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Cardiac defects, nuchal edema and abnormal lymphatic development are not associated with morphological changes in the ductus venosus

Nicole B. Burger [a,*], Monique C. Haak [b], Evelien Kok [c], Christianne J.M. de Groot [a], Weinian Shou [d], Peter J. Scambler [e], Youngsook Lee [f], Eunjin Cho [f], Vincent M. Christoffels [c], Mireille N. Bekker [g]

[a] Department of Obstetrics and Gynecology, VU University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, the Netherlands
[b] Department of Obstetrics, Leiden University Medical Center, Albinusdreef 2 2333 ZA Leiden, the Netherlands
[c] Department of Anatomy, Embryology & Physiology, Academic Medical Center, Meibergdreef 9 1105 AZ Amsterdam, the Netherlands
[d] Riley Heart Research Center, Herman B Wells Center for Pediatric Research, Division of Pediatric Cardiology, Indiana University School of Medicine, 705 Riley Hospital Dr. Indianapolis, Indiana, USA
[e] Department of Molecular Medicine, University College London, Institute of Child Health, Gower Street, London, WC1E 6BT, United Kingdom
[f] Department of Cell and Regenerative Biology, University of Wisconsin-Madison, 1111 Highland Ave. Madison, Wisconsin, USA
[g] Department of Obstetrics and Gynecology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, the Netherlands

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ABSTRACT

Background: In human fetuses with cardiac defects and increased nuchal translucency, abnormal ductus venosus flow velocity waveforms are observed. It is unknown whether abnormal ductus venosus flow velocity waveforms in fetuses with increased nuchal translucency are a reflection of altered cardiac function or are caused by local morphological alterations in the ductus venosus.
Aim: The aim of this study was to investigate if the observed increased nuchal translucency, cardiac defects and abnormal lymphatic development in the examined mouse models are associated with local changes in ductus venosus morphology.
Study design: Mouse embryos with anomalous lymphatic development and nuchal edema (*Ccbe1*$^{-/-}$ embryos), mouse embryos with cardiac defects and nuchal edema (*Fkbp12*$^{-/-}$, *Tbx1*$^{-/-}$, *Chd7*$^{fl/fl}$;*Mesp1Cre*, *Jarid2*$^{-/-NE+}$ embryos) and mouse embryos with cardiac defects without nuchal edema (*Tbx2*$^{-/-}$, *Fgf10*$^{-/-}$, *Jarid2*$^{-/-NE-}$ embryos) were examined. Embryos were analyzed from embryonic day (E) 11.5 to 15.5 using markers for endothelium, smooth muscle actin, nerve tissue and elastic fibers.
Results: All mutant and wild-type mouse embryos showed similar, positive endothelial and smooth muscle cell expression in the ductus venosus at E11.5–15.5. Nerve marker and elastic fiber expression were not identified in the ductus venosus in all investigated mutant and wild-type embryos. Local morphology and expression of the used markers were similar in the ductus venosus in all examined mutant and wild-type embryos.
Conclusions: Cardiac defects, nuchal edema and abnormal lymphatic development are not associated with morphological changes in the ductus venosus. Ductus venosus flow velocity waveforms most probably reflect intracardiac pressure.

1. Introduction

The ductus venosus is an embryonic shunt located at the level of the liver that connects the umbilical vein and the inferior vena cava [1,2]. The function of the ductus venosus is to transport well-oxygenated blood directly to the heart [1,2]. The narrowest part of the ductus venosus has been suggested to function as an active sphincter to regulate the extent of shunting [1,3,4]. This sphincter mechanism would ensure fetal adaptation to hypoxemia or stress.

The phases of ductus venosus flow velocity waveforms correlate in timing to concurrent phases of the cardiac cycle [5]. Altered ductus venosus flow velocity waveforms may reflect changes in volume and pressure in the cardiac chambers [6]. Ductus venosus flow velocity waveforms are therefore considered to reflect cardiac function [6] and are utilized to assess the fetal hemodynamic performance.

