ORIGINAL RESEARCH

Clinical and pathophysiological correlates of basilar artery measurements in Fabry Disease

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ABSTRACT

BACKGROUND AND PURPOSE: Alterations of the Basilar Artery (BA) anatomy have been suggested as a possible Magnetic Resonance Angiography (MRA) feature of Fabry Disease (FD). Nonetheless, no information about their clinical or pathophysiological correlates is available, limiting our comprehension of the real impact of vessel remodeling in FD.

MATERIALS AND METHODS: Brain MRIs of 53 FD subjects (40.7±12.4 years, M/F=23/30) were collected in this single center study. Mean BA diameter and its Tortuosity Index (TI) were calculated on MRA. Possible correlations between these metrics and clinical, laboratory and advanced imaging variables of the posterior circulation were tested. In a subgroup of 20 subjects, a two-year clinical and imaging follow-up was available, with possible longitudinal changes of these metrics and their ability in predicting clinical scores that were also probed.

RESULTS: No significant association was found between MRA metrics and any clinical, laboratory or advanced imaging variable (ρ values ranging from -0.006 to 0.32). At the follow-up examination, no changes were observed over time for mean BA diameter (p = 0.84) and TI (p = 0.70). Finally, baseline MRA variables failed to predict the clinical status of FD patients at follow-up (p=0.42 and 0.66, respectively).

CONCLUSIONS: Alterations of BA in FD lack of any significant association with clinical, laboratory or advanced imaging findings collected in this study. Furthermore, this lack of correlation seems constant over time, suggesting their stability over time. Taken together, all these results suggest that the role of BA dolichoectasia in FD should be reconsidered.

ABBREVIATIONS: CNS = Central Nervous System; FASTEX = FAbry STabilization indEX; FD = Fabry Disease; Gb3 = Globotriaosylceramide; LysoGb3 = globotriaosylsphingosine; MSSI = Mainz Severity Score Index.

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SUMMARY SECTION

PREVIOUS LITERATURE: The search of a reliable Magnetic Resonance (MR) biomarker in Fabry Disease (FD) is an unmet need. Different neuroradiological features have been reported in these patients, but none of them has proved to be, to date, reliably useful for either diagnostic or prognostic purposes. In this context, alterations of the posterior circulation (namely elongation, tortuosity and ectasia, especially of the Basilar Artery (BA) have been proposed as the most prominent neuroradiological findings in FD, but their real prevalence of this finding as well as the possible clinical consequences or pathophysiological correlates is still debated in literature.

KEY FINDINGS: No meaningful increase in the mean BA diameter was observed in our sample. No significant correlation emerged between morphological alterations of the BA and macro- or microstructure of the posterior circulation, as well as any clinical outcome, either at a cross-sectional or at a longitudinal evaluation.

KNOWLEDGE ADVANCEMENT: BA measurement is prone to a high variability both within and between readers, mitigating against the routine use in clinical practice of this feature. Furthermore, the absence of correlation with clinical markers of the disease excludes a possible use of the BA abnormalities as a prognostic biomarker in FD.

INTRODUCTION

Fabry disease (FD) is a rare X-linked lysosomal storage disorder caused by deficient activity of the lysosomal enzyme α-galactosidase A[1], that leads to a progressive lysosomal accumulation of glycosphingolipids, mainly globotriaosylceramide (Gb3) and its deacylated form globotriaosylsphingosine (LysoGb3), in multiple organs, including Central Nervous System (CNS)[2].

Although a relatively new but large amount of evidence suggests the occurrence of a deep and widespread CNS involvement occurring in FD[3–8], brain damage has historically been considered as primarily sustained by cerebral vasculopathy, due to the high incidence of stroke in these patients[9,10]. In this light, and from a neuroradiological standpoint, a lot of interest in the field has been therefore dedicated to the search of an imaging biomarker related to vascular abnormalities in FD[11]. Among these, the dilative arteriopathy of posterior circulation is recognized as one of the main conventional imaging findings in this condition, with reported alterations of the vertebrobasilar system that included elongation, ectasia, tortuosity, and focal aneurismal dilatation[11,12]. In the search for quantitative biomarkers to characterize and standardize the evaluation of these posterior circulation alterations, different measurements of the dolichoectasia of the basilar artery (BA) have been tested, in some cases proposing thresholds to differentiate FD patients from healthy controls[13,14]. However, these evidences are either described in small groups of patients[13,15], and not always confirmed in other studies, with conflicting results reported in literature[11,14,16–18]. Furthermore, also when tested in large and representative groups of patients[14,18–20], neither information about possible association with structural alterations of the posterior circulation territories, nor the possible clinical impact of this alteration, were ever probed, leaving the question on the real impact of this observed change in FD unanswered.

