Expanding the distinctive neuroimaging phenotype of *ACTA2* mutations

Abstract

BACKGROUND AND PURPOSE: Arg179His mutations in *ACTA2* are associated with a distinctive neurovascular phenotype characterized by straight course of intracranial arteries, absent basal 'moyamoya' collaterals, dilatation of the proximal internal carotid arteries and occlusive disease of terminal internal carotid arteries. We now add to the distinctive neuroimaging features in these patients by describing their unique constellation of brain malformative findings that could flag the diagnosis in cases where targeted cerebrovascular imaging has not been carried out.

MATERIALS AND METHODS: Neuroimaging studies from 13 patients with heterozygous Arg179His mutations in *ACTA2* and 1 patient with pathognomonic clinico/radiological findings for *ACTA2* mutation were retrospectively reviewed. Presence and localization of brain malformations and other abnormal brain MRI findings were reported.

RESULTS: Characteristics bending and hypoplasia of the anterior corpus callosum, apparent absence of the anterior gyrus cinguli, radial frontal gyration were present in 100% of the patients, flattening of the pons on the midline and multiple indentations in the lateral surface of the pons were demonstrated in 93% of the patients, and apparent "squeezing" of the cerebral peduncles in 85% of the patients.

CONCLUSIONS: As α-actin is not expressed in brain parenchyma, but only in vascular tissue, we speculate that, rather that a true malformative process, these findings represent a deformation of the brain during development related to the mechanical interaction with rigid arteries during the embryogenesis.

ABBREVIATIONS: EDM= electronic data management systems; MRA= magnetic resonance angiography; SMC= smooth muscle cells. GA= gestational age.

Introduction

The cerebral arteriopathy associated with Arg179His mutations in *ACTA2* is a prototypical example of non-atherosclerotic cerebral arteriopathies, some of which are Mendelian disorders(1). Patients with *ACTA2* mutation have distinctive clinical (multisystem smooth muscle involvement) and angiographic features(2) – specifically, a combination of ectasia

and stenosis, straight arterial course, absence of basal collaterals and more widespread cerebrovascular involvement in comparison with moyamoya disease(2). The diagnosis is highly suggested by these imaging features and has important implications for management of the patient (increased risks associated with arterial instrumentation) and other family members, but also provides important mechanistic insights, that may be more generalisable(1). Previously the imaging phenotype associated with *ACTA2* mutations has been confined to cerebrovascualr abnormalities and associated leukoencephalopathy (3), apart from a single case report of a patient with a dysmorphic corpus callosum(4).
Here we expand the neuroimaging phenotype and describe characteristic brain parenchymal abnormalities that could flag the diagnosis when targeted cerebrovascular imaging has not been performed.

Materials and Methods

We reviewed in detail the brain magnetic resonance imaging (MRI) findings in a cohort of patients with ACTA2 mutations to describe characteristics of brain congenital abnormalities in addition to known neuroangiographic findings previously described(2). Clinically acquired, anonymised brain and cerebrovascular imaging studies from 13 unrelated patients with heterozygous Arg179His mutations in *ACTA2* were retrospectively included from four pediatric hospitals and one general university hospital, with appropriate governance permissions from each site. The patients were selected after search for ACTA2 mutation in the electronic data management systems (EDM) of the centers involved in the study (last 10 years). Inclusion criteria were: confirmed mutation and/or pathognomonic neuroradiological + clinical findings and availability of MRI of diagnostic quality.
This included re-analysis of eight patients already published by Munot et al.(2). All patients had had the genetic diagnosis confirmed within a clinically accredited laboratory, as part of their clinical evaluation. Another patient had no genetic confirmation (lost at follow-up) but showed typical radiological and clinical phenotypes which are considered pathognomonic for *ACTA2* mutation(2,5) and thus was included in the study.
Images were reviewed for quality and co-reported by two pediatric neuroradiologists (F D'A and KC) who reported presence and localization of brain malformations (i.e.: presence of cortical malformations, abnormal shape of the brainstem, abnormal relative size of the midbrain, pons and medulla oblongata, abnormal shape and size of the corpus callosum, abnormal gyration) (Table 1). Magnetic resonance angiography (MRA) and/or angiography,

where available, were also reviewed for typical ACTA2-related cerebrovascular anomalies as described in literature(2).

