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Multi-omics analysis provides novel pathways related to progression of heart failure

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96 **Key Words**

97 Heart failure; machine learning; systems biology; omics

98

99 **Abstract**

100 *Background*

101 Despite major advances in pharmacological treatment for patients with heart
102 failure, residual mortality remains high. This suggests that important pathways
103 are not yet targeted by current heart failure therapies.

104 *Objective*

105 Integration of genetic, transcriptomic and proteomic data in a large cohort of
106 patients with heart failure to detect major pathways related to progression of
107 heart failure leading to death.

108 *Methods*

109 We used machine learning methodology based on stacked generalization framework
110 and gradient boosting algorithms, using 54 clinical phenotype, 403 circulating plasma
111 proteins, 36,046 transcript expression levels in whole blood, and 6 million genomic
112 markers to model all-cause mortality 2,516 patients with heart-failure, from the
113 Systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure study.
114 Results were validated in an independent cohort of 1,738 patients.

115 *Results*

116 Mean age of the patients was 70 (25th-75th percentiles 61-78), 27% was female,
117 median NT-proBNP was 4275 (2360-8486) ng/L and 7% had heart failure with
118 preserved ejection fraction. During a median follow-up of 21 months, 657 (26%) of
119 patients died. The four major pathways with a significant association to all-cause
120 mortality were: 1) the PI3K/AKT (phosphoinositide 3-kinase) pathway; 2) the
121 Mitogen-Activated Protein Kinase Pathway 3) the Ras signaling pathway and 4)
122 EGFR tyrosine kinase inhibitor resistance. Results were validated in an independent
123 cohort of 1,738 patients.

124 *Conclusions*

125 A systems biology approach integrating genomic, transcriptomic and proteomic data
126 identified four major pathways related to mortality. These pathways are related to
127 decreased activation of the cardioprotective ERBB2 receptor which can be modified
128 by neuregulin.

129

130

131 **Condense Abstract**

132 Pathways involved in the progression of heart failure are still poorly understood. In this
133 study we identified four major pathways related to mortality combining multiple large
134 omics-datasets using sophisticated machine learning methodology. We were able to
135 validate these results in a separate cohort. These pathways are related to decreased
136 activation of the cardioprotective ERBB2 receptor which can be modified by
137 neuregulin.

138

139 **Abbreviations**

140

141 ERBB2: Erb-B2 Receptor Tyrosine Kinase 2

142 PI3K: phosphoinositide 3-kinase-protein

143 AKT: kinase B

144 MAPK: Mitogen-Activated Protein Kinase

145 Ras: rat sarcoma

146 EGFR: Epidermal growth factor receptor

147 ACE: Angiotensin converting enzyme

148 ARB: angiotensin receptor blockers

149 HFrEF: Patients with heart failure and reduced ejection fraction

150 HFpEF: Patients with heart failure and reduced ejection fraction

151

152 **Introduction**

153

154 Despite all recent advances in pharmacological treatment of heart failure,
155 residual mortality remains high. This suggest that there are still important
156 pathways not adequately targeted by current guideline therapies¹.

157 Capturing these pathways requires integration of genetic, transcriptomic,
158 proteomics and phenotypic markers, using a systems biology *-omics* approach²..

159 With recent advancements in bioinformatics³, and high-throughput *-omics* data;
160 integration and subsequent interpretation of multiple high-dimensional *-omic*
161 datasets is becoming increasingly feasible tools to unrevealing novel biological
162 insights. Previous efforts revealed putative markers related to pathological lipid
163 abundance⁴ and cancer⁵.

164 Such a deep analysis of heart failure requires an enormous repository of data with
165 robust and reproducible observations that can also be validated in an
166 independent population⁶. BIOSTAT-CHF has already generated multiple new
167 insights partially unraveling processes on single *-omics* data⁷⁻¹⁶. However,
168 integration of multiple *-omics* datasets in BIOSTAT-CHF has never been done
169 before.

170 Here, we present a multi-omics approach integrating phenotypic, proteomic,
171 transcriptomic and genetic datasets using advance machine learning. The primary
172 aim of this study is to identify, validate and understand disease pathways that are
173 associated with progression of heart failure leading to early mortality.

174 **Methods**

175 *Patient population and study design*

176 The Systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure
177 (BIOSTAT-CHF) was designed to identify pathophysiological pathways related to
178 heart failure progression using a systems biology approach on multi-omics data. The
179 design and baseline characteristics of this study have been previously reported¹⁷.
180 Briefly, BIOSTAT-CHF consists of two independent (index and validation) cohorts.
181 Inclusion criteria were similar in both cohorts. The index cohort consisted of 2,516
182 patients with worsening signs and/or symptoms of heart failure, included from 69
183 centers in 11 European countries during 2010–2014. The validation cohort consisted
184 of a comparable cohort of 1,738 patients from six centers in Scotland, UK. Patients
185 were enrolled as in- or out-patient, with a median follow-up in each cohort of 21 (25th
186 and 75th percentiles 15-27] months. The endpoint of interest for the present study
187 was 1-year all-cause mortality. Patient characteristics of both cohorts are presented
188 in supplementary material¹⁷.

