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Primary cilia on endothelial cells : component of the shear stress sensor localized to athero-prone flow areas

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Chapter 1

General Introduction

1.1 Hemodynamics in development and pathology

Mechanobiology is receiving enormous interest in the field of cardiovascular research, because of emerging evidence that it plays a major role in cardiovascular development and pathology. Biomechanical forces that act upon the vessel wall include shear stress and (cyclic) stretch. They modulate endothelial structure and function through mechanosensing/transducing mechanisms, ultimately resulting in regulation of endothelial gene expression (reviewed by Lehoux *et al.*¹). Shear stress is the parallel frictional drag imposed on endothelial cells by the viscous blood. Stretch, which is related to blood pressure, acts perpendicular to the endothelium. Stretch primarily affects the vascular smooth muscle cells, whereas endothelial cells are most responsive to shear stress. The focus of this thesis is on shear stress and the endothelial biosensor for shear stress. Background information is provided for shear stress and its role in cardiovascular development and pathology.

1.1.1 Shear stress

Shear stress is the force per unit area imposed by a fluid in motion on a solid boundary in parallel to the direction of the fluid in motion. In case of the cardiovascular system blood induces shear stress on the endothelium. Shear stress is measured in dyne/cm² or N/m² (= Pascal (Pa), 1Pa = 10 dyne/cm²). When fluid flows through a straight tube flow velocity is highest at the center and drops towards the wall. Shear stress is proportional to the velocity gradient (shear rate) near the wall. This velocity gradient is due to frictional forces between adjacent layers of the fluid and between the fluid and the wall. These forces arise from the viscous properties of the fluid. Viscosity is dependent on the composition of the fluid. Blood is a mixture of erythrocytes, leukocytes, thrombocytes, and plasma. Plasma behaves as a Newtonian fluid, i.e., it has a constant viscosity at all shear rates. Whole blood, on the other hand, is non-Newtonian, i.e., blood viscosity depends on shear rate. Because analyzing the behavior of a non-Newtonian fluid is far more complex than analyzing a Newtonian fluid, whole blood is considered a Newtonian fluid in many computational studies. In vessels with a diameter smaller than 300 μ m, the fluid layer over the endothelium is indeed Newtonian, as a cell-free layer of plasma with reduced viscosity is present near the wall. This is known as the Fåhræus-Lindqvist effect². Steady, laminar flow of a Newtonian fluid in a cylindrical tube with rigid walls develops into a parabolic velocity profile, with the highest velocity at the center of the tube. This type of flow is termed Poiseuille flow. Shear stress can subsequently be calculated by the Hagen-Poiseuille formula: $\tau = 4\mu Q/\pi R^3$, where τ is the shear stress, μ is the blood viscosity, Q is the volumetric flow rate, and R is the lumen radius. However, considering blood flow as Poiseuille flow requires simplifications that are not physiologically relevant, as blood vessels, and in particular the heart, are not straight and rigid tubes. Nevertheless, these simplifications are acceptable to obtain approximations of wall shear stress values³.

With respect to the cardiovascular system the viscosity and pulsatility of blood flow and the curvature of blood vessels should be considered. The Reynolds number (Re), which is dependent on the viscosity, density, and velocity of the blood, and on the lumen radius of the blood vessel, represents the ratio of inertial forces to viscous forces. It is a measure of the stability of flow, i.e., whether it is laminar or turbulent. Inertia is the property of an object to remain at constant velocity unless acted upon by an outside force. When Re is very low (< 1) viscous forces dominate over inertial forces, the flow is laminar and follows the vessel geometry. This is the case in the embryonic system. In the adult system, on the

other hand, inertial forces dominate over viscous forces ($Re \gg 1$). Between a Re of 500 and 1500 flow is still laminar but turbulence can occur, dependent on the vessel geometry (bends and bifurcations) and flow pulsatility⁴. Turbulent flow is characterized by regions of separation, recirculation, and temporal as well as spatial gradients of shear stress and is termed oscillatory (multi- or bidirectional) shear stress. Laminar, non-turbulent flow, is unidirectional and termed steady (veins) or pulsatile (arteries) shear stress. The effect of pulsations on the flow profile is described by the Womersley number (α). In cases in which α is greater than unity, the flow profile will flatten compared to the parabolic poiseuille flow profile. In the embryo, α does not exceed unity. Furthermore, as blood vessels and the heart are not straight tubes the Dean number (Dn) should be taken into account. Dn describes the effect of the curvature of a vessel on the flow profile. With large values of Dn in the adult situation the maximum flow velocity shifts toward the outer wall of the curvature. However, the combination of the strong curvature of the embryonic heart and the low Re results in a small Dn and a shift of the maximum velocity toward the inner curvature^{5,6}.

Shear stress drives gene expression in endothelial cells *ex vivo*^{7,8} and *in vivo*^{9,10}. As a consequence, shear stress plays a crucial role in remodeling of the heart and vasculature. A key component in the endothelial flow response is the shear-responsive gene *Krüppel-like factor-2* (*KLF2* or *LKLF*), a zinc finger transcription factor. *KLF2* expression is confined to areas of high shear stress in the embryonic⁹ and adult cardiovascular¹⁰. *In vitro*, *KLF2* expression is upregulated by high steady or pulsatile shear stress^{8,10} but is downregulated by prolonged oscillatory shear stress¹¹. *KLF2* acts as a switch between the quiescent and activated state of the endothelium through regulation of multiple genes¹². Furthermore, it plays a role in vascular tone regulation by upregulating expression of *endothelial nitric oxide synthase* (*eNOS*), which produces the vasodilator nitric oxide (NO), and downregulating expression of *endothelin-1* (*ET1*), a vasoconstrictor¹⁰. Alterations in shear stress, therefore, result in adjustment of the vessel diameter and subsequent maintenance of shear stress level.

1.1.2 Shear stress and development

Shear stress-related gene expression patterns of *KLF2*, *ET1*, and *eNOS* are already present in early cardiovascular development⁹. Consequently, shear stress is believed to play a substantial role in cardiovascular development. The early embryonic heart is a nearly straight tube that develops into a four-chambered pump through extensive growth and remodeling. This generates changes in luminal diameter and accordingly shear stress. The animal model we use to study shear stress and heart development is the chicken embryo. This is a suitable model as shear stress levels are in the same range as in the human adult arterial system (up to 70 dyne/cm²)¹³. The velocity gradients near the wall and concomitant shear stress can be measured in the beating chicken embryonic heart, *in ovo*, with a high speed optical technique called micro particle image velocimetry (μ PIV). A maximum wall shear stress of 50 dyne/cm², was measured in the outflow tract (OFT) of chicken embryos¹⁴.

The chicken hatches after 21 days, which corresponds to Hamburger and Hamilton stage (HH)¹⁵ 46. At HH10 the heart begins to contract¹⁶, which is just before looping starts (see below). A relatively stable laminar blood flow pattern is present from HH12 on¹⁷ but oscillations in flow still occur due to movement of the cardiac wall. Below, heart development is described correlated to the stages of chicken development, as reviewed by Martinsen¹⁸.

