Atherosclerosis is the major cause of death in the Western world. The mechanism leading to atherosclerotic lesion formation has been tremendously updated in the last decades. From a merely degenerative concept, the pathogenesis of the disease is now well ascertainment to be inflammatory. It is also recognized that the thrombotic complications of the disease are a consequence of the disruption of the atherosclerotic plaque due to the erosion of the endothelium or the rupture of the fibrous cap covering the lipid core within in the plaque (127). The current morbidity and mortality and increasing age of the population require the development of new diagnostic and therapeutic strategies to treat early subclinical disease stages.

In addition to the endothelium, blood vessel walls are composed of many constituents such as matrix base ment, smooth muscle cells, and pericytes. All these components form a cohesive tissue that requires, for proper function, a constant exchange of information via electrical and chemical signals. A key pathway that allows such communication is gap junction (GJ). GJ channels, composed of six connexins to form a hemi channel in the cell membrane, are important for signaling between intracellular and extracellular spaces. Two hemi-adjacent cells can form a full gap junction channel, allowing the direct exchange of ions, small metabolites, and other secondary messenger molecules between cell in contact (101).

**Pathogenesis of Atherosclerosis**

The dysfunction of the endothelium, often induced by hypercholesterolemia and other cardiovascular risk factors, is the initial step of atherosclerosis. Atherosclerotic lesions are typically observed in arteri al vessels with high shear stress such as bifurcations. Endothelial cells (ECs), once activated, recruit mainly monocytes and T-cells (46), but also platelets (118). Platelet rolling is initiated by activation-dependent selectins present on endothelial and platelet surfaces (36). Indeed, mice with platelets lacking P-selectin develop smaller atherosclerotic lesions than mice with wild-type platelets (14). This protection is enhanced when both P- and E-selectins are also lacking (30), illustrating the early role of platelets in the disease. Interestingly, the lack of the integrin α₅β₃ (a subunit of the fibrinogen receptor) in mice exposed to this lesion formation (83). Beside selectins, activated ECs express integrins such as vascular cell-adhesion molecule 1 (VCAM-1) in response to cholesterol accumulation in the intima (23). T-cells and monocytes, expressing both the very late antigen 4 (VLA-4), bind thereafter to endothelium and transmigrate under the influence of chemokines such as macrophage colony-stimulating factor that is produced by low-density lipoprotein (LDL)-stimulated ECs and smooth muscle cells (SMCs) (99). SMCs may also express VCAM-1, promoting further recruitment and retention of mononuclear cells in the intima (72). Once migrated within the plaque, two phenotypes of macrophages appear: inflammatory macrophages and foam cells. The latter cells are characterized by the accumulation of cholesterol in their cytoplasm, resulting from the uptake of oxidized lipoprotein by these cells (113). Besides macrophages, T-cells are the second most important cell population in the atherosclerotic plaque (~10%) (61). They are important determinants in the disease by governing the transition from a latent plaque to a vulnerable plaque (46). CD4+ T-cells and T-cells isolated from atherosclerotic plaques recognize oxidized LDL (114), suggesting a local stimulation of T-cells by monocytes/macrophages in the plaque through major histo-compatibility antigen II. These T-cells express a pro-inflammatory (TIL) phenotype, suggesting that atherosclerosis is a T₃,4-driven pathology (114, 148). T₃,4-type cytokines are predominant in plaques, but II-4, a T₄,2-type cytokine, is also produced by a few cells (37). Thus the initiation of atherosclerotic lesions is induced by many cell types within the vasculature, circulating or resident.

