Chapter

An Overview of Gene Variants of Endothelin-1: A Critical Regulator of Endothelial Dysfunction

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Abstract

Endothelial dysfunction (ED) is an early marker of development of cardiovascular diseases and is closely related to clinical events in patients with atherosclerosis and hypertension. Endothelin-1 (ET-1), a potent vasoconstrictor, and nitic oxide (NO), a potent vasodilator, produced in endothelial cells are leading molecules which regulate vascular function. Failure of the physiological balance between these two molecules, often aggravated by increased production and biological activity of ET-1, commonly reflects endothelial dysfunction. The role of endothelium-derived small molecules like ET-1 (among many) with diverse biological functions continues to fascinate researchers all over the world both for its evolutionary significance and its translational potential in disease biology. Studies on systems genetics in human endothelial cells have provided evidence supporting the possibility that predisposition to complex disease is manifested through noncoding common genetic variants that modify levels of target gene expression in endothelial cells. These studies highlight the importance genetic variants of regulatory molecules secreted by endothelial cells in health and disease. It is unlikely that a single-nucleotide polymorphism (SNP) would directly cause disease, but it would increase the genetic predisposition of individuals and can affect their responses to drugs and medications. The knowledge gained would help in the risk stratification and clinical management of patients with personalized medicine.

Keywords: endothelial dysfunction, endothelin-1, cardiovascular diseases, single nucleotide polymorphisms, haplotype, precision medicine

1. Introduction

Endothelial dysfunction (ED) is a hallmark of many human vascular diseases [1] like peripheral arterial disease, cardiovascular diseases including atherosclerosis and hypertension, stroke, diabetes, chronic kidney failure, tumor growth, and metastasis. Endothelial dysfunction, like many other multifactorial diseases, is caused by a combination of multiple genetic and environmental factors, a large proportion of which remain unexplained. Individual differences in endothelial function and hence susceptibility to diseases might relate not only to different levels of exposure

to risk factors but also to differences in the presence of different risk alleles of genes expressed in vascular endothelium, in different individuals [2]. Genetic regulation of variation in vascular function in different individuals is poorly understood and is largely mystifying. The genetic factors are one of the key determinants in the approach to prevent or treat diseases as envisaged by the Precision Medicine Initiative (PMI) [3] launched in 2015. Single-nucleotide polymorphisms (SNPs) are the most common genetic variation between human beings and key enablers of the concept of personalized medicine. An SNP is a single base substitution occurring at a specific site in the DNA sequence and in at least 1% or more of the population.

The healthy endothelium acts a gatekeeper of cardiovascular health regulating an exchange of fluids, nutrients, and metabolites critical to homeostasis and vascular health. Endothelial dysfunction leads to (i) loss of vascular integrity, (ii) increased expression of adhesion molecules, (iii) pro-thrombotic phenotype, (iv) production of cytokines, and (v) upregulation of human leukocyte antigen molecules [4].

Endothelial cells modulate the underlying vascular smooth muscle compartment by secreting several vasoactive substances [5] that control vascular relaxation and contraction as well as enzymes that control blood clotting, immune function, and platelet adhesion. Two major endothelium-derived factors are nitric oxide (NO) and endothelin-1 (ET-1) that have opposing effects on the function and structure of the vessel wall. Nitric oxide (NO) is a vasodilator, and endothelin (EDN-1) is a potent vasoconstrictor. Both molecules are critical regulators of vascular function. Decrease in NO production and the consequent impaired vasodilation is a hallmark of endothelial dysfunction. Failure of the complex balance between vasodilation brought about by NO and vasoconstriction brought about by ET- 1, because of genetic or acquired disturbances between these two molecules, results in changes in vascular tone and ED, triggering the pathological process of vascular diseases at their primary stage [6].

2. Genetic variation in the study of human disease

The potential of genetic discoveries in unraveling pathophysiological mechanisms and identifying drug targets is widely accepted [7]. The sequence of any two individuals is 99.5% identical, and the genomes of any two individuals differ by approximately 0.1% or less. It is in this tiny fraction of the genome that researchers seek to find the collection of sequence variations that determine susceptibility to disease and its outcome. A resource for cataloging the differences between any two genomes was created with the completion of mapping and sequencing of human genome. Sites in the DNA sequence where individuals differ at a single DNA base are called single-nucleotide polymorphisms (SNPs). As some SNPs predispose individuals to have a certain disease or trait or react to a drug in a different way, they are highly useful in diagnostics and drug development. Single-nucleotide polymorphisms (SNPs) have the potential to improve personalized medicine, and discovery of new SNPS enhance the risk stratification of patients with multifactorial diseases. In a clinical setting, SNP testing is particularly useful in complementing family history and phenotypic risk factors. The basic assumption here is that the affected individuals harbor a significant excess of clinically defined established pathogenic DNA variants as compared with a group of unaffected persons (controls) that are available from large datasets obtained from the general population.

The association of an SNP with a disease in an individual can be studied either directly or indirectly. Searching the entire genome for SNPs for disease association

would be very expensive because it would involve the cost of sequencing the entire genome of several healthy and diseased individuals and comparing the sequences to identify the variants. In the indirect approach, marker SNPs called the "tag SNPs" which represent sets of nearby SNPs on the same chromosome inherited in blocks, and their disease associations are identified. The pattern of SNPs on a block is a haplotype, and a few SNPs are enough to uniquely identify the haplotypes in a block. The HapMap is a map of these haplotype blocks, and the tag SNPs are the specific SNPs that identify the haplotypes. The HapMap reduces the number of SNPs to be scanned in the genome making the indirect approach more efficient and comprehensive.

