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Common variants on *FGD5* increase hazard of mortality or re-hospitalization in heart failure patients from ASCEND-HF trial

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Abstract

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Registration

This was registered as NCT00475852 (https://clinicaltrials.gov/ct2/show/NCT00475852).

Disclosures

DEL is a consultant for Amgen, Janssen, Ortho Diagnostics and DCRI (Novartis) and has participated in clinical trials from Amgen, Bayer, and Janssen. SF is currently a consultant for Janssen and was previously employed by Janssen. TMC was previously employed by Janssen. CDM has participated in clinical studies for Pfizer. WWHT is a consultant to Sequana Medical AG. HG, JL, RS, LKW, JAL, NLP, PB and KFA have nothing to disclose.

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Background—Heart failure (HF) remains a global health burden and patients hospitalized are particularly at-risk, but genetic associates for subsequent death or re-hospitalization are still lacking.

Methods—The genetic sub-study of the Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure (ASCEND-HF) trial was used to perform genome-wide association study (GWAS) and trans-ethnic meta-analysis. The overall trial included the patients of self-reported European ancestry (EA, N=2,173) and African ancestry (AA, N=507). The endpoint was death or HF re-hospitalization within 180 days. Cox models adjusted for 11 *a priori* predictors of re-hospitalization and 5 genetic principal components were used to test the association between single nucleotide polymorphisms (SNP) and outcome. Summary statistics from the two populations were combined via meta-analysis with the significance threshold considered $p<5\times10^{-8}$.

Results—Common variants (rs2342882 and rs35850039 in complete linkage disequilibrium) located in *FGD5* were significantly associated with the primary outcome in both ancestry groups (for EA Hazard ratio [HR] =1.38, $P=2.42\times10^{-6}$; AA HR =1.51, $P=4.43\times10^{-3}$; HR in meta-analysis =1.41, $P=4.25\times10^{-8}$). *FGD5* encodes a regulator of VEGF-mediated angiogenesis and *in-silico* investigation revealed several previous GWAS 'hits' in this gene, among which rs748431 was associated with our outcome (HR=1.20, meta $P<0.01$). Sensitivity analysis proved *FGD5* common variants survival association did not appear to operate via coronary artery disease (CAD) or nesiritide treatment (P >0.05); and the signal was still significant when changing the censoring time from 180 to 30 days (HR=1.39, $P=1.59\times10^{-5}$).

Conclusions—In this multi-ethnic GWAS of ASCEND-HF, SNPs in *FGD5* were associated with increased risk of death or re-hospitalization. Additional investigation is required to examine biological mechanisms and whether *FGD5* could be a therapeutic target.

Registration—This was registered as NCT00475852 (https://clinicaltrials.gov/ct2/show/NCT00475852).

Keywords

acute heart failure; survival; GWAS; *FGD5* ; meta-analysis

1. Introduction

Heart failure (HF) continues to be an enormous public health problem despite the many advances in its treatment over the past 25 years. Acute decompensated HF (ADHF) in particular is a critical issue due to its high mortality, re-hospitalization rates, astronomical costs, and dearth of specific therapies. [1,2] Part of the difficulty which has hampered progress in ADHF is that the patient population, response to therapies, and clinical outcomes in this entity are highly heterogeneous; it is likely that exacerbated HF represents a wide variety of underlying patient, disease, and treatment-response phenotypes. [3–5] A clearer concept of this underlying variability may hold the key to improving outcomes in ADHF and identifying new strategies for intervention. Investigation of genomic factors contributing to ADHF outcomes could thus illuminate underlying pathobiology at work, allowing us to identify patients with different natural histories or response to therapy, elucidate new pathways

to target, or select patient sub-groups for whom existing therapies may be particularly efficacious.

The Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure (ASCEND-HF) trial [6–9] has several characteristics that are advantageous in terms of exploring these critical questions. This randomized clinical trial of nesiritide (recombinant human b-type natriuretic peptide [BNP]) in hospitalized HF patients was the largest study of ADHF performed to date, enrolling a diverse cohort of roughly 7,000 patients worldwide. The patients in ASCEND-HF were well characterized in terms of comorbidities, symptoms, and clinical outcomes. Nesiritide did not significantly affect the primary outcome, which was re-hospitalization for HF or death within 30 days.[9] ASCEND-HF also conducted a genetic sub-study which enrolled roughly 3000 subjects. This dataset is therefore well-suited to evaluate whether there are important genetic factors that predict outcomes after hospitalization with ADHF.[10,11]

Genome-wide association studies (GWAS) have been reported for many complex disorders, including HF and other cardiovascular disease traits that are risk factors for HF.[12] One recent large-scale consortium identified 12 independent loci (e.g., *LPA, ABO* and *BAG3*) underlying the pathogenesis of all-cause HF.[13] Despite this progress, little is known regarding genetic influences on outcomes among those with established HF. A previous study found that 5q22 variants influence HF mortality in a European population,[10] but this locus was only in chronic stable HF (as opposed to acutely exacerbated patients) and has not yet been replicated in other cohorts to our knowledge. To date, no GWAS has analyzed hospitalized HF patients or examined mortality and re-hospitalization among HF patients.

Therefore, in this study we performed a trans-ethnic GWAS to identify novel genetic variants associated with death or re-hospitalization for ASCEND-HF participants, and tested candidate associations of genes or loci identified in previous HF GWAS studies.