Heart development starts at HH7 with the formation of the bilateral endocardial heart tubes. These tubes fuse and form the early embryonic heart tube¹⁹ at HH8. This tube consists of an inner endothelial layer and an outer myocardial layer, which are separated by cardiac jelly, an extracellular matrix (ECM)²⁰. The first stage of looping (C-shape) (reviewed by Männer²¹, Gittenberger-de Groot *et al.*²², Taber²³) occurs from HH10 to HH12/13 and is regulated by several genes essential in left-right signaling in embryos²⁴ but independent from blood flow^{23,25}. At the beginning of the second phase of looping (S-shape), which occurs between HH12/13 to HH18, blood flow is initiated and shear stress becomes a crucial factor. The heart lumen is wide and shear stress is expected to be moderate. Endocardial cushion development (HH12-36) and cardiac septation (HH17-46) separate the heart into four chambers, resulting in local changes in shear stress. The endothelial/endocardial cells overlying the cushions undergo the process of epithelial-to-mesenchymal transformation (EMT) and invade the cushion ECM²⁶. The endocardial cushions, subsequently, develop into the semilunar valves and the AV valves (mitral and tricuspid valves), which are fully developed at HH34 and HH36, respectively. The endocardial cushions cover the narrowest passages of the heart and are, therefore, exposed to high levels of shear stress, which is exemplified by the patterning of *KLF2* expression⁹. Cushion development also contributes to septation. Formation of the interatrial, interventricular, and OFT (aorticopulmonary) septa alters lumen diameter and, consequently, shear stress. All three septa are completed at approximately HH34. However, the atrial septum undergoes another conformational change after birth when the foramen ovale closes. During atrial septum development shunting of blood from the right atrium to the left atrium is always possible due to multiple fenestrations, rendering intricate flow patterns. The ventricular trabeculations start to form at HH16 and become more pronounced over time, affecting shear stress patterning as well.

The pharyngeal arch artery (PAA) system, the connection between the OFT of the heart and the aorta, remodels from a symmetrical to an asymmetrical system. This asymmetric remodeling is dependent on genetic factors and shear stress, as was demonstrated in mouse embryos. The genetic programme induces a morphological change in the OFT, which affects shear stress in the PAAs. The alterations in shear stress regulate the persistence or regression of the PAAs²⁷. Eventually, the PAA system of the chicken embryo includes the brachiocephalic arteries (left and right 3rd), an aortic arch (right 4th), and the ducti arteriosi (left and right 6th)^{28,29}.

Several animal models show that disturbing hemodynamics during development results in congenital heart malformations³⁰⁻³³. The model we use to disturb hemodynamics during chicken embryonic development is the chicken venous clip model³⁰ in which blood flow through the embryo is experimentally-altered through ligation of the right lateral vitelline vein, which is one of the veins that drains blood from the yolk sac vessels into the embryo. Vitelline vessel development is related to intracardiac flow patterns. Blood from specific yolk sac regions follows a specific stage-dependent intracardiac route¹⁷. Venous clip reduces preload³⁴ and shifts blood flow towards the inner curvature of the heart³⁵. A decrease in mean and peak blood flow up till 5 hours after clip is reported for the dorsal aorta³⁴. This is followed by a rapid shift, within 3 hrs, in *KLF2* expression, which is suggestive for an increase in cardiac shear stress and a decrease in aortic shear stress³⁶. Permanent ligation results in a spectrum of congenital cardiovascular anomalies^{30,35} and leads to structural adaptations of the heart that becomes less compliant³⁷⁻³⁹. In conclusion,

venous clip induces shear stress alterations that eventually result in cardiac malformations, demonstrating the importance of hemodynamics on cardiovascular development.

1.1.3 Shear stress and disease: atherosclerosis

Besides the major impact of hemodynamics on development it plays an equally important role in adult life. The main cause of cardiovascular death is a disease of the arterial wall, called atherosclerosis. Atherosclerosis is an inflammatory disease characterized by, e.g., the accumulation of lipids within the arterial wall. Atherosclerotic lesions can lead to ischemia (infarction) of the heart, brain, or extremities. Risk factors for atherosclerosis include elevated and modified low-density lipoprotein (LDL); free radicals caused by smoking, hypertension, and diabetes; genetic susceptibility; elevated plasma homocysteine concentrations; and infectious micro-organisms. Atherosclerosis originates at specific sites in the circulation (see below), where the endothelium is activated or primed for atherosclerosis. At these sites, endothelial cells express adhesion molecules that are responsible for the adherence, migration, and accumulation of monocytes and lymphocytes (reviewed by Ross⁴⁰). In the presence of cardiovascular risk factors, activated endothelium can become dysfunctional, which is the start of lesion formation. Endothelial dysfunction is followed by an inflammatory response, which in turn stimulates migration and proliferation of smooth muscle cells into the lesion. The resulting thickening of the arterial wall is compensated by gradual dilation to maintain lumen diameter, a process termed outward remodeling. Continued inflammation results in invasion of monocytes and lymphocytes from the blood into the lesion, where they proliferate and differentiate, leading to further enlargement of the lesion. The lesion then restructures and becomes covered by a fibrous cap overlying a lipid core and necrotic tissue. At some point further remodeling is not possible and the lesion protrudes into the lumen, resulting in alterations in blood flow^{40,41}.

Although the etiology of atherosclerosis is systemic, lesions develop locally, i.e., exclusively at sites of low and disturbed blood flow and concomitant low and multi- or bidirectional (oscillatory) shear stress. Therefore, the role of shear stress in atherosclerosis has gained enormous interest in the last decade. Low and disturbed flow is invoked by the geometry of the vasculature, i.e., by arterial bifurcations, branch points, and the curvature of arteries. The endothelium plays an essential role in atherogenesis as it translates the mechanical signal into a biological response. The endothelium is differentially affected by high or low shear stress and by unidirectional or bidirectional shear stress, causing the flow-determined occurrence of lesions. Low and bidirectional shear stress correlates with endothelial activation (athero-prone), whereas high and unidirectional shear stress is associated to a quiescent phenotype of endothelial cells (athero-protective)^{42,43}. Athero-prone flow profiles prime the endothelium for atherosclerosis, resulting in atherosclerosis when cardiovascular risk factors are present. Athero-prone shear stress induces formation of reactive oxygen species through uncoupling of the eNOS enzyme⁴⁴ and it increases the permeability of the endothelial layer through disruption of the glycocalyx⁴⁵. It induces inflammatory gene expression profiles⁴, downregulates expression of *KLF2*¹¹ rendering the endothelium in an activated state¹², and induces expression of adhesion molecules⁴⁰.