Using just the tag SNPs, particular regions of chromosomes can be identified that have different haplotype distributions in the two groups of people, those with a disease and those without. The identified regions are scanned in more detail to discover the gene variants in the region that contribute to the disease or determine the response to drugs by affecting drug metabolism pathways, leading to development of more effective tests and interventions.

The 1000 Genomes project was undertaken to provide a comprehensive description of common genetic variation by applying whole-genome sequencing to a diverse set of individuals from multiple populations. In this project, genomes of 2504 individuals from 26 populations were reconstructed by sequencing and 88 million SNPs were genotyped. The resource generated provides insights into processes that shape genetic diversity and advanced understanding of disease biology (The 1000 Genomes project Consortium, Nature 2015 [8]).

3. Candidate gene variations in endothelial dysfunction

A candidate gene in context of gene polymorphisms is one which is presumed to be associated with a particular disease or a phenotypic trait and whose biological functions are derived directly or indirectly from other studies. The role of nitric oxide (NO) and ET-1 in maintaining endothelial homeostasis is well established, and they are the obvious candidate genes of choice for studying endothelial dysfunction. Low levels of NO are associated with impaired endothelial function, and polymorphisms in genes of molecules, factors, and pathways regulating synthesis of nitric oxide in vascular endothelium have been implicated in endothelial dysfunction, the rationale being the impaired bioavailability of endogenous nitric oxide (NO) that underlies vascular disease [2]. They include polymorphisms in the endothelial nitric oxide synthase gene (eNOS gene), NOS3, asymmetric dimethyl arginine gene (*ADMA*), tetrahydrobiopterin gene *BH4*, and the gene encoding the p22phox subunit of NADPH oxidase *(CYB A).* NO in vascular endothelium is synthesized by the enzyme NOS which requires BH4 as a co-factor. NOS is inhibited by ADMA, a naturally occurring product of metabolism found in human circulation and an analog of L-arginine. NO synthesis is inhibited by raised levels of ADMA, and this results in impaired endothelial function. Increased levels of ADMA are found in people with hypercholesterolemia, atherosclerosis, hypertension, chronic heart failure, diabetes mellitus, and chronic renal failure. Reactive oxygen species (ROS) such as superoxide (O_2^-) lead to increased inactivation of NO with the generation of $ONOO^-$ which can lead to protein and DNA damage and subsequently loss of atheroprotective functions of NO. A variant of p22phox subunit of NADPH oxidase, an enzyme responsible for generation of O_2^- in vasculature involved in the production of ROS in vessel wall, has been shown

to be associated with progression of atherosclerosis [9]. Results of polymorphisms studied in other genes whose products have been implicated in endothelial dysfunction have been inconclusive.

4. Endothelin-1 as a candidate gene in human diseases for the study of endothelial dysfunction

Endothelin-1 like nitric oxide is a key regulator of endothelial dysfunction. The beneficial effects in maintenance of healthy endothelium are attributed to increased bioavailability of NO that regulates vascular homeostasis by causing vasodilation and by having antiproliferative, antioxidant, anti-inflammatory properties that inhibit atherogenesis. Conversely, increased synthesis of ET-1 is associated with the disturbance of homeostatic balance with pathological outcomes. The involvement of ET-1 in pathological process of vascular diseases with endothelial dysfunction like hypertension, coronary artery diseases, atherosclerosis, and diabetes is well established now. A knowledge of the mechanisms behind the development of endothelial dysfunction and the role of ET-1 and its gene is of great importance. The selection of ET-1 as a candidate gene is attractive because of its established role in vascular diseases and has assumed importance in the conduct of genetic association studies and SNP profiling in suitable population-based studies [10].

5. Endothelin system

The endothelin system is comprised of:

1. Endothelins (ETs): The 21 amino acid peptide isoforms such as ET-1, ET-2, and ET-3

2. Endothelin receptors (ETRs): The G-protein-coupled receptors for the peptides such as endothelin receptor A (ETRA) and endothelin receptor B (ETRB)

3. The endothelin-converting enzymes (ECEs) such as ECE1 and ECE2

5.1 Endothelin-1

Endothelin-1 was discovered as a potent vasoactive peptide [11] mainly secreted by endothelial cells and playing a role in regulating vascular tone, blood pressure, cell proliferation, and hormone production. It is now known to have diverse biological actions on almost all aspects of physiology and cell function and is increasingly being recognized as a pro-inflammatory cytokine. Because of its vasoconstricting effects on vascular smooth muscle cells (VSMCs) and the resultant increase in arterial blood pressure, the peptide is best known for its role in hypertension. It is a molecule with great clinical relevance with critical roles in neurological function [12], pulmonary physiology [13], chronic kidney disease [14], fluid and electrolyte transport [15], autoimmune disorders [16], cancer biology [17], inflammatory response and sepsis [18], embryogenesis [19, 20], and importantly endothelial dysfunction [21].

5.2 Biosynthesis of endothelin-1

ET-1 peptide is most abundant and widely expressed of the three isoforms such as ET-1, ET-2, and ET-3 [22]. ET-2 and ET-3 exhibit two and six different amino acids, respectively, compared to ET-1. ET-1 has a molecular weight of 2492, a hydrophobic carboxyl terminus, and two intramolecular disulfide bonds near the amino terminus [22]. It is the only isoform thought to be constitutively released from endothelial cells and is synthesized as the result of a series of proteolytic cleavages of the initial gene product – the preproendothelin – an inactive precursor 212 amino acids long. A 17-aa leader sequence targets preproET-1 to the endoplasmic reticulum where it enters the secretory pathway [23]. The precursor peptide is processed by furin-like proteases to biologically inert intermediates pro-endothelin1 and the 38-aa "big ET-1." Endothelin-converting enzyme (ECE) cleaves the bond between Trp [21] and Val[22] [24–26] to generate the mature 21-aa active ET-1 peptide (**Figure 1**).

