Short communication

Formation of the circle of Willis during human embryonic development

Takakuwa

Short title: Formation of the CW

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Abstract

The circle of Willis (CW) is a circulatory anastomosis that supplies blood to the brain and adjacent structures. We examined the timing of formation of CW in 20 Japanese human embryo samples by using 3-dimensional reconstruction of serial histological sections. The CW was closed in 1 (n=6), 2 (n=8), 2 (n=3) and 2 (n=3) samples at Carnegie stages 20, 21, 22, and 23, respectively. The CW was unclosed in 13 samples (unclosed at ACOM alone, 6 samples; ACOM and bilateral P1, 4; left PCOM and right P1, 1; right PCOM and right P1, 1; ACOM and left PCOM, 1). It was difficult to predict whether the circle would close during further development, as such variations frequently exist in adults.

Key Words

Circle of Willis, human embryo, three-dimensional reconstruction

Introduction

The circle of Willis (CW), also called Willis' circle or cerebral arterial circle, is a circulatory anastomosis that supplies blood to the brain and adjacent structures (Eastcott 1994). The CW is composed of the following arteries: left and right anterior cerebral arteries (ACA), anterior communicating artery (ACOM), internal carotid arteries (ICA), posterior cerebral arteries (PCA), posterior communicating arteries (PCOM), and basilar artery (BA). As the CW serves the important function of providing blood supply to the brain, it communicates with multiple collaterals in the cerebral circulation. Willis recognized these anastomoses as a compensatory system to overcome any arterial occlusion. The presence of collateral flow via the PCOM in the CW is associated with a low prevalence of border zone infarcts in patients with unilateral ICA occlusion (Hendrikse et al. 2001). A recent simulation study demonstrated that the CW served as a passive pressure dissipating system under physiologic conditions, implying that the CW and its communicating arteries protect the cerebral arteries and the blood–brain barrier from hemodynamic stress (Vrselja et al. 2014).

The CW has many variations. The complete circle was observed in only 5-28% of adults. Variations at the ACOM, PCOM, and precommunicating segment

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(segment P1) of the PCA are well known. ACOM aplasia or hypoplasia was observed in

5-32% of the total examined adult cases, PCOM aplasia or hypoplasia was observed in

23-81%, and segment P1 aplasia or hypoplasia was observed in 7-15% (Alpers et al.

1959; Eftekhar et al. 2006; Fisher 1965; Fujimoto & Tanaka 1989).

Cerebral aneurysms that result in intracranial hemorrhage (subarachnoid

hemorrhage) frequently occur in the CW. Cerebral aneurysms are associated with

various pathological cerebrovascular conditions responsible for hemodynamic changes

(Dyste & Beck 1989). Variations in the CW have a direct impact on the distribution of

carotid and basilar blood flow and the formation of aneurysms (Horikoshi et al. 2002;

Kayembe et al. 1984; Kirgis et al. 1966; Kwak et al. 1980). There are other

cerebrovascular disorders (Hendrikse et al. 2001; Schomer et al. 1994) and mental

disorders that may also be associated with these variations (Blackburn 1907, 1910).

Padget (1948) was the first person to precisely describe the embryonic

development of the human brain vasculature. In her study, development of the

intracranial artery, including CW, was fully described in 19 human embryonic samples

between Carnegie stages (CS) 13 and 21, one fetal sample (crown-rump length, CRL =

40 mm), and one adult sample. Padget also demonstrated that the circle was unclosed

in 2 samples at CS 18 and CS 19, whereas closed in all 3 samples at CS 20 and all 3

samples after CS 21. Among the 3 samples at CS 20, ACOM had a plexiform

appearance in two samples, and a single main artery in one sample. No other studies

have provided such a precise description of the development of the CW in the

embryonic period of humans to date.

In the present study, we examined the timing of formation of CW in Japanese

human embryo samples by using 3-dimensional (3-D) reconstruction of serial histology

sections.

Materials and Methods

Human embryo specimens

Approximately 44,000 human embryos comprising the Kyoto Collection are

stored at the Congenital Anomaly Research Center of Kyoto University (Nishimura et al.