The animal model we use to study shear stress and atherosclerosis is the apolipoprotein-E-deficient (*Apoe*^{-/-}) mouse. This is a suitable model as *Apoe*^{-/-} mice are hyperlipidemic, develop atherosclerosis spontaneously, i.e., not diet-induced, and have lesions similar to those in man⁴⁶. Lesion formation can be accelerated by feeding *Apoe*^{-/-} mice a high-fat diet⁴⁷ or by experimental induction of low and/or disturbed shear stress⁴⁸.

To gain insight into the participation of specific proteins in atherosclerosis, genetically modified mice are interbred with *Apoe*^{-/-} mice. In this respect, we used Connexin (Cx) deficient (*Gja4*^{-/-}) *Apoe*^{-/-} mice (Chapter 9). Connexins are the structural components of gap junctions that mediate intercellular communication. Endothelial cells possess 3 types of connexins, i.e., Cx 37, 40, and 43⁴⁹, which can form homo- or heteromeric channels. Cx37 was believed to play a role in atherosclerosis after the observation of genetic polymorphisms of the human gene coding for Cx37 in patients with atherosclerosis⁵⁰. Lesion formation is accelerated in *Gja4*^{-/-} *Apoe*^{-/-} mice when compared with *Gja4*^{+/-} *Apoe*^{-/-} mice⁵¹.

1.2 The primary cilium

The differential response of endothelial cells to various velocity patterns is directed by the mechanosensing/transducing machinery. The shear stress sensing apparatus could therefore be involved in pathological alterations of endothelial cells. Recently, primary cilia were shown to function as shear stress sensors of several flow exposed cell types. We hypothesized that primary cilia function as shear stress sensors of endothelial cells. Background information is provided for cilia structure and function.

1.2.1 Ciliogenesis

Cilia are membrane-covered, rod-like cellular protrusions. They contain microtubules, which are a major constituent of the cytoskeleton. Microtubules consist of two subunits, i.e., α - and β -tubulin that form protofilaments by alternating addition of subunits at the plus-ends of the protofilaments. One microtubule contains 13 protofilaments organized in a tubular structure. The template on which microtubules nucleate is γ -tubulin. γ -tubulin is found in the centrosome, which functions as the microtubule organizing center (MTOC) of the cell. The centrosome consists of two interconnected⁵² centrioles, i.e., the mother (oldest) and daughter centriole, and pericentriolar material (PCM) located around the centrioles. γ -tubulin is present in the PCM and in subdistal appendages attached to the mother centriole⁵³. The cytoskeletal microtubules nucleate from the γ -tubulin in both these structures^{54,55}. The microtubules of a cilium, the ciliary axoneme, nucleate on the distal site of a centriole, which is located just beneath the plasma membrane. When a centriole nucleates a cilium it is called a basal body. The axoneme of the basal body consists of 9 triplets of microtubules, whereas the ciliary axoneme has 9 microtubule doublets. Two types of cilia exist, i.e., motile and immotile cilia. If the 9 doublets surround a central pair of singlet microtubules and have axonemal dynein arms, which are responsible for ciliary movement, attached the cilia are motile (9+2). If they lack central microtubules and axonemal dynein arms they are immotile, termed primary cilia (9+0). Nucleation of the central pair of singlet microtubules is dependent on the presence of γ -tubulin within the distal central part of the basal body⁵⁶. The basal body of a motile cilium forms in a centrosome-dependent or independent pathway and migrates towards the cell surface⁵⁷. The basal body of a primary cilium is always the mother centriole of the centrosome⁵⁸, which does not contain γ -tubulin in its distal central part and can, therefore, not nucleate a central pair of microtubules⁵⁶. As the mother centriole functions as the basal body of a primary cilium and as a nucleation site for cytoskeletal microtubules the primary cilium and the cytoskeletal microtubules are physically connected. Due to this connection to the centrosome the occurrence of primary cilia is cell cycle-dependent. Cell surface protruding primary cilia are present during the G0 and G1 phase of the cell cycle, whereas primary

cilia immersed in the cytoplasm can be observed during the S and G2 phase⁵⁸. This is due to ciliary resorption for disassembly in the cytoplasm upon entry into the cell cycle⁵⁹. In contrast, motile cilia are cell cycle independent as they form on terminally differentiated cells⁶⁰. Cells can present multiple motile cilia but only one primary cilium. The immotile primary cilium has sensory functions⁶¹ (see below) and are found on nearly all mammalian cell types⁶². Motile cilia beat in a wave-like manner. The axonemal dynein arms on one microtubule doublet generate force against the adjacent doublet, causing them to slide past one another. The central pair of microtubules regulate dynein movement⁶³. Motile cilia can propel a cell (single-celled organisms) or transport fluid over the surface of the cell, e.g., on epithelial cells lining the airways and reproductive tracts and epithelial cells of the ependyma and choroid plexus in the brain⁶⁴. Two additional types of motile cilia exist, i.e., motile 9+0 cilia and flagella. Motile 9+0 cilia are located on the mammalian embryonic node (homologous to Hensen's node in the chicken and the dorsal lip of the amphibian blastopore), the organizing center of the early embryo, and function in determining left-right asymmetry⁶⁵⁻⁶⁷. In contrast to primary cilia they do contain axonemal dynein arms but as they lack the central pair of microtubules, movement is not regulated, resulting in a twirling motion. The axoneme of flagella is identical to that of motile cilia (9+2) but flagella are longer and cells have only 1 or 2 flagella (spermatozoa and protists)⁶⁰.

Ciliary assembly and maintenance requires microtubule motor-based transport of axoneme subunits from the body of the cell to the ciliary tip as cilia themselves lack the machinery for protein synthesis. This process is bidirectional and is called intraflagellar transport (IFT), which was discovered in the green alga *Chlamydomonas*. Besides the continuous supply of microtubule subunits, which is necessary because of the constant turnover of microtubules at their distal plus-ends, IFT is required for the transport of specialized proteins to the ciliary membrane and the transport of various signals from the cilium to the cell body and vice versa. Microtubule motor proteins move in a single direction between the ciliary membrane and the outer doublet microtubule. Kinesins are responsible for anterograde movement of IFT particles. Dyneins direct retrograde movement of IFT particles and motors, which are thus recycled. Between the cell cytoplasm and the ciliary cytoplasm is a boundary demarcated by transition fibers that controls movement of particles between both compartments. Proteins destined for the cilium contain a specific amino-acid sequence through which they are targeted to the cilium and carried through the transitional zone (reviewed by Rosenbaum and Witman⁶⁸).

Mutations in any of the IFT components lead to defective ciliogenesis. Defects in ciliogenesis or ciliary function are associated to several pathologies. A defect in motile cilia, rendering them immotile, is called primary ciliary dyskinesia (for example Kartagener syndrome). This disease can affect all tissues that possess motile cilia. It encompasses respiratory tract defects, infertility, laterality disturbances, ectopic pregnancies, and hydrocephalus⁶⁹. Defects in primary cilia were associated to human disorders such as Senior-Loken, Alström, Orofaciodigital, Jeune, Usher, Meckel(-Gruber), and Bardet-Biedl syndrome. These syndromes can include anosmia, laterality defects, sensorineural deafness, vestibular impairment, retinitis pigmentosa, and polycystic kidney diseases^{60,68-70}. Ciliopathy phenotypes include obesity, diabetes, hypertension, and cardiac abnormalities⁷¹.

