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Gross anatomical variants of the vasculature of the Göttingen™ minipig

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Abstract

Objectives: The Göttingen™ minipig has become increasingly popular in cardiovascular research, because physiological and anatomical features of the porcine cardiovascular system are comparable to humans. To support refinement of biomedical studies on this animal model we used contrast enhanced computed tomography (CECT) to study the vasculature of the neck, the proximal parts of the limbs and the abdomen for variants.

Methods: 18 pre-adult female Göttingen™ minipigs (16 ± 4 months old) were included in the study. The body weight was 30.8 ± 9.0 kg. Prior to CECT anaesthesia was performed using ketamine, xylazine and azaperone. CECT was performed in prone position on a 64-slice scanner (LightSpeed™ 64, GE Medical Systems, IL, USA) after intravenous injection of 80 ml nonionic iodinated contrast medium (XenetiX™ 350, Guerbet GmbH, Germany) in each pig. Image analysis was performed using Advantage Windows 4.2 (GE Medical Systems, IL, USA) and AMIRA™ (Visage Imaging GmbH, Germany).

Results: The study demonstrated variants in the vasculature of pre-adult Göttingen™ minipigs concerning the linguofacial vein, the subclavian vein, the caudal vena cava, the common iliac vein, the cranial mesenteric artery and the renal arteries and veins.

Conclusion: In individual cases variants of the vasculature can be found in the Göttingen™ minipig which might cause complications when performing manipulations within the vasculature like implantation of e.g. stents, vascular prostheses or catheters.

Key words: vasculature, minipig, variants, computed tomography

Introduction

Due to anatomical and physiological similarities between pigs and humans, the pig is more suitable for medical research than other laboratory animals [1-4]. Already with 25-30 kg body weight the cardiovascular system of pigs is regarded as sufficiently matured to

model the cardiovascular system of humans [5]. Pigs are conform with humans in key parameters of the cardiovascular system: relative heart weight [5], cardiac output [3], systolic and diastolic blood pressure [6-10], coronary anatomy, blood volume, hematocrit and hemoglobin content [1-4]. Due to restrictions in housing and animal care in the 60's the

Göttingen™ minipig was crossbred from the Vietnamese pot-belly pig, Minnesota miniature pig and the German Landrace pig [11]. The cardiovascular system of the Göttingen™ minipig is more resistant to stress compared to the other pig breeds [11-14]. Therefore this animal model has become increasingly popular in cardiovascular research. Recently it could be shown, that the vasculature – luminal diameter and length of arteries and veins – remained constant in 17-21 month old Göttingen™ minipigs, despite of a significant increase of the body weight ($p < 0.05$) over 4 months (from months 17 to 21). Because of this the pre-adult Göttingen™ minipig was suggested for use in *in vivo* studies which require long-term stable vascular morphometric parameters, such as implantation of stents, vein cuffs, heart or venous valves and vascular prostheses [15].

Efficient use of the minipig as laboratory animal for these studies according to the principles of Russel & Burch [16] necessitates a thorough knowledge of the anatomy of the major blood vessels. Unfortunately the classic anatomic description of arterial and venous patterns in textbooks concerning Göttingen™ minipigs is severely limited [6]. However, yet it is the premise on which many surgeons operate. For this reason the study was aimed to gain data about gross anatomical variants of the vasculature in these animals.

Methods

Animals, housing and care

In vivo experiments were approved by the regional office for health and social welfare (LaGeSo) of Berlin and were performed at the Charité University Clinic, Campus Virchow (Berlin, Germany), Department of Experimental Medicine (certified by ISO 9001) using pre-adult female Göttingen™ minipigs ($n=18$, Ellegaard, Denmark). The animals were 16 ± 4 months old and had a body weight of 30.8 ± 9.0 kg. They were cared according to the guidelines of the European so-

cieties of laboratory animal sciences and housed in groups of six in an environmentally controlled room (12/12 h light/dark-rhythm, 15-24 °C, 55 ± 10 % relative humidity).

