European Journal of Medical Genetics 65 (2022) 104424

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

Neurovascular abnormalities in patients with Loeys-Dietz syndrome type III

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ARTICLE INFO

Keywords:
Neurovascular abnormalities
Cranial aneurysm/dissection/tortuosity
Loeys-Dietz syndrome type III
SMAD3 mutation
Aorta aneurysms osteoarthritis syndrome

ABSTRACT

The aim of this article is to describe neurovascular findings in patients with Loeys Dietz syndrome type III and their possible clinical impact. Loeys Dietz syndrome type III, caused by pathogenic *SMAD3* variants, is an autosomal dominant syndrome characterized by aneurysms and arterial tortuosity in combination with osteoarthritis. Neurovascular abnormalities have been described in other heritable aortic syndromes, however, reliable data in Loeys Dietz syndrome type III is missing.

In our tertiary center, all adult patients with confirmed Loeys Dietz syndrome type III are followed in a standardized aorta outpatient clinic including Computed Tomography Angiography (CTA) of the head and neck region at baseline and (tri) yearly during follow-up. We performed an analysis of the neurovascular imaging findings and clinical follow-up. The primary outcome was a combined endpoint of mortality, dissection, cerebral vascular event and intervention. In addition, tortuosity and vascular growth were assessed. In total 26 patients (mean age 38.4 years, 38.5% males) underwent 102 (mean 3.9 (1-8) per patient) neurovascular Computed Tomography Angiography scans between 2010 and 2021. In 84.6% some form of neurovascular abnormality was found. The abnormalities at baseline were aneurysm (26.9%) dissection flap (7.7%), arterial tortuosity (61.5%), arterial coiling (23.1%) and arterial kinking (3.8%). During follow up (mean 8.85 (1-11) years) one patient suffered from sudden death and one patient needed a neuro-radiological intervention. No cerebral bleeding or stroke occurred.

In conclusion, neurovascular imaging in Loeys Dietz syndrome type III patients revealed abnormalities such as aneurysm, tortuosity, coiling and kinking in the vast majority of patients, but clinical events were rare. Neurovascular screening and follow up is advised in all Loeys Dietz syndrome type III patients.

1. Introduction

The Aneurysms-Osteoarthritis Syndrome (AOS) was first described in 2011, and was caused by pathogenic variants in the *SMAD3* gene. The syndrome was classified as Loeys Dietz syndrome type III (LDSIII) (Schepers et al., 2018). This syndrome has an autosomal dominant inheritance pattern with variable clinical expression (Van Der Linde et al., 2012). The *SMAD3* gene is located on chromosome 15q22.2–24.2 and encodes for a protein of the *TGF-β* pathway that is essential for *TGF-β* signal transmission (van de Laar et al., 2011). Heterozygous *SMAD3* variants lead to increased aortic expression of several key players in the *TGF-β* pathway, such as phosphorylated *SMAD2*, *SMAD3* and *TGF-β1* (van de Laar et al., 2011). Activation of the *TGF-β* pathway has also been observed in other diseases with arterial wall anomalies, such as Marfan Syndrome (MFS), bicuspid aortic valve and degenerative aneurysmal aortic disease (van de Laar et al., 2012). LDS exhibits a more aggressive course than the other disorders, with morbidity and mortality typically resulting from complications of aortic/arterial dissections (Loeys, 2006).

Aneurysms, dissections and tortuosity throughout the arterial tree are the main features of LDS III. These features may potentially cause serious complications such as dissection and rupture, which explain the

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https://doi.org/10.1016/j.ejmg.2022.104424
Received 23 September 2021; Received in revised form 31 December 2021; Accepted 8 January 2022
Available online 11 January 2022

reported high mortality rates of up to 34% (Van Der Linde et al., 2012). Early diagnosis, short-interval follow-up imaging and prophylactic surgical intervention have proven beneficial in preventing catastrophic vascular complications or death (van den Hoven et al., 2018).

Neurovascular abnormalities such as tortuosity or aneurysms of the carotid and vertebrobasilar arteries have been described in patients with heritable thoracic aortic diseases. van de Laar et al. (2012) described tortuosity in cerebral arteries in 50% of 45 patients with a *SMAD3* mutation. Although arterial tortuosity is not a classical feature of MFS, vertebral tortuosity is found significantly more often in MFS and LDS patients compared to controls (Rodrigues et al., 2009).

Moreover, in MFS, a higher percentage of vertebral arterial tortuosity was associated with earlier age at first cardiovascular surgery and increased rates of surgical interventions (Bons et al., 2019; Weibel).

Since there are different types of LDS, with variable clinical symptoms, it is important to report in which type neurovascular abnormalities are more prevalent. Evidence about neurovascular abnormalities in patients with a *SMAD3* gene-variant is limited, with mostly retrospective studies and without information about the clinical follow-up of these neurovascular abnormalities (Regalado et al., 2011; Hostetler et al., 2019; Rodrigues et al., 2009).

The aim of this study is to assess the prevalence of neurovascular abnormalities and to investigate their clinical impact in LDS III patients, evaluate changes of cerebral vasculature and abnormalities over time and identify factors associated with these neurovascular abnormalities.

