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The Pronephros; a Fresh Perspective

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Synopsis Contemporary papers and book chapters on nephrology open with the assumption that human kidney development passes through three morphological stages: pronephros, mesonephros, and metanephros. Current knowledge of the human pronephros, however, appears to be based on only a hand full of human specimens. The ongoing use of variations in the definition of a pronephros hampers the interpretation of study results. Because of the increased interest in the anamniote pronephros as a genetic model for kidney organogenesis we aimed to provide an overview of the literature concerning kidney development and to clarify the existence of a pronephros in human embryos. We performed an extensive literature survey regarding vertebrate renal morphology and we investigated histological sections of human embryos between 2 and 8 weeks of development. To facilitate better understanding of the literature about kidney development, a referenced glossary with short definitions was composed. The most striking difference between pronephros versus meso- and metanephros is found in nephron architecture. The pronephros consists exclusively of non-integrated nephrons with external glomeruli, whereas meso- and metanephros are composed of integrated nephrons with internal glomeruli. Animals whose embryos have comparatively little yolk at their disposal and hence have a free-swimming larval stage do develop a pronephros that is dedicated to survival in aquatic environments. Species in which embryos do not have a free-swimming larval stage have embryos that are supplied with a large amount of yolk or that develop within the body of the parent. In those species the pronephros is usually absent, incompletely developed, and apparently functionless. Non-integrated nephrons were not identified in histological sections of human embryos. Therefore, we conclude that a true pronephros is not detectable in human embryos although the most cranial part of the amniote excretory organ is often confusingly referred to as pronephros. The term pronephros should be avoided in amniotes unless all elements for a functional pronephros are undeniably present.

Introduction

A kidney-related article or book chapter commonly starts with: "Human kidney development follows three separate stages: pronephros, mesonephros, and metanephros (Fig. 1A)" (Prentiss and Arey 1917; Bailey and Miller 1921; McCrory 1974; Patten and Carlson 1974; Tuchmann-Duplessis and Haegel 1974; Moore 1988; Vize et al. 1997; Kuure et al. 2000; Cochard 2002; Pole et al. 2002; Hiruma and Nakamura 2003; Ryffel 2003; Nishinakamura 2003; Sadler 2004; Solhaug et al. 2004; Carev et al. 2006; Raciti et al. 2008; Michos 2009; Wessely and Tran 2011; Gerlach and Wingert 2013; Marra and Wingert 2014; Upadhyay and Silverstein 2014; Xing et al. 2014; Hohenstein et al. 2015; Wang and Li 2015). Is this actually true? How sure are we that human embryos pass through a pronephric phase? Doubt on the existence of this structure might be inferred from its vague connotation as "transient," "vestigial" (Goodrich 1930), "nonfunctional," or "aglomerular" (Goodrich 1930; Fraser 1950; Hamilton et al. 1972; Solhaug et al. 2004). Until the 1950s the pronephros, referred to as the first and most primitive embryonic kidney, was actively studied in various species and it recently regained attention because of the establishment of zebrafish and *Xenopus laevis* as vertebrate models to study human urogenital development. These species display a transient but functional pronephros at some stage of their embryonic development (Vize et al. 1997; Kuure et al. 2000; Drummond 2005; Jones 2005; Raciti et al. 2008; Wessely and Tran 2011).

In three to six out of 1000 human live births, the renal system is affected (Schulman et al. 1993;

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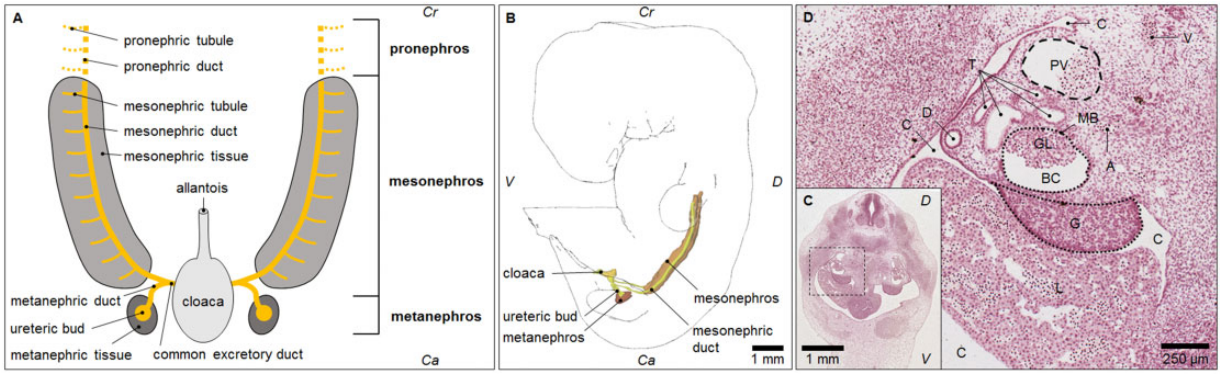

Fig. 1 Organization of kidney development. **A**) Diagram illustrating the three sets of excretory structures as supposed to be present in a human embryo during the fifth week (about 32 days, Carnegie stage 14) (after Moore 1988). Cr, cranial; Ca, caudal. **B**) Reconstruction of the urogenital system of a stage 16 human embryo specimen 6517 (37–42 days), including meso- and metanephros and their ducts (de Bakker et al. 2016). Cr, cranial; Ca, caudal; D, dorsal; V, ventral. **C**) Section through the mesonephric region of a stage 17 human embryo specimen 6521 (42–44 days). D, dorsal; V, ventral. **D**) Enlarged part of the section in C. A, arteriole; BC, Bowman's capsule; C, coelom; D, mesonephric duct; G, gonad; GL, glomerulus; L, liver; MB, Malpighian body; PV, postcardinal vein; T, mesonephric tubules; V, vein.

Sanna-Cherchi et al. 2007). As a possible cause of renal agenesis, Wallace and McCrory suggested that when the pronephros or mesonephros fails to form, the mesonephric duct, ureteric bud, or ureter will be absent (McCrory 1974). Therefore, the pronephros as a model to study human kidney development and disease received increasing interest of researchers (Raciti et al. 2008) and Wessely and Tran (2011) rightly stated in 2011 that "the golden age of pronephros development may just have begun." Because molecular mechanisms in nephron development tend to be similar in all three types of kidneys, a study of the pronephros in species with easy experimental access may indeed be very useful. However, literature on the morphology and development of the renal system in chordates is confusing and the results remain inconclusive. The objective of this study was to clarify the existence of a pronephros in the various vertebrate taxa, especially in humans, by means of a literature review and by exploring histological sections of human embryos between Carnegie Stage 9 and 23 (19–60 days of development).

Background

The pronephros; prone to confusion and inconclusiveness

Since Johannes Müller first discovered the pronephros and its associated excretory duct in frogs in 1829 (Müller 1829, 1830; Balfour and Sedgwick 1878; Vize et al. 1997), and Bidder (1846) identified the glomus (i.e., a large external glomerulus that forms over two to three body segments) as its vascular component in 1846 (Vize et al. 1997; Raciti et al. 2008), many histological studies were performed in a range of species: Amphioxus (Prentiss and Arey 1917), primitive jawless fish (Prentiss and Arey 1917), cartilaginous fish (Kerr 1919; Goodrich 1930; Fraser 1950; Vize et al. 2003; Chimenti and Accordi 2011), bony fish (Prentiss and Arey 1917; Szebenyi 1977; Gilbert 2010; Hiruma and Nakamura 2003; Nishinakamura 2003; Raciti et al. 2008; Chimenti and Accordi 2011), amphibians (Sedgwick 1881; Rabl 1908; Prentiss and Arey 1917; Goodrich 1930; Fraser 1950; Vize et al. 1997, 2003; Wrobel and Suss 2000; Hiruma and Nakamura 2003; Nishinakamura 2003; Raciti et al. 2008; Gilbert 2010; Chimenti and Accordi 2011), reptiles (Goodrich 1930; Fraser 1950; Vize et al. 1997, 2003; Chimenti and Accordi 2011), birds (Balfour and Sedgwick 1878, 1879; Sedgwick 1880, 1881; Gasser 1879; Davies 1950; Goodrich 1930; Vize et al. 1997; Hiruma and Nakamura 2003), and mammals (Goodrich 1930; Vize et al. 1997, 2003). Although the presence of a pronephros in human embryos was already questioned by Fraser in 1950 (Fraser 1950), it remains unsettled whether amniotes, mammals, or humans actually do possess a pronephros in the embryonic stage. This is mainly due to confusing terminology and definitions. For example, the nephrocoel (Kerr 1919; Goodrich 1930), a fluid filled cavity in which the external glomerulus or glomus of the pronephros protrudes, was also referred to as pronephric cavity (Vize et al. 1997), glomerular space (Vize et al. 1997), pronephric chamber (Goodrich 1930; Huettner 1968), nephric chamber (Fraser 1950), or coelomic chamber (Fraser 1950; Davies 1951), depending on the source, era, and background of the author. Even more confusing is

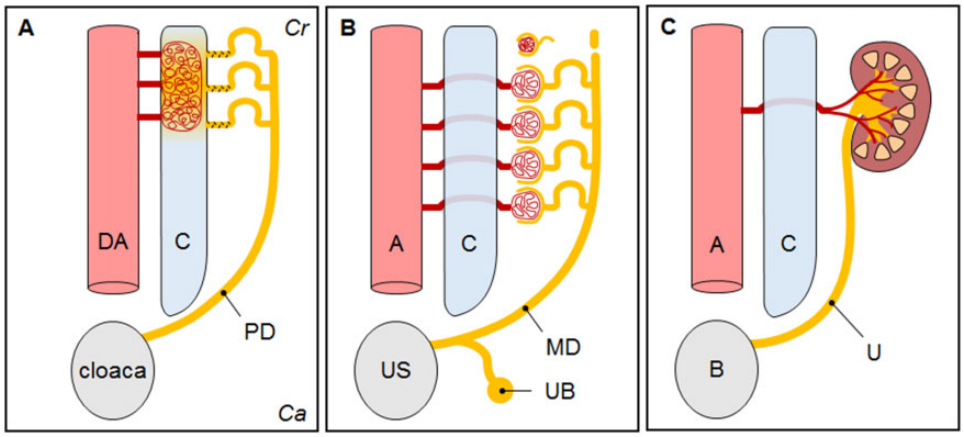

Fig. 2 Schematic drawings of pro-, meso-, and metanephros. **A**) Schematic drawing of the *non-integrated nephron* of a pronephros in frogs. The glomus, a single glomerular unit that is formed over two to three body segments and later becomes more compact, is supplied by arterial sprouts from the dorsal aorta and filters wastes directly in the fluid of the coelomic cavity (Gerth et al. 2005). The glomus is not integrated in the tubules but it lies in the immediate vicinity of them, so there is almost no space between the glomus and nephrostomes. These ciliated nephrostomes collect the coelomic fluid and the primitive urine will be collected through the pronephric tubules via the pronephric duct into the cloaca. See also Fig. 3A. **B**) Schematic drawing of the *integrated nephrons* of a mesonephros. The glomeruli are supplied by arterial sprouts from the aorta that pass behind the coelom and filter wastes into Bowman's cavities. The glomeruli are therefore integrated in the tubules. The collected wastes are transported through the mesonephric tubules toward the mesonephric duct and collected in the urogenital sinus. See also Fig. 3B. The ureteric bud which initiates metanephric development and will become the ureter is an outgrowth of the mesonephric duct. The cranial-most metanephric nephrons degenerate over time. Note that these degenerated nephrons have often been wrongly described as pronephric. **C**) Schematic drawing of a metanephros and its supplying renal artery are situated retro-peritoneal, so dorsal of the coelom. The metanephric nephron with its Bowman's space and extensive tubule is firmly embedded within the renal cortex and renal pyramids (lighter triangles). Urine is collected in the renal pelvis and transported through the ureter toward the bladder. A, aorta; B, bladder; C, coelom; Ca, caudal; Cr, cranial; DA, dorsal aorta; PD, pronephric duct; US, urogenital sinus; U, ureter; UB, ureteric bud; MD, mesonephric duct.

the fact that sometimes one term is used for two different structures. The nephrocoel has also incorrectly been named nephrotome (Fraser 1950; Davies 1951), but nephrotomes are in fact the mesodermal segments that form the precursors of individual pronephric branches. A referenced glossary with short definitions was composed to facilitate better understanding of the literature concerning kidney development (Supplementary data: Kidney development glossary).