**Rupture of the Atherosclerotic Plaque is the Primary Cause of Sudden Cardiac Death**

Acute coronary syndrome (ACS) results mostly of sudden luminal thrombosis (15), which may result from three different pathological processes: plaque rupture, erosion, and calcified nodules (128). Rupture occurs when the fibrous cap overlying a necrotic core is thin and broken, inducing luminal thrombosis. Lesions with erosion may also show a luminal thrombus but are often devoid of necrotic core or the core is isolated from the lumen by a thick fibrous cap (127). Calculated nodules are rarely exposed to thrombus, as they are often devoid of necrotic core. About 55–60% of ACS result from a rupture of the fibrous cap and another 20% from erosion. These two pathological processes usually overlap and are responsible for the unstable plaque phenotype (39, 115), leading to an increased risk of cardiovascular events. Thus, the rupture is considered the primary cause of ACS (128, 129). Therefore, the understanding of the mechanisms leading to the rupture of the fibrous cap is of particular importance in the management of ACS patients. In the next paragraphs, we will discuss the role of connexins in the pathophysiology of atherosclerosis.
nerves are rare and show a calcified plate with superimposed bony nodules resulting in discontinuity of the fibrous cap with overlying luminal thrombus (127). About 55–60% of sudden deaths are caused by plaque rupture, and ~30–35% are due to plaque erosion (127, 129). Therefore, the quality of the plaque is essential for the evolution of the pathology. Plaques may be stable or prone to rupture, depending on the size of the necrotic core, infiltration of the fibrous cap by macrophages, foam cells, and calcification. Thus collagen fibers allow stability of the plaque (186). Interferon-γ, a T helper 1 cytokine, is known to inhibit both collagen production and proliferation of SMCs, the main source of this extracellular matrix (3). Moreover, collagen is degraded by proteases secreted by macrophages, SMCs, and lymphocytes (38, 40, 90). Lastly, the rupture of the fibrous cap allows platelets and leukocytes to be activated by thrombogenic tissue factor (TF) in the necrotic core. Interestingly, the main source of TF are monocytes, responsible for the acute thrombus propagation overlying an unstable plaque (41). T cells also induce the production of TF by macrophages in the plaque (13, 86). Hence, following the formation of the plaque, many cells and processes are responsible for their quality. The plaque rupture is life threatening and leads to thrombosis and myocardial ischemia.

Connexins in the Vasculature

GJs mediate the direct exchange of ions, small metabolites, and other secondary messenger molecules between adjacent cells (101). This allows for synchronization of cell responses in tissues, necessary for the contraction of cardiac and smooth muscle cells, for the transmission of signal between neurons and for the organ development. GJs are formed by connexons and more than 20 different connexins have been described (112). Six connexins form a connexon or hemichannel, two connexons form a functional GJ channel. Many combinations of connexins may assemble into channels, and each type of GJ channel has its proper gating and permeability properties. In addition, the expression pattern of connexins is complex with multiple connexins in one cell type, and this is enhanced by the very short half-life of connexins (~1–5 h), indicating that channels are renewed several times daily (101). This signifies that connexin expression modulation at multiple levels could be a mechanism to regulate intercellular cross-talk.

At least four connexins have been described in the vascular system, connecting ECs and SMCs in the blood vessel wall (26, 48, 49). Expression of connexins varies between blood vessel type (48) and species (26). Thus Cx37 and Cx40 are usually expressed in ECs (145). Cx43 and Cx45 are expressed in SMCs. Furthermore, Cx43 is detected in few arterial ECs exposed to turbulent shear stress and in capillaries (22, 38, 119). Cx37 and Cx40 have been observed in SMCs of small elastic or resistance arteries (44, 79, 87, 123). Cx43 is generally considered as the predominant connexin in the media (105). This has been reported mostly for aorta, an elastic artery (105, 122). However, Cx43 can barely be detected in the media of muscular arteries (31) and of coronary arteries (12, 43, 51, 125, 145). Thus many connexins are present in the vasculature, leading to complex communication exchanges within tissue.

Major Roles of Connexins in the Vasculature

Connexins have many functions in vascular physiology, such as conduction of vasomotor responses and tone among SMCs (20, 26, 33), capillary sprouting, and endothelial repair (65). Specifically, Cx40 is important for blood pressure regulation. Cx40−/− mice are hypertensive (27, 28, 104), possibly due to the requirement of the protein for the transmission of the endothelium-dependent vasodilator response (27). Furthermore, a link between Cx40 expression and renin secretion has been reported. Hence, the plasma titer of renin is increased in Cx40−/− mice due to a modified number and distribution of renin-secreting cells (62). Moreover, a polymorphism of Cx40 is linked to an increased risk of hypertension (35). In contrast, it seems that mice with an endothelial-specific deletion of Cx43 suffer from hypotension and bradycardia (73). Although contradictory results have been described as well (119). Interestingly, Cx43 is thought to be involved in the sensitivity to mechanic stimuli due to overexpression during shear stress, in particular in cardiac valves ECs (29, 58).

