Endothelin receptor polymorphisms in the cardiovascular system: potential implications for therapy and screening

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Abstract Since its discovery in 1988, the endothelin system has been employed in multiple physiological and pathological roles. Endothelin-1 (ET-1) is not only a major regulator of vascular tone and cardiac contractility but also exerts mitogenic effects and is involved in inflammatory responses. ET-1 acts via two endothelin receptors located mainly on smooth muscle and endothelial cells through complex intracellular pathways differing between receptors and cell types. Polymorphisms of the endothelin receptor A have been associated not only with the risk in pulmonary arterial hypertension (PAH), systolic heart failure and systemic hypertension but are also of prognostic significance in dilated cardiomyopathy. Polymorphisms of endothelin receptors might lead to altered endothelin signaling and influence the response to endothelin receptor antagonist therapy in PAH in light of pharmacogenetics. This review will summarize the role of ET-1 within major cardiovascular pathologies and discuss endothelin receptor polymorphisms with special emphasis on potential therapeutic and screening implications.

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Introduction

Endothelin was first described in 1988 by Yanagisawa and was found to be a strong vasoconstrictor but also mediates fundamental cellular processes, such as cell proliferation, fibrosis and inflammation [1–4]. Following the initial description of the endothelin system, there have been multiple experimental and clinical studies employing endothelins in diverse pathologies within the cardiovascular system such as systolic heart failure [5, 6] and pulmonary hypertension [7, 8]. In spite of the extent to which the endothelin system seems involved in cardiovascular pathology, its complexity remains poorly understood. Post-translational modifications of endothelin receptors as well as the existence and relevance of endothelin and endothelin receptor polymorphisms have been studied in several pathologies such as hypertension and dilated cardiomyopathy [9–11]. However, knowledge about relevance of polymorphisms of endothelin receptors in the cardiovascular system especially in sight into antagonist therapy and pharmacogenetics is very limited, and the number of patients studied to date is too small for a formal meta-analysis [11]. Endothelin receptor antagonists are one of the hallmarks of therapy for pulmonary artery hypertension today but are not effective in all patients and furthermore have failed to be of benefit in systolic heart failure for incomplete understood reasons [12–15].

This review will shortly summarize the pathophysiology of the endothelin system with focus on polymorphisms of endothelin receptors within the cardiovascular system.

Physiology of the endothelin system

Endothelin

Endothelins form a family of three peptides, ET-1, ET-2 and ET-3 each located on a distinct chromosome, 6.1 and 20, respectively [16, 17]. ET-1 is the predominant form in vivo and is produced by endothelial cells, whereas ET-2 is mainly expressed in the kidney [18] and ET-3 in the central nervous system and gastrointestinal tract [19, 20]. This review will focus on the role of ET-1.

ET-1 is encoded by EDN1 located on chromosome 6p24.1. Human and mouse EDN1 genes code for a biologically inactive large precursor protein called prepro-endothelin-1 (PPET1). The biologically active form is generated within the cell in a 2-step proteolytic process. A neutral endopeptidase cleaves prepro-ET-1 to generate the still inactive precursor big-ET1. Big-ET 1 is then converted to the 2,492 Da 21-amino peptide ET-1 by endothelin converting enzymes (ECE) [2, 17, 21–24]. Endothelin-1 is mainly synthesized by endothelial cells but genes encoding for endothelin are also found in several other cell types including vascular smooth muscle cells, bronchial epithelium, cardiomyocytes, glial cells, macrophages and glomerular mesangium [25–27].

Stimuli for ET-1 expression and release from endothelial cells are ET-1, angiotensin II, catecholamines, cardiothrophin-1, thrombin, growth factors, cytokines, free radicals, insulin, hypoxia and sheer stress [19, 28–32].

Endothelin synthesis is inhibited by nitric oxide, natriuretic peptides and prostaglandins. Inhibition of synthesis by nitric oxide and prostacyclines is of special interest since these factors are down regulated in pulmonary hypertension [33, 34]. ET-1 is secreted by endothelial cells mostly at the basal side resulting in much higher concentrations within the vascular wall compared to plasma levels [35]. The quasi irreversible feature of binding to its receptors followed by clearance in the pulmonary circulation via endothelin B receptors further reduces plasma levels, and in normal states, there is no or a minimal pulmonary arteriovenous ET-1 gradient. There is quantitatively similar production and release of ET-1 by the lung [32, 36]. ET-1 therefore acts as a para- and autocrine hormone, and it is released continuously mostly from endothelial cells. ET-1 is a major contributor in regulation of vascular tone and has almost unparalleled vasoconstrictive potential [37, 38]. Besides the regulation of vascular tone, ET-1 is pro-inflammatory via induction of cytokines and adhesion molecules such as vascular cell adhesion molecule (VCAN-1), thrombogenic via induction of platelet endothelial cell adhesion molecule (PECAM-1) and mitogenic on VSMC and fibroblasts [39–42].
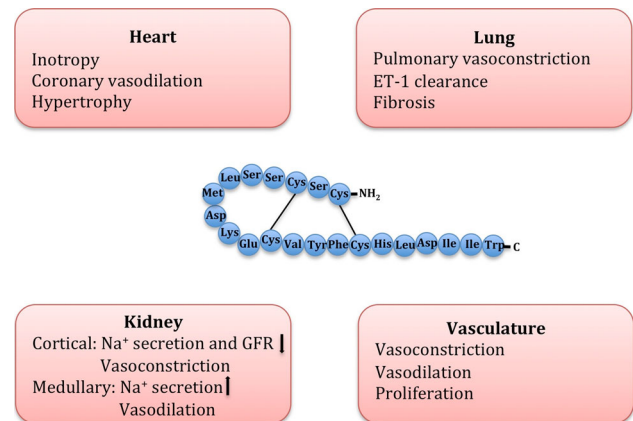

Fig. 1 Endothelin-1 (ET-1) effects on heart, lung, kidney and vasculature. *GFR* glomerular filtration rate

ET-1 exerts positive inotropic effects in healthy hearts, whereas its role in contractility in dilated or hypertrophied hearts is debated [43]. However, it is known that ET-1 triggers cardiac hypertrophy via several pathways including cell cycle regulator mitogen-activated protein kinase (MAPK), vascular growth factor and tumor growth factor [44–46].

