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PRECISION MEDICINE IN DISTINCT HEART FAILURE PHENOTYPES: FOCUS ON CLINICAL EPIGENETICS

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Running head: Epigenetics and precision medicine in heart failure

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Abstract

Heart failure (HF) management is challenging due to high clinical heterogeneity of this syndrome~~,~~ which makes patients responding differently to evidence-based standard therapy established by the current reductionist approach. Better understanding of the genetic~~/~~ and epigenetic interactions may clarify molecular signatures underlying maladaptive responses in HF, including metabolic shift, myocardial injury, fibrosis, and mitochondrial dysfunction. DNA methylation, histone modifications and micro-RNA (miRNAs), which may also be major epigenetic players in the pathogenesis of HF. DNA hypermethylation of the kruppel-like factor 15 (*KLF15*) gene plays a key role in switching the failing heart from oxidative to glycolytic metabolism. Moreover, hypomethylation at H3K9 promoter level of atrial natriuretic peptide (*ANP*) and brain natriuretic peptide (*BNP*) genes also lead to reactivation of fetal genes in man. The role of miRNAs has been investigated in HF patients undergoing heart transplantation, for whom miR-10a, miR-155, miR-31, and miR-92 may be putative useful prognostic biomarkers. Recently, higher RNA methylation was observed in ischemic human hearts, opening the era of "epitranscriptome" in the pathogenesis of HF. Currently, hydralazine, statins, apabetalone, and omega-3 polyunsatured fatty acids (PUFA) are being tested in clinical trials to provide epigenetic-driven therapeutic interventions. Moreover, network-oriented analyses could advance current medical practice~~,~~ by focusing on protein-protein interactions (PPIs) perturbing the cardiac interactome. In this review, we provide an epigenetic map of maladaptive responses in HF patients. Furthermore, we propose the "EPi-transgeneratIonal network mOdeling for STratificatiOn of heaRt Morbidity" (EPIKO-STORM), a clinical research strategy offering novel opportunities to stratify the natural history of ~~disease.~~ HF.

Key words: Heart failure, maladaptive responses, epigenetics, transgenerational effect, precision medicine, artificial intelligence.

1. Introduction

Heart failure (HF) is a complex disease resulting from structural or functional dysfunction of ventricular filling or ejection of blood.[1,2] HF arises as the culmination of a wide range of disease processes, including genetic disorders, ischemic disease, metabolic disease, and hypertension. Coronary atherosclerosis, aging, diabetes, and obesity are major clinical risk factors for HF (**Figure 1**).[1,2] According to the latest guidelines of cardiological societies[3,4], HF patients are classified according to their left ventricular ejection fraction (EF) value in three groups: 1) HF with reduced EF (HFrEF), EF being <40%, 2) HF with mid-range EF (HFmrEF), with EF between 40-49%, and 3) HF with preserved EF (HFpEF), with EF >50%. Usually, physicians additionally stratify HF patients according to the ACC/AHA Heart Failure classification system including stage A-D [3,4] which places HF patients in one of four categories based on heart structural alterations. In detail, stage A patients are at risk for HF but have-not yet developed structural heart changes, e.g. patients with diabetes, coronary heart disease (CHD) without previous myocardial infarction; stage B patients show structural heart disease, e.g. reduced EF, left ventricular hypertrophy, chamber enlargement, but have not yet developed symptoms of HF; stage C patients who have developed clinical HF, whereas stage D patients require advanced intervention, e.g. cardiac resynchronization therapy (CRT), left ventricular assist device (LVAD) and heart transplantation (HTx).[3-6]

This approach implies the need for a deeper knowledge of mechanistic links among genetic and epigenetic events governing the pathophysiology of HF, leading to personalized "precision medicine" therapy of disease. The epigenome comprises a large spectrum of molecular changes leading to a functional reinterpretation of DNA sequence by dynamic activation/repression of specific genes, without changing nucleotide sequence.[7-12] The main epigenetic determinants are DNA and mRNA methylation, post-translational histone/non-histone modifications, and non-coding RNA.[7-12] These molecular signatures can be

meiotically (transgenerational effect) and mitotically transmitted across several generations representing an additional modality for the inheritance of cardiovascular (CV) diseases (**Figure 2**).[13-15]

The goal of our review is to present a picture of putative epigenetic-sensitive mechanisms underlying cardiac remodeling in HF patients. Moreover, we report the main clinical trials testing the role of epigenetic-sensitive drugs (epidrugs) in advancing the HF management. Finally, we propose the "EPi-transgeneratIonal networK mOdeling for STratificatiOn of heaRt Morbidity" (EPIKO-STORM), a clinical research program that may unveil epigenetic changes over time and useful biomarkers and drug targets.

2. A focus on the current therapeutic paradigm of HF

The current therapeutic paradigm for HFrEF patients focuses on reducing congestive symptoms and mortality rate by inhibiting both the adrenergic nervous system (ANS) and the renin-angiotensin-aldosterone system (RAAS).[3-5] The most effective RAAS inhibitors are angiotensin-converting enzyme inhibitor (ACEi), angiotensin receptor blockers (ARBs), mineralcorticoid receptor antagonists (MRAs), and angiotensin receptor-neprilysin inhibitor (ARNI), which can reduce morbidity and mortality in chronic HFrEF patients.[3,4,16] Remarkably, RAAS hyperactivation is a prominent target in treatment of HFrEF and (likely) HFmrEF patients, but not only in order to control congestion. Indeed, RAAS inhibitors have synergicistic action with beta-blockers (BB) that are still the most effective therapy in promoting reverse remodeling and improving beta-adrenergic and heart-rate responsiveness.[16] [17] Moreover, BB reduce mortality and sudden death by 30-40% in patients treated with ACEi[17] [18] by modulating end-organ response to the autonomic storm, and they can also inhibit myocyte apoptosis[18] [19], while vagal stimulation is able to affect interleukin-6 levels in advanced HF.[19] [20] However, BB are not able to provide equal benefit in each patient; for

instance, in the case of bucindolol, two polymorphisms of beta-1 adrenergic receptor, e.g., Arg-389 homozygosis and Gly-389 heterozigosis were associated with positive and negative clinical response to bucindolol, respectively.[20-21] Remarkably, BB are also the most effective therapeutic agents providing changes in myocardial gene expression (epigenetic-like), regulating pathways of contractility and pathologic hypertrophy in HF patients *vs* placebo.[21-22] Currently, no guideline exists for specific treatment of HFpEF, owing to a great clinical heterogeneity of these patients who are complicated by multiple comorbidities as well as different etiology and pathophysiology.[22,23,24] HFpEF patients seem to be less susceptible to conventional pharmacotherapy and many clinical trials did not provide solid evidence about treatments able to alter the natural history of this condition.[3,23,24] Interestingly, the sodium-glucose cotransporter 2 inhibitors (SGLT2i) acting as glucose-lowering agents, e.g. empagliflozin, have been successively applied for management of diabetic patients affected by HFrEF and HFpEF improving hemodynamic parameters and reducing their hospitalization.[3] Although their mechanisms of action are not well understood, a putative epigenetic regulation underlying the protecting CV effect exerted by SGLT2i was found.[25,26] Moreover, SGLT2i might have a putative effect also in man by decreasing RAAS activation.[27,28] Indeed, there is tubule-glomerular feedback in patients with type 1 diabetes before and after administration of empagliflozin.[27,28] The study results disclosed the mechanism by which the RAAS axis is tapered down by the effect of SGLT2i on tubular sodium and glucose reabsorption.[27,28] This evidence was confirmative of what previously observed in animal models.[25,26] Thus, we emphasize the need to further explore if the current approved drugs may restore the aberrant epigenetic-sensitive pathway in pathophysiologically distinct HF conditions.

