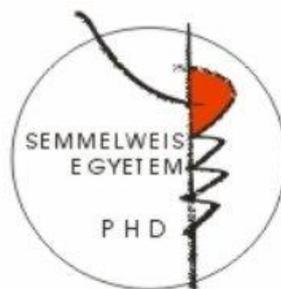


THE MORPHOLOGY OF THE VERTEBROBASILAR SYSTEM

PhD thesis

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INTRODUCTION

The vertebrobasilar system is unique in anatomical terms, as it is the only instance in the human body where a third artery is formed by the merging of two arteries. Asymmetrical vertebral artery (VA) flow and diameter are common findings, and the vertebral system is often left dominant. Unequal VA flow may lead to complex secondary blood flow patterns in the basilar artery (BA), which may cause bending of the artery over time. Increased VA diameter difference, tortuosity and torsion and increased BA curvature and tortuosity may cause oscillating and low wall shear stress (WSS), which plays an important role in the pathogenesis of atherosclerosis. These changes over time may lead to vertebrobasilar insufficiency. Increased white matter hyperintensity (WMH) burden has been considered as an indicator for inadequate blood perfusion on T2 weighted Fluid-attenuated inversion recovery (FLAIR) images. The presence of WMH triples the risk of stroke and doubles the risk of dementia. Previous publications showed significant correlation between vertebrobasilar artery morphology and stroke localization. However, the connection between WMHs and large cerebral artery morphology is not yet fully understood. Furthermore, the genetic and environmental determinants influencing the development of the vertebrobasilar morphological indices are not yet clear.

OBJECTIVES

Our aim was:

- to investigate the intracranial vertebrobasilar morphology in a Caucasian population non-invasively with individually reconstructed 3D vessel models,
- to measure the morphological indices automatically on our models,
- to investigate the influence of the VA dominance on BA geometric indices,
- to investigate whether the BA geometry contributed to the existence and laterality of WMHs,
- to measure the heritability of each measured morphological vessel index using a classical twin study model.

Our hypothesis was:

- that most of the vertebrobasilar morphological changes demonstrated in the Asian population can also be found in a Caucasian population,
- that these changes are influenced by genetic factors,
- that the vertebrobasilar system morphology can influence the location of WMHs found on MRI FLAIR sequences.

In order to investigate these research hypotheses, two studies have been performed on two different patient groups:

- 1) a retrospective analysis of patients who underwent brain MRI (1. Study “Laterality of deep white matter hyperintensity correlates with basilar artery bending and vertebral artery dominance”),
- 2) a prospective healthy twin study (2. Study “Are the Morphological Indices of the Vertebrobasilar System Heritable? A Twin Study Based on 3D Reconstructed Models”).

METHODS

1. Study - Laterality of deep white matter hyperintensity correlates with basilar artery bending and vertebral artery dominance

1.1. Patients

In this research, we retrospectively selected patients who visited the Medical Imaging Centre, Semmelweis University between 2017 and 2018, and underwent non-contrast 3D Time of Flight (TOF) and T2-weighted FLAIR brain MRI. Comorbidities such as hypertension, hyperlipidemia, and diabetes were gathered from the medical chart reports. The exclusion consisted of a medical history of large vessel obstruction, stroke, vasculitis, demyelinating disease, malignancies of the brain, abscess, encephalitis, autoimmune diseases, or migraine as these conditions may also present WMHs. As the development of the basilar curve is a highly age-related and a long-term process, patients younger than 20 years were also excluded. Based on these criteria we included 290 patients in this study.

1.2. Imaging analysis

Image assessment was performed by a single reader blinded to the clinical information. FLAIR images were used to separate patients with and without WMHs into two groups. Separating Deep WMHs from Periventricular WMHs was based on the combination of continuity to the ventricles or if no continuity was seen, a 10 mm distance from the ventricles rule was employed.