189 The study complied with the Declaration of Helsinki and was approved by the
190 participating centres' medical ethics committees. All patients provided written
191 informed consent.

192 BIOSTAT-CHF has generated a large repository of clinical phenotypic data and
193 biological data over the years. Biological data consisted of high-dimensional
194 proteomic, transcriptomic and genetic data.

195

196 *Sample handling*

197 Samples were collected and centrifuged and stored at preferably -80 °C or -70 °C,
198 else -20 °C for a maximum of one month. Shipments from the site to the central
199 biobank were done periodically.

200 Blood was drawn by venapuncture.

201 1) Plastic vacutainer tubes were used to collect approximately 83.5 ml blood.

202 2) Following samples were collected:

203 a. EDTA plasma, 5 6 samples of 10 ml content each,

204 b. Serum with gel, 1 2 sample of 8.5 ml content,

205 c. PAXgene™ Blood RNA tubes, 2 samples of x approximately 2.5 ml content each

206 Details on the entire process of sample handling is described in the Laboratory

207 Handbook in the supplementary data.

208

209 *Phenotypic (clinical) panel*

210 We collected 54 clinical markers in BIOSAT-CHF (supplementary data: *Phenotypic*

211 *parameters*). Phenotypic data consisted of demographic data (e.g., age, sex, medical

212 history, and co-morbidities) and data derived during physical examination (e.g., body

213 mass index, systolic and diastolic blood pressure, and left ventricular ejection fraction

214 [LVEF]). The estimated glomerular filtration rate (eGFR) is calculated by the CKD-

215 EPI formula¹⁸. Clinical data were coordinated by the Trial Coordination Center (TCC),

216 a contract research organization affiliated with the UMCG Groningen. Local

217 investigator collected clinical data and was monitored by TCC. Data was

218 electronically stored in a centralized database at the UMCG.

219

220 *Protein panel from peripheral blood: performed by Olink and locally at center*

221 *laboratories*

222

223 We measured 403 serum/plasma biomarkers (supplementary data: *Protein listings*)
224 from several pathophysiological domains, including markers of inflammation,
225 apoptosis, remodelling, myocyte stress/injury, angiogenesis, endothelial function,
226 and several markers of renal function. The protein biomarker data used for this
227 study have been described in recent papers⁷⁻⁹. In brief, the biomarkers included
228 standard biochemical blood parameters (e.g., hemoglobin, hematocrit, blood urea
229 nitrogen, and heart failure-related markers [NT-proBNP and BNP]). In addition, four
230 biomarker panels comprising each of 92 protein biomarkers provided by the Olink
231 Bioscience analysis service (Uppsala, Sweden) were measured. These respective
232 panels were Cardiovascular II (CVD II), CVD III, Immune response, and Oncology II
233 panels (<https://www.olink.com>). The proteins were profiled using Olink Proseek®
234 Multiplex Inflammatory 96x96 platform. The Proseek® kit uses proximity extension
235 assay technology, whereby oligonucleotide-labelled antibody probe pairs bind to their
236 respective targets. Quantification was achieved using a Fluidigm BioMark™ real-time
237 PCR platform. The platform provides normalized protein expression (NPX, log2-
238 normalised), rather than an absolute quantification.

239

240 *Transcriptomic panel: performed by University of Leicester*

241 Whole blood transcriptomic profiles from 944 patients (626 survivors and 318 non-
242 survivors who died from cardiovascular causes) from the index cohort were obtained
243 using the GeneChip® Human Transcriptomic Array® 2.0 (HTA 2.0) developed by
244 Affymetrix, Inc. (part of Thermo Fisher Scientific). Patients were age- and sex-
245 matched. Details on the protocols and methodology used to assess and confirm the
246 quality of the raw transcriptomic data, the processes used to integrate signals from

247 individual probes on the array to determine the expression levels of each gene and to
248 assess the quality of the summarized RNA expression set data were previously
249 published¹⁹. In total, 36,046 (17,924 protein-coding and 18,122 non-protein-coding)
250 transcripts were analyzed.

251

252 *Genomic panel: performed by University of Leicester.*

253 Both cohorts were processed, genotyped, quality controlled and imputed
254 independently, using identical protocols¹². Genotyping of all patients was performed
255 using the Affymetrix Axiom Genome-Wide UKB WCSG genotyping array. Sample
256 level QC was performed for X chromosome homozygosity (sex mismatch) and
257 identity by descent estimates (relatedness and duplicates). Before imputation,
258 variants were removed if their call rate was <95% for variants with minor allele
259 frequency (MAF) $\geq 5\%$, or <99% with MAF <5%, or a Hardy-Weinberg equilibrium
260 $P < 1 \times 10^{-6}$. Imputation was performed using SHAPEIT2²⁰ and IMPUTE2²¹ with the
261 phase 3 release 1000G reference panel²².

