The presence of a functional collateral circulation can have great importance for clinical symptoms as well as for clinical decision making. When experiencing an acute myocardial infarction, most patients have severe symptoms, call for emergency, and are treated rapidly and effectively given a functioning infrastructure for primary percutaneous coronary interventions. In contrast, other individuals experience few symptoms, and some may not even notice their infarction.

One possible explanation for the attenuated symptoms in the second group of patients is the presence of functional coronary collateral vessels, which are capable of supplying significant amounts of blood into an otherwise completely ischemic myocardium. In fact, the significant variability of native collateral conductance in humans is well established.1

Moreover, the genetic background had been identified as a denominator of the presence of functional collateral vessels in mice as different mouse strains show major differences with regard to the presence of collaterals.2,3

The study by Chalothorn et al, published in this issue of Circulation Research,4 provides a potential explanation for the interindividual variability of the presence of functional collateral vessels. In fact, data from both the peripheral circulation (hindlimb model), as well as from the cerebral circulation of a mouse model support the idea that the absence of the CLIC4 gene, ie, the gene encoding chloride intracellular channel-4, is related to the presence of fewer (approximately one-third) collateral vessels in adult mice. Likewise, the diameter of the collaterals was slightly (roughly 30%) reduced. Of interest, hemizygous CLIC4+/- mice showed an intermediate phenotype with regard to collateral number and size.

**A Genetic Basis for the Presence of Functional Collaterals**

The novel data from Chalothorn et al focus on differences in native collateral vessels. This refers to the process of creating arterio-arterial anastomoses during embryonic and early postnatal life in the absence of any existing pathology, a process described as collaterogenesis. CLIC4+/- mice show reduced collateral density (reduced number and size), thus impaired collaterogenesis. This leads to reduced hindlimb perfusion following femoral artery ligation, and reduced recovery of perfusion. Nevertheless, regional perfusion improves within 1 week thereafter, indicating that arteriogenesis, ie, the growth/remodeling of existing collateral vessels, is not negatively affected by CLIC4 knock-out. These novel data clearly prove that collaterogenesis and arteriogenesis are independent components, which both contribute to the collateral circulation in different ways (Table). It will be a rewarding challenge to identify the underlying components of both collaterogenesis and arteriogenesis in the clinical situation, which ultimately will enable us to use them for risk prediction, patient stratification and clinical decision making.

**Table. Collaterogenesis and Arteriogenesis: Two Distinct Components Defining the Functional Impact of a Collateral Circulation**

<table>
<thead>
<tr>
<th>Collaterogenesis</th>
<th>Arteriogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native Collateral Conductance</td>
<td>Collateral Remodeling</td>
</tr>
<tr>
<td>No. of arterioarterial connections</td>
<td>Size/diameter of arterioarterial connections</td>
</tr>
<tr>
<td>Activated during embryonic and early postnatal period</td>
<td>Activated during adult period secondary to pathological regional perfusion</td>
</tr>
<tr>
<td>Limitation: Determined by genes (eg, CLIC4)</td>
<td>Limitations unknown</td>
</tr>
<tr>
<td>Major species differences known2,3</td>
<td>Species differences unknown</td>
</tr>
<tr>
<td>Driven by VEGF-A6</td>
<td>Driven by VEGF-A, MCP-1, shear stress</td>
</tr>
<tr>
<td>Dependent on endothelial cells4,5</td>
<td>Dependent on endothelial cells and monocytes12</td>
</tr>
<tr>
<td>Possible influence of metabolic or environmental disturbances unknown</td>
<td>Negatively affected by environmental influences (diabetes mellitus etc)10,11,15</td>
</tr>
</tbody>
</table>

**CLIC4: Genetic Basis Controlling Mechanisms of Collateral Growth in Mice**

This study confirms earlier predictions that CLIC4 is involved in vessel formation. Moreover, it underscores the power of the proteomic approach initially used to identify the involvement of CLIC4 in vascular processes.5 Chalothorn et al can add important mechanistic insight into the role of CLIC4 in collaterogenesis. Of importance, CLIC4 acts upstream of other genes relevant for vascular growth including hypoxia-inducible factor-1α, vascular endothelial growth factor-A (VEGF-A), and angiopoietin-2. This aspect is crucial for understanding the contribution of the ion channel CLIC4 to vascular biology. In essence, CLIC4 regulates the availability of VEGF-A, and VEGF-A drives collaterogenes.

**Correspondence to Johannes Waltenberger, MD, PhD, Department of Cardiology, Maastricht University Medical Centre, P. Debyelaan 25, POB 5800, 6202 AZ Maastricht, The Netherlands. E-mail j.waltenberger@mumc.nl**

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See related article, pages 89–98

**Limits to Growth of Native Collateral Vessels**

**Just One Mouse CLIC Away From Unlimited Collateral Perfusion?**

Johannes Waltenberger
An important contribution of Chalothorn et al is that they clearly identify collaterogenesis as a distinct component, which shows some parallels but mostly differences compared to arteriogenesis.

