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**Circulating growth differentiation factor -15 levels are associated with early echocardiographic signs of diastolic function impairment in the STANISLAS cohort: a 20-year follow-up study**

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## **Abstract**

**Aims** Early identification of healthy subjects prone to develop cardiac dysfunction may be instrumental to prevention strategies. Our study aimed to evaluate whether circulating levels of growth differentiation factor-15 (GDF-15) could predict adverse changes in echocardiographic indexes of cardiac structure and function in an initially healthy populational familial cohort with a long follow-up (STANISLAS cohort).

**Methods and results** We evaluated 1679 participants ( $49 \pm 14$  years, 48% males) included in the 4<sup>th</sup> visit (V4) of the STANISLAS cohort with available GDF-15 measurements (Olink proteomic analysis) and echocardiographic parameters. Among them, 1562 had also GDF-15 measurements at the first visit ( $18.2 \pm 1.4$  years before V4). We evaluated both prospective (GDF-15 at V1) and cross-sectional (GDF-15 at V4) associations between GDF-15 levels and echocardiographic variables. In adjusted analyses, higher baseline GDF-15 levels were associated with lower left ventricular (LV) end-diastolic volume and higher E/e' ratio nearly two decades later (both  $p \leq 0.01$ ). The cross-sectional analysis at V4 also found significant associations between GDF-15 and LV end-diastolic volume and higher E/e' ratio, further identifying significant associations with impaired LV strain.

**Conclusions** In the STANISLAS cohort, initial circulating GDF-15 levels were associated with echocardiographic signs of reduced LV size and increased filling pressure almost two decades later. This association was also confirmed by evaluating GDF-15 at the follow-up visit (V4). These findings suggest a potential role for GDF-15 as an easy screening and prognostic tool of diastolic dysfunction in the general population.

**Keywords:** Growth differentiation factor-15; echocardiography; diastolic dysfunction; cohort study

## Introduction

Heart failure (HF) is characterized by significant morbidity and mortality, poor quality of life, and high costs, representing a major and growing global public health issue.<sup>1</sup> The occurrence of overt HF is often preceded by a phase of clinically silent disease (Stages A and B of the American College of Cardiology [ACC]/American Heart Association guidelines [AHA] HF classification), which frequently goes unnoticed and untreated.<sup>2</sup> Imaging techniques, particularly echocardiography, play a key role in detecting preclinical myocardial alterations.<sup>3</sup> However, the routine, widespread use of echocardiography for screening in asymptomatic patients is limited due to constraints such as machine availability, physician time, and high costs. Despite these hurdles, the significant risk of these individuals progressing to symptomatic HF and experiencing clinical events<sup>4, 5</sup> underscores the importance of an early diagnosis in order to implement preventive measures. This concept was further highlighted by recent guidelines emphasizing the need for timely identification and management of subjects with subtle myocardial alterations predisposing to HF development.<sup>2</sup>

Circulating biomarkers, and in particular natriuretic peptides, have been utilized to predict the occurrence of structural or functional abnormalities in the general population,<sup>6</sup> yet their specificity has proven to be limited.<sup>7</sup> Growth differentiation factor-15 (GDF-15), a member of the transforming growth factor  $\beta$  (TGF- $\beta$ ) cytokine superfamily, is secreted in reaction to various stimuli including oxidative stress, proinflammatory cytokines, ischemia, mechanical strain, and neurohormonal activation.<sup>8</sup> Notably, GDF-15 level was associated with diastolic dysfunction in asymptomatic individuals,<sup>9</sup> in obese subjects,<sup>10</sup> and in patients with HF.<sup>11</sup> However, the potential of GDF-15 as a long-term predictor of structural and functional cardiac abnormalities remains underexplored.

The aim of the present study was to assess whether a single measurement of GDF-15 can predict in the long term the development of subclinical yet adverse changes of echocardiographic indexes of structure and function in an initially healthy populational familial cohort. Additionally, we investigated whether these associations remained significant nearly 20 years later (cross-

sectional analysis at V4).

## **Methods**

### *Study population*

The STANISLAS (Suivi Temporaire Annuel Non-Invasif de la Santé des Lorrains Assurés Sociaux) cohort is a single-center familial longitudinal cohort established between 1993 and 1995 in the Nancy region (France) with the primary objective of investigating gene-gene and gene-environment interactions in the transition toward cardiovascular diseases.<sup>12</sup> The enrolled families, consisting of two parents and at least two biological children, were deemed healthy and free of any declared acute and/or chronic illnesses at their first visit (V1). The implementation of the fourth visit (V4) enabled a follow-up of almost 2 decades (mean of  $18.2 \pm 1.4$  years). From 2011 to 2019, 1705 participants of the original cohort underwent their fourth examination (V4) at our hospital, as previously described.<sup>12</sup> The research protocol was approved by the local Ethics Committee (Comité de Protection des Personnes Est III, Nancy, France) and conforms to the guiding principles of the Declaration of Helsinki. The informed written consent was approved previously by the local ethics committee (ClinicalTrials.gov identifier: NCT01391442) and was signed by all participants.

For the present study, 1562 participants attending V1 and 1679 participants attending V4 having GDF-15 measurements and echocardiographic parameters available were included in the analyses. All participants were scheduled to attend the Centre d'Investigation Clinique Plurithématique (CIC-P) Pierre Drouin at Nancy University Hospital at 8 AM after a 12- to 14-hour fast. All subjects underwent echocardiographic examination and blood and urine sampling for laboratory analysis. Medical history, medications, and anthropometric parameters were also recorded. Office blood pressure was measured three times in all participants, at 1-min intervals, using an electronic sphygmomanometer after the participant had rested for at least 10 min and calculated as the mean of the three measurements, as previously described.<sup>13</sup>

### *Echocardiography*

A comprehensive echocardiographic examination was performed only at V4 using standard, commercially available ultrasound equipment (Vivid 9, General Electric Medical Systems, Horten, Norway) with a 2.5 MHz phased-array transducer and reviewed in our central core lab on an Echopac workstation (version 202 or 203, GE Healthcare). All measurements were conducted by experienced echocardiographers, with high intra- and interobserver reproducibility.<sup>14, 15</sup> Chambers quantification was evaluated according to current recommendations.<sup>16</sup> Mitral annular early diastolic velocity ( $e'$ ) was assessed at the septal and lateral sites of the mitral annulus using tissue Doppler imaging. The E/A ratio,  $e'$  mean, and E/ $e'$  mean ratio were calculated.<sup>17</sup> Left ventricular (LV) systolic deformation was assessed from the 3 apical views with measurement of the global LV systolic strain (GLS) corresponding to the maximum absolute value of strain derived from strain curves.

### *Growth-differentiation factor-15 and other laboratory assays*

All samples were collected at the CIC-P de Nancy with minimally traumatic venipuncture. Standardized sample handling procedures enabled the collection of serum and plasma (EDTA, heparin) as well as buffy coat fraction. GDF-15 and N-terminal pro-B-type natriuretic peptide (NT-proBNP) were analysed with the Olink Proseek<sup>®</sup> Multiplex cardiovascular (CVD) panels (CVD II for NT-proBNP and CVD III for GDF-15), using a proximity extension assay (PEA) technology.<sup>18</sup> The platform provides  $\log^2$ -normalized protein expression (NPX) data. Further details about the analytic process are described in the White paper from Olink company (<https://www.olink.com/resources-support/white-papers-from-olink/>). In a subset of participants at V4 (N = 199), GDF-15 was also evaluated using enzyme linked immunosorbent assay (ELISA; Elecsys<sup>®</sup>, Roche Diagnostics; limits of detection, 400- 20000 pg/mL; coefficient of variation: 3.4%-3.8%). A linear regression analysis was conducted on subjects with available measurements from both assays to determine the GDF-15 values in pg/mL corresponding to Olink values.

Glomerular filtration rate (eGFR) was estimated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation. All intra-assay variations were <10%.

Blood DNA of all participants was extracted using Gentra Puregene Blood Kit (Qiagen, Hilden Germany) and stored at -20°C. Genotyping was conducted at CNRGH (Centre National de Recherche en Genomique Humaine, Evry, France) using two chips: the Illumina Global Screening Array (GSA) which is composed of 687 572 intronic and exonic markers and the Illumina Exome Array, which is constituted of 244 330 single nucleotide polymorphisms (SNPs), mostly exonic. All blood-derived bio-samples are stored in a central biobank facility with temperatures between -80 °C and -196 °C (as required, e.g. -196 °C for plasma and serum).

### *Statistical analysis*

The characteristics of the study population were compared across tertiles of plasma GDF-15 concentrations (at both V1 and V4) and reported as means, medians, or proportions (as appropriate). Comparisons of baseline and follow-up characteristics of the three groups were carried out using analysis of variance for continuous variables and Pearson's chi-square or Fisher exact tests for categorical variables, as appropriate.

Normality was assessed using the Shapiro-Wilks test. Variables not normally distributed were log-transformed before the analysis. Multiple linear regression analyses were conducted to assess the relationships between GDF-15 levels (at both V1 and V4) and echocardiographic measures at V4, while controlling for potential confounding factors. Variance inflation factor (VIF) analysis was performed to assess multicollinearity among covariates. The linearity assumption of the relationship between GDF-15 and echocardiographic variables was assessed using restricted cubic splines with five knots at fixed percentiles (5%, 27.5%, 50%, 72.5%, and 95%) of the distribution. The analyses were adjusted using a series of four nested models to progressively account for additional variables:

- Model 1 (M1): Adjusted for age and sex;

- Model 2 (M2): Included all variables in M1, plus current smoking status, body mass index (BMI), and total cholesterol levels;
- Model 3 (M3): Included all variables in M2, plus systolic blood pressure, eGFR and diabetes mellitus (only at V4, since virtually no patients had diabetes at V1);
- Model 4 (M4): all variables in M3, plus NT-proBNP levels.

The same approach was also used to assess the association between the change in GDF-15 between V1 and V4 and echocardiographic variables at V4, after an additional adjustment for baseline GDF-15 value (Model 0, M0). Additionally, we explored the association between NT-proBNP levels at V1 and echocardiographic variables at V4, accounting for confounding factor as in M3. The two-tailed significance level was set at  $p < 0.05$ . All analyses were performed using SAS version 9.4.6 (SAS Institute Inc., Cary, N.C., USA).

