Genetic Variant-Associated Endothelial Dysfunction Behind Small-Vessel Cerebral Circulatory Disorders: A New Pathomechanism Behind Common Cerebral Phenotypes

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Abstract: An increasing body of evidence suggests that different genetic factors, such as angiotensin-converting enzyme (ACE) I/D , angiotensin II type-1 receptor (AT1R) A1166C, methylenetetrahydrofolate reductase (MTHFR) C677T and ENOS G894T variants are associated with an endothelial dysfunction (ED). EDs are relatively new phenomena that are presumed to contribute to vasoregulatory malfunctions at the small-vessel level. Ever more clinical observations indicate that the above genetic variants are also associated with cerebral small-vessel disorders. This article reviews the knowledge available on the roles of ED- associated genetic variants in cerebral circulatory disorders, and suggests that EDs can be causative factors for different common cerebral pathologies such as leukoaraiosis or/and small-vessel infarcts. Newly-developed drugs involving phosphodiesterase type-5 inhibitors, which improve the endothelial functions, may comprise a new approach to the treatment and prevention of small-vessel cerebral circulatory disorders.

Key Words: Leukoaraiosis, small-vessel infarcts, endothelial dysfunction, nitric oxide, endothelin-1, MTHFR C677T, angiotensin-converting enzyme I/D, ENOS G894T, AT1R A1166C, genetic variant, phosphodiesterase type-5 inhibitors.

INTRODUCTION

Normal vasoregulation is of great importance for the maintenance of harmonized blood flow in the brain in response to a wide range of conditions, such as a shift in blood pressure, physical exercise, infection or fever. Although a complex network of systems involving cortical areas, vegetative brain centers, peripheral effector nerves, circulating humoral chemicals and vascular smooth cells take part in the vasoregulation of the brain, the final effectors of this vasoregulatory process are the dilator or constrictor influences of the endothelial cells in order to adapt to the environmental changes. These two antagonistic operations depend on a finely-tuned dynamic balance between nitric oxide (NO) and endothelin-1 (ET-1) produced in the endothelial cells. The former leads to vasodilatation, while the latter has the opposite effect on the vascular smooth muscle cells [1-3]. The NO diffuses out of the endothelial cells and into the underlying smooth muscle cells, where it binds to and activates guanylyl cyclase to produce cyclic guanosine monophosphate (cGMP). The cGMP triggers a response that causes the smooth muscle cells to relax, enhancing blood flow through the blood vessels. The action of NO is demonstrated in Fig. (**1**). The ET-1 exerts its vasoconstrictor effects on the ETB receptor of the vascular smooth muscle cells. However, ETB receptors on the endothelium stimulate the release of NO [1,4].

Hypertension, diabetes mellitus, smoking and chronic cardiac diseases favor an imbalance characterized by an increase in the ratio ET-1/NO. This increased ratio gives rise to further vasoregulatory disturbances and vasoconstriction, which can result in a chronically repeated reduced regional cerebral blood flow [1].

Although a number of genetic factors are presumed to result in an endothelial dysfunction, few such data are available as regards neurological circulatory disorders. This article reviews the knowledge available on endothelial dysfunction-associated genetic variants, which can be presumed to be causative risk factors for neurological circulatory diseases.

ENDOTHELIAL DYSFUNCTION-ASSOCIATED GENETIC VARIANTS

Renin-Angiotensin Sytem

The angiotensin-converting enzyme (ACE) converts angiotensin I into angiotensin II, which exerts its vasoconstrictor effects on the angiotensin II type-1 receptor (AT1R) [5]. The enhanced activation of the AT1R, however, results in an increase in the production of ET-1 and reactive oxygen species (ROS) through nicotine adenine dinucleotide oxidase in the endothelial cells [6]. ROS in turn result in increased synthesis of ET-1 and ET-1 receptors [1]. Therefore, the genetic polymorphisms, which can be associated with an enhanced activity of the angiotensin II-AT1R axis, can result in an endothelial dysfunction.