1.2.2 Primary cilia function

Although the existence of primary cilia is known for more than a century, investigations into their functionality arose in the last decade after they were associated to several human

syndromes. They were demonstrated to function in olfaction, photoreception, chemosensation, and mechanosensation. The mechanosensory function makes primary cilia attractive for our study on blood flow-induced shear stress sensing by endothelial cells. Primary cilia are very sensitive mechanosensors⁷², responding to shear stresses as low as 0.007 dyne/cm². They function as flow sensors of kidney epithelial cells⁷³, nodal cells^{65,66}, osteocytes and osteoblasts occupying the fluid filled cavities (lacunae) in bone matrix^{74,75}, and bile duct epithelial cells⁷⁶. The various ciliary functions, as reviewed by Satir and Christensen⁶⁴ and by Singla and Reiter⁷⁰, are described below.

Olfaction

Primary cilia of olfactory sensory neurons function in olfaction. An odorant binds to a G-protein-coupled receptor (GPCR) localized in the membrane of the primary cilium, producing the second messenger cyclic adenosine monophosphate (cAMP). Elevated levels of cAMP then depolarize the cell by opening a cyclic nucleotide-gated channel, located in the ciliary membrane.

Photoreception

Light photons activate opsin GPCRs located in the ciliary membrane of rod and cone cells of the retina by increasing hydrolysis of cyclic guanosine monophosphate (cGMP), thereby closing cGMP-gated channels.

Chemosensation

The chemosensing function of primary cilia is gaining much interest after the discovery that they play a role in the Hedgehog and Wnt signaling pathways, key regulators of development that have been implicated in stem cell function and carcinogenesis⁷⁷. The Hedgehog receptor Patched 1⁷⁸, its downstream mediators Smoothened⁷⁹ and Gli transcription factors⁸⁰, and the Wnt mediator Inversin⁸¹ are localised in the primary cilium and are dependent on IFT. In neuronal cells, SST₃ somatostatin⁸² and 5-HT₆ serotonin⁸³ receptors were observed in the ciliary membrane. Primary cilia of connective tissue cells, such as chondrocytes and fibroblast, are embedded in the ECM. Chondrocyte primary cilia sense mechanical (see below), physiochemical, and osmotic stimuli through ciliary receptors and ion channels. Platelet-derived growth factor receptor α (PDGFR α) localizes to fibroblast primary cilia and is involved in growth control⁸⁴. In the absence of PDGF the primary cilium functions as a mechanosensor (see below) but in the presence of PDGF it acts as a chemoreceptor.

Whether primary cilia function as chemosensors on the embryonic organizing center is a matter of debate. The node contains two populations of cilia, i.e., motile 9+0 cilia in the center of the node and immotile 9+0 cilia at the periphery of the node. The motile cilia generate a left-ward (nodal) flow⁶⁷. Two hypotheses about the function of cilia in determining left-right asymmetry exist. The flow establishes a gradient of signaling proteins, such as fibroblast growth factor, present in the nodal fluid that (I) induce signaling through activation of receptors present on the nodal cilia⁸⁵ and/or (II) the immotile cilia at the periphery of the node sense the left-ward flow, resulting in asymmetric Ca²⁺ signaling, triggering left-sided gene expression^{65,66} (see below).

Mechanosensation

Besides functioning in chemosensation chondrocyte and fibroblast primary cilia function in mechanosensation. They make contact with the ECM through specific receptors on the

ciliary membrane and can therefore transmit mechanical forces (reviewed by Satir and Christensen⁶⁴). In the ciliary membrane of the fruit fly *Drosophila Melanogaster* and the nematode *Caenorhabditis elegans* transient receptor potential (TRP) ion channels are located that respond to mechanical stimuli. In the cilia of auditory sensory neurons of *D. Melanogaster* they sense vibrations and in cilia of sensory neurons of *C. elegans* they sense touch⁷⁰. In *C. elegans* a mechano-responsive complex of PKD-2 (vertebrate homolog polycystin-2 (PC2)) and LOV-1 (vertebrate homolog polycystin-1 (PC1)) localizes to the cilia of male-specific sensory neurons⁸⁶. PC1 is an integral membrane protein with a large extracellular part, a transmembrane domain, and a C-terminal cytoplasmic tail, whereas PC2 is a Ca^{2+} -permeable, non-selective cation channel that is a member of the TRP channel family⁸⁷. The link between polycystins and primary cilia is conserved from nematodes to mammals. Polycystins play a role in cilia-mediated sensing of another mechanical force: flow-induced shear stress. PC2 is present in the two populations of nodal cilia in mice⁶⁵, strengthening the mechanosensory hypothesis, and both PC1 and PC2 localize to the primary cilium of murine kidney epithelial cells, where they form a mechano-responsive complex⁸⁸. Mutations in one of the polycystin genes in humans cause polycystic kidney disease (PKD). PKD is characterized by clonal expansion of kidney epithelial cells, resulting in cyst formation that causes kidney failure. In addition, both polycystins are present in ciliated osteocytes and osteoblasts⁸⁹ and in ciliated cholangiocytes⁷⁶. In cholangiocytes flow induces ciliary- and polycystin-dependent rises in intracellular Ca^{2+} . Bending of bone primary cilia by flow results in altered gene expression, suggesting they function as mechanosensors, but cilium-mediated mechanosensing in bone is different, as the Ca^{2+} transient upon flow is independent of primary cilia or PC2⁷⁴.

A comparable role for primary cilia in endothelial mechanosensing is likely as (I) primary cilia are also present on endothelial cells⁹⁰⁻⁹³, (II) a similar flow-induced rise in intracellular Ca^{2+} is seen⁹⁴, and (III) both polycystins were detected in these cells⁹⁵.

1.3 Endothelial mechanotransduction

Several cell components, other than primary cilia, have been put forward as potential endothelial shear stress sensors. They are described as sensors because blockage leads to abrogation of the shear stress response. However, they are more likely to be downstream of the shear stress sensor and are, therefore, termed mechanotransducers in this thesis. In this section background information on these mechanotransducing components, as reviewed by Lehoux *et al.*¹, Resnick *et al.*⁷, and Li *et al.*⁹⁶, and the potential pathway from shear stress sensing to gene expression is provided.

1.3.1 Mechanotransduction

Cell-extracellular matrix and cell-cell junction molecules

Shear stress induces a conformational change of integrins, which are $\alpha\beta$ heterodimers involved in cell-ECM interactions. The extracellular domain binds specific ECM components, such as fibronectin, vitronectin, collagen, and laminin. The cytoplasmic domain of integrins is functionally linked to cytoskeletal proteins and to focal adhesion sites. Interference of integrin-ECM interactions abrogates the shear stress response. Another mechano-responsive complex is located at the endothelial cell-cell junction. Platelet endothelial cell adhesion molecule-1 (PECAM-1; also known as CD31), vascular endothelial (VE)-cadherin, and vascular endothelial growth factor receptor 2 (VEGFR 2;

also known as FLK-1 or KDR) form a complex at adherens junctions. This complex is connected to the cytoskeleton via anchoring molecules such as catenins. Activation by shear stress results in activation of the NF- κ B pathway⁹⁷.