The genetic analyses were performed using R (version 3.4.1). A genome-wide association study (GWAS) was conducted to investigate GDF-15 levels at visits V1 (N=1441) and V4 (N=1552), using a linear mixed-effect model that accounted for pedigree data under an additive genetic model, with age and sex as covariates. Ethnicity was not included as a covariate since quality control steps confirmed that all subjects were genetically homogeneous. The statistical significance thresholds were set at  $10^{-7}$  for stringent genome-wide significance and  $10^{-5}$  for exploratory analysis, allowing us to assess potential associations at a relaxed significance level. For heritability estimation, a genetic relationship matrix was used in a linear mixed model, with age and sex used as covariates, to estimate the variance captured by additive genetic effects via average information restricted maximum likelihood analysis. An additional random effect was defined to take into account the common household effect resulting from nuclear families (i.e. parental couple and children aged less than 20 years old). Both models were implemented in the R package “gaston” (version 1.5.7).<sup>19</sup>

## Results



### *Baseline characteristics*

Among the 1562 subjects with available GDF-15 measurements at both baseline and V4, the mean plasma concentration (Olink) at V4 was  $5.85 \pm 0.54$  (**Supplemental Figure 1A**). Three similarly distributed quantiles of baseline (V1) plasma GDF-15 concentrations were created: lower tertile (T1),  $\leq 5.04$  (N= 520); intermediate tertile (T2), 5.05 and 5.42 (N= 521); and higher tertile (T3),  $> 5.42$  (N= 521). Subjects with higher plasma GDF-15 were older, with more comorbidities (higher prevalence of antihypertensive, lipid lowering and diabetes treatments), had higher BMI and blood pressure, lower eGFR and increased concentration of NT-proBNP. Additionally, they showed larger LV mass index (LVMI), increased LV filling pressure at echocardiography (higher E/e' ratio, lower e' wave and E/A ratio) and smaller LV volumes (**Table 1**).

Among the 1679 individuals with available GDF-15 measurements at V4, the mean plasma concentration (Olink) was  $5.83 \pm 0.55$  (**Supplemental Figure 1B**). The characteristics of the population, categorized based on tertiles of plasma GDF-15 concentration at V4 (T1:  $\leq 5.58$ , N= 559; T2: 5.59–6.00, N= 560; T3:  $> 6.00$ , N= 560), were akin to those categorized using GDF-15 values at V1 (**Supplemental Table 1**). Similar findings (with the exception of echocardiography parameters, not recorded at this stage) were observed when examining the population at V1 (N= 1562; mean value:  $5.26 \pm 0.52$ ) according to tertiles of baseline GDF-15 concentration (**Supplemental Table 2**).

The correlation between Olink method and ELISA assay at V4 was high (correlation coefficient = 0.71;  $p < 0.001$ ) (**Supplemental Figure 2**). In the subgroup of participants evaluated with both the assays at V4 (N=199), the mean GDF-15 value was  $819 \pm 395$  pg/mL using ELISA method, and  $6.03 \pm 0.45$  using Olink method. The linear regression model linking Olink and ELISA values categorized GDF-15 tertiles as follows: at V1, T1:  $\leq 706$  pg/mL; T2: 707–919 pg/mL; T3:  $> 919$  pg/mL. At V4, T1:  $\leq 636$  pg/mL; T2: 637–851 pg/mL; T3:  $> 851$  pg/mL.

### *Association between GDF-15 and echocardiographic variables*

In the unadjusted model, higher baseline GDF-15 was associated with increased LVMI ( $\beta \pm$  standard error [SE],  $4.020 \pm 0.950$ ,  $p < 0.0001$ ) and left atrial volume index (LAVi;  $\beta \pm$  SE,  $0.969 \pm 0.375$ ,  $p = 0.010$ ), smaller LV volumes index (end-diastolic:  $\beta \pm$  SE,  $-2.661 \pm 0.632$ ,  $p < 0.0001$ ; end-systolic:  $\beta \pm$  SE,  $-0.990 \pm 0.316$ ,  $p = 0.002$ ), and Doppler markers of LV increased filling pressure ( $e'$  mean:  $\beta \pm$  SE,  $-2.029 \pm 0.148$ ; E/ $e'$  ratio:  $\beta \pm$  SE,  $0.898 \pm 0.090$ ; E wave:  $\beta \pm$  SE,  $-0.031 \pm 0.007$ ; E/A ratio:  $\beta \pm$  SE,  $-0.204 \pm 0.020$ ; all  $p < 0.0001$ ) in the population at V4. Higher baseline GDF-15 concentration remained significantly associated with higher E/ $e'$  ratio and lower LV end-diastolic volume index (LVEDVi) even after multiple adjustments (**Figure 1A; Supplemental Table 3**). Baseline NT-proBNP levels were associated only with E/A ratio ( $\beta \pm$  SE,  $0.031 \pm 0.012$ ,  $p = 0.009$ ) in the long term (**Supplemental Table 4**).

The association between V4 GDF-15 value and V4 echocardiographic variables revealed associations consistent with those identified in the previous analysis using GDF-15 at baseline. Additionally, a significant association was detected between higher GDF-15 and lower LV-GLS ( $\beta \pm$  SE,  $0.501 \pm 0.146$ ,  $p < 0.0006$ ). In the adjusted models, increased GDF-15 was consistently associated with higher E/ $e'$  ratio and E wave, alongside lower LV-GLS, LV volumes and LVMI (**Figure 1B; Supplemental Table 5**).

The spline analysis confirmed the trends observed in our statistical models, demonstrating consistent monotonic associations between GDF-15 levels and the echocardiographic variables highlighted in the regression analysis at both V1 and V4 (**Supplemental Figure 3**).

The assessment of the relationship between longitudinal changes in GDF-15 levels (from V1 to V4) and echocardiographic variables was not significantly more informative compared to the analysis conducted solely at V4. Notably, in the fully adjusted analysis (M4), larger changes in GDF-15 levels remained significantly associated only with lower LV volumes (end-diastolic:  $\beta \pm$  SE,  $-2.489 \pm 0.816$ ,  $p = 0.002$ ; end-systolic:  $\beta \pm$  SE,  $-0.885 \pm 0.408$ ,  $p = 0.030$ ) and LVMI ( $\beta \pm$  SE,  $-2.266 \pm 1.106$ ,  $p = 0.041$ ) (**Supplemental Table 6**).

### *GDF-15 and NT-proBNP*

Variance inflation factor analysis demonstrated that GDF-15 and NT-proBNP, along with all other covariates, had VIF values below the accepted threshold for significance (i.e.,  $<5$ ) at both V1 and V4, indicating that multicollinearity was not a significant concern in the regression models. A head-to-head comparison of GDF-15 and NT-proBNP to assess their respective contributions to predicting echocardiographic outcomes demonstrated that GDF-15 significantly enhanced the predictive value of models for key diastolic function parameters compared to NT-proBNP. Likelihood ratio tests indicated a significant improvement in model accuracy when GDF-15 was added alongside clinical variables and NT-proBNP. Notably, GDF-15 at V1 significantly improved model performance in predicting long-term changes in E/e' ratio and LVEDVi (**Supplemental Table 7**).

### *Heritability estimation and genome-wide association study of GDF-15 levels*

The heritability of circulating plasma GDF-15 was estimated at 26% at V1 and 32% at V4. Common environmental effects related to the nuclear family played no role at V1 but accounted for 8% of the variance at V4. Overall, 74% and 60% of the variance remained unexplained at V1 and V4, respectively (**Supplemental Figure 4**).

For the GWAS analysis on circulating GDF-15 levels, no SNP reached the genome-wide significance threshold (p-value  $< 10^{-7}$ ) at V1, and only one (rs6512265;  $\beta \pm SE$ ,  $-0.098 \pm 0.018$ , p-value =  $3.2 \times 10^{-8}$ ) was significant at V4. The rs6512265 polymorphism is located on chromosome 19 within the LRRC25 gene, close to the boundaries of the GDF-15 gene. When the statistical significance threshold was lowered to  $10^{-5}$ , rs6512265 was found to be significant at both V1 and V4 (**Supplemental Figure 5**).

## **Discussion**

In this study we found that higher circulating levels of GDF-15, initially measured in healthy young adults, were associated with reduced LV size (low LVEDVi) and increased filling pressure (high E/e' ratio) in the long term ( $\approx$ 18 years after). Furthermore, the cross-sectional analysis revealed that the association between increased GDF-15 levels and echocardiographic measures of LV diastolic function impairment remained consistent at the follow-up visit (V4), further identifying an association with lower LV-GLS (**Graphical Abstract**). GDF-15 demonstrated greater predictive value than NT-proBNP in this context, as NT-proBNP was linked only with E/A ratio in the long term. Additionally, the heritability of GDF-15 levels in our population was low (30%), with only one SNP identified as potentially influencing circulating values. This suggests that GDF-15 levels are primarily determined by environmental factors, indicating that its concentrations are more likely acquired than inherited. However, the small sample size and the limited geographic diversity may have constrained our ability to detect relevant genetic associations. Larger studies or more comprehensive genetic models that incorporate gene-environment interactions may be necessary to further explore the genetic influences on GDF-15 levels.

#### *GDF-15 as a biomarker of cardiac dysfunction*

GDF-15 is a cell stress-responsive cytokine expressed with low serum levels in healthy individuals.<sup>20</sup> Conversely, in experimental studies GDF-15 levels markedly increase in response to various conditions of stress or pathology, such as diastolic dysfunction.<sup>9, 11</sup> Previous studies suggested that elevated GDF-15 levels in patients with more advanced stages of LV diastolic dysfunction (regardless of their symptoms) could represent an adaptive response that is outweighed by the severity of the underlying condition.<sup>9</sup> Interestingly, GDF-15 has recently been identified as a mediator of anorexia and cachexia symptoms in patients with advanced HF with reduced ejection fraction (HFrEF), mirroring its role in cancer.<sup>21</sup> In patients with HF with preserved ejection fraction (HFpEF), elevated circulating GDF-15 levels were associated with echocardiographic markers of increased LV filling pressures (i.e., left atrial volume, E/e' ratio) and its severity,<sup>11</sup> reinforcing the

plausibility of GDF-15 as a marker of diastolic (dys)function. Our cross-sectional data provides further data in support of GDF-15 as an effective early marker for detecting preclinical diastolic dysfunction in a wide demographic of healthy individuals, showing a significant association with measures of increased LV filling pressure, namely a higher E/e' ratio and E wave and lower LV volumes.<sup>22</sup> This is particularly noteworthy given that GDF-15's predictive capability remains robust even after adjusting for significant confounders, such as age, sex, BMI, blood pressure, and NT-proBNP. In contrast, a recent study involving the STANISLAS cohort showed that the predictive performance of other biomarkers (brain natriuretic peptide, Galectin-3, N-terminal propeptide of procollagen type III, and soluble ST2) for LV diastolic dysfunction offered only a slight improvement over standard evaluations.<sup>23</sup> Additionally, we found a significant association between increased GDF-15 levels and worsen preclinical systolic dysfunction (GLS). Impaired GLS is a strong predictor of mortality in the general population<sup>24</sup> and has previously been associated with higher circulating GDF-15 levels in HFpEF patients.<sup>25</sup> In aortic stenosis patients, a lower GDF-15 level was able to predict ventricular recovery after transcatheter aortic valve replacement.<sup>26</sup> Similar to diastolic function impairment, it is possible that increased GDF-15 represents an adaptive response to a pathological condition (systolic dysfunction) in its initial stages.