All patients' brain, who had WMHs were divided with a virtual vertical line in the middle along the falx cerebri creating a right and left side. White matter regions were created on each side along large vessels of interest representing their supplied regions (VA, BA and PCA regions) These regions were identified by means of templates based on imaging and anatomical studies (Table 1.).

Table 1. Regions of interest created with anatomical templates along the vertebral, basilar, and posterior cerebral artery. These regions represent the areas these large vessels supply. White matter hyperintensities were analyzed in these regions.

Region	Territories involved
VA region – Vertebral artery	Lower 1/3 of the cerebellum and medulla
BA region – Basilar artery	Upper 2/3 of the cerebellum and pons
PCA region – Posterior cerebral artery	Areas of the parieto-occipital lobe

Each region's WMH burden was compared to its contralateral side. Deep WMH severity classification was based on the age-related white matter changes (ARWMC) score: 0 = no confluence 1 = focal lesions; 2 = beginning confluence; 3 = diffuse involvement of the entire region (Figure 1.).

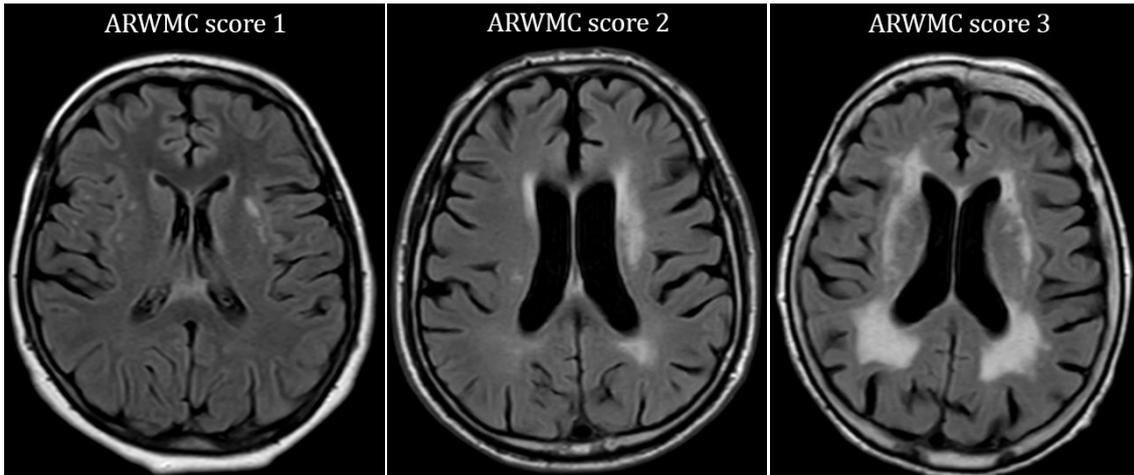


Figure 1.

Deep White matter Hyperintensity severity classification based on the age-related white matter changes (ARWMC) score. (Source: Medical Imaging Centre, Semmelweis University, Budapest, Hungary). Own picture.

The dominant WMH side (laterality) was identified either by higher ARWMC score, or if the score was the same on both sides, by manual counting of the WMH lesions that were ≥ 5 mm. The posterior communicating arteries were also analyzed. We considered these arteries as hypoplastic or occluded if their external diameter was less than 1 mm, or the arteries were absent.

1.3. 3D vascular reconstruction and measurements

We created a 3D reconstruction of the vertebrobasilar system for each patient based on the TOF MR images. We used ITK-SNAP software's (version 3.8.0.) built in semi-automated segmentation tool for reconstruction. For postprocessing all individual 3D mesh models were imported into MeshLab (v2016.12) and smoothed with the Taubin algorithm. Both vertebral arteries were cut after the V3 section which creates a curve around the arc of the atlas. The basilar top with the two branching PCAs were also cut. This method ensured obtaining the same three main arteries for our standardized measurement as in our previous research (Figure 2.). The descriptors of the vertebrobasilar geometry were extracted from the smoothed meshes semi-automatically by using VMTK scripts programmed within a Python environment.