Presence of associated ischemic brain damage was also reported and divided into large territorial infarction of the brain and evidence of watershed infarctions (i.e.: radiological evidence of small vessel disease).

Presence of specific brain malformations found in our cohort was compared with known similar brain malformation patterns and analyzed in view of known embryological knowledge(6) in order to elucidate possible pathogenetic mechanisms.

Review through EDM of the MRI scans and clinical data of all the patients from the main institution (i.e. Great Ormond Street Hospital for Children) with diagnosis of other forms of neurovascular dysplasia (e.g. moyamoya), excluded presence of similar brain abnormalities.

Results

Clinical, radiological and genetic findings in our patients are summarized in Table 1. 13 patients had heterozygous missense Arg179His in *ACTA2* and 1 patients without genetic confirmation had clinical and radiological findings pathognomonic for *ACTA2* mutation (subject 14) and was included in the case series.

- All patients had the neuroangiographic features previously described in *ACTA2* Arg179His mutations, namely: dilatation of the proximal internal carotid arteries, occlusion of the distal internal carotid arteries, straight "broomstick-like" arteries of the circle of Willis and absence of moyamoya collaterals. (Figure 1 E and F). Multiple areas of abnormal signal in keeping with small vessel disease and sometimes frank supratentorial infarctions in a different stage of maturation were commonly observed.

- All patient showed different degree of abnormal brain morphology, namely:

(i) Bending (excessive curvature inferiorly and anteriorly) and hypoplasia (rostrum not well formed and flattened genu) of the anterior corpus callosum (Figure 1 A and B) with relatively normal or mildly hypoplastic posterior corpus callosum. On axial

images, the anterior corpus callosum demonstrates a characteristic “V” shaped appearance (Figure 1 C and D). This finding was present in all (100%) patients.

(ii) Abnormal radial gyration of the frontal lobes and deficient anterior cingulate gyrus (Figure 1 A and B). (100%)

(iii) “Twin Peaks” pons (appreciable in all the patients with exception of patient 1: 93%): pons is flattened with subjective reduction of the anteroposterior diameter noted on the midline and impression of the basilar artery on the anterior surface with a consequent presence of two symmetrical prominences resembling twin mountains (Figure 2). In the parasagittal slice, the patients had multiple indentations on the surface of the pons (Figure 3), probably due to the straightened pontine arterial branches creating compression of the pontine surface (see discussion).

(iv) Apparent “squeezing” of the cerebral peduncles in the midbrain (Figure 2 C and D), that was present in 12/14 patients (85%).

(v) variable degree of horizontal orientation and thickening of the fornix (i.e.: fornix parallel to the corpus callosum) was present in all patients (Figure 1 A and B).

The radiological and clinical findings of the patient without confirmed mutations were similar to the confirmed cases and with the typical *ACTA2* cerebrovascular changes(2).
Interestingly we also found an adult patient with similar and pronounced ACTA2-like cerebrovascular phenotype (patient 15 in table 1), which showed same spectrum of brain abnormalities, straightening of posterior circulation arteries and abnormal radial gyration also involving the posterior temporal lobes (Figure 4). This patient refused genetic testing and did not have pathognomonic clinical findings associated with *ACTA2* mutation. Nevertheless this case is extremely interesting to try to understand the possible pathophysiology of the brain abnormalities related to vascular dysplasia (see discussion).
In eight of the subjects (patient 1, 5, 8, 10, 11, 12, 13 and 15) follow-up MRI was not available. The others had a different evolution of the ischemic cerebral lesions.
In all the patients with follow-up available the abnormalities in the corpus callosum, gyration and brainstem were stable overtime as well as the degree of arteriopathy.

No one of the patients showed cortical malformations such as focal cortical dysplasia, polymicrogyria, agyria or pachygyria, as described in literature(7).