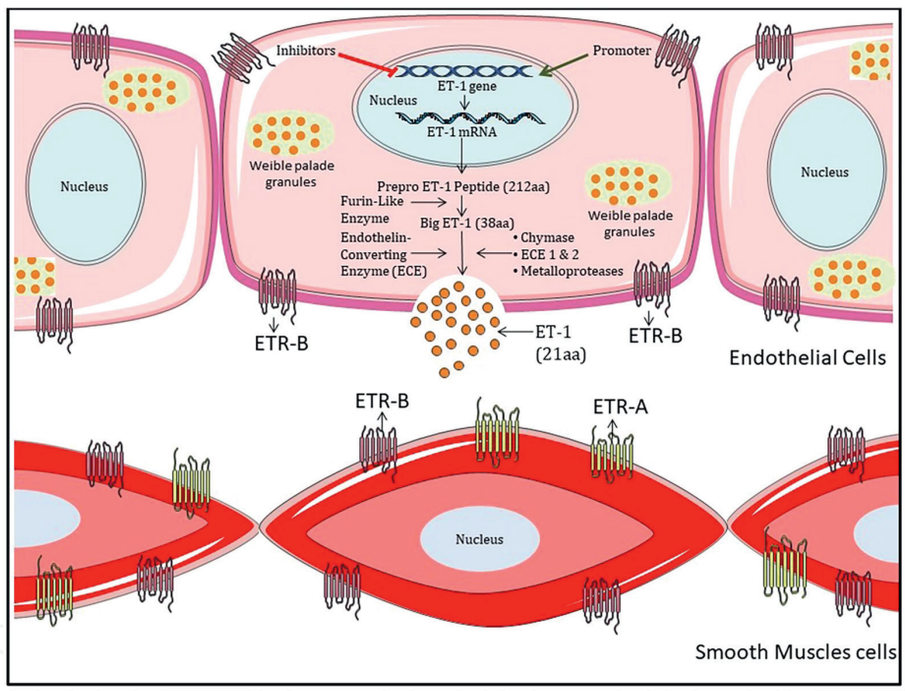

Figure 1.
Schematic representation of endothelin-1 and its biosynthesis and the localization of endothelin receptor subtypes on vascular smooth muscle cells and endothelial cells. Abbreviations: ET-1, endothelin-1; ETR-A, endothelin receptor A; ETR-B, endothelin receptor B; ECE, endothelin-converting enzyme. Figure created by using the Bioservier Medical Art.

ET-1 is synthesized by a dual pathway being released continuously by the secretory vesicles of the constitutive pathway to maintain the vascular tone [27]. They are also stored in Weibel-Palade granules of endothelial cells and released by exocytosis and degranulation in a regulated manner when exposed to pathophysiological stimuli [28].

Under physiological conditions, blood flow appears to regulate ET-1 synthesis and release via the "shear stress receptors" on endothelial cells. This endothelin synthesis is activated in response to major cardiovascular risk factors such as

hyperglycemia [29, 30], hypercholesterolemia [31], arterial hypertension, estrogen deficiency [32], and aging [25], as well as by biochemical and mechanical stimuli.

5.3 Endothelin-converting enzymes

Endothelin-converting enzymes (ECE-1 and ECE-2) are type II membrane-bound zinc metalloproteases that cleave the low-activity precursor, Big ET-1 between Trp21- and Val22 to produce mature ET-1 [33]. The two enzymes have 59% overall homology [24–26, 34–36] but differ in pH for maximal activity. ECE-1 has a pH optimum of 7.0, whereas ECE-2 has an optimum pH of 5.5 for its activity. ECE-2 is 250 times more sensitive to the metalloprotease inhibitor phosphoramidon. In humans, ECE-1 has four isoforms, ECE1a-d [34, 37], derived from a single gene by differential splicing of mRNA transcripts. These isoforms differ only in the aminoacid sequence of N-terminus and show comparable efficiency in catalyzing the cleavage of Big ET-1 into mature ET-1. ECE-1 is the main enzyme responsible for the transformation of big ETs into ETs [38].

5.4 Endothelin receptors

In the vasculature, contraction or vasodilation by ET-1 are mediated by two different receptor subtypes, ET_A and ET_B [39], belonging to the family of heptahelical G-protein-coupled receptors located on vascular smooth muscle cells (VSMCs) and endothelial cells. The endothelin receptor subtypes are distinctively localized. The ETA-receptor subtype mainly mediates the vasoconstrictor activity. The receptor subtype is widely co-localized with ETR-B in vascular smooth muscle of cardiovascular tissues [40, 41], cardiopulmonary [42], central nervous system [43], retina [44], and placenta. However, ETR-A is not expressed on endothelial cells and renal-collecting duct cells. ETRB is highly expressed in the endothelium, and under pathophysiological conditions, the expression of ETB receptor subtype also increases on VSMCs and produces vasoconstriction. ETR-A has high affinity for both ET-1 and ET-2, whereas ETR-B has a similar affinity for all ET isoforms [45]. ETRB has broader effects compared to ETR-A and has roles to play in ET-1 clearance, endothelial cell survival, signaling to NO synthase (eNOS) and NO production, prostacyclin synthesis, and inhibition of ECE-1 [46]. Interaction of ET-1 with its receptors increases intracellular calcium, leading to phosphorylation and activation of myosin light chain to produce vasoconstriction [47] Vasodilatory effect by ET-1 is mediated through ETB receptors on endothelial cells which increase the production of NO and PGI2.

Endothelin receptor antagonists (ERAs) have been developed to block the effects of ET-1 in a variety of cardiovascular conditions. Three main kinds of ERAs exist:

1. selective ETA receptor antagonists (sitaxentan, ambrisentan, atrasentan, BQ-123, and zibotentan), which affect endothelin A receptors.