Membrane structures

Several shear stress responsive components reside in the endothelial cell membrane, such as ion channels, including Na⁺, K⁺ and Ca²⁺ channels, receptors, and caveolae. Tyrosine kinase receptors, such as VEGFR2, are phosphorylated upon exposure to shear stress. Activation is independent of their ligands, suggesting a double role in chemo- and mechanosensation. In addition, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and G-proteins are activated upon the onset of shear stress. G-proteins are also located in caveolae, which are membrane domains that contain signaling molecules. Caveolae are rich in cholesterol and consequently more rigid than other parts of the plasma membrane. Upon shear stress caveolae are directed to the cell surface where they mediate the shear stress response. Another shear stress transducer connected to the plasma membrane is a hydrated polysaccharide coat covering the apical membrane, termed the glycocalyx. The glycocalyx might mediate mechanotransduction as degradation of the glycocalyx results in a reduced shear stress response^{98,99}.

Cytoskeleton

A structure that can tie all the shear stress responsive cell components together is the cytoskeleton. The cytoskeleton rearranges in response to flow and cells elongate along the flow direction¹⁰⁰. The cytoskeleton comprises microtubules, actin microfilaments, and intermediate filaments that are interconnected¹⁰¹, transduce force to various cell compartments, and translocate signaling molecules. Davies¹⁰² proposed the decentralization model of endothelial mechanotransduction, i.e., shear stress is transduced by the cytoskeleton to multiple sites that are directly or indirectly connected to the cytoskeleton¹⁰⁰, which each generate a shear stress response.

1.3.2 Intracellular signaling pathways and gene expression

Several intracellular signaling pathways are activated in response to shear stress, which modulate expression of genes, including those involved in proliferation or growth arrest, inflammation or anti-inflammation. Shear stress modulates the activity of small GTPases (including Ras, RhoA, Rac1, Cdc42, Arf, Rab and Ran), which mediate both cytoskeletal reorganization and transcriptional activation of target genes¹⁰³, through induction of the NF- κ B or MAP kinase cascades. The latter comprises activation of MEKK, MEK, and MAPK, involving ERK1/2, JNK or p38¹. KLF2, the key regulator in shear-induced gene expression, is induced by high shear stress through the MEK5-ERK5-MEF2 and PI3K-nucleolin signaling pathways^{104,105}. Activation of transcription factors such as NF- κ B and KLF2 depends on the pattern of shear stress, i.e., steady, pulsatile, or oscillatory, resulting in a pro- or anti-inflammatory gene profile. NF- κ B regulates expression of VCAM-1, ICAM-1, and E-selectin, these products are involved in vascular inflammation and cell viability. KLF2, on the other hand, is a potential mediator of the anti-inflammatory effects of shear stress, as it modulates pro-inflammatory signaling pathways, and can suppress NF- κ B activation¹⁰⁶.

In conclusion, the exact mechanism of endothelial shear stress sensing remains to be elucidated but the cytoskeleton may play a central role. The cytoskeleton can transduce the

force throughout the cell to the connected shear-responsive sites. A consequence of that would be that blood flow-induced shear stress is sensed by the endothelium from the inside-out. A way to amplify cytoskeletal deformation upon shear stress could be the protrusion of a primary cilium into the lumen. In this thesis the precise relation between endothelial primary cilia and shear stress is described.

1.4 Chapter outline

Chapter 2 describes the distribution of endothelial primary cilia in chicken cardiovascular development. We show that the occurrence of primary cilia on endothelial cells is shear stress-related.

In *Chapter 3* the effect of venous clip on shear stress and the distribution of endothelial primary cilia is investigated. We demonstrate that venous clip appears to alter shear stress levels but not primary cilia distribution.

Chapter 4 describes the role of shear stress in looping of the embryonic chicken heart.

In *Chapter 5* the correlation between primary cilia occurrence and different flow profiles is studied. We show that endothelial primary cilia are induced by flow reversals.

Chapter 6 demonstrates that the endothelial shear stress response is dependent on an intact microtubular cytoskeleton and that primary cilia sensitize the endothelium for shear stress.

Chapter 7 describes a double mechanistic role for endothelial primary cilia in shear stress sensing.

Chapter 8 presents the distribution of endothelial primary cilia in the aortic arch and common carotid artery of wild-type and *Apoe*^{-/-} adult mice. The effect of experimentally-altered shear stress on primary cilia distribution was analyzed by placement of a restrictive cast around an artery. A correlation between the presence of endothelial primary cilia and disturbed flow is demonstrated.

In *Chapter 9* the effect of Cx37 deficiency on primary cilia occurrence in the aortic arch of *Apoe*^{-/-} adult mice is investigated. The absence of Cx37 does not affect the prevalence of primary cilia on endothelial cells.

The obtained results are discussed in *Chapter 10*.

