

# Congenital coronary artery anomalies: a bridge from embryology to anatomy and pathophysiology—a position statement of the development, anatomy, and pathology ESC Working Group

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## Abstract

Congenital coronary artery anomalies are of major significance in clinical cardiology and cardiac surgery due to their association with myocardial ischaemia and sudden death. Such anomalies are detectable by imaging modalities and, according to various definitions, their prevalence ranges from 0.21 to 5.79%. This consensus document from the Development, Anatomy and Pathology Working Group of the European Society of Cardiology aims to provide: (i) a definition of normality that refers to essential anatomical and embryological features of coronary vessels, based on the integrated analysis of studies of normal and abnormal coronary embryogenesis and pathophysiology; (ii) an animal model-based systematic survey of the molecular and cellular mechanisms that regulate coronary blood vessel development; (iii) an organization of the wide spectrum of coronary artery anomalies, according to a comprehensive anatomical and embryological classification scheme; (iv) current knowledge of the pathophysiological mechanisms underlying symptoms and signs of coronary artery anomalies, with diagnostic and therapeutic implications. This document identifies the mosaic-like embryonic development of the coronary vascular system, as coronary cell types differentiate from multiple cell sources through an intricate network of molecular signals and haemodynamic cues, as the necessary framework for understanding the complex spectrum of coronary artery anomalies observed in human patients.

## Keywords

Coronary arteries • Embryology • Congenital heart disease • Pathology • Anatomy

## 1. Introduction

Coronary arteries (CAs) are the blood vessels that nourish the heart muscle. Proper coronary circulation is therefore crucial for myocardial

homeostasis and performance, which is, in turn, necessary to sustain the rest of body functions. Disruption of coronary development during embryogenesis, however, results in coronary congenital defects (including coronary mispatterning, structural vascular defects, and

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anomalous communication of coronary vessels) that alter coronary artery blood flow. Such anomalies may persist after birth, occasionally in association with other cardiac conditions, and can severely affect cardiovascular health due to haemodynamic impairment related to shunting, ischaemia, or even sudden cardiac death, particularly in children–adolescents and young adults.

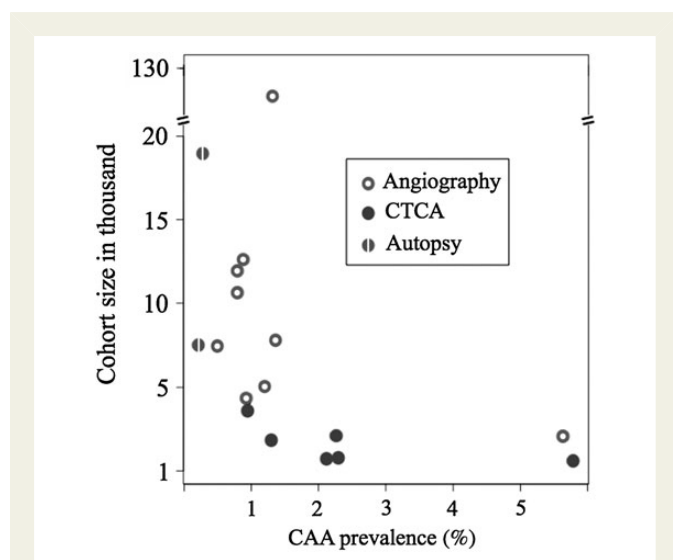
The estimated prevalence of CA anomalies is quite variable, ranging from to 0.21 to 5.79% (Figure 1, see references in Supplementary material online, Table S1) based on angiography, computed tomography (CT), and autopsy databanks. Each of these sources is limited by entry biases and a lack of clear diagnostic criteria of normality vs. abnormality.

Despite the prevalence and clinical relevance of CA anomalies, much is unknown concerning the cellular and molecular mechanisms that control CA embryonic development and their impact on adult coronary pathophysiology. In this document, we review and update knowledge on developmental and physiological aspects of CA formation, and discuss the molecular mechanisms that underlie the embryogenesis of congenital CA anomalies.

## 2. Normal CA anatomy

### 2.1 Normal CA origin

In the normal heart, the two aortic sinuses of Valsalva giving origin to the CA are adjacent to (or facing) the subpulmonary infundibulum. Viewed from the non-coronary (non-facing) aortic sinus, the remaining two sinuses are named right- and left-hand facing sinuses accordingly (Figure 2). This convention allows description of CA origin in malformed hearts that have abnormal locations of the aortic valve relative to the pulmonary valve. Coronary orifices are seldom positioned in the middle of the sinuses; they tend to be located just below, at or just above the level of the sino-tubular junction, and are often not in the midline of the respective sinusal junctions (Figure 2).<sup>1</sup>



**Figure 1** CA anomalies prevalence. Data on the prevalence of CA anomalies [from Supplementary material online, Table S1) were as based on different diagnostic methods [angiography, computed tomography coronary angiography (CTCA), and autopsy].

Viewed from the frontal plane, the right coronary artery (RCA) arises at nearly 90° with respect to the aortic sinus, whereas the left coronary artery (LCA) commonly descends at about 45°, although it could vary from ~90° to 25°. The first ventricular branch from the RCA is the infundibular branch. The left main stem branches into the circumflex (LCX) and left anterior descending (LAD, or interventricular) arteries but a third branch, the intermediate artery (also known as the first diagonal branch or the lateral branch of the LCA), is occasionally found. It is rare but well recognized to find the LAD and LCX arteries arising from separate orifices within the left coronary aortic sinus.

### 2.2 Normal CA course and distribution

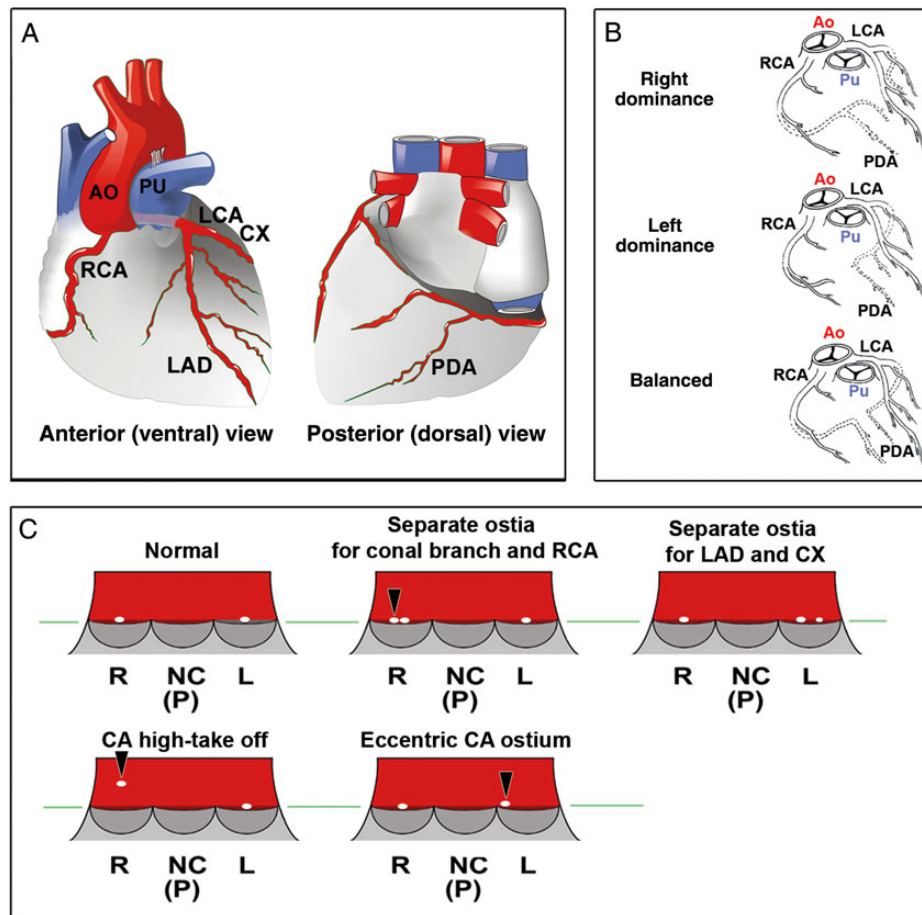
The major RCA and LCA run in the fat-filled atrioventricular and interventricular grooves and give rise to branches supplying the atria and the ventricles. The main descending branches run subepicardially in the interventricular grooves as the LAD from the LCA and the posterior descending CA. In 90% of individuals, the posterior descending arises from the RCA (right dominance), whereas in nearly 10% it is the continuation of the LCX artery (left dominance). However, in the terminology used by investigators in the CA Surgery Study (CASS),<sup>2</sup> and others, RCA dominance is described only when the RCA extends beyond the cardiac crux sending branches to the inferior diaphragmatic surface of the left ventricular wall and the postero-medial papillary muscle of the mitral valve. The atrioventricular node is usually supplied by the dominant artery.<sup>3</sup>