### *Predictive Role of GDF-15*

A significant and unique finding of our study is the association between baseline circulating levels of GDF-15 and an echocardiographic index of LV diastolic impairment approximately two decades in advance. GDF-15 levels have previously been shown to predict the incidence of HF in community-based populations. Specifically, the ARIC (Atherosclerosis Risk in Communities) study revealed that individuals in the highest quartile of GDF-15 concentration had a 70% increased risk of incident HF compared to those in the lowest quartile over a 23-year follow-up.<sup>27</sup> Similarly, in the DAN-MONICA (Danish-Multinational MONItoring of trends and determinants in CARdiovascular disease) cohort, individuals in the highest quartile faced a 36% increased risk compared to those in

the lowest quartile, over a median follow-up of 27 years.<sup>28</sup> In a recent study, circulating GDF-15 showed to significantly improve HF risk prediction compared to a clinical model accounting for risk factors in both HOMAGE (Heart Omics and Ageing) cohort and Framingham Heart Study.<sup>29</sup> Additionally, plasma concentration of GDF-15 were showed to increased gradually with progressing of HF stages (from “at risk” [stage A] to overt HF [stage C and D]).<sup>30</sup> Our finding of a longitudinal association between GDF-15 values and early markers of LV diastolic impairment provides further biological plausibility to the previously demonstrated link between GDF-15 levels and incident HF. Moreover, it extends our knowledge to a critical earlier phase when changes in diastolic function are in their preclinical stages. While we lack a definitive explanation for this association, it could represent the onset of an early cardioprotective response of GDF-15 within initially disturbed metabolic or inflammatory pathways.<sup>31, 32</sup> Indeed, a recent study suggests that HF patients with high plasma GDF-15 levels had increased activation of inflammatory pathways and pathways related to insulin-like growth factor (IGF-1) regulation and bone/tissue remodeling.<sup>33</sup>

The observation that the delta change in GDF-15 was not more informative than the absolute values at V1 and V4 should be interpreted with caution, as only two measurements (18 years apart) may be insufficient to capture the dynamic variation of GDF-15. Interestingly, similar findings were reported in the large population-based DAN-MONICA cohort, where repeated GDF-15 measurements (three timepoints, approximately five years apart) provided only a modest improvement in predicting HF and mortality compared to a single measurement.<sup>28</sup> In contrast, temporal changes in GDF-15 concentrations have shown to improve risk prediction in elderly community populations and in patients with HF.<sup>34, 35</sup> Future studies with frequent serial longitudinal assessments are needed to clarify the temporal dynamics of GDF-15 and its role in risk prediction within population-based settings. Determining the optimal timing of repeat measurements, while accounting for the biological variability of these biomarkers, remains also an important area for further investigation.

### *Clinical perspective*

From a clinical perspective, our findings highlight the potential of GDF-15 as an accessible and practical screening tool for identifying early echocardiographic signs of diastolic dysfunction in the general population. GDF-15 uniquely predicts increased E/E' ratio and lower LVEDVi nearly two decades in advance, allowing for the identification of individuals who may not have specific current clinical findings or history yet silently progress toward overt LV diastolic dysfunction. Recognizing these early alterations, which are associated with an increased risk of evolving into symptomatic HF,<sup>3</sup> can prompt proactive clinical preventive measures, including aggressive risk factor management, to delay or prevent the development of overt cardiac dysfunction.<sup>36</sup> In population-based settings deemed healthy, GDF-15 may be especially valuable due to the limited influence of confounding factors (e.g., CKD, cancer, chronic inflammation)<sup>37</sup> on its predictive power. However, before routine clinical adoption, further studies are needed to assess the cost-effectiveness of GDF-15 testing in standard care. The availability of fully automated assays could further improve the accessibility of GDF-15 measurements for clinicians while reducing associated costs.

Notably, some treatments that improve outcomes in chronic HF showed to do not significantly alter GDF-15 concentrations.<sup>38, 39</sup> Similarly, in subject at risk for HF enrolled in the HOMAGE trial, spironolactone did not consistently modify circulating GDF-15 levels at 9 months follow-up.<sup>40</sup> At present, a novel monoclonal antibody therapy (ponsegromab), aimed at selectively blocking GDF-15 to improve symptoms in patients with HF who exhibit elevated levels of this marker, is under evaluation (GARDEN-TIMI 74 study; NCT05492500). Positive outcomes from this study could pave the way for testing poncegromab in individuals with raised GDF-15 levels but without overt HF, potentially offering new strategies for intervening in the early stages of cardiac dysfunction.

### **Limitations**

Our study has certain limitations. First, a comprehensive echocardiographic evaluation was not performed at V1, preventing us from offering insights into any pre-existing cardiac alterations or their progression. However, the initial cohort consisted of participants who were healthy and free from any reported acute and/or chronic illnesses, making the likelihood of existing cardiac alterations low. Additionally, the absence of intermediate echocardiographic evaluations may have hindered a more thorough assessment of the longitudinal progression of diastolic dysfunction. Our investigation focused solely on individual echocardiographic markers of diastolic function impairment rather than adhering to the comprehensive definition of diastolic dysfunction set forth by current guidelines,<sup>17</sup> given the expected low prevalence of such dysfunction in an asymptomatic population. Moreover, the population-based nature of our cohort limited the evaluation of certain variables. For instance, only a small proportion of participants (309 subjects at V4 and 281 at V1) had measurable tricuspid regurgitation to estimate pulmonary artery systolic pressure, restricting our ability to explore its association with GDF-15. Nevertheless, this approach enabled us to identify at least initial echocardiographic signs of diastolic dysfunction, even without the full spectrum of diagnostic criteria. Several of the echocardiographic measure we collected (E/E' ratio, E/A ratio, LVEF, E wave, etc.) are heart rate, preload, and/or afterload dependent, and may vary over time in a given patient. GDF-15 levels were measured using PEA technology, which provided unitless standardized normalized expression. We relied on a linear equation derived from a small subset of patients at V4 with available GDF-15 ELISA values to estimate standard “classical/mass values”. Additionally, GDF-15 measurements using both methods were not available at V1, preventing us from assessing the correlation between these methods at different time points and potentially introducing measurement bias. While treatments like metformin might have affected GDF-15 levels,<sup>41</sup> their use was limited to a small proportion of patients (1%-3%), likely suggesting a marginal impact on GDF-15 values. Finally, our study population consisted of Caucasian subjects from a specific region in France. This limited geographic diversity, along with the resulting homogeneity in ethnicity and socioeconomic factors, may have introduced confounding effects,



particularly in the evaluation of gene-environment interactions, and limits the generalizability of our findings to other demographic groups. Additionally, the lack of geographic heterogeneity, combined with the small sample size, likely contributed to the limited genetic signal observed in our GWAS analysis, potentially reducing our ability to identify other biologically relevant associations.

## **Conclusions**

Among participants in the STANISLAS cohort, higher initial circulating levels of GDF-15 were associated with early echocardiographic markers of impaired diastolic function almost two decades in advance. This association was also confirmed by evaluating GDF-15 at the follow-up visit (V4). These findings suggest GDF-15's potential role as both a long-term predictor and easy screening tool of diastolic function impairment in the general population, which could be instrumental for possible proactive clinical preventive measures.

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### **Conflict of interest**

J.P.F. has received consulting fees from Boehringer Ingelheim. G.B. reported receiving personal fees from Abbott, AstraZeneca, and Boehringer. P.R. has received grants and personal fees from AstraZeneca, Bayer, CVRx, Fresenius, and Novartis; and personal fees from Grunenthal, Servier, Stealth Peptides, Vifor Fresenius Medical Care Renal Pharma, Idorsia, NovoNordisk, Ablative Solutions, G3P, Corvidia, and Relypsa. G.B. reports receiving personal fees from Abbott, AstraZeneca, and Boehringer. F.Z. reports personal fees from Applied Therapeutics, Bayer, Boehringer, BMS, CVRx, Cardior, Cereno Pharmaceutical, Cellprothera, CEVA, Merck, Novartis, Novo Nordisk, Owkin, Pfizer and Servier, having stock options at G3 Pharmaceuticals and equities at Cereno Pharmaceutical, Cardiorenal and Eshmoun Clinical Research and being the founder of Cardiovascular Clinical Trialists. N.G. reports honoraria from AstraZeneca, Bayer, Boehringer, Lilly, Novartis, NovoNordisk, Roche Diagnostics, NP Medical and Echosens. All other authors have

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### Figure legends

#### **Figure 1 Associations between circulating GDF-15 and echocardiographic indexes of structure and function at V4.**

**A)** Baseline (V1) GDF-15 and **B)** GDF-15 at visit 4 (V4). Adjustments: Model 1, age, gender; Model 2: age, gender, current smoker, body mass index (BMI), total cholesterol; Model 3: age, gender, current smoker, BMI, total cholesterol, systolic blood pressure (SBP); Model 4: age, gender, current smoker, BMI, total cholesterol, SBP and N-terminal pro-B-type natriuretic peptide.

**Abbreviations:** GDF-15, growth differentiation factor-15; LAVi, left atrial volume index; LV-GLS, left ventricular global longitudinal strain; LVEDVi, left ventricular end-diastolic volume index; LVESVi, left ventricular end-systolic volume index; LVEF, left ventricular ejection fraction; LVMi, left ventricular mass index.

#### **Central Figure Summary of the study design and key findings.**

The (+) and (–) signs mean that the variable is positively or inversely associated with growth differentiation factor-15 (GDF-15) levels in the fully adjusted model (age, gender, current smoker, body mass index, total cholesterol, systolic blood pressure and N-terminal pro-B-type natriuretic peptide), respectively. **Abbreviations:** LV, left ventricular; LVEDVi, left ventricular end-diastolic

volume index; LV-GLS, left ventricular global longitudinal strain; LVMI, left ventricular mass index.

**Supplemental Figure 1** Distribution of GDF-15 values (Olink) at visit 1 (panel A) and visit 4 (panel B)

**Supplemental Figure 2** Correlation between Olink method and ELISA assay at visit 4

**Supplemental Figure 3** Spline analysis of the association between GDF-15 levels and key echocardiographic variables at visits 1 (panel A) and visit 4 (panel B)

**Supplemental Figure 4** Variance decomposition of circulating GDF-15

Abbreviations: GDF-15, growth differentiation factor-15; V1, visit 1; V4, visit 4 (V4)

**Supplemental Figure 5** Manhattan plot of the GWAS study analysis of circulating GDF-15 concentration.

The horizontal red and orange lines indicate the statistically significant threshold at  $p$ -value =  $10^{-7}$  and  $10^{-5}$ , respectively. Each point represents a genetic variant. The  $x$ -axis represents the genomic position, and the  $y$ -axis represents the strength of association as represented by  $-\log_{10}(p \text{ value})$ . Sex and age were used as covariates. \*Located on or close to the GDF-15 coding gene. Abbreviations: GDF-15, growth differentiation factor-15; GWAS, genome-wide association; SD, standard deviation.