In the kidney, ET-1 controls glomerular filtration rate but it is debated whether ET-1 predominantly contracts the afferent or efferent arterioles and multiple studies investigated the role of ET-1 on water and sodium reabsorption. Vignon-Zellweger et al. [38] recently reviewed the topic and concluded that ET-1 constricts arterioles via ETA and thereby reduces renal and cortical blood flow but on the other hand enhances medullary blood flow via ETB. Speed et al. also concluded that ET-1 has different effects depending upon the area of production within the kidney. Cortical ET-1 not only causes hypertension via increase in vascular resistance and reducing glomerular filtration but also increases sodium reabsorption. In contrast medullary ET-1 has anti-hypertensive effects via direct inhibition of sodium reabsorption in the collecting duct and the mentioned increased blood flow via ETB [47]. Figure 1 summarizes ET-1 effects.

The various major roles of endothelin-1 in cardiovascular pathologies will be discussed later in this review.

Endothelin receptors

The endothelin receptors A and B (ETA, ETB) were first described in 1990 by Arai et al. [48] and Sakurai et al. [49], respectively. The receptors are members of the heptahelical G-protein-coupled receptor superfamily, range from 45 to 50 kDa in size [19] and are encoded by distinct genes

located on chromosomes 4 and 13 [50]. EDNRA is encoding for ETA and maps to chromosome 4q31.22, includes over 63.97 kb of genomic DNA and eight exons with 4 major haplotype blocks [11]. EDNRB encoding for ETB is located on chromosome 13 and has 7 exons and 6 introns; it is 442 amino acid long and has about 59 kDa molecular mass [51]. The receptors are characterized by 60 % amino acid identity and high preservation across mammalian species (85–90 %) [19, 50]. ETA and ETB consist of a long extracellular amino-terminal domain, seven loops of membrane-spanning domains, and an intracellular carboxy-terminus. The C-terminal tail and the third cytoplasmic loop contain several putative phosphorylation sites. The Asp–Arg–Tyr motif in the second cytoplasmic loop is highly conserved among species and is thought to be involved in coupling to G-proteins [32, 52].

Endothelin receptors expressed on vascular smooth muscle cells (VSMC) have synergistic effects and mediate vasoconstriction and cell proliferation when stimulated [53–56]. ETA is primarily localized on VSMC [57] in systemic and pulmonary vessels, whereas ETB is expressed on endothelial cells and on VSMC. Depending on the effector cell type, two ETB functions are known: vasoconstriction on VSMC and vasodilation on endothelial cells. Under physiological conditions, stimulation of ETB has a mild vasodilatory effect outweighing its vasoconstrictor potential. However, in pathological states, this ratio can be altered in [58, 59]. The receptors have the highest expression in lung and heart but have also been identified in numerous tissues including kidney, intestine, adrenal gland, eye and brain [55]. Within the lung, ETA and ETB are mainly located on smooth muscle cells of alveolar walls with predominant ETB [60], whereas in the large pulmonary arteries, ETA is predominant with an increasing portion of ETB in peripheral smaller vessels [61]. The predominant ET-1 has similar affinity to both ETA and ETB, while the affinity of ET-2 and ET-3 varies among receptors [48, 49]. ETA and ETB are G-protein coupled, and activation by ET-1 leads to activation of phospholipase C (PLC). PLC then leads to formation of inositol triphosphate (IP3) and diaglycerol (DAG). Intracellular Ca^{2+} is increased via stimulation of IP3 receptors from the reticulum as well as opening of dihydropyridine-sensitive voltage-gated transmembrane Ca^{2+} channels resulting in vasoconstriction [55]. DAG leads to phosphorylation of caldesmon (CAD) via protein kinase C and thereby increases the myofilament sensitivity to Ca^{2+}. DAG can also lead to activation of proto-oncogenes such as the MAPK cascade [39, 62].

Stimulation of ETB receptors located on endothelial cells leads to activation of phosphoinositide 3-kinase (P13 K) and downstream activation of protein B kinase/ Akt [63]. The P13 K/Akt pathway is responsible for activation of endothelial nitric oxide synthase (eNOS). NO on the other hand antagonizes ET synthesis via inhibition of prepro-ET-1 transcription [64]. Other vasodilatory effects following ETB activation are Ca^{2+}/calmodulin dependent, cyclooxygenases, prostacyclins and voltage-dependant Ca^{2+} channels [65–69]. Figure 2 summarizes endothelin-1 synthesis and receptor signaling.

Selective blockade of ETA enhances the NO-mediated vasodilation by ETB [70]; selective blockade of ETB consequently leads to vasoconstriction [71]. To achieve optimal inhibition of vasoconstriction in isolated human small pulmonary arteries, combined blockade of both ETA and ETB is required [72]. However, under normal circumstances, ETB does not seem to contribute significantly to pulmonary vascular tone [73, 74], and ETB removal of the endothelium does not affect ET-1 induced vasoreactivity significantly [73]. ETB induces significant vasodilation in coronary arteries of healthy pigs but not in diseased explanted human heart suggesting that under pathological circumstances, the vasodilatory role may be lost, and it even has been reported that stimulation of ETB might be detrimental via inducing cell adhesion and contraction [75, 76].

It has recently been observed that endothelin receptors form homo- and heterodimers in vitro. This could partially explain the complexity of ET-1 responses. The types of dimers (ETA/ETA, ETA/ETB, or ETB/ETB) are associated with different binding density [77]. Also there is a different functional response depending on whether homo- or heterodimers are formed: the binding of ET-1 to homodimers induces a transient increase in Ca^{2+} concentration, while the response mediated by heterodimers has been shown to last for several minutes [78].

Following this short review on the endothelin system, we will discuss its role within three major cardiovascular pathologies and especially the potential importance of endothelin receptor polymorphism: pulmonary hypertension, systolic heart failure and dilated cardiomyopathy as well as systemic arterial hypertension.