The posterior descending CA runs in the posterior interventricular groove, although in a small proportion it branches early from the RCA and runs obliquely along the diaphragmatic surface of the right ventricle before entering the groove. The posterior descending artery gives a series of septal perforating arteries that run in the musculature of the ventricular septum, often connecting with the perforating branches of the LAD. The posterior descending artery usually terminates at the cardiac apex, but may connect with the terminal ramifications of the LAD. Rarely, the terminal branches of the RCA and the LCX artery descend to either side of the interventricular groove in a so-called balanced pattern without a prominent posterior descending artery.<sup>3</sup>

The main stem of the LCA normally courses behind the pulmonary trunk before branching into the LCX and LAD arteries. The latter runs directly into the interventricular groove, and sends diagonal branches to the anterior wall of the left ventricle, and the septal perforating arteries. Arising from the left main stem, the LCX artery passes beneath the left atrial appendage to enter the left atrioventricular groove. In some cases, its course is short, merely supplying the obtuse margin of the left ventricle. In others, it supplies a larger territory, giving branches to the left atrium and ventricle as it courses around the atrioventricular groove. One of its atrial branches supplies the sinus node in ~45% of the population.

A series of atrial arteries, anterior, middle, and posterior, arise from the proximal course of the RCA to supply the atrial musculature. In 55–66% of individuals, the anterior artery is the sinus node artery. A variable number of branches, generally called the right anterior ventricular branches, supply the anterior wall of the right ventricle. Of these, the infundibular, preventricular, and acute marginal branches have specific names.

Normal CA anatomy is graphically summarized in Figure 2. Specific aspects of CA anatomy as related to congenital CA anomalies will be presented in the following sections.



**Figure 2** Normal CA anatomy showing right/left dominance variants and position of coronary ostia. (A) The major right and left CAs are connected to the aortic root (AO). CAs (RCA, right coronary artery; LCA, left coronary artery) run in the atrioventricular grooves giving rise to branches supplying the atria and the ventricles. Main descending branches run subepicardially in the interventricular grooves [e.g. LAD from the LCA and the posterior descending artery (PDA)]. Arising from the left main stem, the circumflex (CX) artery passes beneath the left atrial appendage to enter the left atrioventricular groove. (B) The PDA normally originates from the RCA (90% of individuals, right dominance), whereas in nearly 10% it is the continuation of the CX artery (left dominance). Occasionally, posterior branches originate from both the RCA and LCA (balanced pattern). (C) Coronary orifices are usually positioned at the level of the sino-tubular junction (R, right; L, left; NC-P, non-coronary, posterior); they can also be located just below, at or just above the level of the sino-tubular junction (green line). From left to right, upper row: variation in the normal pattern, displaying two separate RCA and LCA ostia, can occur; the conal branch (usually from the RCA) can open to a separate ostium (arrowhead); LAD and CX arteries can have separate orifices within the L coronary aortic sinus as well. From left to right, lower row: either the RCA or the LCA can arise at a significant distance above the Valsalva sinuses (high take-off, arrowhead); the RCA or LCA ostium can sometimes be located eccentric, close to the commissure (arrowhead).

## 3. Development of coronary blood vessels

### 3.1 The developmental origin of CA: a historical perspective

CAs in amniotic vertebrates, including humans, join the systemic circulation at the aortic root, whereas cardiac veins connect to the general circulation via the coronary sinus (or closely related vascular structures like the common carotid vein in the mouse). In accordance with these anatomical facts, CAs were originally thought to form by *angiogenesis* from the aortic root endothelium.<sup>4</sup> Angiogenesis implies the formation of new vessels from pre-existing ones, mostly by means of controlled endothelial sprouting.<sup>5</sup> Indeed, until the late 1980s, it was thought that

CAs entirely derived from an aortic endothelial outgrowth that would expand to form the complete coronary system, including coronary veins. Further research in avian models<sup>6–9</sup> partially argued against this model, demonstrating that: (i) prospective CA endothelial cells do not bud from the aortic root, but instead grow into the aortic wall from an aortic peritruncal plexus to connect to the systemic circulation, most likely under the guidance of VEGF-C and periaortic cardiomyocytes,<sup>10</sup> and (ii) at least part of the early arterial coronary vascular system forms through a process of *vasculogenesis*, i.e. the coalescence of endothelial progenitor cells (or *angioblasts*, see the next sections for a discussion on the origin of these cells) and subsequent fusion of endothelial cell clusters to form new blood vessels.<sup>11</sup> The relationship between the early coronary vascular plexus and the embryonic endocardium is discussed in the following section.

## 3.2 Diversity of coronary progenitors: (i) the origin of coronary endothelium

Larger CAs comprise an inner endothelial layer (*intima*), a medial wall formed by smooth muscle cells and elastic fibres (*media*), and an outer layer of fibrous tissue (*adventitia*). While the mesodermal nature of coronary endothelium is undisputed, the specific origin of its cellular precursors remains the subject of intense controversy. As discussed below, the embryonic origin of coronary endothelium is not a trivial issue, as it relates to the developmental programmes that control the coordinated differentiation and maturation of distinct coronary vascular elements (e.g. arteries vs. veins). In this context, the endocardium lining the ventricular trabeculae,<sup>12</sup> the aortic root endothelium,<sup>4</sup> liver sinusoids,<sup>13</sup> and the epicardium<sup>8,14</sup> has independently been suggested to give rise to coronary endothelium.