3. Basic mechanisms of epigenetic regulation

The cell-specific patterns of epigenetic modifications, mainly DNA methylation, histone modifications, and miRNAs, are affected by environmental exposures and represent a strong mechanistic link for the etiology of HF (**Figure 3**).[26] [29] DNA methylation is catalyzed by DNA methyltransferases (DNMTs) enzymes, which are classified in DNMT1, 2, or 3 able to add a methyl-group on cytosine carbon-5' position into promoter genes and "CpG islands".[9] Generally, DNA methylation can reduce the accessibility of transcription machinery to target gene promoters leading to lower levels of gene expression.[7] [9] Otherwise, demethylases belonging to 10-11 translocation (TET) family of DNA dioxygenases (TET1/2/3) enzymes can remove the methyl group from the methylated cytosines leading to a possible increase in gene expression.[7] [9]

Histone and non-histone modifications can occur at specific amino acid positions. Among them, histone acetylation and methylation are the most studied. The first is regulated by two families of proteins with the opposing functions: histone acetyltransferases (HATs) and histone deacetylases (HDACs) that add and remove one or more acetyl groups mainly at lysine residues of histone tails.[8] [10] Generally, HATs and HDACs are associated with gene activation and repression, respectively.[8] [10] Histone methylation mainly occurs at the lysine residues and is mediated by lysine methyltransferases (HMTs) with differential turnover rates.[9] [11] Overall, the final effect of varies according to the specific site of methylation and the number of added methyl groups.[9] A plethora of methyltransferases, including SET domain containing proteins, and demethylases, including Jumonji C (JmjC) domain containing, were identified.[9] [11]

Different classes of non-coding RNAs control gene expression at different levels.[10] [12] In detail, microRNAs (miRNAs) are small non-coding RNA molecules (21-22 nucleotides) which bind to specific target mRNAs by blocking their translation or inducing their degradation.[10] Moreover, long non-coding RNAs (lnc-RNAs) are non-coding transcripts that

are >200 nucleotides forming RNA-protein interactions to carry out their functions by modulating chromatin-modifying complexes and interacting with transcription factors.[10] [12]

4. An epigenetic-centered map of cardiac remodeling in HF patients

Myocyte death, cardiac hypertrophy, alteration of extracellular matrix homeostasis, fibrosis, defective autophagy, metabolic abnormalities, and mitochondrial dysfunction are relevant epigenetic-sensitive endophenotypes underlying cardiac remodeling and HF pathogenesis (**Figure 1**).[26] [1]

Consistently, the onset of CV diseases in adult life may be explained by the concept of "persistent memory", for which early environmental insults experienced in prenatal or perinatal life are translated in epigenetic changes persisting across several generations.[12] [14], [27,28] [13-15], [30,31] (**Figure 2**). This effect, known as "transgenerational epigenetic inheritance", is largely supported by preclinical models of CV diseases.[12] [14] [13-15] Recently, a direct correlation between hypermethylation of the sterol regulatory element binding protein 2 (*SREBP2*) gene in fetal aortas and maternal hypercholesterolemia was observed during early atherogenesis.[28] [31] Further long-term causal relationship studies are needed to dissect the extent of epigenetic-sensitive changes and maladaptive responses during pregnancy and translate them in useful biomarkers for CV primary and secondary prevention (**Figure 2**).[29] [30-32]

4.1 Epigenetics of maladaptive responses in HF patients

Epigenetic-sensitive molecular networks are crucial during the early adaptive (compensatory) responses to cell damage that become "maladaptive" after establishment of chronic stress in the heart. We focus on the main intermediate phenotypes (endophenotypes), such as metabolic shift, myocardial injury, fibrosis, and mitochondrial dysfunction, and report the same findings in humans (**Table 1**).

4.1.1 Metabolic shift

A switch in cardiomyocyte energy substrate utilization from fatty acids to glucose is the metabolic hallmark of HF remodeling, resulting in inefficient energy production.[30,31] [33,34] The enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2)-DNA methylatransferase (DNMT) complex could coordinate hypermethylation and downstream silencing of the kruppel-like factor 15 (*KLF15*) gene in 5 ischemic cardiomyopathy (ICM) *vs* 6 non-ischemic cardiomyopathy (NICM) patients with reduced EF.[30] [33] As consequence, EZH2-DNMT complex may be useful to develop personalized approaches to treat ICM by counteracting the fetal-like switch program (anaerobic glycolysis).[30] [33] Moreover, upregulation of histone H3 lysine 9 (H3K9) demethylase, named Jmjd1a and 2b, reduced H3K9me3 and H3K9me2 (repression markers) favoring an open chromatin formation in the promoter regions of atrial natriuretic peptide (*ANP*) and brain natriuretic peptide (*BNP*) genes leading to reactivation of fetal genes in 8 dilated cardiomyopathy (DCM) and 8 ICM patients as compared with 8 nonfailing donor hearts.[31] [34] The first large-scale DNA methylation profile of peripheral blood leukocytes isolated from 27 end-stage ICM patients compared to 20 healthy controls revealed 732 differentially methylated regions (DMRs) in genes related to metabolism and junction cellular components.[32] [35] In detail, hypomethylation of mitochondrial inner membrane protein like (*MPV17L*), a negative regulator of apoptosis, and SLC2A1 solute carrier family 2 member 1 (*SLC2A1*), a glucose transporter protein, were correlated to higher expression levels whereas hypermethylation of plectin (*PLEC*), encoding a protein involved in actin binding and ankyrin binding, corelated with lower protein levels.[32] [35] Further studies with large sample size are needed to validate these results and establish the cause-effect ratio underlying myocardial alterations.

4.1.2 Myocardial injury

A large-scale DNA methylation analysis suggested that a parallel hypermethylation of the lymphocyte antigen 75 (*LY75*) and hypomethylation of the adenosine A2a receptor (*ADORA2A*) genes at promoter level was correlated to downregulation of their transcripts in LV samples isolated from 10 DCM *vs* 10 cardiac transplant patients (controls) undergoing cardiac catheterization controls.[33] [36] *LY75* and *ADORA2A* are genes not previously associated with DCM and could clarify pathogenesis of HF as well as be represent useful biomarkers of cardiomyocyte death.[33] [36] Koczor et al.[34] [37] reported a global trend of DNA hypermethylation resulting in silencing of gene expression in LV samples isolated from 10 DCM patients with respect to 10 nonfailing controls. Importantly, four genes with no previously reported role in DCM resulted crucial in alteration of cell cycle progression and tight junctions, such as thymidine kinase 1 (*TK1*), claudin 5 (*CLDN5*), aurora kinase B (*AURKB*), and butyrophilin-like 9 (*BTNL9*).[34] [37] However, these genes warrant further study to show how these changes may trigger myocardial injuries. For the first time, by using LV samples some authors reported that simultaneous DNA hypermethylation and reduced enrichment of histone 3 lysine 36 tri-methylation (H3K36me3) was correlated to downregulation of double homeobox transcription factor (*DUX4*) gene, involved in reduced cell viability.[35] [38] However, their functional role remains to be elucidated in human end-stage cardiomyopathy and further studies are needed to determine whether these epigenetic features may predict disease severity or normalize with recovery of LV function.

4.1.3 Fibrosis

During LV remodeling, collagen increases and occupies the areas between the myocytes and the vessels. As a result, reparative (adaptive) fibrosis may adversely progress in maladaptive process by influencing tissue stiffness and diastolic ventricular function.[36] [39] By using tissue samples isolated from right atrial appendage of 26 patients undergoing elective cardiac-

bypass surgery, some authors reported that an increasing degree of hypoxia induced a global DNA hypermethylation, *via* DNMT1 and DNMT3B up-regulation, leading to overexpression of collagen 1 and alpha-smooth muscle actin (*α-SMA*) gene.[37] [40] Interestingly, by using siRNA administration to block DNMT3B expression, a reduced collagen 1 and α-SMA gene expression was observed in human primary cardiac fibroblasts suggesting a useful putative drug target.[37] [40] A possible direct causal-effect relationship between hypermethylation and pro-fibrotic phenotype *in vivo* requires further investigation.