2. dual antagonists (bosentan, macitentan, and tezosentan), which affect both endothelin A and B receptors.

3. selective ETB receptor antagonists (BQ-788 and A192621) which affect endothelin B receptors are used in research but have not yet reached the clinical trial stage.

Sitaxentan (withdrawn in 2010 after acute liver failure leading to death), ambrisentan, and bosentan are mainly used for the treatment of pulmonary arterial hypertension (PAH), while atrasentan is an experimental anticancer drug

6. Possible role of endothelin in endothelial dysfunction

It is generally accepted that generation of reactive oxygen species (ROS) and an increased level of oxidative low-density lipoprotein (oxLDL) induces endothelial dysfunction. The receptor for oxidative low-density lipoprotein (oxLDL) is the lectin-like oxidized LDL receptor (LOX-1) found on endothelial cells (**Figure 2**). Under normal conditions, LOX-1 is expressed at a low level on endothelial cells, but it is induced by pro-inflammatory cytokines and under proatherogenic conditions such as hypertension, diabetes, and hyperlipidemia [6]. Angiotensin II and homocysteine that induce oxidative stress also induce LOX-1 expression. Also, LDL is oxidized by oxidative stress, leading to generation of ox-LDL. Binding of oxLDL to its receptor LOX-1 reduces NO production from endothelial cells via generation of reactive oxygen. It also induces the production of superoxide anion and activation of redox-sensitive transcription factor NFkB [48], which in turn upregulates ET-1 as well as adhesive molecules and chemokines promoting endothelial dysfunction.

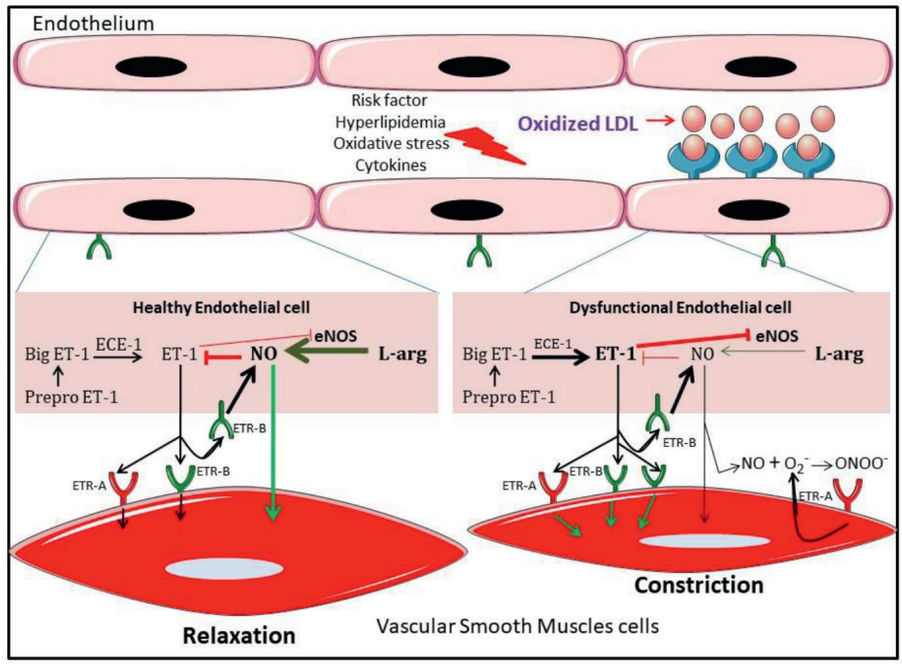

Figure 2.
The role of endothelin in endothelial dysfunction: In a healthy cell (left), the protective role of nitric oxide (NO) signaling pathway predominates. The formation of NO from L-arginine is catalyzed by endothelial NO synthase (eNOS). NO is released from endothelial cells and acts on smooth muscle cells to exert vasodilator and proliferative effects. In a dysfunctional endothelial cell (right), the vascular homeostasis is disrupted via the engagement of endothelial LOX-1 with oxidized LDL (OxLDL) resulting in downregulation of NO and upregulation of NFkB and the endothelin (ET)-1 signaling pathway. ET-1 is released from endothelial cells and acts on smooth muscle cells through the interaction of two types of receptors (ET-1 receptor type A [ETR-A] and ET-1 receptor type B [ETR-B]), both of which mediate vasoconstriction and proliferation. Figure created using Servier Medical Art.

7. Endothelin-1 gene polymorphism

An individual's phenotypic characteristics, including a person's propensity toward complex disorders such as heart disease and cancer at the genetic level, are determined by sequence variations that exist at defined positions within genomes. Sequence variations are tools for understanding human variation and molecular genetics and can be used for gene mapping, definition of population structure, and performance of functional studies. The human genome has a total of over 88 million variants of which 84.7 million are SNPs, 3.6 million short insertions/deletions (indels), and 60,000 structural variants (The 1000 Genomes Project Consortium, Nature, 2015 [8]). A typical genome differs from the reference human genome at 4.1million–5.0 million sites. Realizing the importance of the role of SNPs in human health, many databases like Ensembl Variation Database, A-SNP, HGBase (Human Genic Bi-allelic SEquences), HOWDY (Human organized whole-genome variation Database), and dbSNP have been created for cataloging the variations occurring in human genome. The emergence of genetic variation databases, such as (i) dbSNP and HGV for short genetic variations, (ii) dbVar and DGV for structural variations, (iii) dbGaP for genotype/phenotype interaction studies, and (iv) ClinVar and ClinGen for human variations of clinical significance, facilitates the contemporary identification/discovery of (i) known or novel polymorphisms, (ii) phenotype to genotype associations, and (iii) clinically important human genetic variations.