**Table 1** Characteristics of the population at STANISLAS-V4 according to baseline (V1) GDF-15 value in tertiles

|   | <b>Overall<br/>(N=1562)</b> | <b>Lower Tertile<br/>of GDF-15 at<br/>V1<br/>(N= 520)</b> | <b>Intermediate<br/>Tertile of GDF-<br/>15 at V1<br/>(N= 521)</b> | <b>Higher Tertile of<br/>GDF-15 at V1<br/>(N= 521)</b> | <b>p-<br/>value</b> |
|---|-----------------------------|---|---|--|---------------------|
| <b>GDF-15 (Olink)</b>                   | 5.85 ± 0.54                 | 5.49 ± 0.44   | 5.86 ± 0.40   | 6.20 ± 0.53  | <0.001              |
| <b>Demographics and medical history</b> |                             |   |   |  |                     |
| Age, years                              | 50 ± 14                     | 40 ± 12   | 52 ± 12   | 58 ± 9   | <0.001              |
| Male, n (%)                             | 746 (48)                    | 242 (47)  | 245 (47)  | 259 (50)   | 0.54                |
| BMI, Kg/m <sup>2</sup>                  | 26 ± 5                      | 25 ± 5  | 26 ± 5  | 27 ± 5   | <0.001              |
| Diabetes, n (%)                         | 81 (5)                      | 12 (2)  | 16 (3)  | 53 (10)  | <0.001              |
| Smoker status, n (%)                    |                             |   |   |  |                     |
| - Current                               | 318 (20)                    | 132 (25)  | 96 (18)   | 90 (12)  | 0.002               |
| - Former                                | 523 (33)                    | 113 (22)  | 178 (34)  | 232 (44)   |                     |
| - Never                                 | 721 (46)                    | 275 (53)  | 247 (47)  | 199 (38)   |                     |
| Antihypertensive<br>treatment, n (%)    | 299 (19)                    | 44 (8)  | 109 (21)  | 146 (28)   | <0.001              |
| Lipid lowering therapy, n<br>(%)        | 255 (16)                    | 27 (5)  | 83 (16)   | 145 (28)   | <0.001              |
| Diabetes treatment, n (%)               | 61 (4)                      | 8 (2)   | 13 (3)  | 40 (8)   | <0.001              |
| Metformin use, n (%)                    | 29 (2)                      | 4 (1)   | 9 (2)   | 16 (3)   | 0.03                |
| SBP, mmHg                               | 126 ± 16                    | 122 ± 13  | 126 ± 15  | 130 ± 17   | <0.001              |
| DBP, mmHg                               | 73 ± 9                      | 71 ± 9  | 73 ± 9  | 74 ± 9   | <0.001              |
| Heart rate, bpm                         | 62 ± 10                     | 62 ± 9  | 62 ± 9  | 64 ± 10  | 0.054               |
| <b>Laboratory</b>                       |                             |   |   |  |                     |
| Haemoglobin, g/L                        | 14.7 ± 1.2                  | 14.7 ± 1.3  | 14.6 ± 1.2  | 14.8 ± 1.2   | 0.052               |
| Total cholesterol, g/L                  | 2.1 ± 0.4                   | 2.1 ± 0.4   | 2.2 ± 0.4   | 2.2 ± 0.4  | <0.001              |
| Glycemia, g/L                           | 0.9 ± 0.2                   | 0.9 ± 0.1   | 0.9 ± 0.2   | 1.0 ± 0.2  | <0.001              |

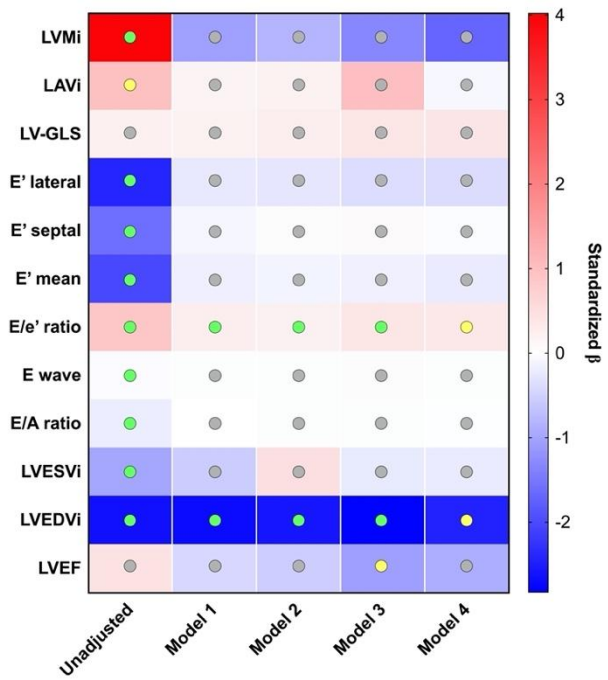
|                                  |                   |                   |                   |                    |        |
|----------------------------------|-------------------|-------------------|-------------------|--------------------|--------|
| eGFR, mL/min/1.73 m <sup>2</sup> | 95 ± 15           | 104 ± 14          | 93 ± 14           | 89 ± 14            | <0.001 |
| Microalbuminuria, mg/l           | 6.07 [3.48; 9.94] | 5.97 [3.41; 9.22] | 6.14 [3.26; 9.82] | 6.19 [3.87; 11.30] | 0.065  |
| NTproBNP (Olink)                 | 3.6 ± 1.0         | 3.3 ± 1.0         | 3.7 ± 1.0         | 3.9 ± 1.0          | <0.001 |
| <b>Echocardiography</b>          |                   |                   |                   |                    |        |
| LVMi, gr/m <sup>2</sup>          | 77 ± 19           | 73 ± 17           | 77 ± 20           | 80 ± 20            | <0.001 |
| LAVi, mL/m <sup>2</sup>          | 23 ± 7            | 22 ± 7            | 23 ± 7            | 23 ± 8             | 0.11   |
| LV-GLS                           | -20.6 ± 3.1       | -20.7 ± 2.9       | -20.7 ± 3.1       | -20.3 ± 3.2        | 0.10   |
| E' lateral, cm/s                 | 12.7 ± 4.0        | 14.7 ± 4.1        | 12.3 ± 3.7        | 10.9 ± 3.5         | <0.001 |
| E' septal, cm/s                  | 9.7 ± 2.9         | 11.2 ± 3.0        | 9.5 ± 2.7         | 8.6 ± 2.4          | <0.001 |
| E' mean, cm/s                    | 11.2 ± 3.2        | 13.0 ± 3.2        | 10.9 ± 2.9        | 9.7 ± 2.6          | <0.001 |
| E/e' ratio                       | 6.5 ± 1.9         | 5.8 ± 1.5         | 6.6 ± 1.8         | 7.2 ± 2.1          | <0.001 |
| E wave, m/s                      | 0.69 ± 0.15       | 0.72 ± 0.15       | 0.69 ± 0.15       | 0.67 ± 0.16        | <0.001 |
| E/A ratio                        | 1.2 ± 0.4         | 1.3 ± 0.4         | 1.1 ± 0.4         | 1.0 ± 0.4          | <0.001 |
| LVESVi, mL/m <sup>2</sup>        | 17 ± 6            | 18 ± 6            | 17 ± 6            | 16 ± 7             | 0.001  |
| LVEDVi, mL/m <sup>2</sup>        | 49 ± 13           | 50 ± 12           | 49 ± 13           | 47 ± 13            | 0.001  |
| LVEF, %                          | 65 ± 7            | 64 ± 6            | 66 ± 6            | 66 ± 7             | 0.006  |

**Abbreviations:** BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; LAVi, left atrial volume index; LV-GLS, left ventricular global longitudinal strain; LVEDVi, left ventricular end-diastolic volume index; LVESVi, left ventricular end-systolic volume index; LVEF, left ventricular ejection fraction; LVMi, left ventricular mass index; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SBP, systolic