Kidney architecture

The basic architecture of a nephron shows that it is one of the best evolutionary conserved structures in the vertebrate kingdom (Fox 1963) and to a certain extent also in several invertebrate clades (Ruppert 1994). Despite the anatomical differences (Figs. 2 and 3) and functions between the three kidney types, the nephron is more or less present in all of them (Wessely and Tran 2011). Each nephron is composed of three components; an initial filtering component (a more or less developed glomerulus), a waste collecting unit (coelom/nephrocoel/Bowman's capsule/Bowman's space), and a nephric tubule specialized for secretion of wastes and reabsorption of solutes and water (Fraser 1950; Sanna-Cherchi et al. 2007). Although each kidney stage differs in overall organization and complexity, they all have the nephron as their basic structural and functional unit (Raciti et al. 2008). Gérard and Cordier (1934a,b) divided nephrons into two types, whether they are in open communication with the coelom (the non-integrated nephron, Fig. 3A), or separated from the coelom (the integrated nephron, Fig. 3B) (Dawson 1925; Lambert 1933; Gérard and Cordier 1934a, 1934b; Fraser 1950). For further details on the terminology used in describing kidney development, see the enclosed glossary (Supplementary data: Kidney development glossary).

Definition of a pronephros

The word "pronephros" is derived from Greek and means "before kidney" (Larsen 1993): the first and most primitive kidney (Hall 1904; Fox 1963). The pronephros develops from mesenchymal buds of *pronephric primordia*, or *nephrotomes* (Vize et al. 1997; Sadler 2004) at the most cranial part of the mesodermal nephrogenic cord (Mathews 1976; Vize et al. 1997; Cochard 2002; Chimenti and Accordi 2011). These buds of pronephric primordia hollow out to

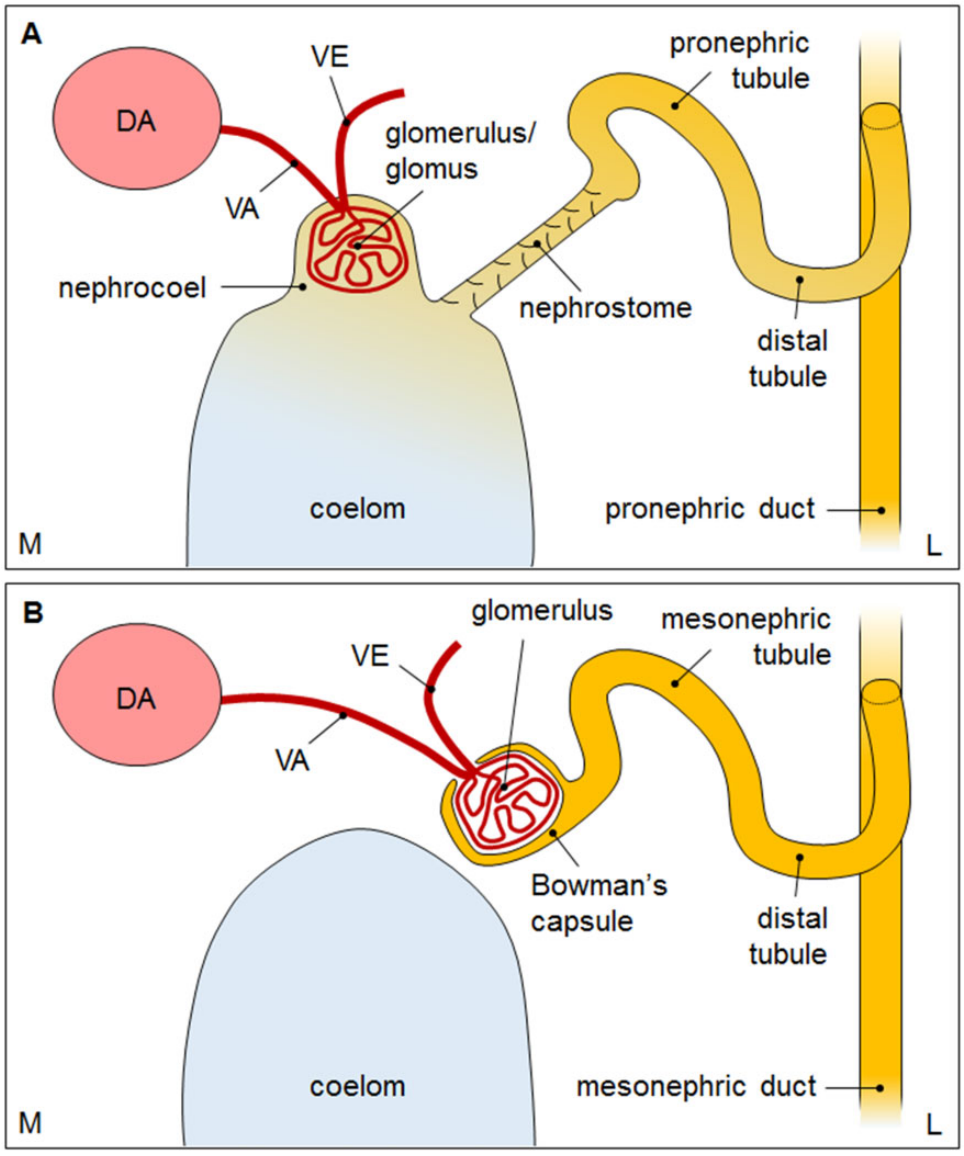

Fig. 3 Detailed architecture of a pronephric and a mesonephric nephron. **A**) Pronephric anatomy: the non-integrated nephron. A typical pronephric nephron, as can be found in amphibian larvae and some teleosts (Fraser 1950), consists of the following functional units; the coelom/nephrocoel with an external glomerulus or glomus, from which a ciliated nephrostome leads into the pronephric tubule that lastly drains into the pronephric duct (Fraser 1920, 1950; Dawson 1925; Lambert 1933; Davies 1950; Nieuwkoop and Faber 1994; Vize et al. 1997; Brandli 1999; Nishinakamura 2003; Raciti et al. 2008; Chimenti and Accordi 2011; Cho et al. 2011; Wessely and Tran 2011). The glomerulus or glomus is not integrated in the tubule. **B**) Mesonephric anatomy: the integrated nephron. The mesonephric tubules develop a Bowman's capsule that encloses a vascularized internal glomerulus supplied by branches of the dorsal aorta (McCrory 1974). So the glomerulus is integrated in the mesonephric tubule. Bowman's capsule, together with the internal glomerulus, constitutes a Malpighian body (Fraser 1950). The Malpighian body is regarded as a typical feature of the mesonephros (Wrobel and Suss 2000). The mesonephros differs from the pronephros by the absence of external glomeruli (Davies 1950, 1951; Nelson 1953; Hamilton et al. 1972; Vize et al. 1997; Wrobel and Suss 2000; Chimenti and Accordi 2011). The mesonephros is formed in all vertebrates, but while it degenerates and relinquishes its function to the metanephros in more advanced vertebrates, it serves as the adult kidney in fish and amphibians (Wessely and Tran 2011). DA, dorsal aorta; L, lateral; M, medial; VA, vas afferens; VE, vas efferens.

form *pronephric tubules* (McCrory 1974; Mathews 1976).

A typical pronephric nephron, as can be found in amphibian larvae and some adult teleosts (Fraser 1950; Hamilton et al. 1972), consists of the following functional units; an *external glomerulus* or *glomus* as vascular component that filters wastes into the *coelom* or *nephrocoel* as waste-collecting unit, from which a ciliated *nephrostome* leads to the *pronephric tubule* that drains into the *pronephric*

duct (Figs. 2A and 3A) (Brandli 1999; Chimenti and Accordi 2011; Cho et al. 2011; Fraser 1920, 1950; Dawson 1925; Lambert 1933; Davies 1950; Nieuwkoop and Faber 1994; Vize et al. 1997, 2003; Nishinakamura 2003; Raciti et al. 2008; Wessely and Tran 2011). Vize et al. (1997) referred to a filtering vascular structure that is one body segment in length as a *glomerulus*, while a vascular structure that extends over multiple body segments is referred to as a *glomus* (Pole et al. 2002). The pronephros proper secretes its filtered wastes from the glomerulus or glomus directly into the *coelom* (Fig. 4A) whereas an anatomically more advanced pronephros filters its waste into a *nephrocoel*, a fluid filled cavity contiguous with the *coelom* into which the *external glomerulus* or *glomus* of the pronephros protrudes (Fig. 4B) (Goodrich 1930; Fraser 1950; Huettner 1968; Vize et al. 1997; Chimenti and Accordi 2011). Although the glomus hangs freely in the coelom, it is intimately associated with the ciliated nephrostomes that transport the coelomic fluid toward the pronephric tubule (Fig. 2B). In contrast, more advanced mesonephric and metanephric nephrons encompass a *Bowman's capsule* or *Bowman's space*, respectively, as waste-collecting unit, which are integrated in the tubule and are therefore called *integrated nephrons* (Davies 1950; Fraser 1950; Vize et al. 1997). Bowman's capsule, together with its internal glomerulus, constitutes a Malpighian body (Fraser 1950) which is regarded as a typical feature of the mesonephros (Wrobel and Suss 2000). The waste-collecting unit of the pronephros on the other hand is *not* integrated in the tubule and is, therefore, referred to as *non-integrated nephron* (Figs. 2B and 3B) (Dawson 1925; Lambert 1933; Davies 1950; Fraser 1950; Vize et al. 1997).