Pathophysiology of the endothelin system and receptor polymorphism in pulmonary artery hypertension

Endothelin system in pulmonary artery hypertension and right ventricular adaption

Pulmonary hypertension (PH) is a multifactorial disease leading to an increase in pulmonary vascular resistance secondary to endothelial dysfunction, vasoconstriction, arterial wall thickening and thrombosis [79]. PH is defined as a sustained increase in pulmonary arterial pressure to more than 25 mmHg at rest with a mean pulmonary
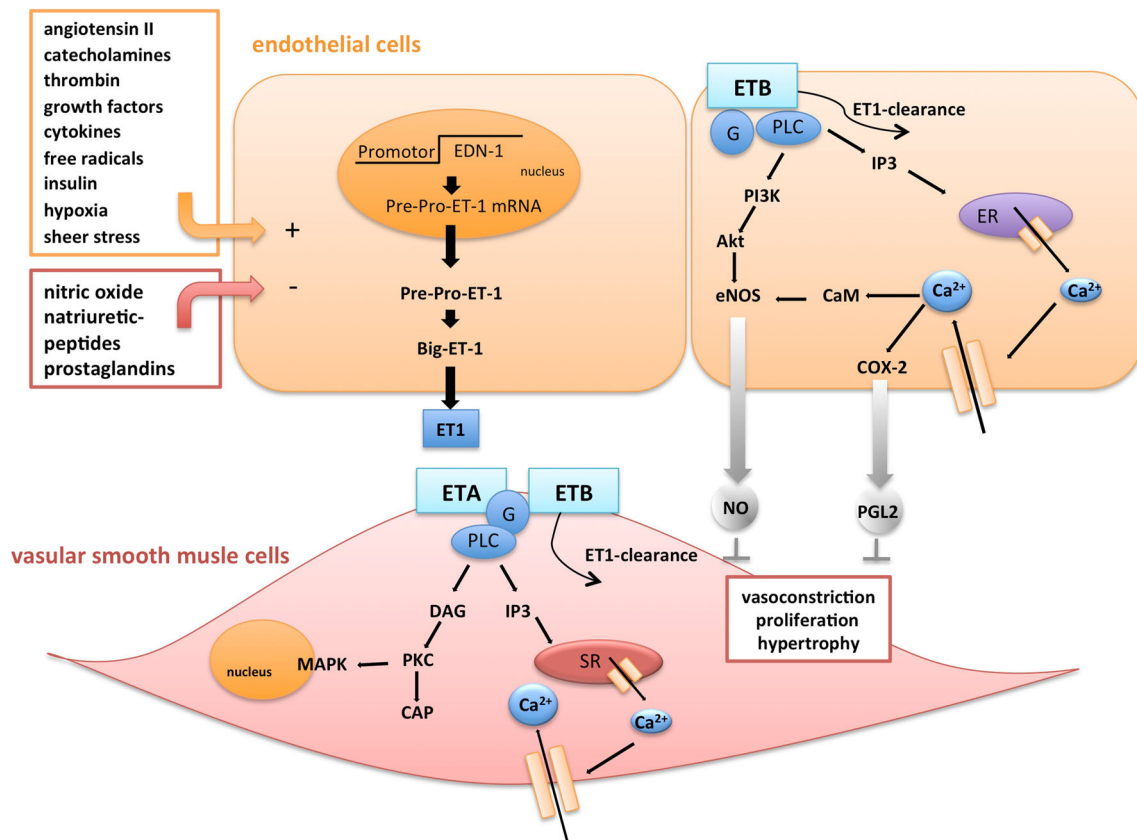
Fig. 2 Endothelin-1 synthesis and signal conduction via its receptors. *EDN-1* endothelin-1 gene, *ET-1* endothelin-1, *ETA* endothelin receptor A, *ETB* endothelin receptor B, *G* G-protein, *DAG* diacylglycerol, *PKC* protein kinase C, *CAP* calponin, *MAPK* mitogen-activated protein kinase, *IP-3* inositol 1,4,5-trisphosphate, *SR* sarcoplasmaic reticulum, *NO* nitric oxide, *PGL2* prostaglandin-2, *P13 K* phosphoinositide-3 kinase, *eNOS* endothelial nitric oxide synthase, *CaM* calmodulin, *COX-2* cyclooxygenase 2

capillary wedge pressure (PCWP) of less than 15 mmHg. The revised WHO classification of PH forms five groups: group I pulmonary artery hypertension (PAH) that includes idiopathic (IPAH), familial (FPAH) and associated with (APAH) such as congenital heart disease and connective tissue disease, drugs and toxin induced, human immunodeficiency virus (HIV), portal hypertension, hemoglobinopathies and myeloproliferative disorders. Group II is classified secondary to left heart disease; group III is associated with lung diseases and/or hypoxemia; group IV is due to chronic thrombotic and/or embolic disease and group V includes miscellaneous causes [15]. Pulmonary hypertension due to left heart failure is much more common than group I PAH and can result from any pathology leading to increased left heart filling pressures, including systolic dysfunction, diastolic dysfunction and valvular heart disease [15]. This review will focus on group I PAH.

The physiological interplay between ET-1, ETA and ETB is significantly altered in PH with increased pulmonary vascular tone. Under such circumstances, the vasodilatory role of ETB seems to be of significance and can be unmasked with selective ETB stimulation with very low dose ET-1 or specific ETB blockade. With higher ET-1 concentrations, the mild vasodilatory effect of ETB is overcome by vasoconstriction [73, 74].

Stewart et al. [80] described higher serum levels of ET-1 and higher arterial-to-venous ratios in patients with IPAH. Lung tissue from PH patients shows altered endothelin receptor expression, increased ET-1 staining and increased pro-pre-endothelin-1 (PPET-1) in pulmonary arteries with a correlation between the amount of ET-1 staining and hemodynamic parameters such as pulmonary vascular resistance [7, 81]. It is unclear whether this phenomenon results from increased ET-1 production, reduced pulmonary clearance or a combination [82]. Serum levels of ET-1 are markers of disease severity and are of prognostic value in pulmonary artery hypertension [80].

Endothelin converting enzymes are necessary to cleave ET-1 from its less active form. In patients with PH, increased levels of endothelin converting enzyme have been found [83]. Besides the idiopathic form, increased ET-1 levels have also been described for other pathologies. In pulmonary hypertension from chronic hypoxia, increase

in ETA was found and treatment with combined ETA/ETB receptor blockade led to amelioration of pulmonary hypertensive changes [84, 85]. Congenital heart disease and chronic thrombembolic pulmonary hypertension are also associated with increased activity of the ET-1 system [82].