1968; Shiota 1991; Yamada et al. 2004). Well-preserved human embryos that were

found to be normal on external appearance were subjected to serial sectioning for

histology. For this study, 20 Japanese human embryos between CS 20 and CS 23 were

selected from the Kyoto Collection. All the selected samples exhibited no overt damage

or anomalies, and the histological specimens were well preserved.

Digitalization of histological sections and 3D reconstruction of CW

Serial sections (transverse sections, 10 μm thick) of whole embryos were scanned and stored as digital data by using a film scanner (CanoScan 9000F, Canon, Tokyo, Japan) at 4800 dpi. Sequential 2-D images were trimmed digitally by using ImageJ 64-bit (ver. 1.46, National Institutes of Health, Bethesda, MD, USA) and saved in Microsoft Windows Bitmap Image files (.bmp) at a resolution identical to that of the original digital data. The cranial arteries, including CW, were segmented on serial digital sections, and 3-D images were computationally reconstructed for examining the morphology by using Amira software (ver. 5.5.0, Visage Imaging, Berlin, Germany) in all samples. The ethics committee of the Kyoto University Graduate School and Faculty of Medicine approved this study (E986).

Results

A total of 20 samples were analyzed. The CW was closed in 1 (n=6), 2 (n=8), 2 (n=3), and 2 (n=3) samples at Carnegie stages 20, 21, 22, and 23, respectively (Figure 1, Table 1). The CW was unclosed in 13 samples, 6 at ACOM alone, 4 at ACOM and bilateral P1, one at right PCOM and right P1, one at left PCOM and right P1, and one at the ACOM and left PCOM (Figure 2). ACOM occasionally had a plexiform

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appearance (Figure 1, reconstruction at CS 22).

Discussion

The study by Padget (1948) remains unmatched in its precision in reporting the

development schedule of the formation of CW during the embryonic period. Thus, the

schedule described in that study is the only one available worldwide. Padget's study

demonstrated that the CW forms a closed circle at CS 20 to coincide with the formation

of the ACOM. The results of our study deviate from Padget's findings in suggesting a

delay of one or two stages in the schedule of formation of the CW. Racial differences

may one possible explanation of the difference in timing noted in the two studies.

Previous studies reviewed the distribution of the CW variation in different populations

(Eftekhar et al. 2006; Hashemi et al. 2013). They are very cautious to the conclusion

that the distributions of the variations of cerebral arterial circle differ in different

populations. Padget has reported many variations in adults samples (Padget 1944), but

did not describe the variation during development (Padget 1948). We speculate that

Padget might exclude samples with such variations in CW in the latter study to

demonstrate the natural development of CW or might not meet such samples as she

described in the limited number of samples.

The ACOM may close in later stages, as its formation is the final step in

completing the CW, according to the previous study by Padget. It is also possible that

the CW remains unclosed at the ACOM until birth, as such a variation is known to exist

in adults (Alpers et al. 1959; Eftekhar et al. 2006; Fawcett & Blachford 1905; Fisher

1965; Fujimoto & Tanaka 1989). A similar interpretation can be considered for samples

in which the CW remained unclosed at the PCOM and P1, suggesting this may remain

so until birth. Previous studies indicate that the blood supply from ICA to PCA through

PCOM, supplying the cerebellum and brain stem, may decrease during embryonic

development owing to the growth of the prosencephalon (cerebrum) (Okahara et al.

2002; Van Overbeeke et al. 1991). The blood supply to the PCA territory is

compensated by the BA. However, an unclosed CW owing to aplasia or hypoplasia of

PCOM results in restricted collateral flows, and, is a recognized risk factor for

cerebrovascular conditions such as ICA occlusion, border zone infarcts, transient

ischemic attacks, and minor strokes (Hendrikse et al. 2001). Variations, in the form of

aplasia or hypoplasia of P1, result in the ICA providing blood supply to the PCA. This

condition is referred to as the fetal posterior cerebral artery (fetal type) (Raamt et al,

2006). In the fetal type of CW, the entire PCA territory depends on the ICA for its blood

supply, while the other areas, including cerebellum and brain stem, depend on the BA

for their blood supply. The communication of the CW at P1 has a significant influence on

intracranial hemodynamics (Wentland et al. 2010). The present study, however,

suggests the possibility that the variations in ACOM, PCOM, and P1 may occur during

embryonic development itself. Such variations may have a significant influence on the

development and growth of the brain, and affect its morphology as well as function

(Raybaud 2010; Wentland et al. 2010). The present study indicates that the CW is

closed around CS 22, one or two stages later than previously reported (Padget 1948).