The pronephros is a relatively large excretory organ in early chordates, such as jawless fish (Prentiss and Arey 1917; Vize et al. 2003), teleosts (Vize et al. 1997, 2003), lungfish (Goodrich 1930; Fraser 1950; Vize et al. 2003), and amphibians (Sedgwick 1881; Rabl 1896; Prentiss and Arey 1917; Goodrich 1930; Fraser 1950; Vize et al. 1997, 2003; Wrobel and Suss 2000; Hiruma and Nakamura 2003; Nishinakamura 2003; Raciti et al. 2008; Gilbert 2010; Chimenti and Accordi 2011). In amphibians, the pronephros functions mainly during their larval stage in an aquatic environment (Fraser 1950; Gaeth et al. 1999; Vize et al. 2003; Chimenti and Accordi 2011). Presence of a pronephros has also been reported in some reptiles (Vize et al. 2003) and birds (Balfour and Sedgwick 1878, 1879; Gasser 1879; Sedgwick 1880, 1881; Davies 1950) like the green sea turtle (Wiedersheim 1890; Davies 1950), crocodilians (Wiedersheim 1890; Davies 1950; Vize et al. 2003), chicken (Davies 1950; Kerr 1919; Sedgwick 1880; 1881; Szebenyi 1977; Vize et al. 2003), and duck (Sedgwick 1880; Mihalkovics 1885; Davies 1950) and a pronephros is commonly assumed to be present in mammalian embryos including humans (Felix 1912; Prentiss and Arey 1917; Fraser 1920; Bailey and Miller 1921; Hoadley 1926; Keith 1933; Abdel-Malek 1950; Hamilton 1952; Torrey 1954; Hamilton et al. 1972; McCrory 1974; Tuchmann-Duplessis and Haegel 1974; Gasser 1975; Moore 1988; Kuure et al. 2000; Hiruma and Nakamura 2003; Sadler 2004, 2015; Solhaug et al. 2004; Carev et al. 2006; Gilbert 2010; Cochard 2012). In a few species, such as the sea lamprey (Prentiss and Arey 1917), lancelet (Prentiss and Arey 1917), hagfish (Prentiss and Arey 1917), lungfish (Prentiss and Arey 1917), and some teleosts like Fierasfer (Goodrich 1930), Zoarces (Goodrich 1930), and Lepadogaster (Guitel 1906), the pronephros remains functional into adulthood.

Pronephros in human embryos?

Existence of a pronephros has often been claimed in human embryos (Felix 1912; Prentiss and Arey 1917; Fraser 1920; Bailey and Miller 1921; Hoadley 1926; Keith 1933; Abdel-Malek 1950; Hamilton 1952; Torrey 1954; Hamilton et al. 1972; McCrory 1974; Tuchmann-Duplessis and Haegel 1974; Gasser 1975; Moore 1988; Kuure et al. 2000; Hiruma and Nakamura 2003; Sadler 2004, 2015; Solhaug et al. 2004; Carev et al. 2006; Gilbert 2010; Cochard 2012) and nowadays still many kidney-related articles or book chapters open with the assumption that human kidney development passes through all three kidney stages. In an era in which study designs were based on the theory that ontogeny recapitulates phylogeny (Smith 1953; Huettner 1968; Hiruma and Nakamura 2003; Solhaug et al. 2004), it could be condoned that the findings of studies on fish and amphibians were projected onto the early stages of human development. According to this refuted theory, the most cranial region of the human mesonephros might have been named "pronephric" (Davies 1950; Fraser 1950). Note also that research on human embryos has always been hampered by their scarcity. Therefore, recent literature is almost always directly or indirectly referring to the extensive study of the human pronephros by Felix in 1912 (Felix 1912). Since 1912, not many researchers specifically studied the human pronephros. Most textbooks are referring to Lauri Saxen's "Organogenesis of the Kidney" (1987). In the corresponding chapter

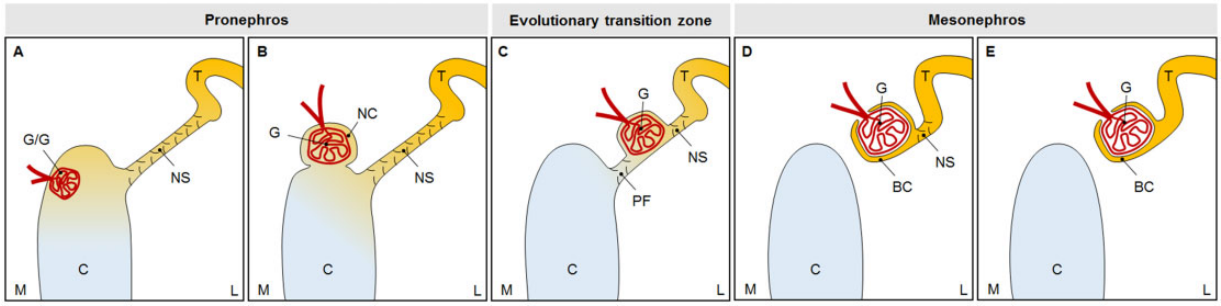

Fig. 4 Different manifestations of pronephric and mesonephric nephrons. The *non-integrated nephrons* in **A** and **B** are purely pronephric as the external glomerulus or glomus hangs freely in the coelom (**A**) or nephrocoel (**B**). The *integrated nephrons* in **D** and **E** are typical mesonephric because the connection with the coelom is lost and the internal glomerulus is enclosed by Bowman's capule. The nephron in **C**, which includes both a peritoneal funnel (PF) and a nephrostome (NS), has been regarded by Vize as mesonephric (Vize et al. 2003). However, to avoid ambiguity we suggest to refer to these nephrons as *intermediate nephrons*. The region in which these nephrons are found has been called the *evolutionary transition zone* (ETZ) by multiple authors (Fraser 1920; Davies 1950; Sedgwick 1881; Renson 1883; Mihalkovics 1885; Hiruma and Nakamura 2003), since the glomerulus is still in contact with the coelom (a typical pronephric feature) and the PF has been regarded both pronephric (Fraser 1950; Davies 1951; Hamilton et al. 1972) as mesonephric (Vize et al. 2003). This intermediate type of nephron might actually represent the gradual evolutionary change from pronephros to mesonephros (Wiedersheim 1890; Davies 1950; Hiruma and Nakamura 2003). C, coelom; G, glomerulus; G/G, glomerulus/glomus; L, lateral; M, medial; NS, ciliated nephrostome, which links the coelom or nephrocoel with the proximal tubule; PF, ciliated peritoneal funnel, which links the coelom to the encapsulated glomerulus (primitive Bowman's capsule); T, tubule.

the author quotes another kidney scientist, Torrey, as his prime source for information on the pronephros, but it turns out that Torrey did not claim at all that human embryos have a pronephros (Torrey 1954; O'Rahilly and Müller 1987). As it appears, the current knowledge of the human pronephros is very limited, since it is based on only a hand full of observations. Already in 2004, Solhaug et al. (2004) stressed the need for studies in human samples. Therefore, we decided to investigate the development of the nephric system in the specimens of human embryos that were available to us.

Materials and methods

Specimens

Images of serial histological sections of 43 human embryos from Carnegie stage 8 (17–19 days) till 23 (56–60 days) from the Carnegie Collection in Silver Spring, MD, USA, were used to study kidney development. Details concerning the used specimens can be found in Table 1 (Streeter 1942, 1945, 1948, 1949, 1951; O'Rahilly and Müller 1987; Lockett 2001; Morgan 2009a; Gasser et al. 2014). The pronephros is said to develop in the third week of human embryonic development and to disintegrate at the end of the fourth week (Felix 1912; Hamilton et al. 1972; McCrory 1974; Tuchmann-Duplessis and Haegel 1974; Gasser 1975; Moore 1988; Kuure et al. 2000; Sadler 2004; Solhaug et al. 2004; Carev et al. 2006). Therefore, more specimens of Carnegie stage 8 (17–19 days), stage 9 (19–21 days), and stage 10 (21–23 days) were incorporated in this study (Table 1). From stage 11 (23–26 days) onward, two specimens per stage were studied. Image acquisition and alignment of the images was done as previously described (de Bakker et al. 2012, 2016).

Research method

Histological sections of human embryos from Carnegie stages 8 (17–19 days) till 23 (56–60 days) were inspected with a focus on the intermediate mesoderm or nephrogenic cord (Hamilton et al. 1972; Tuchmann-Duplessis and Haegel 1974; O'Rahilly and Müller 1987; Sadler 2004), which is the region between the somites (i.e., paraxial mesoderm) and the lateral plate mesoderm from which the urogenital tract develops. Following the previously formulated definition (Fraser 1920, 1950; Dawson 1925; Lambert 1933; Davies 1950; Nieuwkoop and Faber 1994; Vize et al. 1997, 2003; Brandli 1999; Nishinakamura 2003; Raciti et al. 2008; Chimenti and Accordi 2011; Cho et al. 2011; Wessely and Tran 2011), pronephric nephrons were distinguished from mesonephric nephrons by the exclusive presence of external glomeruli in the former (Figs. 2 and 3). All readers are encouraged to study the histological sections of all studied stages by downloading them from our website, http://www.3datlasofhumanembryology.com.

Interactive 3D-PDF

To better understand human kidney development, an interactive 3D portable document format (pdf)

Table 1 Overview of the studied human specimens

CS	Specimen #	Origin[a]	Year	Aquired through	CRL (mm)	Day	Sex	Fixation medium	Staining	P[b]	Z-res (μm)
8	5960	CC	1929	Hysterectomy	1.52	18		Kaiserling	Al. Coch. & eosin	o	5.00
8	7545	CC	1938	No information	Unknown			Formol	Hematoxylin and Eosin	t	6.00
8	7568	CC	1938	No information	Unknown			Formol	Al. Coch.	t	10.00
8	7972	CC	1942	No information	Unknown			Alcohol & Bouin	Hematoxylin and Eosin	s	6.00
8	8671	CC	1949	Hysterectomy	0.61			Alcohol & Bouin	Hematoxylin and Eosin	t	2.70
8	10157	CC	1967	Hysterectomy	1.16	23		Formol	Cason	t	5.34
9	1878	CC	1907	No information	1.38			Formol	Hematoxylin and Eosin	t	10.00
9	3709	CC	1921	No information	1.38	25		Formol	Erythrosin	t	9.08
9	5080	CC	1926	No information	1.50			Formol	Al. Coch.	t	10.00
9	H712	BC	1957	Hysterectomy	1.57			Formalin and Bouin	Hematoxylin and Eosin	t	3.59
9	N509	HDBR	2011	Abortion	2.40			Paraformaldehyde	Hematoxylin and Eosin	t	5.00
10	0391	CC	1907	No information	2.00			Formol	Al. Coch	t	10.00
10	3707	CC	1921	No information	1.50			Formol	I.H.	o	12.50
10	3710	CC	1921	No information	3.60			Formol	H. & or. G.	o	10.00
10	4216	CC	1923	No information	2.00			Formol	Unknown	o	15.00
10	5074	CC	1925	Abortion (EUG)	1.41			Bouin	Alum cochineal (i.e., carmine)	t	4.69
10	6330	CC	1931	No information	1.95	28		Formol	Ehrlich's acid hematoxylin	t	11.63
11	6344	CC	1931	Hysterectomy	2.58	29		Formalin	Alum cochineal (i.e., carmine)	t	18.83
11	6784	CC	1933	Hysterectomy	2.46			Formol	Iron Hematoxylin	t	8.70
12	8505A	CC	1947	Miscarriage	2.86			Formol	Hematoxylin and Phloxin	t	10.32
12	8943	CC	1934	Hysterectomy	3.58			Zenker's Formol	Hematoxylin and Eosin	t	8.22
13	836	CC	1914	Hysterectomy	4.09	32		Mercuric Chlorine	Alum cochineal (i.e., carmine)	t	16.55
13	5541	CC	1927	Miscarriage	4.08	38		Formol	Alum cochineal, eosin	t	10.76
14	6502	CC	1931	No information	5.54			Could be Souza	Hematoxylin and Eosin	t	5.01
14	8314	CC	1945	Hysterectomy	5.16	22		Formol	Azan	t	8.07
15	721	CC	1913	No information	4.79	36		Zenker's Formol	Hematoxylin and Eosin	t	8.69
15	3512	CC	1921	Miscarriage	6.55			Formol	Alum cochineal (i.e., carmine)	t	10.06
16	6517	CC	1931	No information	10.46	39		Corrosive Acetic Acid	Alum cochineal (i.e., carmine)	t	19.13
16	8773	CC	1950	Therapeutic abortion	6.74	39		Bouin	Azan	c	10.73
17	6520	CC	1932	No information	12.21	41		Corrosive Acetic Acid	Alum cochineal (i.e., carmine)	t	17.86