Given this background, this study had two main aims: i) to replicate the methodology used in basilar artery (BA) measurements previously proposed in FD literature and ii) evaluate the suitability of these measurements as severity biomarker in this condition. To fulfill this second major aim, we a) investigated the relation between BA changes and either macro- and microstructure of the posterior circulation territories, as well as the possible associations with clinical and laboratory metrics, to understand both the pathophysiological and biological meanings of posterior circulation alterations in FD, and b) investigated these variables in a longitudinal setting, to evaluate the occurrence of possible changes over time and their meaning in FD and if they can predict the clinical status of the patients.

MATERIALS AND METHODS

Participants

In this retrospective study, part of a larger monocentric framework of CNS involvement in FD, MRI and clinical data were collected from October 2015 to December 2019. From a clinical standpoint, exclusion criteria were the following: age <18 and >65 years, history of major cerebrovascular events and/or other relevant neurological or systemic conditions. Furthermore, patients with artifacts on MRI sequences evaluated in this analysis, incomplete MRI exam or vertebral artery agenesia on MRA were excluded from the study (Figure 1).

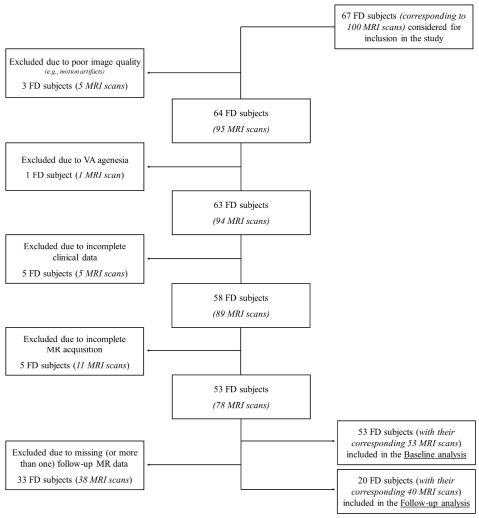

FIG 1. Flowchart showing how the final number of patients included in this study was reached.

FD = Fabry Disease; MRI = Magnetic Resonance Imaging; VA = vertebral artery

Clinical and laboratory variables, collected within 1 week from the MRI scan and retrieved from medical records, included the Mainz Severity Score Index-MSSI-[21] and the FAbry STabilization indEX (FASTEX)[22] scores, as indices of multi-organ damage severity, along with the residual α-GalA activity and LysoGb3 levels. According to the median value of the MSSI score found our group, patients were defined as mildly (if equal or minor to the median value) or severely affected. A similar approach was applied for the neurological sub-score of the MSSI, to identify patients with a less severe or a more pronounced CNS involvement.

This study was approved by the local Ethics Committee "Carlo Romano" (62/10) in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all subjects prior to enrollment.

MRI data acquisition

All participants underwent a standardized MRI protocol on the same 3T scanner (Trio, Siemens Medical Systems, Erlangen, Germany), with the same software version (VB19) and the same 8-channel head coil. The MRI protocol included a Magnetization Prepared RApid Gradient Echo sequence (MPRAGE; 160 axial slices; TR=1900ms, TE=3.4ms, TI=900ms, flip angle=9°, voxel size=1.0×1.0×1.0 mm^3) for the macrostructural evaluation of brain volumes, a 3D Fluid Attenuated Inversion Recovery sequence (FLAIR; 160 sagittal slices; TR=6000ms, TE=396ms, TI=2200ms; voxel size=1.0×1.0×1.0 mm^3) used for the assessment of white matter hyperintensities (WMH), a diffusion-weighted spin echo sequence (TR=7400ms, TE=88ms, flip angle=90°, voxel size=2.2×2.2×2.2mm^3 with 64 directions at b=1000s/mm^2 in addition to nine b=0 s/mm^2) for the evaluation of brain microstructure and a 3D Time of Flight (ToF) Magnetic Resonance Angiography (MRA) sequence (128 slices; TR=22ms; TE=3.86ms; flip angle=18°; voxel size=1.1×0.8×0.8mm^3) used for the posterior circulation anatomy assessment.