PH leads to significant increase in right ventricular (RV) afterload. The upregulated myocardial endothelin system in PH could represent a compensatory mechanism to adapt RV contractility following increased afterload [86]. The RV has to adapt resulting in concentric hypertrophy away from its crescent shape under physiological conditions leading to flattening of the interventricular septum [87]. These adaptive changes of the RV lead to development of left ventricular diastolic dysfunction secondary to the flattened septum. The consequently reduced left ventricular end-diastolic volume contributes to the reduction in stroke volume. Without effective measurements to lower the afterload, the RV will progress to dilation and failure. The reduced perfusion then can lead to RV ischemia even in the absence of coronary artery disease [88]. It is unclear why some patients with PH can maintain normal cardiac outputs over long time periods, while other patients have rapid decompensation and death [87]. The ETA and ETB antagonist bosentan and the selective ETA antagonist ambrisentan are approved for use in group I PAH. In animal studies, endothelin receptor antagonists (ETRAs) are effective in reducing pulmonary artery resistance and vascular remodeling. Bosentan was studied in patients with scleroderma-induced pulmonary hypertension resulting in improvement of 6-minute walk test, improvement of pulmonary hemodynamics, especially cardiac index and pulmonary vascular resistance and increased time to clinical worsening [89]. Following this primary study, the BREATHE-1 trial for PAH associated with connective tissue disease confirmed the initial findings [90].

It has been reported that endothelin receptor antagonists can have a negative inotropic effect in hypertrophied right- and left ventricle and therefore could partially explain the increased rate of edema upon initiation of ETRA therapy [86].

However, it remains unclear why a certain percentage of patients do not respond to endothelin receptor antagonist therapy.

Endothelin receptor polymorphism in PAH

Calabro et al. were the first to study endothelin receptor polymorphisms in idiopathic pulmonary arterial hypertension (IPAH, 34.7 % of patients), PAH due to congenital heart disease (CHD) with systemic to pulmonary shunts (43.9 % of patients) and PAH due to connective tissue disease (CTD, 21.4 % of patients) in a total of 98 Caucasian patients and 100 aged BSA-matched healthy controls. The authors investigated the role of two polymorphisms:

- EDN1: +134del/insA 1
- EDNRA: His323His

For both polymorphisms, no substantial difference in genotype and allele distribution was identified between the PAH and the control group. When analyzed for subgroups, the main finding of the study is an association between His323His TT-genotype in EDNRA and development of IPAH (the TT subtype is significant more frequently expressed in IPAH with 73.5 % carriers compared to CHD or CTD especially when homozygotic for TT compared to TC). In the overall, IPAH cohort patients with the TT-genotype showed higher pulmonary resistances (2 ± 0.6 vs. 2.3 ± 0.6; $p = 0.05$) and a higher pulmonary vascular resistance index (18.8 ± 9.6 vs. 14.2 ± 6.9; $p = 0.01$). A cardiac index < 2 l/min/m^2 is a marker for poor prognosis in PAH [91]; the authors therefore hypothesize that patients with IPAH with the TT-genotype have a more aggressive disease. There was significant correlation of idiopathic disease ($p = 0.01$; OR = 3.8; CI = 1.3–11) and indexed pulmonary vascular resistances ($p = 0.01$; OR = 1.1; CI = 1–1.2) with the TT-genotype. However, no clinical correlation was found in-between the TT-genotype in IPAH likely secondary to limited sample size [92].

Previously it has been reported that the +134ins/delA polymorphism correlates with levels of endothelin-1 ex vivo; however, this was not confirmed in this study with PAH [10]. There was no difference in the +134del/insA polymorphism among different groups of patients [92]. This study of Calabro included 200 age-matched controls but the diversity of the patient's conditions might limit the investigations power as concluded by Cattano et al. [93]; however, the study suggests a possible role for the EDNRA polymorphism in the pathogenesis of PAH but lacks demonstration of an experimental functional role.

The physiological effects of the TT-phenotype are unknown but could include altered gene expression and modified post-transcriptional processing. The polymorphism also has been studied in DCM, and it was hypothesized that His323His could affect splicing leading to production of an altered receptor. Furthermore, the His323His variant might be in linkage disequilibrium with another functional variant [9, 92].

There could be a potential link between specific genotypes in the EDNRA gene and susceptibly for PAH with worse hemodynamic profiles [92]. A failing RV is a major prognostic factor in PH. At this point, it is unclear why the compensatory capacity of the RV is so different among patients with PH.

ETRA therapy in light of pharmacogenetics with focus on barriers for clinical practice

Possibly the described polymorphisms of ETA are linked to responsiveness to endothelin receptor antagonist treatment and could thereby in part explain the different degree of RV recovery or compensation following initiation of therapy. Tailored therapy in light of pharmacogenetics therefore could potentially improve treatment results.

Furthermore, ETRAs have been reported to be negative inotropic in hypertrophied RV and patients with relatively worse RV hypertrophy could have polymorphism promoting stronger hypertrophic effects and therefore would be more sensitive to negative inotropic effects of ETRA therapy.

However, effects of endothelin receptor polymorphisms on cell signaling have not yet been studied. The complex regulation and function of the endothelin system in physiology and pathology remains poorly understood, and therefore, substantial research is required to clarify the relevance of these polymorphisms in human disease especially with focus on potential therapeutic implications. In spite of its promising concept, pharmacogenetics is not yet used widely in clinical settings. Johnson and Cavallari have extensively reviewed current implications of pharmacogenetics with special emphasis on cardiovascular disease [94].

Cardiovascular pharmacogenetics used to focus on implications for already existing therapies but now starts to involve in new drug development as the concept of personalized medicine is emerging [94]. Thus, pharmacogenetics may be used to predict response to therapy as well as drug toxicity. However, to date general acceptance of pharmacogenetics in clinical practice remains low. There are knowledge barriers such as unawareness, uncertainty how to interpret pharmacogenetic test results and necessary actions based on these results. Furthermore, logistical barriers such as turning around time when genetic information is delivered after a treatment decision was made as well as financial barriers of genetic testing. Finally, evidence barriers represent a challenge for pharmacogenetics given lack of randomized controlled trials proving improved outcome following a pharmacogenetic approach. The authors then discuss the following solution approaches: It has been suggested to obtain genetic information at one point in life and then keep available for future use to eliminate turn round time. This is also more cost-effective than genotyping for single nucleotide polymorphisms (SNP) or one gene at a time. In terms of evidence barriers, it has been argued that randomized controlled trials may not be an absolute requirement but rather the little likelihood of causing harm with pharmacogenetics compared to potential benefit [94].

In summary, pharmacogenetics has made great progress in the last decade and the implementation of genetics to tailor therapy in clinical practice appears very promising once above discussed barriers have been overcome. Implementation of endothelin receptor polymorphisms as concept of personalized medicine could be one future example.