(continued)

Table 1 Continued

CS	Specimen #	Origin[a]	Year	Aquired through	CRL (mm)	Day	Sex	Fixation medium	Staining	P[b]	Z-res (μm)
17	6521	CC	1933	No information	10.60			Corrosive Acetic Acid	Alum cochineal (i.e., carmine)	t	10.01
18	4430	CC	1923	No information	15.85		F	Corrosive Acetic Acid	Alum cochineal (i.e., carmine)	t	37.19
18	6524	CC	1933	No information	9.73			Corrosive Acetic Acid	Aluminum Cochineal	t	10.18
19	2114	CC	1918	Hysterectomy	12.59		F	Formalin	Aluminum Cochineal	t	40.75
19	8965	CC	1952	Abortion (EUG)	17.72			Zenker's Formol	Borax Carmine—Orange G	t	60.69
20	462	CC	1910	Miscarriage	15.93		M	Formol	Aluminum Cochineal	t	42.36
20	s2025	AMC	~1975	No information	19.77	50.5	M	Bouin	Haematoxylin-azophloxine	t	30.51
21	4090	CC	1922	Abortion (EUG)	19.43		F	Formol	Alum cochineal (i.e., carmine)	t	99.62
21	7254	CC	1936	Hysterectomy	17.36		M	Bouin	Hematoxylin and Eosin	t	60.12
22	895	CC	1914	Hysterectomy	21.22		F	Formol	Aluminum Cochineal	t	50.52
22	H983	BC	1962	No information	28.00		M	Formalin	HE/trichrome/silver	t	53.00
23	950	CC	1914	Miscarriage	23.79		M	Formalin	Aluminum Cochineal	t	42.71
23	9226	CC	1954	Abortion (EUG)	30.01	56	M	Formol	Azan	t	144.28

Note: CS, Carnegie stage; Year, Year of acquisition; CRL, Calculated crown-rump-length in mm; Day, days post ovulation; *Z*-res, calculated *Z*-resolution in μm.

[a]Origin of the specimen: CC=Carnegie collection: Human Developmental Anatomy Center at the National Museum of Health and Medicine in Silver Spring, MD, USA; BC=Boyd collection: Department of Physiology, Development and Neuroscience, University of Cambridge, UK; AMC=Department of Medical Biology, Amsterdam UMC, University of Amsterdam, The Netherlands; CU=Cambridge University, UK; HDBR=Human Developmental Biology Resource, Institute of Genetic Medicine, International Centre for Life, Newcastle Upon Tyne, UK (Streeter 1942, 1945, 1948, 1949,1951; O'Rahilly and Müller 1987; Lockett 2001; Morgan 2009b; Gasser et al. 2014).

[b]Plane of sectioning: o, oblique; t, transversal; c, coronal.

file was created based on six human embryos covering stage 12 (26–30 days) till stage 17 (42–44 days). The creation of these interactive 3D-PDFs has been described by us before (de Bakker et al. 2012, 2016). This 3D-PDF can be viewed in a recent version of Adobe Reader® (X or higher, freeware, http://www.adobe.com) on MS Windows or MacOS systems, with "javascript" and "playing of 3D content" enabled. We designed a navigation panel with a structure tree, including relevant structures besides the kidneys such as the skin, somites, skeleton, urogenital sinus, cloaca, rectum, and allantois to facilitate the study of the developing renal system including its spatial relations with other organs. Combined with options to select each separate structure and the "show," "transparent," and "hide" buttons, the user is able to show any combination of structures from any chosen visual angle and in any chosen zoom. Using these interactive tools, the user can obtain a good understanding of the spatial relations of the developing renal system within the human embryo. To ease the study of kidney development, six preset views were included per embryonic stage in the lower part of each page.

Results

The stage 8 embryos (17–19 days of development) showed only the three undifferentiated germ layers, i.e., endoderm, mesoderm, and ectoderm. In stage 9 (19–21 days) the mesoderm could be differentiated into axial mesoderm (i.e., the notochordal plate),

paraxial mesoderm (i.e., the somites), and the lateral plate mesoderm. In-between these last two the intermediate mesoderm could be identified (Fig. 5A). In stage 10 (21–23 days; Fig. 5B) and stage 11 (23–26 days; Fig. 5C) the intermediate mesoderm stood out more clearly as a mass of undifferentiated mesenchymal cells. The cranial margin of the nephrogenic cord was first identified in the intermediate mesoderm at the level of the 10th somite of stage 12 human embryos (26–30 days; Fig. 5D, G; Supplementary 3D-PDF). The presence of a very primitive Bowman's capsule around a glomerulus, without a connection to the coelom, qualifies these nephrons as mesonephric (Fig. 5G). Structures with pronephric characteristics (i.e., external glomeruli excreting directly into the coelom or nephrocoel) were not seen in embryos of stage 12 nor in embryos of earlier or later stages.

The mesonephros with its mesonephric duct (Figs. 1D and 5D–O) is present from stage 12 onward and remains present during the embryonic phase of development, at least up to 60 days of development. Already in stage 12 or 13, depending on the specimen, the mesonephric duct makes contact with the urogenital sinus (Supplementary 3D-PDF). In stage 12 (26–30 days) the cranial margin of the mesonephros can be found at the level of the seventh cervical, or first thoracic vertebra, and this margin remains at that level until stage 16 (37–42 days). In stage 17 (42–44 days), this cranial margin reaches the fifth thoracic vertebra (Supplementary 3D-PDF).

In human embryos of stage 14 (31–35 days), the metanephros anlage was first recognized as mesenchymal packaging around the ureteric bud and in stage 15 (35–38 days) the metanephros was clearly present. Mesonephros and metanephros were histologically easily distinguishable from each other, based on the complexity of their nephrons. Due to embryonic growth, the mesonephros shrinks relatively in size, compared with the metanephros (de Bakker et al. 2016). At stage 17 already, the metanephros is found at its adult location at the level of the first lumbar vertebra (Supplementary 3D-PDF). The caudal region of the embryo, including the inferior mesenteric artery, continues to grow into caudal direction, giving the erroneous impression that the kidneys migrate upward during development (de Bakker et al. 2016).

Discussion

The aim of the current study was to clarify by using histological sections whether or not a pronephros exists in human embryos. Based on these sections we can conclude that the pronephros is not detectable in human embryos of 3–4 weeks of development, the time frame in which we expected to find the pronephros according to literature, nor in earlier or later stages (Felix 1912; Fraser 1920; Davies 1951; Hamilton et al. 1972; Tuchmann-Duplessis and Haegel 1974; Moore 1988; Kuure et al. 2000; Sadler 2004; Solhaug et al. 2004; Carev et al. 2006; Gilbert 2010).

A summary of the differences between the three kidney forms is given in Table 2 and Figs. 2, 3, and 4. The most striking difference between pronephros and other kidney types is that the pronephros proper consists of *non-integrated nephrons*, whereas the mesonephros and metanephros consist of only *integrated nephrons* (Figs. 2B and 3B) (Dawson 1925; Lambert 1933; Davies 1950; Fraser 1950; Vize et al. 1997).

The pronephros; a matter of definition?

It has proven to be a challenge to provide clear definitions on the developmental aspects of the pronephros. The pronephros can be defined strictly as consisting of non-integrated nephrons, whereas others like Davies (1951) defined it as "the most cranial part of the amniote excretory organ" (Larsen 1993; Pole et al. 2002; Vize et al. 2003). Early German researchers, and above all by Felix (1912), put the notion forward, that the vertebrate excretory system was made up of three sets of organs, the pronephros, the mesonephros, and the metanephros, which were all laid down along the trunk and succeeded one another in time (Fig. 1A). This notion was long ago shown to be merely a hypothesis for which no real proof has ever been found (Fraser 1950). The idea that vertebrates carry three sets of kidneys has first been explained by the existence of a common ancestral kidney, the archinephros, which became differentiated into pro-, meso-, and metanephros, according to the needs of the animal (Balfour and Sedgwick 1876; Renson 1883; Weldon 1883; Wiedersheim 1890; Field 1891; Price 1897, 1904; Brauer 1902; Borcea 1905; Burlend 1913; Kerens 1907; Kerr 1919; Goodrich 1930; Fox 1963). The obsolete terms holonephros (Price 1897; Brauer 1902; Torrey 1954) and mononephros (Audigé 1910) which have also been used in literature to indicate the entire excretory system (Smith 1943, 1953) have added to the confusion.

The exclusive existence of nephrostomes and peritoneal funnels have long been regarded as typical differences between pronephros and mesonephros.