MRI data analysis

All MRA images were evaluated by three Readers of different expertise: a neuroradiologist resident with 2 years of experience in neuroimaging (Reader A), and two board-certified neuroradiologists with more than 5 (Reader B) and 15 years of experience (Reader C), respectively. Readers evaluated all ToF images independently and blinded to the subjects' demographic and clinical data, to evaluate the reliability of MRA related signs in FD, and a possible effect of the degree of experience in these evaluations. Measures obtained by the most experienced rater (Reader C) were reported in the following sections of the Manuscript and used for all statistical analyses. Furthermore, a subgroup of 15 subjects was randomly selected to assess the intrareader reliability of the investigated MRI measures after a wash-out period of 15 days by Reader A.

According to a previous study[14], two measures were obtained from MRA data: the mean BA diameter and its Tortuosity Index (TI). These two metrics were calculated as follows: using the Multiplanar Reconstruction (MPR) function of a freely available DICOM viewer (Horos, v. 3.3.6, http://horosproject.org), ToF reformats were oriented perpendicularly to the BA at three different points (namely, proximal, central and distal portions) to obtain the corresponding diameters. An average value of these three measurements was then calculated, with the mean BA diameter that was used in all analyses. Using the same MPR function, the coronal plane was selected, with a Maximum Intensity Projection reconstruction and the minimum slab thickness enabling the inclusion of the BA in its entirety. On this image, the linear and curved length of the BA were collected, as a straight line passing from the apex of the BA to the convergence of the vertebral arteries and as a curved line following the vessel along its central portion in its entirety, respectively. These two measurements were then combined to calculate the TI according to the formula "TI = (curved length/linear length) – 1". An example of these measurements is available in Figure 2.

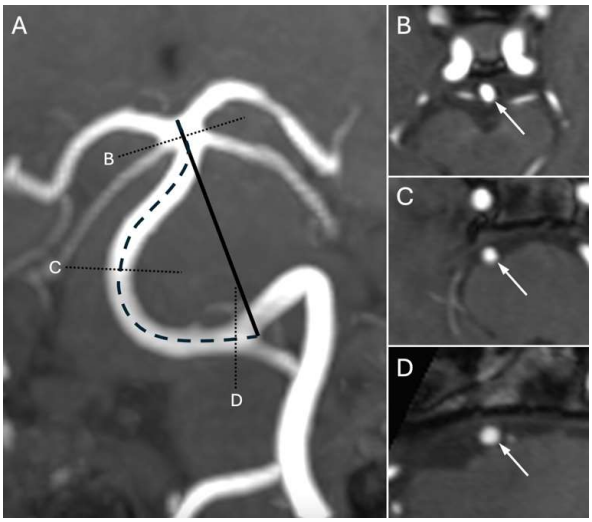

FIG 2. An example of the posterior circulation vessel measurements evaluated in this study.

In the left panel (A), a maximum intensity projection coronal MPR of the ToF sequence acquired in this study showing how the curved (*thick dashed black line*) and linear length (*straight black line*) measures were traced, with these two measures that were combined to calculate the TI. In the right panels, three axial MPR at three different levels of the BA (highest -B, intermediate -C and lower -D- portions, corresponding to the *thin dashed black line* in A) showing where the three axial diameters of this vessel were obtained to calculate the mean BA diameter for each patient.

MPR = Multiplanar Reconstruction; ToF = Time of Flight; TI = tortuosity index; BA = basilar artery

Macro- and microstructural indices of damage in FD were obtained as follows: as a first step, DICOM images were converted into NIfTI format using dcm2niix (https://github.com/rordenlab/dcm2niix), in order to be processed using FSL (https://fsl.fmrib.ox.ac.uk/fsl). For the macrostructural evaluation, brain volumes were segmented using FMRIB's Automated Segmentation Tool (FAST)[23] into their three major components: gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). For all subsequent analyses, GM and WM volumes were considered together, normalized for the total intracranial volume (as the sum of GM, WM and CSF) to take the head size into account. For the microstructural evaluation, diffusion MR images underwent denoising, unringing and eddy current correction, followed by tensor fitting to compute Fractional Anisotropy (FA) maps as the main index of microstructural damage[24–27]. The obtained FA maps were linearly registered to 3D T1-weighted volumes, with these latter that were registered to the standard Montreal Neurological Institute (MNI) space through a two-stage registration process, with a linear followed by a non-linear transformation. The corresponding transformation matrices were then inverted to register an atlas of the vascular territories available in the MNI space to subject-level scans[28,29] and extract brain volumes and mean FA values of posterior cerebral artery (PCA) territory[28,29]. A graphical representation of these processing steps is available in Figure 3.