Anaesthesia

Prior to computed tomography (CECT) anaesthesia was performed. One night before anaesthesia the animals were fasted, but had free access to water. The minipigs were premedicated by intramuscular (i.m.) injection of 0.5 ml atropine (Atropinum sulfuricum, 1 mg/ml, Eifelfango, Germany) and anesthetized by i.m. injection of ketamine (27 mg/kg, Ursotamin™, Serumwerk Bernburg, Germany), xylazine (3.5 mg/kg, Rompun™ TS, Bayer Vital, Germany) and 120 mg/animal azaperone (Stresnil™, Janssen Animal Health, Germany). Additionally an electrolyte solution (Ionosteril™, Fresenius, Bad Homburg, Germany) was continuously infused intravenously (i.v.).

Computed tomography

CECT was performed in prone position on a 64-slice scanner (LightSpeed™ 64; GE Medical Systems, IL, USA). The scan protocol used contrast enhancement with automatic intravenous injection of 80 ml nonionic iodinated contrast medium (XenetiX™ 350, Guerbet GmbH, Sulzbach, Germany 350 mg/ml iodine) in each pig. The scan parameters were standardized (voltage 120 kV, maximal 500 mA with automatic mA-optimization at a noise index of 15, mean mA 490; collimated slice thickness: 64×0.625 mm; total detector width: 55 mm; rotation speed: 0.4 sec; table feed per rotation: 55 mm) and the scan speed was approximately 3 sec for 30 cm scan length in the z-axis. For volumetric assessment 1.25 mm images were reconstructed without overlapping. Image analysis was performed using Advantage Windows 4.2 (GE Medical Systems, IL, USA) and AMIRA™

(Visage Imaging GmbH, Germany). The vasculature of the neck, the proximal parts of the limbs and the abdomen were analyzed for variants in the course and the macromorphological structure of vessels with an inner diameter > 1 mm. According to the angle between the renal arteries (veins) and the aorta (vena cava) (Fig. 3) in direction of the blood flow the course of the renal vessels was classified in horizontal ($\alpha = 90 \pm 5^\circ$), craniocaudal (equivalent to superoposterior in humans, $\alpha > 90 \pm 5^\circ$) and caudocranial (equivalent to posteroposterior in humans, $\alpha < 90 \pm 5^\circ$).

Results

In all animals the subclavian vein was demonstrated as a continuation of the axillary vein which merged to form the respective brachiocephalic vein as the major vein returning blood to the cranial vena cava. The subclavian vein was in all animals bilaterally established in duplicates and in most animals (88.9%, $n=16$) additionally uni-/bilaterally parallelly accompanied by one to two additional vessels with a smaller caliber which also continued in the brachiocephalic vein (see Fig. 1 A-C). The size of the cross sectional area of the additional veins which run in a parallel course to the subclavian vein was 3.2-40.8% of that of the subclavian vein.

Variants were also obvious in the renal vasculature. The renal arteries usually arise

asymmetrically from the lateral surfaces of the abdominal aorta and caudally to the cranial mesenteric artery. Latter was found in one animal (5.6%) in between the origin of the left and right renal arteries (Fig. 2 A).

In 11.1% ($n=2$) of the animals the right renal artery arose from the caudal abdominal aorta directly contralateral to the left renal artery (Fig. 2 C). However, in 55.6% ($n=10$) of the animals the origin of the right renal artery was cranial (Fig. 2 B) and in 33.3% ($n=6$) caudal (Fig. 2 D) to the origin of the left renal artery.

The course of the renal artery varied between caudocranial ($\alpha \leq 90 \pm 5^\circ$; left renal artery 44.4%, $n=8$; right renal artery 22.2%, $n=4$; Fig. 2 D) and craniocaudal ($\alpha > 90 \pm 5^\circ$; left renal artery 0%; right renal artery 11.1%, $n=2$, Fig. 2 A) and was most frequently almost horizontal ($\alpha = 90 \pm 5^\circ$; left renal artery 55.6%, $n=10$; right renal artery 66.7%, $n=12$, Fig. 2 C).