During the last 20 years, most studies have focused on the epicardium as the origin of coronary endothelium.<sup>7–9,14,15</sup> The epicardium is the outermost tissue layer of the heart. It derives from the proepicardium, a cluster of coelomic cells located at the inflow of the developing heart at embryonic day (E) 9.5 in the mouse, or during the fourth week of gestation in humans. Proepicardial cells attach and spread over the surface of the embryonic myocardium, forming a monolayered epithelium (the primitive epicardium) over the originally bare myocardium. Some primitive epicardial cells immediately undergo an epithelial-to-mesenchymal transition (EMT),<sup>8,15</sup> transforming their epithelial phenotype into a mesenchymal one. Thus, after the epicardial EMT a new population of highly invasive mesenchymal cells, normally referred to as epicardial-derived cells (EPDCs), appears.<sup>16</sup>

EPDCs critically contribute to the morphogenesis of the coronary vascular system as they incorporate to the intimal, medial, and adventitial layers of developing coronary blood vessels. However, while the participation of EPDCs in formation of the avian CA endothelium is widely accepted, disagreement persists on the quantitative and qualitative contribution of these cells to coronary endothelium in the mouse. Genetic lineage tracing (i.e. permanent tagging of a defined cell population and its progeny by the permanent expression of a reporter gene) using Cre recombinase under transcriptional control of different epicardial-related gene regulatory elements<sup>17–19</sup> failed to show extensive epicardial differentiation into coronary endothelium.<sup>16</sup> Relevant to this discussion, a study by Red-Horse *et al.*<sup>20</sup> proposes a mechanism for CA development based on the sprouting of the endocardium of the sinus venosus to form the endothelium of coronary veins, followed by reprogramming of these cells into the endothelium of CAs. Such results, which point to the sinus venosus endocardium as the origin of the entire coronary endothelium, have to be reconciled with three novel studies. The first one provides evidence on the contribution of a subset of epicardial progenitors (proepicardial cells) from *Scleraxis* (*Scx*) and *Semaphorin 3D* (*Sema3D*) cell lineages to the murine coronary endothelium.<sup>14</sup> The second study, based on *Nfatc1* genetic tracing, supports an endocardial origin for part of the coronary endothelium, as it shows a major contribution of ventricular endocardial endothelial cells to the arterial, but not the venous, endothelium.<sup>21</sup> The third report provides data indicating that the ventricular coronary capillary bed forms perinatally by endocardial trapping during ventricular wall compaction, thus showing that part of the adult coronary vascular system does not derive from the embryonic coronary outline.<sup>22</sup> Taken together, these findings point to at least three different

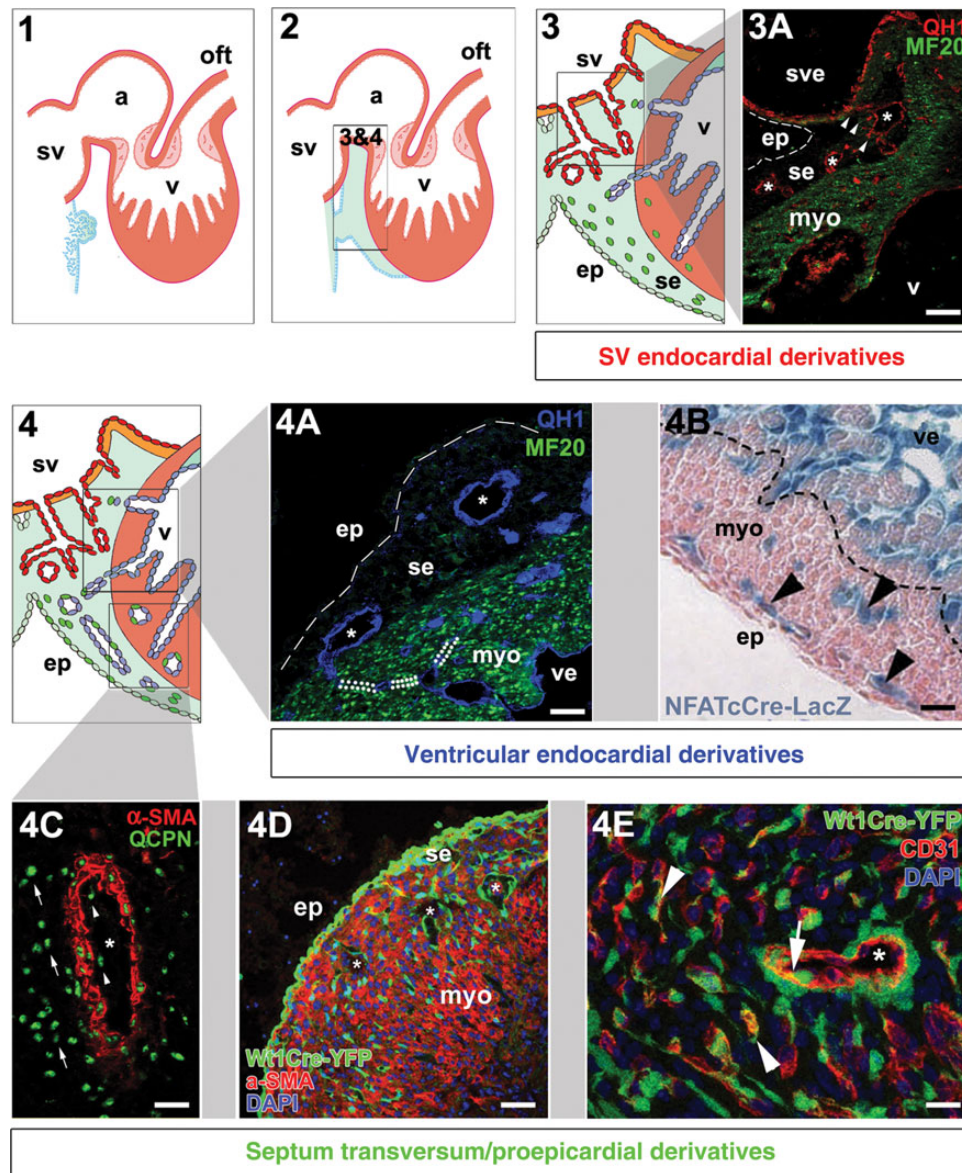
cellular sources of coronary endothelium, i.e. the sinus venosus endocardium, the ventricular endocardium, and cells of the epicardial lineage. A ventricular endocardial source for coronary artery endothelium is further supported by the nuclear translocation of Notch1 intracellular domain (a well-known arterial endothelium fate determinant) in both ventricular endocardial and prospective coronary artery endothelium,<sup>23</sup> as well as by a recent report revealing that endothelial plasticity within the endocardium drives arterial remodelling in infarcted murine hearts.<sup>24</sup> Although the relative importance of each endothelial source in mammalian coronary vascular development needs further evaluation,<sup>25</sup> current evidence suggests that sinus venosus endocardium mostly accounts for the scaffolding of coronary veins, whereas ventricular endocardial sprouts and epicardial-derived endothelial cells are the main contributors of the arterial components of the coronary tree. This model of coronary embryonic development, which is in accordance with the majority of data reported in the literature, reconciles apparently opposed views and is summarized in *Figure 3*. Nevertheless, the controversy on the topic of the origin of coronary endothelium, in particular the relative contribution of the different established progenitor cell sources, remains open.