Discussion

Actin is an abundant protein in eukaryotic organisms and plays an essential role in the protein-protein interactions. The actin protein represents a monomeric subunit of two main varieties of filaments in cells, which make up the cytoskeleton and form part of the contractile apparatus in muscle cells. The mammalian genome comprises six distinct actin isoforms (α-skeletal, α-cardiac, α-smooth; β-cytoplasmic; γ-smooth and γ-cytoplasmic actin) encoded by six different genes (8). α -smooth muscle actin, encoded by *ACTA2*, located on 10q22-q24,(9) is a principal element of the contractile units of vascular smooth muscle cells (SMC) but is not expressed in the brain parenchyma. However α – actin cross reacts with gamma-actin to reinforce the cytoskeleton (10). The arterial phenotype in Arg179His *ACTA2* mutations, with ectasia of large arteries and occlusive disease in small arteries appears to reflect the local influence on the presence or absence of elastin within the arterial wall on the vascular phenotype(2) These arterial features are also observed in the mouse model(11). It is important to note that vascular SMCs derive from the mesoderm in the posterior fossa/brainstem/thalami, and from the neural crest supratentorially (anterior neural plate)(12), however there are no data, to the best of our knowledge, to suggest differences in expression of *ACTA2* related to different embryological origins.

The occlusive disease observed in intracranial vessels is a result of fibrosis, thickening of the vascular wall, flattening and disorganization of the internal lamina and proliferation of SMCs(13). We previously postulated that this renders the arteries more rigid and less deformable(2). Arterial growth follows the contours of brain growth and gyration during the normal development(12) – thus we speculate that in patients with these *ACTA2* mutations the increased rigidity of the intracranial arteries results both in the characteristic “straight” appearance and in the morphological brain changes that we have described, as a consequence of local mechanical effect from these vessels. Interestingly the muscular layers do not appear in the basal perforator vessels until gestational age (GA) 27, and, progressively until term, over the convexity. However studies on rat embryos show that actin expression in the vasculature starts very early(14) and histological specimens in subjects with *ACTA2*

mutations demonstrate that the rigidity is also due to abnormality in elastic and intima laminae(13). Furthermore, although the shape of the corpus callosum is complete by GA 20, this structure enlarges together with the connectivity and the development of the cortex(6); thus it is still possible that interaction with abnormally formed vessels is responsible for the observed deformity despite these difference in embryological age of development. In addition, as the actin cytoskeleton participates directly or indirectly in almost every aspect of neuronal development and function (15) any instability in the cytoskeleton resulting from abnormal cross linkage between actin subtypes could also influence neuronal migration (8). α – actin is not expressed in the brain parenchyma but only in vessels (differently from other isoforms), so it is unlikely that a mutation of this isoform will directly influence brain development, but it is possible that cross-regulation between different isoforms may play a role in subjects with *ACTA2* mutation.

There are other examples of malformative disorders being related to local mechanical factors, for example arachnoid cysts with surrounding brain hypoplasia, and Chiari I malformation in which a small posterior fossa results in distortion and inferior displacement of the cerebellar tonsils; which is why the term "Chiari I deformity" was proposed instead of malformation(16)

In *ACTA2* mutated patients, the abnormal arterial morphology and structural brain abnormalities parallel each other in both supratentorial and infratentorial parenchyma – universally so in the anterior part of the corpus callosum, anterior cingulate gyrus, an abnormal radial frontal gyration and variably in the brainstem. In fact, the characteristic "twin peaks" appearance of the pons in the axial plane seems to be related to impression by the basilar artery as well as the indentation of the lateral pontine surface may be due to the impression by the pontine arteries coming from the basilar artery (Figure 3).
We have observed a particularly extreme example of this in a genetically untested case who shows neuroradiological features similar to ACTA2 mutations (patient 15). In this patient the posterior cerebral arteries are also severely straightened and are associated with abnormal radial gyration involving the posterior temporal lobes and marked brainstem compression (Figure 4). The vascular phenotype present in this case is similar to the others and only described in patients with ACTA2 mutation with the exception of an isolated case report(17) where a mutation was not found and that showed very similar brain images to our patient. Thus, even though it is possible that this patient (despite neurovascular findings) does not

have ACTA2 mutation, we think that the striking association between severe vascular and brain phenotypes in this subject is supportive of our hypothesis that brain abnormalities are secondary to vessel rigidity.

