Omnibus (GEO) database (GSE57345 GPL19052) from the MAGNet consortium human cardiac tissue collection using GEO2R. MAGNet consortium collected and evaluated the cardiac transcriptome by microarray at the time of heart transplant or explant (Das, Morley et al. 2015, Liu, Morley et al. 2015). TIMP1 and LGALS3 were significantly differentially expressed in the MAGNet study (LogFC = -0.69, p-value = 6.32 x 10^-17 and logFC = 0.17, p-value = 8.30 x 10^-6, respectively). As observed in mice, NPPB levels were not differentially expressed between HF cases and control subjects (p-value = 0.32).

We overlaid the top differentially expressed (Table 1) and correlated (Table 2) lists from the heart failure HMDP to identify novel candidate transcripts that were both differentially regulated by isoproterenol and correlated significantly to left ventricular dilation. Cdo1 and Gpmb fit both criteria. While Cdo1 was negatively correlated with left ventricular dilation (probe ID = ILMN_2975345, r = -0.37, p-value = 3.8 x 10^-4), Gpmb was positively correlated with left ventricular dilation (probe ID = ILMN_2614625, r = 0.36, p-value = 5.2 x 10^-11). Gpmb encodes a transmembrane protein expressed in macrophages and has an ectodomain that is shed by its regulatory protein ADAM10 to the extracellular compartment. We chose to follow up on GPNMB after confirming that GPNMB transcript levels were similarly upregulated in failing vs. non-failing hearts by 1.2 fold (p-value= 2.9 x 10^-6) in subjects from the MAGNet cohort.

**GPNMB levels in two mouse models for HF**

To confirm the protein levels of GPNMB in heart failure, we used two widely accepted modes of cardiac injury, isoproterenol (ISO) and transverse aortic constriction (TAC), to induce a heart failure-like state.
in mice. Both models lead to cardiac hypertrophy as measured by heart weight at sacrifice and left ventricular mass estimates by echocardiography (Figure 1). Consistent with our observation in the cardiac transcriptome, GPNMB protein expression in the heart was increased in mice treated with ISO as compared to controls (Figure 2A and 2B). Similarly, GPNMB protein level in the TAC hearts also showed a significant increase as compared with sham animals (Figure 2C and 2D), indicating that there is increased GPNMB cardiac expression in two different HF mouse models.

Furthermore, we measured plasma GPNMB protein levels in the ISO and TAC models. In the ISO model, after 3 weeks of continuous infusion of ISO, plasma levels of GPNMB were lower than in the control group (5.96 ± 2.66 ng/mL in control vs. 3.18 ± 1.08 ng/mL in ISO; p-value = 0.007) (Figure 2C). Although the plasma GPNMB levels in the TAC model compared with the sham surgery group at 4 weeks after surgery were not statistically significantly different due to small sample sizes, there was a trend toward decreased GPNMB levels in the TAC mice (4.19 ± 2.33 ng/mL in Sham vs. 2.22 ± 1.80 ng/mL in TAC; p-value = 0.13) (Figure 2F).