## 3.3 Diversity of coronary progenitors: (ii) the origin of coronary smooth muscle and fibroblasts

EPDCs are generally acknowledged to be the source of smooth muscle cells forming the CA medial wall (*Figure 3*). Although this seems to be the case for all animal models so far studied, a careful evaluation of the literature does not fully endorse the idea of the epicardium as the only source of coronary smooth muscle cells.<sup>9,17–19,26</sup> This is evident when analysing results from genetic tracing of EPDCs using the Cre/LoxP system (see the previous section), which show a proportion of non-labelled cells in the CA wall. This result is frequently attributed to partial Cre driver activity or reporter recombination efficiency (i.e. it is assumed that an incomplete genetic recombination reduces the number of cells expressing the reporter in the same lineage), but alternative origins of coronary smooth muscle cells cannot be excluded. In accordance with this idea, neural crest and second heart field cardiovascular progenitor cells are likely to be alternate sources accounting for these cells.<sup>27,28</sup>

Fibroblasts are the third cell type constituting coronary blood vessels. They are especially abundant in the adventitial, external fibrous layer of large CAs. While several studies have suggested the epicardium as the origin of coronary fibroblasts in the heart,<sup>29–31</sup> fibroblasts from other origins (e.g. bone marrow) also seem to incorporate into the heart, including CAs, during late fetal stages of development.<sup>32,33</sup> Recently, the epicardial origin of cardiac fibroblasts in the infarcted heart has been reported,<sup>34</sup> but further research is needed to ascertain whether coronary adventitial and ventricular interstitial cardiac fibroblasts jointly participate in pathological cardiac fibrosis.

Taken together, studies on the origin of coronary cell types strongly suggest that coronary blood vessels are a developmental mosaic, including cells derived from different embryonic sources. It is also evident that, while many molecular mechanisms regulating CA development have been evolutionary conserved, important differences exist between species. In this regard, it is assumed that the relative evolutionary proximity of mice and humans qualifies the former as an optimal model



**Figure 3** Developmental origins and morphogenesis of CAs. (1–2) Epicardial (ep) formation from the proepicardium (pro) is required for the formation of a primary coronary vascular capillary plexus in the subepicardium (se). At least two endocardial populations contribute to coronary endothelium. The first one (3) derives from the sinus venosus endocardium (sve; 3A, arrowheads) and forms the outline of cardiac veins (3A, asterisks). The second one (4) buds (dotted lines in 4A) from the ventricular endocardium of the trabeculae (ve, separated from the compact myocardium by a dashed line in 4B) and mostly contributes cell to coronary arteries (4A, asterisks; 4B, arrowheads). (4C) Quail-to-chick proepicardial chimeras illustrate septum transversum/proepicardial (ST/PE)-derived cell [positive for the quail nuclear marker QCPN, green dots] incorporation to CAs asterisk]. ST/PE cells contribute to CA endothelium (arrowheads), smooth muscle cells (alpha-smooth muscle actin immunoreactive tissue in red), and adventitial fibroblasts (arrows). ST/PE contribution to mouse CA is evident after crossing *Wt1Cre* and *Rosa26YFP* reporter transgenic lines (4D, E; arteries marked with asterisks; arrows and arrowheads identify ST/PE-derived endothelial cells in CA and capillaries, respectively). Abbreviations: a, atrium;  $\alpha$ -SMA, alpha-smooth muscle actin; CD31, a.k.a. PECAM1, platelet endothelial cell adhesion molecule; DAPI, 4',6-diamidino-2-phenylindole; ep, epicardium; MF20, sarcomeric myosin; myo, myocardium; NFATcCre-LacZ, nuclear factor of activated T-cells/*Rosa26LacZ* offspring; oft, cardiac outflow tract; QCPN, quail pan-nuclear marker; QH1, quail endothelial marker; se, subepicardium; sv, sinus venosus; sive, sinus venosus endocardium; v, ventricle; *Wt1Cre-YFP*, Wilms' tumour suppressor *Cre/Rosa26YFP* offspring. Bars: 3A, 4A, 4D = 50  $\mu$ m; 4B = 20  $\mu$ m; 4C = 30  $\mu$ m; 4E = 15  $\mu$ m. Permission to reprint panel 4B from Wu et al.<sup>21</sup> has been obtained from the publisher via Rightslink (license number 3331981165635).

for the study of coronary blood vessel development in humans. However, avian and human coronary vessels, especially CAs, share a number of important similarities such as the subepicardial course of the main arterial branches, which are intramyocardial in the mouse, suggesting

that specific cellular mechanisms related to CA morphogenesis may differ between phylogenetically close animal models. In summary, comparing different animal models provides unique complementary insights into coronary morphogenesis.

## 4. Congenital CA anomalies

### 4.1 Classification of CA anomalies using anatomical, embryological, and physiopathological criteria

Nowadays, the most commonly used classification of CA anomalies is based purely on anatomical considerations, recognizing three categories: (i) anomalies of origin and course; (ii) anomalies of intrinsic CA anatomy; and (iii) anomalies of termination.<sup>35,36</sup>

A complementary modified classification scheme of CA anomalies advanced by our working group based on developmental considerations is provided in *Table 1*. Our classification aims at providing a link between coronary embryonic developmental mechanisms and CA congenital anomalies; this might be useful to basic scientists, but also to clinicians interested in establishing a pathological rationale between different CA anomalies on the grounds of embryological criteria.

From a pathophysiological viewpoint, there are haemodynamically relevant CA anomalies, which may be associated with shunting or ischaemia, and CA anomalies that are usually not haemodynamically significant. Noteworthy, according to older classification schemes, all CA anomalies connecting to the aorta were referred to as 'minor', since they were believed not to be physiologically relevant, apart from potential technical surgical or angiographic challenges.<sup>37</sup> However, since some so-called minor CA anomalies might be associated with ischaemia,<sup>38</sup> this terminology was abandoned; CA anomalies with potential haemodynamic/clinical implications were labelled as 'malignant', whereas the remainders were designated 'benign' CA anomalies.

In the next section, we will focus on those CA anomalies of potential interest for the clinician, by following this 'embryological–anatomical' classification.

#### 4.1.1 Anomalies of CA connection

##### 4.1.1.1 To the pulmonary artery

This is one of the most serious CA anomalies, with an estimated prevalence of one in 3 000 000 live births.<sup>39</sup> This congenital condition is a known cause of myocardial infarction and sudden death.<sup>40</sup> In the most common form, the LCA connects to the pulmonary artery or trunk (ALCAPA, anomalous origin of the left coronary artery from the pulmonary artery) and the RCA connects normally to the aorta (also known as Bland–White–Garland syndrome).<sup>41</sup> Coronary steal may occur when the low blood pressure in the pulmonary artery causes blood from the abnormal LCA to flow towards the pulmonary artery instead of towards the heart, thus resulting in ischaemia and collateral growth. The extent of the acquired collateral circulation between the two CAs is the major determinant of the degree of ischaemia, severity of clinical presentation, and outcome. Thus, patients with well-established collateral vessels have the 'adult type' of the disease, and those without or with few collaterals have the 'infant type', with early onset of symptoms when pulmonary arterial pressure decreases.<sup>42</sup> Noteworthy, the availability of less invasive diagnostic modalities, such as CT and MRI, has resulted in more frequent identification of this condition in an older cohort.<sup>43</sup> The definitive treatment for ALCAPA is surgical intervention, with direct reimplantation of the anomalous CA into the aorta.

##### 4.1.1.2 To the aorta

Among the various types listed, we will address those clinically relevant types.

