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GENETIC AND ENVIRONMENTAL EFFECTS ON THE MORPHOLOGY AND HEMODYNAMICS OF THE CIRCLE OF WILLIS

PhD thesis

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The List of Abbreviations

ACA: anterior cerebral artery

ACoA: anterior communicating artery

BA: basilar artery

BMI: body mass index

CEA: carotid endarterectomy

CE-MRA: contrast-enhanced magnetic resonance angiography

CI: confidence interval

CST3: cystatin 3

CT: computed tomography

CTA: computed tomography angiography

CoW: Circle of Willis

DBP: diastolic blood pressure

DSA: digital subtraction angiography

DZ: dizygotic

EDV: end diastolic velocity

FTP: fetal-type posterior circulation

GPx4: glutathione peroxidase 4

ICA: internal carotid artery

LACA A1: left anterior cerebral artery A1 segment

LACA A2: left anterior cerebral artery A2 segment

LMCA: left middle cerebral artery

LPCA P1: left posterior cerebral artery P1 segment

LPCA P2: left posterior cerebral artery P2 segment

LPCoA: left posterior communicating artery

LVA: left vertebral artery

MAP: mean arterial pressure

MCA: middle cerebral artery

MFV: mean flow velocity

MRA: magnetic resonance angiography

MRI: magnetic resonance imaging

MZ: monozygotic

OA: ophthalmic artery

OR: odds ratio

PCA: posterior cerebral artery

PCoA: posterior communicating artery

PFN-2: profiling-2

PGF: placental growth factor

PI: pulsatility index

PICA: posterior inferior cerebellar artery

PP: pulse pressure

PSV: peak systolic velocity

RACA A1: right anterior cerebral artery A1 segment

RACA A2: right anterior cerebral artery A2 segment

RI: resistance index

RMCA: right middle cerebral artery

RPCA P1: right posterior cerebral artery P1 segment

RPCA P2: right posterior cerebral artery P2 segment

RPCoA: right posterior communicating artery

RVA: right vertebral artery

SBP: systolic blood pressure

TCCS: transcranial color-coded duplex sonography

TCD: transcranial Doppler sonography

TOF MRA: time of flight magnetic resonance angiography

VA: vertebral artery

VEGF: vascular endothelial growth factor

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1. Introduction

1.1. The anatomy of the Circle of Willis (CoW)

The circulus arteriosus cerebri, an arterial anastomotic polygon located at the skull base is of key importance regarding the maintenance of cerebral perfusion. The arterial anastomotic circuit that connects the carotid and vertebrobasilar circulation was first described by Thomas Willis nearly 400 years ago and was named in his honor “Circle of Willis”. The anastomotic circuit ensures blood supply to the brain through the cerebral and communicating arteries (Figure 1). The two anterior cerebral arteries (ACA) are branches of the internal carotid artery (ICA) and are connected by a single anterior communicating artery (ACoA). The middle cerebral artery (MCA) origins from the ICA bilaterally and is connected to the posterior cerebral artery (PCA) by the posterior communicating arteries (PCoA). The two PCAs origin from the basilar artery (BA). The BA arises from the confluence of the vertebral arteries (VA). The integrity of the CoW seems to be crucial to maintain a compensatory flow in case of cerebrovascular events involving large artery stenosis or occlusion. However, the classical anatomical configuration of the CoW is rarely seen during autopsy and imaging studies. A relevant percentage of the healthy adult as well as paediatric population has hypoplasia or absence of one or more CoW arteries. The etiology of these anatomical variants is unknown.

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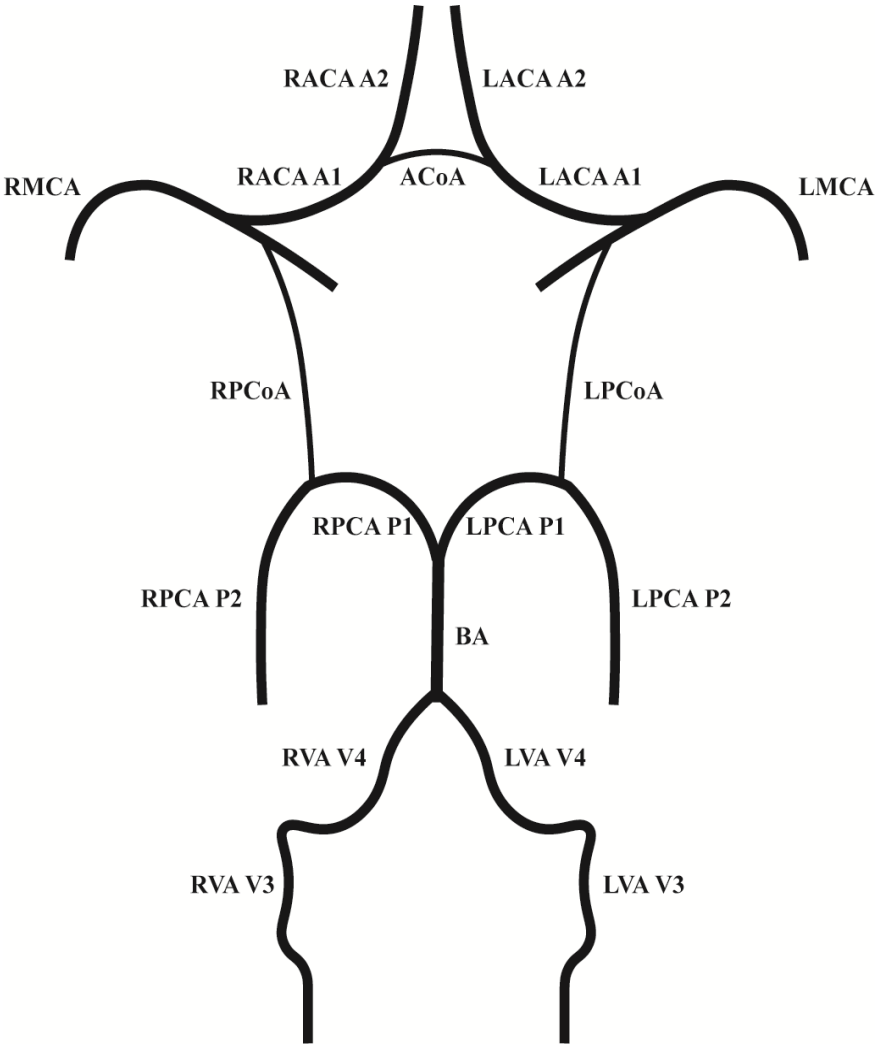

Figure 1. Normal anatomy of the CoW. ACoA: anterior communicating artery, LACA A1: left anterior cerebral artery A1 segment, CoW: Circle of Willis, LACA A2: left anterior cerebral artery A2 segment, LMCA: left middle cerebral artery, LPCA P1: left posterior cerebral artery P1 segment, LPCA P2: left posterior cerebral artery P2 segment, LPCoA: left posterior communicating artery, LVA V3: left vertebral artery V3 segment, LVA V4: left vertebral artery V4 segment, RACA A1: right anterior cerebral artery A1 segment, RACA A2: right anterior cerebral artery A2 segment, RMCA: right middle cerebral artery, RPCA P1: right posterior cerebral artery P1 segment, RPCA P2: right posterior cerebral artery P2 segment, RPCoA: right posterior communicating artery, RVA V3: right vertebral artery V3 segment, RVA V4: right vertebral artery V4 segment (self-made illustration).

1.2. CoW variants

The exact number of variants is impossible to determine, however, certain parts of the CoW show a tendency to appear hypoplastic or absent. The anterior and posterior communicating arteries commonly show variant anatomy. The typical CoW configuration without any hypoplasia or absence was found only in 5-28% [1-6]. Variants of the posterior circulation are more common as compared to the anterior CoW when investigating the anterior and posterior circulation separately [7-10]. Bilateral hypoplastic PCoA was observed in 13-32% being the most common variant amongst all, non-classical configurations as well as in the posterior circulation [1-6, 10]. The prevalence of unilateral hypoplastic PCoA was somewhat lower but also among the most common variants (6-20%) [1-6]. Hypoplasia of the ACoA was the most commonly seen variant anteriorly (0-14%) and hypoplastic ACA A1 segment was observed in 0-10% [1-6, 11]. The more rare variants include fenestrations and duplications mainly in the anterior CoW, with debated significance in intracranial aneurysm formation [12-14]. Conflicting results are reported regarding the fetal-type posterior circulation (FTP) and the role of this variant in the individual predisposition to stroke [15-17]. The FTP is a variant with debated significance, where the PCA P1 segment is supplied by the ICA through the posterior communicating artery. The FTP can be divided into partial and full categories. Partial FTP is defined as a hypoplastic PCA P1 segment and a more dominant ipsilateral PCoA, whereas an absent PCA P1 segment is seen in case of full FTP [18]. The prevalence of fetal-type variants was 3-36% in autopsy and imaging studies [2, 5, 11, 18-31], however, these numbers are highly dependent on the imaging modalities and autopsy methods. Figures 2 and 3 show illustrations of common CoW variants (these images are also referred to in chapter 3.2.1.1., 4.2.1. and 4.2.2.).

The factors determining these variants are still to be elucidated. There is substantial research supporting that these variants show a differences regarding their prevalence among ethnicities.

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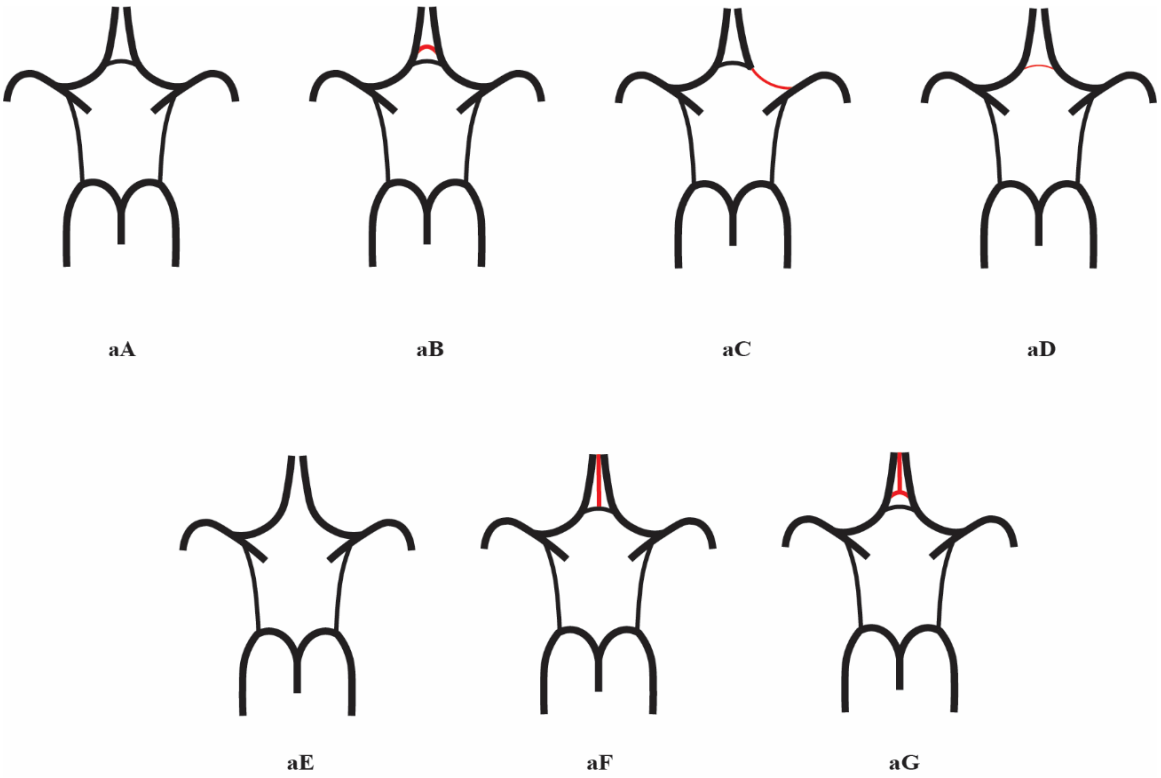

Figure 2. Variants of the anterior part of the CoW. aA: „Normal" variant with single, non-hypoplastic ACoA and ACAs. aB: Two ACoAs. aC: Unilateral ACA A1 segment hypoplasia. aD: hypoplastic ACoA. aE: Absent ACoA. aF: Medial artery of the corpus callosum arising from the ACoA. aG: Two ACoAs with medial artery of the corpus callosum arising from the ACoA. ACA: anterior cerebral artery, ACoA: anterior communicating artery, CoW: Circle of Willis (self-made illustration).

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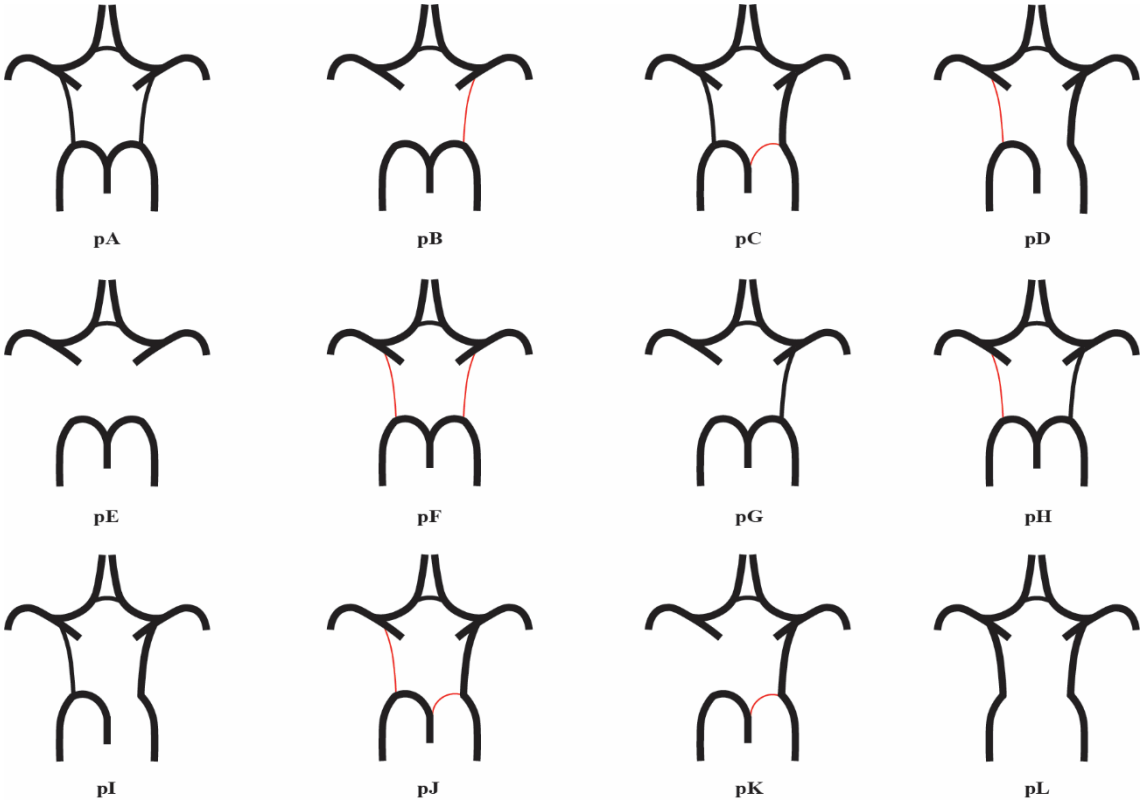

Figure 3. Variants of the posterior CoW. pA: „normal" configuration with non-hypoplastic PCoAs present on both sides. pB: Unilateral PCoA hypoplasia, absent contralateral PCoA. pC: Unilateral fetal-type variant with ipsilateral dominant PCoA and hypoplastic PCA P1 segment. pD: Unilateral fetal-type variant with absent ipsilateral PCA P1 segment and contralateral PCoA hypoplasia. pE: Bilaterally absent PCoAs. pF: Bilaterally hypoplastic PCoAs. pG: Absent unilateral PCoA. pH: Hypoplastic unilateral PCoA. pI: Unilateral fetal-type variant with ipsilateral absent PCA P1 segment. pJ: Unilateral fetal-type variant with ipsilateral hypoplastic PCA P1 segment and contralateral hypoplastic PCoA. pK: Unilateral fetal-type variant with ipsilateral hypoplastic PCA P1 segment and contralateral absent PCoA. pL: Bilateral fetal-type variant with bilaterally absent PCA P1 segments. CoW: Circle of Willis, PCA: posterior cerebral artery, PCoA: posterior communicating artery (self-made illustration).

1.2.1. Ethnical differences in the appearance of CoW variants

There are autopsy studies suggesting ethnical differences in CoW-variations, although results are inconclusive in this field. Significant differences were described in the CoW-variant profile of the USA compared to Morocco, Iran, France and Sri-Lanka and the difference was mostly attributable to complex configurations where both the anterior and the posterior circulation is affected [1]. The complex variants are rare and therefore they could be related to the larger sample size in the USA-studies. In contrast, Eftekhar et al. did not find a significant difference amongst the aforementioned (Moroccan, French, Iranian and North-American) populations [6]. Li et al. investigated the CoW variants in the Chinese population and concluded that the prevalence of compromised CoW was higher in this cohort which may be due to ethnical differences in CoW morphology, although the results were compared to magnetic resonance angiography (MRA) and autopsy studies and therefore, methodological differences could account for this observation [11]. Methodological aspects make the comparison challenging [1]. Post mortem changes in vessel calibre may bias the number of detected hypoplastic vessels. Furthermore, the criteria for hypoplasticity, the cause of death and the gender distribution in the sample may all be relevant factors that influence the results. Therefore, the results urge for thorough investigation of the genetic and environmental factors influencing the formation of variants in the CoW in humans using different imaging methods.

1.3. Imaging of the CoW

1.3.1. Hemodynamic assessment of the CoW using transcranial insonation

The morphology of the CoW can be analysed by magnetic resonance imaging (MRI) and computed tomography (CT), but the hemodynamic parameters of the CoW can exclusively be measured real-time by transcranial insonation such as transcranial Doppler (TCD) or Transcranial Color Coded Duplex Sonography (TCCS) [32].

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1.3.1.1. Basic principles of transcranial insonation

TCD uses pulsed-wave Doppler measurements without the imaging of the intracranial vessels and surrounding structures [33]. Modern TCD equipment combine the power motion Doppler with a single-channel spectral analysis as power-motion Doppler is a helpful tool to identify the CoW-arteries [34]. Using the power motion mode, color-coded flow signals indicating flow direction can be detected in a range of insonation depth [34]. The sonographer can identify the CoW arterial segments based on given flow direction in a given depth on power-motion Doppler mode and choose an accurate depth for single-depth spectral analysis accordingly [34]. Therefore, identification of the vessels with this technique is based on the depth, flow direction and resistance pattern. TCCS is a technique where B-mode imaging is used together with color-Doppler and most often is completed by spectral analysis. Unlike TCD, TCCS allows angle correction and thus, more accurate velocity measurement as well as the investigation of CoW anatomy. Figures 4, 5 and 6 show examples of TCCS examinations.