Deletion of connexins affects also the vasculature development. Indeed, Cx45−/− embryos die between days 9.5 and 10.5 (63) and Cx37−/− females are infertile (109). Cx37−/− mice do not display a noticeable vascular phenotype (34), but when two connexins are lacking, Cx37−/−Cx40−/− pups die perinatally due to severe vascular abnormalities, characterized by local hemorrhages and sorts of hemangiomata (110).

Expression of Connexins by Inflammatory Cells

Atherosclerosis is an inflammatory disease in which immune cells infiltrate the atherosclerotic plaque. These inflammatory cells are mainly blood-borne T-cells and macrophages. They accumulate during the early phases of plaque formation. Adherent murine macrophages are electrically coupled (71), and dye transfer has also been observed between them (82). Interestingly, cellular activation seems to increase GJ coupling, as seen in microglia (32) and human monocytes (31). However, other studies have not been able to detect dye transfer between

Connexins are Expressed in the Vascular Wall

Adherent murine macrophages are electrically coupled (71), and dye transfer has also been observed between them (82). Interestingly, cellular activation seems to increase GJ coupling, as seen in microglia (32) and human monocytes (31). However, other studies have not been able to detect dye transfer between
human or murine monocytes (2, 98), between human monocytes and ECs and between human mononuclear cells and SMCs (31, 86). Various connexins have been found in mononuclear cells: Cx43 is present in mouse macrophage cell lines (2, 7, 16), in activated peritoneal hamster and mouse macrophages (2, 40), and in mononuclear cells stimulated by tumor necrosis factor (TNF)-α or IFN-γ, and Cx47 has been detected in human and mouse monocytes (135).

Lymphocytes form functional GJs. Electrical coupling has been observed after phytohemagglutinin-stimulation (56). Dye-coupling between lymphocytes and ECs has also been reported (91). Cx43 is found in human blood-derived T- and B-cells and CD35+/+ (natural killer (NK)) cells (92). Tonol-derived T- and B-cells express both Cx40 and Cx43 (92). Neutrophils also play a role in atherosclerosis by contributing to the pathogenesis of the disease (93). Neutrophils have been described to express Cx37, Cx40, and Cx43, but not Cx31 (11, 146). After activation by Lipopolysaccharide (LPS) or TNF-α, human neutrophils are able to form morphological, but not dye-coupled, GJs (11). Functional GJs have been shown between ECs and neutrophils, and this was decreased by TNF-α (146). Thus connexins are expressed and GJ channels are functional in blood cells and reveal a communication network between circulating cells and vascular cells.

Expression of Connexins During Atherosclerosis Is Altered and Participates in the Disease Progression

Atherosclerosis is a progressive disease, and Cx37, Cx40, and Cx43 expression patterns change during plaque formation (summarized in FIGURE 1) in murine and human atherosclerotic plaques (84). Here, we will report the role of GJs in atherosclerosis throughout the disease progression.

Endothelial dysfunction initiates the disease

The major risk factors for atherosclerosis are age, hypertension, hyperlipidemia, smoking, and diabetes. These conditions influence endothelium biology. ECs display GJs, and connexin expression is tightly regulated. Deregulation can occur in different pathological conditions that will be discussed here.

Aging seems to induce a general decrease in connexin expression, with Cx40 being relatively undisturbed for a long time (138). Nicotine induces a decrease in Cx43 expression due to an enhanced protein degradation (121).

Hypertension is a cause of ECs dysfunction and a major risk factor of atherosclerosis. Hypertensive rats have a reduced endothelial expression of Cx37 and Cx43, but Cx40 expression is not modified (141). Moreover, carvedilol, a commonly used β-blocker for hypertension and cardioprotection, directly upregulates endothelial Cx43 independently of its antioxidative activity (141). Oxidation products of lipoprotein-derived phospholipids upregulate Cx43 and downregulate Cx37 but do not affect Cx40 expression in murine endothelium of carotid arteries both in vivo and in culture (59). ECs are also sensitive to cholesterol tilters. Increased GJ assembly between hepatoma cells is observed after increasing the cholesterol level or treatment with LDL or apolipoprotein B (85, 86). Besides the effect of cholesterol on Cx43 expression, membrane cholesterol content affects the Cx43 channel. Indeed, neonatal cardiomyocytes are also protected against the heptanol-induced closure of Cx43 GJs with increased membrane cholesterol (5).