1.5 References

1. Lehoux S, Castier Y, Tedgui A. Molecular mechanisms of the vascular responses to haemodynamic forces. *J Intern Med.* 2006;259:381-392.
2. Goldsmith HL, Cokelet GR, Gaehtgens P. Robin Fahraeus: evolution of his concepts in cardiovascular physiology. *Am J Physiol.* 1989;257:H1005-H1015.
3. Katritsis D, Kaiktsis L, Chaniotis A, Pantos J, Efstathopoulos EP, Marmarelis V. Wall shear stress: theoretical considerations and methods of measurement. *Prog Cardiovasc Dis.* 2007;49:307-329.
4. Cunningham KS, Gotlieb AI. The role of shear stress in the pathogenesis of atherosclerosis. *Lab Invest.* 2005;85:9-23.
5. Berger SA, Talbot L, Yao L-S. Flow in curved pipes. *Ann Rev Fluid Mech.* 1983;15:461-512.
6. Wang CY, Bassingthwaite JB. Blood flow in small curved tubes. *J Biomech Eng.* 2003;125:910-913.
7. Resnick N, Yahav H, Shay-Salit A, Shushy M, Schubert S, Zilberman LC, Wofovitz E. Fluid shear stress and the vascular endothelium: for better and for worse. *Prog Biophys Mol Biol.* 2003;81:177-199.
8. Dekker RJ, Van Soest S, Fontijn RD, Salamanca S, de Groot PG, VanBavel E, Pannekoek H, Horrevoets AJG. Prolonged fluid shear stress induces a distinct set of endothelial cell genes, most specifically lung Krüppel-like factor (*KLF2*). *Blood.* 2002;100:1689-1698.
9. Groenendijk BCW, Hierck BP, Gittenberger-de Groot AC, Poelmann RE. Development-related changes in the expression of shear stress responsive genes *KLF-2*, *ET-1*, and *NOS-3* in the developing cardiovascular system of chicken embryos. *Dev Dyn.* 2004;230:57-68.
10. Dekker RJ, van Thienen JV, Elderkamp YW, Seppen J, de Vries CJM, Biessen EAL, van Berkel TJ, Pannekoek H, Horrevoets AJG. Endothelial *KLF2* links local arterial shear stress levels to the expression of vascular-tone regulating genes. *Am J Pathol.* 2005;167:609-618.
11. Wang N, Miao H, Li YS, Zhang P, Haga JH, Hu YL, Young A, Yuan SL, Nguyen P, Wu CC, Chien S. Shear stress regulation of Kruppel-like factor 2 expression is flow pattern-specific. *Biochim Biophys Res Commun.* 2006;341:1244-1251.
12. Dekker RJ, Boon RA, Rondaij MG, Kragt A, Volger OL, Elderkamp YW, Meijers JC, Voorberg J, Pannekoek H, Horrevoets AJG. *KLF2* provokes a gene expression pattern that establishes functional quiescent differentiation of the endothelium. *Blood.* 2006;107:4354-4363.
13. Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. *J Am Med Assoc.* 1999;282:2035-2042.
14. Vennemann P, Kiger KT, Lindken R, Groenendijk BCW, Stekelenburg-de Vos S, ten Hagen TLM, Ursem NTC, Poelmann RE, Westerweel J, Hierck BP. *In vivo* micro particle image velocimetry measurements of blood-plasma in the embryonic avian heart. *J Biomech.* 2006;39:1191-1200.
15. Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. *J Morphol.* 1951;88:49-92.
16. Fishman MC, Chien KR. Fashioning the vertebrate heart: earliest embryonic decisions. *Development.* 1997;124:2099-2117.
17. Hogers B, DeRuiter MC, Baasten AMJ, Gittenberger-de Groot AC, Poelmann RE. Intracardiac blood flow patterns related to the yolk sac circulation of the chick embryo. *Circ Res.* 1995;76:871-877.
18. Martinsen BJ. Reference guide to the stages of chick heart embryology. *Developmental Dynamics.* 2005;233:1217-1237.
19. DeRuiter MC, Poelmann RE, VanderPlas-de Vries I, Mentink MMT, Gittenberger-de Groot AC. The development of the myocardium and endocardium in mouse embryos. Fusion of two heart tubes? *Anat Embryol.* 1992;185:461-473.
20. Markwald RR, Fitzharris TP, Manasek FJ. Structural development of endocardial cushions. *Am J Anat.* 1977;148:85-120.
21. Männer J. Cardiac looping in the chick embryo: a morphological review with special reference to terminological and biomechanical aspects of the looping process. *Anat Rec.* 2000;259:248-262.
22. Gittenberger-de Groot AC, Bartelings MM, DeRuiter MC, Poelmann RE. Normal cardiac development. Chapter 1: 1-14. In: Ultrasound and the fetal heart. Wladimiroff JW, Pilu G, eds. The Parthenon Publishing Group, New York-London, 1996.
23. Taber LA. Biophysical mechanisms of cardiac looping. *Int J Dev Biol.* 2006;50:323-332.
24. Olson EN, Srivastava D. Molecular pathways controlling heart development. *Science.* 1996;272:671-676.
25. Manasek FJ, Monroe RG. Early cardiac morphogenesis is independent of function. *Dev Biol.* 1972;27:584-588.

26. Azhar M, Schultz JJ, Grupp I, Dorn GW, Meneton P, Molin DGM, Gittenberger-de Groot AC, Doetschman T. Transforming growth factor beta in cardiovascular development and function. *Cytokine Growth Factor Rev.* 2003;14:391-407.
27. Yashiro K, Shiratori H, Hamada H. Haemodynamics determined by a genetic programme govern asymmetric development of the aortic arch. *Nature.* 2007;450:285-288.
28. Bergwerff M, DeRuiter MC, Poelmann RE, Gittenberger-de Groot AC. Onset of elastogenesis and downregulation of smooth muscle actin as distinguishing phenomena in artery differentiation in the chick embryo. *Anat Embryol.* 1996;194:545-557.
29. Kirby ML, Waldo KL. Neural crest and cardiovascular patterning. *Circ Res.* 1995;77:211-215.
30. Hogers B, DeRuiter MC, Gittenberger-de Groot AC, Poelmann RE. Unilateral vitelline vein ligation alters intracardiac blood flow patterns and morphogenesis in the chick embryo. *Circ Res.* 1997;80:473-481.
31. Tobita K, Schroder EA, Tinney JP, Garrison JB, Keller BB. Regional passive ventricular stress-strain relations during development of altered loads in chick embryo. *Am J Physiol Heart Circ Physiol.* 2002;282:H2386-H2396.
32. Sedmera D, Pexieder T, Rychterova V, Hu N, Clark EB. Remodeling of chick embryonic ventricular myoarchitecture under experimentally changed loading conditions. *Anat Rec.* 1999;254:238-252.
33. Hove JR, Koster RW, Forouhar AS, Acevedo-Bolton G, Fraser SE, Gharib M. Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis. *Nature.* 2003;421:172-177.
34. Stekelenburg-de Vos S, Ursem NTC, Hop WCJ, Wladimiroff JW, Gittenberger-de Groot AC, Poelmann RE. Acutely altered hemodynamics following venous obstruction in the early chick embryo. *J Exp Biol.* 2003;206:1051-1057.
35. Hogers B, DeRuiter MC, Gittenberger-de Groot AC, Poelmann RE. Extraembryonic venous obstructions lead to cardiovascular malformations and can be embryolethal. *Cardiovasc Res.* 1999;41:87-99.
36. Groenendijk BCW, Hierck BP, Vrolijk J, Baiker M, Pourquie MJBM, Gittenberger-de Groot AC, Poelmann RE. Changes in shear stress-related gene expression after experimentally altered venous return in the chicken embryo. *Circ Res.* 2005;96:1291-1298.
37. Broekhuizen MLA, Hogers B, DeRuiter MC, Poelmann RE, Gittenberger-de Groot AC, Wladimiroff JW. Altered hemodynamics in chick embryos after extraembryonic venous obstruction. *Ultrasound Obstet Gynecol.* 1999;13:437-445.
38. Ursem NTC, Stekelenburg-de Vos S, Wladimiroff JW, Poelmann RE, Gittenberger-de Groot AC, Hu N, Clark EB. Ventricular diastolic filling characteristics in stage-24 chick embryos after extra-embryonic venous obstruction. *J Exp Biol.* 2004;207:1487-1490.
39. Stekelenburg-de Vos S, Steendijk P, Ursem NTC, Wladimiroff JW, Delfos R, Poelmann RE. Systolic and diastolic ventricular function assessed by pressure-volume loops in the stage 21 venous clipped chick embryo. *Pediatr Res.* 2005;57:16-21.
40. Ross R. Mechanisms of disease - Atherosclerosis - An inflammatory disease. *N Engl J Med.* 1999;340:115-126.
41. Slager CJ, Wentzel JJ, Gijzen FJ, Schuurbers JC, van der Wal AC, van der Steen AF, Serruys PW. The role of shear stress in the generation of rupture-prone vulnerable plaques. *Nat Clin Pract Cardiovasc Med.* 2005;2:401-407.
42. Shaaban AM, Duerinckx AJ. Wall shear stress and early atherosclerosis: a review. *Am J Roentgenol.* 2000;174:1657-1665.
43. Dai G, Kaazempur-Mofrad MR, Natarajan S, Zhang Y, Vaughn S, Blackman BR, Kamm RD, Garcia-Cardena G, Gimbrone MA. Distinct endothelial phenotypes evoked by arterial waveforms derived from atherosclerosis-susceptible and -resistant regions of human vasculature. *Proc Natl Acad Sci U S A.* 2004;101:14871-14876.
44. Rabelink TJ, Luscher TF. Endothelial nitric oxide synthase - Host defense enzyme of the endothelium? *Arterioscler Thromb Vasc Biol.* 2006;26:267-271.
45. van den Berg BM, Spaan JAE, Rolf TM, Vink H. Atherogenic region and diet diminish glycocalyx dimension and increase intima-to-media ratios at murine carotid artery bifurcation. *Am J Physiol Heart Circ Physiol.* 2006;290:H915-H920.
46. Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler Thromb.* 1994;14:133-140.
47. Getz GS, Reardon CA. Diet and murine atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2006;26:242-249.
48. Cheng C, de Crom R, van Haperen R, Helderma F, Gourabi BM, van Damme LC, Kirschbaum SW, Slager CJ, van der Steen AF, Krams R. The role of shear stress in atherosclerosis: action through gene expression and inflammation? *Cell Biochem Biophys.* 2004;41:279-294.