2. Methods

2.1. Study population

In 2010 a standard protocol for assessment and follow-up of patients with LDS III was initiated (Fig. 1) at the dedicated outpatient clinic for adult patients with aorta pathology in our tertiary center. Patients were routinely investigated and underwent electrocardiogram, echocardiography and CTA of the thorax and abdomen every year and CTA of the head and neck arteries at baseline and at least every three years. All adult patients with confirmed LDS III, were invited to participate in this observational study between April 2010 and January 2020, with clinical follow-up until September 2020. The study was approved by the medical ethics committee, and was designed, performed and controlled in accordance with current local and international good clinical practice guidelines. Written informed consent was obtained from all patients.

The primary endpoint was any neurovascular event, defined as neurovascular related mortality (proven or high suspicion), neurovascular dissection or rupture, ischemic stroke or cerebral bleeding or need for preventive neurovascular intervention.

The secondary endpoint was presence of neurovascular abnormalities on CT and neurovascular changes over time.

2.2. Data collection

For this observational study, the following information was registered at baseline: medical history, neurovascular abnormalities, neurovascular interventions, aorta- and small vessel abnormalities and interventions, other vascular abnormalities or interventions.

The body surface area (BSA) was calculated using the DuBois and DuBois formula: BSA (m2) = 0.007184 × height $(m)^{0.725}$ × weight $(kg)^{0.425}$. Hypertension was defined as a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg or 'requiring medical therapy'. Dyslipidemia was defined as 'Cholesterol total ≥6.5 and/or LDL ≥4.12' or 'requiring medical therapy'. Smoking was defined as never, current or former. The age at first neurovascular CTA was defined as the baseline of the study. The follow up CTA was defined as the last CTA the patient received. Baseline measurement of the thoracic, abdominal and cerebral vascular diameters was performed using CTA. Cross sectional aortic diameter was evaluated at the aortic annulus, the sinus of Valsalva, the sino-tubular junction, and the ascending aorta using the double oblique measurement method perpendicular to the vessel axis (Bons et al., 2019). A thoracic aorta ≥40 mm was defined as thoracic aortic aneurysm (TAA). An abdominal aorta ≥25 mm was defined as abdominal aortic aneurysm (AAA). A common internal artery ≥25 mm, an iliac internal artery ≥8 mm, a splenic artery ≥6 mm, a hepatic/gastric artery ≥6 mm, a renal artery ≥6 mm, a coeliac artery ≥ 6 mm, a left internal mammary artery (LIMA) ≥ 5 mm and a right

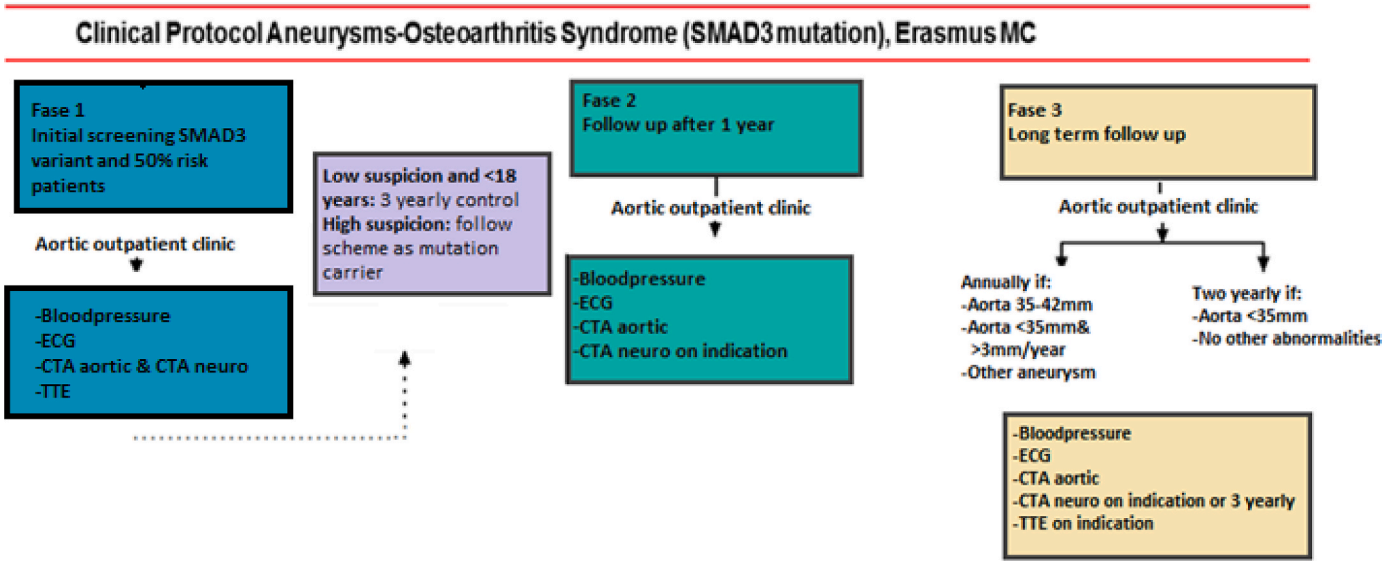

Fig. 1. Clinical protocol of aneurysm-osteoarthritis syndrome (*SMAD3* variant). Legend: CTA aortic = computed tomography angiography of the total aorta, CTA head/neck arteries = computed tomography angiography of the neurovascular arteries, ECG = electrocardiogram, TTE = transthoracic echocardiography.