2. Methods

2.1 Parent Study, Patients, and Endpoints

The methods and results of the ASCEND-HF trial have been previously described.[7,9] Briefly, this multinational clinical trial of 7,141 patients hospitalized for HF randomized participants to nesiritide or placebo. The primary endpoint was all-cause death or recurrent hospitalization at 30 days in the original trial. However, outcome data from the trial was gathered to at least 180 days. Therefore we chose the longer window to realize the clinical value of observation over a longer period of time as well as to optimize power by including more events. Of the overall study participants, 3,097 also gave written informed consent to participate in the genetic sub-study. Patients were eligible to participate in the study if: 1) they were hospitalized for HF occurring within 24 hours before they received their first intravenous treatment for HF, or 2) they had received a diagnosis of ADHF less than 48 hours after hospitalization for another cause and underwent randomization within 24 hours after intravenous treatment for HF. Key exclusion criteria were a high risk of hypotension (systolic pressure <100 mm Hg or 110 mm Hg with the use of intravenous nitroglycerin), other contraindications for vasodilators, treatment with dobutamine (at a dose

≥5 μg per kilogram of body weight per minute), treatment with milrinone or levosimendan within the previous 30 days, persistent uncontrolled hypertension, acute coronary syndrome, normal level of BNP or N-terminal pro-BNP, severe pulmonary disease, end-stage renal disease during receipt of renal replacement therapy, and clinically significant anemia. The current study (genetic analysis of ASCEND-HF sub-study) was approved by the Henry Ford Hospital Institutional Review Board and conformed to the principles of the Declaration of Helsinki. The individual genetic data underlying this article will not be shared due to lack of authority by consent to share. GWAS summary statistics will be deposited into EBI GWAS Catalog for free access.

2.2 DNA Samples and Genotyping

DNA samples were isolated from blood at the Cleveland Clinic genetics lab. These were labeled with de-identified sample ID numbers (unrelated to study ID) and shipped to Henry Ford Hospital where they underwent quantification, plating, and then genotyping. Whole genome SNPs were genotyped using Axiom® Biobank Array (Affymetrix, Santa Clara, CA, USA). We further imputed additional SNP genotypes using Minimac3 on Michigan Imputation Server with 1000 Genomes Phase 3 (version 5) as reference panel.[14] GWAS data quality control was achieved using standard Affymetrix recommended pipeline implemented by the University of Michigan Affymetrix core lab (Ann Arbor, MI, USA). Sample quality assurance excluded samples with 1) missing genotyping rate > 10%, 2) outliers from heterozygosity (i.e., Fst > or < 4 standard deviation from the mean), 3) elevated identity by descent (IBD) relatedness (i.e., PI_HAT > 0.125), or 4) outlier from principal component analysis (PCA) plotting (i.e., scatter plot using first and second PCs). SNP assay quality checking excluded 1) minor allele frequency (MAF) < 0.05, 2) genotyping call rate < 95%, 3) Hardy-Weinberg equilibrium (HWE) p-value < 1×10^{-6}, or 4) imputation score < 0.5. A total of 2,795 patients successfully underwent genome-wide (GW) genotyping. From these a total of 2,680 subjects of European ancestry (EA, N=2,173) and African ancestry (AA, N=507) based on participant self-report had analyzable data of high enough quality for inclusion in the analytic cohort for this project. To show whether self-reported ancestry matches with the genetic ancestry, we projected our samples into the principal component maps of 3 reference populations (i.e., CEU, YRI, and CHB) from 1000 Genome project, using a subset of ~4K common independent SNPs across different populations.[15]

2.3 Statistical analysis

Test of difference for key variables (e.g., age, sex, event rate) in these two groups were performed by independent student t-test, chi-square test, or log-rank test respectively, depending on which data type it belongs to (i.e., continuous, categorical, or survival data). The primary composite endpoint of all-cause death or re-hospitalization was modeled in time-to-event fashion over the entire available follow up period (roughly 180 days). As described previously, adjudicated data were used to identify the presence and timing of endpoints. Covariates used in a previous survival analysis of the ASCEND-HF study[16] were included in multivariable Cox proportional hazards models *a priori*: age, systolic blood pressure (SBP), sodium, blood urea nitrogen (BUN), dyspnea at rest, elevated jugular venous pressure (JVP) noted during qualifying HF event, history of depression treated with medications, history of chronic respiratory disease, history of cerebrovascular disease,

history of hospitalized status for HF within past year (each as yes/no), and creatinine. Nesiritide vs placebo status was not included because treatment had no effect on the endpoint, as previously reported.[9] Patients with missing record for any of these 11 variables were excluded in this analysis. With the 'survival' R package, the relationship between the primary endpoint and selected covariates was examined using Cox regression ('coxph' function), and the proportional hazards assumption was checked by a global test based on the scaled Schoenfeld residual ('cox.zph' function).

2.4 GWAS and Meta-analysis for genetic variants

Population-specific GWAS was firstly conducted for ASCEND EA or AA patients separately to identify common genetic variants as risk to HF survival. We used Cox proportional hazards regression models (assocCoxPH function in R package "GWASTools") to test associations of SNPs with the endpoint (i.e., time-to-death or re-hospitalization) for EA or AA patients in ASCEND-HF trial.[17] In addition to the 11 covariates described above, we also included the top five PCs estimated from the SNP array to control for population stratification. Then we combined the results from EA- and AA-GWAS by trans-ethnic meta-analysis. To estimate overall effect across the two cohorts, summary statistics (*p*-values, sample size, beta estimate, and standard error) from both groups were combined using a fixed-effect model in METAL[18] and a random-effect model (Han and Eskin's) in MetaSoft.[19] A variant was considered replicated if its association *p*-value was <0.05; variants with $p<5\times10^{-8}$ in the meta-analysis were considered to meet GWAS significance. A list of previously reported GWAS hits for HF phenotypes (onset, incidence, or mortality)[10,13,20] in GWAS Catalog (retrieved on May 01 2021) were also interrogated.[21] To better present GWAS result, we generated Quantile-quantile (QQ) plot, Manhattan plot, regional plot, and linkage disequilibrium (LD) block plot using self-written R script, python package "region-plot"[22] or online tool LocusZoom,[23] and Haploview v4.2 software.[24]

2.5 Bioinformatics annotation and post-GWAS analyses

Firstly, for genetic variants residing in the significant loci, we queried against public databases for their functional annotation. These includes GWAS Catalog,[21] eQTL Catalogue (consisting of expression quantitative trait locus [eQTL] from Genotype-Tissue Expression [GTex) and other public datasets),[25,26]regulation databases (HaploReg and RegulomeDB)[27,28], and Phenome-wide association study (PheWAS) databases via OpenGWAS project (variant level) [29] and GWAS ATLAS (gene level)[30] for other pertinent associations or possible mechanisms of action. Biological pathway and interaction network of functional genes were constructed by GeneCards[31] or GeneMANIA.[32] In addition, a few secondary post-GWAS analyses were used in combination to explain our GWAS signals. This included methods to speculate the molecular mechanism (e.g., eQTL identification and their colocalization with GWAS locus, fine-mapping of the causal variant via a Bayesian approach, and identification of associated genes or pathways); the detailed protocol for each analysis is provided in the Supplementary methods.