Statistical analysis

To assess both the inter- and the intrareader agreements, a two-way mixed Intraclass Correlation Coefficient (ICC) analysis with absolute agreement was carried out. According to the literature[30], values greater than 0.9 were interpreted as proxy of an excellent reliability, while values less than 0.5 were indicative of poor reliability. Values ranging from 0.5 to 0.75 and from 0.75 to 0.9 were interpreted ad indices of moderate and good reliability, respectively.

Possible correlations between MRA metrics and demographic (age), clinical (MSSI at baseline, FASTEX at follow-up), laboratory (residual α-GalA activity and LysoGb3 levels) and imaging (global Fazekas score, as well as volumes and FA of the posterior circulation) variables were probed via partial correlation analysis, age and sex corrected, as appropriate.

Possible differences in terms of MRA variables between FD patients with a less pronounced or more severe clinical involvement, either according to the general MSSI scale or its neurological sub-score, were probed via General Linear Model, age and sex corrected.

In the subgroup of subjects with a follow-up MR scan available, possible differences in terms of BA diameters and TI between baseline and follow-up data were probed via paired t-tests. Furthermore, we tested whether MRA variables at baseline were independent predictors of the FASTEX score recorded at the follow-up examination, to investigate the possible prognostic role of these measures, via linear regression analysis. Finally, the possible association between MRA metrics and macro- and microstructural indices of damage of the posterior circulation were tested, similarly to the baseline data, via partial correlation analysis, age and sex corrected.

All statistical analyses were performed using the SPSS (Statistical Package for Social Science, Version 25.0; IBM) software and corrected for multiple comparisons via Bonferroni correction with a statistical threshold equal to 0.0045 (as 0.05/11, given that eleven different variables were probed – age, mean BA diameter, TI, MSSI scale and its neurological sub-score, FASTEX score, residual enzyme activity, LysoGb3 levels, Fazekas score, macro- and microstructural metrics of the PCA vascular territories).

The REMARK checklist for this study can be found in Supplementary Materials.

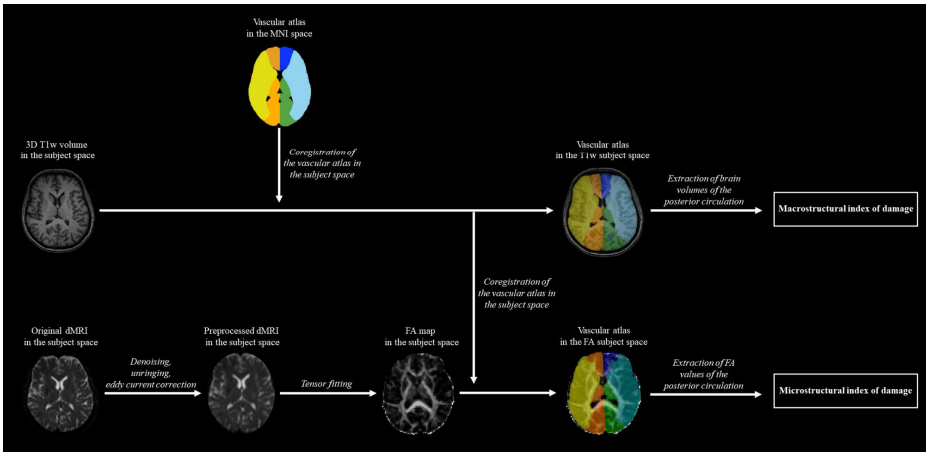

FIG 3. Workflow of the main processing steps performed to obtain macro- and microstructural indices of damage of the posterior circulation.