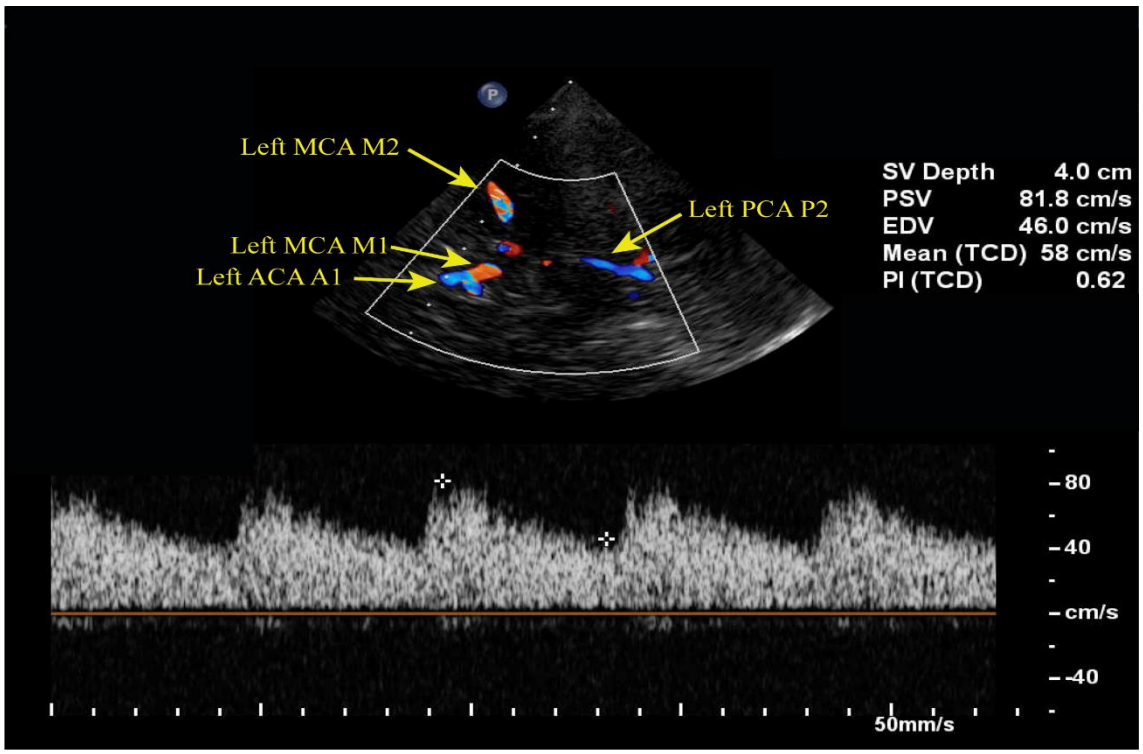

Figure 4. TCCS showing normal flow velocities in the left MCA. ACA: anterior cerebral artery, EDV: end-diastolic velocity, MCA: middle cerebral artery, mean (TCD): mean flow velocity, PCA: posterior cerebral artery, PI (TCD): pulsatility index, PSV: peak systolic velocity, SV depth: sample volume depth, TCCS: transcranial color-coded duplex sonography. Courtesy of Dr. Zsolt Garami.

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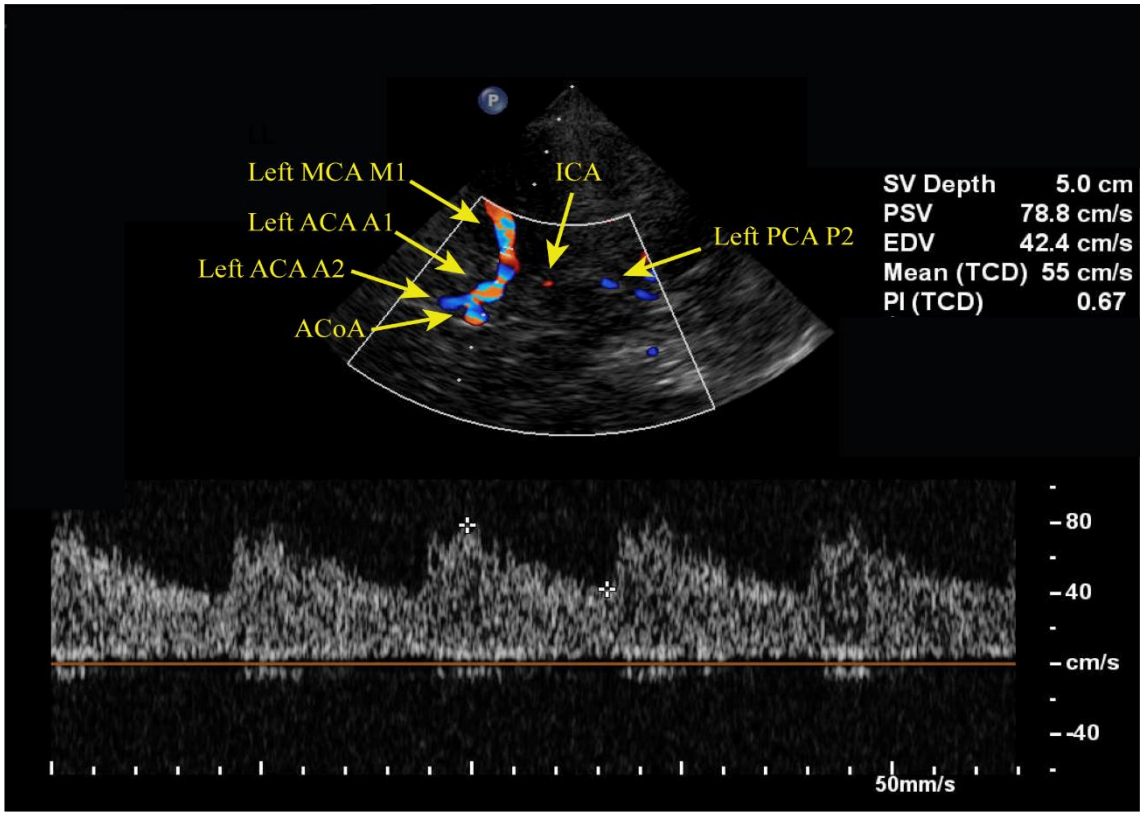

Figure 5. TCCS showing normal flow velocities in the left MCA. ACA: anterior cerebral artery, ACoA: anterior communicating artery, EDV: end-diastolic velocity, ICA: internal carotid artery, MCA: middle cerebral artery, mean (TCD): mean flow velocity, PCA: posterior cerebral artery, PI (TCD): pulsatility index, PSV: peak systolic velocity, SV depth: sample volume depth, TCCS: transcranial color-coded duplex sonography. Courtesy of Dr. Zsolt Garami.

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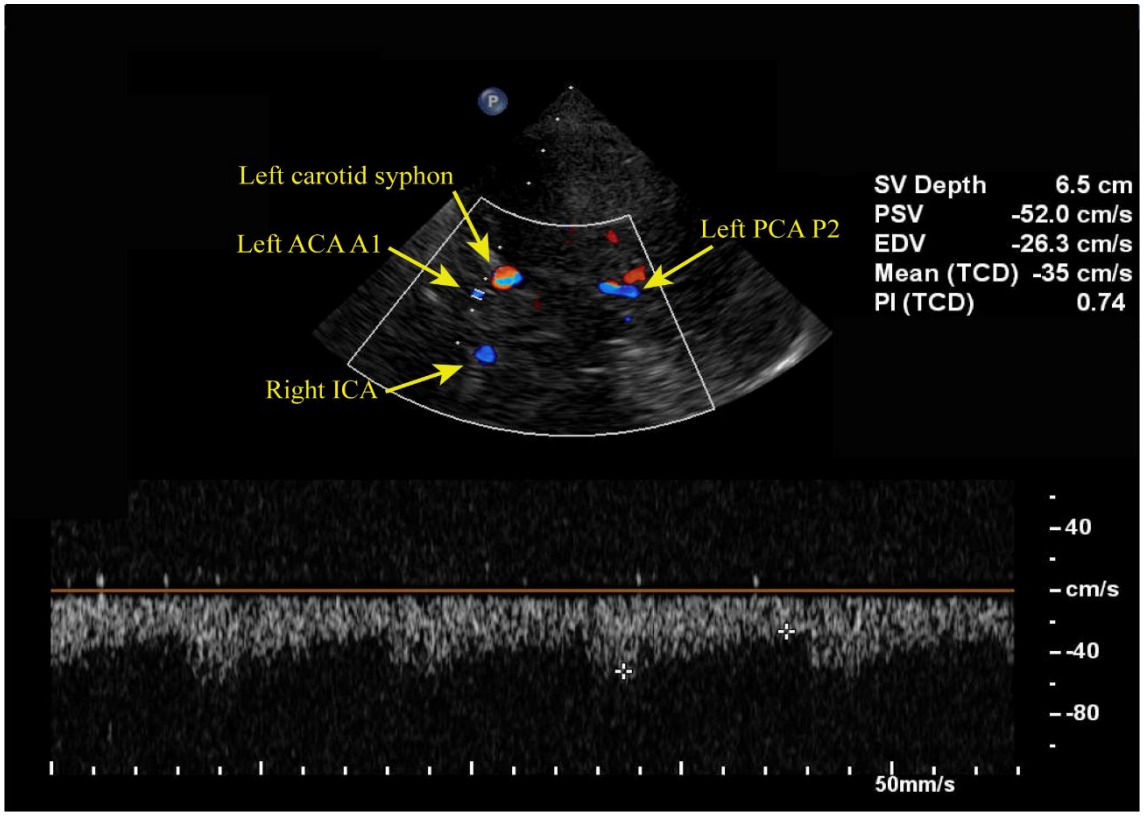

Figure 6. TCCS showing normal flow velocities in the left ACA. ACA: anterior cerebral artery, EDV: end-diastolic velocity, ICA: internal carotid artery, mean (TCD): mean flow velocity, PCA: posterior cerebral artery, PI (TCD): pulsatility index, PSV: peak systolic velocity, SV depth: sample volume depth, TCCS: transcranial color-coded duplex sonography. Courtesy of Dr. Zsolt Garami.

Transcranial insonation utilizes parts of the skull where the bone is thinner (transtemporal window, transorbital window), or naturally absent (transforaminal window), and therefore, Doppler signal from the arteries and veins can be obtained. Transducers with low frequency (usually 2 MHz) are used in order for the ultrasound beam to penetrate the skull. The ophthalmic artery (OA) and the carotid syphon can mainly be detected through the transorbital window, the ACA, MCA, PCA P1 and P2 segments and rarely, the PCoA can be examined through the transtemporal window. The BA and the VA V3 and V4 segments can typically be detected through the transforaminal window. The detection rate through the transtemporal window using transcranial color duplex is very good in case of the ACA (83%), MCA (91%) and PCA (90%) and its P1 (92%) and P2 (94%) segments, but it is poor regarding PCoA (65%) [33, 35-37]. Fair detection rates of the OA (85%) and carotid syphon (67%) were observed with TCCS through the transorbital window,

but the detection rates were excellent when investigated by TCD (100% for the OA and carotid syphon, respectively) [33, 38-42]. Through the transforaminal window, BA was detected in 79–94% [36, 43-45] and VA V4 segment in 89-98% [33, 36, 43, 45].

1.3.1.2. The clinical use of TCD

TCD together with carotid artery ultrasound, MRA or computed tomography angiography (CTA) is a valuable complementary diagnostic method to assess the flow and collateral function in the CoW related to extracranial ICA stenosis [46]. Furthermore, TCD has been used before, during and after vascular surgical procedures that involve clamping of the carotid arteries and consequently affect the CoW flow [47]. These procedures include aortic arch surgery and carotid endarterectomy (CEA) [48, 49]. Preoperative TCD may also assess microemboli and collateral function that is relevant during CEA [50] and intraoperative monitoring may improve clinical outcomes by giving additional real-time information about the decrease of blood flow in the MCA during carotid cross-clamping (and thus, shunt need) and about the presence of microemboli [51]. Configuration of the CoW may also affect the outcome of CEA or may require a shunt to prevent cerebral ischemia [52, 53]. Postoperatively, TCD may be useful in the detection hyperaemia [54]. The real-time function of the CoW can only be examined using transcranial sonographic methods. TCD gives additional information to carotid duplex ultrasound as it can detect the effect of carotid artery stenosis on cerebral hemodynamics and it might be informative when carotid duplex is ambiguous or inconclusive [46]. Figures 7 and 8 show TCD signals in the right MCA at baseline and during CEA.

TCD can detect flow in the OA, ACoA and PCoA if there is flow gradient present in these collaterals [55]. Therefore, these collaterals are visualized better when a lesion is present in the vertebrobasilar or carotid arteries which causes an increased gradient rather than in healthy individuals [55]. Therefore, it may not be an accurate tool to assess the morphology of communicating arteries [55]. Patients with greater than 70% carotid artery stenosis are more likely to have established intracranial collaterals detected by TCD [46]. Anterior circulation ischemic stroke was associated with nonfunctional anterior

collaterals and the prevalence of failing anterior collaterals was greater in patients with stroke related to ICA stenosis than in those with stroke etiology of cardiac emboli [56].

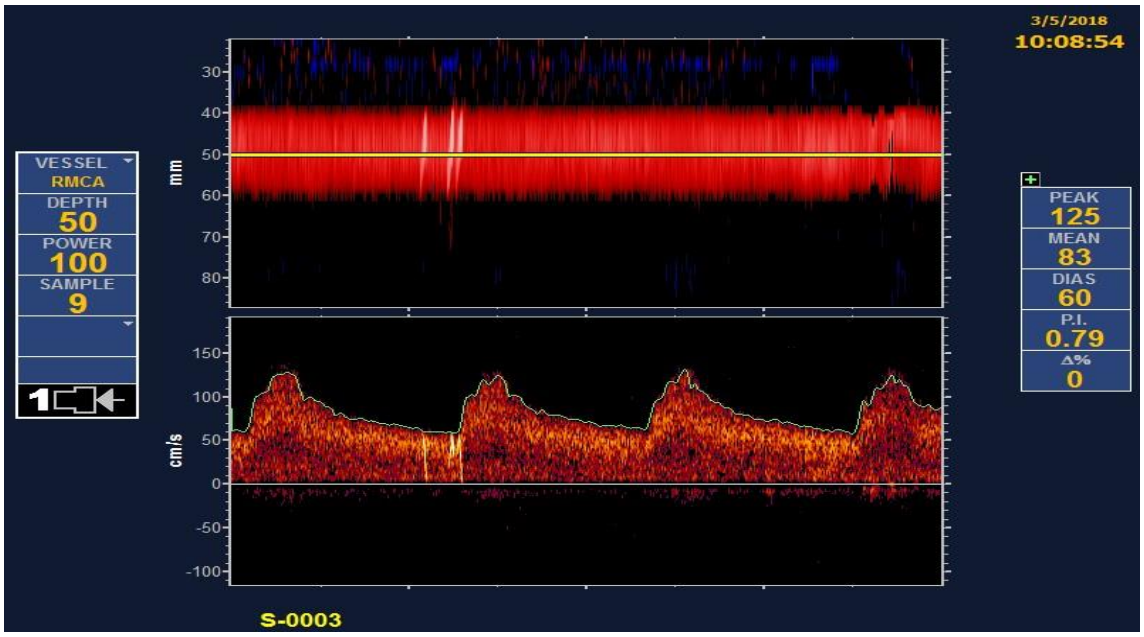

Figure 7. TCD showing normal baseline flow in the right MCA with high-intensity signals related to microemboli. DIAS: end-diastolic velocity, MEAN: mean flow velocity, PEAK: peak systolic velocity, P.I.: pulsatility index, RMCA: right middle cerebral artery, TCD: transcranial Doppler. Courtesy of Dr. Zsolt Garami.

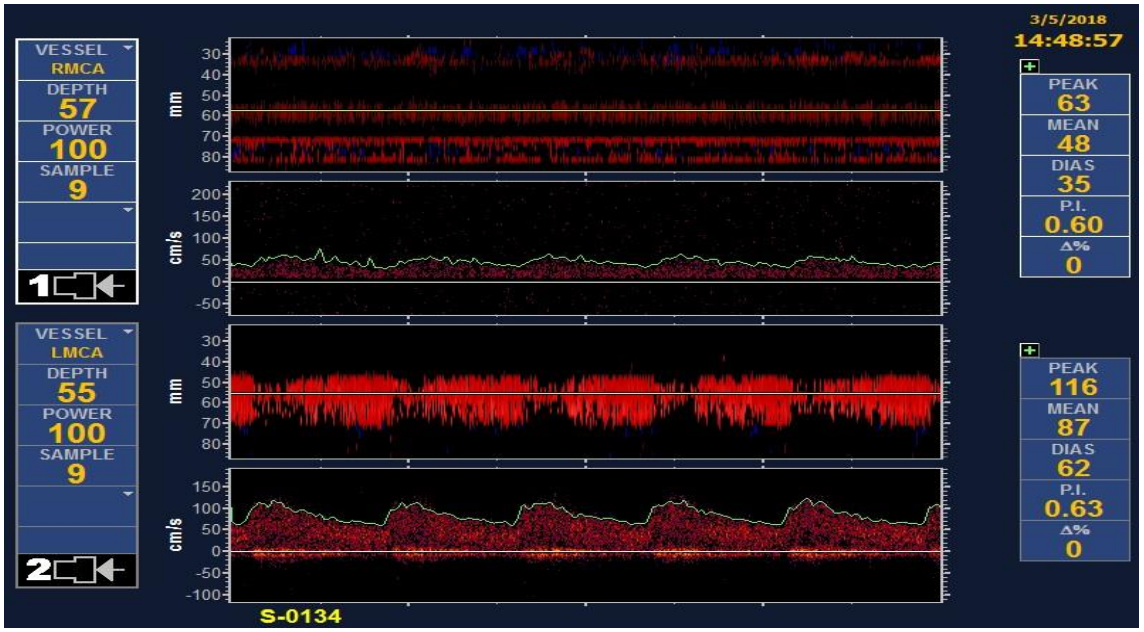

Figure 8. TCD showing decreased flow in the right MCA upon clamping during CEA. CEA: carotid endarterectomy, DIAS: end-diastolic velocity, MEAN: mean flow velocity, PEAK: peak systolic velocity, P.I.: pulsatility index, RMCA: right middle cerebral artery, TCD: transcranial Doppler. Courtesy of Dr. Zsolt Garami.

1.3.1.3. Assessed flow parameters using transcranial insonation

Abnormal flow velocities recorded by transcranial sonography may indicate pathologic conditions. Mean flow velocity (MFV) is the average velocity during the time-velocity waveform [57]:

$$MFV = \frac{PSV + (EDV \times 2)}{3}$$

It is a useful marker of stenosis or occlusion of intracranial vessels and used more often for TCD rather than the absolute PSV and EDV values since it is less influenced by the cardiac output and peripheral resistance [58]. These parameters are illustrated on Figure 9. Resistance index (RI) is calculated by subtracting the EDV from PSV and dividing it by the PSV and is another indicator of vascular resistance distal to the site of insonation [32, 34, 58]:

$$RI = \frac{PSV - EDV}{PSV}$$

Pulsatility index (PI) is used to assess cerebrovascular resistance and is calculated by subtracting EDV from PSV and dividing the value by MFV and is more dependent on the cardiac output than the RI [32, 58]:

$$PI = \frac{PSV - EDV}{MFV}$$

Albeit transcranial sonography is a widespread modality to assess the function of CoW-vessels, their morphology can more precisely be assessed by angiography.