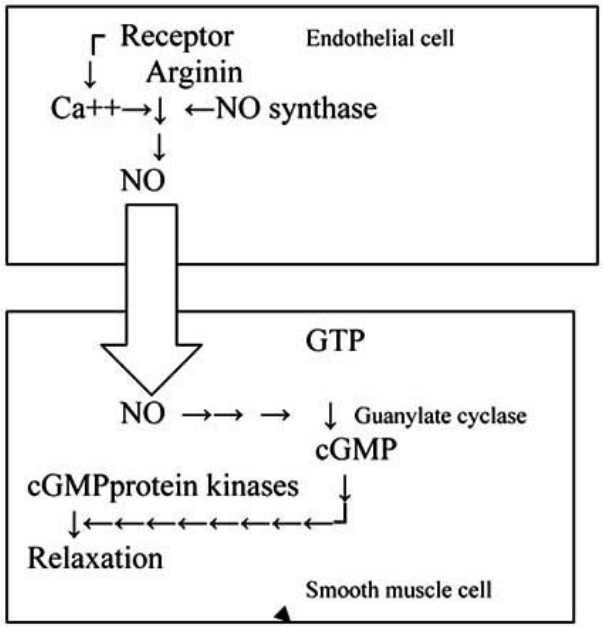

Fig. (1). Action of nitric oxide.

The ACE I/D polymorphism is the main determinant of the level of ACE and thereby of its product, angiotensin II. The ACE D allele is associated with increased levels of ACE and angiotensin II in a dose-dependent way [7]; and the AT1R A1166C variant is related to an enhanced responsiveness of the AT1R itself [8,9]. The AT1R A1166C variant was also demonstrated to be an independent predictor of ROS in heart-failure patients [10]. The levels of plasma protein carbonyls, a marker of oxidative protein modification, were 50-folder higher in heart-failure patients homozygous for the AT1R A1166C variant than in controls [10]. Accordingly, both the ACE D

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allele and AT1R A1166C variants can contribute to endothelial dysfunctions.

Clinical observations have revealed that the presence of the ACE D/D genotype may be a risk factor predisposing to the development of LA or/and cerebral small-vessel infarcts [11-14]. There also data demonstrating that the presence of the ACE D/D genotype in Alzheimer' disease is presumably associated with the severity of the damage to the vascular white matter in the brain [15]. The ACE D/D genotype in combination with the MTHFR 677TT genotype can be a risk factor for LA with or without cerebral infarcts [16]. LA and vascular white matter damage are considered to be small-vessel circulatory disorders and synonymous phenomena in this context.

The AT1R A1166C variant can give rise to small-vessel cerebral infarcts when in combination with the ACE D/D genotype [17]. It can also influence the development of white matter lesions [18]. These data indicate that both the ACE D/D and AT1R 1166C variants can contribute to small-vessel cerebral circulation disorders.

Methylenetetrahydrofolate Reductase C677T Variant

The MTHFR C677T variant, associated with an elevated serum homocysteine level, brings about a reduced production of NO in the endothelial cells, thereby leading to an increased ratio ET-1/NO [19]. The endothelium contributes to the resting tone of cerebral arteries by tonically releasing NO. Dilatations can occur by increased release of NO. The dilator influence of the endothelium can turn to that of constriction when the bioavailability of NO is decreased or ET-1 is released [20].

Clinical studies have led to the findings that the MTHFR C677T variant contributes to the evolution of LA or small-vessel cerebral infarcts when it is in combination with ACE D/D or AT1R 1166C variants [16,21]. It has also been reported that the MTHFR 677T variant can give rise to the development of LA *via* an endothelial dysfunction [22].

Endothelial Nitric Oxide Synthase G894T Variant

The endothelial NO synthase (ENOS) G894T gene polymorphism, is presumably associated with a reduced lifetime and faster turnover of ENOS mRNAs in the endothelial cells [23]. The main task of ENOS is to produce the endothelial derived NO. Accordingly, a reduced lifetime of its mRNA can be hypothesized to lead to a reduced NO level in the endothelial cells.

Clinical data indicate that the ENOS G894T variant may give rise to damage to the white matter in the brain [18].

The studies of endothelial dysfunction-associated genetic factors in cerebral circulatory disorders are listed in Table **1**.

DISCUSSION

Biochemical and clinical studies suggest that the endothelial dysfunction-associated genetic variants give rise to cerebral small-vessel circulatory disturbances such as small-vessel infarcts or/and LA. The expression endothelial dysfunction is a functional term meaning a malfunction in the harmonization of the local circulation of the tissue, taking place at the small-vessel level. Three pathomechanisms have been hypothesized to explain the cerebral manifestation of the endothelial dysfunction.