Thus, the frequency of variations in the CW at CS 23, at the end of the embryonic stage,

and its comparison with the frequency of variations after birth need to be investigated in

future studies.

Acknowledgements

Disclosures

None

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Figure legends

Figure 1. Formation of the CW during Carnegie stages 20 and 23

＊ in CS20; Left and right ACA was not connected. Acom was not formed.
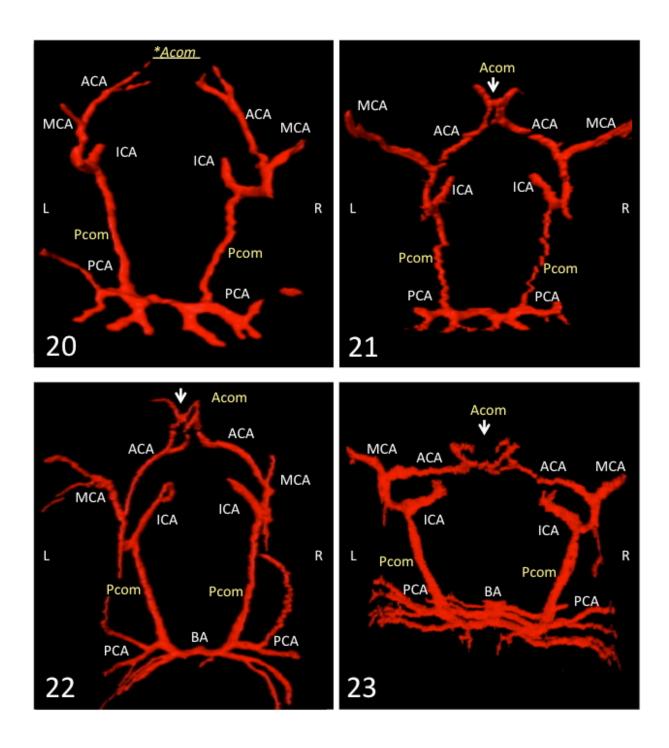
Figure 2. Variations in the CW at Carnegie stage 21.

(A) The circle is unclosed at ACOM and bilateral PCOM.

(B) The circle is unclosed at left PCOM and right P1. ACOM, anterior communicating

artery; PCOM, posterior communicating artery; ACA, anterior cerebral arteries; ICA,

internal carotid arteries (ICA); PCA, posterior cerebral arteries; BA, basilar artery; P1,
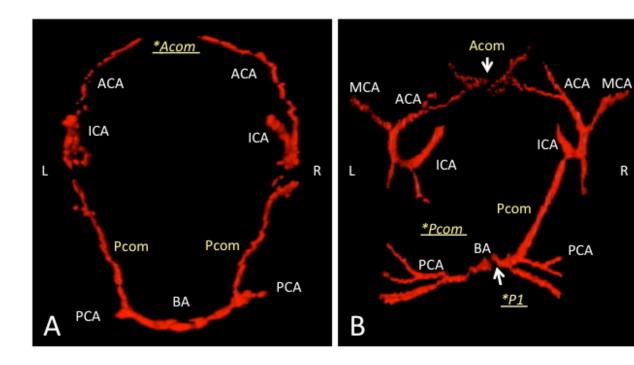
P1 segment of PCA

Table1. Formation of the circle of Willis

Carnegie stage	No of samples	Circle formation		Segments at which the circle was unclosed		
		Closed	Unclosed	ACOM alone	ACOM + b/l P1	PCOM + others
20	6	1	5	4	1	0
21	8	2	6	2	1	ACOM + Lt. PCOM, 1; Lt. PCOM + Rt. P1, 1; Rt. PCOM + Rt. P1, 1
22	3	2	1	0	1	0
23	3	2	1	0	1	0
Total	20	7	13	6	4	3

ACOM, anterior communicating artery; PCOM, posterior communicating artery; P1, P1 segment of posterior cerebral artery; Rt., right; Lt., left; b/l, bilateral