**Table 1 Embryological–anatomical classification of CA anomalies (modified from Angelini<sup>35</sup>)**

A. Anomalies of CA connection
a. to the pulmonary artery (PA)/pulmonary circulation
1. LCA to posterior facing sinus (ALCAPA)
2. LCX to posterior facing sinus
3. LAD to posterior facing sinus
4. RCA to anterior right facing sinus
5. Ectopic connection (outside facing sinuses) of any CA to PA left sinus, trunk, or branch
6. RV
b. to the aorta/systemic circulation
1. Absent left main trunk (split LCA)
2. Anomalous CA ostium location <i>within or near proper aortic sinus</i> of Valsalva: <ul style="list-style-type: none"> <li>• High tubular aorta</li> <li>• Low aorta</li> <li>• Commissural (with acute angle)</li> </ul>
3. Anomalous CA ostium location at <i>improper aortic sinus–wrong sinus</i> : <ul style="list-style-type: none"> <li>• RCA to left sinus</li> <li>• LCA to right sinus</li> <li>• LCX to RCA/ or sinus</li> <li>• LAD to RCA/or sinus</li> <li>• RCA or LCA to posterior sinus with anomalous course: interarterial, prepulmonic, intraseptal, retroaortic, posterior atrioventricular groove or retrocardiac, postero-anterior interventricular groove</li> </ul>
4. Single CA
5. Anomalous CA ostium location <i>outside sino-tubular aorta</i> : <ul style="list-style-type: none"> <li>• LV</li> <li>• Ascending aorta</li> <li>• Aortic arch</li> <li>• Others (innominate artery; right carotid artery; internal mammary artery; bronchial artery; subclavian artery; descending thoracic aorta)</li> </ul>
B. Anomalies of intrinsic CA anatomy
1. CA ostial stenosis or atresia
2. CA ostial dimple
3. CA ectasia or aneurysm
4. Absent CA
5. CA hypoplasia
6. Anomalous CA ramification: <ul style="list-style-type: none"> <li>• Anomalous origin of PD from LAD or septal penetrating branch</li> <li>• Split RCA</li> <li>• Split LAD</li> <li>• Ectopic origin of first septal branch (RCA, right sinus, diagonal, LCX)</li> </ul>
C. Anomalous myocardial/CA interaction
1. Intramural course ('myocardial bridge')
2. Subendocardial course
3. Fistulae from RCA, LCA, or infundibular artery to: RV, RA, coronary sinus, superior vena cava, PA, PV, LA, LV, multiple
4. Inadequate arteriolar/capillary ramifications

*Anomalous coronary ostium location at improper aortic sinus–wrong sinus.* In the setting of anomalous connection of either the RCA to the left coronary sinus or the LCA to the right coronary sinus, the proximal anomalous CA may run anterior to the pulmonary trunk (prepulmonic), posterior to the aorta (retroaortic), septal (subpulmonic), or between

the pulmonary artery and the aorta itself (interarterial). Among them, only those with an interarterial (aorta–pulmonary) course are regarded as hidden conditions at risk of ischaemia and even sudden death.<sup>44</sup> Several mechanisms of ischaemia particularly during exercise have been postulated: (i) increased cardiac output and expansion of the great vessels, with compression of the anomalous vessel coursing between the aorta and the pulmonary artery; (ii) the acute angle take-off of the anomalous vessel with further stretch during exercise, possibly accounting for a flap-like closure of the coronary ostium; (iii) spasm or kinking of the anomalous vessel; and (iv) the course within the aortic wall ('intramural') of the proximal segment of the anomalous vessel.<sup>35,44</sup> This intramural aortic course, originally documented in pathology reports,<sup>44</sup> can explain the imaging feature (angiography and echo) of CA intussusception into the aortic wall; the narrowing of the proximal segment of the anomalous vessel (segmental hypoplasia); and the asymmetrical lateral compression of the anomalous vessel with a slit-like or ovoid rather than circular lumen, particularly during systole and stress. Although both RCA and LCA anomalous connection to the wrong aortic sinus are risky, anomalous LCA connection to the right sinus is considered more malignant because of the larger amount of myocardium at ischaemic risk.<sup>45</sup> Anomalous connection of a CA to the posterior aortic sinus is quite rare and even more exceptionally associated with sudden death.

The anomalous connection of the LCX branch to the RCA or sinus is considered the most frequent CA anomaly with an angiographic incidence of up to 0.67%.<sup>46,47</sup> After the anomalous take-off, the LCX branch shows an abnormal retroaortic course to reach the left atrio-ventricular groove crossing the mitro-aortic fibrous continuity. This anomaly is usually considered benign and an uncertain cause of myocardial ischaemia and sudden death. Thus, its clinical significance should be judged on a case-by-case integrated approach after exclusion of all other possible causes of signs or symptoms.<sup>48</sup>

**Single CA.** In this very rare condition that is seen in 0.0024–0.044% of the population,<sup>49</sup> only one CA connects to the aorta through a single ostium. The single CA may take the course of either an RCA or an LCA and divide shortly from its origin into two or three of the main coronary branches.<sup>50</sup> While single CA may be compatible with a normal life expectancy, when a major CA branch courses between the pulmonary artery and the aorta patients are at increased risk of myocardial ischaemia. In addition, the intrinsic inability to develop proximal collateral branches in the setting of acquired stenosis may be devastating.

Major congenital anomalies of CA connection to the aortic or the pulmonary arterial roots are illustrated in *Figure 4*.

#### 4.1.2 Anomalies of intrinsic CA anatomy

Among these CA anomalies, *CA ostial stenosis or atresia* is a rare condition, with most of the reported cases discovered at angiography and involving the left main CA. Although no lumen can be identified in most of the cases, an almost obliterated lumen has been reported at angiography with disappearance of the LCA between its orifice and the bifurcation.<sup>51</sup> Prominent right-to-left CA collateral vessels are usually detected, but they are inadequate to meet the myocardial oxygen demand. Clinical presentation is usually early in the first year of life.<sup>51</sup>

#### 4.1.3 Anomalies of myocardial/CA interaction

##### 4.1.3.1 Intramural course (or myocardial bridge)

An epicardial coronary artery, usually the LAD branch, may lie deep within the myocardium, thus presenting as an intramyocardial course. The prevalence of myocardial bridge at angiography is lower than at autopsy (0.5–2.5% vs. 15–85%) since many bridges consist of thin

loops of myocardium not causing haemodynamic changes and as such are undetectable by angiography with the typical vessel constriction (i.e. 'milking effect').<sup>52</sup> Coronary bridges are also common in hypertrophic cardiomyopathy, with a frequency of ~25%.<sup>53</sup> Although sudden death has been ascribed to myocardial bridge in young people and athletes,<sup>54</sup> this feature is nowadays classified among the uncertain causes of sudden death.<sup>48</sup> Exercise-induced ischaemia has been attributed to tachycardia, which both increases the myocardial oxygen requirement and reduces the diastolic coronary flow.<sup>54</sup> By intravascular ultrasound clinically significant myocardial bridges are characterized by phasic systolic vessel compression, persistent reduction in diastolic lumen, increased blood flow velocities, retrograde systolic flow, and decreased coronary flow reserve.<sup>55</sup> As far as therapy is concerned, surgical de-bridging and even stent implantation have been successfully carried out in symptomatic cases.