The Single Nucleotide Polymorphism Database (dbSNP) is **a free public archive for genetic variation** developed and hosted by the National Centre for Biotechnology Information (NCBI) in collaboration with the National Human Genome Research Institute (NHGRI). This collection of polymorphisms includes

i. single-base nucleotide substitutions (also known as single-nucleotide polymorphisms or SNPs),

ii. small-scale multi-base deletions or insertions (also called deletion insertion polymorphisms or DIPs), and

iii. retrotransposable element insertions and microsatellite repeat variations (also called short tandem repeats or STRs).

Majority of the genetic variations among individuals are due to SNPs. The association of candidate gene SNPs like those of EDN1 in multifactorial diseases, like endothelial dysfunction which often set the stage for the occurrence of vascular diseases like CAD, is important for the identification of therapeutic targets.

The gene for ET-1 gene locus spans a region of approx. 7.0 kb on short arm of chromosome 6 at 6p24–23 [49, 50]. The gene is composed of five exons (**Figure 3**) that synthesizes a cDNA of 2026 bps. Nucleotide sequences encoding the mature ET-1 are present in the second exon. ExonI has the 5'UTR of mRNA. The upstream promoter region is well conserved.

The endothelin pathway is central to pulmonary vascular function. Several polymorphisms and/or mutations in the genes coding for endothelin (ET)-1 and its receptors correlate with the clinical manifestations of other diseases. The dbSNP contains 15,259 entries for human *EDN1* gene (as on 20.06.2022) which represent

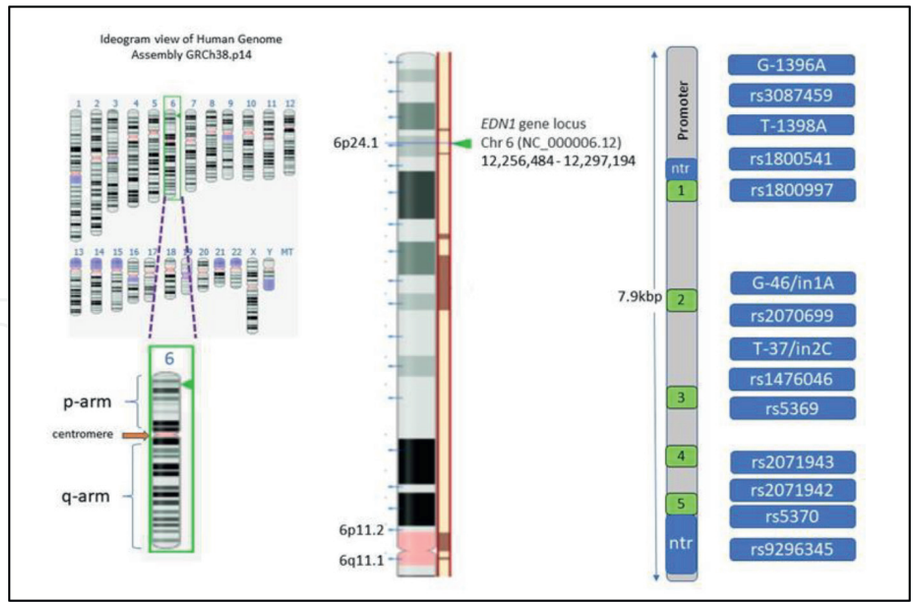

Figure 3.
EDN1 Gene Locus and some of its common gene variants. The EDN1 gene is located on short arm (p-arm) of chromosome 6 and has five exons (green), a 5'non-translated region and a 3'non-translated region of the gene that is transcribed in mRNA. The 5'ntr is located downstream of promoter, while the 3'ntr is located downstream of exon 5. The gray areas of the gene represent the introns that are spliced off in the mature RNA. The rs nos. of commonly studied SNP variants in EDN1 gene are mentioned in blue horizontal bars in extreme right. The complete data from SNPs build 155 are available at https://ftp.ncbi.nlm.nih.gov/snp/ in multiple formats.

all the above categories of variations. Many of these entries are redundant. For example, rs386556298, rs60458956, rs56478068, and rs3730357 have been merged into rs2070699 which represents intron variant c.233 + 30 G > C/G > T variation in the *EDN1* gene. A total of 15 of these as represented in the ClinVar database (Build 155, Jun 16, 2021) have been included in **Table 1** due to the limitation of space in this article.

This is by far the most reported variant of pre-proendothelin-1, and there are 161 publications (21.06.2022) in LitVar for this variant. The third base "**G**" of codon 198 of preproendothelin-1 gene is substituted with "**T**" leading to a change in codon for lysine to arginine. The genotypic variants of rs5370 are GG, GT, and TT. In the ECTIM (**Etude Cas-Témoin de l'Infarctus du Myocarde**) multicenter study [51] comprising of 648 male patients who had survived myocardial infarction and 760 population-based controls, the G/T polymorphism predicting the Lys/Asn change showed that the "T" allele was associated with increase of blood pressure in overweight subjects. This finding was confirmed by the Glasgow Heart Scan Study [51] as well.