262

263 **Statistical Analyses**

264 We used machine learning methods, particularly gradient boosting (with tailored loss
265 functions), with stacked regularization²³ to model all-cause mortality. This method
266 combines multiple -omics data in a non-linear manner by learning how to combine
267 predictions given by models trained on the individual -omics sets into a single
268 coherent output.

269 It is specifically designed, in contrast to standard modern statistical methods
270 (Supplementary data: Benefits of Machine Learning for Multi-omics Analysis), not
271 only to handle high-dimensional -omics data, where the number of patients is

272 significantly smaller than the number of variables ($n \ll p$), but also when different data
273 sources are collectively used to estimate the 'core mechanism' present in all data
274 sources.

275 In brief, we used a combination of stacking generalization framework with multiple
276 gradient boosting classifiers to improve prediction accuracy. For each *-omics* set, we
277 built a 'level-0' model. These level-0 models were subsequently combined to form the
278 level-1 model²³. This allows us to use all data available in each panel (e.g.,
279 phenotype data from all 2,516 patients is used to create the level-0 model of the
280 phenotype panel and the level-0 transcriptomic model was estimated using 944
281 patients). A figure visually summarizing this approach can be seen in the central
282 illustration.

283 One of the challenges in machine learning is tuning the various models'
284 hyperparameters. Typically, each model is optimized separately, leading to a local
285 optimum. We optimize all the models simultaneously using Bayesian Optimization²⁴,
286 achieving a global optimum. To avoid over-fitting, we used stratified cross-validation
287 over the training partition²⁵.

288 To ensure the feature signatures' reliability and robustness, we conducted stability
289 selection²⁶. The complete analysis was repeated 50 times. Receiver- Operating-
290 Characteristics Area-Under-Curves (ROC-AUC) were computed each time and
291 averaged over the repeated analyses in both the index and validation cohort. A
292 permutation (randomization test)²⁷ was used to evaluate the results' statistical
293 validity.

294 We evaluated the model's quality separately in the validation cohort. In the validation
295 cohort transcriptomic data was not measured and did not include the corresponding

296 level-0 model. Nevertheless, our approach can validate the results of the other
297 phenotype, protein, and genomic panels.

298

299 *Pathway enrichment*

300 To identify novel pathways related to mortality, we performed an over-representation
301 analysis. We determined the effect of each variant using Ensembl Variant Effect
302 Predictor²⁸ and converted all selected markers to Ensembl IDs.

303

304 We performed enrichment using Gprofiler for Gene Ontology, KEGG, Reactome,
305 CORUM and WikiPathways pathways. We report corrected *P*-value <0.05 as
306 significant.

307 To test statistical differences between patients with and without activated pathways
308 and their association to uptitration we utilized principal component analysis, using
309 missMDA^{29, 30}, to reduce the dimensionality of the biomarkers present in the
310 pathways, in a similar manner as previous published¹⁰. A weighted score (first
311 principal component) was generated to which each selected biomarker contributed to
312 a greater or lesser extent, based on how much population variance they explain.
313 First, we used the weighted score to identify associated clinical characteristics in a
314 penalized linear regression³¹. Second, we performed a multivariable regression
315 analyses including percentage achieved target doses of Angiotensin converting
316 enzyme (ACE)/angiotensin receptor blockers (ARB), beta-blockers and MRA¹, and
317 age and sex. For this analysis we only included HFREF patients. Last, we associated
318 activation of pathways to all-cause mortality, using cox proportional hazards
319 modeling.

320

321 Data are presented as means \pm standard deviations (SD) when normally distributed,
322 as medians (interquartile range) for skewed variables, and as frequencies
323 (percentage) for categorical variables. Differences between patients who died and
324 those who did not in the index and validation cohort were tested using the Students'
325 independent t-test or Mann-Whitney U test, where appropriate, for continuous
326 parameters. Differences in categorical variables were tested with Chi-Squared tests.
327 We used Python v. 3.8 (www.python.org), with packages Numpy, Scipy, and Scikits-
328 learn for implementing the stacking model and R (version 4.0, R Foundation for
329 Statistical Computing, Vienna, Austria, www.r-project.org) for visualizations.
330

331 **Results**

332 *Clinical characteristics*

333 Data was available for 2,516 patients in the phenotypic and protein panels, 944 in the
334 transcriptomic and 2,470 in the genomic panels in the index cohort (Figure S1A). The
335 validation cohort had data available for 1,738 patients in the phenotypic and protein
336 panels and for 1,693 in the genomic panel (Figure S1B).

337 During a median follow-up of 21 (25th and 75th percentiles 11–32) and 21 (25th and
338 75th percentiles 15–27) months, 657 (26%) and 501 (32%) patients died in the index
339 and validation cohorts, respectively. Baseline characteristics of the patients who died
340 and those who survived in the index and validation cohorts are presented in Table 1.
341 Patients who died in the index cohort were older (73 ± 11 vs 68 ± 12 years; $p<0.001$),
342 had a higher NYHA class (NYHA class III/IV 74% vs 58%; $p<0.001$), and more
343 comorbidities. These differences were similar in the validation cohort (Table 1).