4.1.4 Mitochondrial dysfunction

Higher levels of circulating mitochondrial DNA (mtDNA) can contribute to LV remodeling and poor prognosis of HF patients.[38,39] [41] A differential methylation of some mtDNA genes may trigger cardiomyocyte death by silencing survival pathways (hypermethylation) or upregulating the expression of proteases (hypomethylation).[40] [41] For the first time, Baccarelli et al.[41] [42] measured the levels of mtDNA methylation derived from platelets in genes belonging to ATP-synthesis machinery by using samples isolated from 10 patients at risk of HF *vs* 17 healthy controls; CV patients showed significant hypermethylation in 4 genes, including mitochondrially-encoded cytochrome-c oxidase I (*MT-CO1*), mitochondrially-encoded cytochrome-c oxidase II (*MT-CO2*), mitochondrially-encoded cytochrome-c oxidase III (*MT-CO3*), and mitochondrially-encoded tRNA leucine 1 (UUA/G) (*MT-TL1*).[41] [42] This evidence may clarify the relationship between platelet mitochondria, DNA methylation, and HF suggesting novel predictive non-invasive biomarkers.

4.2"Epitranscriptome": the next frontier in HF patients

The epitranscriptome, known as RNA epigenetic code, refers to a plethora of chemical modifications in different small RNAs representing an additional layer of gene expression regulation[42] as well as novel opportunities to establish CV biomarkers.[43,44] RNA methylation,

mainly on the adenosine base at the nitrogen-6 position (N6-methyladenosine, m6A), has been defined a potential regulatory mechanism underlying the cardiomyocyte adaptive responses.[44] [45] DNA polymorphisms in the fat mass and obesity associated (*FTO*) gene, encoding for a specific m6A-demethylase, are associated with high risk for obesity, metabolic abnormalities, and CV diseases.[45] Recently, a concomitant decrease in FTO levels and increase of m6A content in RNA was observed in ischemic human hearts.[44] [45] Another form of RNA modification is the switch from adenosine to inosine (A-to-I), known as RNA editing, which is mediated by the adenosine deaminase acting on RNA (ADAM) enzymes, as strong contributors to atherosclerosis.[45] [46] Clinical evidence reported an increased A-to-I editing of the mediator complex subunit 13 (MED13) mRNA in blood samples isolated from cyanotic congenital heart pediatric patients (n=19) compared to controls (n=13)[47], suggesting a putative pathogenic[46] and prognostic role.[47] [48] However, there are not studies investigating the role of epitranscriptome in HF patients.

4.3 Epigenetics in patients with advanced HF on waiting list for HTx

HTx is the only available long-term treatment for terminal HF patients.[48] [49] Despite current immunosuppressive regimens, the allograft rejection rate is high and clinically asymptomatic making the diagnosis very difficult.[49] [50] Predictive epigenetic indicators may identify patients at high risk for post-transplant complications. Duong et al.[50] [51] reported that pro-inflammatory miR-10a, miR-155, miR-31, and miR-92a were differentially expressed both in serum and tissue samples isolated from 30 patients with acute allograft rejection respect with 30 healthy controls, suggesting a putative role as useful non-invasive biomarkers. Interestingly, some authors suggested that circulating cell-free (cfDNA) donor-derived DNA might also provide a non-invasive diagnosis of acute rejection.[51] [52] Thus, further clinical studies should focus on DNA methylation levels in cfdDNA molecules, which may represent an early and non-invasive predictive biomarker of HTx complications.

5. Rethinking the transgenerational effect in the stratification of the natural history of HF

Development HF syndrome reflects a long lag time between onset and real clinical manifestations providing a huge opportunity for implementation of inexpensive lifestyle interventions to reduce mortality and morbidity rate.[3,4] Much attention should be given to stage A patients which did not yet develop structural heart changes but are likely in progression of HF.[3] Since numerous epigenomic patterns exist in human adaptive/maladaptive responses (**Table 1**), it is need to also confirm or not if these molecular signatures can track disease progression. These responses are crucial to establish epigenomic assays for assessing prognosis in HF.

Early stages of CV lesions are already set during human fetal development providing epigenetic tracks of vascular damage[~~12,13,28~~ 13,14,30,31]; thus, an optimal experimental strategy should address the putative role of "epigenetic memory" in the insurgence of HF (**Figure 2**). We revisited this concept by proposing the EPi-transgeneratIonal networK mOdeling-STratificatiOn of heaRt Morbidity (EPIKO-STORM) platform, as clinical integrated research program based on "epigenetics memory" and network-based analyses to stratify the natural history of HF. The idea of this proposed platform is to monitor large prospective mother-child cohorts to clarify how, when and where maternal risk factors, e.g. diabetes, dyslipidemias, CHD with no myocardial infarction (stage A) can epigenetically impact early fetal development making a subject more susceptible to HF later in life.

There are many concerns on the potential windows of vulnerability in which detrimental exposure can epigenetically impact crucial nodes (genes or proteins) underlying the development of HF from high risk patients (stage A). We remark three times for which epigenetic-sensitive sensors might play a role in pathogenesis and progression of HF (window 1: "early", <6 months to 15 years; window 2: "later", 15-50 years; window 3: >50 years)

(**Figure 2**). The epigenetic plasticity to environmental risk factors is highly relevant from embryonic development to early post-natal life (window 1). This impacts on individual genetic make-up by modulating putative maladaptive responses when a subject experience the same *in utero* insults (or other) in post-natal life. Moreover, the inherited epigenetic tracks may be further impacted by acquired structural and functional cardiac alterations in adulthood leading to synergic effects in progression of HF (window 2) until conclamate disease (window 3). Much difficult is to determine if epigenetic-sensitive nodes can be further impacted by environmental factors in end-stage failing hearts (window 3). Evidence reported that epigenetic sensors may be useful biomarkers. Whether these sensors can be useful to improve the quality of care and quality of life of HF patients that experience high rate of hospitalization remains undetermined.

A strength of EPIKO-STORM platform would be to combine liquid biopsy approaches and network analysis which can identify non-invasive biomarkers arising from cardiomyocytes death or other common circulating cells (e.g., fibroblasts) at different time points.[52] [53] Indeed, the widely diffuse tissue biopsy from explanted hearts may be altered during handling (degradation, missing oxygenation, innervation and worsening LV function) and difficult to obtain.[53] [54] Since epigenetic-sensitive changes largely vary over time, the possibility of studying these patterns at different time-points during life may be an add value respect with the most frequent cross-sectional analysis (one time-point measure).[54] [55] This strategy is particularly useful to design longitudinal analysis by following the "transgenerational effect" of maladaptive responses in high risk families. In this way, the EPIKO-STORM platform may identify novel circulating epigenetic signatures mirroring the "cardiac" interactome and monitor their variations from the fetal-perinatal stage until childhood and older age. This comprehensive staging approach may provide earlier identification and management of at-risk subjects before the development of HF (**Figure 2**). For this reason, future studies would

greatly benefit from using the EPIKO-STORM platform to provide novel biomarkers for disease staging mainly at two time points: 1) identification of asymptomatic subjects with increased genetic risk or structural/functional dysfunction (primary prevention) and 2) monitoring of patients with severe or end-stage HF (secondary prevention). Overall, this strategy could open an important new avenue of research because the epigenome may represent a drug discovery target for novel HF therapy.