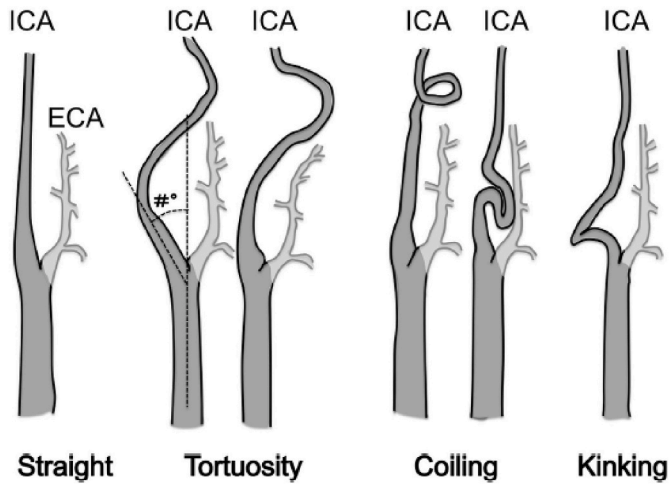

Fig. 2. Overall tortuosity of intracranial vasculature. Schematic drawing of morphological classification of the carotid artery, viewed from the right lateral side
Legend: ICA = internal carotid artery; ECA = external carotid artery. *Obtained permission for publication from Elsevier, Journal of Cranio-Maxillofacial surgery, 'Three-dimensional computed tomographic analysis of variations of the carotid artery', Authors:Tetsuji Nagata, Kazuma Masumoto, Yutaro Hayashi, Yoshiko Watanabe, Yuta Kato, Fuminori Katou.*

internal mammary artery (RIMA) $\geq$ 5 mm were defined as small vessel aneurysm. In the patients with an aneurysm in the intra- and extracranial vasculature the change in diameter in mm's was measured and divided by the number of years between the scans.

2.3. Assessment of cerebral vasculature

See supplemental data: file 1. (Fig. 2)

2.4. Statistical analyses

Continuous data were presented as mean and standard deviation (SD) when normally distributed, and as median with interquartile range (IQR) when skewed. Categorical data were presented as frequencies with percentages. Differences were assessed between patients with and without neurovascular abnormalities. For categorical variables including thoracic aortic aneurysm, abdominal aneurysm, age, height, BSA, hypertension, hypercholesterolemia and smoking the Chi-square test was used. Analyses were performed using IBM SPSS Statistics Viewer (version 25) software. A p-value $\leq$ 0.05 was defined to be statistically significant.

3. Results

3.1. Baseline characteristics

In total, 26 patients with confirmed pathogenic or likely pathogenic *SMAD3* genetic variants were included. These variants in our group were documented in the Leiden Open Variation Database (LVOD), the access numbers are as follows: 0000308567; 0000308566; 0000615471; 0000308563; 0000308554; 0000308555; 0000831232; 0000308564; 0000399285; 29907982; 0000399300. (See supplemental file 2)

The mean age was 38.4 $\pm$ 12.7 years (38.5% males) (Table 1). In total 102 CTA's of the head and neck arteries were performed (mean 3.9 (1-8) per patient). Four patients had only one CTA due to short follow up. The mean follow up between the first and last CTA of the 23 patients with multiple CT scans was 8.85 (1-11) ; ; ; years.

The median height was 177.5 cm (IQR 173.3–187.0), weight 83.3 kg (IQR 72.5–92.8) and BSA 2.0 m^2 (IQR 1.9–2.1). The median systolic blood pressure was 123.0 mmHg (IQR116.0–134.5), diastolic blood pressure 75.5 mmHg (IQR 71.8–82.8), median of the mean arterial blood pressure 93.0 mmHg (IQR 87.0–100.8). Hypertension was found in 12 (46.2%) of the patients. Of the 26 patients 6 (23.0%) were treated with a beta blocker, 6 (23.0%) with an Angiotensin-converting enzyme (ACE) inhibitor, 1 (3.7%) with an Angiotensin II receptor blockers (ARB), 1 (3.8%) with a calcium blocker and in 14 (53.8%) no medication was used. Seven (26.9%) were current, 3 (11.5%) former and 16 (61.5%) never smokers. Cholesterol panel was normal in 19 (73.1%), without use of cholesterol lowering medication. In 4 patients (15.4%) hypercholesterolemia was present of whom 1 (3.8%) received medication. In three patients no information on cholesterol was available.

3.2. Prevalence of abnormalities in cerebral vasculature

At baseline 22 of the 26 patients (84.6%) showed neurovascular abnormalities. Neurovascular aneurysms were diagnosed in 6 patients (23.1%), of whom one patient had 2 aneurysms. The aneurysms were located in the internal carotid artery (5) , the vertebral artery (1) and the middle cerebral artery (1) (Fig. 3). In terms of shape, 5 were saccular, 1 was fusiform and 1 was a pseudo aneurysm (vertebral artery). One patient had two aneurysms bilateral of the internal carotid arteries. Arterial dissection of the vertebral artery was diagnosed in 2 (7.7%) patients at baseline. None of these patients had any symptoms.

The patients having a chronic neurovascular dissection, were both female and had no intervention. The first patient underwent only one CTA of the head and neck arteries at the age of 43 years, due to late inclusion with short follow-up. There was tortuosity of the left internal carotid artery with a focal dilatation, possible because of an earlier dissection. The right internal carotid artery had a small torturous course. The proximal vertebral artery had dilatations, narrowing and on more places a suggestion of dissection flaps on both sides and a suggestion of a small pseudo aneurysm on the left side. More distally a strong tortuous course on both sides of the vertebral artery was found. The intracranial arteries all showed tortuosity. This patient was known to have a normal diameter of the thoracic- and abdominal aorta, a BSA of 1.74 m^2, hypertension without medical treatment, no dyslipidemia and never smoked.