The renal artery from the right side passed the caudal vena cava mostly (77.8%, $n=14$) dorsally (Fig. 2 B) and only in 22.2% ($n=4$) ventrally (Fig. 2 D).

In one animal (5.6%, $n=1$) the point of division of the renal veins into the cranial and caudal branch seemed to be shifted bilaterally close to the caudal vena cava (Fig. 2 E, F).

The angle α between the renal vein and the caudal part of the abdominal vena cava (Fig. 2D) was mostly $\leq 90 \pm 5^\circ$ (left kidney 100%, $n=18$; right kidney 72.2%, $n=13$). On-

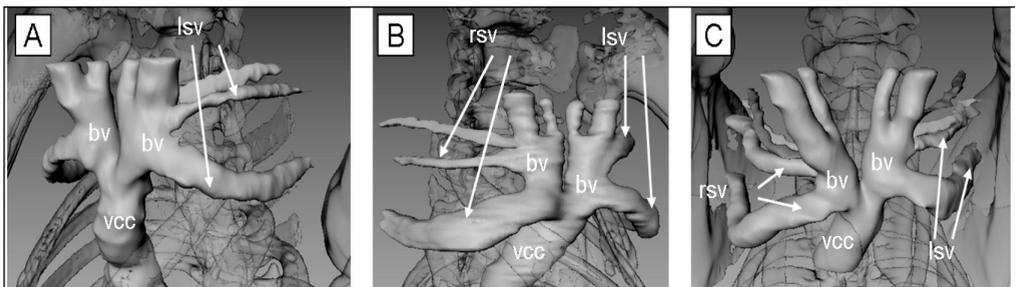


Figure 1: Subclavian vein pairs on the right (rsv) and left (lsv) side of the body continuing in the brachiocephalic vein (bv) and the cranial vena cava (vcc) (A, B, C), and accompanied by one (A, B) to two (C) veins in a parallel course in pre-adult Göttingen™ minipigs; volume rendering; ventrodorsal view.