**Figure 1**

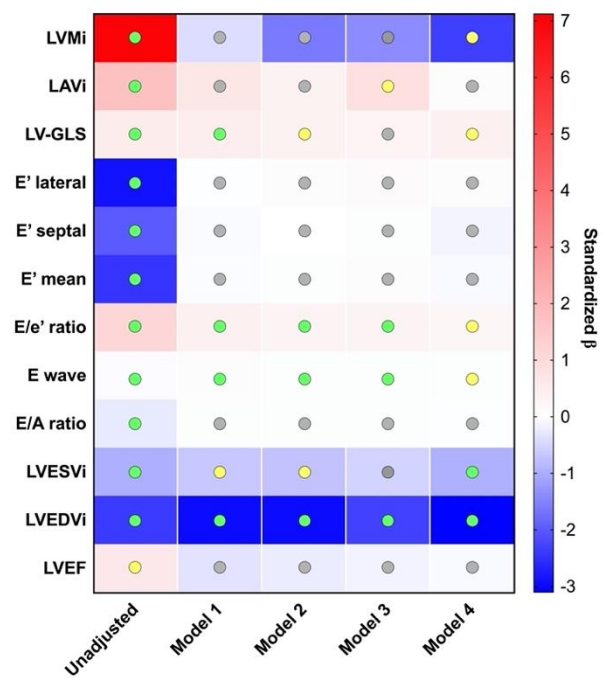
**A**

**GDF-15 at Visit-1**



**B**

**GDF-15 at Visit-4**



**P-value** ●  $\geq 0.05$  ● 0.01 - 0.04 ●  $< 0.01$

**Supplemental Table 1** Characteristics of the population according to GDF-15 tertiles at Visit-4 (V4)

|   | <b>Overall<br/>(N=1679)</b> | <b>Tertile 1 of<br/>GDF-15 at V4<br/>(N= 559)</b> | <b>Tertile 2 of<br/>GDF-15 at V4<br/>(N= 560)</b> | <b>Tertile 3 of GDF-<br/>15 at V4<br/>(N= 560)</b> | <b>P-<br/>value</b> |
|---|-----------------------------|---|---|--|---------------------|
| <b>GDF-15 (Olink)</b>                   | 5.83 ± 0.55                 | 5.28 ± 0.25                                       | 5.70 ± 0.12                                       | 6.42 ± 0.41  | <0.001              |
| <b>Demographics and medical history</b> |                             |   |   |  |                     |
| Age, years                              | 49 ± 14                     | 39 ± 12   | 50 ± 13   | 58 ± 10  | <0.001              |
| Male, n (%)                             | 812 (48)                    | 246 (44)  | 244 (44)  | 322 (58)   | <0.001              |
| BMI, Kg/m <sup>2</sup>                  | 26 ± 5                      | 25 ± 4  | 26 ± 5  | 27 ± 5   | <0.001              |
| Diabetes, n (%)                         | 82 (5)                      | 5 (0.9)   | 17 (3)  | 60 (11)  | <0.001              |
| Smoker status, n (%)                    |                             |   |   |  |                     |
| - Current                               | 358 (21)                    | 118 (21)  | 123 (22)  | 117 (21)   | 0.89                |
| - Former                                | 538 (32)                    | 128 (23)  | 180 (32)  | 230 (41)   |                     |
| - Never                                 | 783 (47)                    | 313 (56)  | 257 (46)  | 213 (38)   |                     |
| Antihypertensive treatment, n (%)       | 303 (18)                    | 30 (5)  | 96 (17)   | 177 (32)   | <0.001              |
| Lipid lowering therapy, n (%)           | 259 (15)                    | 22 (4)  | 72 (13)   | 165 (29)   | <0.001              |
| Diabetes treatment, n (%)               | 61 (4)                      | 2 (0.4)   | 8 (1.4)   | 51 (9)   | <0.001              |
| Metformin, n (%)                        | 29 (2)                      | 1 (0)   | 1 (0)   | 27 (2)   | <0.001              |
| SBP, mmHg                               | 126 ± 15                    | 121 ± 13  | 125 ± 14  | 131 ± 17   | <0.001              |
| DBP, mmHg                               | 72 ± 9                      | 70 ± 8  | 72 ± 9  | 75 ± 9   | <0.001              |
| Heart rate, bpm                         | 63 ± 10                     | 62 ± 9  | 62 ± 9  | 64 ± 11  | 0.005               |
| <b>Laboratory</b>                       |                             |   |   |  |                     |
| Haemoglobin, g/L                        | 14.7 ± 1.2                  | 14.6 ± 1.3  | 14.7 ± 1.1  | 14.9 ± 1.2   | 0.004               |
| Total cholesterol, g/L                  | 2.1 ± 0.4                   | 2.0 ± 0.4   | 2.2 ± 0.4   | 2.2 ± 0.4  | <0.001              |
| Glycemia, g/L                           | 0.9 ± 0.2                   | 0.9 ± 0.1   | 0.9 ± 0.1   | 1.0 ± 0.2  | <0.001              |
| eGFR, mL/min/1.73 m <sup>2</sup>        | 96 ± 15                     | 105 ± 13  | 95 ± 14   | 89 ± 15  | <0.001              |

|                           |                    |                   |                   |                    |        |
|---------------------------|--------------------|-------------------|-------------------|--------------------|--------|
| Microalbuminuria, mg/l    | 6.07 [3.53; 10.00] | 5.59 [3.22; 9.60] | 5.69 [3.42; 8.86] | 7.08 [4.13; 12.30] | 0.002  |
| NTproBNP (Olink)          | 3.6 ± 1.0          | 3.2 ± 0.9         | 3.6 ± 0.9         | 3.9 ± 1.1          | <0.001 |
| <b>Echocardiography</b>   |                    |                   |                   |                    |        |
| LVMi, gr/m <sup>2</sup>   | 76 ± 19            | 71 ± 16           | 76 ± 19           | 82 ± 21            | <0.001 |
| LAVi, mL/m <sup>2</sup>   | 23 ± 7             | 22 ± 6            | 22 ± 7            | 24 ± 8             | <0.001 |
| LV-GLS                    | -20.6 ± 3.1        | -20.8 ± 2.7       | -20.7 ± 3.1       | -20.1 ± 3.3        | 0.001  |
| E' lateral, cm/s          | 12.9 ± 4.1         | 14.9 ± 4.1        | 12.6 ± 3.8        | 11.1 ± 3.5         | <0.001 |
| E' septal, cm/s           | 9.9 ± 2.9          | 11.4 ± 2.9        | 9.6 ± 2.7         | 8.7 ± 2.6          | <0.001 |
| E' mean, m/s              | 11.4 ± 3.2         | 13.1 ± 3.1        | 11.1 ± 2.9        | 9.9 ± 2.7          | <0.001 |
| E/e' ratio                | 6.5 ± 1.9          | 5.8 ± 1.5         | 6.6 ± 1.7         | 7.1 ± 2.1          | <0.001 |
| E wave, m/s               | 0.70 ± 0.15        | 0.73 ± 0.15       | 0.70 ± 0.14       | 0.67 ± 0.16        | <0.001 |
| E/A ratio                 | 1.2 ± 0.4          | 1.4 ± 0.4         | 1.2 ± 0.4         | 1.0 ± 0.4          | <0.001 |
| LVESVi, mL/m <sup>2</sup> | 17 ± 6             | 18 ± 6            | 17 ± 6            | 17 ± 8             | 0.001  |
| LVEDVi, mL/m <sup>2</sup> | 49 ± 13            | 51 ± 12           | 48 ± 13           | 48 ± 14            | 0.001  |
| LVEF, %                   | 65 ± 7             | 65 ± 6            | 65 ± 6            | 65 ± 7             | 0.046  |

**Abbreviations:** BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; LAVi, left atrial volume index; LV, left ventricle; LVEDVi, left ventricular end-diastolic volume index; LVESVi, left ventricular end-systolic volume index; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SBP, systolic blood pressure.



**Supplemental Table 2** Characteristics of the population according to GDF-15 tertiles at baseline (Visit-1, V1)\*

|   | <b>Overall<br/>(N=1562)</b> | <b>Tertile 1 of<br/>GDF-15 at V1<br/>(N= 520)</b> | <b>Tertile 2 of<br/>GDF-15 at V1<br/>(N= 521)</b> | <b>Tertile 3 of<br/>GDF-15 at V1<br/>(N= 521)</b> | <b>p-value</b> |
|---|-----------------------------|---|---|---|----------------|
| <b>GDF-15 (Olink)</b>                   | 5.26 ± 0.52                 | 4.77 ± 0.30                                       | 5.23 ± 0.11                                       | 5.79 ± 0.42                                       | <0.001         |
| <b>Demographics and medical history</b> |                             |   |   |   |                |
| Age, years                              | 32 ± 14                     | 22 ± 13   | 34 ± 13   | 39 ± 10   | <0.001         |
| Male, n (%)                             | 747 (48)                    | 242 (47)  | 245 (47)  | 260 (50)  | 0.50           |
| BMI, Kg/m <sup>2</sup>                  | 23 ± 4                      | 21 ± 4  | 23 ± 4  | 24 ± 4  | <0.001         |
| Smoker status, n (%)                    |                             |   |   |   |                |
| - Current                               | 241 (15)                    | 22 (4)  | 62 (12)   | 157 (30)  | <0.001         |
| - Former                                | 259 (17)                    | 42 (8)  | 108 (21)  | 109 (21)  |                |
| - Never                                 | 783 (47)                    | 313 (56)  | 257 (46)  | 213 (38)  |                |
| Antihypertensive treatment, n (%)       | 10 (0.6)                    | 1 (0.2)   | 2 (0.4)   | 7 (1.3)   | 0.37           |
| Lipid lowering therapy, n (%)           | 18 (1.2)                    | 2 (0.4)   | 2 (0.4)   | 14 (2.7)  | 0.001          |
| Diabetes treatment, n (%)               | 1 (0.1)                     | 0 (0.0)   | 0 (0.0)   | 1 (0.2)   | 1.00           |
| SBP, mmHg                               | 122 ± 13                    | 120 ± 11  | 123 ± 13  | 124 ± 14  | <0.001         |
| DBP, mmHg                               | 71 ± 11                     | 67 ± 11   | 72 ± 11   | 74 ± 11   | <0.001         |
| Heart rate, bpm                         | 68 ± 12                     | 71 ± 12   | 67 ± 12   | 67 ± 10   | <0.001         |
| <b>Laboratory</b>                       |                             |   |   |   |                |
| Haemoglobin, g/L                        | 14.3 ± 1.2                  | 14.2 ± 1.1  | 14.2 ± 1.2  | 14.5 ± 1.3  | 0.008          |
| Total cholesterol, g/L                  | 2.1 ± 0.4                   | 1.9 ± 0.4   | 2.1 ± 0.4   | 2.2 ± 0.4   | <0.001         |
| Glycemia, g/L                           | 0.9 ± 0.1                   | 0.9 ± 0.1   | 0.9 ± 0.1   | 0.9 ± 0.1   | 0.77           |
| eGFR, mL/min/1.73 m <sup>2</sup>        | 92 ± 14                     | 96 ± 13   | 92 ± 13   | 89 ± 14   | <0.001         |
| NTproBNP (Olink)                        | 3.2 ± 0.9                   | 3.1 ± 0.9   | 3.3 ± 0.9   | 3.4 ± 1.0   | <0.001         |

\*In visit 1, echocardiographic variables, microalbuminuria and the prevalence of diabetes

**were not investigated.**

**Abbreviations:** BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SBP, systolic blood pressure

**Supplemental Table 3** Unadjusted and adjusted linear regression between concentration of GDF-15 at baseline (V1) and echocardiographic variables at Visit-4

|            | Unadjusted            |                   | Adjusted Model 1      |                   | Adjusted Model 2      |               | Adjusted Model 3      |              | Adjusted Model 4      |              |
|------------|-----------------------|-------------------|-----------------------|-------------------|-----------------------|---------------|-----------------------|--------------|-----------------------|--------------|
|            | Beta ± SE             | p-value           | Beta ± SE             | p-value           | Beta ± SE             | p-value       | Beta ± SE             | p-value      | Beta                  | p-value      |
| LVMi       | <b>4.020 ± 0.950</b>  | <b>&lt;0.0001</b> | -1.052 ± 0.942        | 0.26              | -0.822 ± 0.995        | 0.41          | -1.332 ± 1.372        | 0.33         | -1.704 ± 1.427        | 0.23         |
| LAVi       | <b>0.969 ± 0.375</b>  | <b>0.010</b>      | 0.180 ± 0.415         | 0.66              | 0.202 ± 0.444         | 0.65          | 1.00 ± 0.632          | 0.87         | -0.097 ± 0.659        | 0.88         |
| LV-GLS     | 0.216 ± 0.164         | 0.19              | 0.201 ± 0.179         | 0.26              | 0.273 ± 0.191         | 0.15          | 0.378 ± 0.276         | 0.17         | 0.387 ± 0.284         | 0.17         |
| E' lateral | <b>-2.423 ± 0.189</b> | <b>&lt;0.0001</b> | -0.249 ± 0.166        | 0.13              | -0.270 ± 0.176        | 0.12          | -0.379 ± 0.208        | 0.07         | -0.400 ± 0.216        | 0.06         |
| E' septal  | <b>-1.613 ± 0.137</b> | <b>&lt;0.0001</b> | -0.103 ± 0.124        | 0.41              | 0.031 ± 0.131         | 0.81          | 0.064 ± 0.156         | 0.68         | -0.040 ± 0.162        | 0.80         |
| E' mean    | <b>-2.029 ± 0.148</b> | <b>&lt;0.0001</b> | -0.184 ± 0.122        | 0.13              | -0.127 ± 0.130        | 0.33          | -0.163 ± 0.155        | 0.29         | -0.228 ± 0.161        | 0.16         |
| E/e' ratio | <b>0.898 ± 0.090</b>  | <b>&lt;0.0001</b> | <b>0.264 ± 0.093</b>  | <b>0.005</b>      | <b>0.206 ± 0.097</b>  | <b>0.033</b>  | <b>0.375 ± 0.137</b>  | <b>0.006</b> | <b>0.362 ± 0.142</b>  | <b>0.011</b> |
| E wave     | <b>-0.031 ± 0.007</b> | <b>&lt;0.0001</b> | 0.013 ± 0.008         | 0.10              | 0.012 ± 0.008         | 0.16          | 0.020 ± 0.010         | 0.06         | 0.015 ± 0.011         | 0.15         |
| E/A ratio  | <b>-0.204 ± 0.020</b> | <b>&lt;0.0001</b> | 0.000 ± 0.018         | 0.99              | 0.004 ± 0.020         | 0.82          | 0.008 ± 0.022         | 0.72         | -0.016 ± 0.023        | 0.49         |
| LVESVi     | <b>-0.990 ± 0.316</b> | <b>0.002</b>      | -0.573 ± 0.339        | 0.09              | 0.503 ± 0.364         | 0.17          | -0.236 ± 0.497        | 0.63         | -0.231 ± 0.517        | 0.65         |
| LVEDVi     | <b>-2.661 ± 0.632</b> | <b>&lt;0.0001</b> | <b>-2.716 ± 0.679</b> | <b>&lt;0.0001</b> | <b>-2.604 ± 0.729</b> | <b>0.0004</b> | <b>-2.829 ± 0.978</b> | <b>0.004</b> | <b>-2.458 ± 1.015</b> | <b>0.016</b> |
| LVEF       | 0.440 ± 0.329         | 0.18              | -0.433 ± 0.359        | 0.23              | -0.556 ± 0.384        | 0.15          | <b>-1.067 ± 0.497</b> | <b>0.032</b> | -0.902 ± 0.516        | 0.08         |