The second patient who had a neuro-vascular dissection underwent 5 neurovascular CTA's in total and her age at baseline was 42 years. The neurovascular findings were stable over time. The CTA's described a normal diameter of the carotid artery, fusiform dilatation of the V1 segment of the right vertebral artery, with a dissection of a short segment. Normal diameter of the left vertebral artery and the intracerebral arteries. This patient was known with a normal diameter of the thoracic aorta, an aneurysm of the right internal mammary artery of 6 mm, a BSA of 1.84 m^2, no hypertension, no dyslipidemia and never smoked.

The most prevalent neurovascular abnormality in our patient population was neurovascular arterial tortuosity (see Figure 2), which was found in 61.5% of all the patients. In 23.1% arterial coiling and in 3.8% arterial kinking was found. A distribution in categories of the tortuosity was made on the different arteries, the bilateral internal carotid artery, the bilateral vertebral artery and the basilar artery, see Table 2.

Table 1
Baseline characteristics and neurological findings of adult patients with proven *SMAD3* gene variant.

patient	sex	BSA@	Hyper-tension*	Age diag-nosis(y)	Gene variants	Age first CTA head neck (y)	Follow up (y)	Amount of neuro CT's	Neurovascular findings		Neurovascular intervention	Thoracic aorta Findings/ intervention		Abdominal aorta Findings/intervention		Small vessel findings	Small vessel intervention
									Aneurysm/ shape (1,2) **	Overall tortuosity#		TAA@	Aortic surgery	AAA##	AAA## Intervention	Small vessel aneurysm	Small vessel intervention
1	F	1,74	No	29	c.861delG, p. Arg288fs	29	7	5	aneurysm ICA left (1) 2 mm	1	No	No	No	No	No	Saccular aneurysm splenic artery	coiling splenic artery
2	F	1,87	Yes	54	c.861delG, p. Arg288fs	54	22	5	No	3	No	Yes	Yes	No	No	Aneurysm distal splenic artery Aneurysm proximal splenic artery	coiling distal splenic artery
3	F	1,71	Yes	59	c.859C>T, p. Arg287Trp	59	8	3	No	1	No	Yes	No	No	No	No	
4	M	2,10	Yes	31	c.859C>T, p. Arg287Trp	31	8	4	aneurysm ICA left (1) and right (1)	1	Yes	Yes	Yes	No	No	No	
5	F	1,88	Yes	46	c.859C>T, p. Arg287Trp	46	11	8	No	2	No	Yes	No	Chronic dissection distal aortic	No	Aneurysm splenic artery Aneurysm coeliac artery, iliac communis artery right, splenic artery, hepatic artery left	coiling splenic artery
6	M	1,96	No	19	c.859C>T, p. Arg287Trp	22	9	3	No	0	No	Yes	Yes	No	No	No	
7	F	1,82	No	66	c.859C>T, p. Arg287Trp	66	8	6	aneurysm MCA left (1)	2	No	No	No	No	No	No	
8	F	2,08	No	40	c.859C>T, p. Arg287Trp	40	12	6	No	1	No	Yes	Yes	No	No	No	
9	M	2,04	Yes	63	c.859C>T, p. Arg287Trp	63	9	4	No	1	No	Yes	Yes	No	No	Aneurysm gastroduodenal artery, renal artery right	No
10	M	2,42	Yes	31	c.859C>T, p. Arg287Trp	36	11	3	No	2	No	Yes	Yes	No	No	No	
11	F	2,14	No	35	c.859C>T, p. Arg287Trp	40	12	4	No	0	No	Yes	Yes	No	No	Aneurysm thoracic internal artery right	Coiling thoracic internal artery right
12	F	2,17	No	30	c.401-6G>A, r.400_401insACAG	30	5	2	aneurysm ICA left 3 mm (1)	1	No	No	No	No	No	Aneurysm splenic artery, one saccular, one fusiform	No
13	F	1,74	Yes	36	c.859C>T, p. Arg287Trp	43	8	1	Pseudo aneurysm and dissection flap VA left	2	No	No	No	No	No	No	
14	F	1,83	Yes	45	c.859C>T, p. Arg287Trp	46	12	5	No	1	No	Yes	Yes	No	No	No	

(*continued on next page*)

Table 1 (*continued*)