**Table 2** Top correlated transcripts with isoproterenol-induced left ventricular dilation

| innm_id | symbol | cor  | p-value |
|---------|--------|------|---------|
| ILMN_2698449 | Drt  | 0.433 | 2.6E-05 |
| ILMN_2768087 | Col6a1 | 0.429 | 3.1E-05 |
| ILMN_2636424 | Itgb1 | 0.414 | 6.2E-05 |
| ILMN_2818294 | Srpz2 | 0.410 | 7.3E-05 |
| ILMN_2883952 | 1810015A11Rik | 0.410 | 7.3E-05 |
| ILMN_2887408 | Gai3 | 0.406 | 8.6E-05 |
| ILMN_2597831 | Cacna1c | -0.403 | 9.9E-05 |
| ILMN_2748402 | Col5a1 | 0.398 | 1.2E-04 |
| ILMN_2603958 | 9130427A09Rik | 0.391 | 1.6E-04 |
| ILMN_2721149 | Arf11 | 0.389 | 1.8E-04 |
| ILMN_2946873 | D030070L09Rik | 0.388 | 1.9E-04 |
| ILMN_2638256 | Tlx16 | -0.379 | 2.7E-04 |
| ILMN_1259388 | Col6a1 | 0.378 | 2.8E-04 |
| ILMN_2717163 | Ctsk | 0.378 | 2.8E-04 |
| ILMN_2811421 | Matk | 0.377 | 2.9E-04 |
| ILMN_2782964 | Enpp1 | 0.376 | 3.1E-04 |
| ILMN_2664660 | Aldh5a1 | -0.375 | 3.2E-04 |
| ILMN_2696063 | Spp1 | 0.374 | 3.4E-04 |
| ILMN_3136651 | Sparc | 0.373 | 3.4E-04 |
| ILMN_1232884 | Sphk1 | 0.371 | 3.7E-04 |
| ILMN_2750201 | 1700023I07Rik | 0.370 | 3.8E-04 |
| ILMN_2975345 | Cdo1 | -0.370 | 3.8E-04 |
| ILMN_2641956 | Naxb | -0.370 | 3.9E-04 |
| ILMN_2833163 | BC064033 | 0.369 | 4.0E-04 |
| ILMN_2613601 | 2010001M09Rik | -0.367 | 4.3E-04 |
| ILMN_1231851 | Enpp1 | 0.365 | 4.6E-04 |
| ILMN_2953515 | Aldh3b1 | 0.365 | 4.7E-04 |
| ILMN_1223552 | Fbn1 | 0.365 | 4.8E-04 |
| ILMN_2645526 | Abcc8 | -0.363 | 5.1E-04 |
| ILMN_2616455 | Gpnnmb | 0.363 | 5.2E-04 |
| ILMN_1214571 | Cd109 | 0.361 | 5.5E-04 |
| ILMN_1225835 | Mfap5 | 0.360 | 5.6E-04 |
| ILMN_2702704 | Nduv1 | -0.359 | 5.9E-04 |
| ILMN_2725484 | Padi4 | 0.359 | 6.0E-04 |
| ILMN_2691951 | Polydom | 0.358 | 6.2E-04 |
| ILMN_1221611 | Pitpn | 0.357 | 6.3E-04 |
| ILMN_1228485 | Csnk2a2 | -0.356 | 6.6E-04 |
| ILMN_2838317 | Ppil3 | 0.356 | 6.6E-04 |
| ILMN_1221800 | Gabpa | 0.356 | 6.6E-04 |
| ILMN_2646254 | 1700102P08Rik | -0.356 | 6.6E-04 |
| ILMN_3022719 | Wiz | -0.355 | 6.8E-04 |
| ILMN_2453695 | Urod | -0.353 | 7.5E-04 |
| ILMN_2671170 | Gm128 | 0.352 | 7.6E-04 |
| ILMN_3116885 | Gpr137b | 0.352 | 7.7E-04 |
| ILMN_2487358 | Ef3A6 | 0.351 | 8.1E-04 |
| ILMN_2671755 | Ceecam1 | 0.351 | 8.1E-04 |
| ILMN_2492500 | Zfhx1a | 0.351 | 8.1E-04 |

**GPNMB levels in human HF From the METSIM study**

We measured plasma GPNMB levels in 119 HF subjects and 270 non-HF controls from the METSIM study. Patients’ baseline characteristics are listed in Supplemental Table 3. The distribution of plasma GPNMB did not reveal normality in both control and HF groups, thus we used log GPNMB in these analyses. As observed in the ISO mice, there were significantly lower plasma GPNMB levels in patients with HF compared with non-HF controls (GPNMB 1.20 ± 0.26 ng/mL in control vs. 0.74 ± 0.40 ng/mL in heart failure, p-value < 0.0001) (Figure 2G). To prevent bias due to an age difference between HF cases and controls, we performed sensitivity analysis confirming that our results were not affected by age differences between the groups (p-value < 0.001). GPNMB level, age, BMI, history of HTN and DM, eGFR and LDL-C were significantly associated with HF (Table 3) and were included in the multivariate analyses. The association between GPNMB and HF remained significant in the multivariate analyses (OR = 0.86 [0.82-0.90], p-value < 0.001). In a subset of HF cases, where proBNP levels were available, GPNMB and proBNP were found to be independent (r =0.028, p-value =0.863), suggesting that measurement of GPNMB in plasma of HF patients may provide additional prognostic value or reflect different clinical or biological states from those associated with proBNP elevation (Figure 3).