**Adjustments:** Model 1, age, gender; Model 2: age, gender, current smoker, body mass index (BMI), total cholesterol; Model 3: age, gender, current smoker, BMI, total cholesterol, systolic blood pressure (SBP), estimated glomerular filtration rate (eGFR); Model 4: age, gender, current smoker, BMI, total cholesterol, SBP, eGFR and N-terminal pro-B-type natriuretic peptide.

**Abbreviations:** GDF-15, growth differentiation factor-15; LAVi, left atrial volume index; LVEDVi, left ventricular end-diastolic volume index; LVESVi, left ventricular end-systolic volume index; LVEF, left ventricular ejection fraction; LV-GLS, left ventricular global longitudinal strain; LVMi, left ventricular mass index; SE, standard error.

**Supplemental Table 4** Adjusted linear regression between concentration of NT-proBNP at baseline (V1) and echocardiographic variables at Visit-4.

|            | <b>Beta ± SE</b> | <b>p-value</b> |
|------------|------------------|----------------|
| LVMi       | 1.088 ± 0.628    | 0.084          |
| LAVi       | 0.435 ± 0.271    | 0.109          |
| LV-GLS     | 0.054 ± 0.118    | 0.651          |
| E' lateral | 0.041 ± 0.107    | 0.702          |
| E' septal  | 0.059 ± 0.081    | 0.468          |
| E' mean    | 0.055 ± 0.080    | 0.489          |
| E/e' ratio | 0.077 ± 0.062    | 0.211          |
| E wave     | 0.009 ± 0.005    | 0.076          |
| E/A ratio  | 0.031 ± 0.012    | 0.009          |
| LVESVi     | 0.113 ± 0.232    | 0.627          |
| LVEDVi     | -0.380 ± 0.462   | 0.412          |
| LVEF       | -0.327 ± 0.240   | 0.173          |

**Adjustments:** age, gender, current smoker, body mass index, total cholesterol, systolic blood pressure.

**Abbreviations:** LAVi, left atrial volume index; LVEDVi, left ventricular end-diastolic volume index; LVESV, left ventricular end-systolic volume index; LVEF, left ventricular ejection fraction; LV-GLS, left ventricular global longitudinal strain; LVMi, left ventricular mass index; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SE, standard error.

**Supplemental Table 5** Unadjusted and adjusted linear regression between concentration of GDF-15 and echocardiographic variables at Visit-4

|            | Unadjusted            |                   | Adjusted Model 1      |                   | Adjusted Model 2      |                   | Adjusted Model 3      |              | Adjusted Model 4      |                   |
|------------|-----------------------|-------------------|-----------------------|-------------------|-----------------------|-------------------|-----------------------|--------------|-----------------------|-------------------|
|            | Beta ± SE             | p-value           | Beta ± SE             | p-value           | Beta ± SE             | p-value           | Beta ± SE             | p-value      | Beta ± SE             | p-value           |
| LVMi       | <b>7.118 ± 0.853</b>  | <b>&lt;0.0001</b> | -0.422 ± 0.932        | 0.65              | -1.627 ± 0.936        | 0.08              | -1.405 ± 0.971        | 0.15         | <b>-2.335 ± 0.985</b> | <b>0.018</b>      |
| LAVi       | <b>1.724 ± 0.332</b>  | <b>&lt;0.0001</b> | 0.665 ± 0.402         | 0.10              | 0.358 ± 0.412         | 0.39              | <b>0.865 ± 0.431</b>  | <b>0.045</b> | 0.034 ± 0.429         | 0.94              |
| LV-GLS     | <b>0.501 ± 0.146</b>  | <b>0.0006</b>     | <b>0.473 ± 0.173</b>  | <b>0.006</b>      | <b>0.353 ± 0.173</b>  | <b>0.042</b>      | 0.270 ± 0.180         | 0.14         | <b>0.394 ± 0.183</b>  | <b>0.032</b>      |
| E' lateral | <b>-2.876 ± 0.172</b> | <b>&lt;0.0001</b> | -0.018 ± 0.168        | 0.92              | 0.067 ± 0.169         | 0.69              | 0.113 ± 0.177         | 0.52         | 0.029 ± 0.180         | 0.87              |
| E' septal  | <b>-2.015 ± 0.123</b> | <b>&lt;0.0001</b> | -0.050 ± 0.124        | 0.69              | -0.009 ± 0.124        | 0.94              | 0.006 ± 0.129         | 0.96         | -0.123 ± 0.131        | 0.35              |
| E' mean    | <b>-2.460 ± 0.133</b> | <b>&lt;0.0001</b> | -0.048 ± 0.122        | 0.69              | 0.011 ± 0.122         | 0.93              | 0.037 ± 0.127         | 0.77         | -0.066 ± 0.129        | 0.61              |
| E/e' ratio | <b>1.115 ± 0.080</b>  | <b>&lt;0.0001</b> | <b>0.407 ± 0.091</b>  | <b>&lt;0.0001</b> | <b>0.312 ± 0.092</b>  | <b>0.0007</b>     | <b>0.294 ± 0.096</b>  | <b>0.002</b> | <b>0.238 ± 0.098</b>  | <b>0.015</b>      |
| E wave     | <b>-0.036 ± 0.007</b> | <b>&lt;0.0001</b> | <b>0.032 ± 0.008</b>  | <b>&lt;0.0001</b> | <b>0.028 ± 0.008</b>  | <b>0.001</b>      | <b>0.028 ± 0.008</b>  | <b>0.001</b> | <b>0.018 ± 0.008</b>  | <b>0.037</b>      |
| E/A ratio  | <b>-0.258 ± 0.018</b> | <b>&lt;0.0001</b> | 0.006 ± 0.018         | 0.76              | 0.003 ± 0.019         | 0.89              | 0.002 ± 0.019         | 0.94         | -0.024 ± 0.019        | 0.22              |
| LVESVi     | <b>-0.983 ± 0.288</b> | <b>0.0007</b>     | <b>-0.680 ± 0.338</b> | <b>0.044</b>      | <b>-0.753 ± 0.345</b> | <b>0.029</b>      | -0.536 ± 0.362        | 0.14         | <b>-0.966 ± 0.366</b> | <b>0.008</b>      |
| LVEDVi     | <b>-2.395 ± 0.577</b> | <b>&lt;0.0001</b> | <b>-2.958 ± 0.676</b> | <b>&lt;0.0001</b> | <b>-2.986 ± 0.692</b> | <b>&lt;0.0001</b> | <b>-2.319 ± 0.725</b> | <b>0.001</b> | <b>-3.104 ± 0.734</b> | <b>&lt;0.0001</b> |
| LVEF       | <b>0.626 ± 0.300</b>  | <b>0.037</b>      | -0.338 ± 0.358        | 0.35              | -0.241 ± 0.369        | 0.51              | -0.151 ± 0.387        | 0.70         | 0.070 ± 0.394         | 0.86              |

**Adjustments:** Model 1, age, gender; Model 2: age, gender, current smoker, body mass index (BMI), total cholesterol; Model 3: age, gender, current smoker, BMI, total cholesterol, systolic blood pressure (SBP), estimated glomerular filtration rate (eGFR), diabetes mellitus (DM); Model 4: age, gender, current smoker, BMI, total cholesterol, SBP, eGFR, DM and N-terminal pro-B-type natriuretic peptide.

**Abbreviations:** GDF-15, growth differentiation factor-15; LAVi, left atrial volume index; LVEDVi, left ventricular end-diastolic volume index; LVESV, left ventricular end-systolic volume index; LVEF, left ventricular ejection fraction; LV-GLS, left ventricular global longitudinal strain; LVMi, left ventricular mass index; SE, standard error.

**Supplemental Table 6** Unadjusted and adjusted association between change in concentration of GDF-15 (V4-V1) and echocardiographic variables at Visit-4