##### 4.1.3.2 Fistulae

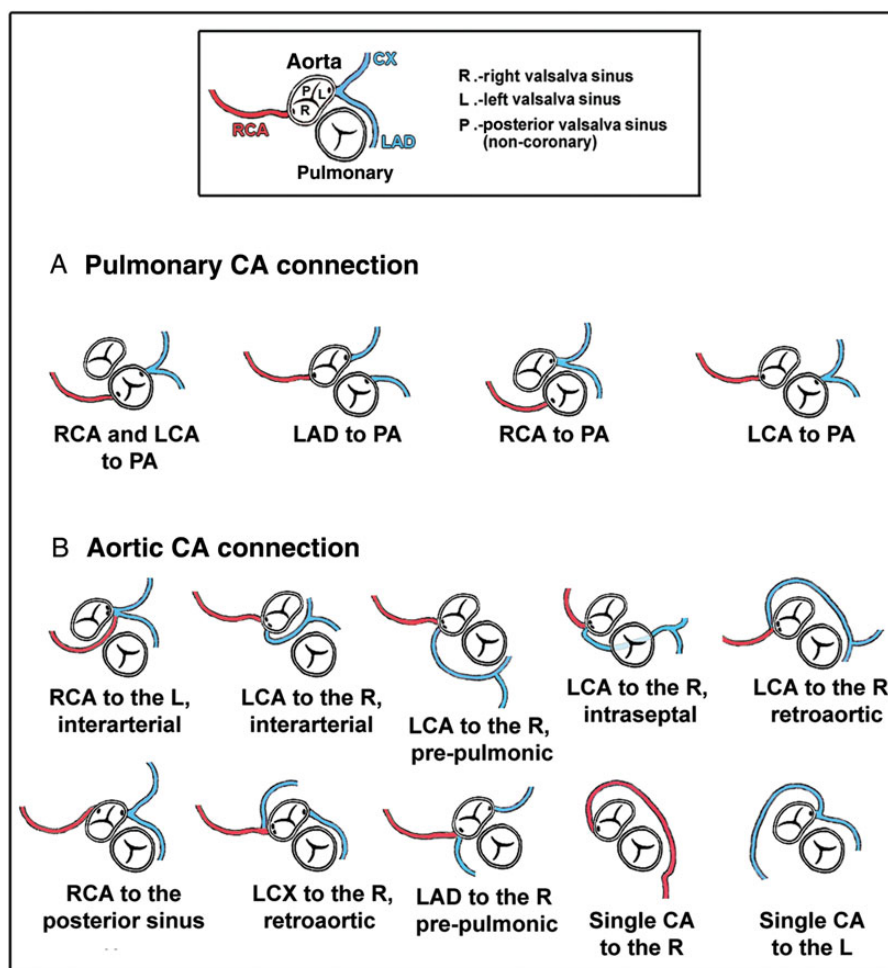
In this condition, a communication exists between one or two CA(s) and either a cardiac chamber or any of the great vessels (the coronary sinus, the superior vena cava, and the pulmonary artery or veins).<sup>56,57</sup> It is seen in ~0.1–0.2% of all patients who undergo selective coronary angiography.<sup>56</sup> The most common site of drainage is the right ventricle, followed by the right atrium and the pulmonary artery. Fistulae draining into the left atrium or left ventricle are observed in <10% of cases. The haemodynamic consequences of CA fistulae depend mainly on the resistance (which is determined by fistula size, tortuosity, and length) and on the site of drainage. The blood flow from the CA to a venous structure or right-sided cardiac chamber occurs throughout the cardiac cycle; with larger fistulae, a diastolic 'coronary steal' may exist, drawing blood away from the normal coronary tree, leading to symptoms and signs of myocardial ischaemia. If the fistula drains to the systemic venous circulation, a left-to-right shunt develops with volume overloading both ventricles. When the drainage site is the left atrium or pulmonary vein, a left-to-left shunt with left heart volume overload occurs. Small fistulae in asymptomatic children should be followed up for signs of increasing size and flow because of their tendency to grow with age. It is recommended to close them early in symptomatic or asymptomatic patients with large, haemodynamically significant CA fistulae. Treatment options include surgical ligation, either isolated or in association with CA bypass grafting, and interventional closure with occlusion coils, umbrellas, vascular plugs, and covered stents.<sup>57</sup>

Recommendations for anomalies of CA connection and for coronary arterio-venous fistula have been recently developed within the ACC/AHA guidelines for the management of adults with congenital heart diseases.<sup>58</sup>

## 4.2 CA anomalies associated with other congenital heart defects

Although this not the focus of the current document, it is important to mention that any of the aforementioned CA anomalies can coexist with congenital heart malformations and may complicate management, both prior to and after surgical repair. The anomaly can involve features such as origin, course, and communication.

In certain heart malformations, such as complete transposition and double outlet right ventricle, an abnormal position of the aortic root is common. Although CAs connect to the aortic sinuses that are adjacent to the pulmonary valve, they frequently have abnormal patterns, some of which may require special attention when planning to relocate them in surgical repair. In complete transposition, single CA or



**Figure 4** Normal and anomalous CA–truncal connection. (A) Anomalous CA connection to the pulmonary artery. Both LCA and RCA to the pulmonary artery; LAD to the pulmonary artery; RCA to the pulmonary artery; LCA to the pulmonary artery. (B) Anomalous CA connection to the aorta. Top line: RCA to the L coronary sinus with interarterial course; LCA to the R coronary sinus with interarterial course; LCA to the R coronary sinus with prepulmonic course; LCA to the R coronary sinus with intraseptal course; LCA to the R coronary sinus with retroaortic course. Bottom line: RCA to the P non-coronary aortic sinus; LCX to the RCA or R coronary sinus with retroaortic course; LAD to the R coronary sinus; single CA connected to the R coronary sinus; single CA connected to the L coronary sinus.

abnormal CA coursing between the great arteries is more common in hearts with side-by-side or anterior–posterior relationships of the arterial trunks.<sup>59–61</sup> Furthermore, not all patients have a differentiated pulmonary and systemic arterial trunk. Patients with persistent truncus arteriosus have coexisting significant CA abnormalities. They may contribute to high operative mortality rate and may be a cause of late sudden death. Surgical procedures should be planned to protect CA in the region of the right ventricular outflow tract below the truncus; CA obstruction (either ostial or luminal) need to be addressed as a separate issue during surgical procedures.<sup>62</sup>

Irrespective of their connection to the aorta, the main CA can show abnormalities in their epicardial courses. This has surgical implications that vary for each case. For example, when an aberrant (or accessory) left CA crosses the right ventricular outflow tract in a heart with Fallot's tetralogy, this vessel should be avoided when resecting or incising into musculature to reconstruct the tract.

Abnormal communications between a cardiac chamber and the CA system (fistulae) occur most frequently in hearts with atresia of the right or left ventricular outflow tracts and an intact ventricular septum.

The most common sites of communication are the right ventricle in cases of pulmonary atresia, and the left ventricle in hearts with aortic atresia. Remarkably, the fistulous communications in hearts with pulmonary atresia are usually to the RCA or the LAD branch, and there may be multiple connections.<sup>63</sup> The luminal aspect of the CA shows characteristic myointimal hyperplasia that may lead to occlusions. When associated with occlusions or atresia of the proximal portion, the coronary circulation may become dependent on perfusion through the fistulae.<sup>64</sup>

## 5. Cellular and molecular bases of CA anomalies

### 5.1 Animal models in the study of coronary development and disease

Research on the development of human coronary blood vessels has been strongly influenced by the anatomy of the adult coronary vascular



system. For obvious reasons, our current understanding of coronary blood vessel formation is based on the use of animal models, providing a dynamic approach to coronary embryonic development and crucial information on the origin, fate, and patterning of CA. This approach, however, requires a careful identification of the similarities and differences in cardiac anatomy of different animal species (Table 2, see also Ostadal<sup>65</sup>), as well as the impact of intraspecies variability (including genetic background effects in defined animal breeds) in research animal models. This latter aspect is of particular importance in the mouse, as the genetic background of mouse strains frequently used for the development of transgenic models (e.g. C57BL/6) has been shown to be responsible for unusual anatomical CA patterns.<sup>66</sup> Knowledge of the embryonic development of human coronary vessels thus depends on plausible extrapolation of results derived from animal experimentation. The following sections provide a systematic analysis and discussion of relevant animal experimental models for the study of anomalous coronary development. Basic cellular and molecular mechanisms of CA development have been summarized in Figure 5.