7.1 Pulmonary arterial hypertension

Endothelial dysfunction is believed to be one of the first triggers initiating the process of abnormal vascular remodeling in pulmonary arterial hypertension (PAH) [52]. K198N (rs5370) polymorphism in the endothelin 1 gene (EDN1) has been demonstrated to associate with blood pressure reactivity and can result in greater endothelin-1 (ET-1) synthesis which may favor the development of PAH and affect its course of progression [53]. The influence of EDN1 gene variants on susceptibility to pulmonary arterial hypertension remains uncertain. However, a meta-analysis of

S.No.	Variant	Alleles	Coding/noncoding	Position	Flanks (25 nt. on either side of the SNP site)	Amino acid
1	rs376892399	G>A, T	Coding/synonymous	g.8658 c.246	5' CTTTGGATAA TAGGCACGTT GTTCC[G/A/T] 3' TATGGACTTG GAAGCCCTAG GTCCA	Pro 82=P[CCG] > P [CCA]
2	rs 10478695	C>A,G,T	Intron	g.5406	5' AAGGAGCTCC AGAAACAGGT AGGCA[C/A/G/T] 3' GCTCGTTGAC TTGTAAGTCT CGGAA	
3	rs147381256	G>A	Coding/missense	g.8650 c.238	5' CTITCTCTCT TTGGATAATA GGCAC[G/A] 3' TTGTTCCGTA TGGACTTGGA AGCOC	Val 79 lle V[GTT] > I [ATT]
4	rs145546137	A>C	Coding/missense	g.9056 c.480	5' GGAAAAAGTG TATITATCAG CAGTT[A/C] 3' GTGAGAGGAA GAAAAATCAG AAGAA	Leu 160 Phe L[TTA] > F[TTC]
5	rs183694577	G>A,C	Coding/missense	g.7087 c.106	5' GCTCAGCGCG GTGGGTGAGA ACGGC[G/A/C] 3' GGGAGAAACC CACTCCCAGT CCACC	Gly36Arg G[GGG] > R[AGG]
6	rs 150035515	G>A,T	Coding/synonymous	g.7071 c.90	5' CAGTCTTAGG CGCTGAGCTC AGCGC[G/A/T] 3' GTGGGTGAGA ACGGCGGGGA GAAAC	Ala30 = A[GCG] > [GCA]
7	rs148565651	C>T	Coding/synonymous	g.7122 c.141	5' CCACTCCCAG TCCACCCTGG CGGCT[C/T] 3' CGCCGGTCCA AGCGCTGCTC CTGCT	LEU47 = L[CTC] > L[CTT]
8	rs1561693994	G>C	Coding/missense	g.7165 c.184	5' CTCCTGCTCG TCCCTTGATGG ATAAA[G/C] 3' AGTGTGTCTA CTTCTGCCAC CTGGA	Glu62Gln E[GAG] > Q[CAG]
9	rs149316725	C>A	Coding/missense	g.9093 c.517	5' AAAAATCAGA AGAAGTTCAG AGGAA[C/A] 3' ACCTAAGACA AACCAGGTAA GAGGG	His173Asn H[CAC] > N[AAC]
10	rs1064796796	G>A	Coding/missense	g.7169 c.188	5' TGCTCGTCCC TGATGGATAA AGAGT[G/A] 3' TGTCTACTTC TGCCACCTGG ACATC	Cys63Tyr C [TGT] > Y [TAT]
11	rs587777231	A>G	Coding/missense	g.8683 C.271	5' GTATGGACTT GGAAGCCCTA GGTCC[A/G] 3' AGAGAGCCTT GGAGAATITA CTICC	Lys91Glu K [AAG] > E[GAG}
12	rs587777232	C>A	Coding/missense	g.7211 C.230	5' CTGGACATCA TITGGGTCAA CACTC[C/A] 3' CGAGTAAGIC TCTAGAGGGC ATTGT	Pro77His P[CCC] > H[CAC]
13	rs587777233	T>A	Coding/missense	g.7172 c.191	5' TCGTCCCTGA TGGATAAAGA GTGTG[T/A] 3' CTACTTCTGC CACCTGGACA TCATT	Val64Asp V[GTC] > D[GAC]

14	rs587777234	T>G	Coding/stop_gained	g.8661 c.249	5' TGGATAATAG GCACGTTGTT CCGTA[T/G] 3' GGACTTGGAA GCCCTAGGTC CAAGA	Tyr83Termination Y[T A T] > * [TAG]
15	rs5370	G>T	Coding/missense	c.594	5' TTCATGATCC CAAGCTGAAA GGCAA[G/T] 3' CCCTCCAGAG AGCGTTATGT GACCC	Lys198Asn K[AAG] > N [AAT]

rs5370: Codon 198: Lys198Asn or K198N (G > T Transversion in Exon 5 Position g.10727 of RefSeqGene NG_016196.1 and position c.594 of transcript variant 1 NM_001955.5 and position c.591 of transcript variant 2 NM_001168319.2).

Table 1.
15 SNP variants reported in Clinvar database having pathophysiological significance are mentioned with their rs I'd, allelic variation, their occurrence in coding vs. noncoding region in the gene, their coordinates, and 25 nucleotide upstream and the downstream flanking sequences and the amino acid change associated with SNPs in the exonic/coding region. SNPs mentioned here are from transcript variant 1 (NM_001955.5) and the corresponding genomic RefSeq EDN1 gene (NG_016196.1). Among 15 SNPs, 5 are benign, 3 are likely benign, 3 are of uncertain significance, and 4 are pathogenic. One of the SNP rs5370 G > T has multiple submitters. The complete data from SNPs build 155 are available at https://ftp.ncbi.nlm.nih.gov/snp/ in multiple formats.

a total of 17 articles with 2631PAH subjects and 5139 controls and 5 candidate gene variants that also included rs5370 SNP of EDN1 gene for susceptibility to PAH showed a significant association between "T" allele carriers and risk of developing PAH [54]. Another large-scale genomic analysis to examine the interaction of ET-1 pathway polymorphisms and treatment response of patients with PAH treated with ET receptor anatagonists (ERAs) showed that these polymorphisms of the ET-1 pathway may influence the clinical efficacy of ERAs [55].