6. Advanced omics technologies and bioinformatic tools to identify novel useful epigenetic biomarkers in HF

6.1 Omics platforms and longitudinal analysis of epigenetic tracks in HF patients

Since next generation sequencing (NGS) tools become more rapid and less expensive, researchers are turning toward unbiased large-scale analyses on multiple omics data to better understand, identify, and treat HF patients (**Table 2**).[55-58] [56-59] Genomics enlarged the range of HF susceptibility genes and improved the performance of screening tests but offered a knowledge at a limited resolution level.[55] [56] Transcriptomics, proteomics and metabolomics may provide insight into disease origin and may be possible clinical applications useful to provide a more accurate prediction of the long-term clinical course of a patient.[56-59] [57-59] On the other hand, only epigenome-wide association studies (EWAS) integrated with current clinical approaches may better clarify the mechanistic role of molecular sensors modulating the gene-environment interplay in CV patients (**Figure 3**).[60-63] In **table 1**, we reported some platforms which may powerfully aid to: 1) discriminate patients with common clinical phenotypes (deep-phenotyping), 2) identify more penetrant causal-effect endophenotypes, and 3) customize drug therapies.[60] Despite increasing advance, the use of epigenomics approaches also presents several limitations mainly owing to the confounding effects related to the heterogeneity of the whole cardiac tissue.[64,65] Indeed, experimental approaches should

major investigate circulating cells and their products in blood samples (liquid biopsy strategy), including endothelial progenitor cells (EPCs), cfDNA, and circulating histones. In this way, we can provide specific and non-invasive biomarkers for diagnosis and prognosis of many CV diseases.[52] [53] For this aim, prospective population studies will strongly help us to find novel targets for risk evaluation, diagnosis and treatment strategies.[62-64]

6.2 Advanced bioinformatic tools to identify protein-protein interactions (PPIs)

Network analyses are useful to advance our CV clinical practice.[65-68] [66-70] Respect with the current reductionist approach, network analyses focus on human diseases as consequence of alterations in gene-gene interactions rather than a single defect in a single gene.[65-68] [66-70] This approach may aid to clarify the complex effects of multiple exposures on molecular networks perturbing the cardiac interactome at individual level.[65-68] [66-70] Network-oriented analyses may capture crucial nodes (genes, proteins or other) or disease modules (subgroups of gene products with the same or similar functions) by using specific criteria measuring network topology.[65-68] [66-70] A large panel of quantitative platforms is available including protein-protein interactions (PPIs) networks, in which pathogenic proteins are the nodes linked by physical and functional interactions (e.g., GenePanda, DIAMOnD) regulatory networks, thereby interactions between a transcription factor and its target gene are investigated (e.g., PANDA) as well as co-expression networks, in which genes with concordant expression profiles are correlated (e.g., SWIM and WGCNA).[65-68] [66-70] Some of these applications, mainly PPI networks, were applied to identify crucial nodes underlying the heart remodeling in patients.[69-72] [71-74] Of note, by using machine learning algorithms to predict PPIs or predictive models from clinical data stored in big consortia, we may provide an in-depth understanding of disease as well as gain the goals of precision medicine and personalized therapy of HF.[73,74] [75,76] However, enthusiasm around artificial intelligence analysis should be

tempered as the road from bench to bed is complex and time-consuming owing to their "black box" nature, also (**Figure 4**). This intrinsic criticism means that we will never aware about the logic criteria used by the algorithms to make the final decision, thus physicians can only make final decisions according to current guidelines, own experience and intuition. To potentially accelerate this direction, it is needed to apply rigorous scientific methodology testing the artificial intelligence tools in clinical practice.

7. The current and future contribution from epigenetics to precision medicine of HF

To date, a low number of basic findings has been effectively translated in HF clinical practice, such as BNP, N-terminal pro-B-type natriuretic peptide (NT-proBNP), creatine phosphokinase (CPK), and high-sensitive cardiac troponin (hscTn), which are well established indicators of myocardial injury/fibrosis and its progression.[3] Nevertheless, an untimely detection and inter-individual differences in the baseline features still exist[~~75~~ 77], thus, the current clinical practice would benefit from more accurate non-invasive biomarkers that might be a realty in the future clinical practice of HF. In this context, interest is growing about nerve growth factor (NGF), that is expressed by ischemic myocytes, as well as circulating progenitor cell (CPC) dysfunction in diabetes which seems to be a key factor in microcirculation disruption.[~~76,77~~ 78,79]

7.1 Stratification of the natural history of disease in different HF phenotypes

Nowadays, evidence regarding epigenetic-sensitive causal pathways are lacking in HFrEF, HFmrEF, and HFpEF. We reported some examples of epigenetic signatures in different HF phenotypes. Recent evidence reported that levels of circular RNA, named circRNA MICRA, were lower in the subgroup of post-MI patients associated with reduced EF after 4 months from measurements with respect to HFmrEF or HFpEF groups.[~~78~~ 80] This suggests MICRA as potential predictive biomarker for HFrEF phenotype after MI.[~~78~~ 80] CRT is associated with

improved performance and survival in patients with advanced HF. A clinical trial reported that HFrEF patients, classified as "responders" to CRT showed an overexpression of 19 circulating miRNAs associated with improvement of systolic function.[79] [81] In contrast, non-responders patients showed a downregulation of 6 different circulating miRNAs. This suggests that a specific miRNA profile may be useful as predictor of response to CRT in HFrEF.[79] [81] By using peripheral blood and cardiac cells, some authors performed the first epigenome-wide association study in HFmrEF patients.[80] [82] As a result, DNA hypomethylation of the natriuretic peptide A and B (*NPPA* and *NPPB*) genes at promoter level was reported in 41 HFmrEF patients *vs* 31 controls leading to upregulation of ANP and BNP proteic products, respectively.[80] [82] The key message of this study is that detection of DNA hypomethylation in *NPPA/NPPB* genes in peripheral blood may mirror the cardiac epigenome and represent a potential diagnostic biomarker of HFmrEF patients.[80] [82] The high phenotypic heterogeneity of HFpEF is a major challenge to identification of this patients and the development of new therapies.[81] [83] Recent evidence suggested that miRNAs might discriminate between HFrEF and HFpEF phenotypes, as well as provide a better insight in their differential pathophysiology of disease.[82,83] [84,85] In detail, differential expression of miR-30c, miR-146a, miR-221, miR-328, and miR-375, miR-125a-5p, miR-190a, miR-550a-5p, and miR-638 may improve the predictive value of BNP as well as the differentiation of HFpEF from HFrEF supporting additional diagnostic strategies.[82,83] [84,85] We noted that the most of clinical findings about discerning HF phenotypes are related to miRNAs, likely because they are stable circulating molecules and their detection is non-invasive, thus representing ideal biomarkers of disease and therapeutic strategies.[84] [86]

7.2 Personalized therapy

Could the reversal of aberrant epigenetic networks be an add value to the current evidence-based guidelines in HF management? Moreover, which subgroups of patients may have some

benefits from an epidrug rather than another? And in which doses? Monotherapy or polytherapy? These are crucial questions that are still waiting a response from the research community. Several evidence reported that some classes of epidrugs may act as immunosuppressive agents by modulating the activity of T-reg cells in post-transplant patients.[6] However, there are not clinical trials investigating the role of epidrugs in preventing organ reject after Htx. In **Table 3**, we reported the currently available trials which investigated the effect of monotherapy/polytherapy epidrugs in HF management. Preclinical evidence suggests that "pleiotropic" effects of statins, as HMG-CoA reductase inhibitors, may improve survival in ischemic and non-ischemic HF by regulating the autonomic nervous system through angiotensin II and nitric oxide modulation.[85] [87] Statins act as epidrugs by inhibiting the HDAC activity thus shifting the histone balance toward an acetylated state, marker of active transcriptional activity.[86] [88] However, preliminary human studies reported mixed results. Indeed, Horwich al.[85] [89] reported that short-term statin treatment did not result in a significant decrease in autonomic nervous system activation in HF patients.[87] In contrast, a large metanalysis of 13 randomized trials reported that administration of lipophilic statins (e.g., atorvastatin) significantly decreased all-cause mortality, hospitalization for worsening HF, and low-density lipoprotein cholesterol, independently from age, baseline LVEF, and cause of HF.[88] [90] Thus, there is no evidence to recommend their routine use in HF therapy. Interestingly, a dietary supplementation with fish-oil-derived long-chain omega-3 polyunsaturated fatty acids (PUFA) may have hypotriglyceridemic, anti-inflammatory, anti-arrhythmic, and anti-thrombotic effects by altering the global DNA methylation profile in blood leukocytes.[89] [91] The large-scale, randomized, double-blind study (the GISSI Heart Failure project) demonstrated that 1 g per day of omega 3 is associated with a small reduction (9%) in mortality and admissions to hospital for CV events in HF patients.[90] [92]