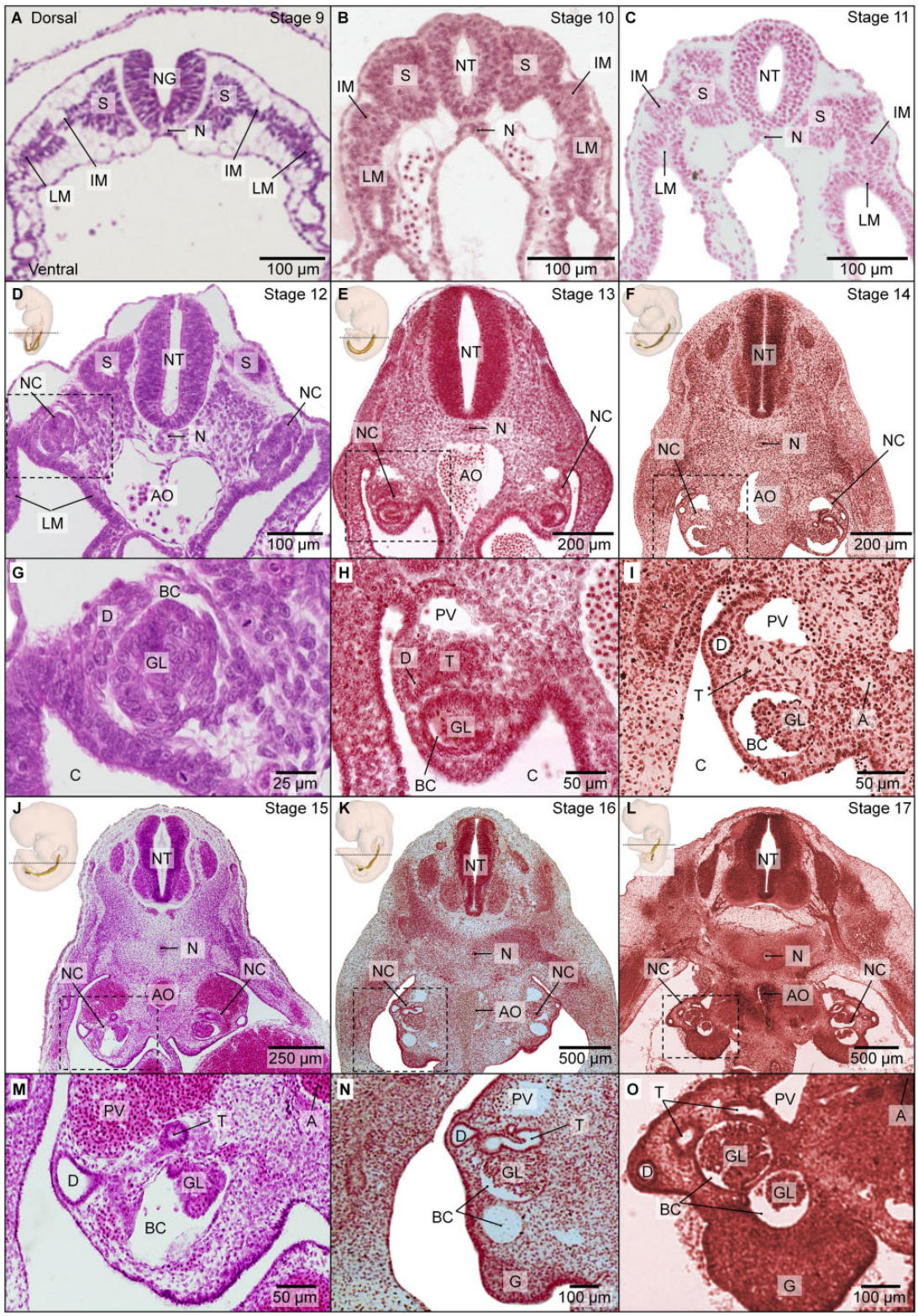

Fig. 5 Histological features of human nephrogenic development. **A**) Transverse section through the caudal region of a stage 9 (26–30 days) human embryo specimen H712. The intermediate mesoderm (IM) is still hard to discern from somite (paraxial mesoderm) and lateral plate mesoderm (LM) **B**) Transverse section through the caudal region of a stage 10 (21–23 days) human embryo specimen 5074. The intermediate mesoderm is recognizable as a clump of undifferentiated mesenchymal cells. **C**) Transverse section through the caudal region of a stage 11 (23–26 days) human embryo specimen 6344. The intermediate mesoderm is still undifferentiated. **D**) Transverse section through the caudal region of a stage 12 (26–30 days) human embryo specimen 8943. The nephrogenic cord (NC) is now present. **E**) Transverse section through the mesonephric region of a stage 13 (28–32 days) human embryo specimen 836.

Table 2 Similarities and differences between the excretory organs

	Pronephros	Evolutionary Transition zone	Mesonephros	Metanephros
Nephron	Non-integrated	Intermediate	Integrated	Integrated
Filtering component	External glomerulus/ glomus/coelomic epithelium (Vize et al. 1997)	Intermediate glomerulus	Internal glomerulus	Definitive internal glomerulus
Waste collecting unit	Coelom/nephrocoel	Primitive Bowman's capsule	Bowman's capsule	Bowman's space
Ciliated peritoneal funnel	Absent	Present	Absent	Absent
Nephrostome	Connected to coelom/ nephrocoel	Absent/connected to Bowman's capsule	Absent/connected to Bowman's capsule	Absent
Architecture	Segmental	Segmental	Segmental	Branched
Complexity	Simple	Intermediate	Intermediate	Advanced
Collecting duct	Pronephric duct	Pro-/mesonephric duct	Mesonephric duct	Ureteric bud
Cellular	Parietal epithelial cells not yet described in the pronephric glomerulus (Wessely and Tran 2011)			Parietal epithelial cells present in Bowman's space (Wessely and Tran 2011). Dedicated cell types like pericytes and mesangial cells to form the filtration barrier (Vize et al. 2003).

However, literature remains inconclusive on which of the two features is typically pronephric or mesonephric, due to returning terminology issues. Peritoneal funnels and nephrostomes are both ciliated tubules, which complicates the distinction between the two (Kerr 1919; Goodrich 1930). A ciliated *nephrostome* links the waste collecting unit [i.e., the coelom (Fig. 4A), nephrocoel (Fig. 4B), or Bowman's capsule (Fig. 4D)] to the proximal nephric tubule (Vize et al. 2003), whereas a narrow ciliated *peritoneal funnel* links the coelom to the encapsulated glomerulus, the precursor of Bowman's capsule (Fig. 4C). The ciliated nephrostome is always present in the non-integrated nephron of a pronephros (Fig. 4A, B) but can also be present in a mesonephros (Fig. 4D). As such, it does not discriminate between the two. The gradually ligating wide connection between coelom and nephrocoel in the pronephros has sometimes even been regarded as a peritoneal funnel, but we recommend not to use this term when there are no cilia present.

The nephron in Fig. 4C, which includes both a peritoneal funnel and a nephrostome, as found in embryos of ruminants such as sheep and cattle, has been identified by Vize in the anterior-most tubules of the mesonephros (Davies 1951; Wintour et al. 1996; Vize et al. 2003). To avoid ambiguity, we suggest to refer to these anterior-most *intermediate nephrons* (i.e., neither non-integrated nor integrated nephrons) as an *evolutionary transition zone* between

Fig. 5 F) Transverse section through the mesonephric region of a stage 14 (31–35 days) human embryo specimen 6502. **G**) Enlarged part of the section in D. A very primitive glomerulus is recognizable, surrounded by a primitive Bowmans capsule. Bowmans capsule is not in contact with the coelom, also not on the adjacent and subsequent sections. The mesonephric duct is not yet lumenized at this stage. **H**) Enlarged part of the section in **E**. The glomerulus and Bowman's capsule still appear in a primitive stage. The mesonephric duct becomes lumenized. **I**) Enlarged part of the section in **F**. The definitive morphology of the mesonephros is recognizable. The glomerulus and Bowman's capsule together constitute the Malpighian body. The mesonephric duct and tubule are well defined and lumenized. At this stage the mesonephros can be assumed to be in function. Between stage 14 and stage 17 (L, O) the histological features of the mesonephros remain constant. **J**) Transverse section through the mesonephric region of a stage 15 (35–38 days) human embryo specimen 721. **K**) Transverse section through the mesonephric region of a stage 16 (37–42 days) human embryo specimen 6517. **L**) Transverse section through the mesonephric region of a stage 17 (42–44 days) human embryo specimen 6520. **M**) Enlarged part of the section in J. **N**) Enlarged part of the section in K. Two Bowman's capsules are present in this section. Also note the clear presence of a gonadal ridge. **O**) Enlarged part of the section in L. Two glomeruli can be appreciated in this section. A, arteriole; AO, aorta; BC, Bowman's capsule; C, coelom; D, mesonephric duct; G, gonadal ridge; GL, glomerulus; IM, intermediate mesoderm; LM, lateral plate mesoderm; N, notochordal plate (CS 9, 10, 11) or notochord (CS 12, 13, 14); NC, nephrogenic cord; NG, neural groove; NT, neural tube; PV, postcardinal vein; S, somite; T, mesonephric tubule. We encourage the readers to study the histological sections of all presented stages. All stacks of sections can be downloaded from http://www.3datlasofhumanembryology.com.

pro- and mesonephros (Sedgwick 1881; Renson 1883; Mihalkovics 1885; Fraser 1920; Davies 1950; Hiruma and Nakamura 2003) because the waste collecting unit is still in contact with the coelom (a typical pronephric feature) and the peritoneal funnel has confusingly been regarded as both pronephric (Goodrich 1930; Fraser 1950; Davies 1951; Hamilton et al. 1972) as well as mesonephric (Vize et al. 2003). Therefore, we do not consider the peritoneal funnel as an exclusive feature of pronephros or mesonephros, but advocate this characteristic to be a reflection of a gradual evolutionary change from pronephros to mesonephros, as Wiedersheim (1890) already postulated in 1890 based on his observations in crocodile and turtle embryos (Davies 1950; Hiruma and Nakamura 2003).

Pronephros in amniotes

In amniote embryos that have no free swimming larval stage and are supplied with a large amount of yolk or develop within the body of the parent (i.e., elasmobranchii, reptilia, aves, and mammalia) the pronephros is usually not present or incompletely developed, and therefore functionless (Sedgwick 1881; Rabl 1896; Fraser 1950). However, functional pronephroi with external glomeruli, ciliated nephrostomes, and three to four well-differentiated tubules have been reported in some reptilia, e.g., turtles and crocodiles (Wiedersheim 1890; Davies 1950; Vize et al. 2003). Nevertheless, since the main observation in reptiles date back to Wiedersheim's work from 1890, it is advisable to reinvestigate these observations following the correct definition of a pronephros.

Based on a range of different definitions, the pronephros has often been described in birds (Balfour and Sedgwick 1876, 1878; Gasser 1879; Sedgwick 1880, 1881; Mihalkovics 1885; Rabl 1896; Kerr 1919; Goodrich 1930; Davies 1950; Huettner 1968; Patten and Carlson 1974; Szebenyi 1977; Vize et al. 1997; Hiruma and Nakamura 2003), but this pronephros is almost never regarded as functional (Sedgwick 1880, 1881; Rabl 1896; Goodrich 1930; Abdel-Malek 1950; Davies 1950; Hamilton 1952; Huettner 1968; Patten and Carlson 1974; Vize et al. 1997; Hiruma and Nakamura 2003). At the utmost, the avian pronephros is considered to have particular minor functions like secreting some wastes into the body cavity (Needham 1931; Waddington 1938; Jacob et al. 1977; Vize et al. 1997), because the tubules are not hollow (Huettner 1968). Nevertheless, detailed descriptions of the external glomeruli in avian pronephros are given by Gasser (1879), Balfour and Sedgwick (1876, 1878), Sedgwick (1880, 1881), and Mihalkovics (1885).

The pronephros in mammals, including human, has most often been considered as vestigial and non-functional (Sedgwick 1880; Rabl 1896; Prentiss and Arey 1917; Goodrich 1930; Fraser 1950; Hamilton et al. 1972; Cochard 2002; Nishinakamura 2003; Vize et al. 2003; Sadler 2004; Solhaug et al. 2004; Gilbert 2010; Chimenti and Accordi 2011), or not present at all (Guitel 1906; Davies 1951). On the other hand, there have also been authors describing a pronephros in embryos, not only of humans, as stated above, but also in mice (Kuure et al. 2000; Nishinakamura 2003; Kobayashi et al. 2007) and other mammals (Fraser 1920; Goodrich 1930; Davies 1951; Vize et al. 1997, 2003; Pole et al. 2002). The most cranial part of the amniote excretory organ is then often confusingly referred to as transient, vestigial (Goodrich 1930), nonfunctional, or aglomerular (Goodrich 1930; Fraser 1950; Hamilton et al. 1972; Solhaug et al. 2004) pronephros (Fraser 1920, 1950; Goodrich 1930; Davies 1951; Larsen 1993). Although in the absence of distinctive morphological characteristics, no real distinction can be made between remnants of an incomplete, vestigial pronephros and the gradually degenerating cranial nephrons of the mesonephros (Hoadley 1926; Keith 1933; Abdel-Malek 1950; Hamilton 1952; Hiruma and Nakamura 2003), the term pronephros should be avoided in amniotes (Fraser 1950). The elements for a functional pronephros, including a fully developed external glomerulus, hollow ciliated nephrostomes and hollow pronephric tubules, are undeniably never present.