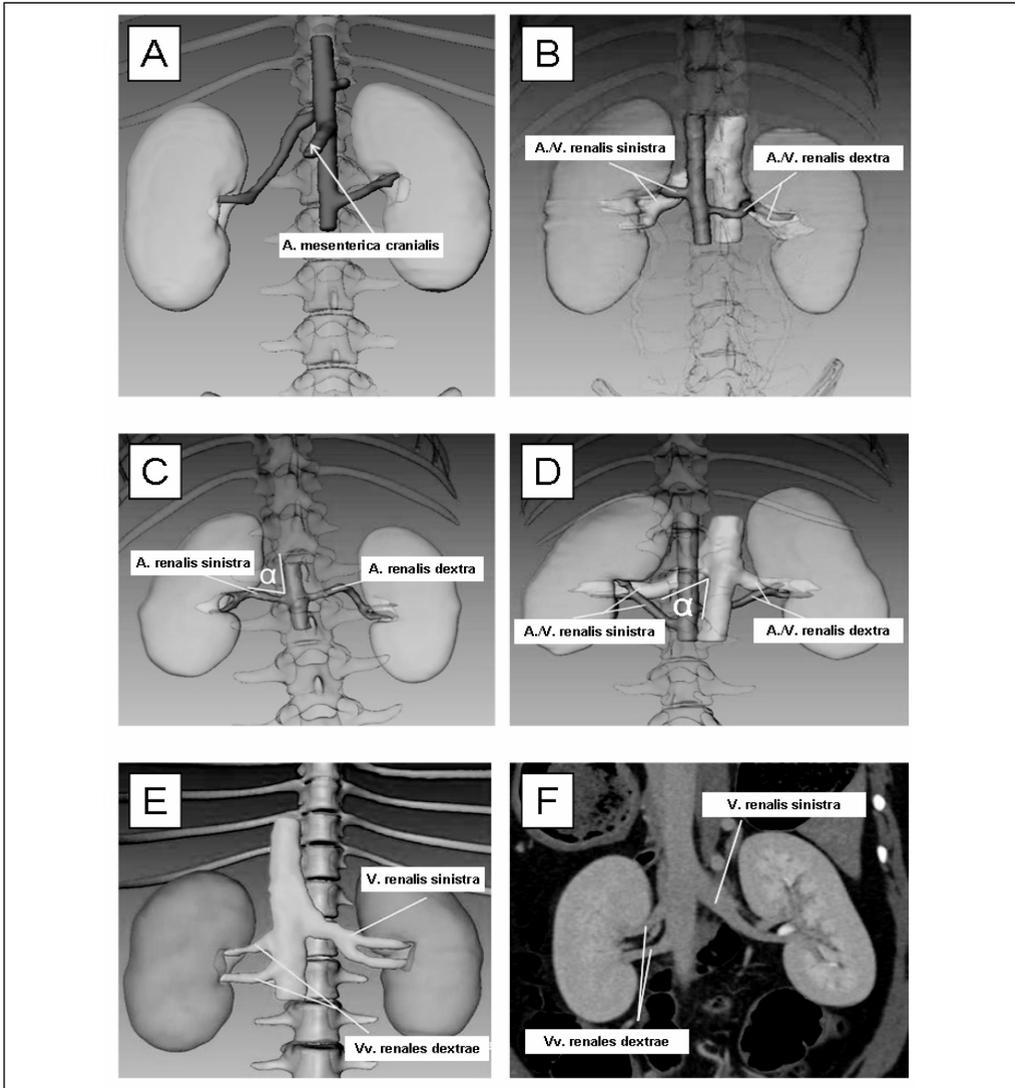


Figure 2: Variants in the course and macromorphological structure of the vasculature in the abdomen (arteries in dark gray, veins in light gray, α angle between the renal arteries resp. veins and the abdominal aorta resp. Vena cava) of pre-adult Göttingen™ minipigs; (A) caudally shifted origin of the cranial mesenteric artery, (B, C, D) course of renal arteries and veins; (E) medially shifted branching sites of the renal veins, volume rendering (A-E) and (F) contrast enhanced computed tomography; (A, E) ventrodorsal view and (B, C, D) dorsoventral view.

ly on the right kidney an α of $90 \pm 5^\circ$ was found (27.8%, n=5).

In some animals a single vein was split within the regular course only for a short distance into two parts which thereafter merged together and formed the single vein again. This phenomenon was noted in 38.9% (n=7) of the animals at the zone of division of the

caudal vena cava into the left and right common iliac vein (Fig. 3 A, B), and in one animal (5.6%, n=1) also at the linguofacial vein (Fig. 3 C). The vein splitting was accompanied by a reduction of the cross section area of the blood vessel in both venous split segments (V. linguofacialis sinistra -6.5%, V. cava caudalis -24.7%, V. iliaca communis -8.1%).

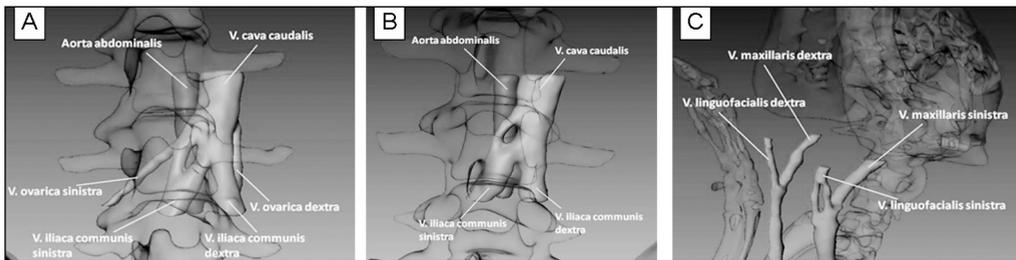


Figure 3: Partial splitting of veins in the pre-adult Göttingen™ minipig; left common iliac vein (A), caudal vena cava (B) and linguofacial vein (C); A and B: ventrodorsal view, C: dorsoventral view; volume rendering.