T1w = T1-weighted; MNI = Montreal Neurological Institute; dMRI = diffusion Magnetic Resonance Imaging; FA = Fractional Anisotropy

RESULTS

A final number of 53 FD subjects (40.7±12.4 years, M/F=23/30), all with classic or late-onset pathogenic mutation according to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), all under ERT (44/53, 83.0%) or chaperone therapy (5/53, 9.4%) with the exception of only 4 patients (4/53, 7.6%), were included in this study (Figure 1). For a subgroup of 20 patients (42.1±10.1 years at baseline, M/F=9/11) a follow-up MR scan was also available (mean follow-up time=28±8 months). Demographic and clinical data of the subjects included in the analyses are available in Table 1.

The ICC analysis of the BA diameter showed a moderate agreement between the less experienced Reader and the remaining two (0.69

and 0.72, respectively), with this value that increased to 0.87 (suggesting a good, but not excellent, agreement) between the two more experienced Readers. On the other hand, the ICC analysis of the TI proved a poor reliability of this measure between the less experienced Reader and the remaining two (0.34 and 0.46, respectively). Similarly to the findings of the BA diameter analysis, this value increased to a moderate agreement (0.65) between the two more experienced Readers. Finally, the intra-reader ICC analysis showed a moderate agreement (0.65) for the mean BA diameter, with an excellent one (0.99) for the TI. Results of this analysis can be found in Supplementary Materials.

Table 1: Demographic and clinical data of the subjects.

	Age (years)	*Sex (M/F)*	*Residual enzyme activity (μmol/L blood/h)*	*LysoGb3 (ng/mL)*	*MSSI*	*FASTEX*
Patients included in the Baseline analysis *(n=53)*	40.7±12.4	23/30	1.79±1.65	5.5±8.3	12 [4 – 52]	6 [1 – 13]
Patients included in the Follow-up analysis *(n=20 – first time point)*	42.1±10.1	9/11	2.1±1.7	7.8±12.4	13 [5 – 47]	18 [1 – 13]
Patients included in the Follow-up analysis *(n=20 – second time point)*	44.6±9.8	9/11	2.1±1.7	7.8±12.4	13 [5 – 47]	8 [1 – 14]

Ages, residual enzyme activity and LysoGb3 levels are reported as mean ± standard deviation.

MSSI and FASTEX scores are presented as median values [with ranges].

LysoGb3 = globotriaosylsphingosine; MSSI = Mainz Severity Score Index; FASTEX = FAbry STabilization indEX

In the whole group of FD patients, we found a mean BA diameter equal to 3.3±0.5 mm, with this metric that showed no significant correlation with age ($p = 0.01$, $\rho = 0.35$). Mean curved and linear length of the BA were 29.8±5.0 mm and 28.7±4.4 mm, respectively, with a resulting mean TI of 0.04±0.04, not showing a significant correlation with age ($p=0.06$, $\rho=0.26$). Patients with a more severe disease (according to the MSSI) were not different in terms of mean BA diameter ($p=0.08$) or TI ($p=0.03$) compared to subjects with a relative multi-organ preservation. Similarly, FD patients with a more pronounced CNS involvement were not different compared to subjects with a less severe neurological phenotype for any of the two tested MRA variables ($p=0.19$ and $p=0.32$, respectively).

When the possible pathophysiological and clinical counterparts of mean BA diameter changes were probed, no significant correlations were found between this value and global Fazekas score ($p = 0.18$, $\rho = 0.19$), as well as macro- and microstructural metrics of the PCA vascular territories, expressed respectively as volumes ($p = 0.51$, $\rho = 0.09$) and mean FA values ($p = 0.85$, $\rho = -0.03$). Similarly, no significant correlations were found between TI and global Fazekas score ($p = 0.81$, $\rho = 0.03$), mean FA values ($p = 0.09$, $\rho = 0.24$) and volumes ($p = 0.22$, $\rho = - 0.17$).

When the possible clinical counterpart of posterior circulation abnormalities were probed, no significant correlations were found between BA diameter and MSSI neurological sub-score ($p = 0.18$, $\rho = 0.19$), FASTEX score ($p = 0.06$, $\rho = 0.27$), residual enzyme activity ($p=0.40$, $\rho =-0.12$) or LysoGb3 levels ($p=0.03$, $\rho =0.31$), while a significant correlation was found with the general MSSI scale ($p=0.003$ 0, $\rho =-0.40$). Similarly, no correlations emerged between TI and MSSI scale ($p=0.34$, $\rho =0.14$), MSSI neurological sub-score ($p=0.45$, $\rho =0.11$), FASTEX score ($p=0.19$, $\rho =0.19$), residual enzyme activity ($p=0.44$, $\rho =-0.11$) or LysoGb3 levels ($p=0.68$, $\rho =-0.06$).