Ultrasound examination of the ductus venosus is increasingly used in daily prenatal obstetrical care [7,8]. Abnormal ductus venosus flow velocity waveforms in the first trimester of pregnancy are related to an increased risk for chromosomal abnormalities, cardiac defects, increased nuchal translucency (NT) and adverse pregnancy outcomes [2,8–16].

* Corresponding author.
E-mail addresses: n.burger@vumc.nl (N.B. Burger), m.c.haak@lumc.nl (M.C. Haak), e.kok0602@gmail.com (E. Kok), cj.degroot@vumc.nl (C.J.M. de Groot), wshou@iu.edu (W. Shou), p.scambler@ucl.ac.uk (P.J. Scambler), youngsooklee@wisc.edu (Y. Lee), echo36@wisc.edu (E. Cho), v.m.christoffels@amc.uva.nl (V.M. Christoffels), m.n.bekker-3@umcutrecht.nl (M.N. Bekker).

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Table 1
Number of mouse embryos examined per embryonic day.

	Embryonic day	Number of mouse embryos
Mouse embryos with a lymphatic defect (mouse model group I)		
Ccbe1$^{+/+}$	15.5	4
Ccbe1$^{-/-}$	15.5	4
Mouse embryos with a cardiac defect with nuchal edema (mouse model group II)		
Tbx1$^{+/+}$	14.5	2
Tbx1$^{-/-}$	14.5	3
Fkbp12$^{+/+}$	11.5–13.5	5
Fkbp12$^{-/-}$	11.5–13.5	5
Jarid2$^{+/+}$	14.0	1
Jarid2$^{-/-NE+}$	14.0–14.5	4
Chd7$^{+/+}$	15.5	2
Chd7$^{-/-}$	15.5	2
Mouse embryos with a cardiac defect without nuchal edema (mouse model group III)		
Tbx2$^{+/+}$	12.5	3
Tbx2$^{-/-}$	12.5	3
Fgf10$^{+/+}$	13.5	2
Fgf10$^{-/-}$	13.5	6
Jarid2$^{+/+}$	14.5	1
Jarid2$^{-/-NE-}$	14.5	5

The causal mechanism of abnormal first-trimester ductus venosus flow velocity waveforms in fetuses with increased NT is unknown.

Cardiac failure has been suggested to explain altered ductal flow velocity waveforms [2,9,10,14]. But abnormal ductal flow velocity waveforms cannot be attributed to a specific cardiac defect that could influence the hemodynamic status [17,18]. Signs of fetal cardiac failure are rarely seen in fetuses with increased NT [19,20] and conflicting evidence on altered intracardiac velocities exists [21,22].

Another theory to clarify abnormal ductus venosus flow velocity waveforms is a local morphological alteration in the ductus venosus. A morphological study of the ductus venosus in embryos with cardiac anomalies and nuchal edema, the morphological equivalent of increased NT, is currently lacking. We tested the hypothesis that nuchal edema, cardiac defects and abnormal lymphatic development are related to local changes in ductus venosus morphology, such as altered endothelial expression or disturbed contributions of smooth muscle cells, and histological construction of the ductus venosus tissue. The morphology of the ductus venosus was examined in three different categories of mutant mouse models; mouse embryos with (i) abnormal lymphatic development and nuchal edema, (ii) cardiac defects with nuchal edema and (iii) cardiac anomalies without nuchal edema.

2. Material and methods

2.1. Embryos

Mouse embryos were analyzed from embryonic day (E) 11.5 to 15.5. These embryonic stages coincide with initial lymphatic developmental processes and the presence of nuchal edema. Cardiovascular development is largely completed at E15.5. These embryonic stages correlate with the timing of the visibility of nuchal edema in human fetuses between 10 and 14 weeks gestational age.