49. Gabriels JE, Paul DL. Connexin43 is highly localized to sites of disturbed flow in rat aortic endothelium but connexin37 and connexin40 are more uniformly distributed. *Circ Res*. 1998;83:636-643.
50. Chanson M, Kwak BR. Connexin37: a potential modifier gene of inflammatory disease. *J Mol Med*. 2007;85:787-795.
51. Wong CW, Christen T, Roth I, Chadjichristos CE, Derouette JP, Foglia BF, Chanson M, Goodenough DA, Kwak BR. Connexin37 protects against atherosclerosis by regulating monocyte adhesion. *Nat Med*. 2006;12:950-954.
52. Yang J, Adamian M, Li TS. Rootletin interacts with C-Nap1 and may function as a physical linker between the pair of centrioles/basal bodies in cells. *Mol Biol Cell*. 2006;17:1033-1040.
53. Fuller SD, Gowen BE, Reinsch S, Sawyer A, Buendia B, Wepf R, Karsenti E. The core of the mammalian centriole contains gamma-tubulin. *Curr Biol*. 1995;5:1384-1393.
54. Doxsey S. Re-evaluating centrosome function. *Nat Rev Mol Cell Biol*. 2001;2:688-698.
55. Uzbekov R, Prigent C. Clockwise or anticlockwise? Turning the centriole triplets in the right direction! *FEBS Lett*. 2007;581:1251-1254.
56. McKean PG, Baines A, Vaughan S, Gull K. Gamma-tubulin functions in the nucleation of a discrete subset of microtubules in the eukaryotic flagellum. *Curr Biol*. 2003;13:598-602.
57. Hagiwara H, Ohwada N, Takata K. Cell biology of normal and abnormal ciliogenesis in the ciliated epithelium. *Int Rev Cytol*. 2004;234:101-141.
58. Vorobjev IA, Chentsov YS. Centrioles in the cell cycle. I. Epithelial cells. *J Cell Biol*. 1982;93:938-949.
59. Rieder CL, Jensen CG, Jensen LC. The resorption of primary cilia during mitosis in a vertebrate (PtK1) cell line. *J Ultrastruct Res*. 1979;68:173-185.
60. Dawe HR, Farr H, Gull K. Centriole/basal body morphogenesis and migration during ciliogenesis in animal cells. *J Cell Sci*. 2007;120:7-15.
61. Praetorius HA, Spring KR. A physiological view of the primary cilium. *Annu Rev Physiol*. 2005;67:515-529.
62. Wheatley DN, Wang AM, Strugnell GE. Expression of primary cilia in mammalian cells. *Cell Biol Int*. 1996;20:73-81.
63. Smith EF, Lefebvre PA. The role of central apparatus components in flagellar motility and microtubule assembly. *Cell Motil Cytoskeleton*. 1997;38:1-8.
64. Satir P, Christensen ST. Overview of Structure and Function of Mammalian Cilia. *Annu Rev Physiol*. 2007;69:377-400.
65. McGrath J, Somlo S, Makova S, Tian X, Brueckner M. Two populations of node monocilia initiate left-right asymmetry in the mouse. *Cell*. 2003;114:61-73.
66. Yost HJ. Left-right asymmetry: Nodal cilia make and catch a wave. *Curr Biol*. 2003;13:R808-R809.
67. Nonaka S, Tanaka Y, Okada Y, Takeda S, Harada A, Kanai Y, Kido M, Hirokawa N. Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. *Cell*. 1998;95:829-837.
68. Rosenbaum JL, Witman GB. Intraflagellar transport. *Nat Rev Mol Cell Biol*. 2002;3:813-825.
69. Eley L, Yates LM, Goodship JA. Cilia and disease. *Curr Opin Genet Dev*. 2005;15:308-314.
70. Singla V, Reiter JF. The primary cilium as the cell's antenna: signaling at a sensory organelle. *Science*. 2006;313:629-633.
71. Davenport JR, Yoder BK. An incredible decade for the primary cilium: a look at a once-forgotten organelle. *Am J Physiol Renal Physiol*. 2005;289:F1159-F1169.
72. Liu W, Xu S, Woda C, Kim P, Weinbaum S, Satlin LM. Effect of flow and stretch on the [Ca²⁺]_i response of principal and intercalated cells in cortical collecting duct. *Am J Physiol Renal Physiol*. 2003;285:F998-F1012.
73. Praetorius HA, Spring KR. Bending the MDCK cell primary cilium increases intracellular calcium. *J Membrane Biol*. 2001;184:71-79.
74. Malone AM, Anderson CT, Tummala P, Kwon RY, Johnston TR, Stearns T, Jacobs CR. Primary cilia mediate mechanosensing in bone cells by a calcium-independent mechanism. *Proc Natl Acad Sci U S A*. 2007;104:13325-13330.
75. Malone AMD, Anderson CT, Tummala P, Stearns T, Jacobs CR. Primary cilia mediate PGE₂ release in MC3T3-E1 osteoblasts. *MCB*. 2006;3:207-208.
76. Masyuk AI, Masyuk TV, Splinter PL, Huang BQ, Stroope AJ, Larusso NF. Cholangiocyte cilia detect changes in luminal fluid flow and transmit them into intracellular Ca²⁺ and cAMP signaling. *Gastroenterology*. 2006;131:911-920.
77. Moon RT, Bowerman B, Boutros M, Perrimon N. The promise and perils of Wnt signaling through beta-catenin. *Science*. 2002;296:1644-1646.