Figure 2.

3D reconstructed model of the vertebrobasilar system. After locking all models in the same position, the Vascular Modeling Tool Kit (VMTK) application using Python scripts identifies and measures the left (tan) and right vertebral artery (red) and the basilar artery (blue). Own picture.

We set a difference of 0.3 mm or higher in diameter to define the dominance between the two VAs. Volume, curvature, torsion, and tortuosity was also measured on both VAs. The angle of the vertebrobasilar junction was measured between the two VAs. On the BA, we measured the length, volume, curvature, and cross-section. We further grouped patients based on a two-step process. First, we connected the confluence of the VAs and the basilar top with a straight line. Second, we separated each patient based on if the BA deviated to the left or to the right, while also measuring the extent of the deviation.

1.4. Statistical analysis

All analysis were adjusted for age and gender. A descriptive analysis (mean, standard deviation, and percentages) was calculated for the demographic data and the measured morphological indices in R studio (v.1.1.463). The occurrence of comorbidities between the WMH and the control group was analyzed using Fisher's Exact Test. We used Mann-

Whitney U test in the analysis of all morphological parameters as they did not show normal distribution. VA dominance was analyzed in conjunction with VA region WMH laterality (Fisher's Exact test) and each VA measured parameters (Mann-Whitney U test). The bending direction of the BA was analyzed in conjunction with BA region WMH laterality (Fisher's Exact test) and BA measured parameters (Mann-Whitney U test). We used the Spearman correlation to analyze the directional relationship between VA dominance and the bending direction of the BA. We performed 5 logistic regression analysis for white matter lesions in the PCA regions in SPSS v2.4 to determine variables, that were predictors for WMHs. The 5 logistic regression analysis was based on, whether the posterior communicating artery was occluded, or hypoplastic. Each regression model included VA dominance, bending direction of the BA and all the measured BA parameters.

2. Study - Are the Morphological Indices of the Vertebrobasilar System Heritable? A Twin Study Based on 3D Reconstructed Models

2.1. Patients

Two hundred healthy Caucasian twins (100 pairs) were randomly selected for our study from the Hungarian Twin Registry. These patients underwent T2-weighted FLAIR and 3D TOF MRI. Self-reported questionnaires were used to maximize the accuracy of zygosity classification and to collect a detailed medical history and risk factors. Exclusion criteria consisted of pregnancy, claustrophobia, or intervention in the vertebrobasilar system. We recorded exercise, smoking, alcohol consumption, body weight, height, BMI, hypertension, diabetes, and hyperlipidemia. Former and current smokers were included in the smoking group.

2.2. 3D Reconstruction

We used the same 3D reconstruction method described previously.

2.3. Statistical analysis

A descriptive analysis (mean, standard deviation, and percentages) for the risk factors and vertebrobasilar parameters was calculated with SPSS Statistics v2.4. ACE univariate genetic modeling was performed with the RStudio version 1.3.1093 and OpenMx 2.18. The phenotypic variance of the different morphological parameters was decomposed into heritability (A), shared (C), and unshared (E) environmental effects (ACE analysis). A heritability estimate was calculated using within-pair correlation between MZ and DZ twins with 95% confidence intervals (CI). We compared the saturated model (correlation matrix without zygoty) and the ACE model (correlation matrix including zygoty) for each parameter. If the two model greatly differed the results were considered only as informative. To investigate whether the anthropometric or cardiovascular risk factors influenced the morphological indices beyond age and sex, bivariate regression analyses were performed. Based on these regression models, covariates were added to further adjust the heritability models (Model 1 and Model 2). For each morphological phenotype, Model 1 was only adjusted for age and sex, while Model 2 was additionally corrected for all the risk factors with significant relationship. The best fitting ACE models were chosen based on comparing $2 \times \log$ -likelihoods and on the Akaike and Bayesian information criterion (AIC and BIC).