Pronephros in anamniotes

Animals whose embryos have comparatively little yolk at their disposal (mesolecithal) pass through a free swimming larval stage and develop a pronephros (Sedgwick 1881; Rabl 1896; Goodrich 1930), dedicated to water excretion (Vize et al. 2003) and survival in aquatic environments (Howland 1916; Prentiss and Arey 1917; Raciti et al. 2008). In general, all anamniote embryos have well-developed pronephroi (Brauer 1902; Howland 1916, 1921; Prentiss and Arey 1917; Kerr 1919; Goodrich 1930; Armstrong 1932; Fales 1935; Holtfreter 1944; Fraser 1950; Fox 1963; Jaffee 1963; Christensen 1964; Huettner 1968; Nieuwkoop and Faber 1994; Vize et al. 1997, 2003; Kuure et al. 2000; Wrobel and Suss 2000; Nishinakamura 2003; Gilbert 2010; Wessely and Tran 2011), except for most sharks and rays (Kerr 1919; Goodrich 1930; Vize et al. 1997).

In basal chordates, e.g., Amphioxus, Cyclostomes, and Dipnoi, the pronephros functions generally as the adult kidney (Prentiss and Arey 1917; Hamilton et al. 1972; Vize et al. 2003; Bertrand and Escriva 2011). In bony fish and amphibians, the pronephros functions as the embryonic kidney. However, in some Dipnoi (Prentiss and Arey 1917) and Teleosts, e.g., Fierasfer (Goodrich 1930), Zoarces (Goodrich 1930), and Lepadogaster (Guitel 1906), the pronephros remains functional through adulthood, often alongside the functional mesonephros (Hamilton et al. 1972). The number of pronephric nephrostomes differs between species. Anurans generally have three nephrostomes between coelom and pronephric tubules, most urodeles have two and teleosts usually have only one nephrostome connecting to its single pronephric tubule (Vize et al. 1997).

Evolutionary aspects of kidney development

We sought to verify the existence of a pronephros in the different vertebrate taxa to get a grasp on the evolutionary aspects of the three subsequent kidney forms (see Fig. 6 and Supplementary Table S1). Although evolution has provided more advanced vertebrates with complex adult kidneys, these species continue to utilize simple evanescent kidneys during embryogenesis (Vize et al. 1997). Basal vertebrates with simple adult kidneys use even more uncomplicated versions during early developmental stages (see also Figs. 6 and 7 and Supplementary Table S1) (Vize et al. 1997). In the end it is much easier to form a pronephros, than it would be to form a more complex meso- or metanephros in a short period of time. The advantages of a simple temporary kidney to serve the free swimming larva are obvious: borrow time for a complex kidney to form. The same genes are involved in the development of all three vertebrate kidney forms (Carroll and Vize 1996; Heller and Brandli 1997; Kuure et al. 2000; Nishinakamura 2003). Among these genes are Pax2 (Dressler et al. 1990; Dressler and Douglass 1992; Carroll and Vize 1996; Heller and Brandli 1997; Kuure et al. 2000; Bouchard et al. 2002; Nishinakamura 2003; Kobayashi et al. 2007), Pax8 (Bouchard et al. 2002; Kobayashi et al. 2007), Tbx2 (Cho et al. 2011), BMP (Gilbert 2010), Hey1 (Cho et al. 2011), Gremlin (Cho et al. 2011), Xlim1 (Nishinakamura 2003), and WT1 (Carroll and Vize 1996; Heller and Brandli 1997; Kuure et al. 2000; Nishinakamura 2003). No genes have yet been identified that are exclusively involved in pronephric development. This strong genetic conservation of kidney organogenesis (Kuure et al. 2000) ironically hampers differentiation between pro-, meso, and metanephros on a genetic level and also substantiates the theory that pro- and mesonephric development represents merely a gradual evolutionary transition from external- to internal glomerulus.

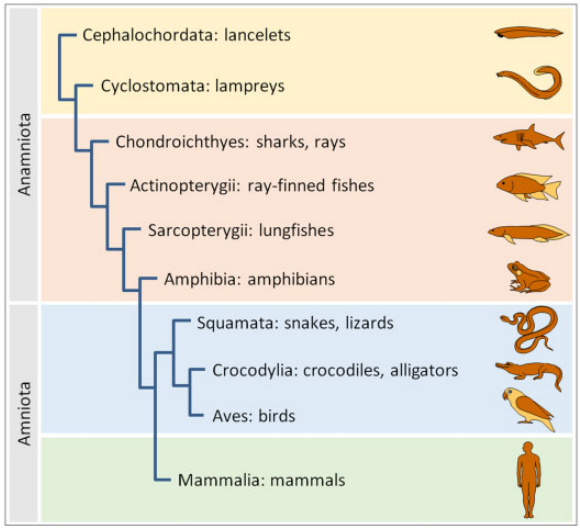

Fig. 6 Overview of the appearance of a pronephros in animal species presented as evolutionary cladogram. For details and references, see Supplementary Table S1. (Cephalochordata and Cyclostomata): pronephros functioning as adult kidney. (Chondroichthyes, Actinopterygii, Sarcopterygii, and Amphibia): pronephros functioning in the larval stage. However, the pronephros seems to be absent in those Elasmobranchii that have no larval stage, and in the Amniota which develop within the body of the parent (Fraser 1950). (Squamata, Crocodylia, and Aves): Although a pronephros has been described in embryos of some of these animals (Supplementary Table S1), the term pronephros should be used with much restraint (Fraser 1950). Further research in these species is needed to clarify the contradictions that appeared in the literature as a result of the use of different definitions. (Mammals): no pronephros is present.

The main tool of vertebrates to survive in varying circumstances, from fresh to salt water and from desert to rain forests, is the renal system which provides the vertebrates to either excrete large amounts of water or retain as much water as possible. The three vertebrate kidney forms are suitable for different habitats and are used in diverse combinations by the vertebrates with specific physiological requirements in the various stages of their life (Raciti et al. 2008). Water excretion seems to be the most common characteristic for species that show pronephros development (Vize and Smith 2004). This is in line with Frasers theory, that the pronephros seems to be absent in those Elasmobranchii that have no larval stage, and in the Amniota which develop within the body of the parent (Fraser 1950).

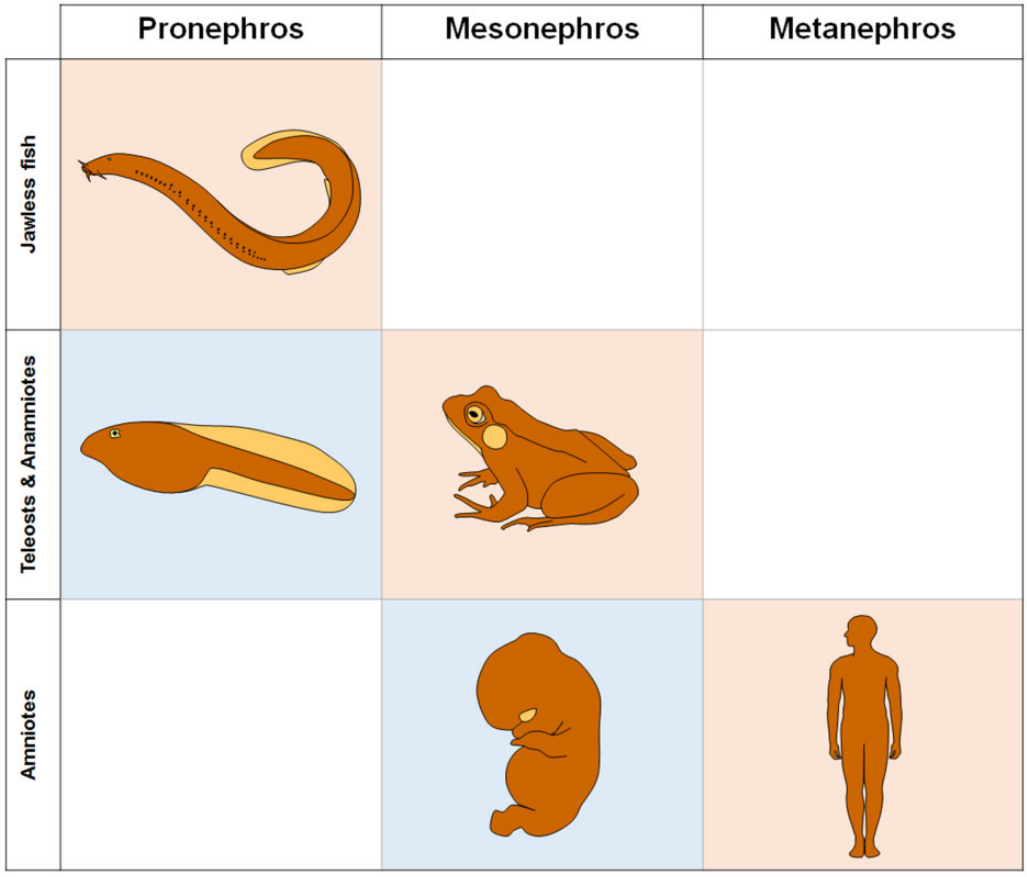

Fig. 7 General concept for renal evolution. Vertical columns: different kidney forms (i.e., pro-, meso-, and metanephros). Horizontal rows: different classes of species. In most jawless fish, like Amphioxus and hagfish, the pronephros remains functional through adulthood, often alongside a functional mesonephros. Larvae of teleosts and anamnia (e.g., tadpoles) generally pass a pronephric stage, while adult specimens (e.g., frogs) use a mesonephros for secretion. Embryos of amniotes (e.g., humans) do not pass a pronephric stage, but do use the mesonephros during the embryonic phase and the metanephros through fetal development, in childhood and in adulthood. The used kidney form thus gradually shifts from simple pronephric kidney as used by adult jawless fish, via the intermediate mesonephric kidney in more basal vertebrates toward the intricate metanephric kidney as used by more advanced vertebrates.

As mentioned before, the need for a pronephros also depends on the amount of yolk available for the embryo. Embryos supplied with large amounts of yolk (macrolecithal) generally show less developed pronephric tubules, whereas embryos with comparatively little yolk at their disposal develop extensive and functional pronephroi (Sedgwick 1880; Rabl 1896; Fraser 1950). The placenta also influences the degree of kidney development. The mesonephros is less developed in species that exhibit an intimate relation between extraembryonic membranes and placenta (e.g., humans and mice), whereas species with less effective placental systems (e.g., pigs) show better developed mesonephroi (Nelson 1953; Carlson 1988; Vize et al. 1997). Thus, in the presence of a yolk sac or a placenta as efficient waste disposal systems, kidney development is not essential for waste disposal or osmotic regulation prior to birth (Vize et al. 2003). It can therefore be reasoned that the evolutionary appearance of the yolk sac and placenta featured the gradual disappearance of the pronephros in more advanced vertebrates. It would be interesting to study the presence of a pronephros in egg laying mammals (Prototheria) since Fraser stated that Marsupiala (Metatheria) do develop a pronephros that functions in the larval stage (Supplementary Table S1). To better grasp the evolutionary development of the pronephros, more research is also needed to dispel the ambiguity about the presence of a pronephros in egg laying amniotes, i.e., birds and reptiles (Supplementary Table S1).