Cx37 and Cx40 are abundant in murine endothelium. Although Cx43 is mostly absent in murine aortic endothelium, it is abundant in ECs localized at the downstream edge of the ostia of branching vessels and at flow dividers, regions where the blood flow is turbulent and experienced shear stress (38), but the ECs covering advanced plaques no longer express Cx37 and Cx40 (64,142). These observations have been confirmed by in vitro studies, showing a positive correlation between Cx43 expression and disturbed flow patterns (22, 24, 29). For example, the expression of endothelial Cx43 increases in response to oscillatory shear stress in vitro, but the pressure does not affect Cx43 expression (66).

The correlation between coronary heart disease and both Type 1 and Type 2 diabetes mellitus has been recognized by epidemiological data (42, 132). Diabetes mellitus markedly suppresses Cx37 GJs in cardiomyocytes. Interestingly, simvastatin reverses this effect (108). In contrast, endothelial Cx37 and Cx40 are downregulated in diabetic-induced ApoE−/− mice, and simvastatin exacerbates the downregulation (53). Coronary ECs of diabetic mouse show lowered protein levels of Cx37 and Cx40, but not Cx43, and a reduction of GJ communication (81).

As described before, shear stress increases Cx43 expression in ECs and decreases Cx37 expression by inhibition of c-Jun N-terminal kinase (JNK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and promotes PI3K/Akt/Erk1/2 signaling (136). In contrast, simvastatin upregulates Cx43 and downregulates Cx37 (64).

Finally, ECs express VEGF. VEGF downregulates Cx37 and Cx43 (64). In contrast, endothelial Cx43 increases in response to oscillatory shear stress in vitro, but the pressure does not affect Cx43 expression (66). Oxidation products of lipoprotein-derived phospholipids upregulate Cx43 and downregulate Cx37 but do not affect Cx40 expression in murine endothelial cells. Thus a large variety of factors regulates Cx40 expression in ECs, Cx37 and Cx43 expression in endothelium is clearly influenced by atherosclerosis.

Tissue hypoxia, due to ischemia and the subsequent reoxygenation by pharmacological- or coronary intervention-induced reperfusion increases the production of superoxide that has different biological effect. Hypoxia/reoxygenation of ECs inhibits GJ communication. Moreover, abrupt reoxygenation of ECs reduces protein kinase A activity and reduces electrical coupling. This effect is prevented in Cx40−/− mice, signifying that the abrupt reoxygenation targets Cx40, compromising arteriolar function (10). C-reactive protein (CRP) is a conserved protein present in low level in humans. But, in response to infection, cancer, tissue injury, CRP titer increases rapidly up to 1,000-fold (95). CRP is associated with coronary atherosclerotic disease (1, 102) and is a predictive marker for cardiovascular risk (57). It is at present not clear whether CRP is only a risk marker or rather a functional risk mediator (126). Nevertheless, CRP influences the expression of endothelial Cx43 (131). CRP decreases Cx40 expression, as shown in human umbilical vein endothelial cells (HUVECs) (139).

Activated ECs secrete large amounts of cytokines that activate monocytes and cause monocyte transmigration. CRP increases the expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) in ECs (140). As a result, CRP enhances leukocyte-EC adhesion (141). ECs express CRP receptors (CRP-R). CRP-R expression is increased in atherosclerotic plaques in vivo and in cultured ECs stimulated with IL-1, TNF-α, and LPS (58). CRP-R expression is associated with inflammation (143).

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influences the gene expression profile of human vascular endothelial cells and enhances monocyte adhesion to ECs (131). Moreover, CRP upregulates Cx43 mRNA expression, as shown by microarray analysis (131).