344

345 *Multi-omics mortality model*

346 Our final risk prediction model, combining phenotypic, proteomic, transcriptomic and
347 genomic data achieved a significant ROC-AUC value of 0.81 ± 0.02 in the stratified
348 cross-validated part of the index cohort and 0.85 ± 0.03 in the validation cohort (Figure
349 S2), both $p<0.001$ in permutation tests (Supplementary Figure S3A).

350 This optimal model consisted of 60 markers per panel with a total of 240 markers, all
351 associated to mortality and closely related to each other (Table S1; Figure S3B). The
352 relative importance of the top-15 markers for each dataset and in the level-1 model is
353 visualized in Figure 1. The direction of the association between each marker and
354 mortality is presented in the spider plot of Supplementary Figure S4.

355

356 *Enrichment*

357 We performed overrepresentation analysis, using 180 (60 proteomic, 60
358 transcriptomic and 60 genomic; Suppl Table S1) selected markers from our level-1
359 model set against all the markers in our dataset. We found that there were 177
360 pathways significantly overrepresented (Figure S5A; Suppl Table S2) These
361 pathways were associated with immunological processes (e.g. immune system
362 process [GO and Reactome], inflammatory response [GO]), involved various cell
363 surface receptor signaling pathways (e.g. cell surface receptor signaling pathway
364 [GO:BP], cytokine-cytokine receptor interaction [KEGG]). Among the most significant
365 pathways were the closely related phosphatidylinositol 3-kinase (PI3K)/protein kinase
366 B(Akt) signaling pathway, Mitogen-activated protein kinase (MAPK) Akt signaling
367 pathway, Ras signaling pathway, and EGFR tyrosine kinase inhibitor resistance.
368 (Suppl Table S1). **Suppl. Figure S5B** visualizes the close relation between the
369 markers (n=18) and their connection to the pathways.

370 Although not directly connected in the pathways, markers from the cytokine-cytokine
371 receptor interaction pathways are also involved. IL1RL1 is involved in negative
372 regulation of the PI3K/AKT network and TNFRSF6B in turn is upregulated by
373 PI3K/AKT-dependent pathway. The strongest of these connecting markers were
374 GDF15-ERBB2 and VEGFR2-S1PR1-ERK1/2-PKC-alpha complexes These
375 complexes are located at the intersection of PI3K-Akt, MAPK and EGFR tyrosine
376 kinase pathways (suppl Figure S5C).

377 Clinical parameters associated with activation of the selected pathways are
378 presented in Table 2A. These characteristics are comparable to the features selected
379 in the phenotype panel of our level-1 model. History of renal disease and peripheral
380 oedema are most significant related to the pathways, but diabetes also plays a role.

381 Based on these clinical parameters, we could reasonably predict which patients
382 would have activation of pathways (C-statistic 0.75 (95% confidence interval [CI]
383 0.74-0.77). Pathways are significantly less activated in patients with HFrEF
384 compared to patients with higher ejection fractions ($p < 0.0001$). Please note that the
385 measurement of pathway activation is performed at baseline and BEFORE up-
386 titration. Mean ACE-inhibitor dose of the group of patients with activated pathways
387 ($n=892$) was 42.8 (± 37.6)% of the target dose, compared to 55.5 (± 38)% in the
388 patients who did not have activated pathways ($n=1208$). Patients with activated
389 pathways had higher risk of dying, with hazard rate of 2.67 (95%CI 2.25-3.16),
390 respectively, all $p < 0.0001$ and age sex corrected.

391 **Discussion**

392 This systems biology multi-omics approach integrating genomic, transcriptomic and
393 proteomic data identified 4 major pathways in the progression of heart failure: PI3K-
394 Akt, MAPK, Ras signaling pathways, and EGFR tyrosine kinase inhibitor resistance.
395 These pathways were identified using 18 of the 180 significant markers in our model
396 spread over all biomarker panels. Activation of these signalling pathways were
397 strongly and independently associated with higher mortality.

398 Interestingly, these pathways are known to be strongly related to each other, the
399 Ras/Raf/MAPK pathway cascade is able to stimulate angiogenesis through changes
400 in expression of genes directly involved in the formation of new blood vessels^{32, 33}.

401 Signaling through the Ras/Raf/MAPK also regulates a variety of cellular functions
402 that are important for tumorigenesis. Ras also interacts with PI3K/AKT and EGFR
403 tyrosine kinase inhibitor pathways^{34, 35}.

404 MAPK is activated in response to a wide variety of extracellular stimuli and induces
405 changes in critical intracellular processes promoting cell growth, apoptosis and
406 transformation. It can transduce multiple extracellular signals through various
407 receptors, such as hypertrophic signals mediated by G protein-coupled receptors,
408 transforming growth factor- β signals mediated by receptor serine/threonine kinases,
409 and insulin-like growth factor-I (IGF-1) signals mediated by receptor tyrosine kinase³⁶
410 PI3K/AKT is important in mediating signals of cell growth and proliferation. It plays an
411 important role in regulating cardiac growth, myocardial angiogenesis, glucose
412 metabolism, and cell death in cardiac myocyte^{37, 38}.