Regarding polytherapy, a completed randomized trial demonstrated that isosorbide dinitrate (ISDN) combined with a global inhibitor of DNA methylation, as hydralazine, did not exert beneficial effects in HFpEF with respect to controls[~~91~~ 93], whereas this polytherapy is already part of the HFrEF (stage C) standard of the care.[3] As mentioned, numerous studies based on epigenetic therapy have no positive results and one reason may be that all these agents are not specific enough but universal, therefore the drugs cannot selectively target the pathogenic epigenetic changes in HF patients. In this way, engineered CRISPR/Cas9 systems and, others innovative systems, are being tested in *vivo* to direct specific epigenetic players to target genes providing novel strategies to establish epigenetic therapy in humans.[~~92~~ 94] Also, we included a number of clinical trials, generated from the website https://clinicaltrials.gov/, that are ongoing or completed in the last ten years but currently without any results (**Supplementary Table 1, Supplementary Table 2**).

8. New perspectives

Despite clinical epigenetics is still in its infancy, it promises to become a new avenue to dissect HF phenotypes at molecular level. Nowadays, tissue heterogeneity has not been fully explored with specific focus in the setting of HF. Remarkably, it is needed to assess if methylation changes in circulating leukocytes may reflect myocardial processes providing reliable biomarkers. Also, to ensure rigor and reproducibility when designing association studies, avoiding contamination, both rigorous sample ~~and sampling considerations,~~ accounting for analytical variability~~,~~ and a better understanding of biological variability and tissue specificity of these epigenetic signatures~~,~~ are desiderable. Artificial intelligence might offer novel opportunities to bridge these gaps but, today, we are still far from clinical application. Current clinical research in HF points to the hypothesis that complex molecular networks are regulated by genetic-epigenetic interplay and activated in the failing

myocardium in response to the original triggering cause, leading to re-expression of fetal genes, upregulation of fibrosis-related genes, and others maladaptive responses. The epigenome regulates the expression of early maladaptive responses, and recent evidence suggests that part of the chromatin remodeling may be altered by diet and other environmental risk factors. Several epigenetic-sensitive changes may enlarge opportunities to explain the missing hereditability in HF (**Table 1**). Moreover, gene polymorphism investigation may also improve our understanding of epigenetics. For example, the insertion/deletion of intron 16 in the *ACE* gene acts as marker for a functional polymorphism that provides gene-environment interactions leading to possible explanations for mixed findings in clinical setting.[93,95]

Most of clinical evidence arose from DNA methylation profile performed in LV tissue samples and circulating miRNAs. Obviously, investigations in human tissues are restricted because genome sequencing needs a relatively large amount of biospecimens, which are not easily available from living patients. Despite increasing advance, the clinical use of epigenomics approaches also presents several limitations mainly owing to the confounding effects related to the heterogeneity of the whole cardiac tissue. Indeed, experimental approaches should major investigate if tissue-related epigenetic signatures may be reflected in circulating cells and their products in blood samples to provide non-invasive biomarkers (liquid biopsy strategy).[65,80,53] Thus, we are still not able to identify epigenetic signatures discriminating the different HF phenotypes (HFrEF, HFpEF, HFmrEF).

Since the reversible nature respect with genetic mutations, epigenetic-sensitive changes are useful drug targets tested in several clinical trials. The BETonMACE ongoing phase III trial (NCT02586155) is assessing the ability of apabetalone in reducing both inflammation and adhesion molecule gene expression in post-ACS diabetic patients with low high-density lipoprotein C (**Table 3**). Importantly, apabetalone is highly selective for the second bromodomain (BD2) within the bromodomain and extraterminal proteins (BETs), which bind

to acetylated histones and transcription factors providing specific biological effects with potentially major benefits for high-risk CV patient by maintaining a safety profile.[94] [96] Major efforts should be done to identify in which type of cells apabetalone can exert its beneficial effect to avoid side-effects; however, it would be interesting to investigate if this epidrug might be also effective in prevention or management of HFpEF that results associated with inflammation, hypertension and metabolic dysfunction (~~T2D~~ type 2 diabetes and obesity).[3] Indeed, it should be noted that the epigenome is highly spatio-temporal specific and each epigenetic change can regulate a cassette of downstream genes; therefore, the use of epidrugs is not totally specific leading to a range of putative side-effects representing one of the main current challenge in clinical trials. In this highly complex and dynamic environment, current investigation methodology based on matching of clinical, biochemical and instrumental variables, to draw study population profile is no more fit for perspective research. Now, all omics platforms are restricted to a bench approach in CV field. If adoption of big data analysis with deep computing or artificial intelligence will supply this gap remains a challenge question. In this way, we propose the EPIKO-STORM, a longitudinal clinical platform combining transgenerational effect and network-oriented analyses to gain personalized "precision medicine" therapy of HF patients.

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Legends to figures:

Figure 1. Epigenetics, maladaptive responses and heart failure. A plethora of epigenetic-sensitive mechanisms could be crucial pathogenic determinants leading to many diseases associated with heart failure. When environmental insults persisting over time, a chromatin remodeling may trigger a switch from adaptive *vs* maladaptive responses, including metabolic shift, myocardial damage, fibrosis, and mitochondrial dysfunction. These molecular perturbations could be related to different cardiac phenotypes, including HFrEF, HFmrEF, and HFpEF.

Abbreviations: CHD: Coronary Heart Diseases; DCM: Dilated Cardiomyopathy; HFrEF: Heart Failure Reduced Ejection Fraction; HFpEF: Heart Failure Preserved Ejection Fraction; HFmrEF: Heart Failure Mid-Range Ejection Fraction; ICM: Ischemic Cardiomyopathy.

Figure 2: **Epigenetics of maladaptive responses in HF patients and EPIKO-STORM**.

At the top of picture, we design an epigenetic-centered map about molecular networks underlying maladaptive responses in HF patients (see the text for more details). Moreover, we emphasize the importance of studying these epigenetic sensors early during the fetal development and progressively later in life to trace longitudinal dynamic trajectories useful to HF precision medicine and personalized therapy. In particular, we propose a program of clinical research, named EPIKO-STORM, focusing on the potential impact of transgenerational effect in identifying novel non-invasive epigenetic biomarkers able to monitor each molecular variation from the fetal-perinatal stage to childhood, older age, and subsequent generations. It poses major attention to detect maternal risk factors and early epigenetic-sensitive signatures of cardiac remodeling in stage A patients.

Abbreviations: CHD: coronary heart disease; EPIKO-STORM: EPi-transgeneratIonal network mOdeling-STratificatiOn of heaRt Morbidity; HF: heart failure, MI: myocardial infarction.

Figure 3: Full-spectrum of epigenomic tools in clinical research programs. The advance of NGS techniques has enlarged the opportunities for measurement of epigenetic-sensitive changes in human samples, such as DNA methylation, histone modifications, and microRNAs. These molecular signatures can be detected in PBMNCs, whole blood, saliva, plasma/serum, and different tissues. By using these applications, we can provide a huge amount of big data that have to be analyzed through potent bioinformatic algorithms, such as network-oriented analyses. The ultimate goal is to identify the individual interactome in heart failure patients to improve personalized therapy of disease.