1. In consequence of the endothelial dysfunction, some areas of the brain, and particularly the arterial border zones between the metabolic units supplied by a single arteriola, suffer hypoxia and therefore a lacunar infarct will evolve.
2. An other possibility is that the same arterial border zones between the arterioles suffer only slight ischemia, which causes not a complete infarct, but vascular demyelization. A complete metabolic hypothesis has been proposed for this process [24].
3. The third possibility is that the endothelial dysfunction causes a wide range of vasoregulatory problems in general (a hypotension crisis, and fast changes in blood pressure under different physiological conditions) these then leading to hypoperfusion in the main arterial border zones between the large arteries.

The local cerebral vasoregulation and the neurovascular coupling are especially important in the dynamic and harmonized blood supply of the brain. Neurovascular coupling means that local chemicals such as NO, CO_2, adenosine, aspartate, glutamate, adenosine monophosphate, cyclic guanosine monophosphate, cyclic adenosine monophosphate and lactate produced by the active neurons and glia cells, change the local microcirculation in accordance with the necessary demand. The rapid responses of the endothelial

Table 1. Studies of Endothelial Dysfunction-Associated Genetic Variants in Cerebral Circulatory Disorders

Authors	Study Design	Findings
Amar *et al*. [14]	182 patients with memory problems	The ACE D/D genotype occurred more frequently in patients with white matter lesions (LA and infarcts).
Hassan *et al*. [11]	84 patients with lacunar syndromes, subjects with LA were compared with the ones without LA.	ACE D/D genotype was a risk factor for LA in lacunar syndromes.
Sierra *et al*. [12]	60 hypertensive patients, subjects with LA were compared with the ones without LA	ACE D/D genotype was a risk factor cerebral white matter lesions in essential hypertension (p<0.022, OR:4.4)
Tian *et al*. [15]	93 patients with Alzheimer' Disease; the severity of LA was analysed for ACE I/D variant.	ACE D/D genotype was a risk factor for damage in cerebral white matter in Alzheimer' disease.
Szolnoki *et al*. [16]	229 patients with LA was compared with 362 control subjects	ACE D/D genotype in combination with MTHFR 677TT genotype was a risk factor for leukoaraiosis with or without cerebral infarction.
Szolnoki *et al*. [17]	308 ischaemic stroke patients, 272 controls	ACE D/D genotype combine with at least AT1R A1166C allele was a risk factor for cerebral small-vessel infarcts.
Henskens *et al*. [18]	93 hypertensive patients	ENOS G894T variant was a risk factor for cerebral white matter damage.
Hassan *et al*. [22]	172 patients with cerebral small-vessel disease, 172 controls.	MTHFR C677T variant can be risk factor for leukoaraiosis via endothelial dysfunction
Szolnoki *et al*.[21]	357 ischemic stroke patients, 263 contols without stroke	MTHFR 677TT variant in combination with AT1R A1166C allele was a risk factor for cerebral small-vessel infarcts.
Szolnoki *et al*. [13]	689 ischaemic stroke patients, 652 stroke free controls	ACE D/D genotype in combination with the MTHFR C677T allele was a risk factor for small-vessel cerebral infarcts.

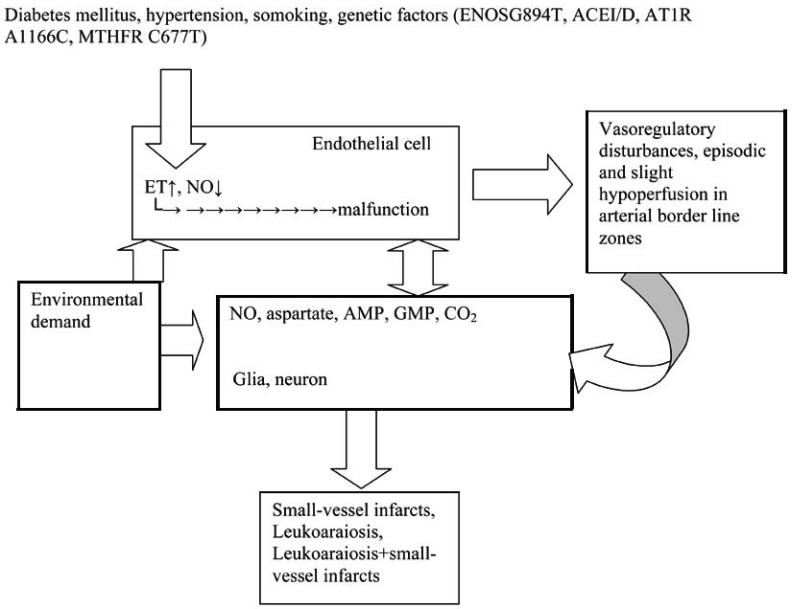

Fig. (2). Model of cerebral small-vessel circulatory disorders facilitated by endothelial dysfunction-associated genetic factors (the scheme of neurovacular coupling).