patient	sex	BSA@	Hyper-tension*	Age diag-nosis(y)	Gene variants	Age first CTA head neck (y)	Follow up (y)	Amount of neuro CT's	Neurovascular findings		Neurovascular intervention	Thoracic aorta Findings/ intervention		Abdominal aorta Findings/intervention		Small vessel findings	Small vessel intervention
									Aneurysm/ shape (1,2) **	Overall tortuosity#		TAA@	Aortic surgery	AAA##	AAA## Intervention	Small vessel aneurysm	Small vessel intervention
15	F	1,95	No	42	c.859C>T, p. Arg287Trp	42	11	4	No	1	No	No	No	No	No	aneurysm CIA	No
16	M	1,99	No	20	c.859C>T, p. Arg287Trp	18	7	4	No	0	No	Yes	Yes	No	No	No	
17	M	2,33	No	28	c.859C>T, p. Arg287Trp	28	11	4	No	2	No	No	No	No	No	No	
18	M	2,33	No	28	c.859C>T, p. Arg287Trp	31	12	5	No	1	No	Yes	No	No	No	No	
19	M	2,17	Yes	40	c.859C>T, p. Arg287Trp	42	11	6	aneurysm ICA right 6 mm (2)	2	No	Yes	Yes	No	No	Aneurysm splenic artery, hepatic artery, gastric artery, LIMA, mesenteric superior artery, thoracic intercostal artery	Stenting splenic artery, hepatic artery, gastric artery. Coiling LIMA, thoracic intercostal artery
20	F	2,06	Yes	45	c.741-742delAT, p. Thr247fs	49	12	6	No	1	No	Yes	Yes	No	No	Aneurysm coeliac artery	No
21	F	1,84	No	41	c.1045G>C, p. Ala349Pro	42	7	5	Dissection flap VA right	1	No	No	No	No	No	Aneurysm RIMA and LIMA	No
22	M	2,06	No	30	exon 6 deletion, c.659_871del	32	12	2	No	1	No	Yes	Yes	No	No	No	
23	F	2,01	No	25	c.1102C>T, p. Arg368*	29	6	2	No	1	No	No	No	No	No	No	
24	M	2,28	Yes	48	c.1102C>T, p. Arg368*	54	7	1	No	1	No	Yes	No	No	No	No	
25	F	1,91	No	23	15q22.3q23 microdeletion including SMAD3	30	8	1	No	0	No	Yes	No	No	No	No	
26	F	2,00	Yes	45	c.76C>T, p.Gln26*	45	1	1	No	1	No	No	No	No	No	No	
Total 26	38,5% men	Mean BSA 2.0 m^2 (+,2)	46.2% Hyper-tension or medical therapy	Mean age 38.4 (+12.7) years		Mean age first CTA 40,1 (+12,4)	Median follow up 9,0 years IQR [7.0–12.0]	Median amount of CTA's head/ neck 4.0 IQR [2,0–5,3]	7 (26.9%) patients with neuro (pseudo) aneurysms/ dissections	22 (84.6%) patients with neuro vascular tortuosity	1 neuro-vascular interven tion	17 (65.4%) patients with TAA	12 (46,2%) patient with aortic surgery	1 (3.8%) patients with abdominal aneurysm	0 (0%) patients with abdominal interven-tion	10 (38,5%) patients with small vessel aneurysm	5 (19,2%) patients with small vessel intervention

Legend: @BSA (body surface area in m2), *hypertension (systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg or 'requiring medical therapy'); **aneurysm shape (1 = saccular, 2 = fusiform), ICA = internal carotid artery; MCA = middle cerebral artery; PCOM = posterior communis artery; VA = vertebral artery; #overall tortuosity (0 = straight, 1 = tortuosity, 2 = coiling, 3 = kinking); @TAA = thoracic aortic aneurysm, if aorta diameter is > 40 mm; ##AAA = abdominal aortic aneurysm, if aorta diameter is > 25 mm; Small vessel aneurysm if a common internal artery ≥25 mm, an iliac internal artery ≥8 mm, a splenic artery >6 mm, a hepatic/gastric artery >6 mm, a renal artery >6 mm, a coeliac artery is > 6 mm, a LIMA/RIMA > 5 mm; CIA = common iliac artery; LIMA = left internal mammary artery, RIMA = right internal mammary artery.