2.6 Additional analyses for *FGD5* locus

To check the independence of *FGD5* common variants' effect on death or re-hospitalization, we also performed several sensitivity analyses to evaluate potential mediation or interaction

by history of coronary artery disease (CAD; yes/no) or treatment arm (nesiritide or placebo). For conditional analysis, we added CAD or treatment arm as one additional covariate to the base model above. For the interaction analysis, we further added to that model an interaction term (CAD*SNP or treatment*SNP). The summary statistics for main effect of SNP and the interaction effect of SNP*CAD or SNP*treatment were used to determine whether there was evidence for any dependence of *FGD5* effects on CAD history or treatment arm. To visualize the impact of the locus on outcome Kaplan-Meier (KM) survival curves were generated for ASCEND-HF patients stratified by rs2342882 genotypes (T/T, T/G, and G/G) and the difference in survival hazard by three genotype groups was tested by the log-rank test in R. Lastly, we also examined whether the censoring time window (30 days versus 180 days) affect the impact of FGD5 common SNPs on HF survival (death or re-hospitalization). The same model and covariates were included when testing for 30-days survival. The Bonferroni method was used to correct for multiple testing (in total 36 tests) in the additional analyses.

3. Results

In total 2,680 acute HF patients were included in this analysis (2,173 EA and 507 AA). As shown in Table 1, there were 618 endpoint events (death or re-hospitalization), including 502 (23%) in the EA group and 116 (23%) in the AA group ($P>0.05$, log-rank test for hazard difference between two populations). As expected, many variables (i.e., age, sex, BMI, SBP, creatinine, ejection fraction, history of myocardial infarction (MI) or CAD, history of atrial fibrillation, and history of hypertension) were statistically different across self-identified race ($P<0.001$, Table 1). Cox model fitting of primary endpoint for the prespecified 11 covariates (from ASCEND-HF rehospitalization score) are included in Supplementary material online, Table S1. The global test supported the proportional hazard assumption in both EA and AA group ($P > 0.05$). In addition, principal component maps of our sample and 1000 Genome reference populations together showed their self-reported ancestry matches well with the genetic ancestry (Supplementary material online, Figure S1).

No genomic inflation was observed from the QQ plots for population specific GWAS or overall meta-analysis (Supplementary material online, Figure S2). Two loci reaching GWAS significance were identified for AA (18q22.1) or meta-analysis (3p25.1), as shown in Manhattan plot (Supplementary material online, Figure S3) and Table 2. In addition, another locus (5q21.3) was suggestively significant ($P<1\times10^{-6}$) with moderate signal in both EA and AA population, but meta-analysis of its strongest SNP (rs293652, in an intergenic region) did not meet the predetermine genome-wide significance threshold (fixed-effect $P=7.40\times10^{-8}$; HR=1.46; 95% confidence interval [CI] 1.27–1.67). The AA-GWAS signal on chromosome 18 was not replicated in EA population ($P>0.05$) and did not pass heterogeneity test. As a comparison, the signal on chromosome 3 was supported by both populations (fixed-effect $P=4.25\times10^{-8}$, HR=1.41, 95% CI 1.24–1.59), and the lead SNP rs2342882 is located in an intron of *FGD5*, a protein-coding gene that is a possible regulator of VEGF-mediated angiogenesis. Figure 1 displays KM curves for the total cohort divided by genotype at rs2342882, revealing that worse survival is associated with the G allele (log-rank p value 0.00003).

Among 14 GWAS hits reported previously for related HF phenotypes (e.g. survival), we found two SNPs (rs660240 from *CELSR2*, and rs1556516 from *CDKN2B-AS1*) that were associated with death or HF re-hospitalization, at nominal significance in EA, AA or their combination (Supplementary material online Table S2). However, in our study neither rs660240 nor rs1556516 was more significantly associated with the primary endpoint than the *FGD5* common variants.

We performed additional investigation (*in silico* and experimental) to characterize possible genetic mechanisms of rs2342882 and the 3p25.1 locus. Figure 2 shows a detailed view of this locus using overall ASCEND-HF genetic data, while Figure S4 (Supplementary online material) gives locus-zoom plots from ASCEND-HF EA and AA samples, respectively. Five common SNPs located in *FGD5* introns had $P<0.001$ in discovery and clustered into two LD blocks in both EA and AA (though the correlation between block 1 and block 2 is stronger in EA than in AA; Figure 3A and Figure 3B). Table 3 provides predicted annotation of these *FGD5* common variants from public databases. Bayesian fine mapping supported rs2342882 and rs35850039 as the most likely causal SNPs in this locus (posterior probability >0.5; Supplementary material online, Table S3). While rs2342882 and rs35850039 are not reported in previous GWAS (i.e., according to records in GWAS Catalog retrieved at May 01 2023) and their roles in regulation are ranked as category 5 in RegulomeDB (i.e., minimal evidence for transcription factor binding or DNase peak), they were significant as eQTLs for *FGD5* in fat tissue and for mitochondrial ribosomal protein S25 (*MRPS25*) in monocytes (from the eQTL Catalogue; Supplementary material online, Table S4). Two other SNPs in the locus, rs748431 ($P=6.58\times10^{-4}$ [EA], $P=0.71$ [AA], meta-analysis HR=1.20 and $P=3.34\times10^{-3}$) and rs34991912 ($P=6.28\times10^{-4}$ [EA], $P=0.43$ [AA], meta-analysis HR=1.18 and $P=5.91\times10^{-3}$) are reported in previous GWAS for CAD,[33,34] are also significant as eQTLs for *FGD5* (skin tissue) and *MRPS25* (atrial appendage), and are rated category 4 by RegulomeDB (Table 3). Integration of our meta-analysis data and public eQTL data (Supplementary material online, Table S5, Figure S5–8) showed this GWAS locus has potential colocalization with eQTL signals for *FGD5* expression in fat tissue, and eQTL signals for *MRPS25* expression in monocyte cell infected by influenza A virus (IAV). On the other hand, our own experiments in whole blood RNA sequencing (RNA-seq) from 87 chronic heart failure patients showed none of the candidate SNPs affected expression of their nearby (± 1Mb) genes (Supplementary material online, Table S6) and multi-marker analysis (i.e., MAGMA) in FUMA identified no significant gene-sets (false discovery rate [FDR] >0.05; Supplementary material online, Table S7). For completeness we also annotated the top four replicated SNPs from other GWAS hits in 5q21.3 region (Supplementary material online, Table S8), and these did not seem to have potential genetic functional impact similar to that of the *FGD5* locus.