|            | Adjusted Model 0      |                   | Adjusted Model 1      |               | Adjusted Model 2      |              | Adjusted Model 3      |              | Adjusted Model 4      |              |
|------------|-----------------------|-------------------|-----------------------|---------------|-----------------------|--------------|-----------------------|--------------|-----------------------|--------------|
|            | Beta ± SE             | p-value           | Beta ± SE             | p-value       | Beta ± SE             | p-value      | Beta ± SE             | p-value      | Beta ± SE             | p-value      |
| LVMi       | <b>6.892 ± 1.049</b>  | <b>&lt;0.0001</b> | -0.101 ± 1.057        | 0.92          | -1.540 ± 1.057        | 0.15         | -1.311 ± 1.094        | 0.23         | <b>-2.266 ± 1.106</b> | <b>0.041</b> |
| LAVi       | <b>1.790 ± 0.410</b>  | <b>&lt;0.0001</b> | 0.903 ± 0.453         | 0.047         | 0.574 ± 0.462         | 0.21         | <b>1.114 ± 0.481</b>  | <b>0.021</b> | 0.271 ± 0.478         | 0.57         |
| LV-GLS     | <b>0.590 ± 0.183</b>  | <b>0.001</b>      | <b>0.449 ± 0.199</b>  | <b>0.023</b>  | 0.248 ± 0.198         | 0.21         | 0.134 ± 0.205         | 0.51         | 0.254 ± 0.209         | 0.22         |
| E' lateral | <b>-2.250 ± 0.204</b> | <b>&lt;0.0001</b> | -0.025 ± 0.185        | 0.89          | 0.107 ± 0.186         | 0.56         | 0.171 ± 0.193         | 0.38         | 0.076 ± 0.196         | 0.70         |
| E' septal  | <b>-1.578 ± 0.148</b> | <b>&lt;0.0001</b> | -0.029 ± 0.138        | 0.84          | 0.043 ± 0.138         | 0.75         | 0.074 ± 0.143         | 0.61         | -0.062 ± 0.145        | 0.67         |
| E' mean    | <b>-1.926 ± 0.158</b> | <b>&lt;0.0001</b> | -0.042 ± 0.136        | 0.76          | 0.056 ± 0.135         | 0.68         | 0.099 ± 0.140         | 0.48         | -0.012 ± 0.141        | 0.93         |
| E/e' ratio | <b>0.899 ± 0.098</b>  | <b>&lt;0.0001</b> | <b>0.369 ± 0.103</b>  | <b>0.0004</b> | <b>0.251 ± 0.104</b>  | <b>0.016</b> | <b>0.222 ± 0.108</b>  | <b>0.040</b> | 0.167 ± 0.110         | 0.13         |
| E wave     | <b>-0.028 ± 0.008</b> | <b>0.001</b>      | <b>0.028 ± 0.009</b>  | <b>0.001</b>  | <b>0.024 ± 0.009</b>  | <b>0.008</b> | <b>0.025 ± 0.009</b>  | <b>0.009</b> | 0.013 ± 0.009         | 0.16         |
| E/A ratio  | <b>-0.210 ± 0.021</b> | <b>&lt;0.0001</b> | -0.005 ± 0.020        | 0.81          | -0.004 ± 0.021        | 0.83         | -0.005 ± 0.021        | 0.83         | -0.031 ± 0.021        | 0.15         |
| LVESVi     | -0.655 ± 0.353        | 0.06              | -0.697 ± 0.379        | 0.07          | -0.705 ± 0.386        | 0.07         | -0.463 ± 0.404        | 0.25         | <b>-0.885 ± 0.408</b> | <b>0.030</b> |
| LVEDVi     | <b>-1.510 ± 0.705</b> | <b>0.032</b>      | <b>-2.574 ± 0.759</b> | <b>0.0007</b> | <b>-2.461 ± 0.773</b> | <b>0.002</b> | <b>-1.695 ± 0.807</b> | <b>0.036</b> | <b>-2.489 ± 0.816</b> | <b>0.002</b> |
| LVEF       | 0.521 ± 0.368         | 0.16              | -0.101 ± 0.404        | 0.80          | -0.037 ± 0.413        | 0.93         | 0.070 ± 0.432         | 0.87         | 0.274 ± 0.440         | 0.53         |

**Adjustments:** Model 0, baseline value of GDF-15; Model 1, age, gender; Model 2: age, gender, current smoker, body mass index (BMI), total cholesterol; Model 3: age, gender, current smoker, BMI, total cholesterol, systolic blood pressure (SBP), estimated glomerular filtration rate (eGFR), diabetes mellitus (DM); Model 4: age, gender, current smoker, BMI, total cholesterol, SBP, eGFR, DM and N-terminal pro-B-type natriuretic peptide.

**Abbreviations:** GDF-15, growth differentiation factor-15; LAVi, left atrial volume index; LVEDVi, left ventricular end-diastolic volume index; LVESVi, left ventricular end-systolic volume index; LVEF, left ventricular ejection fraction; LV-GLS, left ventricular global longitudinal strain; LVMi, left ventricular mass index; SE, standard error.

**Supplemental Table 7** Log-likelihood comparisons and likelihood ratio test results for GDF-15 and NT-proBNP in predicting echocardiographic outcomes at visits 1 and visit 4

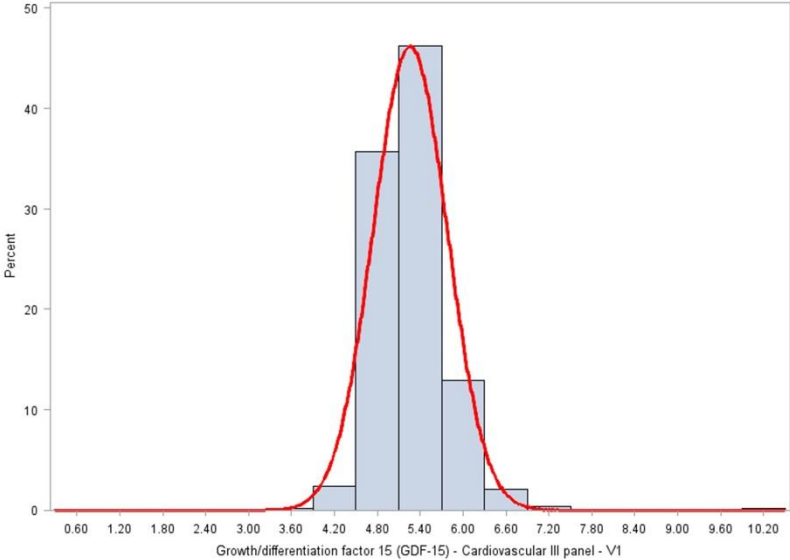
| <b>Visit 1</b> |   |                          |  |                             |   |
|----------------|---|--------------------------|--|-----------------------------|---|
|                | Log-likelihood<br>GDF15 & NT-<br>proBNP | Log-likelihood<br>GDF-15 | p-value<br>(GDF-15 vs. GDF-<br>15 & NT-proBNP) | Log-likelihood<br>NT-proBNP | p-value<br>(NT-proBNP vs. GDF-<br>15 & NT-proBNP) |
| LVMi           | -3625.2                                 | -3625.6                  | 0.34   | -3625.9                     | 0.23  |
| LAVi           | -2857.4                                 | -2858.0                  | 0.29   | -2857.4                     | 0.88  |
| LV-GLS         | -1949.1                                 | -1949.1                  | 0.90   | -1950.1                     | 0.17  |
| E' lateral     | -1806.8                                 | -1809.6                  | <b>0.018</b>                                   | -1806.9                     | 0.81  |
| E' septal      | -2037.6                                 | -2037.7                  | 0.72   | -2039.3                     | 0.06  |
| E' mean        | -1791.7                                 | -1792.7                  | 0.15   | -1792.7                     | 0.16  |
| E/e' ratio     | -1678.9                                 | -1678.9                  | 0.75   | -1682.2                     | <b>0.011</b>                                      |
| E wave         | 490.6                                   | 490.5                    | 0.13   | 491.6                       | 0.15  |
| E/A ratio      | -160.8                                  | -167.9                   | <b>&lt;0.0001</b>                              | -161.0                      | 0.48  |
| LVESVi         | -2728.1                                 | -2728.1                  | 0.97   | -2728.2                     | 0.65  |
| LVEDVi         | -3287.1                                 | -3288.1                  | 0.18   | -3290.1                     | <b>0.015</b>                                      |
| LVEF           | -2733.7                                 | -2734.4                  | 0.24   | -2735.2                     | 0.08  |
| <b>Visit 4</b> |   |                          |  |                             |   |
| LVMi           | -6955.9                                 | -6967.1                  | <b>&lt;0.0001</b>                              | -6958.7                     | <b>0.017</b>                                      |
| LAVi           | -5463.5                                 | -5507.5                  | <b>&lt;0.0001</b>                              | -5463.5                     | 0.94  |
| LV-GLS         | -3753.7                                 | -3759.5                  | <b>0.001</b>                                   | -3755.9                     | <b>0.032</b>                                      |
| E' lateral     | -3602.9                                 | -3615.7                  | <b>&lt;0.0001</b>                              | -3603.3                     | 0.35  |
| E' septal      | -4114.8                                 | -4117.7                  | <b>0.016</b>                                   | -4114.8                     | 0.87  |
| E' mean        | -3551.7                                 | -3560.2                  | <b>&lt;0.0001</b>                              | -3551.8                     | 0.61  |
| E/e' ratio     | -3089.6                                 | -3094.0                  | <b>0.003</b>                                   | -3092.5                     | <b>0.015</b>                                      |
| E wave         | 912.7                                   | 891.7                    | <b>&lt;0.0001</b>                              | 910.5                       | <b>0.036</b>                                      |
| E/A ratio      | -463.5                                  | -485.7                   | <b>&lt;0.0001</b>                              | -464.3                      | 0.22  |
| LVESVi         | -5227.0                                 | -5243.9                  | <b>&lt;0.0001</b>                              | -5230.5                     | <b>0.008</b>                                      |
| LVEDVi         | -6372.9                                 | -6386.8                  | <b>&lt;0.0001</b>                              | -6381.9                     | <b>&lt;0.0001</b>                                 |
| LVEF           | -5362.5                                 | -5366.4                  | <b>0.005</b>                                   | -5362.5                     | 0.85  |

**Models adjusted for** age, sex, body mass index, cholesterol, estimated glomerular filtration rate, smoking status, systolic blood pressure, and diabetes (V4 only).

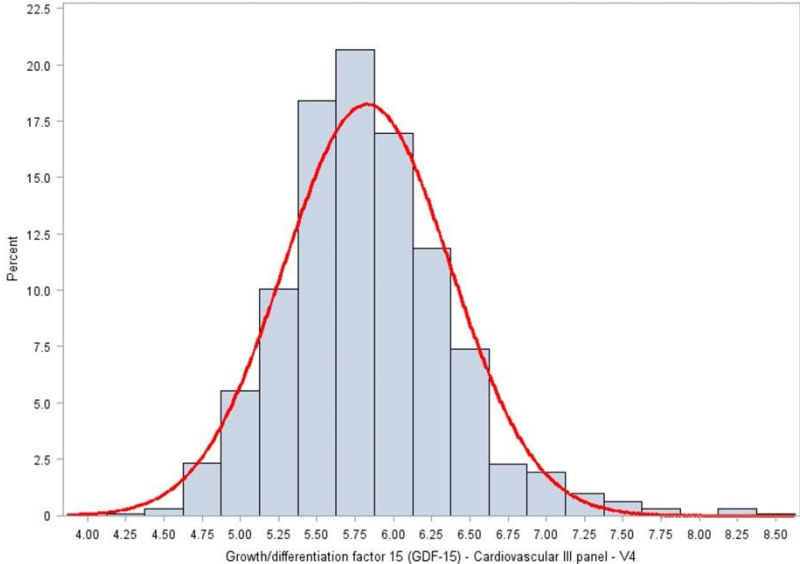
**Abbreviations:** GDF-15, growth differentiation factor-15; LAVi, left atrial volume index; LVEDVi, left ventricular end-diastolic volume index; LVESVi, left ventricular end-systolic volume index; LVEF, left ventricular ejection fraction; LV-GLS, left ventricular global longitudinal strain; LVMi, left ventricular mass index; NT-proBNP, N-terminal pro-B-type natriuretic peptide