7.2 Essential hypertension

There are several reports connecting this SNP variant to hypertension. Our own studies (unpublished) like those of Wiltshire et al. [43] have found no sufficient data supporting the association between K198N polymorphism with high blood pressure, systolic blood pressure, lipid levels, and insulin resistance or metabolic syndrome. In other studies, subjects with high endothelin-1 levels were shown to have an increased risk of low-renin hypertension [56]. Rs5370 variant of EDN1 has been associated with low-renin hypertension and increased aldosterone/renin ratios in individuals of African descent, but not in whites [57]. This study also provided the first evidence of a potential association between the *EDN1* rs5370 SNP and the risk of subclinical hyperaldosteronism in subjects of African descent. These investigators also assessed the effect of EDN1 rs5370 on systolic BP curves, but they did not see an effect. They also observed a significant association of salt-sensitive BP and rs5370, even with adjustment for sex, since an earlier study [58] had reported sex differences in the relationship between systolic BP and a haplotype of *EDN1.* In rheumatoid arthritis, hypertension is quite common and has been reported to be associated with the endothelin-1 (ET-1) gene locus (EDN1) in some groups, such as the Afro-Caribbean but not in the general population. Some other groups where hypertension-related high levels of plasma ET-1 in RA have been observed are the obese and individuals with low-renin states. A study [59] that evaluated the potential association of EDN1 gene locus and serum ET-1 levels with hypertension in patients with RA showed an increase in the prevalence of T-T haplotype carriers.

7.3 Preeclampsia

Preeclampsia (PE) is an often-fatal pathology characterized by hypertension and proteinuria at the 20th week of gestation that affects 5–10% of the pregnancies. [46]. Risk factors for the development of PE include obesity, insulin resistance, and hyperlipidemia that stimulate inflammatory cytokine release and oxidative stress leading to endothelial dysfunction (ED). Normal pregnancy course includes variations in hemodynamics, in which placenta allows the exchange of nutrients and waste disposal between mother and fetus. During the stage of establishment of maternal-fetal interface when the extravillous throphoblasts from placenta conquer the maternal decidua, the maternal spiral arteries from the decidua go through a process of remodeling, where they are upgraded from low-capacity high-resistance into high-capacity low-resistance vessels. PE is characterized by an impaired invasion of fetal trophoblasts which causes a reduced remodeling of the maternal spiral arteries eventually leading to a decrease in blood flow to the placenta. Consequently, the mother develops hypertension, usually at the end of the second or third trimester of

gestation, to increase the blood flow. The polymorphism rs5370 in EDN1 was shown to be associated with susceptibility to preeclampsia [60]. In another study [61], markedly increased risk of early onset of PE was shown to be related to the C allele polymorphism rs5370 in *EDN1.*

7.4 Glaucoma

ET-1 has been suggested to have a role to play in optic neuropathy observed in glaucoma [62]. Associations between polymorphisms of endothelin (ET-1) and endothelin receptors (ER) A and B genes with the occurrence of glaucoma were investigated by Ishikawa et al. [63] in Japanese patients. For the rs5370 ET-1 polymorphism that involved a transversion of G/T in exon 5, the Lys-Lys (GG) genotype tended to be more frequent than in open-angle glaucoma patients.

7.5 Diabetic retinopathy (DR)

Diabetic retinopathy (DR) is the result of impaired NO pathway that affects the vusculature of the retina. Several candidate genes have been studied for their role in diabetic retinopathy, but only a fraction of them have been shown to be associated with DR. Many studies have provided evidence in support of the role of endothelin (ET) system in the pathophysiology of DN. However, studies on K198N variant have revealed that the "T" (Asn) allele actually has a protective role against DR in a Chinese population with type 2 diabetes [64]. Yet another study by Maja Seruga [65] showed that the EDN1 rs5370, rs1476046, and rs3087459 polymorphisms of EDN1 gene are not risk factors for DN in Caucasians with T2DM.

7.6 Childhood primary nephrotic syndrome

ET-1 levels are raised in children with first episode of nephrotic syndrome (FENS), pointing toward endothelial dysfunction [66]. Also, children with steroid resistance have a greater risk of endothelial dysfunction [67]. The rs5730 SNP of EDN1 gene might play a disease-modifying role and susceptibility to childhood primary nephrotic syndrome (CPNS) [68]. Plasma Cholesterol, a hallmark of NS, seems to be associated with the genetic variations within the human ET-1 gene. The other EDN1 SNPs associated with CPNS include rs1630736 and rs10478694 (3A/4A) and rs9296344 [69]. In a case-control study, it was found that GG genotype was more frequent in steroid-sensitive NS group compared to the steroid-resistant NS group and was associated with hypertension. This group also showed a better response to steroid therapy [70]. The study by Hashemi et al. [71], however, did not find any association of rs5370 G > T variant with nephrotic syndrome in children.

7.7 Asthma

EDN1 has been reported to be implicated in the pathophysiology of asthma. In a study on 342 families from UK and 100 families from Norway, rs5370 along with 10 other EDN1 variants rs1800541, rs1800542, rs1476046, rs1800543, rs5369, rs1794849, rs1626492, rs1629862, rs1630736, and rs4714383 were genotyped, and a strong association was found in both the populations for rs5370 and rs1800541 located in

the upstream region of EDN1 gene [72]. However, literature results on the genetic association of EDN1 in asthma are inconsistent.

7.8 As risk predictors in cancer

Ma et al. analyzed the genotypes of angiogenesis-related genes in 180 patients with nasopharyngeal carcinoma (NPC) using Sequenom MassARRAY and found that EDN1-rs1800541, rs2071942, and rs5370 can be used as risk predictors of radiation-induced oral mucositis, xerostomia, and myelosuppression, respectively.

Auriculocondylar Syndrome and Question Mark Ears.

rs587777231 [Lys91Glu].

rs587777232[Pro71His].

rs587777233[Val64Asp],

rs587777234 [Tyr81 Termination (stop gained)].