Pathophysiology of the endothelin system and receptor polymorphism in dilated cardiomyopathy and heart failure

Endothelin system in systolic heart failure

Dilated cardiomyopathy (DCM) is characterized by biventricular chamber enlargement and systolic dysfunction that can result in progressive systolic heart failure. DCM is frequently caused by acquired factors such as ischemia, infectious agents, autoimmune disorders, alcohol excess, chemotherapeutic drugs or nutritional deficiencies [95].

The presence of left ventricular dilation and systolic dysfunction when other causes of dilated cardiomyopathy are ruled out are the hallmarks of idiopathic dilated cardiomyopathy (IDC) [96]. Inherited genetic variations form the subgroup of familial dilative cardiomyopathy (FDC). The genetic base includes a variety of genes coding for proteins compromising the nuclear envelope, the cardiac sarcomere, ion channels, transcription factors and the dystrophin-associated cytoskeletal complex [97]. DCM then potentially leads to heart failure where the endothelin system has been studied in the last two decades.

In systolic heart failure, not only plasma ET-1 levels are increased but also the myocardial endothelin axis with increased receptor expression and endothelin synthesis [5, 98]. Plasma levels of ET-1 and its precursor ET-1 are independent predictors of outcome in systolic heart failure [99, 100].

ETA and ETB receptors are expressed on cardiomyocytes and coronary vasculature. Cardiomyocytes express predominately ETA, while cardiac fibroblasts express both receptor subtypes equally [101]. ET-1 induces myocardial hypertrophy and remodeling via multiple routes including MAPK, vascular endothelial growth factor and TGF-beta pathways [44–46]. Bosentan has been shown to reduce cardiac fibrosis and hypertrophy in systolic heart failure [102, 103]. On the other hand cardioprotective effects of ET-1 have been described. Zhao et al. reported that ET-1 might prevent excessive apoptosis after cardiac overload following aortic banding [38, 104].

The effect of ET-1 and ETRAs on contractility is debated and seems to differ in healthy, hypertrophied and failing hearts [38].

- In **healthy hearts**, ET-1 has a small positive inotropic effect mediated by an increase in intracellular calcium

and increased sensitivity of myofilaments to calcium via the PLC/IP3 pathway as discussed earlier [105, 106]. This small positive inotropic effect is not affected by bosentan in healthy hearts [102].

- In **hypertrophied hearts**, Piuhola et al. [102] describe a significant role for ET-1 in maintaining cardiac function. In this setting, ET-1 and ETA are significantly upregulated and blocking the endothelin system has significant negative inotropic effects. ETRAs have negative inotropic effects in hypertrophied RV [86].
- In **systolic heart failure**, McCarthey et al. [43] also described a negative inotropic effect of ET-1 that was antagonized by use of a specific ETA blocker. ET-1 has also been described as negative inotropic via ETB receptors and consecutive activation of Na^+/Ca^+ exchanger that is known to be overexpressed in systolic heart failure [107, 108].

The described negative inotropic effect of endothelin receptor antagonists in hypertrophied LV and RV could explain the increased rate of edema upon initiation of ETRA therapy in 25 % of patients with group I PAH [86, 102]. Despite a significant reduction in pulmonary artery pressure, there was no improvement in cardiac index described after ETRA use possibly explained by negative inotropy in this setting [109].

At this point, it is unclear why edema develops in a certain percentage of patients on ETRA therapy and potential effects on the RV need to be studied in human and experimental settings [86].

Endothelin receptor polymorphism in DCM and heart failure

Based on the evident role of the endothelin system in cardiovascular pathology, genetic variations have been studied [110, 111]. The first study on an association between endothelin receptor polymorphisms and DCM was part of the CARDIGENE group studies to identify genetic risk factors for idiopathic dilated cardiomyopathy. The following polymorphisms were investigated in French Caucasian patients [112]:

- EDN-1: +138A insertion on exon 1, +61G/T polymorphism on exon 5
- EDNRA: −231A/G and +1363 C/T on exon 8
- EDNRB: +30 A/G on exon 4

A total of 433 patients with DCM LVEF < 40 % and 400 age- and gender-matched controls were included in the study. The ET-1 and ETB polymorphism did not reveal significant results but analysis of the exon 8 +1,363 C/T polymorphism in the endothelin A gene (EDNRA) showed significantly different genotype frequencies in patients and controls ($p = 0.02$) as well as distribution of allele frequencies ($p = 0.045$). This association with the T allele and the disease was secondary to an increased frequency of T/T homozygoty among DCM patients. Therefore, the polymorphism seems to have a recessive mode of action in idiopathic dilative cardiomyopathy. The odds ratio for the disease in patients with the T/T genotype in EDNRA was 1.9 (95 % CI 1.2–3.01; $p < 0.006$). While there was significantly increased risk in the development of DCM, there was no association between the polymorphism (CC, CT and TT) and disease severity as assessed by LVEF, left ventricular dilation or cardiac transplantation [112].

Following the identification of EDNRA polymorphism as a risk factor for the development of DCM, Herrmann et al. investigated differences in DCM (LVEF < 56 %) related cardiac phenotypes and/or clinical outcomes. Authors genotyped 125 patients with idiopathic DCM for the following polymorphisms:

- EDN-1: T1370G, K198 N (G/T), +138ex1 ins/del
- EDNRA: H232H (C/T), G+70C
- EDNRB: L277L (G/A)

The EDNRA polymorphism His323His was found to be associated with a significantly shorter cumulative survival despite no baseline differences in the severity of heart failure [9]. The odds ratio to die within 2 years was 5.5 (95 % CI 1.4–2.1, $p = 0.013$) for T allele carriers. The His323His polymorphism was identified as predictor of survival independent of the known predictors NYHA class, left ventricular function and end-diastolic diameter [9].