Discussion

The study could demonstrate variants in the vasculature of pre-adult Göttingen™ minipigs concerning the linguofacial vein, the subclavian vein, the caudal vena cava, the common iliac vein, the cranial mesenteric artery and the renal arteries and veins. The variants were analysed by CECT which is widely accepted for diagnosis of variants in the vasculature [17-22]. The sensitivity of the method is 100% for variants of the renal vasculature [23-24].

It was apparent that the gross vascular pattern is unique for each individual, much the same way as a fingerprint is.

In pigs usually the subclavian vein can be found in pairs on both sides of the body, whereas in humans this phenomena is a rare event [25]. The additional veins which were found in a parallel course to the subclavian vein duplicates might be due to a proximal shift of the anastomosis of these veins with the continuing vessel. For example the anastomosis site of the suprascapular vein with the continuing vessel, which is usually formed with the axillary vein, might have shifted more proximally to the brachiocephalic vein. The same might be possible for the anastomosis site of the pre-scapular branch of the superficial cervical vein. Due to the smaller calibre of the additional veins in a parallel course to the subclavian vein interventional approaches like the implantation of catheters [26-27] or of port systems [26, 28] via the subclavian vein might get complicated.

A range of variants was also found in the renal vasculature. In the pig the renal vein is usually singular to each kidney and connects the kidney to the caudal vena cava. Upon entering the kidney the renal vein divides into a cranial branch which receives the blood from the cranial portion of the kidney and a caudal branch which receives blood from the caudal kidney portion. A medial shift of the branching site of the renal vein into separate veins for the caudal and cranial kidney portion as seen in one animal is well known also in humans [29]. Also similar to humans in all animals the left renal vein passed the aorta ventrally [30] in a caudocranial course and formed anastomosis with the vena cava cranially to the contralateral vein.

Additionally comparable to humans the right renal artery passed the vena cava dorsally and the hilus of the left kidney was positioned cranial or almost on the same level as the origin of the renal artery on the aorta [31]. Latter resulted in a caudocranial or rectilinear course of the renal arteries which arise from the aorta caudal of the cranial mesenteric artery and instead of entering the kidney at the hilus they usually pierce the upper or lower part of the organ. In only one animal the origin of the cranial mesenteric artery was caudal of that of a renal artery. This is also known as a rare event in humans [20, 32]. The cranial mesenteric artery develops in the early embryonic phase from the contributions of the 9th, 10th, 11th, 12th, and 13th ventral segmentals and descends then from the site of origin to the final adult position. This caudal “wandering” closely parallels the

descent of the organs supplied by the artery [32] and might have been prolonged in the respective animal.

In almost 40% of the animals a segmental splitting of veins was noted causing vein fenestration. This phenomenon is frequently described for human beings and pigs especially at the fusion site of the iliac veins to the vena cava [33-37]. The fenestrations are supposed to be determined already in the embryonic phase when the origins, the shape and the course of blood vessels are fixed by the assembly of the mesodermal coat [38] and they are thought to be abnormalities caused by inhibited maturation processes [32, 36]. The early blood vessels form a capillary plexus in the yolk sac. When blood first flows through the capillary plexus, the network is rapidly remodelled by hemodynamic changes into a functional circulatory system composed of arteries, veins and capillaries [35, 39]. In the venous system the hemodynamic changes are not as intense as in the arterial system. It is assumed that for this reason blood vessel fenestration can be found much more often in veins than in the arteries [35]. The segmental vein splitting resulted in two parallel veins with a calibre much smaller than that of the non-splitting vein. This decrease of the vessel calibre can complicate surgical interventions like the intravenous implantation of catheter systems via the iliac or subclavian vein [40].

It was conspicuous that the vasculature variants were found only in singular animals and were not accompanied by clinical signs. The variants are not regarded as pathological events but may complicate interventional and surgical approaches [21, 41-43]. Because in one pig many of the vasculature variants were found in cumulation (medial shift of the renal vein division site, mesenteric artery arising from the abdominal aorta between the origins of the left and right renal arteries, and vein fenestration), a genetic background for these variants might be possible. However, the results demonstrated that the vasculature of the Göttingen™ minipig can exhibit variants which have to be kept in mind when us-

ing this animal model in biomedical approaches.

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