The apparent absence/definition of a segment of the anterior cingulate and the frontal radial pattern are both likely associated with a callosal abnormality at that level, which itself probably translates into the axial callosal V-shape. The horizontal fornix (or rather, the fornix that is parallel to the anterior callosum) results from an abnormally developed septum pellucidum (i.e. too thick), which itself, may well relate to the abnormal cingulate(6).
The brain malformation features do not appear to have a clear clinical correlate in Arg179His *ACTA2* patients; epilepsy is rare, other than in the context of brain ischemia, and intellectual outcomes again seem related to brain injury rather than to developmental abnormalities "*per se*". However the extreme reproducibility of the brain phenotype could represent an asset in the diagnosis when neuroangiographic studies are not available; for instance in patient 8 standard MRI sequences showed typical bending of corpus callosum and radial frontal gyration that triggered the addition of an MRA sequences which confirmed the radiological diagnosis.

These observations contribute to defining the distinctive neuroradiological features of *ACTA2* mutations, as well to shedding light on mechanisms, both genetic and mechanical, that result in structural changes to the brain and its vasculature.

Conclusion

We describe a characteristic and potentially pathognomonic (in specific clinical context) brain phenotype in patients with *ACTA2* mutations and/or the typical clinical and neurovascular picture. A possible explanation for these brain imaging findings, that can be helpful in the diagnosis, is a mechanical effect on the brain parenchyma during development by abnormal rigid vessels with possible contribution of cross-regulation between different actin isoforms.

Figures and figure legend

Figure 1: Supratentorial abnormalities in ACTA2 patients. Upper row: sagittal T2 weighted images (WI) (A), sagittal T1 WI (B) show typical hypoplasia and bending of the anterior corpus callosum with associated abnormal radial gyration of the frontal lobes and deficient anterior cingulate gyrus (white arrows). In patient 7 there is horizontal orientation of the fornix (black arrow in B), which is also markedly thick. Middle row: axial T1 WI (C and D) demonstrates characteristic "V" shaped anterior corpus callosum (black circle). Lower row: magnetic resonance angiography maximum intensity projection antero-posterior view (E and F) shows typical neurovascular abnormalities in patients with ACTA2 mutation.

Figure 2: Infratentorial malformations in ACTA 2 mutation. Upper row: axial T2 WI (A and B) at the level of the pons. The "twin peaks" sign is demonstrated: pons is flattened with reduction of the antero-posterior diameter and impression of the basilar artery on the anterior surface with consequent presence of two symmetrical prominences resembling twin mountains. Middle raw, axial T2 WI at the level of the cerebral peduncles (C and D) show mild cranio-caudal elongation of the midbrain with reduction of the latero-lateral diameter and "squeezing" of the cerebral peduncles. Right column: normal for comparison.

Figure 3: In parasagittal slice the patients showed multiple indentation on the surface of the pons. We speculate that these may be due to the stretching of straightened pontine arterial branches.

Figure 4: Neuroradiological findings in adult patient without confirmed ACTA2 mutation (patient 15). Sagittal T2 WI (A), axial T2 WI at the level of the pons (B) and of the midbrain (C) show marked callosal anterior bending (dotted arrow in A), "twin peaks" pontine sign (arrow in B) and reduction of the latero-lateral diameter of the midbrain with squeezed cerebral peduncle. D: sagittal T2 WI demonstrates marked basilar impression on the pons and anterior bending of the midbrain; parasagittal right slice (E) demonstrates indentation of the lateral surface of the pons (dotted black arrow) and straight course of the posterior cerebral arteries (white arrow). Axial T2 WI (F) at the level of the proximal segment of the posterior

cerebral arteries (ponto-mesencephalic junction) shows marked compression of the brainstem at that level related to straightening of the arteries (white arrows) and radial gyration of the posterior temporal lobes (black dotted arrows).