**DISCUSSION**

In the present study, we analyzed global cardiac transcriptomic data from the Heart Failure-HMDP study as a strategy to identify novel plasma biomarkers for heart failure. We found that cardiac transcripts of established HF plasma biomarkers, including TIMP1 and galectin-3, were differentially expressed in ISO-treated mouse hearts and correlated with left ventricular dilation compared to the control group. We identified Gpnnmb as an attractive candidate based on similar properties and confirmed its upregulation in the MAGNet human heart failure transcriptome collection. Next, we confirmed the upregulation of GPNMB protein levels in ISO and TAC mice. Subsequently, we examined plasma GPNMB levels in mice treated with ISO and TAC. We found significantly lower levels of circulating GPNMB in the ISO model and a trend toward decrease in the TAC model. This was a surprising finding not explained by a known mechanism. We also investigated whether lower levels of circulating GPNMB were found in human HF patients. Similar to our observation in mice, circulating plasma GPNMB levels were also lower in patients with HF from the METSIM study compared to the control group.

GPNMB has been shown to play a role in promoting tissue regeneration after muscle, kidney, liver and cerebral ischemia reperfusion injury by regulation of immune/inflammatory responses and suppressing fibrosis (Abe, Uto et al. 2007, Furochi et al. 2007a,b, Nakano, Suzuki et al. 2014, Nagahara, Shimazawa et al. 2015). Previous studies using different cardiac injury models have shown that cardiac tissue levels of GPNMB generally increased in response to stress. These include the desmin knockout mouse model (Psarras, Movridis et al. 2012), the Thieier’s murine encephalomyelitis virus-induced acute viral myocarditis model (Omura, Kawai et al. 2014), and the myocardial infarction rat and mouse models (Järve et al. 2017). In the myocardial
infarction model, GPNMB mRNA transcript was up-regulated 17-fold in the peri-infarct (PI) area in the rat and 300-fold in the mouse at 24 hr and 7 days after myocardial infarction, respectively. Approximately 50% of the CD68+ macrophages expressed GPNMB (Jarve et al. 2017). Similar to these publications, we observed an upregulation of Gpnmb by isoproterenol on average across the HMDP mouse strains, in the MAGNet human heart failure transcriptome data and two different cardiac injury models, isoproterenol and transverse aortic constriction, in C57BL/6J mice.

The exact mechanism by which GPNMB exerts its effect on the heart is not clear. Increased GPNMB expression is seen following injury in multiple organs including the heart (Jarve et al. 2017) and kidney (Zhou, Zhuo et al. 2017) and GPNMB could be playing organ specific roles in wound healing. In this regard, it was observed in a study comparing

![Figure 1](image1.png)

**Figure 1** Isoproterenol and transverse aortic constriction induced cardiac remodeling characteristics among C57BL/6J mice. LVID denotes left ventricular internal dimension during diastole. CON denotes control. ISO denotes isoproterenol infusion at 30 mg/kg/day for 21 days. TAC denotes transverse aortic constriction for 28 days.