78. Rohatgi R, Scott MP. Patching the gaps in Hedgehog signalling. *Nat Cell Biol.* 2007;9:1005-1009.
79. Corbit KC, Aanstad P, Singla V, Norman AR, Stainier DY, Reiter JF. Vertebrate Smoothed functions at the primary cilium. *Nature.* 2005;437:1018-1021.
80. Haycraft CJ, Banizs B, ydin-Son Y, Zhang Q, Michaud EJ, Yoder BK. Gli2 and Gli3 localize to cilia and require the intraflagellar transport protein polaris for processing and function. *PLoS Genet.* 2005;1:e53.
81. Morgan D, Eley L, Sayer J, Strachan T, Yates LM, Craighead AS, Goodship JA. Expression analyses and interaction with the anaphase promoting complex protein Apc2 suggest a role for inversin in primary cilia and involvement in the cell cycle. *Hum Mol Genet.* 2002;11:3345-3350.
82. Handel M, Schulz S, Stanarius A, Schreff M, Erdtmann-Vourliotis M, Schmidt H, Wolf G, Holtt V. Selective targeting of somatostatin receptor 3 to neuronal cilia. *Neuroscience.* 1999;89:909-926.
83. Brailov I, Bancila M, Brisorgueil MJ, Miquel MC, Hamon M, Verge D. Localization of 5-HT(6) receptors at the plasma membrane of neuronal cilia in the rat brain. *Brain Res.* 2000;872:271-275.
84. Schneider L, Clement CA, Teilmann SC, Pazour GJ, Hoffmann EK, Satir P, Christensen ST. PDGFRalpha signaling is regulated through the primary cilium in fibroblasts. *Curr Biol.* 2005;15:1861-1866.
85. Tanaka Y, Okada Y, Hirokawa N. FGF-induced vesicular release of Sonic hedgehog and retinoic acid in leftward nodal flow is critical for left-right determination. *Nature.* 2005;435:172-177.
86. Gonzalez-Perrett S, Kim K, Ibarra C, Damiano AE, Zotta E, Batelli M, Harris PC, Reisin IL, Arnaout MA, Cantiello HF. Polycystin-2, the protein mutated in autosomal dominant polycystic kidney disease (ADPKD), is a Ca²⁺-permeable nonselective cation channel. *Proc Natl Acad Sci U S A.* 2001;98:1182-1187.
87. Weimbs T. Polycystic kidney disease and renal injury repair: common pathways, fluid flow, and the function of polycystin-1. *Am J Physiol Renal Physiol.* 2007;293:F1423-F1432.
88. Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Lil XG, Elia AEH, Lu WN, Brown EM, Quinn SJ, Ingber DE, Zhou J. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nature Genetics.* 2003;33:129-137.
89. Xiao Z, Zhang S, Mahlios J, Zhou G, Magenheimer BS, Guo D, Dallas SL, Maser R, Calvet JP, Bonewald L, Quarles LD. Cilia-like structures and polycystin-1 in osteoblasts/osteocytes and associated abnormalities in skeletogenesis and Runx2 expression. *J Biol Chem.* 2006;281:30884-30895.
90. Bystrevskaya VB, Lichkun VV, Antonov AS, Perov NA. An ultrastructural study of centriolar complexes in adult and embryonic human aortic endothelial cells. *Tissue Cell.* 1988;20:493-503.
91. Briffeuil P, Thibaut-Vercruyssen R, Ronveaux-Dupal MF. Ciliation of bovine aortic endothelial cells in culture. *Atherosclerosis.* 1994;106:75-81.
92. Gallagher BC. Primary Cilia of the Corneal Endothelium. *Am J Anat.* 1980;159:475-484.
93. Iomini C, Tejada K, Mo W, Vaananen H, Piperno G. Primary cilia of human endothelial cells disassemble under laminar shear stress. *J Cell Biol.* 2004;164:811-817.
94. Helmlinger G, Berk BC, Nerem RM. Pulsatile and steady flow-induced calcium oscillations in single cultured endothelial cells. *J Vasc Res.* 1996;33:360-369.
95. Ong ACM, Ward CJ, Butler RJ, Biddolph S, Bowker C, Torra P, Pei Y, Harris PC. Coordinate expression of the autosomal dominant polycystic kidney disease proteins, polycystin-2 and polycystin-1, in normal and cystic tissue. *Am J Pathol.* 1999;154:1721-1729.
96. Li YS, Haga JH, Chien S. Molecular basis of the effects of shear stress on vascular endothelial cells. *J Biomech.* 2005;38:1949-1971.
97. Tzima E, Irani-Tehrani M, Kiosses WB, Dejana E, Schultz DA, Engelhardt B, Cao G, DeLisser H, Schwartz MA. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature.* 2005;437:426-431.
98. Florian JA, Kosky JR, Ainslie K, Pang ZY, Dull RO, Tarbell JM. Heparan sulfate proteoglycan is a mechanosensor on endothelial cells. *Circ Res.* 2003;93:E136-E142.
99. Mochizuki S, Vink H, Hiramatsu O, Kajita T, Shigeto F, Spaan JA, Kajiya F. Role of hyaluronic acid glycosaminoglycans in shear-induced endothelium-derived nitric oxide release. *Am J Physiol Heart Circ Physiol.* 2003;285:H722-H726.
100. Helmke BP, Davies PF. The cytoskeleton under external fluid mechanical forces: Hemodynamic forces acting on the endothelium. *Ann Biomed Eng.* 2002;30:284-296.
101. Fuchs E, Karakesisoglou I. Bridging cytoskeletal intersections. *Genes Dev.* 2001;15:1-14.
102. Davies PF. Flow-mediated endothelial mechanotransduction. *Physiol Rev.* 1995;75:519-560.
103. Tzima E. Role of small GTPases in endothelial cytoskeletal dynamics and the shear stress response. *Circ Res.* 2006;98:176-185.

104. Parmar KM, Larman HB, Dai G, Zhang Y, Wang ET, Moorthy SN, Kratz JR, Lin Z, Jain MK, Gimbrone MA, Garcia-Cardena G. Integration of flow-dependent endothelial phenotypes by Kruppel-like factor 2. *J Clin Invest.* 2006;116:49-58.
105. Huddleson JP, Ahmad N, Lingrel JB. Up-regulation of the KLF2 transcription factor by fluid shear stress requires nucleolin. *J Biol Chem.* 2006;281:15121-15128.
106. Helderma F, Segers D, de Crom R, Hierck BP, Poelmann RE, Evans PC, Krams R. Effect of shear stress on vascular inflammation and plaque development. *Curr Opin Lipidol.* 2007;18:527-533.