Table 2: Results of the MRI analyses.

	Fazekas score	*Basilar artery diameter*	*Tortuosity Index*	*Normalized volumes of the PCA territories*	*FA values of PCA territories*
Patients included in the Baseline analysis *(n=53)*	0 [0 – 2]	3.3±0.5	0.04±0.04	0.07±0.003	0.20±0.01
Patients included in the Follow-up analysis *(n=20 – first time point)*	1 [0 – 2]	3.4±0.6	0.04±0.05	0.07±0.003	0.20±0.01
Patients included in the Follow-up analysis *(n=20 – second time point)*	1 [0 – 3]	3.4±0.6	0.05±0.05	0.08±0.004	0.20±0.01

Basilar artery diameters are expressed in millimeters, while all other variables are adimensional.

All results are reported as mean ± standard deviation, with the only exception of Fazekas scores that are presented as median values [with ranges].

FA = fractional anisotropy; PCA= posterior cerebral artery

In the subgroup of subjects with available follow-up MRI scans, MRA metrics showed a substantial stability over time, without a significant difference between baseline and follow-up examination for both mean BA diameter ($p = 0.84$) and TI ($p = 0.70$). Furthermore, the linear regression analysis showed that neither the BA diameter ($p = 0.42$, $\beta = 0.20$, [95% CI = -0.47 - 1.06]) nor the TI ($p = 0.66$, $\beta = 0.11$, [95% CI = -7.81 - 11.92]) at baseline proved to be an independent predictor of the FASTEX score at follow-up. Finally, no correlations were found between mean BA diameter values and both macro- and microstructural metrics of the PCA vascular territories, expressed respectively as volumes ($p = 0.82$, $\rho = 0.06$) and mean FA values ($p = 0.62$, $\rho = 0.13$). A similar result was found when the TI was correlated with volumes ($p = 0.99$, $\rho = -0.002$) and mean FA values ($p = 0.13$, $\rho = 0.37$) of the posterior circulation.

A complete list of the results of the MRI evaluation with linear measurements collected by the three readers are shown in Table 2.

DISCUSSION

This study investigated the presence of morphological alterations of the vertebrobasilar system in FD patients applying several measurements previously proposed in literature, failing to demonstrate any significant correlation between morphological alterations of the BA and macro- or microstructure of the posterior circulation, as well as any clinical outcome, either at a cross-sectional or at a longitudinal evaluation.

The search of a reliable MR biomarker in FD is an unmet need. Different neuroradiological features have been reported in these patients, but none of them has proved to be, to date, reliably useful for either diagnostic or prognostic purposes[11]. These include the pulvinar sign, that went from being considered as pathognomonic of FD to being today accepted as a rare non-specific sign of this condition[31,32], and the common, but extremely aspecific, WM hyperintensities, also in the light of some recent literature showing widespread microstructural WM changes extending beyond conventional MRI hyperintensities[3,5,8]. In this context of absence of reliable imaging biomarkers, alterations of the posterior circulation (namely elongation, tortuosity and ectasia, especially of the BA) have been proposed, and relatively accepted, as the most prominent neuroradiological findings in FD. These have been hypothesized to be related to an accumulation of Gb3 within the arterial smooth muscle cells, that may cause a change in the nitric oxide pathway which in turn may lead to a progressive malfunction of the media layer with direct repercussions on vessel anatomy[33,34]. The real prevalence of this finding is still debated in literature, due to the rarity of the disease, as well as the lack of standardization about acquisition protocols and/or image processing. Furthermore, there is still a lack of information about the possible (if any) clinical consequences or pathophysiological correlates of this alteration. This study fits into this framework with the aim of evaluating in a large sample whether morphological measurements of the BA in FD are reliable between readers of different expertise and then investigating correlation between changes in BA diameters and clinical data and macro-, or microstructural changes in the posterior circulation both cross-sectional and over time.