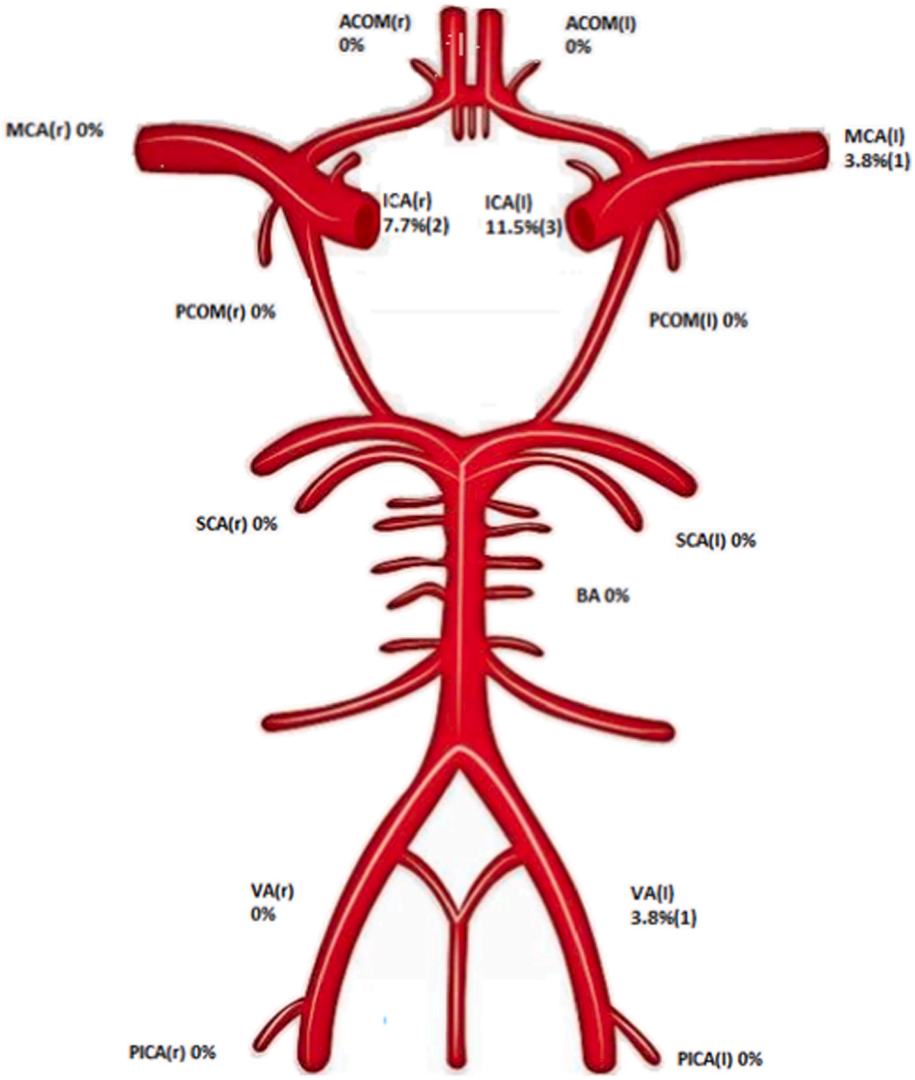

Fig. 3. Occurrence of aneurysms per neurovascular artery, the circle of Willis in adult patients with proven *SMAD3* gene mutation. Percentage of occurrence per neurovascular artery.Legend: ICA(l/r)-internal carotid artery(left/right); ACOM(l/r) = anterior communicating artery(left/right), MCA(l/r) = middle cerebral artery(left/right); PCOM(l/r) = posterior communicating artery(left/right), SCA(l/r) = superior cerebellar artery(left/right); BA = basilar artery; PICA(l/r) = posterior inferior cerebellar artery(left/right); VA(l/r) = vertebral artery(left/right).

Table 2
Overall tortuosity of neurovascular arteries in adult patients with proven *SMAD3* gene variant. Overall tortuosity in all patients and shown in arteries with overall tortuosity divided in straight (normal), tortuosity, coiling and kinking.

	Overall	ICA left	ICA right	VA left	VA right	BA
Straight	4 (15.4)	5 (19.2)	9 (34.6)	4 (15.4)	6 (23.1)	9 (34.6)
Tortuosity	16 (61.5)	18 (69.2)	13 (50.0)	11 (42.3)	10 (38.5)	17 (65.4)
Coiling	6 (23.1)	3 (11.5)	5 (19.2)	8 (30.8)	7 (26.9)	1 (3.8)
Kinking	1 (3.8)	1 (3.8)	0 (0.0)	4 (15.4)	4 (15.4)	0 (0.0)

Legend: Data is displayed as frequency and (percentage), ICA = internal carotid artery, VA = vertebral artery, BA = basilar artery.

3.3. Change of neurovascular abnormalities over time

During a mean of mean 8.85 (1-11) ; ; ; years follow up, in 4 (15.4%) patients there was progression in the neurovascular abnormalities. In one patient, who was already known with 2 aneurysms of the carotid artery on both sites, a new aneurysm of 3 mm in the left carotid artery was observed after 2 years of follow-up. Two patients had a change of the vertebral artery, one from straight to tortuosity and the other from tortuosity to coiling. One patient had a change from tortuosity to coiling in the overall view. No change in diameters was observed in the patients known with an aneurysm.

3.4. Prevalence of clinical events

During follow-up, there were 2 clinical events: one patient needed a neurovascular intervention, and one patient suffered from sudden death of unknown cause, no autopsy was performed. Below we will describe these cases in further detail.

The patient who needed a neurovascular intervention, was a male of 35 years with a Tyron David (valve sparing aortic root replacement) operation at the age of 32. He had a BSA of 2.10 m^2, no dyslipidemia, was current smoker and used ACE-inhibition for hypertension. At baseline, the patient was known with 2 asymptomatic aneurysms of the carotid siphon on both sites, measuring both 7 mm. These aneurysms remained stable during two years of follow-up. However, a third aneurysm of 3 mm occurred and was located also at the left side of the carotid artery in a different part. The left vertebral artery showed coiling, but there was a normal course of the vertebral artery on the right site and the basilar artery. Because there was progression noticed of the third aneurysm, 2 flow diverters were placed in the carotid artery on both sides. No complications occurred.

The patient who died suddenly, was a female patient of 67 years, she was found death lying in front of her bed, no autopsy was performed and the cause of death remains unknown. This patient was known to have a stable aorta sinus of Valsalva of 40 mm and a stable aorta ascending of 38 mm during 8 years of follow-up. The last CTA of head and neck arteries showed a sharp angle at the entrance of the left vertebral artery,

with a further normal aspect of the vertebral arteries and on both sides a normal aspect of the carotid arteries. She was known with an unchanged fusiform dilatation of the basilar artery of 7 mm, no signs of a basilar artery aneurysm and no signs of an intracranial aneurysm. Furthermore this patient had a normal BSA (1.88 m^2), was known with hypertension treated with a beta blocker, had no dyslipidemia and never smoked. The cardiovascular history revealed atrial fibrillation for which 2 electrocardioversions in the past and use of apixaban for stroke prevention. Other relevant medical history included arthrosis of the cervical vertebral column.