Colombo et al. then for the first time studied the effect of two-combined polymorphisms in EDN-1 and EDNRA in 122 Italian patients with systolic heart failure LVEF < 40 % of ischemic or idiopathic etiology:

- EDN1: Lys198Asn (G/T)
- EDNRA: His323His (T/C)

In the present study, the ET-1 Lys198Asn polymorphism in patients homozygotous for Asn/Asn represents an OR of 4.3 (95 % CI 1.7–11.1, $p = 0.001$) for systolic heart failure. The percentage of Asn/Asn in the systolic heart failure group was 13.1 % versus 3.6 % in the control group ($p = 0.005$), and Asn/Asn was identified as an independent risk factor for the development of systolic heart failure ($p = 0.03$) [113]. The His232His polymorphism was found to be distributed equally among the groups what seems to be contradictory to the description of His323His as an independent risk factor for survival in DCM. This might be related to the chosen control group of patients hospitalized for elective valve replacements but LVEF > 50 %. However, Lys198Asn and His323His together were found to be a major risk factor for the development of systolic heart failure. After stratification for His323His, the Lys198Asn

was significantly associated with the presence of heart failure in T/T carriers (43.7, 35.9, 20.3 vs. 60.0, 37.0, 3.0 %, $p = 0.0009$ for Lys/Lys, Lys/Asn, Asn/Asn, patients vs. controls) with an OR of 8.6 (95 % CI 1.5–48.1, $p = 0.005$). There were no differences in allele distribution among ischemic and idiopathic etiology of systolic heart failure [113]. The Asn/Asn SNP was also associated with significant lower LVEF 48.0 ± 0.8 vs. 39.8 ± 2.3 %, $p = 0.0006$) but no significant difference in left ventricular end-diastolic diameter (LVDD) and left ventricular end-systolic diameter (LVSD). When both EDN-1 Asn/Asn and EDNRA T/T SNPs were simultaneously present, LVEF and LVDD/LVSD were significantly reduced compared to Lys and C-allele carriers. Asn/Asn and TT double genotype was found to be an independent predictor of LVEF [113].

Genetic variations of the endothelin system seem to play an important role in development and deterioration of systolic heart failure although the exact mechanisms are not understood mainly in lack of functional studies. The identified SNP Lys198Asn leads to G/T transversion and a change in amino acid sequence that is, however, not located in the regulatory region of EDN-1 but in a region coding for preproendothelin-1. Colombo et al. also conclude similar to Hermann et al. that the SNP might not be functional itself but could be in linkage disequilibrium with a functional polymorphism located within the regulatory side [113]. Lys198Asn has previously been associated with higher ET-1- and blood pressure levels but controversially did not directly affect big-ET-1 production [114–116]. Therefore, the real functional significance of the SNP remains unclear [113]. His323His is located on the sixth exon of the EDNRA gene that codes for the sixth membrane-spanning domain and the third extracellular loop of the protein. The polymorphism substitutes a thymine (T) for a cytosine (C) which does not alter the amino acid sequence in contrast to the Lys198Asn. However, His323His could influence gene transcription or affect splicing leading to production of an altered receptor but as per Herman et al. [9] the existence of another functional variant is more likely. At this point, there are only clinical association studies available for both the +1,363 C/T on exon 8 and the His323His SNP in EDNRA.

It is unknown how a potentially altered receptor expression would influence ET-1 signaling in the cardiovascular system especially in pathologic states. His323His was not identified as a risk factor for the development of systolic heart failure but as an independent prognostic marker for survival in DCM [9]. Particularly interesting is the greater combined effect of Lys198Asn and His323His polymorphism with significantly higher risk of development of HF and lower LVEF representing two altered steps in endothelin effect: ET-1 production and receptor activation [113]. Genetic variations of the endothelin system

therefore are not only associated with the risk of development of systolic heart failure but are of prognostic value raising the question of potential therapeutic interventions.

Charron et al. [112] discuss that a possible loss of function of ETA caused by polymorphisms could have negative effects in systolic heart failure when the endothelin system might be needed to maintain cardiac function. On the other hand, ETRA therapy can ameliorate DCM phenotypes and improve cardiac function and survival in experimental settings supporting a gain of function theory [117, 118].

The paradox disappointment of ETRAs in systolic heart failure and the potential role of endothelin receptor polymorphisms

The failure of ETRAs in clinical use for heart failure seems paradox to date especially given initial extensive evidence for potential benefit. In 1997, Muldner et al. used bosentan in a post-infarction rat model of heart failure and showed markedly increased survival. They described decrease in preload and afterload as well as an increase in cardiac output, decreased left ventricular (LV) hypertrophy, LV dilatation and cardiac fibrosis. The speculation was made that chronic treatment with ETRAs might be beneficial in systolic heart failure and could improve long-term survival [103]. Following this, one small human study used bosentan as adjunct to established heart failure therapy and was able to show short-term benefits on hemodynamic parameters [119]. Unfortunately, these very promising results could not be translated in clinical long-term use. Two large trials on the use of bosentan in symptomatic systolic heart failure were prematurely stopped because of an increase in adverse events and no improvement of symptoms [12, 13]. Following this, the non-selective intravenous antagonist tezosentan and ETA selective darusentan both demonstrated favorable hemodynamic effects with increased cardiac index and reduced pulmonary and systemic vascular resistance in a dose dependent manner in heart failure [120, 121]. However, both drugs did not provide any beneficial effect in chronic heart failure, and specific ETA-receptor antagonists seem to have the same lack of efficiency as non-selective drugs [122, 123].

While ETRAs disappointed for use in systolic heart failure, there was rising evidence for beneficial effects in pulmonary hypertension and a possible benefit from ETRAs was suspected for patients with pulmonary hypertension from left ventricular dysfunction [82]. In 2008, disappointing results were published; bosentan did not provide any measurable hemodynamic benefit and was associated with more frequent adverse events, requiring drug discontinuation in this subgroup of patients [123].

Table 1 Polymorphism of the endothelin receptor A in cardiovascular pathology

SNP	Located	Reference	Studied pathology and effect
H323H (C/T)	EDNRA exon 6	Calabro et al. [106]	PAH:
			T/T phenotype correlates with IPAH OR 3.8 (95 % CI 1.3–11; $p = 0.01$) and PVRI OR 1.1 (95 % CI 1.0–1.2; $p = 0.01$)
			T/T phenotype associated with higher PVR (18.8 ± 9.6 vs. 14.2 ± 6.9; $p = 0.01$) and lower CI (2 ± 0.6 vs. 2.3; $p = 0.05$)
		Herrmann et al. [12]	DCM:
			T/T phenotype is independent prognostic predictor with significant worse cumulative survival
			OR for death in 2 years 5.5 (95 % CI 1.4–2.1; $p = 0.013$)
H323H (C/T)/ Lys198Asn	EDNRA exon 6 EDN-1 exon 5	Colombo et al. [126]	DCM (ischemic/idiopathic):
			Homozygotous state for both Asn/Asn and T/T associated with OR of 8.6 (95 % CI 1.5–48.1, $p = 0.005$) for DCM, significantly lower LVEF, significantly higher LVDD and LVSD
+1,363 (C/T)	EDNRA exon 8	Charron et al. [125]	DCM:
			T/T phenotype associated with increased risk of DCM
			OR 1.9 (95 % CI 1.2–3.01; $p < 0.006$)
			No association to disease severity
		Nicaud et al. [124]	HTN:
			T/T phenotype associated with higher SBP $p = 0.043$ and pulse pressure $p = 0.004$
		Benjafield et al.	HTN:
			C-allele associated with hypertension OR 1.6 (95 % CI 1.1–2.4; $p = 0.019$)
rs 5335 (C+70G)	EDNRA exon 8	Rahman et al. [15]	HTN:
			SNP associated with higher DBP at night, OR for HTN 1.19 (CI 1.00–1.41) per G-allele, each C-allele is associated with 1.5 % lower MAP at night
			The SNP counts for 2.5 % of genetic BP variation
		Nicaud et al. [124]	HTN:
			G-allele associated with higher systolic BP ($p = 0.008$)