VEGF-A is involved not only in driving collaterogenesis but also in arteriogenesis. VEGF-A thereby plays a crucial role in both presence and condition of collateral vessels. VEGF-A action is controlled on three independent levels:

1. Genetic determinants of the availability of VEGF-A secondary to CLIC4 expression (novel data)
2. Pathophysiological induction of VEGF-A to promote arteriogenesis (reviewed elsewhere)
3. Environmental limitations of VEGF-A action (VEGF resistance in the presence of diabetes mellitus and other negative influences, see below)

Open Issues Regarding the Role of CLIC4 in Collateral Formation

It is unquestionable that the genetic background determines the presence of collaterals in mice. The novel data by Chalothorn et al underscore this fact and provide CLIC4 deficiency as a rationale genetic basis for reduced collaterogenesis. It will be important to learn whether the previously observed differences in collateralization among different mouse strains can be explained by differences in CLIC4 expression or whether other genetic differences account for the different phenotypes. Even more important is the question of whether CLIC4 is playing a similar role in the human situation. Are there genetic abnormalities in the “poor collateralizers” related to polymorphisms or different expression levels of CLIC4? Or is CLIC4 just the tip of the iceberg and there are other players of similar relevance?

Whereas arteriogenesis is largely monocyte-driven, the role of monocytes in CLIC4-dependent collaterogenesis remains to be investigated. CLIC4−/− mice have an endothelial phenotype with fewer preformed collateral vessels present, however, other cell types such as monocytes have not been looked at in this context. Monocytes are of special interest because of their paracrine release of growth factors including VEGF-A and their contribution to arteriogenesis. It will certainly be of interest to study the involvement of monocytes in collaterogenesis of CLIC4−/− mice and to investigate whether these cells are functioning properly. Likewise, the role of pericytes, resident progenitor cells, and circulating endothelial cells and their homing ability remain to be investigated in the absence of CLIC4.

Potential Future Algorithms for Predicting Collateral Function

The classic determinants of collateral growth are shear stress, the availability of certain growth factors, and monocyte-related paracrine processes. There are 2 potential reasons for poor or inadequate collateralization (“poor collateralizers”), namely (1) an unfavorable genetic basis; and (2) metabolic or other pathological disturbances:

1. Reduced levels of CLIC4 predispose for poor collateral function as collaterogenesis is impaired in the mouse. Because this CLIC4-related phenotype is very evident in mice, its existence and relevance remains to be proven in humans. There may be other genetic reasons underlying poor collateralization, such as polymorphisms related to reduced CD44 expression or others. It will be important to validate these and other genetic characteristics of reduced collateral development and test them in the clinical situation.

2. It has recently been demonstrated that metabolic influences can have a negative impact on arteriogenesis. This is true for diabetes mellitus, where monocyte dysfunction and VEGF resistance limit arteriogenesis. Likewise, monocyte dysfunction and VEGF resistance can be found in hypercholesterolemia and smoking, predicting impaired arteriogenesis. Although the process of arteriogenesis is understood to some extent, the determinants of collaterogenesis are still poorly understood. Nevertheless, defects in collaterogenesis may be crucial keys to understand the variability in collateral function in the clinical situation, and this may be negatively affected by metabolic diseases as well. What about the number of collaterals in individuals from diabetic mothers, thus, where collaterogenesis took place in the presence of a diabetic milieu and where the collateral circulation matured under pathological conditions?

It will be an important task to dissect both genetic and metabolic denominators of collateralization and to determine their relative, as well as their individual, impact on patients with atherosclerotic disease, who would benefit from the presence of functional collateral vessels.

Lessons for Implementing Therapeutic Arteriogenesis in Patients

Therapeutic arteriogenesis, previously called therapeutic angiogenesis, is defined as a therapeutic intervention aiming in improving regional tissue perfusion. Both growth factor protein application and growth factor gene transfer have been applied, focusing on VEGF-A and fibroblast growth factor-2 and -4, but placental growth factor, hepatocyte growth factor, VEGF-B, VEGF-C, VEGF-D, and VEGF-E are interesting candidates as well. Often, therapeutic angiogenesis has been used to describe therapeutic arteriogenesis, because it is not always possible to discriminate between arteriogenesis and angiogenesis in a given complex situation. For clarity of the concept, I prefer to use therapeutic arteriogenesis to indicate the therapeutic stimulation of arteriogenesis.

Genetic testing of potentially suitable patients before an intervention could be beneficial for the outcome of that intervention, namely to better predict the collateral potential, ie, the outcome of the proarteriogenic intervention. One might argue that stimulating therapeutic arteriogenesis would be more promising, if there were more collateral connections to start, ie, if collaterogenesis was more advanced or more complete. Large randomized clinical trials to stimulate therapeutic arteriogenesis had failed in the past largely because of the variability of the results, likely related to the heterogeneity of the study population and possibly attributable to the inclusion of nonresponders. If it was possible to identify or stratify this subgroup beforehand (eg, polymorphism for CLIC or other genes limiting collaterogenesis and/or the
arteriogenic potential), the outcome of such a proarteriogenic intervention could be more predictable and its efficiency could be demonstrated more reliably. This does not exclude that the therapeutic stimulation of arteriogenesis might even result in enhancing the number of arterioarterial connections, ie, recapitulated collaterogenesis during adulthood, at least, if the genetic background (eg, high CLIC4 levels) would allow this to happen.

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**References**


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Johannes Waltenberger

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