We also examined which phenotypes have been reported (PheWAS queries) of the *FGD5* candidate SNPs (Supplementary material online, Table S9), which indicated that rs2345882 may moderately affect cholesterol in large high-density lipoproteins (HDL) particles, and that rs748431 may affect multiple cardiovascular phenotypes (e.g., blood pressure and CAD) and that rs2345882 may moderately affect cholesterol (high-density lipoproteins particles). Examining the entire gene, GWAS signals from *FGD5* were mostly observed in blood pressure phenotypes (e.g., systolic blood pressure, diastolic blood pressure, and

hypertension) and appeared highly enriched in the cardiovascular domain (Supplemental Table S10). At gene network level (Supplementary material online, Figure S9), FGD5 was predicted with strong inter-connection with a few vascular endothelial growth factors (VEGFA, VEGFD, and VEGFC) and their receptors (KDR and FLT4). In summary, a wide range of supporting data revealed multiple types of evidence indicating a possible functional impact of these genetic variants on gene and protein function, that they are associated with multiple cardiovascular phenotypes relevant to HF (particularly including blood pressure) and that it most likely is operating via VEGF related pathways.

We further performed multiple sensitivity analyses. Since *FGD5* has GWAS hits for CAD we examined whether there was evidence for mediation or interaction of the SNP outcome association with history of CAD. We also tested whether treatment arm had any influence on the association of *FGD5* common SNPs with the primary endpoint. We also test whether its effect changed when shortening the follow-up time from 180 to 30 days. We used rs23422882 as index for LD block 1, and rs784431 as index for LD block 2 and retested the association with death or re-hospitalization in Cox models. Results are summarized in Table 4. The conditional analyses on CAD or treatment arm did not significantly alter the association of rs23422882 (both Meta $P<5\times10^{-8}$) or rs784431 (both Meta $P<0.01$) with our primary outcome. In addition, no interaction effects (for SNP*CAD, or SNP*nesiritide) were statistically significant in any group after multiple testing corrections (all $P > 0.001$), though CAD interaction in meta-analysis was closest with $P= 0.035$, or 0.0596, respectively for two candidate SNPs. The effect of both SNPs on 30-days survival phenotype were both moderately significant ($P<0.0001$ for rs2342882 and $P<0.05$ for rs748431). Taken together this suggests the genetic effect of *FGD5* common SNPs on clinical outcomes in acute HF patients is independent of both CAD history and ASCEND-HF treatment arm, and its effect is stable from 30 days to 180 days.

4. Discussion

The experiments described here attempted to better define the genetic underpinnings of HF death or re-hospitalization after HF exacerbation, with the goal of identifying novel genes or pathways critical to progression or exacerbation of HF in this setting. Our multi-ancestry GWAS identified two regions with signals shared across two ethnic groups tested, including one (3p25.1) that reached the genome-wide significance threshold in meta-analysis. Further analysis and functional annotations pointed towards the *FGD5* gene as a likely candidate. This is the first GWAS of acute decompensated HF that we are aware of, and one of few HF genetic studies to focus on death or re-hospitalization as the primary composite endpoint. *FGD5* as a susceptibility gene for worsening HF is plausible given its role in cardiac development and growth factor pathways for angiogenesis.

FGD5 (FYVE, RhoGEF and PH Domain Containing 5) is a protein-coding gene that may regulate proangiogenic action of VEGF in vascular endothelial cells.[35] Multiple biological studies have linked its role to vascular function in human or mice,[36,37] and the function of this protein family was summarized recently.[38] Several published GWAS provide evidence of *FGD5* involvement in at least 12 different traits or diseases, with most SNP associations in cardiovascular diseases (i.e., blood pressure, CAD, hypertension). A common variant

(rs748431) and a rare loss-of-function mutation (Glu322*) were recently reported to be important risk factors for CAD or pediatric heart disease, respectively.[33,39] Interestingly, even after adjusting for the influence CAD, we still found evidence for moderate impact of rs748431 on death or re-hospitalization in ASCEND-HF and there was no significant interaction with CAD. Moreover, two of the SNPs of interest in *FGD5* (rs2342882 and rs35850039) were not found to be associated in the previously noted in the previous GWASs of CAD,[33,34] and did not appear in GWAS Catalog searches of May 01 2020).[21] Both Bayesian fine-mapping and SNP-level PheWAS analysis also supported the different roles of rs2342882 from rs748431. Together these data possibly suggest a distinct genetic association of *FGD5* for HF compared to CAD. Furthermore, our eQTL and colocalization analyses provided additional insights of *FGD5* locus and its possible impact in HF.