3.5. Factors associated with neurovascular abnormalities

In our study population there was no difference in the amount of neurovascular aneurysms between males (30.8%) and females (30.8%). No significant difference was found in patients with and without neurovascular aneurysm in having TAA (7.7% vs 57.7%, p = 0.028), AAA (0.0% vs 3.8%, p = 1.0), or small vessel aneurysm (15.4% vs 23.1%, p = 0.369). Osteoarthritis was present in 16 (61.5%) patients and 3 (11.5%) of them had a neurovascular aneurysm. There was no significant difference (p = 0.369) in osteoarthritis in patients with or without neurovascular abnormalities. There was also no significant difference in sex, age, hypertension, dyslipidaemia, smoking, BSA or BMI, as is shown in Table 3.

In 15 (57.7%) patients of the same family, the same pathogenic variant on chromosome *R287W, 859C>T (SMAD3 ex 9)* (heterozygous form) was found. Only 2 patients with this pathogenic variant had a neurovascular aneurysm. No difference was found between specific pathogenic variant and neurovascular aneurysm.

Table 3
Prevalence of neurovascular aneurysms in adult patients with *SMAD3* gene variant.

	With cerebral aneurysm n = 6	Without cerebral aneurysm n = 21	p-value
TAA, n(%)	2 (7.7)	15 (57.7)	0.302
AAA, n(%)	0 (0.0)	1 (3.8)	1.00
Small vessel aneurysm, n(%)	4 (15.4)	6 (23.1)	.369
Osteoarthritis, n(%)	3 (11.5)	13 (50.0)	.369
Hypertension, n (%)	3 (11.5)	11 (42.3)	1.00
Dyslipidemia, n(%)	0 (0.0)	4 (15.4)	1.0
Smoking			
current, n(%)	2 (7.7)	5 (19.2)	.755
former, n(%)	1 (3.8)	2 (7.7)	
never, n(%)	3 (11.5)	13 (50.0)	
BSA* (m2)	1.9 (1.74–2.17))	1.9 (1.71–2.42)	.206
BMI* (kg/m2)	25.2 (20.2–30.6)	26.1 (±4.2)	.408
Age*(years)	40.3 (29–68)	40.1 (18–63)	.419
Gender* male, n(%)	2 (7.7)	8 (30.8)	.668
female, n(%)	5 (19.2)	11 (42.3)	

Legend: Data is displaced as frequency (percentages). P value is displaced as Fisher's Exact test. *mean (standard deviation) using the T-test. TAA = thoracic aortic aneurysm, AAA = abdominal aortic aneurysm. Hypertension: systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg or 'requiring medical therapy'. Dyslipidemia: 'Cholesterol total ≥6.5 mmol/L and/or LDL ≥4.12 mmol/L' or 'requiring medical therapy'. Smoking: never, current or former. Age of the first CTA head and neck arteries was defined as the baseline of the study.

4. Discussion

To the best of our knowledge, we conducted the first prospective study with clinical and imaging evaluation on neurovascular abnormalities in LDS III patients. We found at a mean age of 38.5 years in 84.6% of the patients neurovascular imaging abnormalities, including two dissections. During a mean follow-up of 8.85 years, two patients (7.7%) suffered from an event: one unexplained sudden death and one progression of aneurysm formation needing preventive neurovascular intervention. However, no cerebrovascular hemorrhage or ischemic stroke occurred.

Intra- and extracranial aneurysms were found in 26.9% of our patients. Previous studies on neurovascular aneurysms in Loeys–Dietz Syndrome, described different percentages of aneurysms. In a cohort of 62 LDS III patients (Hostetler et al., 2019), 13% had an intra- and extracranial aneurysm, while in a cohort of 25 LDS type I patients (Rodrigues et al., 2009) 32% had an intra- and cranial aneurysm. In a retrospective study (Kim et al., 2016) the prevalence of intra- and extracranial aneurysms in patients with different connective tissue diseases was described: 14% in MFS patients, 12% in different types of EDS patients and 28% in different types of LDS patients. Although no differentiation in types of LDS was mentioned in this study, the percentage of cerebral aneurysms seems similar to our study. Furthermore, a higher prevalence of aneurysm is found in LDS compared to Marfan- and Ehlers Danlos syndrome. With a prevalence of almost one third of the LDS type III patients, it seems advisable to screen for these abnormalities at diagnosis.

In our study, asymptomatic cerebral dissection was seen in 7.7% of LDS III patients. In a cohort of LDS type I patients (Rodrigues et al., 2009) this was reported to be 12% (3/25). Kim et al. (2016) found cerebral dissections in 2% (2/99) of Marfan syndrome patients and in 2% (1/47) of EDS patients. Notably they reported no cerebral dissection in LDS at selection and there was no follow-up. In a cohort of 62 LDS III patients, one patient suffered from a neurovascular hemorrhage due to dissection (Hostetler et al., 2019). Overall, neurovascular dissections in LDS III occur in 7–12% but only rarely seems associated with actual clinical impact.

Diedrich et al. (2011) reported higher arterial tortuosity in the familial aneurysm and connective tissue syndromes cases than the negative controls. In our study the vast majority of LDS III patients (84.6%) had neurovascular arterial tortuosity, while this is not commonly observed in the general population (Rinkel, 2005). Rodrigues et al. (2009) even reported arterial tortuosity in all 25 LDS type I patients. Arterial tortuosity seems more prevalent in LDS than in patients with Marfan syndrome (Diedrich et al., 2011).