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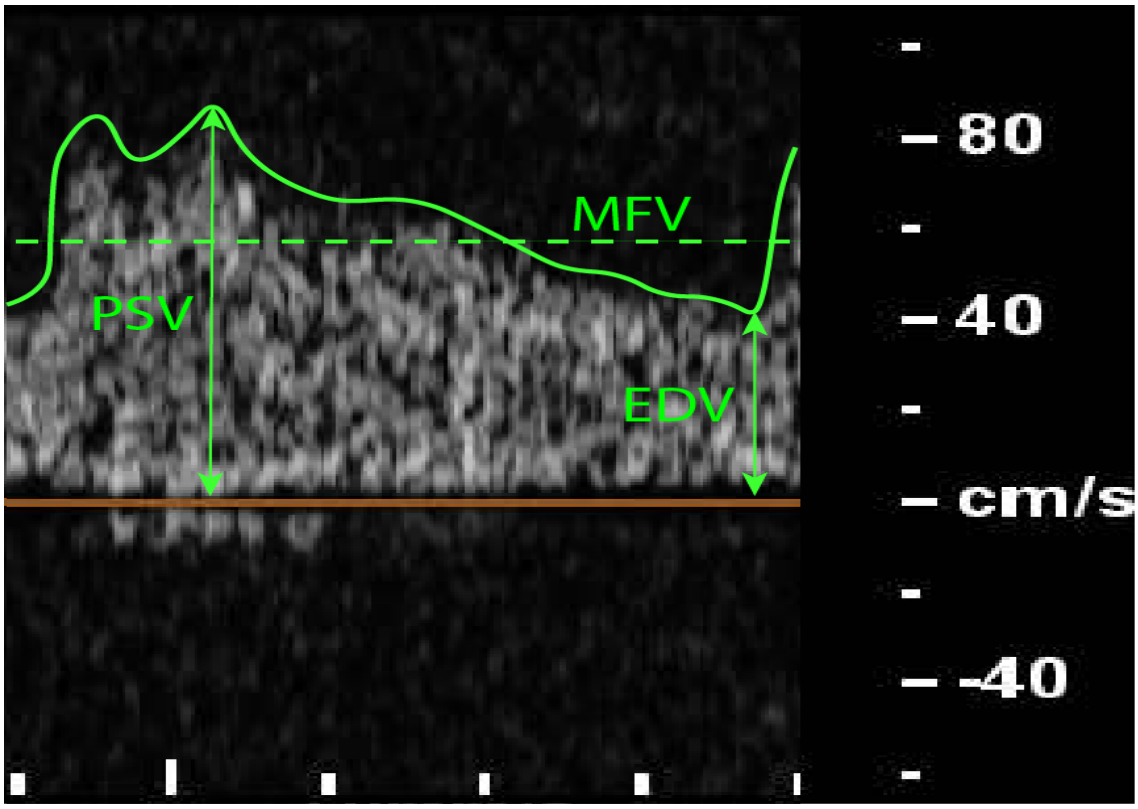

Figure 9. Illustration of measured TCD and TCCS parameters during one cardiac cycle. EDV: end-diastolic velocity, MFV: mean flow velocity, PSV: peak systolic velocity (self-made illustration).

1.3.2. Digital subtraction angiography (DSA)

DSA is the gold standard in the imaging of CoW and is used as a reference modality compared to CTA [59-61], MRA and transcranial ultrasound [37, 62, 63], however, it is an invasive method with risk for complications [64] which requires the administration of contrast media and uses high doses of ionizing irradiation. Another limitation of DSA from a functional aspect is that non-functional collaterals may falsely appear as functional because of the abrupt local increase in the arterial pressure upon the administration of contrast media. Therefore, it is mainly reserved for those patients who are planned to go through vascular interventions and is usually not used as a first-choice diagnostic imaging method as CTA has taken over its role in the acute setting. DSA may also elucidate underlying vessel pathology if there is a disagreement among the other imaging results obtained with CTA or MRA and transcranial ultrasound.

1.3.3. CTA

CTA has also been a popular technique to evaluate CoW variants retrospectively in a research setting [11, 65-67]. CTA uses ionizing irradiation and requires the administration of contrast media which are the most important limiting factors and disadvantages of this modality. This is the reason why CTA is not used for research purposes on healthy (volunteer) populations. On the other hand, CTA is a commonly used modality in stroke patients in an acute setting as the vasculature of the neck as well as the brain and its arteries can be examined quickly with less motion artefacts. Therefore, CTA is commonly used in acute and preoperative settings, allowing to obtain a detailed image on the brain supplying arteries quickly. The non-visualized segments of CoW have a lower prevalence using CTA as compared to MRA because of the better spatial resolution and the absence of flow-dependency. However, compared to the gold standard DSA, the sensitivity of CTA depicting non-hypoplastic CoW vessels is somewhat lower and it is poor regarding the imaging hypoplastic CoW vessels (see chapter 1.3.5.).

1.3.4. MRA

MRA has been extensively used in the research of CoW variants in both healthy individuals as well as patients. MRI does not require ionizing radiation, which is why it is a superior method over CT in healthy volunteers. Furthermore, no contrast agent is needed to visualize vessels with time of flight magnetic resonance angiography (TOF-MRA) technique. On the other hand, TOF-MRA has the disadvantage of long acquisition time and therefore, motion artefacts may appear. Furthermore, visualization of the arteries is highly flow-dependent and therefore, arterial segments with low or turbulent flow may not appear on the image. Consequently, if the blood pressure difference between the anterior and posterior CoW is too low, the PCoA may appear as absent. The relatively low spatial resolution may also be a limiting factor of this imaging method. Despite, it is comparable with contrast-enhanced magnetic resonance angiography (CE-MRA) and CTA. Great efforts are made continuously to improve the imaging of the arteries with TOF MRA and technical advances may eliminate the limiting factors in the near future [68, 69]. Alfke et al. compared each segment of the CoW with CE-MRA and TOF MRA protocols and found that the delineation of the right ACA A1 segment was significantly

better with CE-MRA compared to TOF MRA [70]. No significant difference was observed in any other CoW vessels implying that these imaging methods are comparable and TOF may be utilized when contrast agent administration is not possible, although there was a tendency towards better delineation with CE-MRA [70]. Since CE-MRA has the risk of gadolinium accumulation in the brain and bone tissue even in patients with normal renal function [71], this risk must be taken into consideration in case of gadolinium contrast administration in healthy volunteers.

MRA has been extensively used to determine the prevalence of CoW variants and results are comparable with autopsy studies, although the frequency of "absent" PCoA may be higher because of the aforementioned artefacts [9, 21, 72-75]. Furthermore, MRA is a tool to examine the relation between CoW variants and other neurological conditions such as intracranial aneurysms [76], small vessel disease [77], white matter disease [78, 79], migraine [80, 81] and unilateral thalamic infarction [82]. The CoW variants, such as hypoplasia of one or more vessels are relevant in patients with ICA stenosis or occlusion [23] and CEA because of the possibility of additional pathway for blood flow in these conditions. The CoW may ensure pathways for compensatory flow and the communicant arteries are important regarding shunt need during CEA and regarding its clinical outcomes [53, 83, 84]. MRA has also been used to assess CoW variants in neonates to get a better understanding of the developmental origin of CoW variants. The results are controversial as preterm infants tend to have a considerably higher prevalence of CoW variants compared to adults in one study [85], while another study found higher prevalence of complete CoW in preterm infants compared to term-borns [86].

1.3.5. Comparison of the CoW imaging modalities

Each of the above mentioned modality has its advantages and disadvantages. Combining these techniques usually improves the diagnostic value [87]. Collaterals are difficult to detect with MRA or CTA only. MRA may underestimate the presence of collaterals [88] because of its flow-dependent nature and limited resolution, but its combination with TCD increases the sensitivity, although it still does not reach the sensitivity of the gold standard DSA (92% and 88% for the presence of ACoA and PCoA, respectively, in combined MRA and TCD) [62].

Hetzel et al. applied TCD monitoring during carotid compression and compared the results with the appearance of interhemispheric crossover-flow through the ACoA during temporary balloon occlusion of the ICA [89]. More than 60% decrease in the contralateral MCA MFV and more than 50% increase in the contralateral ACA MFV correlated well with the grade of interhemispheric cross-over flow detected on angiography (positive predictive value 84% for MFV decrease in the MCA and 96% for MFV increase in the ACA, respectively) [89]. Sensitivity and specificity of TCCS without carotid compression test was 98% and 100% for the detection of collateral flow through the ACoA and 84% and 94% for the PCoA, respectively, when compared to DSA [37]. Another study compared the collaterals identified on MRA to those detectable with TCCS under manual carotid artery compression. The sensitivity of MRA was 85% and 87% for the two readers and the specificity was 81% and 67%, respectively [90]. Orosz et al. applied a mathematical model based on autopsy data on the CoW vessels and compared it to in vivo TCCS findings. They demonstrated that functional and non-functional collaterals differ in diameter and flow velocities and depend on the hemodynamic gradient in the collateral vessel (pressure difference between the two ends of the communicating arteries) [91]. When compared to CTA, TCD had a sensitivity of 76% in a small population (n=15) with cerebral ischemia [92].

Importantly, there are some positive and negative features of TCD and TCCS that have to be mentioned. The B-mode in TCCS is useful in the visualisation of vessel anatomy because of the anatomical landmarks and because of the use of color Doppler. Flow velocity measurements are more accurate with TCCS compared to TCD since angle correction is possible with TCCS but not with TCD [93, 94]. On the other hand, the TCCS machine is less portable and more expensive and the TCCS probe is larger and requires a larger insonation window which may lead to the inability to obtain the image. Despite this technical difficulty, Krejza et al. demonstrated that the rate of successful insonation is comparable using TCD and TCCS in healthy individuals [95]. Still, TCD has become more widespread, for example in an intraoperative setting such as during carotid artery surgery.

Through the B-mode and color image, CoW anatomy can be assessed which allows TCCS to detect certain CoW variants. However, assessing the hypoplasia or absence of primary collaterals, that is PCoA and ACoA, is a great challenge especially in healthy individuals,

partly because of the poor resolution, partly because only functioning arteries are visualised. The threshold for collaterals detected by TCCS as functional is between 0.4 and 0.6 mm on autopsy studies [96], but TCCS is not suitable to assess hypoplasia or absence the same way as MRA or CTA does, as the diameter cannot be accurately measured by TCCS. However, other branching anomalies in the CoW and FTP can be assessed using TCCS. In fact, MRA may miss a substantial number of collaterals that are potentially functional [90].

TCD or TCCS have not been proven to be superior methods to CTA or MRA regarding the imaging of CoW vessels, but on the other hand, they provide valuable complementary information on real-time hemodynamics that none of the latter modalities do [92]. The sonographer and interpretational skills, the bone windows and the time between the TCD and CTA studies have a great influence on the agreement of the two modalities [92]. The spatial resolution of CTA is greater than that of MRA, furthermore, CTA is not flow-dependent as compared to MRA. CTA post-processed with volume-rendering has been compared to the gold standard DSA [59]. Sensitivity of the detection of non-hypoplastic vessels and absent vessels was 97.9% and 96.6%, respectively. However, the sensitivity was poor, 52.6% regarding the detection of hypoplasia, mostly attributable to the communicating arteries [59]. CTA may overestimate the presence of collaterals due to overlapping veins or adjacent arteries [59]. On the other hand, CTA is highly accurate in the visualisation of FTP compared to DSA [61].

The choice of modality becomes of key importance in case of acute cerebrovascular events. Recognizing CoW-variants can add valuable information to the diagnostic procedure as well as surgical planning.

1.4. The clinical relevance of cerebrovascular variants

1.4.1. The association between cerebral ischaemia and CoW variants

Hypoplasia or aplasia of the communicating arteries may predispose to hemodynamic ischemic stroke in patients with severe stenosis or occlusion of the ICA [28, 97, 98]. An appropriate flow in the collateral arteries is of great importance regarding the occurrence and extent of cerebral ischemia. The collateral flow in the CoW has been widely investigated in patients with ICA occlusion or stenosis, and intact collaterals seem to have key importance in maintaining cerebral perfusion in this patient group [28, 72, 99-103] as the collaterals may compensate for the restricted flow in the stenotic vessel, which is the major artery ensuring cerebral blood supply [102, 104, 105]. Varga et al. found in a recent CTA study that the prevalence of brain ischemia detected on CT was increased in patients with more hypoplastic or absent segments of the CoW [66]. ICA stenosis was the only independent predictor of CoW variants, furthermore, ACA A1 hypoplasia showed a significantly higher rate in patients with more than 90% ICA stenosis [66]. Waaijer et al. also found significantly higher prevalence of hypoplasia or absence in the anterior CoW in patients with symptomatic ICA stenosis [67]. As for haemodynamic aspects, non-functional anterior and posterior collaterals had a higher prevalence in patients with anterior circulation stroke compared to patients with asymptomatic carotid artery disease [56]. Variations of the CoW were associated with greater risk of stroke in case of hypoplastic or absent segments of the CoW. Prospective studies have confirmed that incomplete anterior CoW is significantly associated with anterior circulation stroke in patients without prior cerebrovascular disease and the risk is even higher if incomplete posterior CoW is present simultaneously [106]. Accordingly, ischemic cerebral changes associated with CoW variants are often detected in clinical practice as shown in Figure 10. The association between stroke and CoW variants is a key issue, but other neurological pathological states may also be related to CoW variants.

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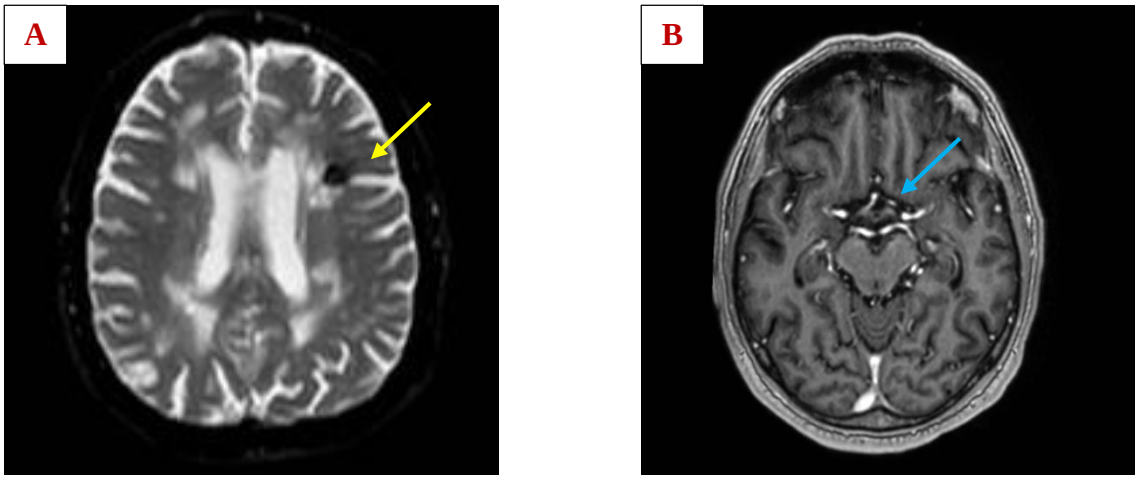

Figure 10. ADC map (A) of a 68-year-old male patient shows subacute ischemic changes at the frontal horn of the left lateral ventricle (yellow arrow). CE-MRA (B) shows left ACA A1 hypoplasia (blue arrow). ACA: anterior cerebral artery, ADC: apparent diffusion coefficient, CE-MRA: contrast-enhanced magnetic resonance angiography. Image from the Department of Radiology, Semmelweis University.

1.4.2. Fetal type posterior circulation (FTP) and its association with cerebrovascular pathologies

Not only the communicating arteries are of crucial significance regarding collateral flow in the CoW. An especially important location in the posterior CoW is the PCA P1 segment. FTP may predispose to cerebral ischemia. According to the study of Arjal et al., having partial FTP means a 3-fold risk for ischemic stroke [15]. No significant difference was observed when comparing patients with normal PCA, partial and complete FTP regarding the demographics and severity of stroke and the involved intracranial vessels [16]. However, complete FTP was significantly associated with large-vessel stroke etiology as compared to partial FTP and non-variant PCA which could be explained by the lack of thalamic perforators contralaterally [16]. Other authors described an association between posterior circulation ischaemia and FTP when a hypoplastic vertebrobasilar system is present simultaneously [107], as shown in Figure 11. FTP was not associated with increased risk for PCA territory infarct in another study [17]. Thus, results on the clinical relevance of the FTP variant remain inconclusive.

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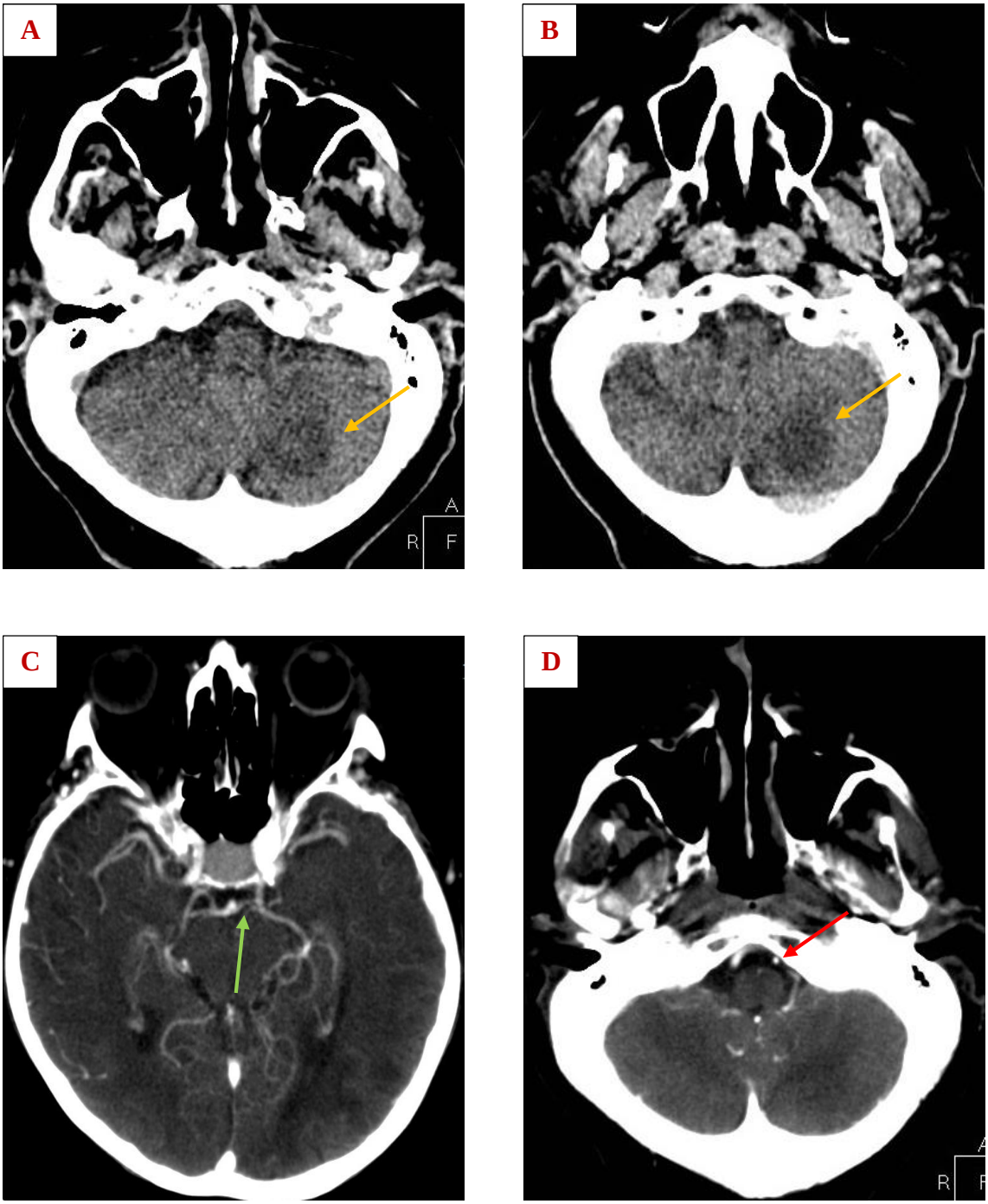

Figure 11. Unenhanced CT (A, B) shows subacute ischemic changes in the left cerebellar hemisphere (orange arrows) in a 71-year-old male patient. CT angiography (C, D) shows left PCA P1 hypoplasia (light green arrow) and left VA V4 segment hypoplasia (red arrow). CT: computed tomography, PCA: posterior cerebral artery, VA: vertebral artery. Image from Department of Radiology, Semmelweis University.