SNP single nucleotide polymorphism, *PAH* pulmonary arterial hypertension, *IPAH* idiopathic pulmonary arterial hypertension, *PVRI* pulmonary vascular resistance index, *CI* cardiac index, *DCM* dilated cardiomyopathy, *HTN* hypertension, *DBP* diastolic blood pressure, *LVDD* left ventricular end-diastolic diameter, *LVSD* left ventricular end-systolic diameter, *MAP* mean arterial pressure, *SBP* systolic blood pressure

Kaluski et al. used the term "the hemodynamic paradox" for the ETRA group. These drugs have impressive initial effect on hemodynamics but no measurable long-term effect in heart failure. In addition, there is no sustained improvement in morphological or functional cardiac parameters [123]. In the majority of studies, both hemodynamic and toxic ETRA effects were directly dose related and after investigating wide dose ranges investigators chose lower doses to avoid toxicity. Directly following ETRA use, there is immediate endothelin overproduction resulting in significant increase in serum levels that usually resolves within hours [124]. Described adverse effects of ETRA use such as anemia, lower extremity edema and hypotension do not seem to be related to direct toxic effects of increased endothelin or potential interaction with neurohormonal and inflammatory pathways. Kaluski et al. speculated that the drugs or their metabolites might have direct drug toxicity such as headaches associated with high dose tezosentan therapy [123].

In 2011, Handoko et al. wrote an editorial named "The rise and fall of endothelin receptor antagonists in congestive heart failure" addressing the paradox of the failure of ETRAs to provide any clinical benefit despite promising results in multiple animal studies [14].

The authors especially commented on the results of Jiang et al. who have conducted similar experiments on the use of bosentan in an animal model of ischemic heart failure as published in 1997 by Muldner et al. Controversially, they were not able to show any benefit from bosentan in cardiac as well pulmonary remodeling and no improvement of RV hemodynamic parameters was shown in ischemic heart failure [125]. Handoko et al. [14] postulate that these results probably will end the discussion on the role of ETRAs in systolic heart failure. However, there is a recent report on 17 patients with NYHA III–IV symptoms treated with bosentan for pulmonary hypertension from left ventricular failure. Nine patients responded

to therapy with improved hemodynamics and 4 became eligible for heart transplantation and 3 for left ventricular assist device implantation. In 6 patients, bosentan was discontinued because of hypotension, increased liver enzymes or acute decompensation of heart failure. The authors concluded that Bosentan decreased pulmonary pressures and PVR in the majority of patients with PH secondary to systolic HF, thereby allowing them to be considered candidates for heart transplantation [126].

Nonetheless unfortunately ETRAs have failed to be beneficial in large clinical trials leading to even more adverse outcomes in heart failure patients treated with ETRA possibly secondary to too high dosages but the exact reason for the failure of ETRAs in systolic heart failure is unknown to the best of our knowledge [122, 127].

However, EDNRA polymorphisms could potentially identify patients who could profit from ETRA therapy to antagonize an over active endothelin system in support of the gain of function theory. Nevertheless, this is only a speculative thought pending functional studies on the actual effect of EDRNA polymorphisms in receptor function and cell signaling in response to ET-1.

Although in a different setting, the endothelin-1 Lsy198Asn polymorphism has recently also been associated with transplant free survival in children with hypoplastic left heart syndrome [128]. Therefore, polymorphisms of the endothelin system could be useful as screening tools to identify high-risk patient populations and potentially identify those who might benefit from endothelin receptor antagonist therapy.

Table 1 summarizes identified endothelin receptor polymorphism and their clinical implications.

Pathophysiology of the endothelin system and receptor polymorphism in essential hypertension

Endothelin system in essential hypertension

Essential hypertension is defined as increased blood pressure of unknown etiology leading to a higher risk of cardiac, renal and cerebral events [129]. Interestingly, ET-1plasma levels were found to be predictors for the development of hypertension in a Japanese population [130]. African American hypertensive patients are known to have a salt-sensitive hypertension with particularly activated endothelin system [131].

ET-1 is a very strong vasoconstrictor and EDNRA was found to be overexpressed in arteries of hypertensive patients, and increased ET-1 production in hypertension might lead to an enhanced EDNRA-dependant vasoconstriction and consequently ET-1-induced hypertrophic remodeling of small arteries [2, 132–134]. Thus, dual

endothelin receptor blockade has been shown to decrease peripheral vascular resistance and blood pressure in healthy humans [135] but selective receptor blockade leads to different effects depending on the model of hypertension in animal studies [136, 137]. In vascular tissue of deoxycortisone acetate (DOCA)- salt hypertensive rats and Dahl salt-sensitive rats, ET-1 protein and mRNA were found to be increased contributing to the development of hypertension [138, 139]. ETA-receptor antagonist therapy and non-selective ETA/ETB-therapy have been shown to prevent hypertension induced tissue injuries in salt-sensitive animal models [140, 141].

In a different mouse model of proendothelin-1 overexpression, hypertrophic remodeling of the vascular wall was characterized by increased inflammation caused by enhanced expression of vascular cell adhesion molecules, presence of macrophages and activation of pro-inflammatory transcription factors promoting endothelial dysfunction [142]. Interestingly, these mice have increased aortic expression of ETB as compensatory mechanism and no significant hypertension. Upon exposure to a high-salt diet, these mice have a greater increase in blood pressure than wild-type mice and worsened endothelial dysfunction. These effects are lessened with ETA antagonists and worsened with ETB antagonists. This study clearly shows the different effect of ETA and ETB on vascular tone and enhances the important role of ET-1 in vascular pathology [143].