Given known interaction of FGD5 with the VEGF and their receptors (e.g., VEGFA-VEGFR2 signaling),[36,40,41] it is tempting to speculate that are acting via altered expression/activity of FGD5 in endothelial cells and thus affecting vascular function (which is known to be important in HF) via the VEGF pathway.[42] Unfortunately there is no public eQTL data available in vascular endothelial cells, but our findings of eQTL and GWAS colocalization for the SNPs of interest on *FGD5* expression in fat and skin tissues indicate a possible link of this locus to *FGD5* expression, perhaps indicating that this is a generalized effect and thus includes vascular endothelial cells or perhaps act via fat tissue specifically given the emerging role of obesity in HF.[43] Interestingly, another recent study suggested importance of *FGD6* regulatory variants on *VEGFC* function in human endothelial cells.[44] On the other hand, the association of the *FGD5* locus with *MPRS25* expression in atrial tissue (and also in monocytes) could suggest a different mechanism perhaps via myocardial energetics. While intriguing, all this remains speculation until more direct evidence in relevant tissue is available.

From a clinical and pathophysiological perspective, the above discussion translates fairly directly into possible mechanisms for FGD5 acting on several known aspects of acute HF pathophysiology.[45] As already noted above, endothelial function is known to impact HF, including in the decompensated setting. More broadly, blood pressure may be key, perhaps through endothelial function (it is long known that VEGF activity is important in endothelium-mediated vasorelaxation)[46] but also possibly due to fluid shifts or perhaps via neovascularization. It is also well established that medications targeting the VEGF pathway for the purpose of cancer treatment are considered anti-angiogenic and have a significant rate of inducing hypertension and even HF.[47,48] Moreover, recent work has reinforced the importance of this relationship in the setting of decompensated HF. For example, in one study of over 1000 hospitalized HF patients, lower soluble VEGF receptor levels in plasma were independently associated with higher risk of cardiovascular death. Even more recently was a parallel finding for lower VEGF signaling (in this case lower plasma VEGF-C levels) being associated with greater fluid retention and worse post-discharge clinical outcomes in 237 patients hospitalized for decompensated HF.[49] While speculative, our data and the existing publications suggest that perhaps alterations in FGD5 function may influence VEGF pathway activity in the setting of acute HF, and that impaired VEGF signaling causes elevations in blood pressure and worse endothelial function, both obviously adverse in a

decompensated HF setting. Further interrogation of the role of FGD5 in the setting of HF as well as the links to VEGF pathway and pathophysiology of acute HF is still needed.

We did not find support for the previous GWAS 'hit' reported for chronic HF mortality (5q22).[10] This could be due to differences in the patient and disease phenotype investigated (exacerbated HF vs. stable HFrEF), differences in the endpoint selected (mortality vs. composite of mortality and HF hospitalization) or insufficient power. We modeled time to death or re-hospitalization because we felt this phenotype was clinically most relevant for a hospitalized HF cohort and most consistent with the parent study design, which had a primary endpoint of death or re-hospitalization. In contrast, the previously published GWAS focused on all-cause mortality, an endpoint that the current study was underpowered to evaluate. The difference of genetic findings between our study (3p25.1 and *FGD5*) and Smith et al (5q22 and *SLC25A46*) may also indicate the heterogenous nature of HF; and the gene-level PheWAS results (related to GWAS signals) highlighted different disease domains for *FGD5* (cardiovascular) compared to *SLC25A46* (respiratory, Supplemental Table S10). Coincidently, our meta-analysis identified a second peak on 5q21 that nearly reached the genome-wide significance threshold. Nevertheless, it is still far from 5q22 locus and the included SNPs are not in any degree of LD with the 5q22 reported SNP (i.e., rs9885413). On the other hand, we did find two loci (in *CELSR2* and *CDKN2B-AS1*) that were previously published as susceptibility genes for incident HF [13] which were associated with death or re-hospitalization in our study. Given the heterogeneity of HF phenotypes, there is a likely a need to study incident, prevalent and exacerbated HF separately,[50] and genetic risk scores for these related phenotypes (e.g., onset and survival) could help understand shared genetic architecture and potentially improve risk stratification or sub-setting of HF phenotypes.[51]

There are some limitations of our study worth noting. First, as noted above, our study is insufficiently powered for mortality alone. However, the composite of death or rehospitalization for HF is a widely accepted clinical endpoint, and despite being a composite outcome is likely to help identify disease specific genetic associations that could be missed by analysis of all-cause mortality alone. While external validation would be ideal, we are not aware of any similar acute HF studies with genome-wide data against which we could perform validation. If anything, use of patient samples from different ancestry groups would have biased our analysis towards the null because of differences in LD structure; as a result, we may not have identified all existing genetic associations, and external validation of *FGD5's* association with HF mortality is important. Second, while we can hypothesize regarding the mechanism of potential impact using previous literature, *in silico* prediction, or resources regarding *FGD5*, our study does not directly add to mechanistic understanding. However, our post-GWAS annotations leveraging accumulated biological knowledge to prioritize functional genes layered on top of known genetic data may be more powerful than association testing alone in defining the salient genes and/or pathways to be validated in vitro or in vivo. Another way potentially forward is to explore intermediate endpoints such as blood pressure, urine output, or symptom severity,[12] which may have greater power to detect differences and help to understand intermediate steps in the pathophysiology. Ultimately, additional translational investigation is needed to fully illuminate mechanisms and identify potential novel interventions for HF.

In conclusion, we have performed unbiased genomic analyses of ASCEND-HF trial and identified common variants in *FGD5* associated with increased risk of death or hospitalization. The findings regarding *FGD5* in this study are corroborated by previous GWAS reports and functional annotation, together providing reasonably strong evidence of its relevance to cardiac function and progression of HF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ADHF	Acute decompensated heart failure
ASCEND-HF	Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure
SNP	single nucleotide polymorphism
HR	hazard ratio
EA	European Americans
AA	African Americans
LD	linkage disequilibrium
CAD	coronary artery disease
GWAS	genome-wide association studies
eQTL	expression quantitative trait locus
PheWAS	Phenome-wide association studies

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What is New?

- First genome-wide association study of clinical outcomes after hospitalization for acute heart failure.
- A novel candidate gene for acute heart failure clinical outcomes was identified, *FGD5*, which may act via VEGF pathway and blood pressure.

What are the clinical implications?