Abbreviations: DNMT: DNA Methyltransferase; HPLC: High Performance Liquid Chromatography; MiRNAs: MicroRNAs NGS: Next Generation Sequencing; PBMNCs: Peripheral Blood Mononuclear Cells.

Figure 4: Clinical practice of HF: a role for artificial intelligence? Despite limitations, current reductionist approach based on clinical evaluation, imaging, surgery and physician-patient fidelity remains irreplaceable for effective outcomes in HF management. Artificial intelligence applications might support physicians in decision-making but, nowadays, are challenged by intrinsic criticisms. Despite artificial intelligence is a "black box", we need to merge these platforms in large clinical trials in order to test their possible use in clinical practice.

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Table 1. Some examples of epigenetic signatures leading maladaptive responses in HF patients.

Epigenetic mechanism	Region	Sample size	Sample source	Platform	Molecular effect	Signaling pathway	Phenotypic effect
			Tissue biopsy				
DNA hypermethylation of *KLF15* [33]	Promoter	5 ICM with reduced EF *vs* 6 NICM patients (M; 49-70 years)	LV biopsy	llumina Infinium Human Methylation450 Beadchip	Silencing of gene expression	Cardiac switch from an oxidative metabolism *vs* anaerobic glycolysis	Fetal-like phenotype
Demethylation of H3K9 at *ANP* and *BNP* [34]	Promoter	8 ICM and 8 DCM patients with reduced EF and *vs* 8 non failing controls (M and F; 45-60 years)	LV biopsy	ChIP assays	Activation of gene expression	Cardiac switch from an oxidative metabolism *vs* anaerobic glycolysis	Fetal-like phenotype
DNA hypermethylation of *LY75*	Promoter	10 non-ischemic, idiopathic DCM patients with reduced EF *vs* 10 healthy subjects (M and F; 53-61 years)	LV biopsy	Infinium Human Methylation 27 platform	Silencing of gene expression	Collagen-binding receptor and G protein-coupled receptor	Myocardial damage
DNA hypomethylation of *ADORA2A* [36]							
DNA hypermethylation of *TK1*, *CLDN5*, *AURKB*, *BTNL9* [34]	Promoter	10 DCM patients with reduced EF *vs* 10 healthy subjects (M and F; 50-60 years)	LV biopsy	MeDIP	Silencing of gene expression	Cell cycle progression and tight junctions	Myocardial damage
DNA hypermethylation and reduced enrichment of H3K3me3 at *DUX4*	Promoter	4 patients undergoing HTx *vs* 4 healthy controls (M and F; 41-	LV biopsy	MeDIP; ChIP	Downregulation of gene expression	Reduced cell viability.	Myocardial damage

		64 years)					
[38]							
DNA hypermethylation of *α-SMA* and *collagen 1* [40]	Not defined	26 patients undergoing cardiac-bypass surgery (case-only, M and F; 60-80 years)	RA biopsy	Quantitative real-time PCR; Western blotting; RNA interference	Activation of gene expression	Increased collagen deposition	Fibrosis
				Liquid-based assays			
Hypermethylation of mtDNA at *MT-CO1*, *MT-CO2*, *MT-CO3* and *MT-TL1* [42]	Not defined	Platelet-derived mtDNA from patients at high-risk of HF (M and F; 22-83 years)	Peripheral blood	Bisulfite-PCR pyrosequencing	Not defined	Cardiac cell death	Myocardial damage
DNA hypomethylation of *MPV17L*, *SLC2A1*	Promoter	27 ICM patients with reduced EF *vs* 20 healthy controls (M and F; 39-95 years)	PBMNCs	RRBS	Higher levels of transcription Lower levels of transcription	Apoptosis, glucose uptake, and cytoskeleton	Metabolic unbalance
DNA hypermethylation of *PLEC* [35]							
				Combination of tissue and liquid-based assays			
DNA hypermethylation of LY75 [80]	Promoter	18 DCM patients and 8 healthy controls	LV biopsy and peripheral blood	Infinium human methylation 450	Lower levels of transcription	Cardiac development and muscle function	Myocardial damage

Abbreviations: α–SMA: Collagen 1 And Alpha-Smooth Muscle Actin; ADORA2A: Hypomethylation Of The Adenosine A2a Receptor; ANP: Atrial Natriuretic Peptide; AURKB: Aurora Kinase B; BNP: Brain Natriuretic Peptide; BTNL9: Butyrophilin-Like 9; CLDN5: Claudin 5; DCM: Dilated Cardiomyopathy; DUX4: Double Homeobox Transcription Factor; EF: Ejection Fraction; HF: Heart Failure; H3K9: Histone 3lysine 9; H3K3: Histone 3 Lysine 9; Htx: Heart Transplantation; ICM: Ischemic Cardiomyopathy; KLF15: Kruppel Like Factor 15; LV: Left Ventricle; LY75: Lymphocyte Antigen 75; MeDIP: Methylated DNA Immunoprecipitation; MPV17 Mitochondrial Inner Membrane Protein Like; Mtdna: Mitochondrial DNA; MT-CO1: Mitochondrially Encoded Cytochrome C Oxidase I; MT-CO2: Mitochondrially Encoded Cytochrome C Oxidase II; MT-CO3: Mitochondrially Encoded Cytochrome C Oxidase III; MT-TL1: Mitochondrially Encoded Trna Leucine 1 (UUA/G); NICM: Non-Ischemic Cardiomyopathy; PBMNCs:Peripheral Blood Leukocytes; PLEC: Plectin; RA: Right Atrial; SLC2A: Glucose Transporter Protein; RRBS: reduced representation bisulfite sequencing; SLC2A1:Solute Carrier Family 2 Member 1; TK1: Thymidine Kinase 1.

Table 2. ~~Selected~~ Examples of omics studies in HF patients.

OMICS	STUDY TYPE	SAMPLE SIZE	SAMPLE SOURCE	PLATFORM	FINDINGS
Genomics [56]	Meta-analysis of 4 prospective cohorts from CHARGE Consortium	23,821 cohort participants	Peripheral blood	Affymetrix 6.0; Illumina 370CNV; Affymetrix 500K and 50K; Illumina 550K v3	2 SNPs were associated with incident HF (1 in European-ancestry and 1 in African-ancestry participants)
Transcriptomics [57]	Case-only	43 HF patients	Endomyocardial biopsy samples	Human Genome U133 Plus 2.0 Array from Affymetrix	Transcriptome from tissue biopsy contains sufficient information to discriminate poor from good prognosis
Proteomics [58]	HOMAGE study	Discovery set with 286 cases (HF) and 591 controls; Replication set with 276 cases (HF) and 280 controls	Plasma	Olink Proseek Multiplex CVD II, III	Increased biomarkers of inflammation, apoptosis, matrix remodeling and impaired blood pressure control and metabolism
Metabolomics [59]	CATHGEN study	HFpEF cases (N=282); HFrEF controls (N=279); controls (N=191)	Plasma	LC-MS/MS	Increased long chain acylcarnitines in states of inefficient β-oxidation differentially elevated in HFpEF and HFrEF

Abbreviations: CATHGEN: CATHeterization GENetics; CHARGE: Heart And Aging Research In Genomic Epidemiology; HFpEF: Heart Failure Preserved Ejection Fraction; HFrEF: Heart Failure Reduced Ejection Fraction; HOMAGE: Heart 'omics' in AGEing; LC-MS/MS: Liquid Chromatography With Mass Spectrometry; SNPs: Single Nucleotide Polymorphisms.

Table 3. Epigenetic therapy in HF clinical trials.