413 EGFR tyrosine kinase inhibitor resistance pathway is important in the treatment of
414 various cancers. These treatments report a high risk of cardiotoxicity. Although a
415 mechanistic explanation for the cardiotoxicity of EGFR tyrosine kinase inhibitor is not

416 fully understood, disruption of ErbB family receptors impairs downstream signaling to
417 Ras-ERK and PI3-kinase-Akt pathways and normal cardiac myocyte stress
418 response^{39, 40}. The association between these pathways and cardiotoxicity became
419 apparent after the presentation of phase 3 trials on the use of trastuzimab (herceptin)
420 in patients with breast cancer. Unexpectedly, an increased risk of the development of
421 congestive heart failure was observed in patients treated with trastuzumab^{41, 42}. This
422 finding was explained by the ERBB signaling pathway. Activation of the ERBB 2 and
423 4 receptors leads to downstream activation of the phosphatidylinositol 3-kinase–AKT
424 (PI3K/AKT) and mitogen-activated protein kinase (MAPK) pathways⁴³, which on the
425 one hand promotes proliferation of tumor cells, but on the other hand promotes
426 cardiomyocyte survival. These mechanisms are supported by data from cardiac
427 myocyte–specific ERBB2– and ERBB4– conditional knockout mice, who developed a
428 cardiomyopathy by 8 to 12 weeks of life⁴⁴. In other words, stimulation of the ERBB-2
429 and -4 receptors seem to exert cardioprotective effects through activation of the PI3K
430 and MAPK pathways⁴⁵. Importantly, the ERBB receptors can be stimulated by
431 neuregulin and parenteral administration of recombinant human neuregulin-1 to
432 patients with stable chronic heart failure resulted in an increase in stroke volume and
433 cardiac output^{46–49}.

434

435 **Future perspectives and limitations**

436 With the present study, we showed the role and opportunities of systems biology in
437 unravelling underlying pathology of complex diseases which is attracting increasing
438 attention in the field of in cardiology^{50–53}. However, as far as we know, there has not
439 yet been a study using this advanced methodology in such a data-rich cohort with the
440 ability of validating the results⁵⁴.

441 This comprehensive picture of markers involved in the pathophysiological disease
442 processes underlying all-cause mortality already yielded a potential future therapeutic
443 intervention target, that is currently in the early phase of development for the
444 treatment of patients with heart failure and a reduced ejection fraction. Literature also
445 suggests that neuregulins might also be beneficial in patients with preserved ejection
446 fraction and other cardiovascular diseases^{55, 56}.

447 The transcriptomic panel consisted of 944 patients selected from the index cohort
448 and matched on age and sex¹⁹. This is an extensive transcriptomic dataset, but
449 unfortunately, data was measured in a pre-selected group of patients from the index
450 cohort and none from the validation cohort. The selection of patients was not random
451 and skewed towards cardiovascular mortality. Transcriptomic markers are therefore
452 better suited to predict cardiovascular mortality. This might explain the lower
453 contribution of the markers from the transcriptomic panel in our combined systems
454 biology model. The absence of this panel had no impact on the (level-0) model
455 development and validation of the other panels because our methods are able to
456 handle changes in data-sources. Also, despite the rigorous selection process, the
457 effects of patient selection cannot be determined.

458 Unfortunately, because of the nature of this study, we are not able to draw causal
459 conclusions on the pathways we found. However, it is apparent that when developing
460 the models for predicting mortality so many markers from all panels are
461 independently selected that are associated with outcome.

462 BIOSTAT-CHF was carried out between 2010 and 2015 and treatments were based
463 on the guidelines that were applicable at that particular time⁵⁷. They did not include
464 treatment with sacubitril/valsartan and SGLT-2 inhibitors. Therefore, theoretically,
465 additional use of sacubitril-valsartan and SGLT2-inhibitors might have yielded

466 different results. Although there is limited data on the interaction between these
467 treatments and the activated pathways⁵⁸⁻⁶¹. It is unknown to what extent our results
468 would have been different.

469 Due to the relatively low percentage of patients in the index cohort (7%) and the
470 validation cohort (34%), we were not able to discriminate between HFpEF and HFrEF
471 ¹⁷.

472

473 **Clinical Implications**

474 Treatment of heart failure with reduced ejection fraction has tremendously improved
475 over the past decades. With the current therapies, life expectancy for a 70-year old
476 patient with HFrEF has increased by 5 years⁶². Nevertheless, residual mortality
477 remains high, even in well-treated patients. This implies that our current therapies do
478 not adequately target all disease pathways that are related to its progression. The
479 present study identified pathways that remained to be activated in patients with
480 HFrEF despite treatment with ACE-inhibitors, angiotensin receptor blockers,
481 mineralocorticoid receptor antagonists and beta-blockers. Although the current
482 analysis does not show causality, these data might stimulate to identify potential
483 novel treatments for HFrEF, targeting the pathways that were identified in the present
484 study. This might further improve outcomes of patients who remained to have a high
485 risk of early mortality.