Activated ECs are at the crossroad of inflammation and hemostasis. Sepsis is a life-threatening state defined by an infection associated with a systemic inflammatory response syndrome (SIRS). In a model of rat peritonitis induced by cecal ligation and puncture, Cx40 expression increases in aortic ECs (100). In contrast, connexin expression and intercellular communication decrease in Cx43–/– mice induced by LPS (111). Apparently, changes in connexin expression depend on the inflammatory model used as well as on the source of infection. Growth factors have an important role in connexin biology. Vascular endothelial growth factor (VEGF) impairs GI communication in ECs (115). The disruption of Cx43 GJs is due to Cx43 internalization and phosphorylation (120). Moreover, epidermal growth factor decreases the GI coupling in early passage HUVECs (136). In contrast, transforming growth factor (TGF-β) increases Cx43 synthesis (68, 70) but decreases Cx37 expression in HUVECs (70). In epithelial cells, TGF-β increases Cx37 expression via p38 and phosphorylating GSK-3 kinase (PI3K)/Akt signaling pathways (116). Finally, basic fibroblast growth factor (bFGF) increases Cx43 expression and GI communication, and antibodies against bFGF abolish intercellular coupling and decrease Cx43 expression during wound healing (94). Finally, ECs are very sensitive to tumor necrosis factor alpha (TNF-α), which activates ECs by promoting expression of adhesion molecules. Thus treatment of ECs with TNF-α decreases the expression of Cx37 and Cx40 but does not change Cx43 expression (123). In keratinocytes, TNF-α increases Cx43 expression, and inhibition of c-Jun NH2-terminal kinase (JNK) abolishes this expression (117). Activation of ECs (52, 123) or SMCs (84) with LPS, TNF-α, IL-1α, or IL-1β reduces the GI communication. Finally, TNF-α induces closure of myoendothelial GJs in co-culture of human ECs and SMCs (54). This modifies both gathing and permeability of GJs.

Thus a large range of stimuli modify GI expression and enhance endothelial dysfunction, leading to atherosclerosis initiation.

**Leukocytes infiltrate the arterial intima**

As described above, TNF-α induces changes in expression of connexins in ECs that affect the migration of leukocytes in different inflammatory pathologies. Thus monocytes and lymphocytes are recruited early in atherosclerosis. A role for connexins in leukocytes migration studied in different inflammatory models and results are conflicting (19, 25). Thus diapedesis of neutrophils is increased or not by using connexin-mimetic peptides or pharmacological channel blockers (19, 103, 146). Using same modulators, monocytes transmigration is decreased (31), but lymphocytes transmigration is only modestly affected (91). Connexin expression is modulated during atherosclerosis. Endothelial Cx37 and Cx40 expression is considerably reduced in low-density lipoprotein receptor (LDLR)–/– mice on a cholesterol-rich diet for several months (64). Moreover, Cx37 is present in monocytes and macrophages in early and late atheroma (64) but also in medial SMCs beneath advanced atherosclerotic lesions (64).

So, ECs covering the advanced plaque do not express Cx37 anymore, but this connexin is still expressed on recruited macrophages (64). Atherosclerosis in Cx37–/– ApoE–/– mice under high-cholesterole diet is exacerbated (135) in both descending aorta and aortic sinuses. The transmigration of mononuclear cells is due to the presence of Cx37 on themselves and not to the expression of Cx37 on ECs, as shown by adoptive transfer. Furthermore, the adhesion of mononuclear cells to activated ECs monolayer is increased when Cx37 is lacking in mononuclear cells. The anti-adhesive effect is due to extracellular release of ATP through hemi-channels. Finally, one Cx37 polymorphism in macrophages affects their adhesiveness (135).

Besides Cx37, a specific deletion of Cx40 in ECs to circumvent the hypertensive phenotype of the systemic Cx40 deletion increases lesion development when atherosclerosis-susceptible ApoE–/– mice were fed with a diet rich in cholesterol.
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high-cholesterol diet (18). Altogether, all these results underline the importance of GJ communication for and during leukocyte infiltration in atherosclerosis.

**Smooth muscle cell migration in the growing phase**

During the growing phase, SMCs transmigrate from the media to the intima. There, they continue to grow and produce components of the extracellular matrix, leading to a strong fibrous cap covering the original plaque. In general, this cover safely isolates the plaque from the blood. Macrophage and SMCs filled with lipids die within the plaque and release lipids. This process generates the typical lipid core of atherosclerotic lesions.

At this stage, two qualities of plaque exist. Plaques with a thin cap, prone to rupture, are composed of a large lipid core and numerous macrophages. Their phenotype is dependent on macrophage activity. Macrophage foam cells produce pro-inflammatory cytokines, amplifying inflammatory response that, in turn, induces macrophage proliferation and lipid uptake. Moreover, activated macrophages produce matrix metalloproteinases (MMPs) that degrade extracellular matrix and weaken the fibrous cap.