The fate of the pronephros

In both Teleost and Ganoid fish, the pronephric filtration unit relinquishes its excretory task to the

mesonephros (Vize et al. 2003), which leaves a lymphoid organ with hematopoietic function (Hansen and Kaattari 1996; Vize et al. 1997, 2003). In the zebrafish, colonization of the pronephros by hematopoietic stem cells begins at 32 h post-fertilization (Bertrand et al. 2008). Further research is needed to grasp the process which underlies the pronephric transition from excretory to lymphoid organ. Formation of the mesonephros occurs around 10 days post-fertilization in zebrafish, during post-embryonic metamorphosis from larva to juvenile (Diep et al. 2015). The mesonephric nephrons will form on top of the pronephric tubules and will later fuse with them. The amphibian pronephros degenerates during metamorphosis (Vize et al. 2003). By stopping the metamorphosis process, through blocking thyroid function (Hurley 1958; Fox and Turner 1967) or by thyroid- or hypophysectomy (Fox 1963), degeneration of the pronephros can be inhibited (Vize et al. 2003). How the degeneration of the pronephros exactly occurs remains however unclear, because in neotene amphibians (e.g., Caudata like Axolotl or Olm salamanders) that stay in their larval phase, the pronephros is also only described in early life (Duellman and Trueb 1994). Some authors proposed apoptosis as key element in this process (Ellis and Youson 1990; Pole et al. 2002; Vize et al. 2003; Chimenti and Accordi 2011), others suggested autolysis followed by phagocytotic activity of reticular macrophages and autophagic bodies (Fox 1970; Chimenti and Accordi 2011) or the breakup of rudiments into mesenchyme (Fraser 1950). Another argument could be that due to differential growth the pronephric remnants become untraceable when the pronephros stops developing after the embryonic stage and other organs expand toward their adult size.

Conclusion

The aim of this study was to clarify the presence of a pronephros in human embryos. With our referenced glossary and extensive literature survey we strived to clarify the definitions used in studies on kidney development. The pronephros proper consists of *non-integrated nephrons*, whereas the mesonephros and metanephros consist of only *integrated nephrons*. We observed that the pronephros as such is not detectable in human embryos. The peritoneal funnel is not entitled as exclusive feature of pronephros or mesonephros. Intermediate nephrons represent the gradual evolutionary change from pronephros to mesonephros, since the glomerulus is still in contact with the coelom (a typical pronephric feature) and the presence of a peritoneal funnel has been regarded both pronephric as mesonephric. Environmental conditions (i.e., life of water) and the appearance of the yolk sac and placenta affected the gradual disappearance of the pronephros in more advanced vertebrates. Thus, as Elizabeth Frazer already stated in 1950, the term pronephros does not apply to human or even mammalian embryos, and should be used with much restraint in other amniotes.

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Supplementary data

Supplementary data are available at *ICB* online.

References

Abdel-Malek ET. 1950. Early development of the urogenital system in the chick. J Morphol 86:599–626.

Armstrong PB. 1932. The embryonic origin of function in the pronephros through differentiation and parenchyma–vascular association. Am J Anat 51:157–88.

Audigé J. 1910. Contribution à l'étude des reins des poissons téléostéens. Arch Zool Exp Gén 4:275–624.

Bailey FR, Miller AM. 1921. Textbook of embryology. 4th ed. New York (NY): William Wood and Company.

Balfour FM, Sedgwick A. 1876. On the origin and history of the urogenital organs of vertebrates. J Anat Lond 10:17–48.

Balfour FM, Sedgwick A. 1878. On the existence of a rudimentary head kidney in the embryo chick. Proc R Soc 27:443–6.

Balfour FM, Sedgwick A. 1879. On the existence of a head–kidney in the embryo chick, and on certain points in development of the Müllerian duct. Quart J Micr Sci 19:1–20.

Bertrand JY, Kim AD, Teng S, Traver D. 2008. CD41+ cmyb+ precursors colonize the zebrafish pronephros by a novel migration route to initiate adult hematopoiesis. Development 135:1853–62.

Bertrand S, Escriva H. 2011. Evolutionary crossroads in developmental biology: amphioxus. Development 138:4819–30.

Bidder FH. 1846. Vergleichend-anatomische und histologische untersuchungen über die geschlechts und harnwerkzeuge der nackten amphibian. Dorpat, Estland: E.J. Karow.

Borcea I. 1905. Recherches sur le Système Uro-génitale des Elasmobranches. Arch Zool Exp Gén 4:199–484.

Bouchard M, Souabni A, Mandler M, Neubuser A, Busslinger M. 2002. Nephric lineage specification by Pax2 and Pax8. Genes Dev 16:2958–70.

Brandli AW. 1999. Towards a molecular anatomy of the *Xenopus* pronephric kidney. Int J Dev Biol 43:381–95.

Brauer A. 1902. Beiträge zur Kenntnis der Entwicklung und Anatomie der Gymnophionen. III. Die Entwicklung der Excretionsorgane. Zool Jahrb Anat 16:1–176.

Burlend TH. 1913. The Pronephros of *Chrysemys marginata*. Zool. Jahrb. Bd. 26:1–90.

Carev D, Krnic D, Saraga M, Sapunar D, Saraga BM. 2006. Role of mitotic, pro-apoptotic and anti-apoptotic factors in human kidney development. Pediatr Nephrol 21:627–36.

Carlson BM. 1988. Patten's foundations of embryology. New York (NY): McGraw-Hill Book Company.

Carroll TJ, Vize PD. 1996. Wilms' tumor suppressor gene is involved in the development of disparate kidney forms: evidence from expression in the *Xenopus* pronephros. Dev Dyn 206:131–8.

Chimenti C, Accordi F. 2011. Morphology and ultrastructure of the pronephros of Testudo hermanni Gmelin, 1789 (chelonian reptiles). Anat Histol Embryol 40:142–8.

Cho GS, Choi SC, Park EC, Han JK. 2011. Role of Tbx2 in defining the territory of the pronephric nephron. Development 138:465–74.

Christensen AK. 1964. The structure of the functional pronephros in larvae of *Ambystoma opacum* as studied by light and electron microscopy. Am J Anat 115:257–77.

Cochard LR. 2002. Netter's atlas of human embryology. Teterboro (NJ): Icon Learning Systems.

Cochard LR. 2012. Netter's atlas of human embryology. Philadelphia: Saunders Elsevier.

Davies J. 1950. The pronephros and the early development of the mesonephros in the duck. J Anat 84:95–103.

Davies J. 1951. Nephric development in the sheep with reference to the problem of the ruminant pronephros. J Anat 85:6–11.

Dawson A. 1925. Glomerular versus tubular activity in the mesonephros of *Necturus*: elimination of iron salts. Amer J Physiol 71:679–87.

de Bakker BS, de Jong KH, Hagoort J, de Bree K, Besselink CT, de Kanter FEC, Veldhuis T, Bais B, Schildmeijer R, Ruijter JM. 2016. An interactive three-dimensional digital atlas and quantitative database of human development. Science 354:aag0053.

de Bakker BS, de Jong KH, Hagoort J, Oostra RJ, Moorman AF. 2012. Towards a 3-dimensional atlas of the developing human embryo: the Amsterdam experience. Reprod Toxicol 34:225–36.

Diep CQ, Peng Z, Ukah TK, Kelly PM, Daigle RV, Davidson AJ. 2015. Development of the zebrafish mesonephros. Genesis 53:257–69.

Dressler GR, Deutsch U, Chowdhury K, Nornes HO, Gruss P. 1990. Pax2, a new murine paired-box-containing gene and its expression in the developing excretory system. Development 109:787–95.

Dressler GR, Douglass EC. 1992. Pax-2 is a DNA-binding protein expressed in embryonic kidney and Wilms tumor. Proc Natl Acad Sci U S A 89:1179–83.

Drummond IA. 2005. Kidney development and disease in the zebrafish. J Am Soc Nephrol 16:299–304.

Duellman WE, Trueb L. 1994. Biology of amphibians. London: The John Hopkins University Press.

Ellis LC, Youson JH. 1990. Pronephric regression during larval life in the sea lamprey, *Petromyzon marinus* L. A histochemical and ultrastructural study. Anat Embryol (Berl) 182:41–52.

Fales DE. 1935. Experiments on the development of the pronephros of *Ambystoma punctatum*. J Exp Zool 72:147–73.

Felix W. 1912. The development of the urinogenital organs. In: Keibel F, Mall FP, editors. Manual of human embryology. 2 ed. Philadelphia: J. B. Lippincott Company. p. 752–979.

Field HH. 1891. The development of the pronephros and segmental duct in amphibia. Bull Mus Comp Zool Harv 21:201–340.

Fox H. 1963. The amphibian pronephros. Q Rev Biol 38:1–25.

Fox H. 1970. Tissue degeneration: an electron microscopic study of the pronephros of *Rana temporaria*. J Embryol Exp Morphol 24:139–57.

Fox H, Turner SC. 1967. A study of the relationship between the thyroid and larval growth in *Rana temporaria* and *Xenopus laevis*. Arch Biol (Liege) 78:61–90.

Fraser EA. 1920. The pronephros and early development of the mesonephros in the cat. J Anat 54:287–304.

Fraser EA. 1950. The development of the vertebrate excretory system. Biol Rev 25:159–87.

Gaeth AP, Short RV, Renfree MB. 1999. The developing renal, reproductive, and respiratory systems of the African elephant suggest an aquatic ancestry. Proc Natl Acad Sci U S A 96:5555–8.

Gasser E. 1879. Beitrag zur Entwicklung des Urogenitalsystems bei den Hühnerembryonen. S B Naturwiss Ges Marburg 58–66.

Gasser RF. 1975. Atlas of human embryos. Hagerstown: Harper and Row Publishers.

Gasser RF, Cork RJ, Noe A, Lockett E, Wilson E. 2014. The Virtual Human Embryo—The endownment for human development, Inc. [Internet]. Computer Imaging Laboratory, Louisiana State University Health Sciences Center, New Orleans, USA. Available from: https://www.prenatalorigins.org/virtual-human-embryo/.

Gérard P, Cordier R. 1934. Esquisse d'une histophysiologie comparée du rein des vertébrés. Biol Rev 9:110–31.

Gérard P, Cordier R. 1934b. Recherches d'histophysiologie comparée sur le pro-et le mésonephros larvaires des Anoures. Z Zellforsch 21:1–23.

Gerlach GF, Wingert RA. 2013. Kidney organogenesis in the zebrafish: insights into vertebrate nephrogenesis and regeneration. Wiley Interdiscip Rev Dev Biol 2:559–85.

Gerth VE, Zhou X, Vize PD. 2005. Nephrin expression and three-dimensional morphogenesis of the *Xenopus* pronephric glomus. Dev Dyn 233:1131–9.

Gilbert SF. 2010. Developmental biology. 9th ed. Sunderland (MA): Sinauer Associates, Inc.

Goodrich ES. 1930. Studies on the structure and development of vertebrates. London: Macmillan Press.

Guitel F. 1906. Recherches sur l'anatomie des reins de quelques Gobiésocidés. Arch Zool Exp Gén 4:505–698.

Hall RW. 1904. The development of the mesonephros and the Müllerian ducts in amphibia. Bull Mus Comp Zool 45:31–123.

Hamilton HL. 1952. Lillie's development of the chick. New York: Henry Holt.

Hamilton WJ, Boyd JD, Mossman HW. 1972. Human embryology, prenatal development of form and function. Cambridge: Heffers Printers Limited.