![Figure 2](image2.png)

**Figure 2** GPNMB levels in Isoproterenol, transverse aortic constriction (TAC) heart failure mouse models and in patients with heart failure. A. Western blot analysis of GPNMB expression in C57BL/6J mouse heart lysates after ISO treatment. B. Graphic representation of Western blot analysis for the ISO model. C. GPNMB plasma levels comparison between control and ISO-treated mice. For the ISO model, mice were anesthetized with intraperitoneal ketamine as a surgical anesthetic agent, and osmotic minipumps were implanted subcutaneously. D. Western blot analysis of GPNMB expression in C57BL/6J mouse heart lysates after TAC surgery. GAPDH was used as housekeeping control. E. Graphic representation of Western blot analysis for the TAC model. F. GPNMB plasma levels comparison between Sham and TAC. For the TAC model, midsternal incision was made to expose transverse aorta between truncus anonymous and the left carotid artery. G. GPNMB levels in HF patients and controls from the METSIM study.
GPNMB-deficient DBA/2J mice and their coisogenic DBA/2J-GPNMB + relatives, that GPNMB appeared to confer increased risk of adverse ventricular remodeling with left ventricular dilation and a decrease in fractional shortening after myocardial infarction (Järve et al. 2017). Because GPNMB has been implicated in endothelial adhesion and transendothelial migration (Shikano, Bonkobara et al. 2001), Jarve et al. postulated that GPNMB-deficiency may impair trans-endothelial migration of monocytes from blood to cardiac tissue. Indeed, elevated numbers of monocytes with the proinflammatory Ly6C(CD11b) phenotype were identified in the blood and bone marrow of GPNMB-deficient mice (Järve et al. 2017). In contrast, the same adverse impact of GPNMB on cardiac remodeling was not observed after isoproterenol (Järve et al. 2017). This could be related to the fact that isoproterenol infusion is associated with decreased inflammatory infiltrate compared to an acute injury such as myocardial infarction that is associated with an intense inflammatory infiltrate in the heart. Moreover, previous studies have suggested that GPNMB serves as an inflammatory stop signal in HF that inhibits the activation of T lymphocytes by binding syndecan 4 (Chung, Sato et al. 2007), a proteoglycan that is up-regulated in chronic HF (Takahashi, Negishi et al. 2011) and has been previously shown to adversely influence cardiac remodeling (Kojima, Takagi et al. 2001). If true, increased consumption of GPNMB or lower circulating levels of GPNMB could be indicative of more severe HF. Taken together, whether GPNMB expression is deleterious to cardiac remodeling may depend upon the mode of injury, type of inflammatory response present, and local cellular expression vs. circulatory levels of GPNMB ectodomain. Additional studies using different cardiac injury models, examining inflammatory response and sites of GPNMB action are needed to fully delineate GPNMB’s relationship with cardiac injury and remodeling.

The observation of directionally opposite changes in biomarker abundance in tissue vs. plasma is especially intriguing. GPNMB, also known as osteoactivin, is a highly-glycosylated type I trans-membrane protein of 572 amino acids that has an integrin and a heparin binding motif, an endosomal sorting signal in the cytoplasmic domain, and a polycystic kidney disease domain of unknown function. It is localized to the cell surface and phagosomal membranes. There is also a secreted variant of the protein that results from ectodomain shedding following cleavage by the metalloprotease ADAM10, such that the cleaved extra-cellular domain circulates as an apparently biologically active fragment (Hoashi, Sato et al. 2010, Rose, Annis et al. 2010). Of note, PKC and Ca (2+) intracellular signaling pathways regulate ectodomain shedding from the largely Golgi-modified form of GPNMB in melanocytes (Hoashi, Sato et al. 2010). Ectodomain fragments of GPNMB act as a growth factor to induce matrix metalloprotease-3 (MMP-3) expression via the ERK pathway in fibroblasts in C2C12 myoblast cultures (Furochi et al. 2007a). The GPNMB ectodomain, released following ADAM10 cleavage of GPNMB from the surface of breast cancer cells, is capable of inducing endothelial cell migration (Rose, Annis et al. 2010). Our observations of increased GPNMB levels in the heart associated with decreased circulating GPNMB levels likely represent changes of GPNMB processing involving cleavage or binding in the setting of HF.