On the other hand, stable plaques, with larger fibrous cap and reduced lipid core, are less prone to rupture, and thus the stabilization of the plaque has become a potentially new therapeutic goal.

The expression of Cx43 differs in intimal SMCs and depends on the stage of the disease. Its expression is increased at early stage but decreased in advanced human plaques (8). Cx43 expression is also decreased between intimal SMCs in advanced lesions in the hypercholesterolemic rabbit (97) and in LDLR−/− mice (64). In addition, Cx43 mRNA is present in foam cells of human and rabbit atherosclerotic carotid arteries (97, 98). The reduction of Cx43 expression inhibits the formation of atherosclerotic lesions in mice models. Cx43+/− LDLR−/− mice (under cholesterol-rich diet) display reduced atherosclerosis lesions, with smaller lipid core and fewer macrophages and T-cells despite normal leukocyte blood counts (67). In addition, lesions have thicker fibrous caps containing more SMCs and interstitial collagen. Interestingly, activation of monocyctic cells, which are associated with the release of MMP-2 and -3, increases GJ communication through Cx43 channel (31). Enhanced MMP release may favor plaque rupture. Interestingly, T(α)1 cells secrete more MMPs than T(α)0 or T(α)2 (6). Moreover, both Cx43 expression and dye-coupling between macrophages and T-cells increase in T(α)1 CD4+ T-cells compared with T(α)0 and T(α)2 (98), pointing out a potent role of connexins in atherosclerotic plaque quality. Thus Cx43 targeting potentially favors plaque stabilization besides its action on plaque development (133).

Finally, Cx43 expression is enhanced in intimal thickening after acute vascular injury (16, 74, 96, 144). Interestingly, the time course of restenosis changes when the Cx43 is reduced (16, 74, 130). Somehow results are opposed, depending on whether the Cx43 expression is systematically reduced, leading to a reduced neointima formation (16), or deleted in SMCs only, leading to an increased neointimal formation (74). It should, however, be kept in mind that the specific deletion of Cx43 in SMCs reduces the expression of Cx43 in aortic endothelium (74), and specific deletion of Cx43 in ECs reduces Cx43 coding mRNA in the adjacent vascular smooth muscle layer (73). This reflects the complex GJ interactions between different cell types. Similar interactions are observed in Cx43−/− mice, supporting a tight relationship between different connexins in ECs.

Recently, it has been described that SMCs of pig coronary arteries show a heterogeneous phenotype, with both spindle-shaped SMCs (S-SMCs) and thomboïd SMCs (R-SMCs) (47). S-SMCs are predominant in the normal media, whereas R-SMCs are present in stent-induced intimal thickening. R-SMCs have an increased proliferative, migratory, and proteolytic phenotype and are involved in vascular tissue repair and restenosis. S-SMCs express Cx40, but R-SMCs do not. R-SMCs display higher Cx43 expression levels and more cell-to-cell coupling than S-SMCs. Moreover, S-SMCs treated with platelet-derived growth factor-BB acquire an R phenotype, with an increased expression of α-smooth muscle actin. This platelet-derived growth factor-BB-induced phenotypic change is prevented by a reduction of Cx43 expression by an antisense oligonucleotide (17). Therefore, Cx43 may also be an attractive target for local delivery strategies aimed at reducing restenosis.

**Connexins as Cardiovascular Risk Markers and Therapeutic Targets**

There is increasing attention to genetic variation in connexin genes and their relationship to cardiovascular disease. A Cx37-1019C polymorphism has been reported to be a potential prognostic marker for carotid atherosclerotic plaque development in a Swedish population (9) and coronary artery disease in a Taiwanese and Swiss population (134, 139). In addition, a Chinese study showed that out of three studied polymorphisms in the Cx37 gene, the C allele contributes to an increased risk of coronary artery disease (48). In contrast, the Cx37-1019T polymorphism has been associated with acute myocardial infarction in a Japanese and Sicilian population (50, 77, 78, 137). Finally, the Cx-1019T polymorphism appears in a prospective study of death after acute coronary syndrome (68). The association of the polymorphism with CAD or MI has not been found in an Irish study group (52). Moreover, the Cx37-1019T polymorphism is not associated with carotid artery disease (47). It is also known to be linked to hypertension.