- Genetic variation in this *FGD5* could help explain varying risk of exacerbation or progression across patients with heart failure.
- FGD5 or related pathways could be a target for novel interventions aimed at acute heart failure.

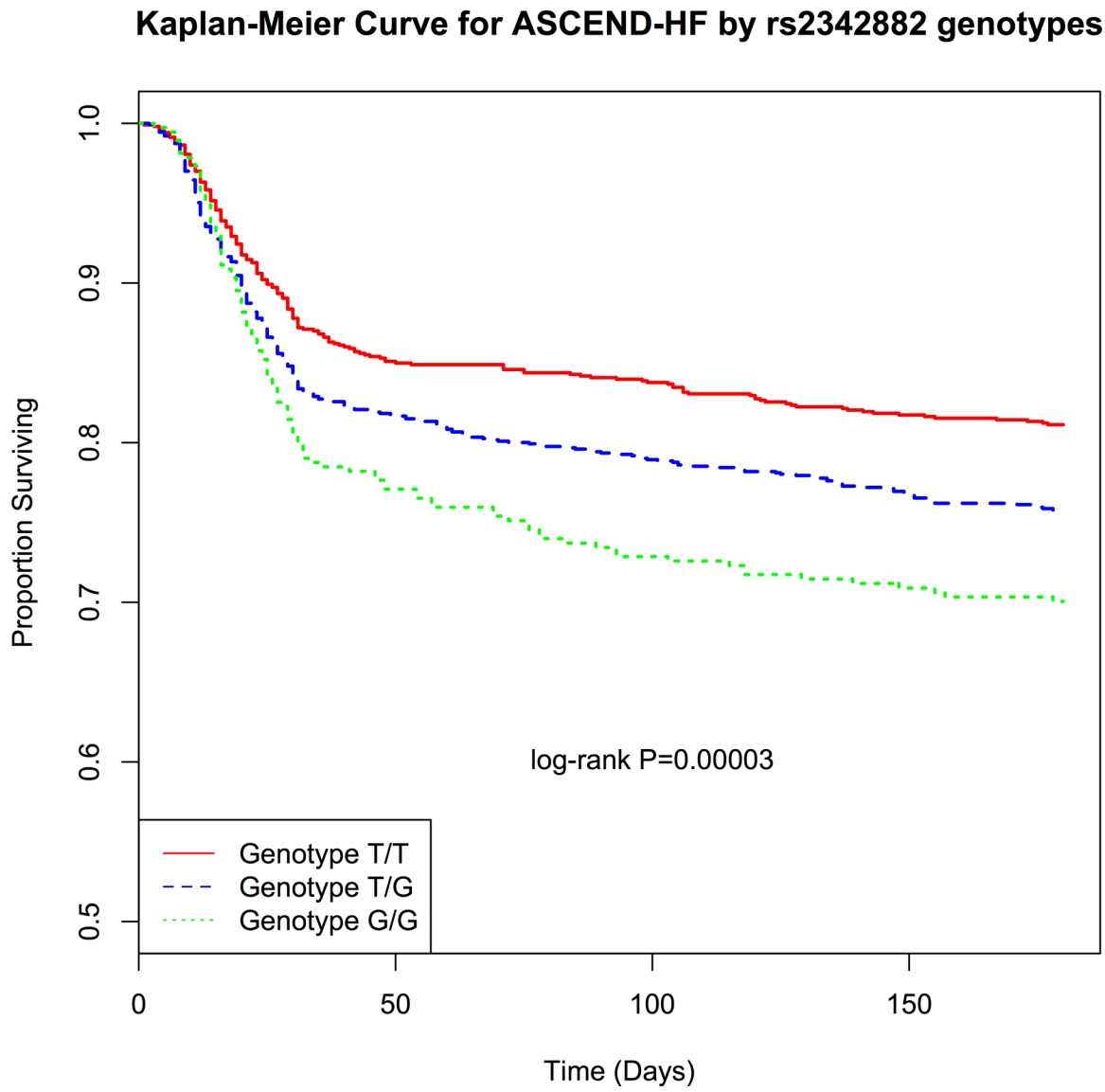

Figure 1. Kaplan-Meier curves for ASCEND-HF patients by rs2342882 genotypes.

Heart failure patients in ASCEND-HF trial (n=2680) was stratified by rs2342882 genotypes (T/T, T/G, and G/G; G is risk allele). Survival time is in days.

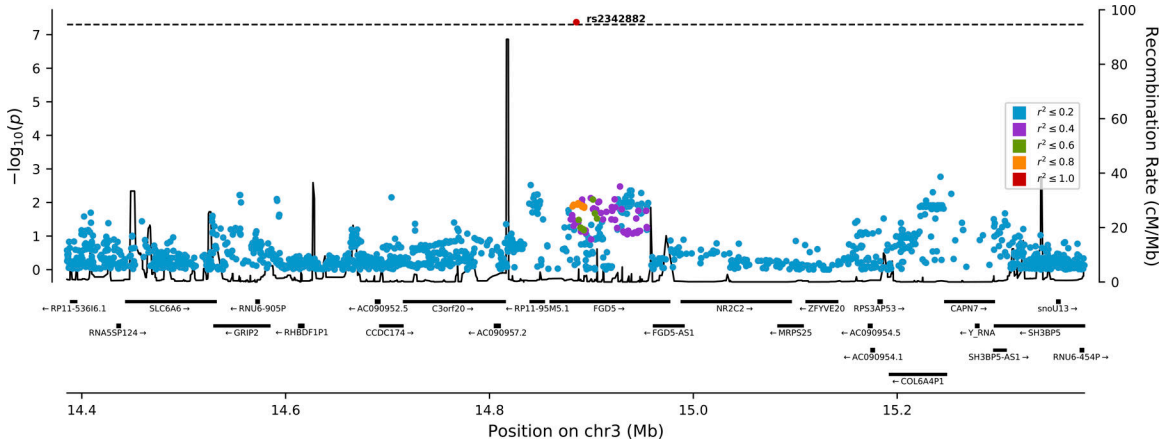

Figure 2. Visualization of 3p25.1 locus by regional plot.