Transcriptome data in the HMDP showed that transcript levels of GPNMB and ADAM9 were positively correlated (r = 0.22, p-value = 0.029). Inhibition of ADAM9, a sheddase of ADAM10, reduced the amount of ADAM10 enzyme in the medium while increasing membrane-bound ADAM10 (Moss, Powell et al. 2011). We postulate that recruitment of GPNMB-expressing monocytes to the heart occurs along with elevated levels of ADAM9, leading to increased ADAM10 shedding and decreased active ADAM10 at the cell surface, thereby decreasing GPNMB cleavage by membrane-bound ADAM10 and lowering circulatory levels of GPNMB. Alternatively, the endosomal regulation of GPNMB by PKC and Ca (2+) intracellular signaling pathways may determine cell surface expression, ectodomain shedding and circulating levels of GPNMB. Additional studies will be required to fully understand the directionally opposite changes in biomarker abundance in tissue vs. plasma.

Due to random selection rather than matched selection, our human controls were not properly matched to the heart failure cohort by demographics and comorbidities. Therefore, we cannot conclude based

| Variables                  | Univariate analysis | Multivariate analysis |
|---------------------------|---------------------|-----------------------|
|                           | OR(95% CI)          | P-value               | OR(95% CI)          | P-value               |
| GPNMB, ng/ml              | 0.865 (0.834-0.896) | <0.001                | 0.863 (0.824-0.904) | <0.001                |
| Age, years                | 1.306 (1.233-1.384) | <0.001                | 1.277 (1.182-1.379) | <0.001                |
| Body mass index kg/m²     | 1.188 (1.124-1.256) | <0.001                | 1.142 (1.057-1.233) | 0.001                 |
| Hypertension              | 6.173 (3.703-10.309)| <0.001                | 2.922 (1.286-6.643) | 0.010                 |
| Diabetes mellitus         | 13.699 (6.536-28.571)| <0.001               | 6.711 (2.128-21.277)| 0.001                 |
| eGFR, mL/min/1.73 m²      | 0.975 (0.960-0.989) | 0.001                 | 0.994 (0.971-1.017) | 0.603                 |
| LDL-c, mg/DL              | 0.972 (0.965-0.980) | <0.001                | 0.991 (0.980-1.002) | 0.097                 |

GPNMB: glycoprotein non-metastatic melanoma protein B; eGFR: estimated glomerular filtration rate; LDL-c: low density lipoprotein cholesterol; OR: Odds ratio; CI: confidence interval.
on our human data alone that lower GPNMB levels are an independent heart failure risk factor. However, our experiments in mice, using matched littermate controls, supports our claim that GPNMB may be a useful independent heart failure biomarker. Finally, we found that GPNMB levels in plasma were independent of proBNP levels, suggesting that GPNMB may be predictive of outcomes based on properties that are dissimilar to the most commonly used biomarker for HF. This characteristic of GPNMB may add a prognostic value to existing clinical practice and, therefore, warrant confirmatory investigation in a larger human cohort. Additional biomarkers that ascertain various properties of HF may be important additions to the full evaluation of HF susceptibility.

In conclusion, we report a proof of concept study illustrating the application of systems genetics data for the identification of biomarkers for HF. We have identified GPNMB as a promising novel plasma biomarker for heart failure based on our preliminary data in two HF mouse models and in human samples. The molecular mechanisms for which GPNMB are implicated in HF warrant further investigation. Additional candidate biomarkers await full characterization toward the goal of distinguishing disease manifestation and progression, precise risk assessment and tailored therapy.

ACKNOWLEDGMENTS
This work was supported in part by the American Heart Association Grant 13SDG16400052 (AHV), NIH grants K08 HL133491 (JJW), HL123295, HL114437, GM053275 (ISS), HL129178, HL137241 (AD) HL30568 (AIL), Academy of Finland (ML), The Finnish Heart Disease Foundation (ML) and a research grant Taipei Veterans General Hospital-National Yang-Ming University Excellent Physicin Scientists Cultivation Program, No. 105-V-A-008 to YLT. SCC is a postdoctoral fellow supported by UPLIFT; UCLA Postdocs’ Longitudinal Investment in Faculty (Award # K12 GM106996) and the David Geffen School of Medicine.

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Communicating editor: F. Pardo-Manuel de Villena