During a mean of 8.85 (1-11) ; ; ; years follow up, in 15.4% of our patients there was progression in the neurovascular abnormalities, from changes in arterial tortuosity to developing a new aneurysm. No change of the diameters was observed in the patients known with an aneurysm. It was to be expected that extreme tortuosity in the shape of kinking, could develop into an aneurysm or a dissection. We did not see extreme tortuosity developing in an aneurysm during follow up. A novel finding from our study is that arterial tortuosity, although prevalent in this population, remained reasonable stable over time.

A neurovascular clinical event was found in 7.7% during follow up, one patient needed a neurovascular intervention and one patient died of unknown cause. Of course we cannot prove this event was of neurovascular origin. It could also be caused by an aortic rupture, knowing that this patient had a stable aorta dilatation for 8 years. No proven neurovascular hemorrhage or ischemic stroke was observed in our patient group. In a retrospective study of LDS III patients of Regalado et al. (2011) 9.5% of the patients had a neurovascular event, such as rupture, or hemorrhage but they also included an aneurysm as an event. A

retrospective review of Hostetler et al. (2019) found at baseline in 6.5% of LDS III patients a neurovascular event, three individuals who underwent surgical repair of intracranial aneurysms and one individual died at the age of 56 years from subarachnoid hemorrhage. All the study groups are small, with less events if the studies contained more patients, pointing towards possible bias in including more severally ill patients. Larger prospective multicenter studies are clearly warranted.

It was to be expected that patients with TAA, AAA or small vessel aneurysm caused by a defective *SMAD3* gene, would also develop aneurysms in the neurovascular arteries. Therefore, we sought to investigate possible associations with the prevalence of TAA/AAA/small vessel aneurysm. However, we found no difference in prevalence of TAA, AAA or small vessel aneurysm, which is in concordance with the study of Regalado (Regalado et al., 2011) et al., who described that *SMAD3* variants were not found in patients with a combination of TAA and intracerebral aneurysms or a combination of TAA, intracerebral aneurysms and AAA. On the other hand Loeys et al. (Bart et al., 2008) described that vertebral and carotid artery dissection and cerebral bleeding have been observed in LDS III patients who had dilatation of the aortic root.

We did not find any significant difference in baseline characteristics between LDS III patients with and without neurovascular abnormalities. The difference in the amount of patients with a neurovascular aneurysm and TAA (11.5%) and without neurovascular aneurysm and TAA (57.7%) was not significant, probably because of our small study group and also here it is clear that larger studies are warranted. There was also no statistical difference in patients with (0.0%) and without (3.8%) neurovascular aneurysm and an AAA or with (23.0%) and without (15.4%) neurovascular aneurysm and small vessel aneurysm. Miyazawa (Miyazawa et al., 2007) et al. also found only 7% of patients known with an intracranial aneurysm to have AAA. Age, multiplicity of intracranial aneurysms, size of intracranial aneurysms, and current smoking were the independent risk factors they found, of having AAA and an intracranial aneurysm. We did not find these risk factors, maybe this was influenced by the higher mean age of the patients of Miyazawa et al. of 76.7 years, whereas in our study the mean age was 38.4 years.

Since the clinical implications with possible risk of these abnormalities is yet unknown, we believe it is important to follow up these patients. Therefore, at least one CTA of the head and neck arteries should be done at the time of diagnosis and since our study did show development of new aneurysms, in our opinion, CTA's of the head and neck arteries should be repeated every 2–5 years, depending on the initial findings. Preferably the imaging surveillance is performed by specialized neurologists or neuro-radiologists. More research and longer follow-up is needed to investigate the clinical relevance of these imaging findings and have better information on when to perform preventive treatment to reduce the risk for possible cerebral hemorrhage or ischemic stroke.

5. Study limitations

The main limitation of this study is the small sample size of patients with LDS type III (*SMAD3*). That was inevitable, as this syndrome is only recently discovered and relatively unknown. In our outpatient clinic we have another group of 14 patients (mean age of 23 years) with a high suspicion of LDS III. They decided not to do genetic testing yet, because of possible problems with their life insurance. These patients are relatives of patients in our study group (often children). Of these 14 patients, nine do have clinical features of LDS III. Neurovascular abnormalities were seen in five and thoracic aortic dilatation in 4 of these 9 patients, of whom 2 already underwent ascending aorta replacement. In the future hopefully we can include these patients in our cohort. Although the follow-up period is not short with 8.85 years, a longer follow-up is clearly needed to get better insight in the clinical relevance of our imaging findings.

6. Conclusion

The vast majority of LDS III patients have neurovascular tortuosity and a quarter has developed a neurovascular aneurysm or dissection, however clinical events were relatively rare. Larger prospective follow-up studies are warranted to determine progression over time and the clinical relevance of the observed neurovascular abnormalities.

Data availability

Data are available to the corresponding author.

Author statement

JR, FK, AE and SD conceived the study. SD analyzed the clinical data, and drafted the manuscript with critical input from JR, CT and DL. JR supervised the project. AE and PJD performed the CTA research analyses. PB supported the study with clinical patient information. IL and JS supported the study with genetically patient information and filled in data in the Leiden Open Variation Database (LVOD). All authors discussed the results and critically revised the manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical approval

Written informed consent was obtained from all patients.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

We would like to thank all the patients for participating in our study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2022.104424.

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