Endothelin receptor polymorphism in hypertension

About 20–40 % of the total variation in blood pressure is attributed to genetic influences especially at night [11, 144]. Rahman et al. have studied common genetic variations of EDNRA in ambulatory blood pressure. One common single nucleotide polymorphism (SNP) was selected from each of the 4 EDNRA haplotype blocks:

- rs1801708 (referred to in previous studies as G-231A) from 5'untranslated region
- rs1568136; from intron 2
- rs5333 SNP (referred to in previous studies as H323H) from exon 6
- rs5335 SNP (previously referred to as C p 211GorC p 70) from exon 8 [11].

There was a significant association between the rs5335 (C+70G) SNP and mean and diastolic blood pressure (DBP) during night and marginally less significant association with daytime systolic blood pressure (SBP), DBP and mean pressure. These associations appeared to be codominant with each C-allele counting for a percentage of lower blood pressures especially at night. Each of the phenotypes had a 1–2 % contribution to the variability of

overall blood pressure what appears to be a rather small number. The CC genotype was associated with 34.7 % of hypertension within the carriers, the CG genotype with 37.9 % and the GC genotype with 42.4 %. The odds ratio for hypertension was 1.19 (95 % confidence interval 1.00–1.41; $p = 0.05$) per G-allele carried. This study of Rahman et al. proved that natural variations of the endothelin signal transduction pathway have significant effects on blood pressure; carriers of the SNP have a 20 % higher risk of hypertension [11].

Nicaud et al. studied the C+1363T and rs5335 (C+70 G) among other SNPs in 773 males who where controls in the ECTIM myocardial infarction study. The +1363T SNP C/T substitution was associated with modestly higher SBP ($p = 0.043$) and carrier of the G-allele of rs5335 had a trend toward higher SBP ($p = 0.008$) due to strong association between the two SNP on EDNRA exon 8. No association of other SNPs with hypertension including ETB was found in this study [110]. Benjafield et al. [145] also studied the C+1363T SNP and found an association with hypertension in 155 hypertensive patients and 245 controls.

The endothelins and consequently endothelin receptors are major regulators of vascular tone and as previously addressed ETA is overexpressed in patients with hypertension [133]. Rahman et al. [11] conclude that variation in the 3' untranslated region of EDNRA is a significant determinant of blood pressure possibly via an effect on arterial compliance.

ETRAs: potential role in treatment of hypertension

Bosentan was studied by Krum et al. [146] in hypertensive patients and could achieve blood pressure control similar to treatment with the ACE inhibitor enalapril without activation of the rennin-angiotensin system or the sympatric nervous system. Darusentan, a selective ETA antagonist, has also been proven effective in treatment of essential hypertension without major side effects [147]. Despite the promising results for blood pressure control, ETRAs were not perused for routine treatment for essential hypertension because of potential severe side effects like liver toxicity, pulmonary vascular dilation and development of lower extremity edema also in light of lack of superiority to established regimens like ACE inhibitors [137].

In resistant hypertension, darusentan was used in a small, randomized, placebo-controlled study and resulted in significant blood pressure reduction after 10 weeks of treatment without significant adverse effects [148]. Following this, a larger trial with 379 patients confirmed the findings with substantial reduction in blood pressure with addition of darusentan to triple therapy compared to placebo [149]. Later a phase 3 clinical trial failed to show improvement of office blood pressure over placebo and led

to cessation of darusentan by the manufacturer [150]. The use of ETRAs on essential and resistant hypertension seems to have failed for lack of superiority to established agents like ACE inhibitors and increased overall risk of adverse events.

However, ETRAs might be beneficial in selective groups of patients. For example, hypertension has a very high incidence in patients after solid organ transplantation [151]. Plasma endothelin levels increase after heart and kidney transplantation and are associated with allograft rejection [152, 153]. Myocardial expression of ET-1 is also increased post-transplant and seems associated with ischemic injury and subsequent development of coronary vasculopathy [153]. Calcineurin inhibitors used for immunosuppression also increase endothelin-1 plasma levels contributing to hypertension and promoting renal dysfunction after transplantation [154]. ETRAs have been used in experimental settings after cardiac transplantation with improved survival and reduced vasculopathy independent of immunosuppression [155]. Furthermore, preclinical after renal transplantation ETRAs have been shown to inhibit cyclosporin-induced hypertension and limited end-organ damage [156]. Recently, ambrisentan was proven save for use in combination with tacrolimus and mycophenolate [151].

It is now compelling to speculate if patients carrying endothelin receptor polymorphisms associated with hypertension are especially prone to development of post-transplant hypertension? If so would these patients particularly benefit from ETRA therapy not only in light of hypertension but also protection against nephropathy and vasculopathy?

Conclusion

The endothelin system is of vast importance in cardiovascular physiology and plays major roles in its pathologies. The interplay between endothelins and their receptors varies between cell types and changes in states of pathology. Polymorphisms of EDNRA have been associated with the development of group I pulmonary hypertension, dilated cardiomyopathy and essential hypertension. The polymorphisms are not only risk factors for development of these cardiovascular pathologies but are of prognostic value in systolic heart failure. Awareness for genetic variations therefore is crucial in understanding and treating pathology offering potentially valuable screening tools. The studies conducted to date have several limitations; one major is that they were mainly conducted in Caucasian European populations, and the possibility of interracial differences in relevance of endothelin receptor polymorphisms has not yet been addressed. Endothelin receptor

antagonists are of great value in treatment of group I pulmonary hypertension but unfortunately have not proven to be beneficial in systolic heart failure despite promising preclinical results for incompletely understood reasons. Nevertheless, despite the effectiveness of ETRAs in PAH, it remains unclear why some patients do not respond to treatment. Endothelin receptor polymorphisms might alter response to antagonist therapy, and patients might benefit from tailored therapy in light of pharmacogenetics. ETRAs might be of benefit in patients with systolic heart failure carrying polymorphisms with potentially hyperactive endothelin system, and moreover, endothelin receptor polymorphism might be useful as screening tools for adverse outcome in these patients? The use of ETRAs for post-transplant hypertension might be especially effective in carriers of endothelin receptor polymorphism prone to hypertension?

At this point, basic science studies need to clarify functional effects of endothelin receptor polymorphism to understand how endothelin signaling is altered and clinical studies should evaluate ETRA therapy with respect to pharmacogenetics.

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