Auriculocondylar syndrome (ACS) is a rare disorder which affects facial development with a small chin micro-gnatha) and a malfunction of the joint that connects the lower jaw (mandible) to the skull—a condition referred to as mandibular hypoplasia. Another feature of this disorder is malformed outer ears that have a characteristic shape caused by a split that separates the upper ear from the earlobe (question-mark ears or QMEs). Ref. [73] identified a homozygous substitution in a furin cleavage site of the EDN1 proprotein in ACS-affected siblings born to consanguineous parents by whole-exome sequencing (WES). Four mutations (S.No. 11–14 in the ClinVar Table) were identified in the EDN1 gene, one of which resulted in a stop codon and the other three resulted in missense mutations. These mutations also had different modes of inheritance, suggesting that the degree of residual EDN1 activity differed depending on the mutation. These findings provided support for the hypothesis that ACS and QMEs are uniquely caused by disruption of the EDN1-EDNRA signaling pathway which is important in the development of the lower jaw. The four variants are classified in ClinVar database as pathogenic having clinical manifestations.

rs1800997: 3A/4A (+138 5'UTR locus of exon1 ins/del A) polymorphism.

(Formerly rs10478694)

This SNP contains an adenine insertion at position +138, 5′ untranslated region (UTR) and exon 1 of the ET-1 [74]. The genotypes are as follows: the mutant form (4A/4A), wild type (3A/3A), and the heterozygote (3A/4A) [53]. Some studies have reported increased plasma levels of ET-1 in individuals who have a mutant genotype [53, 75]. Studies have reported different allele distributions among patients with pulmonary artery hypertension (PAH), idiopathic pulmonary artery hypertension (IPAH), and coronary heart disease (CHD), with the control group [76]. They showed a significant increase of alleles containing the 3A form in patients with hypertension [76]. Some researchers have shown that there is no significant association between this SNP and the development of hypertension [77]. This SNP, although, not reported in ClinVar database has been shown to be associated with high expression levels of ET-1 both in vitro (Popowski) and in vivo (Abhishek Kumar, 2021), and the high expression levels associated with the homozygous mutant form 4A/4A were hypothesized to be deleterious to cyanotic children with severe pulmonary hypertension [75].

Other variants not reported in ClinVar Database but reported in literature as heritable risk variants in many cardiovascular disorders such as hypertension, coronary artery disease, ventricular arrhythmia, and other related disorders are shown in **Figure 3**.

8. Summary and conclusions

Endothelial dysfunction is multifactorial, and ET-1 is a key regulator of ED. The genetic factors that modulate individual susceptibility to multifactorial diseases are common, functionally different forms of genes (polymorphisms), that have modest effects on physiology and disease biology at individual level but, because of their high frequency of occurrence in the population, can be associated with a high attributable risk. By definition, a mutation results in a significant phenotype, whereas an SNP, which represents a stable change in the genome, possesses mild or no phenotypic changes. SNPs whether they relate clinical end points or intermediate phenotypes such as endothelial dysfunction require careful analysis. Available evidence in literature suggests that most of the susceptibility genes from common diseases do not have a primary etilogical role in predisposition to disease, but rather act as response modifiers to exogenous factors such as stress, environment, disease, and drug intake. A better characterization of the interactions between environmental and genetic factors constitute a key issue in understanding of the pathogenesis of multifactorial diseases. For example, risk factors like oxidative stress, hyperlipidemia, and cytokines disrupt the vascular homeostasis in a dysfunctional endothelial cell leading to the production of ET-1 and consequent pathophysiological changes. Also, results from two independent studies ECTIM and Glasgow Heart Scan Study [53] on ET-1, BMI, and Blood Pressure suggested that obesity is a crucial factor influencing the association between the ET-1/Lys198Asn polymorphism and BP levels. Obesity, predominantly governed by complex social and environmental factors, might enhance expression of ET-1 gene possibly through an upregulation by insulin, which is known to stimulate ET-1 production [78]. In common diseases, genetic effects can be considerably amplified in the presence of triggering factors and gene-environment interaction is a central concept in multifactorial diseases.

The potential usefulness of SNPs in medicine is unprecedented. Obtaining a detailed family history is often considered standard in clinical practice for characterizing the inherited component of individual's disease risk. SNPs allow us to look closely at the footprints of past generations of the families. SNPs of the endothelin-1 gene axis have the potential to help us in dissecting the genetic component of complex diseases like cardiovascular diseases of which vascular dysfunction is an early manifestation. Susceptibility to disease in such cases depends on the cumulative contribution of multiple genetic risk factors. SNPs provide the potential to interpret genetic risks associated with complex polygenic disorders by developing models based on quantitative genetic theory to analyze and compare family history and SNP-based models [79, 80].

The most difficult task will be to consider the implementation of SNPs in clinical decision-making, particularly as it relates to providing recommendations for interventional or preventional measures, based on the concept of "risk."

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List of abbreviations

ET-1	Endothelin-1
ETRA	Endothelin Receptor A
ETRB	Endothelin Receptor B
ECEs	Endothelin-Converting Enzymes
ERAs	Endothelin Receptor Antagonists
NO	Nitric Oxide
eNOS	Endothelial NO Synthase
OxLDL	Oxidized LDL
SPH	Severe Pulmonary Hypertension,
CHD	Congenital Heart Disease
SNPs	Single Nucleotide Polymorphisms,
5'UTR	5'Untranslated Region
In/del	Insertion/Deletion
WES	Whole-Exome Sequencing
PE	Preeclampsia
DR	Diabetic Retinopathy
FENS	First Episode Of Nephrotic Syndrome
CPNS	Childhood Primary Nephrotic Syndrome
ACS	Auriculocondylar syndrome
QMEs	Question-Mark Ears

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