Different knockout and one knockdown mouse models were investigated and compared to wild-type (control) embryos (see Table 1). In the human clinical situation, increased NT is not related to a specific type of cardiac defect, but is associated with a spectrum of cardiac abnormalities. Therefore, multiple different mutant mouse models with lymphatic abnormalities or various cardiac defects with and without the presence of nuchal edema were studied.

First, to examine the ductus venosus in mouse embryos with a lymphatic defect, *Ccbe1*$^{-/-}$ embryos [23] were analyzed. *Ccbe1*$^{-/-}$ embryos display absent lymphatic structures and increased nuchal thickening, as described earlier [23]. Second, to study the ductus venosus in various mouse models with cardiac malformations and nuchal edema, we have analyzed (i) *Tbx1*$^{-/-}$ embryos, showing abnormal development of the cardiac outflow tract, ventricular septal defects and aortic arch anomalies [24,25], (ii) *Jarid2*$^{-/-NE+}$ embryos, displaying ventricular septal defects, non-compaction of the ventricular wall and double outlet right ventricle [26], (iii) *Fkbp12*$^{-/-}$ embryos showing myocardial non-compaction, large ventricular septal defects, hypertrophic trabeculae, deep intertrabecular recesses and thinner left ventricular wall [27] and (iv) *Chd7*$^{fl/fl}$;*Mesp1Cre* embryos, demonstrating ventricular septal defects [28] and a variety of pharyngeal arch artery defects [29]. Third, to investigate the ductus venosus in diverse mutant mouse models with heart anomalies but without the presence of nuchal edema, we have examined (i) *Tbx2*$^{-/-}$ embryos [30], showing enlarged and dilated ventricles, small endocardial cushions and outflow tract septation defects, such as double outlet right ventricle [31], (ii) *Fgf10*$^{-/-}$ embryos, displaying abnormal direction of the ventricular apex and absent pulmonary arteries and veins [32] and (iii) *Jarid2*$^{-/-NE-}$ embryos [26], showing ventricular septal defects, non-compaction of the ventricular wall and double outlet right ventricle [26]. *Jarid2*$^{-/-}$ embryos showed nuchal edema in some embryos and normal nuchal thickness in other

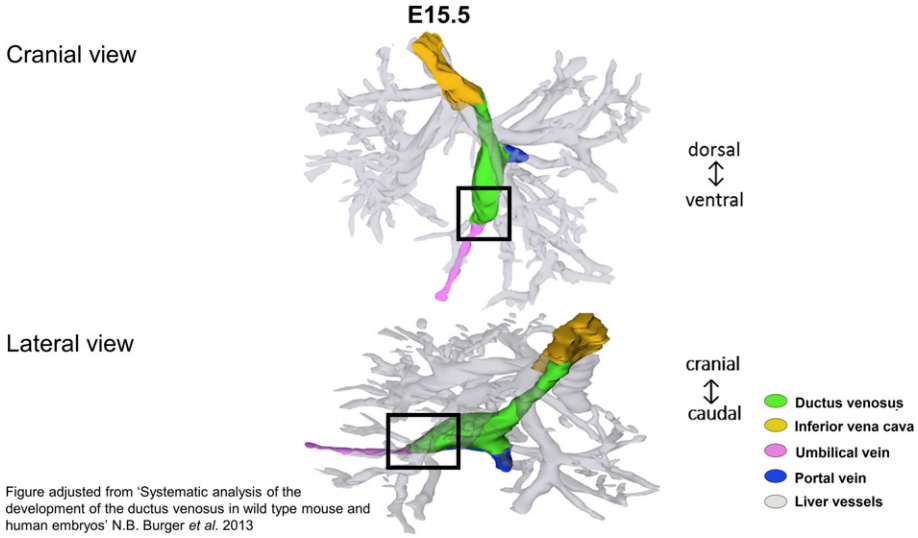

Fig. 1. Three-dimensional view of the ductus venosus and its adjacent vessels. The black box represents the area that is shown in Figs. 2–4.