Supplemental Figure 1

**A** **Visit 1**

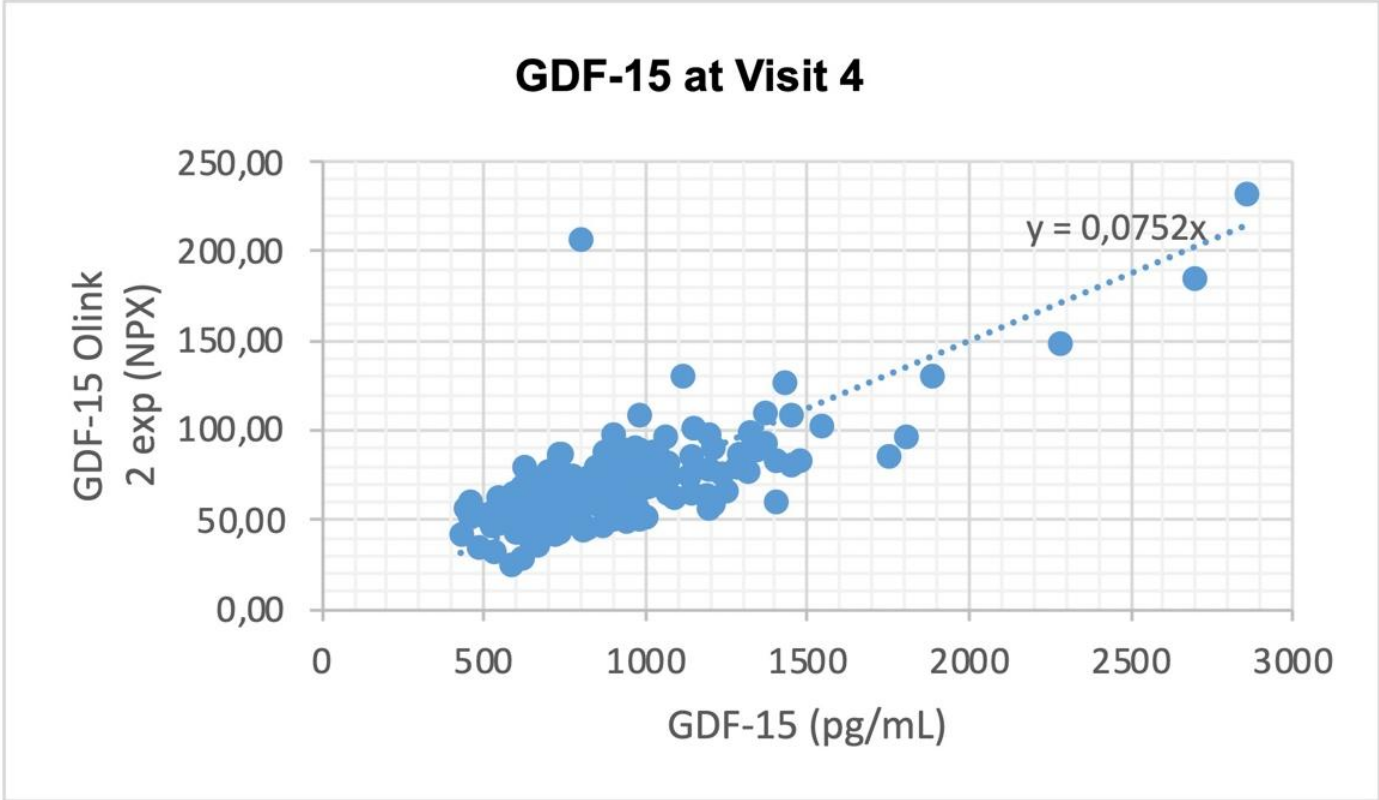


**B** **Visit 4**





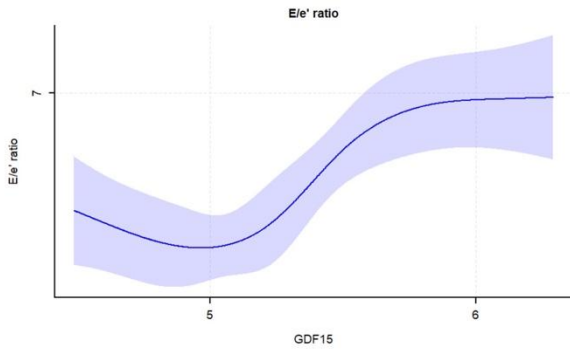
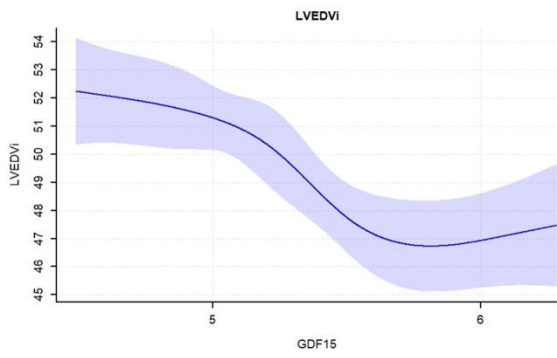
Supplemental Figure 2



### Supplemental Figure 3

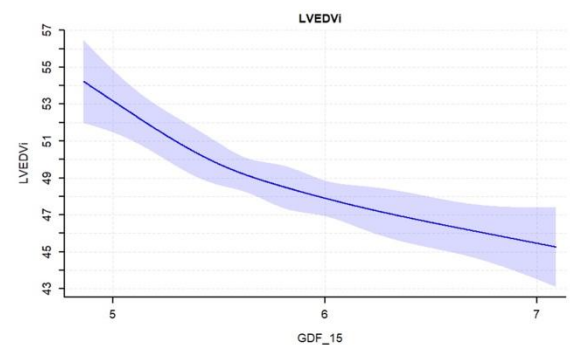
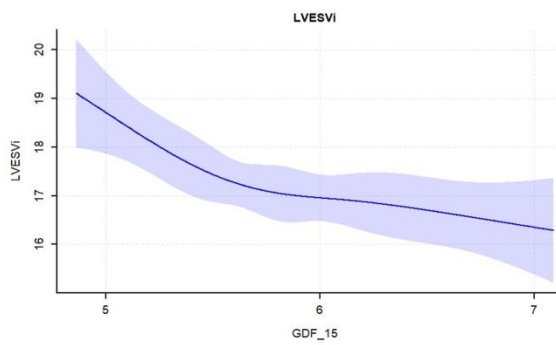
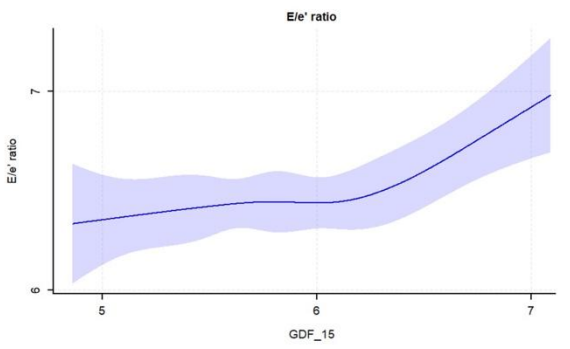
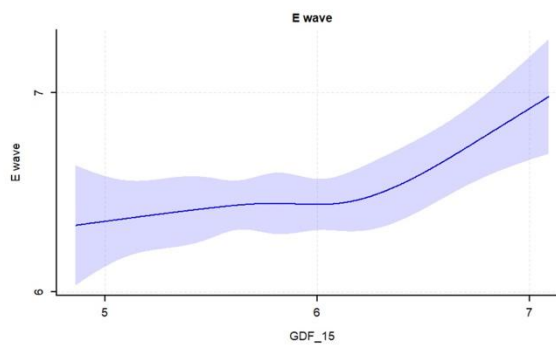
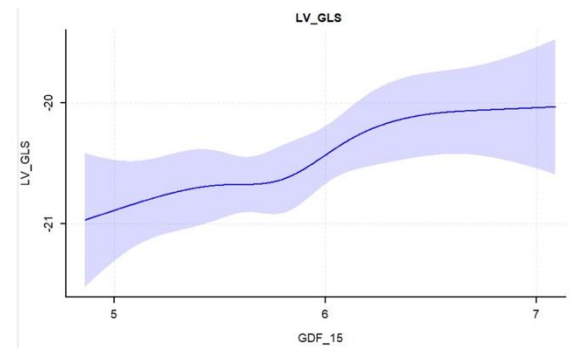
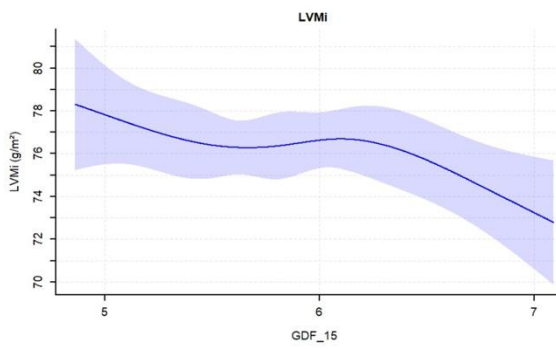
**A**

**Visit 1**



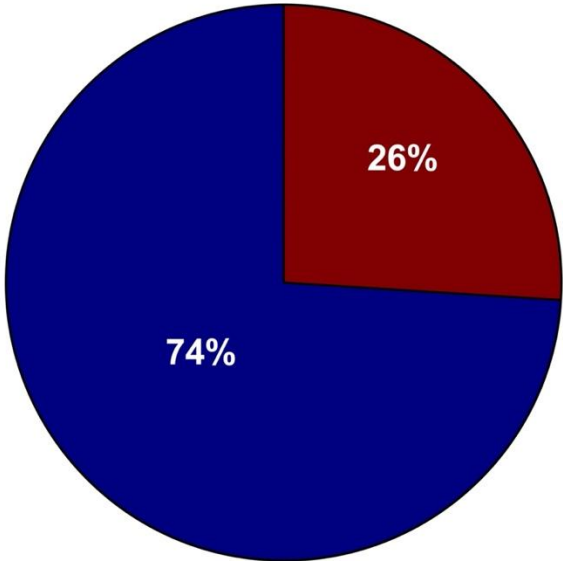
**B**

**Visit 4**



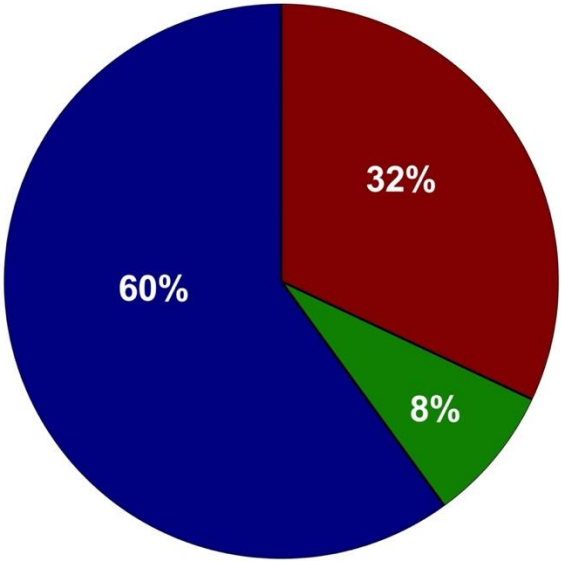
Supplemental Figure 4

GDF-15 at V1



GDF-15 at V4

- Heritability
- Family effect
- Residual effect



Supplemental Figure 5