In contrast with previous literature[14], no meaningful increase in the mean BA diameter was observed in our sample. Several explanations can be found for this result, with the first being the use of a different software for image evaluation (i.e., a freely available in our study vs a commercially available in the one conducted by Manara and colleagues). Furthermore, in our study a correction for multiple comparisons was employed to reduce the possibility of type I errors. Finally, ToF sequences in this study were acquired at 3T, compared to those acquired at 1.5T, with the known better quality of these images acquired at higher filed strength[35]. It is important to stress that a reliable biomarker for both diagnostic and prognostic purposes must be easily accessible and reproducible across centers, characteristics that do not seem to emerge for BA evaluation in the replicative section of our study. Indeed, technical validation is one of the crucial steps in the roadmap to follow to identity a possible biomarker[36]. Furthermore, also reproducibility is a potential issue, given that measurements performed using different equipment, different software, or operators, or at different sites and times, should not show any significant variation in order to be to be considered as reliable biomarker[36]. In this light, our results suggest that the BA measurement is prone to a high variability both within and between readers, further mitigating against the routine use in clinical practice of this MR feature.

Beside these consideration on BA diameters and tortuosity, advocating against the interpretation of posterior circulation changes as a typical feature of FD, we investigated the possible pathophysiological meaning of vessel abnormalities and failed to find any possible association between BA measurement and both macro- and microstructural changes of the posterior circulation parenchymal territories. It has been widely demonstrated that in cerebrovascular conditions, MRI is very sensitive in highlighting alterations both in the brain macrostructure (as in stroke[37,38]), and microstructure (as in cerebral small vessel disease[39,40]). Absence of correlation with these indices of damage, indirectly supported by a MR perfusion study that demonstrated the absence of an increased flow in the posterior circulation areas[41], was also found at the longitudinal analysis, where a lack of significant changes over time in BA metrics was also coupled to a substantial stability of GM and WM structures in the posterior circulation. If the stability over time of BA diameter and posterior GM volumes is in line with previous evidences[42,43], a recent study showed the occurrence of changes in the Apparent Diffusion Coefficient (ADC) maps over time[44]. Nonetheless, it is noteworthy to mention that in this study maps derived from a more comprehensive technique as DTI were used, with ADC values that are known to have a relatively lower reliability compared to FA[45]. Furthermore, and differently from previous studies, all acquisition performed in our study were carried out using the same scanner, with the same software version, coil and acquisition protocol, thus virtually nullify any possible bias deriving from these technical issues. Nonetheless, as significant WM microstructural damage is known to occur in this condition[3,5,8,46] future longitudinal studies are warranted to more deeply understand the role of their changes over time in this condition, given that dMRI has indeed proven to be a sensitive biomarker for monitoring the progression of WM damage[47].

Finally, the absence of correlation with clinical and biochemical markers of the disease, either at a cross-sectional and longitudinal evaluation, exclude a possible use of the investigated vessel abnormalities as a prognostic biomarker.

Despite its several strengths, this study presents some limitations, with the first residing in the lack of a control group that prevented us to define the exact extent of how much the BA diameter might have differed in FD patients. Nevertheless, our thorough examination of possible clinimetric counterparts of these posterior circulation changes does not rely on the evaluation of a control group, with the inclusion of a longitudinal analysis that even further mitigates this limitation. Another possible setback of this study might derive from the exclusion of patients with stroke and TIA, potentially leading to a selection bias towards patients with less pronounced brain involvement.

Nonetheless, it is noteworthy to remember stroke is associated with an increased BA diameter also in subjects without FD[48], a result that also partly question whether the previously observed increased BA diameter in FD might be related to a "general" vasculopathy, as the one found in subjects without FD affected by a stroke. Finally, another intrinsic limitation of this Work resides in the challenges derived from measuring vessels on MRA without intensity normalization, or without procedures for vessel wall identification[14].

In conclusion, our results suggest that evaluation of the posterior circle anatomy in FD might not provide a useful or reliable biomarker in this condition. These metrics show a low reliability within thew same reader and between readers of different expertise, lack of macro- and microstructural brain correlates and do not show significant association with clinical findings, neither at a cross-sectional nor at a longitudinal evaluation. Therefore, the search for a new, possible quantitative, diagnostic and prognostic neuroradiological biomarker in FD remains an urgent need.

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SUPPLEMENTAL FILES

Supplemental Table 2: Results of the ICC analyses.

	Basilar artery diameter	*Tortuosity Index*
Reader A vs Reader B	0.69	0.34
Reader A vs Reader C	0.72	0.46
Reader B vs Reader C	0.87	0.65
Intrareader analysis (Reader A)	0.65	0.99