1.4.3. Further diseases possibly associated with CoW variants

Other neurological conditions have been investigated in relation to CoW variants as well, emphasizing the significance of CoW variants. The relationship between CoW variants and intracranial aneurysms has been intensively researched [76, 108]. A higher incidence of cerebral artery aneurysms has been observed in CoW variants as well as a higher recurrence rate following endovascular procedures [109]. There might be an association between the variant CoW anatomy and white matter disease [78, 79, 110-112], migraine with aura and mesial temporal sclerosis [113, 114]. Interestingly, the causative role of cerebrovascular variants in hypertension was assumed warranting further research in this field [115].

Translational research and studies in humans have contributed significantly to our knowledge on the origin and the consequences of CoW variants, but certain details are still to be elucidated.

1.5. Previous results on the genetic background on CoW variants

1.5.1. Animal studies

Animal studies conducted on rodents provided important information regarding the genetic background of CoW variants. Recently, Faber et al. examined genetic, environmental and potential stochastic factors on the PCoA morphology in mouse strains with different genetic background [116]. A great variation of PCoA diameter and status (unilateral, bilateral or absent) exists between these strains indicating that the naturally occuring genetic constellation may be (at least, partly) responsible for the PCoA variants [116, 117]. Importantly, the differences in PCoA configuration were not only observed between strains, but also within some strains indicating the role of random, stochastic factors besides genetic effects [116]. This was underlined by the fact that low birth weight did not have a significant impact on the PCoA constellation, indicating that in utero random or stochastic factors may be responsible for the inter-individual differences within one strain [116]. Besides in-utero stochastic factors, the role of unique environmental factors has also been investigated. In order to make conclusions on potential environmental factors that contribute to changes in PCoA anatomy, the authors

also examined mice with mutations that predispose to obesity, hyperlipidemia, metabolic syndrome, type 2 and type 1 diabetes mellitus [116]. Aging and hypertension decreased PCoA diameter significantly, while the other aforementioned cardiovascular risk factors did not have a significant impact on PCoA variants [116]. Du et al. emphasized the inheritance of ACoA variants, mainly from the mother's side [118]. The prevalence of incomplete ACoA increased from 35% in the first generation to 63% in the fifth generation by selectively breeding gerbils with incomplete ACoA [118]. Similar tendency (although less marked differences) was observed regarding the PCoA. The ACoA morphology of offspring showed similarity with that of the maternal ACoA pattern in 60.4% [118], however this does not allow any conclusion on heritability. Li et al. identified four genes related to CoW variants that were selected from a pool of vasculogenesis and angiogenesis related genes in gerbils, however, the association between these genes and the CoW phenotypes cannot certainly be established based on these results [119].

Other authors investigated the possible effects on CoW formation during embryogenesis on a molecular level. The vascular endothelial growth factor (VEGF) has an important role in endothelial cell proliferation and the Notch-signaling pathway is needed for the maturation and differentiation of vascular smooth muscle cells [120]. Notch-signaling deficient vascular smooth muscle cells may predispose to aberrant ACoA formation [120-122].

Luna et al. investigated the association between placental growth factor (PGF) deficiency and CoW development in mice and described smaller ACA diameter and less collaterals compared to the non-affected animals [123]. The results could have important clinical implications regarding preeclampsia where decreased PGF levels are suspected [123].

1.5.2. Twin and family studies in humans

In humans, twin and family study design provides a possibility to assess the role of heritability in the development of the CoW variants. Twin studies are based on the assumption that monozygotic (MZ) twins share almost 100% of their genes, whereas dizygotic (DZ) twins share in average 50% of their genes just like non-twin siblings.

Applying the so called ACE statistical model allows conclusions not only on heritability, but also on the proportion of common and individual environmental factors affecting a given phenotype (“A” stands for additive genetics, “C” stand for common environment and “E” stands for the individual, unshared environment). The “C” component usually refers to the shared household (see in chapter 3.2.3.). This decomposition is not possible in family studies, meaning a great advantage for twin studies. Instead, family studies use a ratio to suggest the risk of relatives developing the same phenotype.

Recently, a family study conducted on families with intracranial aneurysm concluded that incomplete PCoA occurred almost 3-times more often in the index families compared to the controls (odds ratio, OR 2.8, 95%, confidence interval, CI 1.8-4.3) and that the prevalence of this variant was significantly higher within one family [108]. ORs were less in all other examined CoW variants (OR 1.7 95% CI 0.8–3.4 for classical CoW, OR 1.1 95% CI 0.5–2.5 for A1 asymmetry, OR 1.5 95% CI 0.8–3.1 for FTP and OR 2.2 95% CI 1.6–3.0 for any variation) [108].

According to our knowledge, no twin study has investigated the morphology of CoW variants and their heritability previously.

2. Objectives

Although the importance of vascular variants is discussed and widely researched, their developmental background remains unclear. A number of other stochastic and environmental factors might affect the formation of CoW variants. Thus, assessing the genetic versus environmental origin of CoW variants could also provide knowledge of genetic predispositions for offspring and could help to understand the role of modifiable environmental factors. Through twin studies it is possible to assess whether heritability plays a significant role in the formation of the variants of CoW or they are influenced mostly by other (environmental or stochastic) factors. Twin studies provide the ideal study model to assess the contribution of genetic versus environmental factors to a certain phenotype. The phenotypes we chose to investigate were the CoW variants assessed by MRA on a Hungarian twin sample and by TCCS on an Italian twin sample as well as the intracerebral hemodynamic factors assessed by TCD in the Hungarian sample and TCCS in the Italian twin sample. Heritability on the CoW morphology alone does not provide enough information, which is why we aimed to study both morphology and hemodynamics of the CoW in healthy individuals. Hypothetically, if the heritability of CoW variants in humans is high, then genetic factors have to be considered. Possibly, in this case studies on the possible benefit of screening could be investigated for offspring of individuals with CoW-variants, if our results are confirmed. On the contrary, if environmental factors dominate over genetics regarding CoW variants, then prevention should be emphasized with focus on the relevant environmental factors that show an association with CoW variants. Accordingly, the aim of our study was to analyse the impact of environmental and genetic factors on CoW morphology and hemodynamic parameters in Caucasian twin samples assessed by MRA, TCD and TCCS.

3. Methods

3.1. Subjects

Two independent samples were involved to investigate the morphology and hemodynamics of the CoW variants and their genetic and environmental background. Our first study population consisted of healthy Caucasian twins who are members of the Hungarian Twin Registry [124]. These twin pairs underwent MRA and TCD studies. Italian twins were involved in the second study who underwent TCCS. The second project was based on an international collaboration between the Hungarian Twin Registry and the Italian Twin Registry [125]. Members of the Hungarian Twin Registry attended the measurements and helped with data collection.

3.1.1. Sample of the MRA and TCD study

120 Hungarian twins attended the study (60 pairs), of whom 38 pairs were MZ and 22 were DZ after the exclusion of 4 twin pairs. These twin pairs had to be excluded either because they did not show up at the scheduled appointment for MRI (3 pairs) or because of claustrophobia (1 pair).

3.1.2. Sample of the TCCS study

64 Italian twins (32 twin pairs), 19 MZ pairs and 13 DZ pairs were analysed. The study population consisted of the members of the Italian Twin Registry who did not have any medical complaints and were older than 18 years of age [124, 126].

3.2. Study designs

3.2.1. Design of the MRA and TCD study

The investigation was approved by the regional ethical committees (Regional/Local Committee of Science and Research Ethics Borsod-Abaúj-Zemplén, Heves and Nógrád County, approval number: 1046-260/2014, 2014/09/22 as well as Semmelweis University Regional and Institutional Committee of Science and Research Ethics 189/2014, 2014/10/21). All participating Hungarian twins signed an informed consent. The tenets of the Declaration of Helsinki were followed. A multiple self-reported questionnaire

based on a seven-part, self-reported response was used to maximize the accuracy of zygosity classification [127]. Detailed history and risk factors were obtained by a questionnaire, collecting data on body weight and height, body mass index (BMI), smoking, hypertension, diabetes mellitus and hypercholesterolemia. Both former and current smokers were included in the smoking group.

The twins underwent 3D TOF MRA of the cerebral arteries if the MRI study was not contraindicated. Patients were excluded with any of the following conditions: pregnancy or positive pregnancy test, breast-feeding, immunosuppressive or immunomodulant therapy including systemic steroids in the past 30 days, chemotherapy within 1 year, major surgery within 2 months, transfusion or blood products received within 2 months. Moreover, pacemaker, implantable cardioverter-defibrillator or other implanted devices, magnetic metal foreign bodies, claustrophobia and aphasia were regarded as exclusion criteria. The studies were performed in part in the Borsod-Abaúj-Zemplén County University Teaching Hospital in Miskolc, Hungary and partly at Semmelweis University Magnetic Resonance Research Centre. Members of a twin pair were examined the same day by a professional operator.

3.2.1.1. MRA study

T1-weightened coronal, T2-weightened sagittal, axial trace-weighted diffusion, axial apparent diffusion coefficient, axial proton density and axial T2 dark fluid images of the brain were obtained. TOF MRA was obtained on the cranial vessels. No contrast agent was applied. The 3D multi-slab TOF MRAs were reconstructed using maximal intensity projection (MIP). At Borsod-Abaúj-Zemplén County University Teaching Hospital measurements were performed on a Siemens Magnetom Verio 3 Tesla scanner (Siemens Healthcare GmbH, Erlangen, Germany). The following imaging parameters were used to obtain the TOF MRA images: RT/ET 21/3.43 ms, flip angle 18°, 768 x 768 matrix, 0.5 mm slice thickness. At the second study location on a Philips Ingenia 3 Tesla scanner (Philips Healthcare, Best, The Netherlands) was used with the following TOF MRA imaging parameters: RT/ET 23/3.45 ms, flip angle 18°, 552 x 332 matrix, 1.2 mm slice thickness.

Evaluation of the CoW was made both on MIP and multi-slice MR images. A single observer performed all measurements in the project. This observer was blinded to zygosity and all clinical information.

We investigated CoW variants from two points of view [11, 23, 90]. First, we analysed each vessel of the CoW and determined if they were non-hypoplastic, hypoplastic or absent. Vessel diameters were measured on transverse slices of the 3D multi-slab TOF MR-angiograms. We used a forced-choice method to define hypoplasia and absence of the vessels, which means that non-visible vessels were defined as absent [90]. Visible arteries with a diameter under 0.8 mm were defined as hypoplastic [23]. Secondly, we categorized the anterior and posterior CoW variants separately according to their appearance. We used the morphological classification described by Hartkamp et al. and Li et al. as a basis [11, 23]. However, we made some modifications in their categories because some were not applicable to our findings. Figures 2 and 3 show our classification of the anterior and posterior CoW variants.

3.2.1.2. Hemodynamic assessment using TCD

TCD was performed on the Hungarian twins by the same investigator with the same ultrasound system (Multi-Dop T, Compumedics Germany GmbH, Singen, Germany) with a 2 MHz transducer. Through the temporal and transforaminal windows, MFV and PI were recorded bilaterally from the M1 segment of the MCA, the ACA A1 segment, P1 segment of the PCA and the BA. Flow direction was determined on all the aforementioned vascular segments, which was normal in all individuals.

3.2.2. Design of the TCCS study

The TCCS investigations were conducted at the Neurosonological Laboratory of the University of Padua in 2014 on Italian twins. All study subjects underwent TCCS and a personal interview. Exclusion criteria were pregnancy, acute infectious disease, history of neurosurgical intervention, twin pairs dysgenesis, as in the latter case gender-dependent environmental factors and chromosome X effects may influence the results. Prior to the examination, each participant signed an informed consent, after having been

verbally informed about the course and purpose of the study. During the research, the principles of the Helsinki Declaration were followed. For each subject, a complete history was obtained, body height and body weight were recorded, and BMI was calculated. Peripheral systolic blood pressure (SBP) and diastolic blood pressure (DBP) was obtained on each individual and pulse pressure (PP) and mean arterial pressure (MAP) was calculated.

3.2.2.1. Hemodynamic assessment using TCCS

TCCS was performed always by the same investigator with the same ultrasound system (Toshiba Aplio XG) with a 2-4 MHz transducer (transducer type: PLT-30BT). Through the transtemporal and transforaminal windows, PSV as well as EDV were recorded from the M1 segment of the MCA, the A1 segment of the ACA, P1, P2a and P2p segments of the PCA, the BA and the V4 segment of the VA. The reported PCA velocity values were actually the mean PSV and EDV of the P1, P2a and P2p segments. The MFV and PI were calculated from the PSV and EDV values. Flow direction was determined on all the aforementioned segments. Normal flow direction was observed in all cases and therefore this trait was not included in further statistical analysis. Anatomical variations of the CoW were recorded; in particular, the missing segments of the CoW were registered as well as those variants where the origin of a given vessel was in an unusual location.

3.3. Statistical analysis

IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, N.Y., USA) and Microsoft Excel 2016 was used for all statistical analysis.

3.3.1. MZ-DZ comparison

3.3.1.1. Continuous variables

We tested whether the variables fulfil the assumptions of the independent-samples t-test in both the MRA-TCD and the TCCS study in order to compare the means of the variables

between the MZ and DZ group. Histograms were obtained on the continuous variables in the MZ and DZ group separately and Shapiro-Wilk test was performed in order to test for normal distribution of the continuous data. If the data showed non-normal distribution in either the MZ or the DZ group, then transformation of the data was performed by obtaining inverse, square, square root, cube root, base-10 logarithm and natural logarithm of the variables. If the transformed data showed normal distribution and non-significant p-values of the Shapiro-Wilk test in both groups, then the homogeneity of variance was tested using Fisher's F-test. If the homogeneity of variances assumption was not violated, then independent samples t-test was performed. We used Welch-test to compare means between variables in the two groups with normal distribution but significantly different variances. These variables included the right MCA EDV, the left ACA EDV, the right PCA PI and the BA PI in the TCCS study.

Accordingly, we used the reciprocal values of the BMI in the MRA-TCD study to perform the parametric independent samples t-test when comparing means between the MZ and DZ groups. In the TCCS study, base-10 logarithmic transformation for BMI and reciprocal transformation for SBP was applied, respectively. Reciprocal values of left and right MCA PI were used when comparing the means between the MZ and DZ groups in the TCCS study.

If the assumption of normal distribution remained violated after data transformation, then we compared the MZ and DZ groups regarding the continuous variable using the non-parametric Mann-Whitney U-test. This was applied when comparing the MZ and DZ groups regarding age in both the MRA-TCD and the TCCS study as the assumptions for parametric tests were violated. Furthermore, the same test was used when comparing means of left VA PI between MZ and DZ groups as this variable showed non-normal distribution between the groups and remained similar after transformation.

3.3.1.2. Dichotomous variables

Fisher's exact test was applied to investigate differences between MZ and DZ twins regarding sex, smoking, diabetes, hypertension and hypercholesterolemia in the MRA-TCD study as well as the difference between the MZ and DZ groups regarding sex in the TCCS study.

3.3.2. Comparison of TCCS hemodynamic parameters between men and women

When comparing the measured TCCS parameters between men and women, we used a similar approach regarding statistical analysis as for the MZ-DZ comparisons (see detailed description in 3.2.3.1.). The variables that did not have normal distribution included BA PSV, left VA EDV, right MCA PI, left MCA PI, left PCA PI and left VA PI. Normal distribution was obtained by square root transformation of the BA PSV and left PCA PI as well as through reciprocal transformation of the left MCA PI and base-10 logarithm transformation of left VA EDV values. All of these transformed variables showed homogeneity of variances between men and women except for the left PCA PI, where Welch-test was used for the comparison of means. Welch-test was also applied for the non-transformed right MCA MFV values as the homogeneity of variances assumption was violated when comparing means between genders.

The right MCA PI and the left VA PI showed non-normal distribution even after transformation. Non-parametric Mann-Whitney U-test was used to compare the means between men and women regarding these variables.

3.3.3. Correlations of the TCCS parameters

Correlations between the TCCS parameters and age, BMI, SBP, DBP, PP and MAP were conducted. If both correlated variables showed normal distribution, then Pearson's correlation was conducted. In all other cases we used Spearman's correlation. The variables that did not show normal distribution included age, SBP, PP, right PCA EDV, right VA PSV, right VA MFV, right MCA PI, left MCA PI, right VA PI, left VA PI and BA PI.

3.3.4. The twin study concept

3.3.4.1. Heritability and concordance rates

Twin studies aim to determine the genetic and environmental basis of a certain phenotypic variance. The concept of twin studies is based on the assumption that MZ twins share almost 100% of their genes on average, whereas DZ twins share in average only 50% of

their genes similarly to non-twin siblings. Under this assumption the following equations can be made, where A stands for additive genetic effects (heritability), E stands for unique, non-shared environmental effects, C stands for common environmental effects and D stands for dominant genetic effects (such as epistasis), rMZ stands for MZ intra-pair correlations and rDZ stands for DZ intra-pair correlations:

variance (A) / total variance (P) = a2 = 2 (rMZ - rDZ)

variance (E) / total variance (P) = e2 = 1 - rMZ

variance (C) / total variance (P) = c2 = 2rDZ - rMZ

variance (D) / total variance (P) = d2 = 2rMZ - 4rDZ

The contribution of these parameters to the phenotypic variance would be calculated as following (assuming that MZ and DZ genetic resemblance is 100% and 50%, respectively):

Variance = A + D + C + E

MZ covariance: A + D + C

DZ covariance: ½ A + ¼ D + C

However, the 4 unknowns cannot be determined using 3 equations, and therefore, A, C, D, and E components cannot be determined at once. Either the C or the D component has to be assumed to equal zero. Usually the D component is fixed to zero and the following equation is used to determine the heritability (additive genetic component behind phenotypic variance) [128]:

2×(rMZ-rDZ)

This is called raw heritability, which we determined regarding continuous hemodynamic parameters measured in TCCS tests. MZ and DZ phenotypic intra-pair correlations (rMZ and rDZ) were counted using Pearson's correlation coefficient.

However, if rMZ is much higher than 2rDZ, then this formula is not correct and no other formula can be used to determine heritability of a trait. If the value of rMZ is between 2rDZ and 4rDZ then the role of dominant genetic effects has to be assumed and the

following formula has to be used to determine the heritability (additive genetic effects) [129]:

$$4rDZ\text{-}rMZ$$

However, raw heritability does not provide any information on the shared and non-shared environmental component. If MZ twins have more similar phenotypes than DZ twins, then there is a bigger contribution to the phenotypic variance by genetic effects. Furthermore, twins share the same early environment and therefore, common and non-common environmental factors can be estimated using ACE-model, where A stands for additive genetics, C stands for common shared environmental factors and E stands for unique environmental factors. In the current study, this detailed analysis was not possible due to the small study population.