Regional association plot for 3p25.1 locus, centering on index SNP rs2342882 (+/− 500Kb), with each dot representing $-\log_{10}$(p-value) from meta-analysis of EA (n=2173) and AA group (n=507) in ASCEND-HF trial. Linkage disequilibrium (i.e., r^2) relative to index SNP was estimated using overall ASCEND-HF samples (n=2680). Mb stands for megabase.

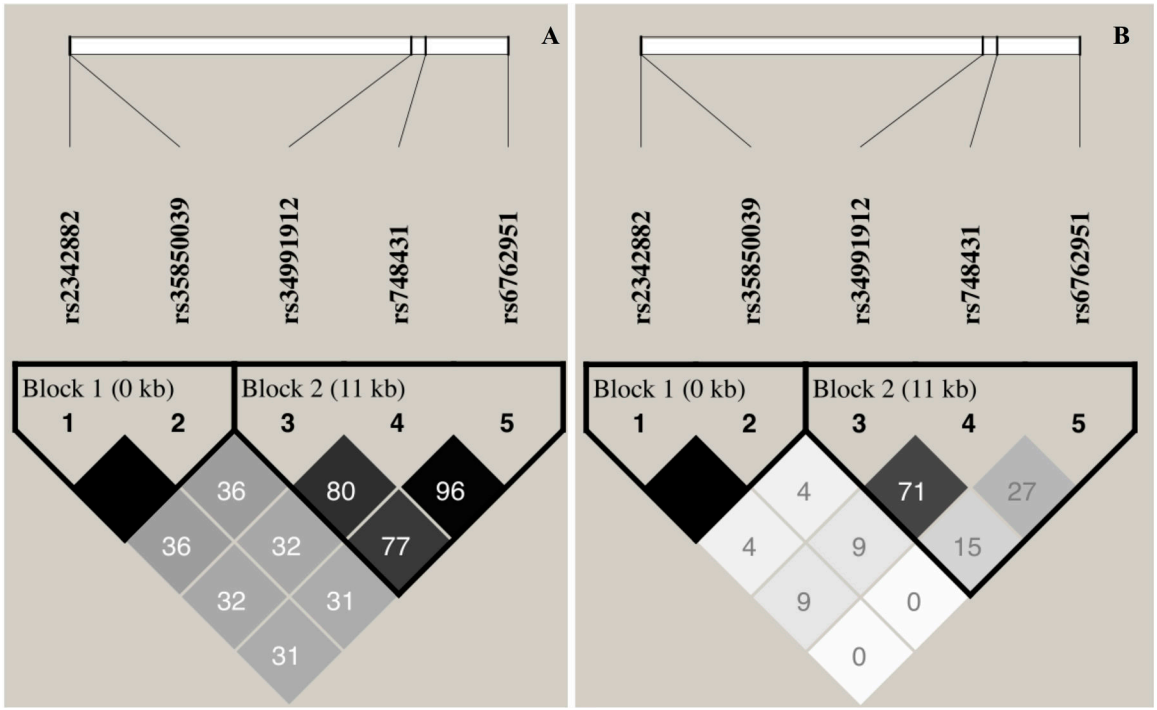

Figure 3. Linkage disequilibrium pattern among *FGD5* common SNPs.

A) Haploview plot for *FGD5* common SNPs in ASCEND-HF EA group (n=2173). B) Haploview plot for *FGD5* common SNPs in ASCEND-HF AA group (n=507). Numbers in each cell are pairwise r^2 between two SNPs.

Table 1.

Cohort characteristics for patients from ASCEND-HF trial

Variables	Data1 ASCEND-HF EA	Data2 ASCEND-HF AA	*P* for difference
Sample size	2173	507	
Study design	RCT	RCT	
Nesiritide treatment (%)	1077 (49.6)	259 (51.1)	0.485
Age in years (mean ± SD)	68.6 ± 12.7	58.1 ± 14.0	<0.001
Female sex (%)	679 (31.2)	198 (39.1)	<0.001
BMI (kg/m^2) (mean ± SD)	30.0 ± 7.0	33.1 ± 8.9	<0.001
SBP (mmHg) at baseline (mean ± SD)	126.5 ± 18.8	130.2 ± 21.7	<0.001
Creatinine (umol/L) (mean ± SD)	120.7 ± 48.3	129.1 ± 51.9	0.001
No. of death or re-hospitalization (%)	502 (23.1)	116 (22.9)	0.800
All-cause death (%)	219 (10.1)	31 (6.1)	0.001
Rehospitalization (%)	283 (13.0)	85 (16.8)	
Follow-up days (mean ± SD)	151 ± 73	143 ± 76	0.025
Ejection fraction at enrollment (mean ± SD)	32.4 ± 13.6	27.4 ± 13.4	<0.001
HFrEF (%)	1219 (56.1)	332 (65.5)	<0.001
History of MI/CAD (%)	1378 (63.4)	213 (42.0)	<0.001
History of diabetes (%)	868 (39.9)	230 (45.4)	0.029
History of atrial fibrillation (%)	1123 (51.7)	113 (22.3)	<0.001
History of hypertension (%)	1601 (73.7)	459 (90.5)	<0.001

Abbreviations: EA for European Americans, AA for African Americans, RCT for randomized clinical trial, SD for standard deviation, BMI for body mass index, SBP for systolic blood pressure, HFrEF for heart failure with reduced ejection fraction (<40%), MI for myocardial infarction, CAD for coronary artery disease.

Table 2.