## 5.2 Cellular determinants of anomalous CA development

There are several key developmental events whose alteration can lead to CA anomalies:

- (i) CA stems connection to the cardiac arterial pole. Disruption of this process would cause anomalies of CA connection to the aorta.
- (ii) Differentiation of coronary cell progenitors (including the possibility of abnormal epicardial EMT). Disruption of this process could result in the reduction of embryonic coronary progenitor cell numbers and their precocious or delayed maturation.
- (iii) Interaction between coronary vessels and the myocardium. Altered coronary–myocardium interaction would have an impact on the patterning of CAs over the ventricular chambers.

**Table 2 Adult ventricular angio- and myoarchitecture in various vertebrate taxa**

Group	Types of ventricles
Cartilaginous fishes	Compact layer often thick and vascularized, CA present in both the compact and trabecular layers
Bony fishes	Highly variable, ranging from entirely spongy ventricle without CA in icefish to mostly compact fully vascular one (tuna) depending on life style
Frogs	Mostly entirely avascular trabeculated ventricle with CA present in the atrioventricular canal and conus; thin vascular compact layer in the large species
Reptiles (including crocodiles)	Mixed type (compact and trabeculated zone). CA present in both compact layer and trabeculae
Birds	Entirely compact, fully vascularized
Mammals	Entirely compact, fully vascularized

The situation is described for the mature adults (modified from Ostadal<sup>65</sup>).

## 5.3 Molecular determinants of anomalous CA development

Anomalous coronary development may arise from the disruption of a myriad of molecular and cellular mechanisms, as evidenced by the phenotypic analysis of animal models for the study of cardiovascular development. From a developmental perspective, molecular signals either are provided by or target distinct cell populations.<sup>16</sup> For the sake of simplicity, in this section, we will focus on the analysis of signalling molecules and transcription factors pivotal to CA embryogenesis, identifying the affected cell mechanisms for each case.

### 5.3.1 Molecules participating in the determination of coronary progenitor cell fate

The *Wilms tumour suppressor gene* (*Wt1*), a Zinc-finger transcription factor, is involved in the regulation of epicardially secreted retinoic signalling. *Wt1*-dependent disruption of retinoic acid synthesis in the epicardium directly or indirectly down-regulates the expression of Pdgf receptors  $\alpha, \beta$  in EPDCs, interfering with coronary smooth muscle progenitor cell differentiation.<sup>67</sup> Other important cues for coronary blood vessel development are provided by the Notch/Delta cell-to-cell signalling pathway, which is necessary to promote arterial endothelium fate during coronary blood vessel development.<sup>23,68</sup> Down-regulation of the nuclear transcription factor *Coup-tfl1*, a Notch repressor,<sup>69</sup> and up-regulation of *Ephrinb2* expression in CA progenitor cells also correlate with CA endothelial specification, although the underlying genetic mechanism controlling this process is unknown.<sup>20</sup> Finally, myocardium-secreted Fgfs and Vegf participate in the regulation of coronary endothelial cell fate and vascular assembly.<sup>70</sup> These two growth factors are closely linked, as Vegf has been reported to be dependent on myocardial Fgf-induced *Hedgehog* activity.<sup>71</sup>

### 5.3.2 Molecules involved in the regulation of epicardial EMT

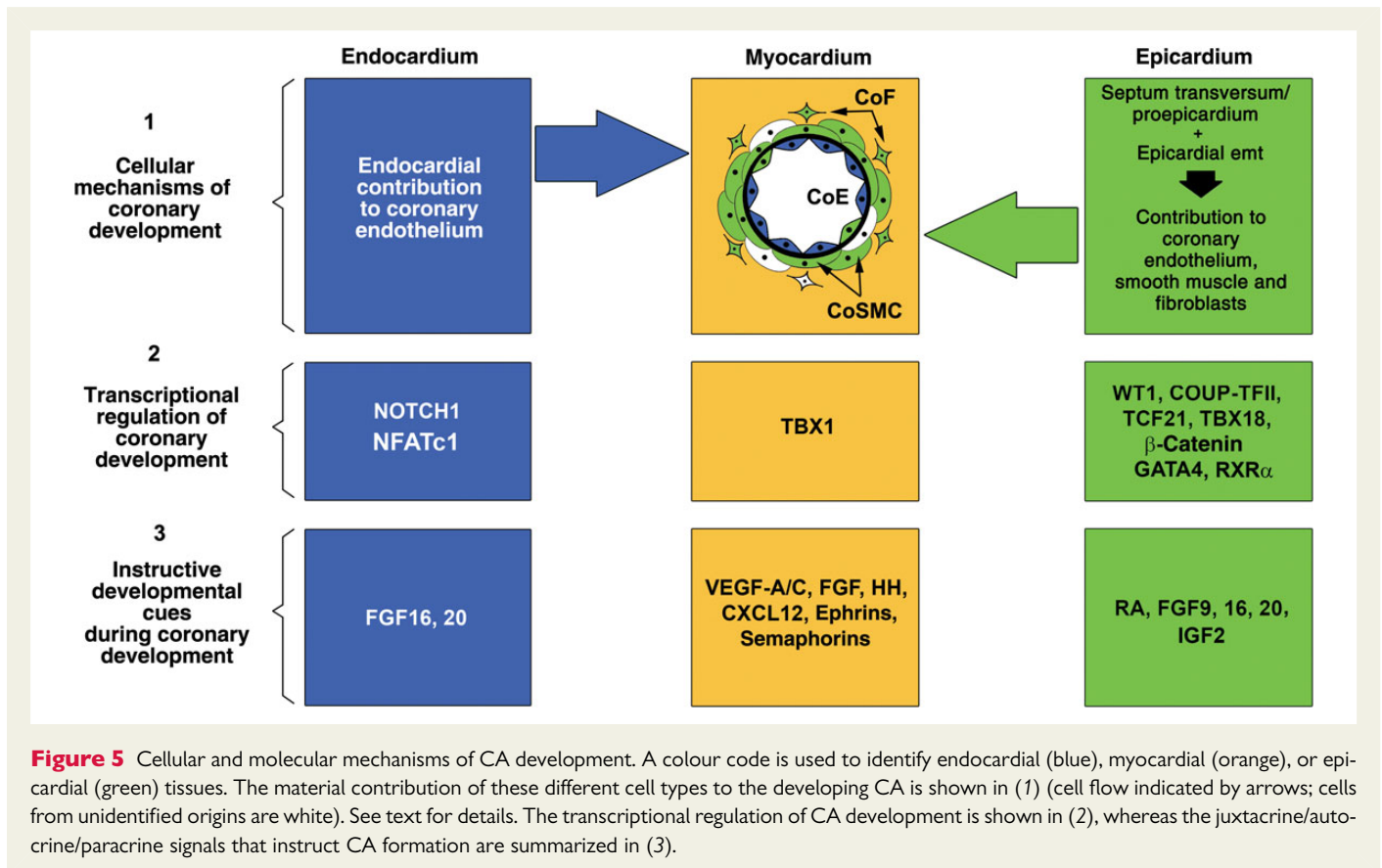
An example of this type of molecule is also provided by *Wt1*, which is known to be involved in the control of the cadherin repressor *Snail*,<sup>72</sup> and Wnt/ $\beta$ -Catenin-dependent signalling,<sup>73</sup> both of which are thought to be required for epicardial EMT. Animal models for defective *Wt1* or  $\beta$ -Catenin signalling display severe CA anomalies, including abnormal CA patterning and arterio-ventricular communications, and die at mid-gestation.<sup>17,67</sup> However, the molecular signals that trigger epicardial EMT genetic programme remain to be elucidated.