The similarity between co-twins was estimated with concordance rate in case of categorical variables (which were used in the current study for CoW variants) [130].

We determined the concordance rates of anterior and posterior CoW morphology separately in the MRA study and for the whole CoW in the TCCS study. Pairwise concordance rates were estimated by the following formula [131]:

$$\frac{n\ concordant\ twin\ pairs}{n\ concordant\ twin\ pairs + n\ discordant\ twin\ pairs}$$

A pair of twins was defined as concordant if both members of the pair had any kind of variant CoW anatomy. Historically, twin pairs where both members are unaffected regarding a given phenotype (which correspond to CoW variants in this work) are not considered concordant. Typically, concordance rates in twin studies are calculated on the presence or absence of a disease, for example schizophrenia [132], diabetes mellitus [133] or rheumatoid arthritis [134]. Concordance rate is an expression of the probability of one twin having the investigated phenotype if his or her co-twin is affected. Therefore, we did not consider twin pairs as concordant if both members had normal CoW anatomy. In discordant twin pairs, one member of the twin pair had classical CoW anatomy and the other member had variant anatomy in the CoW. Both anterior and posterior CoW parts were investigated separately according to these categories in the MRA and TCD study, whereas the CoW as a whole was investigated in the TCCS study.

Chi-square test of homogeneity was conducted in both the MRA-TCD and the TCCS sample to assess proportions of the CoW variants between the MZ and DZ groups. The test yielded non-significant results regarding both samples indicating that the MZ and DZ groups were homogenous regarding the proportion of CoW variants.

3.3.4.2. Statistical analysis in MZ discordant twins in the MRA-TCD study

We examined the intra-pair differences regarding cardiovascular risk factors as well as TCD parameters in MZ twins discordant to anterior or posterior CoW variants. Members of a twin pair were regarded as non-independent groups. Accordingly, we chose statistical tests for this purpose which do not assume independent samples.

Since BMI was not normally distributed across members of the MZ discordant twin pair, we used reciprocal transformation in order to use a parametric test. The inverted BMI values were re-tested for normality and homogeneity of variance and paired t-test was used as the transformed values fulfilled the criteria for paired t-test. Similarly, paired t-test was used regarding the majority of TCD parameters MRA-TCD study. Some hemodynamic parameters were not normally distributed and remained non-normally distributed after transformation. Non-parametric Wilcoxon-signed rank test was performed in these parameters. These TCD parameters included RPCA MFV and LACA PI. Dichotomous variables were compared within the MZ discordant twin pairs using McNemar’s test.

4. Results

4.1. Clinical characteristics

4.1.1. MRA and TCD study in Hungarian twins

The median age was 49.5 and 55.0 years in the MZ and DZ group, respectively. There was no significant difference regarding average age between the two groups (p=0.26). Similarly, no significant difference was demonstrated between the MZ and DZ groups regarding smoking, diabetes and hypertension, however, a significant difference was observed regarding BMI (p=0.01) and hypercholesterolemia (p=0.01) between the two groups. Table 1 shows the characteristics of the MRA and TCD study in the Hungarian study population.

Table 1. Characteristics of the MRA and TCD study population. BMI: body mass index, DZ: dizygotic, MRA: magnetic resonance angiography, MZ: monozygotic, TCD: transcranial Doppler. Data are shown as median±interquartile range with continuous variables. § indicates that the comparison was made on transformed data with independent samples t-test and † indicates the results of the non-parametric Mann-Whitney U-test. P-values for dichotomous variables are calculated by Fisher's exact test. Significant results are marked with asterisk.

	Total (n=120)	MZ (n=76)	DZ (n=44)	p-value
Zigosity (*n*MZ:*n*DZ)	76:44	-	-	-
Sex (male:female)	38:82	26:50	12:32	0.54
Age (years)	52.0±20.8	49.5±25.0	55.0±14.0	0.26†
BMI	25.5±6.2	24.8±6.6	26.7±5.7	0.01§*
Smoking *n*(%)	24(20.0)	16(13.3)	8(6.7)	0.81
Diabetes *n*(%)	10(8.3)	4(3.3)	6(5.0)	0.17
Hypertension *n*(%)	28(23.3)	15(12.5)	13(10.8)	0.25
Hypercholesterolemia *n(%)*	15(12.5)	5(4.2)	10(8.3)	0.01*

4.1.2. TCCS study in Italian twins

The data of 19 MZ (38 persons) and 13 DZ Italian twin pairs (26 persons) were analysed after the exclusion of two MZ twin pairs due to inadequate acoustic window. The median age was 43.0 years in both the MZ and the DZ twins. There was no significant difference between MZ and DZ twins in terms of sex and age. Conversely, there was a significant difference in BMI across zygosity (p=0.01). No significant difference was observed regarding SBP, DBP, PP and MAP between the two groups. Table 2 shows the characteristics of the TCCS study population.

Table 2. Characteristics of the Italian TCCS twin study population. BMI: body mass index, DBP: diastolic blood pressure, DZ: dizygotic, MAP: mean arterial pressure, MZ: monozygotic, SBP: systolic blood pressure, PP: pulse pressure, TCCS: transcranial color-coded duplex sonography. Data are shown as mean±standard deviation with continuous variables that have normal distribution (DBP, PP, MAP) and as median±interquartile range with continuous variables that have non-normal distribution (age, BMI, SBP). § indicates that the comparison was made on transformed data with independent samples t-test and † indicates the results of the non-parametric Mann-Whitney U-test. All other p-values for continuous variables were calculated on non-transformed data with independent-samples t-test. P-values for dichotomous variables are calculated by Fisher's exact test.

	Total	MZ	DZ	p-value
Zigosity (number of twin pairs)	32	19	13	-
Sex (male:female)	23:41	14:24	9:17	1.00
Age (years)	43.0±23.0	43.0±22.0	43.0±20.0	0.57†
BMI	23.0±4.0	22.0±4.0	25.0±4.0	0.01§*
SBP	128.0±25.0	129.0±24.0	128.0±30.0	0.54§
DBP	81.2±11.9	81.5±12.3	80.7±11.5	0.80
PP	49.2±11.1	49.8±11.5	48.3±10.8	0.59
MAP	97.6±13.5	98.1±13.4	96.8±13.7	0.71

4.2. Results of the MRA and TCD study

4.2.1. The prevalence of CoW variants

Table 3 and 4 show the prevalence of variants of the anterior and posterior CoW. The most common anterior CoW configuration was the “normal” form of the CoW without hypoplasticity or absence in the ACA and anterior communicating artery (ACoA) (aA, 45.0%) followed by hypoplastic ACoA (aD, 25.8%) and absent ACoA (aE, 10.8%). Among posterior CoW configurations bilaterally absent PCoA (pE, 22.5%) was the most commonly observed variant. Normal morphology (pA, 16.7%), bilaterally hypoplastic PCoA (pF, 15.0%) and unilaterally hypoplastic PCoA (pH, 11.7%) were also among the most common configurations. The total percentage of unilateral fetal configuration (pC, pD, pI, pJ, pK) was seen in 10.9%. No significant difference was observed regarding anterior and posterior CoW variants shown in Figures 2 and 3 between the MZ and DZ groups with one exception: bilaterally hypoplastic PCoA (pF variant) appeared in a significantly higher rate in the DZ group ($p=0.02$).

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Table 3. Variants of the anterior CoW. aA: „Normal" variant with single, non-hypoplastic ACoA and ACAs. aB: Two ACoAs. aC: Unilateral ACA A1 segment hypoplasia. aD: hypoplastic ACoA. aE: Absent ACoA. aF: Medial artery of the corpus callosum arising from the ACoA. aG: Two ACoAs with medial artery of the corpus callosum arising from the distal ACoA. ACA: anterior cerebral artery, ACoA: anterior communicating artery, CoW: Circle of Willis, DZ: dizygotic, MZ: monozygotic.

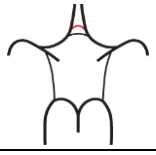
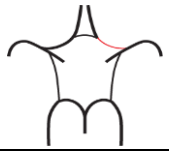
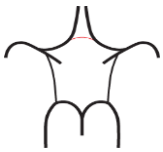
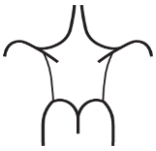
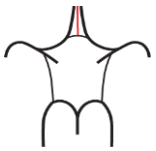
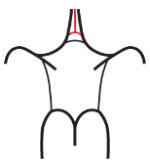
Variant		*n* (total)	% (total)	*n* (MZ)	% (MZ)	*n* (DZ)	% (DZ)
aA		54	45.0	33	43.4	21	47.7
aB		9	7.5	6	7.9	3	6.8
aC		6	5.0	4	5.3	2	4.5
aD		31	25.8	19	25.0	12	27.3
aE		13	10.8	8	10.5	5	11.4
aF		6	5.0	5	6.6	1	2.3
aG		1	0.1	1	1.3	0	0.0

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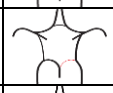
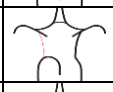
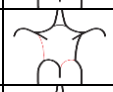
Table 4. Variants of the posterior CoW. pA: „normal" configuration with non-hypoplastic PCoAs and PCA P1 segments present on both sides. pB: Unilateral PCoA hypoplasia, absent contralateral PCoA. pC: Unilateral fetal-type variant with ipsilateral dominant PCoA and hypoplastic PCA P1 segment. pD: Unilateral fetal-type variant with absent ipsilateral PCA P1 segment and contralateral PCoA hypoplasia. pE: Bilaterally absent PCoAs. pF: Bilaterally hypoplastic PCoAs. pG: Absent unilateral PCoA. pH: Hypoplastic unilateral PCoA. pI: Unilateral fetal-type variant with ipsilateral absent PCA P1 segment. pJ: Unilateral fetal-type variant with ipsilateral hypoplastic PCA P1 segment and contralateral hypoplastic PCoA. pK: Unilateral fetal-type variant with ipsilateral hypoplastic PCA P1 segment and contralateral absent PCoA. pL: Bilateral fetal-type variant with bilaterally absent PCA P1 segments. CoW: Circle of Willis, DZ: dizygotic, MZ: monozygotic, PCA: posterior cerebral artery, PCoA: posterior communicating artery.

Variant		*n* (total)	% (total)	*n* (MZ)	% (MZ)	*n* (DZ)	% (DZ)
pA		20	16.7	14	18.4	6	13.6
pB		11	9.2	7	9.2	4	9.1
pC		4	3.3	2	2.6	2	4.6
pD		2	1.7	1	1.3	1	2.3
pE		27	22.5	20	26.3	7	15.9
pF		18	15.0	7	9.2	11	25.0
pG		14	11.7	9	11.8	5	11.4
pH		14	11.7	9	11.8	5	11.4
pI		2	1.7	2	2.6	0	0.0
pJ		2	1.7	1	1.3	1	2.3
pK		3	2.5	1	1.3	2	4.6
pL		3	2.5	3	4.0	0	0.0

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4.2.2. Concordance and discordance rates and TCD results

Table 5 shows the concordance and discordance rates of the anterior and posterior CoW variants according to the morphological classification. MZ concordance was not higher than DZ concordance in anterior and posterior variants suggesting the dominance of environmental factors on CoW configurations. Figures 12 and 13 show an example of differences in CoW configurations in MZ twins.

Table 5. Concordance rates of the anterior and posterior anatomy of the CoW. C: concordant twin pairs, CoW: Circle of Willis, D: discordant twin pairs, DZ: dizygotic, MZ: monozygotic.

				Twin A		Number of C/D	Pairwise concordance rate C/(C+D)
				Normal CoW	Variant CoW		
Anterior CoW	Twin B	MZ	Normal CoW	11	**13**	**6/21**	0.22
			Variant CoW	**8**	**6**		
		DZ	Normal CoW	7	**3**	**7/8**	0.47
			Variant CoW	**5**	**7**		
Posterior CoW	Twin B	MZ	Normal CoW	2	**4**	**25/11**	0.69
			Variant CoW	**7**	**25**		
		DZ	Normal CoW	2	**1**	**17/3**	0.85
			Variant CoW	**2**	**17**		

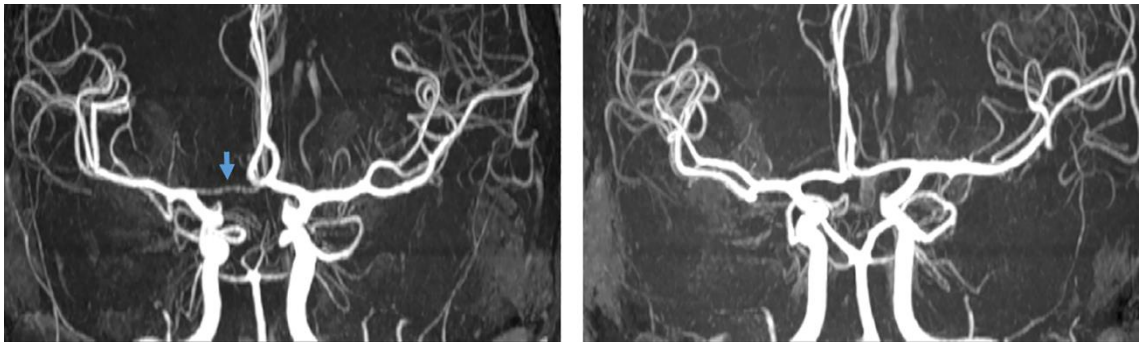

Figure 12. Differences in anterior CoW variants of a monozygotic twin pair. Twin A has hypoplastic ACA A1 segment on the right side (arrow). Twin B has normal anterior CoW configuration. ACA: anterior cerebral artery, CoW: Circle of Willis. Image from Department of Radiology, Borsod-Abaúj-Zemplén County University Hospital.

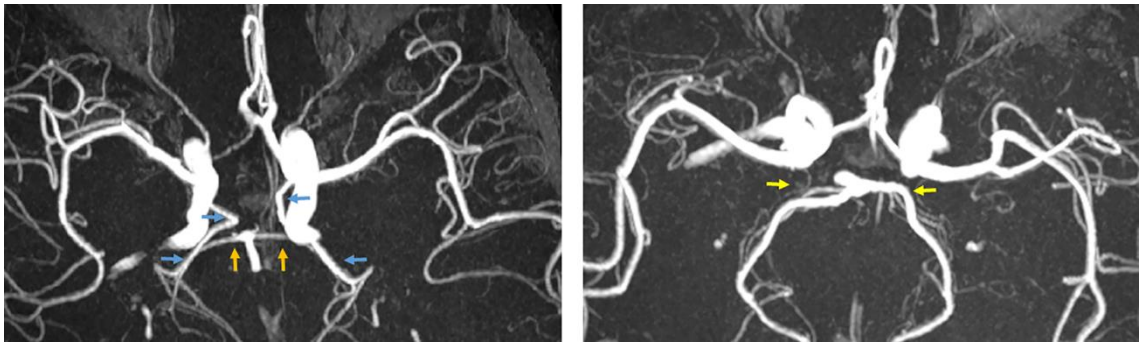

Figure 13. Differences in posterior CoW variants of the same monozygotic twin pair as shown on Figure 12. Twin A has bilateral fetal configuration (blue arrows) with absent PCA P1 segments on both sides. The orange arrows in the left picture point to the superior cerebellar arteries. Twin B has bilaterally absent PCoAs. The yellow arrows in the right picture show the anterior choroidal arteries. CoW: Circle of Willis, PCA: posterior cerebral artery, PCoA: posterior communicating artery. Image from Department of Radiology, Borsod-Abaúj-Zemplén County University Hospital.

4.2.3. Intra-pair differences regarding cardiovascular risk factors and cerebral hemodynamics in MZ twins discordant to CoW morphology

Out of our MZ twin group, 24 twin pairs were discordant regarding anterior or posterior CoW variants. We analysed which environmental effect could have a significant impact on the development of anterior or posterior CoW variants in discordant MZ twin pairs. Smoking, hypertension and hypercholesterolemia did not differ significantly between MZ discordant twins with variant and classical CoW configuration (Table 6). Flow parameters, such as MFV and PI did not show a significant difference within twin pairs of the MZ discordant group in any segment of the CoW (Table 7) in our healthy subjects. In a few individuals some CoW hemodynamic parameters could not be measured and in this case the twin pair had to be excluded from the analysis of the variable, however, all of the 24 twin pairs were included in the analysis regarding the majority of TCD variables.

Table 6. Discordant monozygotic twins: intra-pair comparison of cardiovascular risk factors in twins with and without CoW variants. BMI: body mass index, IQR: interquartile range, CoW: Circle of Willis. P-value calculation with paired t-test on transformed data is indicated with §. P-values for dichotomous variables are calculated by McNemar's test. N/A indicates that the test was not applicable.

	Normal anterior or posterior CoW	Variant anterior or posterior CoW	p-value
BMI, median±IQR	23.8±7.4	23.9±7.2	0.42§
Smoking, *n*(%)	7(29.2)	5(20.8)	0.50
Diabetes, *n*(%)	0(0.0)	1(4.2)	N/A
Hypertension, *n*(%)	4(16.7)	4(16.7)	1.00
Hypercholesterolemia, *n*(%)	1(4.2)	1(4.2)	1.00

Table 7. Monozygotic discordant twins: intra-pair comparison of TCD parameters. BA: basilar artery, LACA: left anterior cerebral artery, LMCA: left middle cerebral artery, LPCA: left posterior cerebral artery, MFV: mean flow velocity, PI: pulsatility index, RACA: right anterior cerebral artery, RMCA: right middle cerebral artery, RPCA: right posterior cerebral artery TCD: transcranial Doppler. Data are shown as mean±standard deviation with continuous variables that have normal distribution and as median±interquartile range with continuous variables that have non-normal distribution (RPCA MFV, LACA PI). † indicates the results of the non-parametric Wilcoxon-signed rank test. All other p-values were calculated on non-transformed data with independent-samples t-test.

	Twin with normal anterior or posterior CoW	Twin with variant anterior or posterior CoW	p-value
RMCA MFV (cm/s)	67.35±16.02	72.58±12.44	0.11
RMCA PI	0.85±0.16	0.84±0.13	0.58
RACA MFV (cm/s)	51.44±7.94	53.56±9.63	0.67
RACA PI	0.93±0.18	0.91±0.17	0.77
RPCA MFV (cm/s)	45.00±8.50	40.50±5.25	0.35†
RPCA PI	0.81±0.13	0.96±0.09	0.16
LMCA MFV (cm/s)	70.15±12.67	73.54±12.46	0.30
LMCA PI	0.81±0.15	0.80±0.16	0.74
LACA MFV (cm/s)	49.67±8.14	53.22±8.90	0.37
LACA PI	0.87±0.14	0.85±0.13	0.13†
LPCA MFV (cm/s)	40.00±4.76	39.71±5.71	0.85
LPCA PI	0.85±0.12	0.94±0.21	0.26
BA MFV (cm/s)	43.71±8.19	43.41±7.41	0.89
BA PI	0.85±0.20	0.86±0.12	0.88

4.3. Results of the TCCS study

4.3.1. Hemodynamic parameters in MZ and DZ twins

In case of the right MCA PSV, significant difference was found between the two groups, while there were no significant differences in the other vessels with regard to PSV, MFV, EDV and PI between MZ and DZ groups. Tables 8 and 9 show the hemodynamic parameters in each segment of the CoW and the differences across zygosity.