Table1: Demographics, clinical presentation and neuroimaging findings

Table legend:

*PDA: patent ductus arteriosus. PFO: persistent foramen ovale. TIA: transient ischemic attacks. PCA: posterior cerebellar arteries. Distinctive ACTA2 cerebrovascular features: dilatation proximal internal carotid arteries, occlusion of the distal internal carotid arteries, a straight course of arteries of the circle of Willis, absence of moya-moya collaterals. *: genetic test not performed and clinical context non-pathognomonic for ACTA2 mutation.*

	Age / Gender/ ethnicity	Main Clinical Findings	Typical ACTA 2 neurovascular abnormalities	bending / hypoplasia anterior corpus callosum	abnormal radial gyration frontal lobes	cortical malformations	absence anterior cingulate gyrus	"twin peaks pons"	"squeezed" midbrain	paramedian indentation of pontine surface	Large territorial infarction(s)	watershed infarctions
Patient 1	23 m/ female/ pakistani	PDA, congenital mydriasis, pulmonary hypertension, right hemiparesis	yes	yes	yes	no	yes	no	no	no	yes	yes

Patient 2	4 y/ female/ white	PDA, congenital mydriasis, bilateral hemiparesis, swallowing difficulties	yes	yes	yes	no	yes	yes	yes	yes	no	yes
Patient 3	9 y/ female/ white	PDA, multiple TIAs, hypocontractile bladder, left hemiatrophy of toes and foot	yes	yes	yes	no	yes	yes	yes	yes	yes	yes
Patient 4	10 y/ male/ white	PDA, congenital mydriasis, unilateral vocal cord paresis, cardiac arrest in newborn period	yes	yes	yes	no	yes	yes	yes	yes	no	yes
Patient 5	10 y/ male/ white	PDA, right femoral artery occlusion, dilatation aortic arch, pulmonary hypertension	yes	yes	yes	no	yes	yes	no	yes	yes	yes
Patient 6	4 y/ female/ arabic	PFO, thrombophilia, dystonic left hemiparesis, possible seizures	yes	yes	yes	no	yes	yes	yes	yes	no	yes
Patient 7	3 y/female/ white	Aneurysmal PDA, congenital mydriasis, dilatation of ascending aorta	yes	yes	yes	no	yes	yes	no	yes	no	yes
Patient 8	1 y/ female/ white	PDA, congenital mydriasis, pulmonary hypertension, deceased for sepsis	yes	yes	yes	no	yes	yes	yes	yes	no	yes
Patient 9	1 y/ male/ arabic	PDA, congenital mydriasis, bulbar palsy, pulmonary hypertension	yes	yes	yes	no	yes	yes	yes	yes	no	yes
Patient 10	4 y/ female/ white	congenital mydriasis, developmental and speech delay	yes	yes	yes	no	yes	yes	yes	yes	yes	yes
Patient 11	8 y/ female/ arabic	PDA, congenital mydriasismidriasis, hands clumsiness, recurrent TIA	yes	yes	yes	no	yes	yes	yes	yes	no	yes
Patient 12	11y/ female/ white	PDA, Aortic dissection, congenital catharacts, cognitive decline	yes	yes	yes	no	yes	yes	yes	yes	no	yes
Patient 13	6y / male/ white	PDA, congential mydriasis, left hemiparesis	yes	yes	yes	no	yes	yes	yes	yes	yes	yes
Patient 14	3 y/female/ white	PDA, congential mydriasis, recurrent TIAs	yes	yes	yes	no	yes	yes	yes	yes	no	yes
Patient 15*	33y/ female/ black	pseudobulbar palsy, left sided piramidal weakness , swallowing difficulties (symptoms started when she was 10 y)	yes	yes	yes (also abnormal posterior temporal gyration in relation with streigthening of PCAs)	no	yes	yes	yes	yes	no	yes (few foci)

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