RESULTS

1. Study - Laterality of deep white matter hyperintensity correlates with basilar artery bending and vertebral artery dominance

290 patients with TOF and FLAIR MRI sequences were involved (Table 2.). The average age of the whole cohort was 52.4 ± 17.6 years and 59.3% of them were female (118 male / 172 female). The WMH group consisted of 204 patients (average age was 57.6 ± 16.7 years; 57.8% female) and the control group consisted of 86 patients (average age 40.2 ± 13.0 years, 62.0% female). The median age of the WMH group were significantly higher ($\tilde{x}=62$) compared to the control group ($\tilde{x}=37$, $p < 0.001$ - Mann-Whitney U test). Both hypertension and diabetes were significantly more common in the WMH group

compared to the control group (p=0.004, OR= 8.75 and p<0.001, OR= 4.08, respectively - Fisher's Exact test).

Table 2. General demographic data and risk factors between our different groups.

	Whole Cohort (n=290)	Control group (n=86)	Deep WMH group (n=204)
General demographic data			
Age (year, Mean±SD)	52.4	40.2	57.6
Sex (Male:Female)	118 : 172	32: 54	86 / 118
Risk factors (n)			
Hypertension	126	11 (8.73%)	115 (91.26%)
Diabetes	38	4 (10.52%)	34 (89.47%)
Hyperlipidemia	18	4 (22.22%)	14 (77.78%)
Basilar artery length (mm)	24.2	22.74	24.87
Basilar artery diameter (mm)	3.61	3.54	3.67
Basilar artery area (mm ²)	10.56	10.04	10.81
Basilar artery volume (mm ³)	256.93	229.32	269.73
BA deviation from centerline (mm)	2.03	1.43	2.29
Basilar artery tortuosity (%)	7.17%	5.05%	8.15%
Basilar artery torsion (%)	15.32%	14.57%	15.67%
Left vertebral artery diameter (mm)	2.87	2.81	2.90
Right vertebral artery diameter (mm)	2.68	2.66	2.69
VA dimension difference (mm)	0.76	0.69	0.79
Vertebral artery angle (°)	66.16	66.03	66.22
Left vertebral artery curvature (%)	12.31%	11.97%	12.47%
Right vertebral artery curvature (%)	12.83%	12.42%	13.02%
Left vertebral artery tortuosity (%)	12.92%	9.76%	14.39%
Right vertebral artery tortuosity (%)	11.03%	8.23%	12.32%
Left vertebral artery torsion (%)	13.86%	13.46%	14.04%
Right vertebral artery torsion (%)	12.73%	17.87%	10.03%

As expected, left VA dominance was more common (Table 3.). In contrast, vertebra artery curvature ($\tilde{x}=0.15$, $p<0.001$ - Mann-Whitney U test) and torsion ($\tilde{x}=4.9$, $p=0.002$ - Mann-Whitney U test) was significantly higher in the non-dominant VA. We found that the VA region WMH burden was significantly higher on the non-dominant hemisphere of the VA ($p=0.006$, $OR=0.13$ - Fisher's Exact test). VA angles were similar between groups WMH/Control and Left dominant/Right dominant VA. 66 patients had less than 0.3 mm difference between the two vertebral artery cross-section and were classified as even. These patients showed no correlation with cerebellar WMH laterality.

Table 3. Laterality of dominant vertebral artery.

	Whole Cohort	Control group	Deep WMH group
	(n=290)	(n=86)	(n=204)
Side of dominant vertebral artery			
Right side (>0.3mm difference)	84	26	58
Left side (>0.3mm difference)	139	39	100
Classified as even	66	20	46

VA dominance and bending direction of the BA correlated inversely ($p<0.001$, $r=-0.56$ - Spearman correlation), which has already been reported in previous publications. There were 3 times more patients with the right bending BA and left dominant VA in the whole cohort (Table 4.). We found that the tortuosity ($\tilde{x}=0.05\%$, $p=0.019$ - Mann-Whitney U test), the length ($\tilde{x}=24.13$, $p=0.005$ - Mann-Whitney U test) and mean cross-sectional area of the BA ($\tilde{x}=10.73$, $p=0.007$ - Mann-Whitney U test) were significantly higher in the WMH group. BA region WMH dominance was significantly higher on the opposite side of the BA curve ($p=0.002$, $OR=0.06$ - Fisher's Exact test).