Table 8. Characteristics of our study population and measured PSV and EDV values. ACA: anterior cerebral artery, BA: basilar artery, BMI: body mass index, DZ: dizygotic, EDV: end-diastolic velocity, MCA: middle cerebral artery, MZ: monozygotic, PCA: posterior cerebral artery, PSV: peak systolic velocity, VA: vertebral artery. Data are shown as mean±standard deviation with continuous variables. ※ indicates Welch-test. All other p-values were calculated on non-transformed data with independent-samples t-test. Significant p-values are indicated by asterisk.

	Grand mean	MZ	DZ	p-value
Right MCA PSV	100.00±23.61	94.95±24.11	107.13±21.32	0.04*
Left MCA PSV	108.50±22.53	105.02±22.95	113.43±21.39	0.15
Right ACA PSV	92.39±18.41	94.49±19.95	89.80±16.33	0.34
Left ACA PSV	84.79±21.83	86.20±23.59	82.84±19.42	0.55
Right PCA PSV	74.51±14.69	74.63±15.42	74.36±13.95	0.94
Left PCA PSV	73.56±19.05	75.85±20.44	70.46±16.90	0.28
Right VA PSV	60.77±14.38	61.98±14.53	59.05±14.27	0.43
Left VA PSV	63.22±16.44	63.82±16.05	62.37±17.26	0.74
BA PSV	77.73±18.52	78.72±19.31	76.28±17.57	0.61
Right MCA EDV	39.50±14.00	37.39±13.51	41.08±9.30	0.20※
Left MCA EDV	42.60±12.75	40.52±13.60	45.56±10.99	0.12
Right ACA EDV	33.98±8.44	34.04±8.81	33.91±8.15	0.95
Left ACA EDV	32.00±16.00	33.10±12.52	31.89±9.20	0.66※
Right PCA EDV	27.45±7.92	27.19±8.17	27.81±7.74	0.77
Left PCA EDV	25.86±8.9	27.61±9.55	23.58±7.63	0.08
Right VA EDV	22.76±6.55	23.41±6.45	21.88±6.70	0.37
Left VA EDV	24.73±7.38	24.69±7.88	24.79±6.75	0.96
BA EDV	29.72±8.11	30.04±8.89	29.26±7.02	0.71

Table 9. Characteristics of our study population and measured MFV and PI values. ACA: anterior cerebral artery, BA: basilar artery, DZ: dizygotic, MCA: middle cerebral artery, MFV: mean flow velocity, MZ: monozygotic, PCA: posterior cerebral artery, PI: pulsatility index, VA: vertebral artery. Data are shown as mean±standard deviation with continuous variables that have normal distribution and as median±interquartile range with continuous variables that have non-normal distribution (right MCA PI, left MCA PI, right PCA PI, left VA PI, BA PI). § indicates that the comparison was made on transformed data with independent-samples t-test and † indicates the results of the non-parametric Mann-Whitney U-test. ※ indicates Welch-test. All other p-values were calculated on non-transformed data with independent-samples t-test.

	Grand mean	MZ	DZ	p-value
Right MCA MFV	59.27±15.24	56.58±16.38	63.10±12.79	0.10
Left MCA MFV	64.56±15.32	62.02±15.99	68.19±13.80	0.12
Right ACA MFV	52.64±10.0	52.73±9.83	52.54±10.30	0.94
Left ACA MFV	50.13±14.0	51.04±15.51	48.87±11.79	0.55
Right PCA MFV	43.13±8.92	43.0±9.10	43.3±8.90	0.89
Left PCA MFV	41.95±12.42	43.93±13.63	39.21±10.12	0.14
Right VA MFV	36.18±9.86	37.48±10.63	34.27±8.45	0.20
Left VA MFV	37.54±9.83	37.68±9.97	37.32±9.81	0.89
BA MFV	46.04±11.65	46.80±12.65	44.93±10.15	0.53
Right MCA PI	0.99±0.21	0.97±0.27	1.05±0.17	0.68§
Left MCA PI	1.00±0.22	1.04±0.33	0.96±0.18	0.29§
Right ACA PI	1.10±0.18	1.11±0.20	1.08±0.17	0.45
Left ACA PI	1.07±0.23	1.08±0.24	1.06±0.21	0.82
Right PCA PI	1.11±0.25	1.12±0.27	1.09±0.22	0.65※
Left PCA PI	1.15±0.26	1.10±0.28	1.22±0.22	0.07
Right VA PI	1.03±0.30	1.00±0.32	1.10±0.26	0.13
Left VA PI	1.02±0.26	1.01±0.19	1.03±0.30	0.88†
BA PI	1.02±0.25	1.02±0.28	1.03±0.23	0.95※

4.3.2. Hemodynamic parameters in relation to gender

Significant difference was observed between men and women regarding the PI measured in the right ACA and the left and right VAs: higher PI was observed in women in these cases. The difference between PI, PSV, EDV, and MFV of the other vessels was not significant between men and women. Sex differences in haemodynamic parameters are shown in Tables 10 and 11.

Table 10. PSV and EDV values in relation to gender. ACA: anterior cerebral artery, BA: basilar artery, BMI: body mass index, DZ: dizygotic, EDV: end-diastolic velocity, MCA: middle cerebral artery, MZ: monozygotic, PCA: posterior cerebral artery, PSV: peak systolic velocity, VA: vertebral artery. Data are shown as mean±standard deviation with continuous variables that have normal distribution and as median±interquartile range with continuous variables that have non-normal distribution (BA PSV, left VA EDV). § indicates that the comparison was made on transformed data with independent samples t-test. All other p-values were calculated on non-transformed data with independent-samples t-test.

	Men	Women	p-value
Right MCA PSV	94.36±25.31	103.21±22.26	0.15
Left MCA PSV	105.10±19.92	110.45±23.93	0.37
Right ACA PSV	88.18±19.88	94.60±17.45	0.21
Left ACA PSV	79.90±22.41	87.68±21.25	0.18
Right PCA PSV	73.36±15.10	75.16±14.62	0.65
Left PCA PSV	69.18±20.73	76.03±17.84	0.18
Right VA PSV	56.37±12.49	63.14±14.91	0.08
Left VA PSV	59.29±18.51	65.33±15.03	0.17
BA PSV	78.00±31.00	80.80±29.00	0.47§
Right MCA EDV	38.63±13.68	39.07±11.12	0.89
Left MCA EDV	42.18±13.04	42.84±12.74	0.85
Right ACA EDV	35.47±10.08	33.18±7.44	0.33
Left ACA EDV	32.53±12.55	32.62±10.47	0.98
Right PCA EDV	27.36±8.03	27.51±7.98	0.95
Left PCA EDV	25.97±10.16	25.80±8.29	0.94
Right VA EDV	23.79±6.46	22.18±6.60	0.36
Left VA EDV	25.40±11.00	23.50±11.00	0.88§
BA EDV	30.65±8.94	29.18±7.67	0.50

Table 11. MFV and PI values in relation to gender. ACA: anterior cerebral artery, BA: basilar artery, BMI: body mass index, DZ: dizygotic, MCA: middle cerebral artery, MFV: mean flow velocity, MZ: monozygotic, PCA: posterior cerebral artery, PI: pulsatility index, VA: vertebral artery. Variables are shown as mean ± standard deviation. Data are shown as mean±standard deviation with continuous variables that have normal distribution and as median±interquartile range with continuous variables that have non-normal distribution (right MCA PI, left MCA PI, left PCA PI, left VA PI). § indicates that the comparison was made on transformed data with independent samples t-test. ※ indicates Welch-test. † indicates the results of the non-parametric Mann-Whitney-U test. All other p-values were calculated on non-transformed data with independent-samples t-test. Significant p-values are indicated by asterisk.

	Men	Women	p-value
Right MCA MFV	57.21±17.08	60.45±14.17	0.42※
Left MCA MFV	63.15±14.63	65.38±15.82	0.58
Right ACA MFV	53.04±12.97	52.42±8.02	0.85
Left ACA MFV	48.71±15.20	50.97±13.39	0.55
Right PCA MFV	42.69±9.30	43.39±8.80	0.77
Left PCA MFV	39.79±13.30	43.23±11.86	0.30
Right VA MFV	34.59±8.17	37.07±10.68	0.34
Left VA MFV	36.29±10.28	38.23±9.63	0.45
BA MFV	45.63±11.82	46.27±11.69	0.83
Right MCA PI	0.96±0.24	1.02±0.19	0.10†
Left MCA PI	0.95±0.24	1.03±0.21	0.48§
Right ACA PI	1.00±0.15	1.14±0.18	0.01*
Left ACA PI	1.00±0.24	1.10±0.22	0.10
Right PCA PI	1.10±0.25	1.11±0.26	0.82
Left PCA PI	1.06±0.21	1.17±0.20	0.34※
Right VA PI	0.92±0.24	1.10±0.31	0.02*
Left VA PI	1.00±0.28	1.05±0.25	0.03†*
BA PI	0.99±0.17	1.07±0.17	0.07

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4.3.3. Hemodynamic parameters in relation to age, BMI and blood pressure

There was a significant inverse correlation between age and PSV and EDV for all vessels examined except for the PCA. The results were similar regarding the MFV where the association was non-significant only for RACA MFV and RPCA MFV. Correlation between age and PI was significant in case of the right MCA, right VA and BA. The BMI also showed an inverse correlation with these parameters, and this was significant in case of the right MCA PSV, right ACA PSV, right and left PCA PSV, PSV of the right and left VA, BA PSV, left PCA EDV, right VA EDV, BA EDV, right and left PCA MFV, right VA MFV, BA MFV and left VA PI. Inverse correlations were observed between most of the measured intracranial hemodynamic parameters and SBP, but this was only significant for the right VA EDV and MFV. The correlation between SBP and PI was positive in the majority of CoW vessels. DBP showed inverse correlations with all hemodynamic parameters. Significant relationships were observed between the DBP and left VA PI, left MCA PSV and PI. Inverse correlation was observed between the PP and PSV, EDV and MFV values, whereas PI showed positive correlation with PP. Right VA EDV and MFV correlated significantly with PP. Inverse association was found between the measured intracranial haemodynamic parameters and MAP, of which right VA MFV and left MCA PSV was significant. The correlations between intracranial hemodynamic parameters and age, BMI and blood pressure values are shown in Tables 12 to 15.

Table 12. Correlation between PSV values and age, BMI, SBP and DBP, PP and MAP. BA: basilar artery, corr: correlation, DBP: diastolic blood pressure, LACA: left anterior cerebral artery, LMCA: left middle cerebral artery, LPCA: left posterior cerebral artery, LVA: left vertebral artery, MAP: mean arterial pressure, PSV: peak systolic velocity, PP: pulse pressure, RACA: right anterior cerebral artery, RMCA: right middle cerebral artery, RPCA: right posterior cerebral artery, RVA: right vertebral artery, SBP: systolic blood pressure. Pearson's correlation where both variables were normally distributed, Spearman's correlation for non-normally distributed variables. Significant p-values are indicated by asterisk.

	Corr. with age	p-value	Corr. with BMI	p-value	Corr. with SBP	p-value	Corr. with DBP	p-value	Corr. with PP	p-value	Corr. with MAP	p-value
RMCA PSV	-0.52	<0.01*	-0.29	0.03*	-0.19	0.13	-0.21	0.09	-0.76	0.55	-0.22	0.09
LMCA PSV	-0.42	<0.01*	-0.13	0.33	-0.22	0.08	-0.26	0.04*	-0.13	0.32	-0.27	0.04*
RACA PSV	-0.28	0.04*	-0.30	0.03*	-0.01	0.95	-0.15	0.27	0.06	0.63	-0.13	0.34
LACA PSV	-0.44	<0.01*	-0.22	0.09	-0.12	0.36	-0.16	0.21	-0.03	0.84	-0.15	0.24
RPCA PSV	-0.16	0.21	-0.32	0.02*	-0.04	0.76	-0.08	0.55	-0.02	0.90	-0.09	0.52
LPCA PSV	-0.19	0.13	-0.32	0.01*	0.06	0.66	-0.03	0.80	0.05	0.71	-0.02	0.89
RVA PSV	-0.40	<0.01*	-0.37	<0.01*	-0.18	0.15	-0.18	0.15	-0.16	0.22	-0.22	0.09
LVA PSV	-0.37	<0.01*	-0.36	0.01*	-0.12	0.37	-0.20	0.11	0.07	0.57	-0.18	0.16
BA PSV	-0.39	<0.01*	-0.33	0.01*	-0.11	0.38	-0.10	0.45	-0.04	0.78	-0.11	0.37

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Table 13. Correlation between EDV values and age, BMI, SBP and DBP. BA: basilar artery, BMI: body mass index, corr: correlation, DBP: diastolic blood pressure, EDV: end diastolic velocity, LACA: left anterior cerebral artery, LMCA: left middle cerebral artery, LPCA: left posterior cerebral artery, LVA: left vertebral artery, MAP: mean arterial pressure, PP: pulse pressure, RACA: right anterior cerebral artery, RMCA: right middle cerebral artery, RPCA: right posterior cerebral artery, RVA: right vertebral artery, SBP: systolic blood pressure. Pearson's correlation where both variables were normally distributed, Spearman's correlation for non-normally distributed variables. Significant p-values are indicated by asterisk.

	Corr. with age	p-value	Corr. with BMI	p-value	Corr. with SBP	p-value	Corr. with DBP	p-value	Corr. with PP	p-value	Corr. with MAP	p-value
RMCA EDV	-0.51	<0.01*	-0.18	0.18	-0.17	0.18	-0.10	0.48	-0.12	0.33	-0.11	0.38
LMCA EDV	-0.40	<0.01*	0.01	0.94	-0.15	0.23	-0.02	0.88	-0.20	0.11	-0.07	0.57
RACA EDV	-0.34	0.01*	-0.20	0.15	-0.05	0.72	-0.07	0.59	-0.04	0.76	-0.08	0.56
LACA EDV	-0.45	<0.01*	-0.15	0.26	-0.09	0.50	-0.03	0.84	-0.11	0.41	-0.06	0.64
RPCA EDV	-0.15	0.25	-0.18	0.19	-0.04	0.76	0.01	0.92	-0.15	0.25	-0.02	0.87
LPCA EDV	-0.24	0.07	-0.28	0.04*	0.11	0.41	0.05	0.72	0.04	0.75	0.05	0.70
RVA EDV	-0.42	<0.01*	-0.48	<0.01*	-0.28	0.03*	-0.18	0.17	-0.29	0.03*	-0.25	0.06
LVA EDV	-0.39	<0.01*	-0.08	0.53	-0.03	0.83	-0.02	0.89	0.04	0.74	-0.02	0.85
BA EDV	-0.49	<0.01*	-0.34	<0.01*	-0.13	0.29	-0.11	0.40	-0.08	0.52	-0.13	0.30

Table 14. Correlation between MFV values and age, BMI, SBP and DBP. BA: basilar artery, BMI: body mass index, corr: correlation, DBP: diastolic blood pressure, LACA: left anterior cerebral artery, LMCA: left middle cerebral artery, LPCA: left posterior cerebral artery, LVA: left vertebral artery, MFV: mean flow velocity, MAP: mean arterial pressure, PP: pulse pressure, RACA: right anterior cerebral artery, RMCA: right middle cerebral artery, RPCA: right posterior cerebral artery, RVA: right vertebral artery, SBP: systolic blood pressure. Pearson's correlation where both variables were normally distributed, Spearman's correlation for non-normally distributed variables. Significant p-values are indicated by asterisk.

	Corr. with age	p-value	Corr. with BMI	p-value	Corr. with SBP	p-value	Corr. with DBP	p-value	Corr. with PP	p-value	Corr. with MAP	p-value
RMCA MFV	-0.53	<0.01*	-0.24	0.06	-0.19	0.15	-0.16	0.22	-0.09	0.49	-0.17	0.18
LMCA MFV	-0.44	<0.01*	-0.06	0.67	-0.18	0.15	-0.14	0.27	-0.16	0.22	-0.17	0.18
RACA MFV	-0.24	0.07	-0.22	0.12	-0.02	0.87	-0.07	0.64	0.01	0.95	-0.06	0.67
LACA MFV	-0.47	<0.01*	-0.19	0.15	-0.10	0.46	-0.09	0.49	-0.08	0.52	-0.10	0.42
RPCA MFV	-0.15	0.25	-0.28	0.03*	-0.02	0.85	-0.01	0.95	-0.05	0.72	-0.03	0.82
LPCA MFV	-0.26	0.05*	-0.33	0.01*	0.06	0.63	-0.01	0.92	0.03	0.84	-0.02	0.90
RVA MFV	-0.45	<0.01*	-0.46	<0.01*	-0.28	0.03*	-0.21	0.09	-0.26	0.04*	-0.27	0.03*
LVA MFV	-0.41	<0.01*	-0.24	0.06	-0.07	0.56	-0.12	0.33	0.06	0.64	-0.11	0.38
BA MFV	-0.46	<0.01*	-0.36	<0.01*	-0.14	0.26	-0.10	0.42	-0.08	0.53	-0.14	0.28

Table 15. Correlation between PI values and age, BMI, SBP and DBP. BA: basilar artery, BMI: body mass index, corr: correlation, DBP: diastolic blood pressure, LACA: left anterior cerebral artery, LMCA: left middle cerebral artery, LPCA: left posterior cerebral artery, LVA: left vertebral artery, MAP: mean arterial pressure, PI: pulsatility index, PP: pulse pressure, RACA: right anterior cerebral artery, RMCA: right middle cerebral artery, RPCA: right posterior cerebral artery, RVA: right vertebral artery, SBP: systolic blood pressure. Pearson's correlation where both variables were normally distributed, Spearman's correlation for non-normally distributed variables. Significant p-values are indicated by asterisk.

	Corr. with age	p-value	Corr. with BMI	p-value	Corr. with SBP	p-value	Corr. with DBP	p-value	Corr. with PP	p-value	Corr. with MAP	p-value
RMCA PI	0.27	0.03*	-0.05	0.69	-0.01	0.96	-0.07	0.59	0.10	0.42	-0.03	0.82
LMCA PI	0.20	0.13	-0.20	0.13	0.02	0.91	-0.25	0.05*	0.28	0.03	-0.12	0.35
RACA PI	0.13	0.33	-0.02	0.88	0.04	0.78	-0.06	0.66	0.16	0.25	-0.02	0.88
LACA PI	0.23	0.07	-0.02	0.87	0.06	0.64	-0.12	0.36	0.22	0.08	-0.05	0.68
RPCA PI	0.11	0.42	-0.04	0.76	0.03	0.84	-0.14	0.27	0.20	0.12	-0.10	0.42
LPCA PI	0.15	0.26	0.14	0.30	0.01	0.96	-0.07	0.59	0.12	0.37	-0.03	0.80
RVA PI	0.24	0.05*	0.23	0.08	0.17	0.19	0.04	0.74	0.22	0.08	0.10	0.42
LVA PI	0.12	0.34	-0.38	<0.01*	-0.13	0.31	-0.26	0.04*	0.03	0.83	-0.19	0.13
BA PI	0.28	0.03*	0.18	0.18	0.18	0.16	0.05	0.68	0.23	0.07	0.13	0.32

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4.3.4. Concordance and discordance rates

Concordance rates regarding CW variants could be estimated in the total of 17 MZ twin pairs as two MZ twin pairs had to be excluded from this analysis due to bad acoustic window. A normal CoW anatomy was observed in most of MZ and DZ twins (76.5% and 92.3%, respectively). Of the detectable CoW variants, the majority of individuals had a missing ACA, but this variant showed significant differences between MZ and DZ ($p = 0.044$). There was no significant difference in the other variations depending on the zygosity. In the MZ group, fetal configuration was seen in one case, VA terminating in the left posterior inferior cerebellar artery (PICA) was observed in two cases. Absent right ACA was registered in four cases (11.8%) and absent left ACA was seen in one case. These variations were not detectable in the DZ group. Right VA hypoplasia was observed in the DZ group in one individual and right VA terminating in the ipsilateral PICA was also detected in one case, the latter two variants were not detected in the MZ group. Concerning the concordance of the MZ and DZ twins, the presence of variations showed no genetic influence as MZ concordance was not considerably higher (0.14) compared to DZ concordance (0.00). The concordance rates are shown in Table 16.