cells must be the effectors of this process. Hence, an endothelial dysfunction can presumably lead to a malfunction of the process [6]. The extent of the endothelial dysfunction, the environmental demand and the local anatomy of the microcirculation are the main parameters which determine whether small-vessel cerebral infarcts or LA or both will evolve. There may also be a possibility that the only result will be a reduction in functional cortical power (a cognitive dysfunction or an executive dysfunction).

A hypothetical model of cerebral small-vessel circulatory disorders facilitated by endothelial dysfunction-associated genetic factors is illustrated in Fig. (**2**).

SUMMARY AND THERAPEUTIC APPROACH

In summary, the endothelial dysfunction-associated genetic variants, such as the ENOS G894T, ACE I/D, AT1R1166C and MTHFR 677TT variants, may give rise to different and common pathological phenotypes of the brain (small-vessel infarcts or LA).

The phosphodiesterase type-5 inhibitors (PDE5Is) are currently at the center of the therapy of endothelial dysfunction-associated circulatory diseases such as an erectile dysfunction or pulmonary hypertension [25,26]. They can improve the endothelial function by several routes. The effects of NO can occur within seconds, because of the normal turnover of cGMP is high: a rapid degradation to GMP by a phosphodiesterase constantly balances the production of cGMP. The PDE5Is inhibit this cyclic phosphodiesterase, thereby increasing the amount of time that cGMP level remain elevated. They can increase the production of ENOS and NO in the endothelial cells, and they can also increase the number of circulating endothelial progenitor cells [27]. This kind of drug treatment may be of great perspective in endothelial dysfunction-associated cerebral circulatory disorders, and appears to demand testing in a well-designed drug study in homogenous patient groups. Recent results exhibit that PDE5Is improves dynamic vascular function in the brain [28]. The administration of folic acid resulting in a reduced serum homocysteine level and improved endothelial function may be a therapeutic possibility too [29]. The angiotensin-converting enzyme inhibitors and AT1R blockers may yield an alternative therapeutic approach, by reducing the activity of angiotensin II-AT1R axis. A combination of these possibilities may also be arisen. The therapeutic approaches are shown in Fig. (**3**).

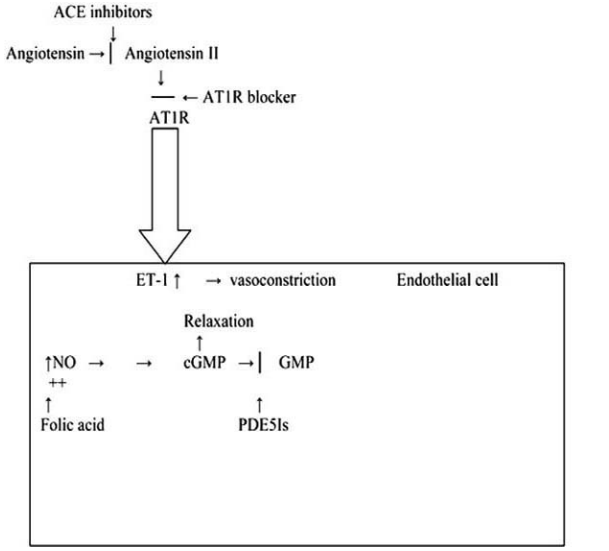

Fig. (3). Therapeutic approaches of endothelial dysfunction.

Accumulating knowledge on endothelial dysfunction associated-genetic variants may help in the choice of the population at higher risk for these cerebral disorders. The genetic variants that are associated with endothelial dysfunctions may be candidate risk factors for small-vessel infarcts or/and LA. The hypothesis concerning endothelial dysfunctions, genetic variants and cerebral circulatory disturbances calls for further studies. The association hypothesized between the endothelial dysfunction-associated genetic variants and cognitive malfunctions may likewise be an interesting new research field [14].

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