Table 4. Bending direction of the basilar artery between our different groups.

	Whole Cohort (n=290)	Control group (n=86)	Deep WMH group (n=204)
Bending direction of the BA			
Right curve	143	36	107
Left curve	41	7	34
Classified as even	106	43	63

Logistic regression which included the whole cohort showed age ($b=0.56$, $p<0.001$, $OR=1.06$) and hypertension ($b=1.33$, $p<0.001$, $OR=1.23$) as predictors for PCA region WMH burden severity. From the 290 patients: 72 patients' posterior communicating artery was detectable on both sides (25%), 56 patients (19%) had left hypoplastic/ occluded and 67 patients (23%) had right hypoplastic/occluded posterior communicating arteries. 95 patients (33%) had hypoplastic/occluded posterior communicating arteries on both sides. Out of these 95 patients 73 had WMHs in the PCA regions. Logistic regression including the aforementioned 73 patients revealed basilar deviation from the centerline ($b=0.316$, $p=0.013$, $OR=1.37$) as a predictor for PCA region WMH laterality.

2. Study - Are the Morphological Indices of the Vertebrobasilar System Heritable? A Twin Study Based on 3D Reconstructed Models

In this study, of the 200 twins (100 pairs), 134 were MZ (67 pairs) and 66 were DZ (33 pairs). The average age was 49.6 (SD: ± 14.4) and 56.0 (SD: ± 15.2) years in the MZ and DZ groups, respectively. The two groups were significantly different in age ($p = 0.004$). The male to female ratio was 44:90 (67% female) in the MZ group and 23:43 (65% female) in the DZ group. No significant difference was observed between the MZ and DZ groups regarding anthropometric variables and risk factors. Table 5. shows the risk factors and the measured characteristics of our population.

Table 5. Demographic, clinical characteristics, and vessel morphological measurements by zygosity. *: Indicates a significant difference between the mono- and dizygotic groups. BMI: body mass index; MZ: Monozygotic; DZ: Dizygotic.

	Total	MZ	DZ	<i>p</i>
Zygosity (<i>n</i> pairs)	100	67	33	-
Age	51.66	49.57	56	0.004 *
Sex (F:M)	132:67	90:44	43:23	-
Does weekly exercise	63.00%	68.65%	51.51%	0.02 *
Alcohol consumption once a week	54.00%	52.23%	59.09%	0.29
Ever Smoked	27.00%	28.35%	24.24%	0.60
Height (cm)	167.95	167.90	168.05	0.91
Weight (kg)	72.75	72.52	73.22	0.74
BMI (kg/m ²)	25.74	25.56	26.12	0.41
Diagnosed with diabetes	8.50%	9.70%	6.06%	0.41
Diagnosed with hypertension	30.50%	30.60%	30.30%	0.95
Diagnosed with dyslipidemia	24.50%	24.62%	24.24%	0.97
Basilar artery length (mm)	24.08	23.77	24.73	0.14
BA diameter (calculated mm)	3.42	3.39	3.49	0.23
Basilar artery area (mm ²)	9.43	9.23	9.84	0.17
Basilar artery volume (mm ³)	218.28	210.47	234.49	0.05
Basilar artery curvature (mm)	2.57	2.51	2.68	0.06
Basilar artery tortuosity (%)	6.37%	6.03%	7.08%	0.35
Basilar artery torsion (%)	11.29%	10.59%	12.57%	0.19
Left vertebral a. diameter (mm)	2.44	2.44	2.45	0.92
Right vertebral a. diameter (mm)	2.36	2.36	2.36	0.91
VA dimension difference (mm)	0.75	0.71	0.82	0.22
Left vertebral artery curvature (%)	7.79%	7.88%	7.61%	0.42
Right vertebral artery curvature (%)	8.10%	8.14%	8.04%	0.77
Left vertebral artery tortuosity (%)	11.77%	11.12%	13.10%	0.23
Right vertebral artery tortuosity (%)	11.57%	10.71%	13.35%	0.07
Left vertebral artery torsion (%)	12.34%	12.64%	11.73%	0.51
Right vertebral artery torsion (%)	12.62%	12.75%	12.35%	0.78