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Table 16. The concordance of the detected variants in the MZ and DZ groups. C: concordant twin pairs, CoW: Circle of Willis, D: discordant twin pairs, DZ: dizygotic, MZ: monozygotic.

			Twin A		Number of C/D	Pairwise concordance rate C/(C+D)
			Normal CoW anatomy	Variant CoW anatomy		
Twin B	MZ	Normal CoW anatomy	10	**4**	**1/6**	0.14
		Variant CoW anatomy	**2**	**1**		
	DZ	Normal CoW anatomy	11	**1**	**0/2**	0.00
		Variant CoW anatomy	**1**	**0**		

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4.3.5. Intra-pair correlations of hemodynamic variants

For most hemodynamic parameters, we found a low to moderate raw heritability. The highest heritability was found in left PCA EDV, right PCA PSV and left ACA PI (0.45, 0.52 and 0.56, respectively). Low heritability (0.00 to 0.20) was observed in the rest of the measured hemodynamic parameters, such as left ACA PSV, right and left VA PSV, BA PSV, right MCA EDV, left ACA EDV, right PCA EDV, left ACA MFV, right PCA MFV, right and left VA MFV, BA MFV, right MCA PI and BA PI. In the remaining hemodynamic parameters, heritability could not be estimated. The heritability of hemodynamic parameters is shown in Table 17 and 18.

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Table 17. MZ and DZ correlation of PSV and EDV values, their p-value and raw heritability. ACA: anterior cerebral artery, BA: basilar artery, DZ: dizygotic, EDV: end-diastolic velocity, MCA: middle cerebral artery, MZ: monozygotic, PCA: posterior cerebral artery, PSV: peak systolic velocity, VA: vertebral artery. Significant p-values are indicated by asterisk. N/A means that the heritability formula was not applicable.

	MZ		DZ		Raw heritability (h^2)
	correlation	p-value	correlation	p-value	
Right MCA PSV	0.77	0.05*	-0.01	0.97	N/A
Left MCA PSV	0.94	0.02*	0.09	0.81	N/A
Right ACA PSV	0.49	0.26	-0.02	0.96	N/A
Left ACA PSV	0.61	0.14	0.51	0.13	0.20
Right PCA PSV	0.56	0.20	0.30	0.40	0.52
Left PCA PSV	0.86	0.01*	-0.35	0.32	N/A
Right VA PSV	0.10	0.83	0.26	0.48	0.00
Left VA PSV	0.17	0.72	0.51	0.13	0.00
BA PSV	0.23	0.62	0.57	0.09	0.00
Right MCA EDV	0.60	0.15	0.60	0.15	0.00
Left MCA EDV	0.97	<0.01*	-0.16	0.67	N/A
Right ACA EDV	0.51	0.24	0.07	0.86	N/A
Left ACA EDV	0.63	0.13	0.53	0.11	0.20
Right PCA EDV	-0.22	0.64	0.29	0.42	0.00
Left PCA EDV	0.95	<0.01*	0.35	0.33	0.45
Right VA EDV	0.49	0.27	<-0.01	0.99	N/A
Left VA EDV	0.40	0.38	-0.21	0.56	N/A
BA EDV	-0.08	0.86	-0.18	0.62	N/A

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Table 18. MZ and DZ correlation of MFV and PI values, their p-value and raw heritability. ACA: anterior cerebral artery, BA: basilar artery, DZ: dizygotic, MCA: middle cerebral artery, MFV: mean flow velocity, MZ: monozygotic, PCA: posterior cerebral artery, PI: pulsatility index, VA: vertebral artery. Significant p-values are indicated by asterisk. N/A means that the heritability formula was not applicable.

	MZ		DZ		Raw heritability (h^2)
	correlation	p-value	correlation	p-value	
Right MCA MFV	0.71	0.07	-0.23	0.52	N/A
Left MCA MFV	0.98	<0.01*	-0.06	0.62	N/A
Right ACA MFV	0.50	0.25	0.04	0.92	N/A
Left ACA MFV	0.60	0.15	0.52	0.13	0.16
Right PCA MFV	-0.15	0.75	0.45	0.41	0.00
Left PCA MFV	0.91	<0.01*	0.03	0.93	N/A
Right VA MFV	0.21	0.65	0.17	0.64	0.08
Left VA MFV	0.23	0.62	0.13	0.72	0.02
BA MFV	0.10	0.83	0.35	0.32	0.00
Right MCA PI	0.39	0.39	0.29	0.41	0.20
Left MCA PI	0.42	0.34	-0.13	0.71	N/A
Right ACA PI	0.33	0.48	-0.03	0.93	N/A
Left ACA PI	0.76	0.05*	0.48	0.16	0.56
Right PCA PI	0.46	0.30	-0.33	0.36	N/A
Left PCA PI	0.67	0.10	-0.06	0.87	N/A
Right VA PI	0.44	0.32	-0.06	0.88	N/A
Left VA PI	0.53	0.22	0.06	0.87	N/A
BA PI	-0.03	0.96	0.58	0.08	0.00

5. Discussion

A relevant percentage of healthy individuals has CoW configuration different from the classical anatomy. Earlier results proposed that the CoW variants may predispose to cerebrovascular ischemia. Therefore, we aimed to determine the background of CoW variants. If a trait has low heritability, then the genetic contribution to that phenotype is less and therefore, stochastic and/or environmental factors have to be considered. Preventive strategies may help to eliminate these factors and consequently, the risk phenotype. Although accurate heritability was not possible to determine in our study, the concordance and discordance rates suggest that CoW variants seem to be determined by environmental factors rather than genetic effects as MZ intra-pair resemblance regarding CoW variants was not higher than DZ resemblance (Figures 14 and 15). The presence of anterior and posterior CoW variants on MRA was not significantly associated with current BMI, hypertension and hypercholesterolemia suggesting that these factors do not affect the development of cerebrovascular variants. Neither was a significant difference present regarding cerebral blood flow in MZ twins discordant to CoW variants indicating that these variants may not alter the flow in healthy individuals. To the best of our knowledge, our TCCS study is the first ultrasound-based study conducted on twins reporting data on the heritability of CoW variations in humans. Beyond the presence of the variants, the hemodynamic flow parameters of the intracranial vessels showed a low genetic influence. The right MCA PI showed low raw heritability in line with the previous study of our working group which has shown that MCA PI was mainly influenced by environmental factors and that MCA MFV showed moderate heritability (30.6%) [135]. Moreover, we investigated more intracranial vessels in this work and demonstrate that left ACA PI and right PCA PSV show also a moderate raw heritability. Therefore, we investigated environmental factors which might be associated with the development of CoW hemodynamics. Yet, our study did not demonstrate any significant association between the CoW variations investigated by TCCS and the presence of hypertension which may be due to the relatively young age of our study subjects. Albeit, as expected, a negative correlation between age and MFV, and a positive correlation between age and PI was found in the CoW in accordance with previous results in the literature, indicating increasing cerebrovascular resistance with age [136]. Moreover, we failed to find any relationship between BMI, hypertension, hypercholesterolemia and the presence of CoW

variants on MRA. Despite the lack of association found, our findings suggest that environmental, mostly lifestyle-related factors affecting flow parameters have a considerable influence beyond stochastic factors. According to previous results in the literature, the possibly relevant environmental factors include smoking, BMI and alcohol consumption. Smoking influences the cerebrovascular resistance [137]. Lower MCA MFV values were associated with higher BMI [138]. Furthermore, curvature of the vessels, known environmental risk factors influencing cardiac output, blood pressure and carotid atherosclerosis also determine CoW hemodynamics. Since our findings demonstrate that genetic factors have lower influence, proper lifestyle interventions might play a considerable role in the regulation of cerebrovascular flow. Further prospective studies are necessary to clarify the role of specific environmental factors on cerebral blood flow especially in patients with impaired flow due to severe carotid artery stenosis.

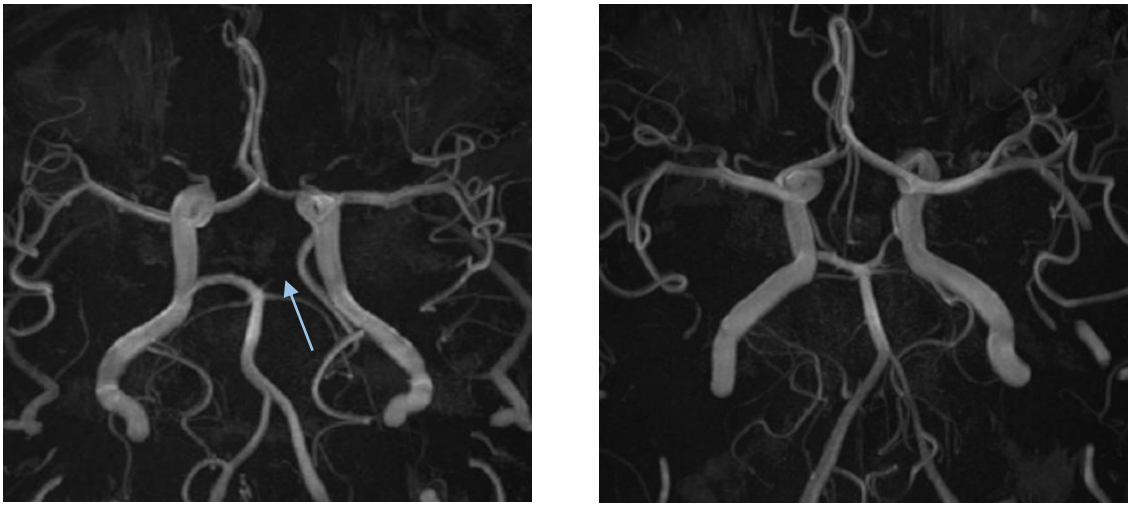

Figure 14. TOF-MRA of the CoW in a discordant DZ twin pair. The left PCA P1 segment of twin A is not visualised and therefore is categorized as absent (arrow). Intact left PCA P1 segment in twin B. CoW: Circle of Willis, PCA: posterior cerebral artery, TOF MRA: time of flight magnetic resonance angiography. Image from MR Research Centre, Semmelweis University.

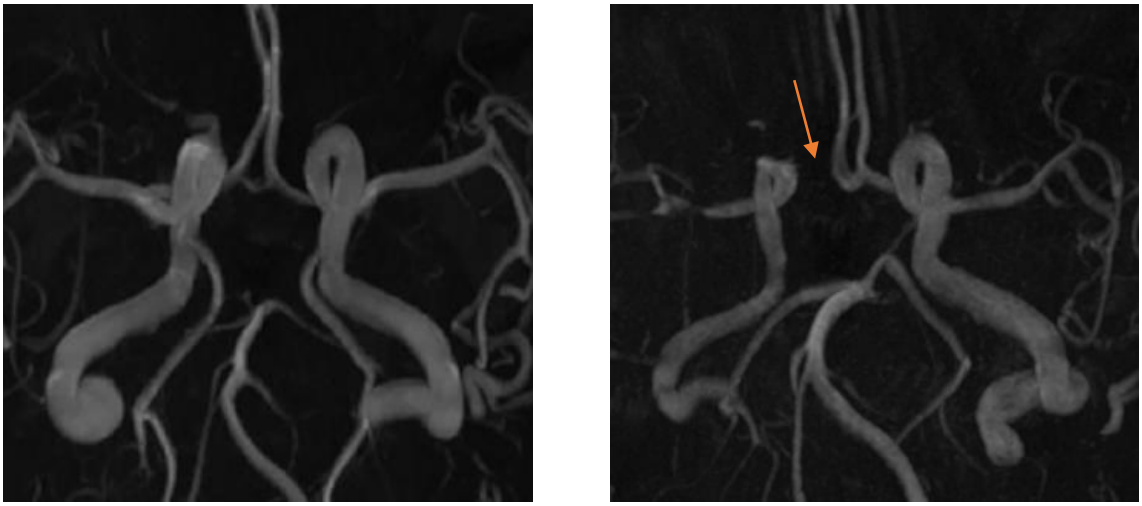

Figure 15. TOF MRA of the CoW in a discordant MZ twin pair. Twin B has non-visualised right ACA A1 segment (arrow) and right PCoA, categorized as absent, whereas those are intact in twin A. ACA: anterior cerebral artery, PCoA: posterior communicating artery, TOF MRA: time of flight magnetic resonance angiography. Image from MR Research Centre, Semmelweis University.

Recently, heritability of CoW variants was assessed for the first time in humans in frame of a family study [108]. According to the results of the meta-analysis, the prevalence of variant PCoA was almost 3-fold higher within families with intracerebral aneurysms (OR 2.8, 95% CI 1.8-4.3). No such association was found regarding ACA A1 asymmetry, FTP, and the classical morphology [108]. Comparing these findings to our results is challenging due to the different study designs and because our study included healthy twin individuals. Apparently, the pathogenesis of intracranial aneurysms is multifactorial. A number of predisposing factors, such as gender differences [139, 140] and local hemodynamics [141-143] have to be considered in this patient group besides the CoW variants. For example, FTP and female gender together and female gender alone was an independent predictor of intracranial aneurysms, but FTP alone did not predict this vascular pathology [144]. Moreover, the sex-related differences in the prevalence of CoW variants may contribute to the complexity of this vascular pathology, as FTP was found to be more common in women [139]. Therefore, all these factors may bias the heritability results of CoW variants.

5.1. The role of cardiovascular risk factors

What environmental factors may be behind CoW variants is yet to be examined. Our results show that environmental factors may determine CoW anatomy, however, we could not confirm any significant association between classical cardiovascular risk factors at study time and the presence of CoW morphological variants examined by TOF MRA. The reason may be that our volunteer twin population consisted of healthy individuals. Results on the association of cardiovascular risk factors and CoW variants are controversial in the literature. The association of CoW variants and changes in CoW hemodynamics with aging is supported by several results but is more ambiguous regarding other cardiovascular risk factors and CoW variants.

5.1.1. Age and CoW

Differences in CoW configuration among age groups were described, with incomplete CoW being more common with increasing age [9, 21, 145-147]. Krabbe-Hartkamp et al. found that the diameter of CoW-vessels decreased with age [21]. Both Zaninovich et al. and El-Barhoun et al. found that more common variants of the CoW such as bilateral absent PCoA showed a clear tendency to appear more commonly with age using TOF MRA [145, 146]. Besides these variants, few of the more common variants showed the same tendency in the anterior [21] and posterior CoW [146]. This tendency was not observed regarding rare variants. It is possible that some degree of genetic predisposition to the rare genetic variants exists but atherosclerosis, arterial stiffening and decreased cardiac output may all be responsible for the compensatory changes in CoW vessel diameters and structure [148, 149]. Our population was relatively young (49.5 and 55.0 years in the MZ and DZ group, respectively) and therefore, the prevalence of CoW variants may not be high enough for comparison. Our findings could also be explained by the small population size, which limited us to perform more detailed heritability analysis. Increased risk for stroke in presence of a CoW variant can be enhanced by decreased blood flow. Studies conducted in an older population indicated a decreasing MFV with increased age in the arteries of the CoW, especially in the posterior circulation [150] which is in line with our findings but also for additional arteries. The underlying

fact is that plasma viscosity is higher in the elderly [151] and the stiffness of the arteries is increased which leads to a decreased elasticity and therefore higher amplitude pulse waves [152, 153]. Moreover, comorbidities, e.g. heart failure [154], and changes in size of intracranial vessels [155] may contribute to reduced brain flow. Significantly decreased MFV and increased PI values were described with increasing age in healthy individuals in a TCD study [136], which is in line with our results. We found negative correlation of age with MFV and its positive correlation with PI in most of the CoW vessels and these associations were significant in most of the CoW-vessels. Increased cerebrovascular resistance, declined vasomotor reserve, changes in cardiac autonomic functions and baroreflex sensitivity could account for the observed age-dependent differences in cerebral hemodynamics [156].

It is noteworthy that in our TCCS study population of Italian subjects there was an overall low rate of CoW variations, as a „normal" CoW occurred in most cases (76.5% of MZ and 92.3% of DZ), which could be in line with previous observations of a high ethnical variance of these intracranial variations. However, most likely methodological aspects account for the low prevalence of CoW variants.

5.1.2. Gender and CoW

Gender is another important and widely researched factor in the association with CoW variants and cerebral blood flow. Although we did not investigate gender differences in CoW variants with MRA, we compared MFV and PI amongst genders in the TCCS study and found that only the PI of the RACA, and the right and left VA differed significantly but not the flow parameters in the rest of the CoW arteries. Regarding the CoW-morphology, Zaninovich et al found that the prevalence of complete CoW differed significantly between men and women above the age of 39 years and overall between the two genders when considering all age groups with women having a greater percentage of complete CoWs. The prevalence of complete CoW decreased with age in both men and women, suggesting that age combined with other environmental factors may have a significant impact [146]. Unilateral FTP and ACA A1 segment variants were significantly more common in women than men whereas variants of the PCoA were more common in men in the Chinese population with a family history of stroke [11].

Gender differences in CoW-morphology could have clinical importance as stroke tends to show differences between males and females regarding demographics, aetiology and fatality. The prevalence and incidence of stroke is greater in men and women get their first stroke years later compared to men [157]. As for the aetiology, women tend to have more cardioembolic strokes whereas large and small vessel disease is a more common stroke aetiology in men [157] which is in line with complete CoW being more prevalent in elderly women. Cerebral infarction, intracranial haemorrhage and stroke of undetermined cause have greater incidence in men but subarachnoidal haemorrhage shows a tendency to appear more often in women [157]. Fatality [157] and disability after stroke [158] is higher in women. The lower stroke incidence in women could be explained by the protective effects of estrogens which disappears after menopause [159] and differences in comorbidities. Another important cerebrovascular pathology related to CoW-variants that show gender differences is intracranial aneurysm [139]. Women have FTP significantly more commonly compared to men whereas men tend to have a significantly greater prevalence of classical CoW in patients both with and without intracranial aneurysms [139].