Type of study	Epidrug	Participants	HF phenotype	Objectives	Phase/ Status	Ref.
			Monotherapy			
Controlled, randomized (NCT00233480)	Atorvastatin	27	HF	• to investigate potential beneficial effects of statins in reducing sympathetic nervous system activation, myocardial remodeling, and immune activation in HF.	Phase 4/ Completed	[89]
Controlled, randomized (NCT00336336)	PUFA or Rosuvastatin	6975	HF	• To demonstrate that long term administration of PUFA or rosuvastatin is more effective than the corresponding placebo in HF patients, treated at the best of recommended therapies.	Phase 3/ Completed	[92]
Metanalysis of 13 randomized trials	Atorvastatin (10 trials), simvastatin (2 trials), and pitavastatin (1 trials)	1,532	HF	• to systematically review all randomised controlled trials evaluating the effects of lipophilic statins in HF patients.	N/A	[90]
			Polytherapy			
Controlled, Randomized (NCT01516346)	Hydralazine (DNA methylation inhibitor) And/or ISDN	54	HF, CHF	• To test the effect of prolonged therapy (24 weeks) with isosorbide dinitrate ± hydralazine on arterial wave reflections.	Phase 2/ Completed	[93]
Controlled, randomized (NCT02586155)	Apabetalone	2425	T2D	• to determine whether inhibition of BETs in high-risk T2D patients with CHD increases the time to major adverse cardiovascular events	Phase 3	[96]

Abbreviations: BETs: Bromodomain And Extraterminal proteins; CHD: Coronary Artery Disease; CHF: Congestive Heart Failure; ISDN: Isosorbide Dinitrate; N/A: Not Available; NCT: Number Of Clinical Trial, PUFA: Polyunsaturated Fatty Acids; T2D: Type 2 Diabetes.

- The goal of this review is to highlight as failing hearts undergo epigenetic-sensitive maladaptive responses.
- Network-oriented analysis and artificial intelligence can advance clinical practice in HF management.
- The EPIKO-STORM is a platform which might improve precision medicine of HF.

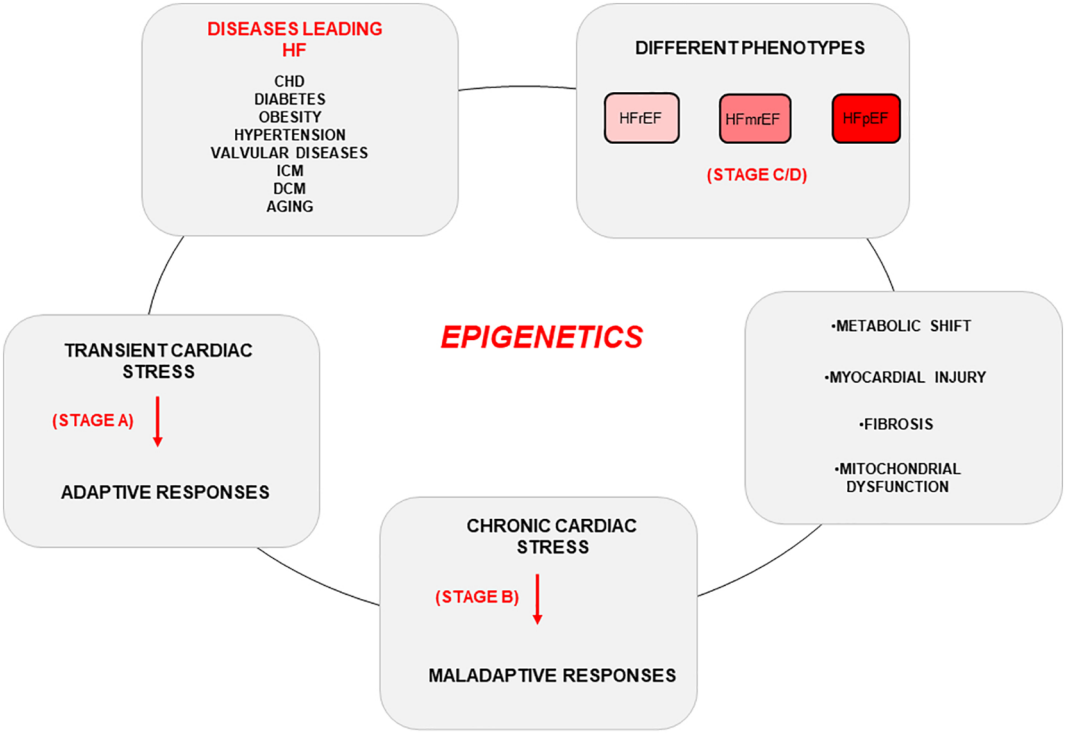
DISEASES LEADING HF
CHD
DIABETES
OBESITY
HYPERTENSION
VALVULAR DISEASES
ICM
DCM
AGING
DIFFERENT PHENOTYPES
HFrEF
HFmrEF
HFpEF
(STAGE C/D)
•METABOLIC SHIFT
•MYOCARDIAL INJURY
•FIBROSIS
•MITOCHONDRIAL DYSFUNCTION
CHRONIC CARDIAC STRESS
(STAGE B)
MALADAPTIVE RESPONSES
TRANSIENT CARDIAC STRESS
(STAGE A)
ADAPTIVE RESPONSES
EPIGENETICS

Figure 1

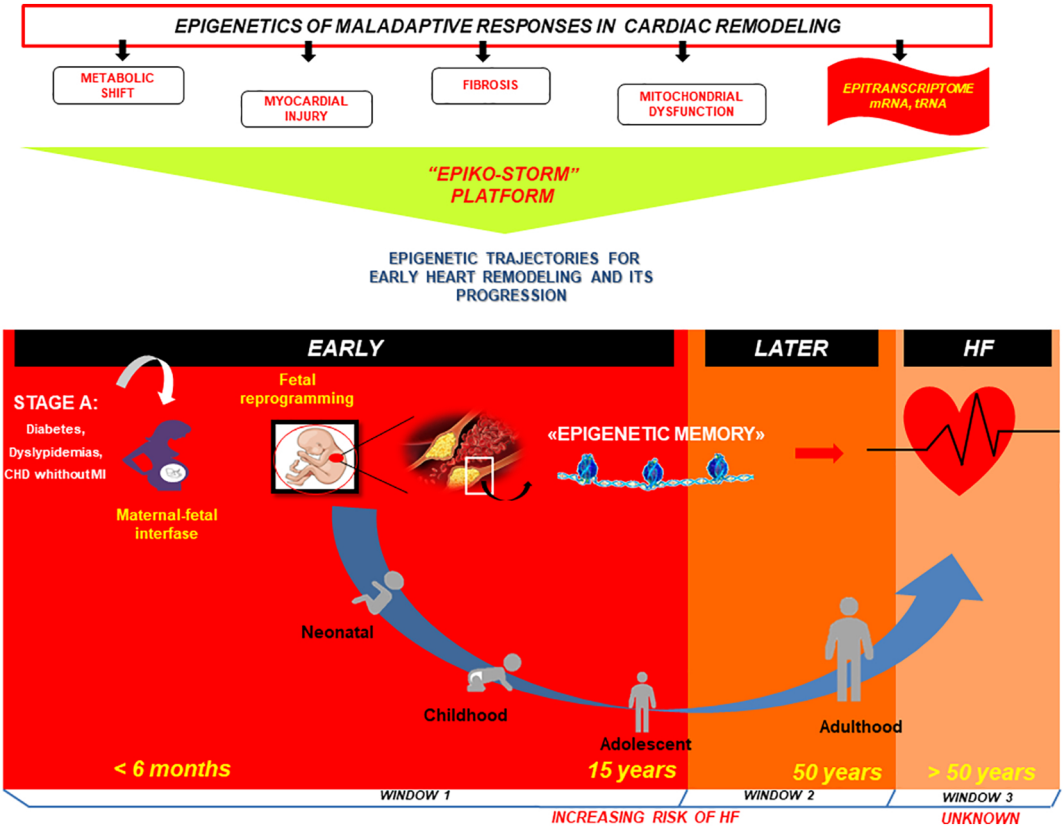
EPIGENETICS OF MALADAPTIVE RESPONSES IN CARDIAC REMODELING
METABOLIC SHIFT
MYOCARDIAL INJURY
FIBROSIS
MITOCHONDRIAL DYSFUNCTION
EPITRANSCRIPTOME mRNA, tRNA
"EPIKO-STORM" PLATFORM
EPIGENETIC TRAJECTORIES FOR EARLY HEART REMODELING AND ITS PROGRESSION
EARLY
LATER
HF
STAGE A:
Diabetes,
Dyslypidemias,
CHD whithoutMI
Maternal-fetal interfase
Fetal reprogramming
«EPIGENETIC MEMORY»
Neonatal
Childhood
Adolescent
Adulthood
< 6 months
15 years
50 years
> 50 years
WINDOW 1
WINDOW 2
WINDOW 3
INCREASING RISK OF HF
UNKNOWN