Hansen JD, Kaattari SL. 1996. The recombination activating gene 2 (RAG2) of the rainbow trout *Oncorhynchus mykiss*. Immunogenetics 44:203–11.

Heller N, Brandli AW. 1997. Xenopus Pax-2 displays multiple splice forms during embryogenesis and pronephric kidney development. Mech Dev 69:83–104.

Hiruma T, Nakamura H. 2003. Origin and development of the pronephros in the chick embryo. J Anat 203:539–52.

Hoadley L. 1926. Developmental potencies of parts of the early blastoderm of the chick. III. The nephros, with special reference to the pro- and mesonephic portions. J Exp Zool 43:197–222.

Hohenstein P, Pritchard-Jones K, Charlton J. 2015. The yin and yang of kidney development and Wilms' tumors. Genes Dev 29:467–82.

Holtfreter J. 1944. Experimental studies on the development of the pronephros. Rev Can Biol 3:220–50.

Howland RB. 1916. On the effect of removal of the pronephros if the amphibian embryo. Proc Natl Acad Sci U S A 2:231–4.

Howland RB. 1921. Experiments on the effect of removal of the pronephros of *Ambystoma punctatum*. J Exp Zool 32:355–84.

Huettner AF. 1968. Fundamentals of comparative embryology of the vertebrates. New York (NY): The Macmillian Company.

Hurley MP. 1958. The role of the thyroid in kidney development in the tadpole. Growth 22:125–66.

Jacob HJ, Jacob M, Christ B. 1977. Die Ultrastructure der externen Glomerula. Ein Beitrag zur Nierentwicklung bei Hühnerembryonen. Verh Anat Ges 71:909–12.

Jaffee OC. 1963. Cellular differentiation in the anuran pronephros. Anat Rec 145:179–82.

Jones EA. 2005. *Xenopus*: a prince among models for pronephric kidney development. J Am Soc Nephrol 16:313–21.

Keith A. 1933. Human embryology and morphology. Baltimore: William Wood. p. 406–29.

Kerens B. 1907. Recherches sur les premières phases du développement de l'Appareil excréteur des Amniotes. Arch Biol 22:493–648.

Kerr JG. 1919. Textbook of embryology. Vol. II. London: Macmillan and Co.

Kobayashi H, Kawakami K, Asashima M, Nishinakamura R. 2007. Six1 and Six4 are essential for Gdnf expression in the metanephric mesenchyme and ureteric bud formation, while Six1 deficiency alone causes mesonephric-tubule defects. Mech Dev 124:290–303.

Kuure S, Vuolteenaho R, Vainio S. 2000. Kidney morphogenesis: cellular and molecular regulation. Mech Dev 92:31–45.

Lambert P. 1933. Sur l'existence d'un gradient de perméabilité dans les néphrons ouverts des Urodèles. C R Soc Biol 114:1370–2.

Larsen WJ. 1993. Human embryology. New York: Churchill Livingstone.

Lockett E. 2001. Histology file of the Carnegie Collection of Human Embryology. Developmental Anatomy Center at the National Museum of Health and Medicine in Silver Spring, MD, USA.

Marra AN, Wingert RA. 2014. Roles of Iroquois transcription factors in kidney development. Cell Dev Biol 3:1000131.

Mathews WW. 1976. Atlas of descriptive embryology. 2nd ed. New York: Collier Macmillan Ltd.

McCrory WW. 1974. The normal embryologic development of the kidney: a basis for understanding structural abnormalities. Birth Defects Orig Artic Ser 10:3–11.

Michos O. 2009. Kidney development: from ureteric bud formation to branching morphogenesis. Curr Opin Genet Dev 19:484–90.

Mihalkovics G. 1885. Untersuchungen über die Entwicklung des Harn- und Geschlechtsapparates der Amnioten. Int Mschr Anat Hist 2:41–62.

Moore KL. 1988. The developing human: clinically oriented embryology. Michigan: Saunders.

Morgan L. 2009a. Icons of life: a cultural history of human embryos. Berkeley (CA): University of California Press.

Morgan LM. 2009b. Chapter 4. Inside the production factory. In: Icons of life: a cultural history of human embryos. Berkeley (CA): University of California Press.

Müller F. 1830. De glandulararum secernentium structura pentitiori earumque prima formatione in homine atque animalibus commentatio anatomica. Lipsiae.

Müller J. 1829. Ueber die wolffschen körper bei den embryonen der frösche und kröten. Arch Anar Phys Jhrg 65–70.

Needham J. 1931. Chemical embryology. London: Cambridge University Press.

Nelson OE. 1953. Comparative embryology of the vertebrates. New York (NY): Blackiston.
Nieuwkoop PD, Faber J. 1994. Normal table of *Xenopus laevis* (Daudin). 4th ed. New York (NY): Garland Science.
Nishinakamura R. 2003. Kidney development conserved over species: essential roles of Sall1. Semin Cell Dev Biol 14:241–7.
O'Rahilly R, Müller F. 1987. Developmental stages in human embryos, including a revision of streeter's "horizons" and a survey of the Carnegie collection. Washington: Carnegie Institution of Washington.
Patten BM, Carlson BM. 1974. Foundations of embryology. 3rd ed. New York (NY): McGraw-Hill Book Company.
Pole RJ, Qi BQ, Beasley SW. 2002. Patterns of apoptosis during degeneration of the pronephros and mesonephros. J Urol 167:269–71.
Prentiss CW, Arey LB. 1917. A laboratory manual and textbook of embryology. Philadelphia: W.B. Saunders Company.
Price GC. 1897. Development of the excretory organs of myxinoid, *Bdellostoma stouti* Lockington. Zool Jahrb 10:205–26.
Price GC. 1904. Further study of the development of the excretory system in *Bdellostoma stouti*. Amer J Anat 4:117–38.
Rabl C. 1896. Über die Entwicklung des Urogenitalsystems der Selachier. Morphol Jahrb 24:632–767.
Rabl H. 1908. Über die entwicklung der vorniere bei den vögeln, nach untersuchungen am kiebitz (*Vanellus cristatus* M.). Arch Mikrosk Anat 72:731–815.
Raciti D, Reggiani L, Geffers L, Jiang Q, Bacchion F, Subrizi AE, Clements D, Tindal C, Davidson DR, Kaissling B, et al. 2008. Organization of the pronephric kidney revealed by large-scale gene expression mapping. Genome Biol 9:R84.
Renson G. 1883. Recherches sur le rein cephalique et le corps de Wolff chez les Oiseaux et les Mammifères. Arch F Micro Anat. Bd. 22.
Ruppert EE. 1994. Evolutionary origin of the vertebrate nephron. Amer Zool 34:542–53.
Ryffel GU. 2003. What can a frog tell us about human kidney development. Nephron Exp Nephrol 94:e35–43.
Sadler TW. 2004. Langman's medical embryology. 9th ed. Philadelphia: Lippincott Williams & Wilkins.
Sadler TW. 2015. Langman's medical embryology. 13th ed. Philadelphia: Wolters Kluwer Health.
Sanna-Cherchi S, Caridi G, Weng PL, Scolari F, Perfumo F, Gharavi AG, Ghiggeri GM. 2007. Genetic approaches to human renal agenesis/hypoplasia and dysplasia. Pediatr Nephrol 22:1675–84.
Schulman J, Edmonds LD, McClearn AB, Jensvold N, Shaw GM. 1993. Surveillance for and comparison of birth defect prevalences in two geographic areas—United States, 1983–88. MMWR CDC Surveill Summ 42:1–7.
Sedgwick A. 1880. Notes and Memoranda: On the development of the structure known as the 'glomerulus of the head-kidney' in the chick. J Cell Sci s2–20:372–4.
Sedgwick A. 1881. Memoirs: On the early development of the anterior part of the Wolffian duct and body in the chick, etc. J Cell Sci s2–21:432–68.
Smith HW. 1943. Lectures on the kidney, Porter Lectures, Series IX. Lawrence, Kansas: University extension division, University of Kansas.
Smith HW. 1953. From fish to philosopher. Boston: Little, Brown.
Solhaug MJ, Bolger PM, Jose PA. 2004. The developing kidney and environmental toxins. Pediatrics 113:1084–91.
Streeter GL. 1942. Developmental horizons in human embryos: description of age group XI, 13 to 20 somites, and age group XII, 21 to 29 somites. Contrib Embryol 30:211–45.
Streeter GL. 1945. Developmental horizons in human embryos: description of age group XIII, embryos about 4 or 5 millimeters long, and age group XIV, period of indentation of the lens vesicle. Contrib Embryol 31:27–63.
Streeter GL. 1948. Developmental horizons in human embryos: description of age groups XV, XVI, XVII, and XVIII, being the third issue of a survey of the Carnegie collection. Contrib Embryol 32:133–203.
Streeter GL. 1949. Developmental horizons in human embryos; a review of the histogenesis of cartilage and bone. Contrib Embryol 33:149–68.
Streeter GL. 1951. Developmental horizons in human embryos: description of age groups XIX, XX, XXI, XXII, and XXIII, being the fifth issue of a survey of the Carnegie collection. Contrib Embryol 34:165–96.
Szebenyi ES. 1977. Atlas of developmental embryology. Cranbury (NJ): Associated University Press.
Torrey TW. 1954. The early development of the human nephros. Contrib Embryol Carnegie Inst Wash Publ 35:175–97.
Tuchmann-Duplessis H, Haegel P. 1974. Illustrated human embryology. Vol. 2. Organogenesis. New York (NY): Springer.
Upadhyay KK, Silverstein DM. 2014. Renal development: a complex process dependent on inductive interaction. Curr Pediatr Rev 10:107–14.
Vize PD, Seufert DW, Carroll TJ, Wallingford JB. 1997. Model systems for the study of kidney development: use of the pronephros in the analysis of organ induction and patterning. Dev Biol 188:189–204.
Vize PD, Smith HW. 2004. A homeric view of kidney evolution: a reprint of H.W. Smith's classic essay with a new introduction. Evolution of the kidney. 1943. Anat Rec A Discov Mol Cell Evol Biol 277:344–54.
Vize PD, Woolf AS, Bard JBL. 2003. The kidney, from normal development to congenital disease. Orlando (FL): Academic Press.
Waddington CH. 1938. The morphogenetic function of a vestigial organ in the chick. J Exp Biol 15:371–6.
Wang Z, Li M. 2015. Evolution of the urinary proteome during human renal development and maturation. Adv Exp Med Biol 845:95–101.
Weldon WFR. 1883. Note on the early development of *Lacerta muralis*. Quart J Micr Sci 23:134–44.
Wessely O, Tran U. 2011. *Xenopus* pronephros development—past, present, and future. Pediatr Nephrol 26:1545–51.

Wiedersheim R. 1890. Über die entwicklung des urogenitalapparates bei krocodilen ind schildkröten. Anat Anz. 5:337–44.

Wintour EM, Butkus A, Earnest L, Pompolo S. 1996. The erythropoietin gene is expressed strongly in the mammalian mesonephric kidney. Blood 88:3349–53.

Wrobel KH, Suss F. 2000. The significance of rudimentary nephrostomial tubules for the origin of the vertebrate gonad. Anat Embryol (Berl) 201:273–90.

Xing L, Wen J-G, Frøkiær J, Djurhuus JC, Nørregaard R. 2014. Ontogeny of the mammalian kidney: expression of aquaporins 1, 2, 3, and 4. World J Pediatr 10:306–12.