Altogether, the expression of Cx37 is decreased in CAD or MI has not been found in an Irish study group (52). A Cx37-1019C polymorphism has been associated with acute myocardial infarction (45). In contrast, the Cx37-1019T polymorphism has been associated with an increased risk of coronary artery disease (134, 139). In a rat model, the same benefit was observed for the Cx37-1019T polymorphism as for the Cx37-1019C polymorphism. The decrease of LDL cholesterol levels (142). The duration of this effect was not assessed.

Along with this, studies have also shown that Cx37 expression is linked to hypertension (12), with Cx37. Moreover, Cx37 expression is increased in intimal thickening after acute vascular injury (16, 74, 96, 144). Interestingly, the time course of restenosis changes when the Cx43 is reduced (16, 74, 130). Somehow results are opposed, depending on whether the Cx43 expression is systematically reduced, leading to a reduced neointima formation (16), or deleted in SMCs only, leading to an increased neointimal formation (74). It should, however, be kept in mind that the specific deletion of Cx43 in SMCs reduces the expression of Cx43 in aortic endothelium (74), and specific deletion of Cx43 in ECs reduces Cx43 coding mRNA in the adjacent vascular smooth muscle layer (73). This reflects the complex GJ interactions between different cell types. Similar interactions are observed in Cx43−/− mice, supporting a tight relationship between different connexins in ECs.

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Of note, these results demonstrate the importance of connexins in atherosclerosis and hypertension and their role as potential prognostic markers. However, it also clearly illustrates the need for studies in larger populations and for well defined clinical end-points.

Statins (inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase) have been shown to be effective in reducing cardiovascular risk, mainly by lowering cholesterol levels (76, 88). Importantly, studies have shown that statins also inhibit, in a dose-dependent manner, Cx43 expression in human vascular cells and reduced GJ communication within the prescribed pharmacological range (133). The pleiotropic role of statins has also been studied in mice. Indeed, mice are resistant to the lipid-lowering effect of statins, allowing the discrimination between pleiotropic and cholesterol-lowering effects in plaque stability (75). Hence, statins reduce Cx43 expressions in plaques of LDLR–/– mice (67). Moreover, statins reverse the decreased expression of Cx43 in aortic tissue specimens of LDLR–/– mice (67). It should be noted that the deletion of Cx43 in aortic sections of LDLR–/– mice results in a decreased macrophage infiltration is observed compared with controls (140). Furthermore, a decreased macrophage infiltration is observed in LDLR–/– mice with reduced Cx43 expression (Cx43–/–) (17) and studies of LDLR–/– mice with peritoneal macrophages display a decreased chemotactic activity for SMCs. In addition, Stress in intima-media thickness, carotid artery compliance, or brachial artery flow-mediated dilation (all markers of subclinical atherosclerosis) in young Finnish adults (21). Cx40 has been linked to hypertension in men (35).

Along with medical therapy, mechanical intervention on arteries is often necessary. Despite new techniques in percutaneous coronary intervention, such as drug-eluting stents (DES), arterial occlusion caused by neointimal hyperplasia (in-stent restenosis) or thrombosis remain an important problem (89). Carotid balloon distension injury in animal models induces marked endothelial denudation, leukocyte infiltration, and activation of medial SMCs. In a rat model of balloon acute injury, an increased expression of Cx43 is measured in SMCs within the lesion media first and later in the neointima as well (144). In a porcine model, Cx40 is downregulated and Cx43 is markedly upregulated in stent-induced intimal thickening (17). After denudation injury and 28 days of healing, expression of Cx40 returns to that of controls, but expression of Cx37 and Cx43 is increased compared with controls (140). Furthermore, a decreased macrophage infiltration is observed in LDLR–/– mice with reduced Cx43 expression (Cx43–/–) 7 days after balloon injury, and Cx43–/– LDLR–/– peritoneal macrophages display a decreased chemotactic activity for SMCs. In addition, less SMC infiltration and proliferation is observed in

**Conclusion**

In this review, we have summarized an emerging role of connexins in all sorts of cardiovascular disease. Studies on knock out mice have unequivocally demonstrated the role of connexins in atherosclerosis and balloon-induced restenosis. Future challenges now include the development of specific connexin-targeting drugs for therapeutic applications.

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