Top SNPs associated with time to death or HF hospitalization in the ASCEND-HF in the multi-ancestry GWAS

SNP	Chr	Gene function	Ref/Effect allele	ASCEND-EA			ASCEND-AA			Meta-analysis*			
				EAF	HR (95% CI)	*P*	EAF	HR (95% CI)	*P*	HR (95% CI)	P-FE	I^2	P-Heter	P-RE
rs2342882	3p25.1	*FGD5* (intron)	T/G	0.39	1.38 (1.21–1.58)	2.42E-06	0.29	1.51 (1.14–2.00)	4.43E-03	1.41 (1.24–1.59)	**4.25E-08**	0	5.88E-01	5.12E-08
rs293652	5q21.3	Intergenic	G/C	0.22	1.39 (1.20–1.61)	7.89E-06	0.06	2.15 (1.41–3.27)	3.79E-04	1.46 (1.27–1.67)	7.40E-08	72.5	5.65E-02	8.67E-08
rs5008759	18q22.1	*DSEL-AS1*	T/C	0.16	1.01 (0.85–1.20)	8.84E-01	0.11	2.95 (2.03–4.28)	**1.17E-08**	1.23 (1.05–1.43)	1.11E-02	96.2	3.25E-07	7.27E-07

*Meta-analysis was done for fixed-effect model in METAL and random-effect model (Han and Eskin's) in MetaSoft, respectively. Abbreviations: SNP for nucleotide polymorphism, Chr for chromosome, Ref for reference, EAF for effect allele frequency, EA for European Americans, AA for African Americans, HR for hazard ratio, CI for confidence interval, P-FE for fixed-effect p-value, P-Heter for heterogeneity test p-value, I^2 for I-square heterogeneity statistic, P-RE for random-effect p-value. P value < 5×10^{-8} is in bold. All HRs were adjusted for 11 clinical covariates and top five principal components from SNP array.

Table 3.

Statistical association and functional annotation of *FGD5* common SNPs

SNP ID	Association summary statistics						Function annotation			
	HR (EA)	P (EA)	HR (AA)	P (AA)	HR (Meta)	P (Meta)*	eQTL Catalogue†	HaploReg	RegulomeDB‡	GWAS Catalog
rs2342882	1.38	2.42E-06	1.51	4.43E-03	1.41	4.25E-08	*FGD5* (fat), *MRPS25* (monocyte_IAV)	Enhancer histone marks ADRL, 5 altered motifs	0.416 (5)	NA
rs35850039	1.38	2.42E-06	1.51	4.43E-03	1.41	4.25E-08	*FGD5* (fat), *MRPS25* (monocyte_IAV)	Enhancer histone marks ADRL	0.554 (5)	NA
rs34991912	1.25	6.28E-04	0.89	4.26E-01	1.18	5.91E-03	*MRPS25* (Heart_Atrial_Appendage), *FGD5* (Skin_Sun_Exposed_Lower_Leg)	Promoter histone marks BLD, enhancer histone marks, multiple motifs changed	0.609 (4)	CAD, SBP
rs748431	1.26	6.58E-04	0.95	7.13E-01	1.20	3.34E-03	*MRPS25* (Heart_Atrial_Appendage)	Promoter histone marks BLD, enhancer histone marks, multiple motifs changed	0.609 (4)	CAD
rs6762951	1.26	7.00E-04	1.15	3.05E-01	1.17	1.03E-02	NS	Promoter histone marks BLD, enhancer histone marks, multiple motifs changed	0.184 (7)	NA

*Meta-analysis p-values were estimated using fixed-effect model in METAL.

†Only eGene in specific tissue or cell with p-value <0.0001 is shown, and NS for non-significant; terms in parenthesis are tissue or cells: Heart_Atrial_Appendage means heart tissue from atrial appendage; monocyte_IAV means monocyte cell infected with influenza A virus (IAV); Skin_Sun_Exposed_Lower_leg means skin tissue at lower leg with sun exposed).

‡Scores and its rank provided in RegulomeDB: 4, TF binding + DNase peak; 5, TF binding or DNase peak; 7, Other. Abbreviations: SNP for single nucleotide polymorphism, HR for hazard ratio; EA for European Americans, AA for African Americans, eQTL for expression quantitative trait loci, CAD for coronary artery disease, SBP for systolic blood pressure. NA means no available data.

Table 4.

Sensitivity analyses for *FGD5* common SNPs

		ASCEND-EA		ASCEND-AA		Meta-analysis	
Model*	**Test**	**HR**	**P**	**HR**	**P**	**HR**	**P†**
Base + SNP (180 days)	rs2342882	1.38	2.42E-06	1.51	4.43E-03	1.41	4.25E-08
Base + SNP + CAD	rs2342882	1.39	2.04E-06	1.50	4.80E-03	1.41	3.76E-08
Base + SNP + Nesiritide	rs2342882	1.38	2.76E-06	1.52	3.97E-03	1.41	4.42E-08
Base + SNP + CAD + SNP*CAD	rs2342882*CAD	0.84	2.30E-01	0.51	2.30E-02	0.75	3.50E-02
Base + SNP + NES + SNP*NES	rs2342882*Nesiritide	1.01	9.70E-01	0.91	7.50E-01	0.99	9.20E-01
Base + SNP (180 days)	rs748431	1.26	6.58E-04	0.95	7.10E-01	1.20	3.34E-03
Base + SNP + CAD	rs748431	1.27	5.94E-04	0.95	7.30E-01	1.20	2.97E-03
Base + SNP + Nes	rs748431	1.26	7.81E-04	0.96	7.60E-01	1.20	3.45E-03
Base + SNP + CAD + SNP*CAD	rs748431*CAD	0.79	1.10E-01	0.73	3.04E-01	0.78	5.96E-02
Base + SNP + NES + SNP*NES	rs748431*Nesiritide	0.96	7.90E-01	1.17	6.01E-01	1.00	9.80E-01
Base + SNP (30 days)	rs2342882	1.34	6.22E-04	1.59	5.37E-03	1.39	1.59E-05
Base + SNP (30 days)	rs748431	1.21	2.54E-02	1.04	8.17E-01	1.17	3.63E-02

*Base in the model referred to all variables (11 clinical variables and 5 genetic principal components) selected as covariates in the discovery analysis.

†Meta-analysis p-values were estimated using fixed-effect model in METAL. Abbreviations: HR for hazard ratio; EA for European Americans, AA for African Americans, CAD for coronary artery disease, NES for nesiritide, SNP for single nucleotide polymorphism.