486

487 **Conclusions**

488 Integrating –genomic, transcriptomic, proteomic, and clinical data from a large cohort
489 of patients with heart failure, identified pathways related to progression of heart
490 failure leading to early mortality. The strongest pathways were related to the ERBB

491 receptors and their downstream effects on the PI3K and MAPK pathways leading to
492 cardioprotective effects. Neuregulin, a ligand of the ERBB receptors is currently in
493 the early phase of clinical development in patients with heart failure and a reduced
494 ejection fraction.

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496

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655 **AUTHOR CONTRIBUTIONS**

656 All authors met all four ICMJE criteria for authorship, gave final approval of the
657 version to be published and agree to be accountable for all aspects of the work in
658 ensuring that questions related to the accuracy or integrity of any part of the work are
659 appropriately investigated and resolved.

660 Specifically, regarding the following aspects, specific authors substantially
661 contributed to: Conception/design: WO, JPBP, TM, EL and AAV.

662 Acquisition of data: SDA, JGC, KD, GF, FZ, CCL, LLN, MM, NJS, PP, RAdB, DJvV,
663 and AAV. Analysis of data: WO, JPBP, TM and EL.

664 Interpretation of data: WO, JPBP, TM, JEE, SMF, IES, JTr, ALK, CPN, MN, SPRR,
665 JTi, EL and AAV.

666 Drafting the work: WO, JPBP, TM, JEE, SMF, IES, JTr, EL and AAV.

667 Revising the work critically for important intellectual content: WO, JPBP, TM, JEE,
668 SMF, IES, JTr, ALK, CPN, MN, SPRR, JTi, SDA, JGC, KD, GF, FZ, CCL, MM, NJS,
669 PP, RAdB., DJvV., LLN, NvR, MN, AKG, ES, AHZ, CSL, EL and AAV.

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Tables and Figures

674 **Table 1 – Baseline demographics index and validation cohort**

	Index			Validation		
	Alive	Died	p-value	Alive	Died	p-value
N	1859 (74%)	657 (26%)		1214 (75%)	401 (25%)	
Age (years)	68 (11.9)	73 (11.2)	<0.0001	73 (10.5)	78 (9.7)	<0.0001
Sex (Males)	1370 (74%)	476 (72%)	0.57	801 (66%)	270 (67%)	0.66
LVEF (%)	31 (9.8)	32 (12.5)	0.03	41 (13)	41 (13.3)	0.63
BMI (kg/m²)	28 (5.5)	27 (5.5)	0.001	29 (6.4)	28 (6.1)	<0.0001
Ischemic heart disease	946 (51%)	412 (63%)	<0.0001	776 (64%)	286 (71%)	0.008
Heart failure hospitalization in last year	531 (29%)	263 (40%)	<0.0001	301 (25%)	130 (32%)	0.003
Myocardial Infarction	657 (35%)	306 (47%)	<0.0001	575 (48%)	223 (56%)	0.006
DM	577 (31%)	242 (37%)	0.007	367 (30%)	155 (39%)	0.002
COPD	279 (15%)	157 (23.4%)	<0.0001	191 (16)	104 (26%)	<0.0001
History of renal disease	402 (22%)	294 (45%)	<0.0001	491 (41%)	241 (61%)	<0.0001
NYHA I	50 (2.76%)	6 (0.94%)	<0.0001	15 (1)	1 (0)	<0.0001
NYHA II	711 (39.28%)	157 (24.69%)		575 (47)	92 (23)	
NYHA III	853 (47.13%)	375 (58.96%)		516 (43)	200 (50)	
NYHA VI	196 (10.83%)	98 (15.41%)		108 (9)	107 (27)	

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678 **Table 2A – Results of penalized linear regression of pathway activation and**
679 **clinical parameters from the index cohort; B results of multivariable analysis of**
680 **pathway activation on drug up-titration levels, in patients with HFrEF from the**
681 **index cohort**

A	ESTIMATE	STD.ERROR	T.VALUE	P.VALUE
(INTERCEPT)	-4,7563087	1,01691898	-4,6771756	0,00012623
AGE (YEARS)	0,04469118	0,00851974	5,24560508	1,8524E-05
AF	0,50231254	0,1549551	3,24166518	0,00127384
DM	0,50748659	0,16363381	3,10135525	0,0021004
RENAL FAILURE	2,04916681	0,1666139	12,2988945	1,0177E-33
DBP (MMHG)	-0,0258009	0,00614469	-4,1988893	6,3187E-05
PULMONARY CONGESTION	0,11316459	0,08586542	1,31792964	0,1878186
OEDEMA	0,92160151	0,10160816	9,07015225	2,8708E-17
HEPATOMEGALY	0,6255319	0,2211534	2,82849773	0,00496272