We calculated age- and sex-adjusted parameter estimates for additive genetic (A), common environmental (C), and unique environmental influences (E) on the different measured parameters by structural equation modeling. The within-pair correlation in MZ twins was higher than in DZ for the BA length (0.616 vs. 0.288) and BA volume (0.646 vs. 0.016).

The age- and sex-adjusted additive genetic effect, within the most parsimonious model, accounted for 63% (95% CI: 45.7–75.2%) of the variance of the BA length and 60.1% (95% CI: 42.4–73.2%) of the variance of the BA volume. Unshared environmental effects accounted for 37% (95% CI: 24.8–54.3%) of the variance of the BA length and 39.9% (26.8–57.6%) of the variance of the BA volume. Although the within-pair correlation was higher in MZ twins than DZ for the VA diameter difference, left and right VA tortuosity, the ACE model differed significantly for these parameters from the saturated model, therefore, these results were only informative. The right VA curvature was moderately influenced by additive genetic factors (21%; 95% CI: 0–42.1%), and it was highly determined by the unshared environment (78.4%; 95% CI: 57.2–100%). No heritability was found for the rest of the measured parameters. There was no within-pair correlation for either MZ and DZ twins for the left and right VA torsion, therefore, the majority of the variances could be attributed to unshared environmental effects. The final ACE models were corrected for age, sex, sport activity, alcohol, smoking, diabetes, hypertension, dyslipidemia, height, weight, and BMI. We constructed models where we only corrected for age and sex and compared the two types of models for all the examined variables. From the fitted ACE models, we identified several significant environmental variables in our models, and two of them (smoking, height) were consistent across similar variables, therefore, we could conclude that smoking and height had a significant effect on the dimensions of the BA, and smoking may also affect the VA (we could only fit the ACE model on the left VA, therefore, we do not have regression data about the right side).

CONCLUSIONS

In conclusion, we created a standardized and semi-automated technique to recreate and to measure the morphology of the vertebrobasilar system. Our data suggests that the severity of WMH burden is mainly influenced by age and hypertension, while the localization of the WMHs (or laterality) is mainly influenced by vessel morphology. In the VA region WMH burden was significantly higher on the non-dominant VA side. In the BA region WMH burden was significantly higher on the opposite side of the BA curve. Increased BA curvature with increased infratentorial lateral WMH burden may be a signal for inadequate blood flow and chronic ischemia. Our data showed that BA morphology may only effect PCA region WMHs when the posterior cerebral communicating arteries are hypoplastic or occluded. Further studies are needed to better understand the supratentorial effects of the BA morphology. VA dominance and the bending direction of the BA showed an opposite directional relationship meaning that a left dominant VA may lead to a right-side bending BA over decades. The length and volume of the BA showed moderate genetical influence. However, most of the measured morphological indices were influenced by shared and unshared environmental factors, which may highlight the complex hemodynamic background of the vertebrobasilar system. These findings support further collaborative initiatives to localize the specific genes involved in the vertebrobasilar system's development and regulation.

The potential clinical relevance of these findings is that it might play a role in the development of detecting intracranial hemodynamic disturbances and it may be used in future stroke prevention methods. However, further studies are needed to elucidate the associations between vertebrobasilar morphology and WMH severity.

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