### 5.3.3 Molecules required for endocardial outgrowth and incorporation in embryonic coronary vessels

It has been proposed that hypoxia gradients in the ventricular walls activate myocardial Vegf and promote endocardial sprouting into the ventricular walls to contribute to CA development.<sup>21</sup> In this regard, several authors have suggested that cell membrane-bound Ephrins<sup>20</sup> and Semaphorins<sup>14</sup> provide attractive or repulsive cues to the developing coronary endothelium, thus patterning its growth over the myocardium. This remains a largely under-researched but important aspect of coronary vascular development, as it links coronary morphogenesis to the growth and compaction of the ventricular walls.

### 5.3.4 Molecules with a role in the spatial patterning (myocardial/aortic) of CAs

The stabilization of the primitive embryonic CA endothelial network into a mature, definitive coronary tree is dependent on medial coronary smooth muscle cell differentiation and endothelial–smooth muscle cell molecular cross-talk. Retinoic acid and Vegf have been reported to



prevent coronary smooth muscle cell differentiation before an extensive coronary capillary network has formed.<sup>74</sup> Alteration of this process<sup>28</sup> is likely to affect the morphology and viability of early CA blood vessels. In parallel, the structural organization of cardiomyocytes also plays a role in the patterning and mural location of CAs as shown by experiments disrupting myocardial cell polarity,<sup>75</sup> indicating that coronary blood vessels and myocardium developmental programmes are inextricably related.

## 5.4 Haemodynamically significant CA anomalies: a link to embryonic cell mechanics

### 5.4.1 Anomalous CA connection

From a developmental perspective, the 'proximal' aorta and the 'distal' cardiac chamber coronary vascular plexus are two distinct, developmentally independent elements of the coronary system; any genetic factor preventing the correct assembly of these two elements is likely to result in CA anomalous connection. In accord with this model, abnormal differentiation of myocardium at the base of the pulmonary trunk might contribute to the abnormal connection of CAs, since this tissue has been proposed to be refractory to blood vessel growth, potentially marking a non-coronary subpulmonary myocardial domain. In support of a regionally instructive myocardial model, mice mutant for *Tbx1*, a crucial gene for development of the arterial pole of the heart and DiGeorge syndrome candidate gene, shows common arterial trunk with a reduced subpulmonary domain and anomalous patterning of CA connections, displaying predominantly right-sided CA connections and frequent convergence of right and

left stems.<sup>76,77</sup> It has also been shown that distal coronary patterning is unaltered by the anatomical rotation of the aortic root in a case of transposition of the great arteries.<sup>78</sup> Thus, it seems that it is the specific location of the aortic root in the cardiac base what ultimately determines the path followed by coronary stems with respect to the aortic and pulmonary roots, the proximity of the coronary sinuses to the anterior interventricular and atrioventricular grooves probably contributing to the specific location of the two main coronary stems.<sup>79</sup> Alternative mechanisms for anomalous CA connections to the proper aortic sinuses could involve the disruption of coronary arterio-venous growth coordination, critical for normal embryonic development of the coronary vascular tree.<sup>20,21</sup> Both scenarios may also account for the pathogenesis of anomalous CA connection to the opposite aortic sinus of Valsalva (see the next section). The aortic root is nevertheless important in defining the connections of the LCA and RCA stems to the proper coronary Valsalva sinuses of the aorta. Interestingly, the CXCL12/CXCR4 signalling axis has been recently implicated in proximal CA connection with the aortic endothelium.<sup>80</sup> Changes in vascular density around the aortic trunk, which can be modulated by hypoxic domains and local VEGF availability,<sup>81</sup> induce physiological vascular hyperfusion, reducing the number of vessels but increasing the calibre of the resulting ones.<sup>82</sup> In addition, formation of the coronary ostia, marked by apoptosis at the aortic wall,<sup>83</sup> is required for the connection of the periaortic coronary vascular ring to the aortic sinuses. All these events can mechanistically explain CA anomalies affecting the number of ostia, the location of CA connections with respect to the aortic valve sinuses (there is a high incidence of coronary ostia anomalies in bicuspid aortic valves<sup>84</sup>), and even the number of CAs.

### 5.4.2 Single CA and anomalous CA ramification

As pointed out, endothelial hyperfusion can result in the reduction of coronary blood vessels, something that can also arise if EPDCs do not develop properly. Failure of one of the two CAs to muscularize can be a factor explaining the absence of a major coronary vessel, since embryonic CA remodelling is followed by the stabilization of definitive CAs, a process mediated by arterial muscularization. This latter event is physiologically delayed by the inhibition of coronary smooth muscle cell differentiation exerted by the synergistic effect of retinoic acid (a biologically active form of vitamin A) and VEGF.<sup>74</sup> Interestingly, although the recruitment of periendothelial cells is an early event during coronary morphogenesis, the full differentiation of these cells into mature coronary smooth muscle cells only takes place after the connection of the primary CA plexus to the aorta, progressing rapidly from the coronary ostia at the aortic root towards the most distal, ventricular coronary branches.<sup>85</sup>

The factors that determine the common course of CA around the atrioventricular canal and the characteristic ventricular branches are unknown. Some authors have proposed that the formation of the main CA tracts correlates with the areas where the epicardium and associated subepicardial matrix first form,<sup>86</sup> a view which is consistent with the subepicardial association of cardiac fat and CAs, two tissues that, at least partially, originate from the embryonic epicardium.<sup>87</sup> An additional mechanistic explanation is the existence of low oxygen tension domains in the myocardium producing high levels of pro-vascular growth factors like VEGF or FGF2.<sup>70</sup> However, an integrated model by which the coronary tree is spatially patterned is still missing.

### 5.4.3 Anomalous myocardial–CA interaction: myocardial bridges and fistulae

It could be argued that abnormal myocardial–coronary interaction, such as that described in *Vangl2* mutants, which display disrupted cell polarity,<sup>75</sup> is a logical explanation for the formation of CA anomalies like myocardial bridges and fistulae. Anomalies in the transmural (epicardial to endocardial) distribution of myocardial growth factors or signalling molecules can also affect the growth of the ventricular wall, and compromise the specific location of embryonic coronary vessels with respect to the ventricular lumen.

## 6. Summary

Advances in our understanding of the embryonic development of coronary blood vessels and the cellular and molecular mechanisms regulating this process have provided insights into the developmental mechanisms and genetic pathways underlying CA anomalies. In particular, in this position statement, we have reviewed data indicating that coronary blood vessels form from a variety of mesodermal cell sources and should thus be regarded as a developmental mosaic. Moreover, we have discussed how signalling and haemodynamic cues provide a framework for understanding the wide spectrum of CA anomalies observed in human patients. Ongoing analysis of the contribution of different cell types and signalling pathways to the establishment, stabilization, and patterning of CAs will further decipher the impact of disrupted embryonic development in the aetiology of CA anomalies with clinical implications.

## Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

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