3RD HEART TONE	1,08070836	0,24867644	4,34584144	1,4971E-05
NYHA	0,46018285	0,1218538	3,77651632	0,0001757
ORTHOPNEA	0,4279144	0,18211088	2,34974645	0,01983737

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B	ESTIMATE	STD.ERROR	T.VALUE	P.VALUE
(INTERCEPT)	-4,1464678	0,70156016	-5,9103524	4,483E-09
% ACE/ARB TARGET DOSE	-1,5333152	0,29227872	-5,2460721	1,8458E-07
% BB TARGET DOSE	-0,1246111	0,37693053	-0,3305942	0,7410107
% MRA TARGET DOSE	0,62659359	0,34549083	1,81363305	0,06999255
AGE (YEARS)	0,06647023	0,00962646	6,90495126	8,254E-12
SEX (FEMALE)	-0,6968047	0,27129458	-2,5684432	0,0103399

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Figure 1 – The 15 most predictive variables of mortality in each panel

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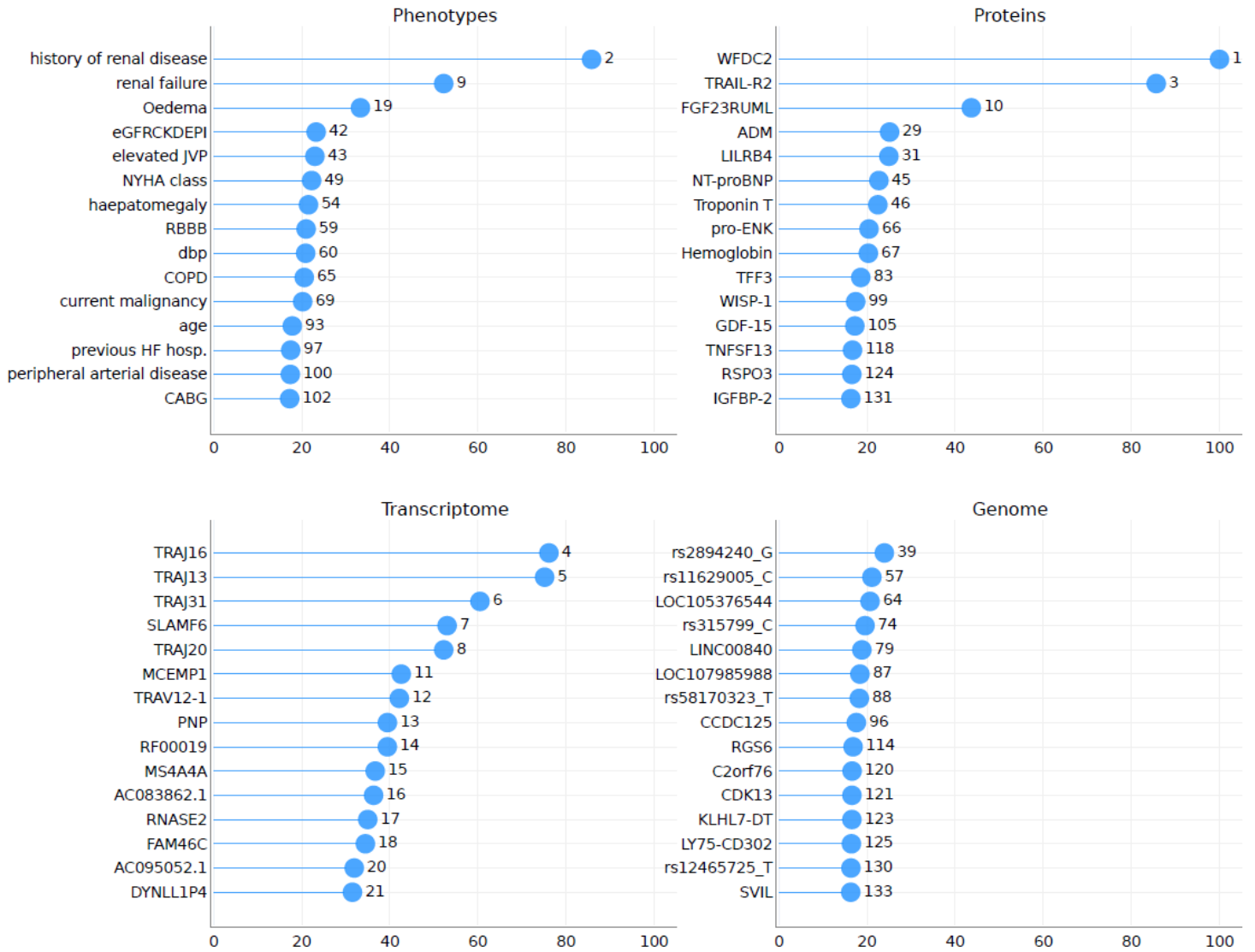
For every panel the top 15 markers are shown in the lollipop plots including their