Figure 2

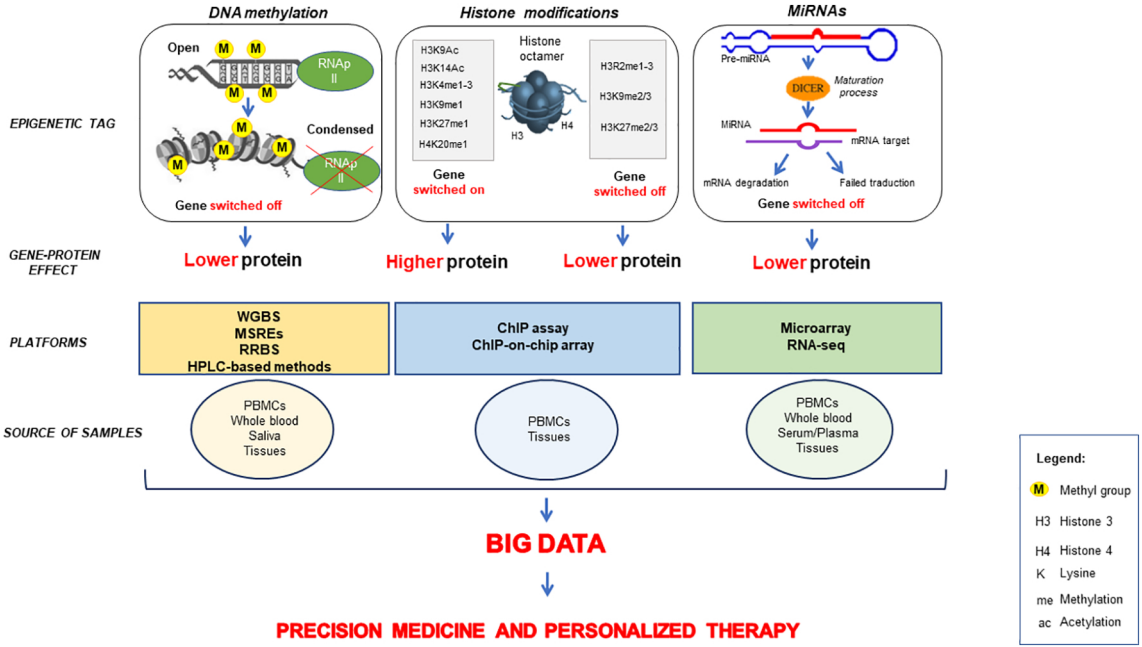
DNA methylation
Histone modifications
MiRNAs
EPIGENETIC TAG
Open
RNAp II
Condensed
Gene switched off
H3K9Ac
H3K14Ac
H3K4me1-3
H3K9me1
H3K27me1
H4K20me1
Histone octamer
H3
H4
H3R2me1-3
H3K9me2/3
H3K27me2/3
Gene switched on
Gene switched off
Pre-miRNA
DICER
Maturation process
MiRNA
mRNA target
mRNA degradation
Failed traduction
Gene switched off
GENE-PROTEIN EFFECT
Lower protein
Higher protein
Lower protein
Lower protein
PLATFORMS
WGBS
MSREs
RRBS
HPLC-based methods
ChIP assay
ChIP-on-chip array
Microarray
RNA-seq
SOURCE OF SAMPLES
PBMCs
Whole blood
Saliva
Tissues
PBMCs
Tissues
PBMCs
Whole blood
Serum/Plasma
Tissues
BIG DATA
PRECISION MEDICINE AND PERSONALIZED THERAPY
Legend:
M Methyl group
H3 Histone 3
H4 Histone 4
K Lysine
me Methylation
ac Acetylation

Figure 3

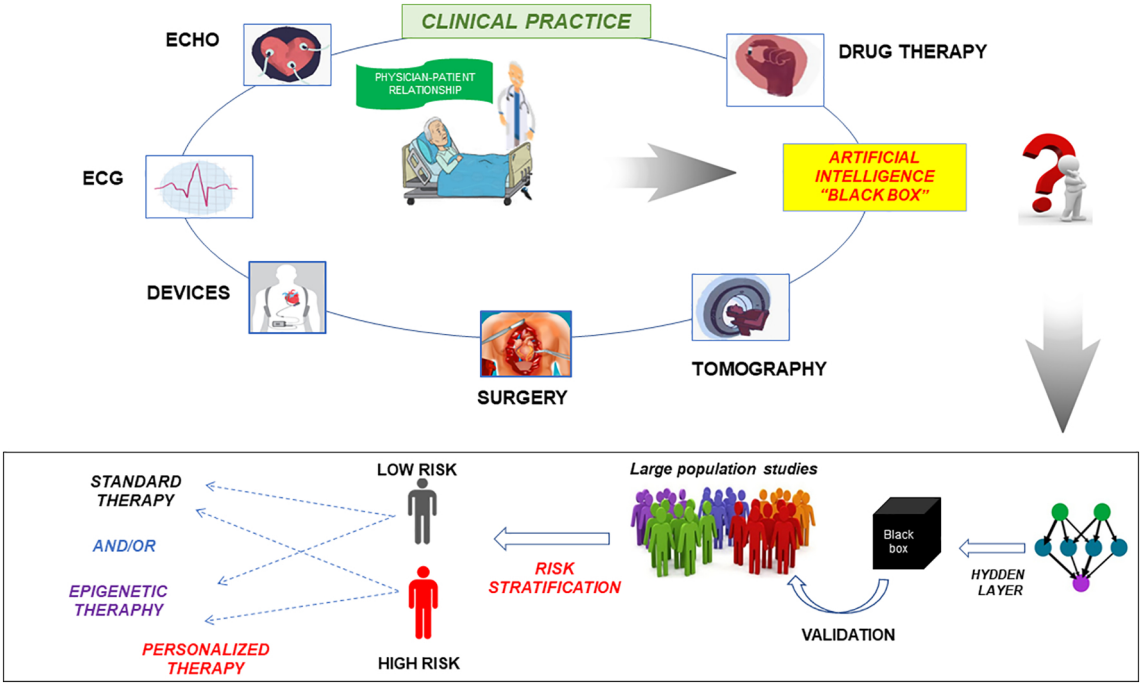
CLINICAL PRACTICE
ECHO
DRUG THERAPY
PHYSICIAN-PATIENT RELATIONSHIP
ECG
ARTIFICIAL INTELLIGENCE "BLACK BOX"
DEVICES
TOMOGRAPHY
SURGERY
STANDARD THERAPY
AND/OR
EPIGENETIC THERAPHY
PERSONALIZED THERAPY
LOW RISK
HIGH RISK
RISK STRATIFICATION
Large population studies
Black box
VALIDATION
HYDDEN LAYER

Figure 4