5.1.3. Hypertension and CoW

Our results do not support the relation between morphological CoW variants or flow parameters in the CoW and hypertension which is probably attributable to the small and mostly healthy study population. Significant relationships were only observed between EDV and MFV of the right VA and SBP, as well as between DBP and left VA PI, left MCA PI and left MCA PSV. On the contrary, interesting results and hypotheses are described in the literature regarding the association between hypertension and CoW variants. A recent animal study showed that chronically elevated blood pressure and aging decreased PCoA diameter in inbred mice but obesity, hyperlipidaemia, metabolic syndrome and diabetes mellitus did not affect this arterial diameter [116]. Increased blood pressure leads to decreased diameter of resistance vessels [160, 161]. However, recently a reversed hypothesis was discussed, namely that the decrease of arterial luminal diameter is a cause and not a consequence of hypertension [162]. The VAs are crucial in this regard since they provide blood supply to the rostral ventrolateral medulla, which is responsible

for sympathetic activity in response to blood pressure decrease-associated metabolic changes. According to the Cushing's mechanism or selfish brain hypothesis of hypertension, the narrowed VA may lead to increase of blood pressure as a compensatory response [162-164]. Supporting this hypothesis, Warnert et al. found that incomplete posterior CoW and VA hypoplasia, but not incomplete anterior CoW were significantly more prevalent in the hypertensive population [165]. Individuals with incomplete CoW were 3.1 (95% CI, 1.6–6.1) times more likely to have hypertension [165]. Using phase-contrast MRI, total and regional cerebral blood flow was significantly lower and cerebrovascular resistance was significantly higher in hypertensive compared to normotensive individuals. They suggested that the increase in cerebrovascular resistance may have a causative role in hypertension in line with the selfish brain hypothesis as cerebrovascular resistance was increased in borderline patients but not muscle sympathetic nerve activity, indicating that cerebrovascular resistance changes occur before the sympathetic response [162, 165]. Also, the prevalence of VA hypoplasia with incomplete CoW was significantly higher in this patient group.

5.1.4. ICA stenosis and CoW variants

ICA stenosis is a pathologic condition where intact CoW collaterals are of key importance regarding survival [99]. According to our results, the presence of CoW variants does not influence the cerebral flow among healthy individuals with non-pathologic circumstances, underlying the fact that the clinical manifestation of the presence of CoW variants is expected in low cerebral blood flow states, such as in ICA stenosis or occlusion [56, 166]. Determining the prevalence of CoW-variants in patients with ICA-stenosis in our population was not possible since only three patients with less than 70% stenosis were included. Recent results pointed out the association between compromised CoW and brain ischemia in a population with high-grade ICA stenosis [66]. Especially the failure of the anterior collateral pathway may predispose to ischemic events [56, 66, 67]. Theoretically, significant ICA-stenosis or occlusion may lead to compensatory increased flow via the ACoA and PCoA as patients with this condition tended to have more complete CoWs, probably as a result of a remodelling process in the collaterals [23]. However, it is not clear retrospectively whether this favourable collateral pathway was

present originally and protected against cerebral ischemia as a consequence of ICA stenosis or the collateral pathway evolved as a conclusion of decreased cerebral flow [23]. Studies evaluating CoW collateral vessel diameters in patients with CEA and carotid artery stenting surgery support the latter assumption [167, 168]. CoW collateral vessel diameters decreased in size after CEA in patients with unilateral carotid artery stenosis [167, 168]. No change was observed in patients with ICA stenosis and contralateral ICA occlusion but in these patients, all CoW vessels had an increased diameter already preoperatively, except for the ACA A1 on the non-operated side [167]. The changes in ACA A1 calibre are debated after CEA because of the different findings probably caused by methodological aspects such as different modalities (CTA versus MRA), different populations and the time between the surgery and the postoperative imaging study [167-169]. Although there are discrepancies amongst these results, one observation is common: the CoW is a dynamically changing arterial structure most likely affected by local hemodynamics, which supports our findings on the dominance of environmental factors.

5.2. Genetic effects on the CoW in animal models

Previous studies investigating genetic effects on CoW variants conducted on animal models have shown controversial results. Some species of rodent CoW configuration is highly inherited with intensive selective breeding [119]. Li *et al* identified 4 genes in gerbils that could possibly play a role in the formation of CoW-variants [119]. The presence of glutathione peroxidase 4 (GPx4) gene was associated with complete anterior and posterior communicating arteries. The levels of cystatin 3 (CST3), alpha stimulating guanine nucleotide binding protein (GNAS) and profiling-2 (PFN-2) were significantly higher in the absence of PCoA and in complete ACoA variants [119]. Another study, also conducted on gerbils, investigated CoW variants through generations and concluded that configurations of ACoA are heritable, mainly from the mothers side which was not shown in case of posterior variants [118]. This way an offspring of rodent without anterior communicating artery was created which was significantly more prone to ischemic stroke [118]. On the other hand, Beckmann et al described great individual differences among mice within the same strain [170] and in other studies it was shown that environmental factors like growth factor deficiency during pregnancy might influence the development

of intracranial vessels in mice [123]. The differences mainly involve the presence of PCoA [170], which corresponds with our findings in humans. In spite of this evidence related to CoW formation in animals, no human data are available to date. Since our findings do not support genetic predisposition to CoW variants, we speculate whether epigenetic effects could have a more significant influence.

5.3. Possible stochastic and epigenetic effects on CoW variants

5.3.1. Possible epigenetic effects on CoW variants

Epigenetic effects at early age, such as DNA methylation, histone modifications and non-coding RNAs, should be considered in the development of CoW variants and cerebrovascular diseases [171]. Epigenetic effects play a substantial role in the evolution of different phenotypes as described by Waddington's epigenetic landscape metaphor [172]. According to this theory, the development of cells can be regarded as a pathway. Originally, the development is determined by the genes but during the developmental process, the cell comes to junctions and has several new pathways to go on. The decision that the cell makes is strongly affected by the environment of the cell. There are favoured pathways to go on, which are highly stable and which result in the manifestation of common phenotypes and are of key importance regarding evolution. Although the cell might choose another pathway because of the current environmental conditions and this might result in a different phenotype [172]. It is possible that the various CoW variants are the results, at least partly, of a similar process. The difference experienced between MZ twin individuals can be explained with epigenetic effects, for example DNA-methylation or post-translational histone modifications in utero. These are interactions between genes an environment that can lead to changes in phenotypes. Although twins share the same uterus, it does not necessarily mean that they share the same in-utero environment since the fetoplacental unit might be unique in each member of the twin pair [173]. Hypoxia and metabolic effects could have an influence on CoW variants already at intrauterine development and in adult life. Maternal alcohol consumption during pregnancy might alter the cerebrovascular response to hypercapnia in newborn lambs [174], and therefore, the intrauterine environment may be essential regarding the regulation of cerebral blood flow later in extrauterine life. Moreover, alcohol intake alters

the cerebrovascular autoregulation acutely [175]. Changes in the volume of the amniotic fluid, blood flow and haemodynamic circumstances during brain development was shown to influence calibres of the intracranial vessels [176-178]. The findings of Hartkamp et al. underline the importance of hemodynamic and metabolic factors later in adult life as patients with ICA stenosis of 70-99% or occlusion of the ICA tend to have complete CoW on the side of the stenosis or occlusion [23] and this corresponds with the results of a study conducted on patients who underwent CEA or stenting which demonstrated that one month after the operation (once the need for collateral supply decreased), collaterals of the CoW became reduced in size [168].

Term and preterm birth [86], intrauterine circumstances and certain diseases during pregnancy may also influence CoW configurations of the fetus. Preeclampsia leads to deficiency in PGF which has an impact on cerebrovascular development in mice: unilaterally hypoplastic CoW and decreased number of collaterals were noted in PGF-/- mice [123]. Thus, the intrauterine environment affects the vasculature of the fetus and maternal diseases can also cause epigenetic effects on the fetus (fetoplacental unit) which might lead to cerebro- or cardiovascular diseases in the offspring later in life [173, 179]. These results stimulate further research regarding the presence of CoW variations early in life. Future studies should investigate whether circumstances of delivery (for example, cardiotocography monitoring or data regarding Caesarean section) are associated with the presence of CoW variants.

There are genetic processes that initiate development of cerebral arteries but as the occipital lobe grows in human brain its functional demand leads to haemodynamic changes and thus CoW variations may arise as consequence of these effects [177]. This is in line with the concept of angioadaptation which is an adaptive change of the vessel wall structure and its continuous interaction with the environment due to haemodynamic, metabolic and molecular inputs [180, 181]. Down- and upregulation of VEGF, expression of certain integrines and gestational age can also influence the development of cerebrovascular structure [86, 182]. Thus, the presence of variants could depend heavily on the development of the arteries. Furthermore, there might be developmental interactions between the CoW and the skull structure as described by Jamniczky et al [183]. The findings of Faber et al. in mice underline the importance of random stochastic factors on PCoA variants in the developmental phase since low birth weight was not

significantly associated with the presence of CoW variants [116]. Aging and hypertension, but not other cardiovascular risk factors influenced PCoA diameter significantly and the PCoA configuration differed amongst mice strains with different genetic background [116]. Thus, it is suggested that genetic and stochastic factors are the primary determinants of PCoA configuration which then is further affected by environmental factors later in life [116].

5.3.2. Stochastic factors influencing CoW variants

The role of environmental factors in CoW configuration is emphasized based on our findings, however, we failed to identify any association between those and the presence of CoW variants when investigating the possible environmental factors individually. This may partly be explained by the relatively young and healthy population, and partly by stochastic factors. These are random molecular effects that can occur and change the gene expressions on the cellular level even if the environmental background is the same, leading to phenotypic differences in MZ twins where the genetic constellation is nearly the same [184].

Little attention has been given to stochastic factors influencing CoW variants in the literature, especially in humans. However, Faber et al. observed great differences in the number of PCoA within the same mouse strains. The PCoA constellation showed significant variation in mice of the same strain, age and postnatal environment and was not associated with intrauterine growth restriction [116]. According to Faber et al, stochastic factors may be responsible similarly for anatomic variations in other body parts. For example, the palmar arch shows great anatomic variation and asymmetry between the two limbs [185, 186]. Thus, stochastic factors may influence the development of the CoW or the CoW configuration later on in life that can be responsible for the high number of MZ discordant twins which we observed in our study.

5.4. Limitations

5.4.1. Limitations of the MRA study

Our study has limitations that have to be acknowledged. The relatively small population in both the MRA-TCD and the TCCS study may influence our results which limited us to perform complex genetic analysis identifying the common and unique environmental factors due to low power (ACE modelling). The comparison of concordance rates alone does not allow conclusions regarding heritability. A high concordance does not mean that the phenotype is heritable and conversely, low concordance rates do not exclude genetic effects [187]. Our imaging method (TOF MRA) can be regarded as a limitation factor of this study because the longer acquisition time increases the motion artefacts on a TOF MRA image. Contrast enhanced MRI has better resolution and is more sensitive to detect the CoW variants, but it is an invasive procedure and requires contrast administration which is ethically not acceptable in a healthy volunteer population. Moreover, previous studies found no significant difference between CE-MRA and TOF MRA regarding displaying intracranial arteries [70, 188]. Detecting ACA, MCA and PCA with TOF MRA has a sensitivity of 94-100% compared with the gold standard method, DSA. The value is lower (65%) in case of anterior and posterior communicating arteries [189]. This may explain why in our study population absent ACoA and PCoA were the most common variants. TOF MRA is useful in the diagnosis of steno-occlusive disease as sensitivity and specificity of TOF MRA of the MCA compared to conventional angiography was 88% and 97% respectively [190]. Furthermore, different MRI scanners were used on two study locations in order to increase our study population which may bias our results.

5.4.2. Limitations of the TCCS study

TCCS may be used to determine CoW flow velocities and certain variants, however, its limitations have to be mentioned. For obvious reasons, no previous studies compare TCCS to CTA on healthy individuals. However, Brunser et al. assessed the sensitivity and specificity of conventional TCD in the diagnosis of acute intracranial obstruction or occlusion in comparison with CTA, which were 81.8% and 94%, respectively [191]. Their results indicate great differences in specificity depending on which region is

examined. Specificity in the MCA was 95.6%, whereas that in the posterior circulation was 57.1% [191]. TCD and TCCS are highly operator-dependent examinations and this is one of the main disadvantages of these methods. TCCS is not only useful for the determination of flow velocities, but also B-mode image can be obtained. Applying color-coded Doppler on the B-mode image helps locating the vessels and velocities can be calculated using spectral analysis [63]. The MCA M1 and M2, ICA C1, ACA A1, PCA P2, BA and VA can be evaluated, but the visualization of PCA P1 segment and PCoA is challenging [63]. Another limitation of this method is the possibility of inadequate bone windows. An insufficient bone window was observed in two individuals, however, these cases were excluded from the study. We tried to decrease this bias by the involvement of a very experienced and trained senior neurologist.

False positive findings are certainly possible upon TCCS examination, either because the study is highly operator-dependent, or because of non-easily accessible bone windows. The TCCS studies were performed by an experienced neurosonographer. The cases with inadequate bone window were excluded. The right ACA was not found in four cases, while the left ACA was not detected in one case. A fetal configuration was seen in one case. According to previous results in the literature, ACA was depicted in 83% of cases through the temporal window [33]. PCA P1 and P2 segments were depicted in 92% and 94% of the cases, respectively [33]. In certain anatomic situations, the division branches may be difficult to identify with TCCS because of its resolution inferior to TOF MRA. Vuillier et al. compared the appearance of MCA M1 segment between TCCS and TOF MRA [192]. The MCA M1 division was classified as monopod, bipod and tripod. TCCS had a sensitivity of 78% and a positive predictive value of 87% regarding the classification of MCA M1 division, although the authors used power Doppler. Furthermore, ultrasound contrast agent was applied in 54% of the patients in this study in order to get a better understanding of CoW anatomy [192]. Therefore, sensitivity and positive predictive values are expected to be lower in a study where no contrast agent and power Doppler analysis was made, such as in our study.

Despite the above mentioned limitations, we chose TCCS because of its advantages that include the non-invasiveness, the lack of ionizing irradiation and contrast administration, which was an important consideration when choosing our method to detect the CoW variants and velocities on a young, generally healthy population. Although TOF MRA

detects the collaterals with great sensitivity, TCCS is more useful in the detection of potentially functional collaterals [90]. We also considered that TCCS was the most time- and cost-effective method for our study.

The small number of cases may affect our results, and due to the small sample we have determined a raw heritability of hemodynamic parameters in the CoW that cannot distinguish between shared and non-shared environmental effects that the univariate ACE model can. Although the number of individuals with CoW variants was lower than expected, our main goal was not to compare the absolute number of cases with variant versus normal CoW anatomy. Our main goal was to determine MZ concordance, in other words, the similarity within one twin pair. Therefore, even non-variant CoW provided important information for us. All investigations were performed by one person, and therefore, inter-observer variability was not an issue.

6. Conclusion

Our study is the first to investigate genetic versus environmental background of the CoW variants with MRI and TCCS in frame of a twin study. Our results show that anatomical variants of the CoW, which commonly occur and might predispose to cerebrovascular events might be shaped and influenced by environmental or stochastic factors, however, our results do not allow to draw conclusions on heritability. Few intracranial hemodynamic parameters showed moderate raw heritability in our TCCS study, but the majority of these recorded parameters showed low heritability underlining the importance of environmental and/or stochastic factors. Anterior and posterior CoW variants were not associated with current BMI, smoking, hypertension and hypercholesterolemia and altered blood flow measured by TCD. Since increasing evidence supports that CoW variants are risk factors of cerebrovascular ischaemia, determining their origin is essential to develop preventive strategies. Our results should stimulate further research on the origin of CoW variants on larger twin populations with the goal to determine heritability using the ACE-model. Further studies should be stimulated to elucidate which epigenetic and environmental factors are responsible for these variants, which might play a role in stroke prevention and management of patients with CoW variants and decreased cerebral blood flow.

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7. Summary

CoW variants have recently received great attention because they are commonly seen in healthy individuals. Increasing evidence supports the hypothesis that they might predispose to cerebrovascular ischemia. However, the factors that determine CoW variants are not established and the previous results in this field are sparse and conflicting. Previous animal studies mainly emphasize the genetic origin, whereas most retrospective studies in human stroke patients support the plasticity of CoW-vessels adapting to pathological conditions, such as ICA stenosis. Therefore, our aim was to determine whether CoW variants and CoW hemodynamics are associated with genetic or environmental factors in healthy twin samples, which are the most ideal cohorts for detecting genetic and environmental effects on a certain phenotype. We investigated two separate populations of twins. One of the groups underwent TOF MRA and TCD, whereas the other group was examined using TCCS. DZ concordance rates were higher than MZ concordance rates regarding CoW variants identified on TOF MRA and TCCS implying that genetics has a negligible role in CoW morphology. Hemodynamic parameters measured by TCD were not influenced by CoW variants identified on TOF MRA indicating that the presence of these variants does not affect cerebral blood flow in healthy individuals. Raw heritability of hemodynamic parameters was low with TCCS. Although detailed heritability analysis was not possible on our sample, our results support the dominance of environmental factors in the formation of CoW variants, however, we were not able to identify any cardiovascular risk factors as a predictor of CoW variants. The number of MZ twins discordant for CoW variants was high, therefore, we suggest that stochastic factors also have an influence on the CoW. Consequently, our results stimulate further research on the origin of CoW variants with the goal of further specifying the environmental factors that affect CoW morphology.

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8. Összefoglaló

A Willis-kör (CoW) anatómiai variációi napjainkig intenzív kutatás tárgyát képezik, mivel egyre több eredmény támasztaja alá azt a hipotézist, hogy ezen vaszkuláris variánsok cerebrális isémiára hajlamosítanak. Ugyanakkor a CoW anatómiai variációit befolyásoló faktorok nem ismertek, valamint kevés eredmény áll rendelkezésünkre ezen a területen, amelyek ellentmondóak. Korábbi állatkísérletek a CoW variánsok genetikai eredetére következtettek, míg a humán, főleg sztrókban és az arteria carotis interna sztenózisában szenvedő betegcsoportban végzett retrospektív kutatások a CoW plasztikus és patológiás állapotokhoz adaptálódó jellegét hangsúlyozzák. Kutatásunk célja annak eldöntése volt, hogy a CoW variánsait és hemodinamikáját egészséges ikrekben genetikai vagy környezeti faktorok határozzák-e meg. A tanulmányozott ikerpopulációk előnye, hogy ezen csoportok a legideálisabbak genetikai és környezeti faktorok egy adott fenotípuson kiváltott hatásának vizsgálatára. Két különböző ikerpopulációt tanulmányoztunk. Az egyik populáció TOF MRA és TCD vizsgálatokon esett át, míg a másik populáción TCCS vizsgálatot végeztünk. A DZ konkordancia ráták a TOF MRA-val leképezett elülső és hátsó CoW variánsok esetében nagyobbnak adódtak a DZ, mint az MZ csoportban, jelezve a genetikai hatások elhanyagolható szerepét a CoW variánsok kialakulásában. A CoW-variánsok jelenléte TOF MRA-val vizsgálva nem befolyásolta a TCD-vel mért hemodinamikai paramétereket egészséges ikrekben. A hemodinamikai paraméterek nyers öröklődése TCCS-sel mérve alacsonynak adódott. Jóllehet a részletes öröklődés analízis elvégzése ezen a mintán nem volt lehetséges, de eredményeink a CoW variánsok kialakulásában a környezeti hatások dominanciáját támogatják, ugyanakkor eredményeink nem erősítették meg a vizsgált környezeti faktorok összefüggését a CoW variánsokkal. Mivel a diszkordáns MZ ikrek aránya magas volt, sztochasztikus, véletlenszerű hatások szerepe valószínűsíthető a CoW variánsok kialakulásában. További prospektív vizsgálatok szükségesek az egyes konkrét környezeti faktorok és a CoW-morfológia összefüggésének vizsgálatára.

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10. Bibliography of own publications

10.1. Publications related to the current PhD thesis

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