688

overall ranking. The overall importance ranking was calculated by scaling all relative

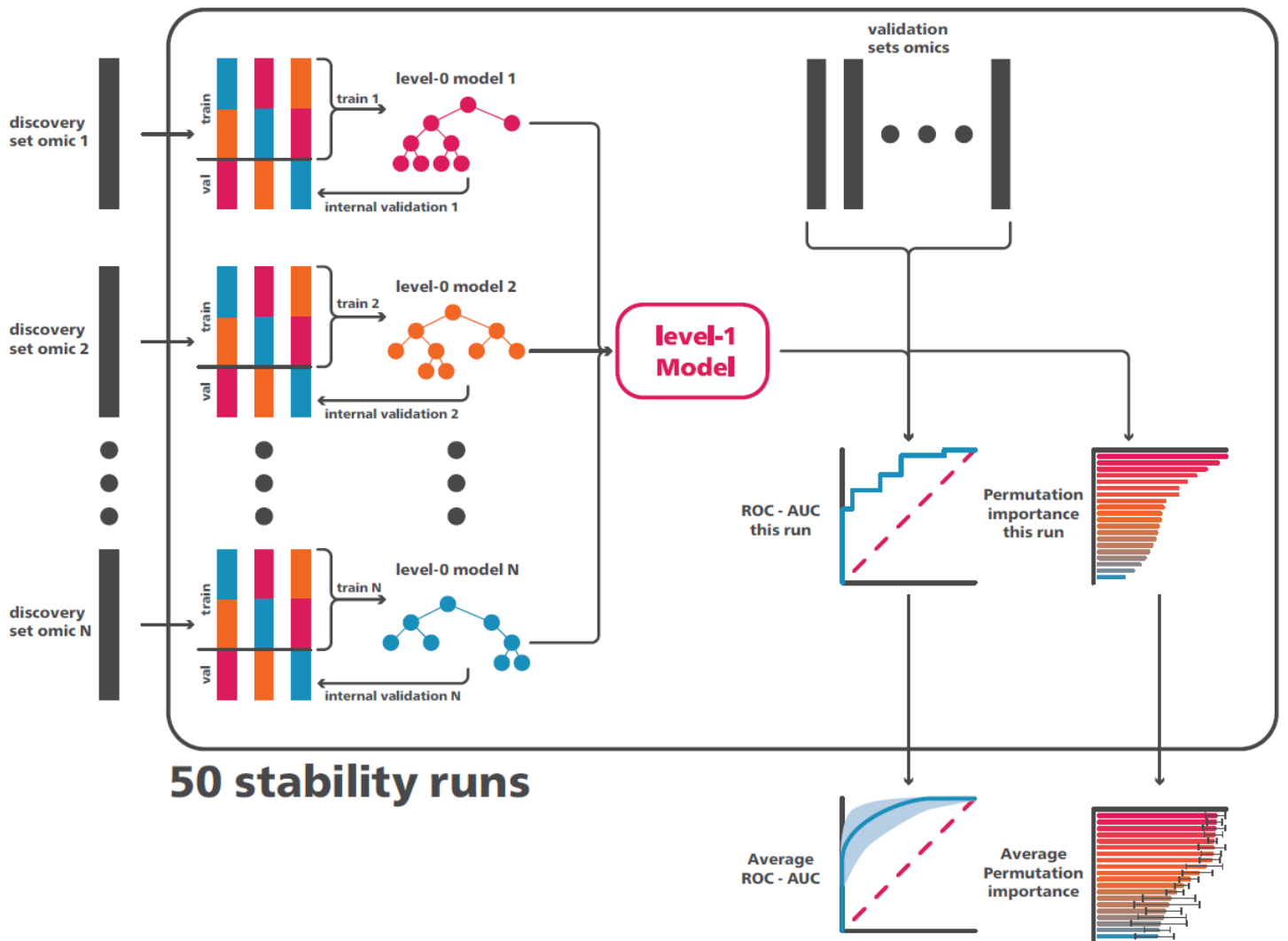
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importance to the importance of each panel.



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692 **Central Illustration**

693 **Machine learning multi-omics approach**

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696 **Clinical Perspectives section**

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698 Treatment of heart failure with reduced ejection fraction has tremendously improved
699 over the past decades. With the current therapies, life expectancy for a 70-year old
700 patient with HFrEF has increased by 5 years⁶². Nevertheless, residual mortality
701 remains high, even in well-treated patients. This implies that our current therapies do
702 not adequately target all disease pathways that are related to progression of disease.
703 The present study identified pathways that remained to be activated in patients with
704 HFrEF despite treatment with ACE-inhibitors, angiotensin receptor blockers,
705 mineralocorticoid receptor antagonists and beta-blockers. Although the current
706 analysis does not show causality, these data might stimulate to identify potential
707 novel treatments for HFrEF, targeting the pathways that were identified in the present
708 study. This might further improve outcomes of patients who remained to have a high
709 risk of early mortality.