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ABSTRACT

BACKGROUND AND PURPOSE: Pathogenic variants in the *ACTA2* gene cause a distinctive arterial phenotype that has recently been described to be associated with brain malformation. Our objective was to further characterize gyral abnormalities in patients with *ACTA2* pathogenic variants as per the 2020 consensus recommendations for the definition and classification of malformations of cortical development.

MATERIALS AND METHODS: We performed a retrospective, multicentric review of patients with proved *ACTA2* pathogenic variants, searching for the presence of malformations of cortical development. A consensus read was performed for all patients, and the type and location of cortical malformation were noted in each. The presence of the typical *ACTA2* arterial phenotype as well as demographic and relevant clinical data was obtained.

RESULTS: We included 13 patients with *ACTA2* pathogenic variants (Arg179His mutation, $n = 11$, and Arg179Cys mutation, $n = 2$). Ninety-two percent (12/13) of patients had peri-Sylvian dysgyria, 77% (10/13) had frontal dysgyria, and 15% (2/13) had generalized dysgyria. The peri-Sylvian location was involved in all patients with dysgyria (12/12). All patients with dysgyria had a characteristic arterial phenotype described in *ACTA2* pathogenic variants. One patient did not have dysgyria or the characteristic arterial phenotype.

CONCLUSIONS: Dysgyria is common in patients with *ACTA2* pathogenic variants, with a peri-Sylvian and frontal predominance, and was seen in all our patients who also had the typical *ACTA2* arterial phenotype.

ABBREVIATIONS: MCD = malformation of cortical development; PMG = polymicrogyria

Heterozygous, autosomal dominant, pathogenic variants in the *ACTA2* gene encoding α-2 smooth-muscle actin that replace the arginine at protein position 179 (Arg179) with either histidine, leucine, cysteine, or serine cause a multisystemic smooth-muscle dysfunction syndrome (Online Mendelian Inheritance in Man, 613834; https://www.omim.org). Actin is found in the contractile apparatus of muscle and the cytoskeleton. There are 6 distinct isoforms (α-skeletal, α-cardiac, α-smooth, β-cytoplasmic, γ-smooth, γ-cytoplasmic actin) encoded by 6 different genes.[1] Alpha smooth-muscle actin encoded by the *ACTA2* gene constitutes the major contractile unit of the vascular smooth muscle expressed by blood vessels.[2,3] As part of this *ACTA2* syndrome, a distinctive arterial phenotype was described by Munot et al,[4] with the phenotype further expanded by D'Arco et al[5] to include brain malformative features such as a radial orientation of frontal lobe gyri, flattening of the pons, and bending and hypoplasia of the anterior corpus callosum. Following the diagnosis of an unusual malformation of cortical development (MCD) in our index patient (Online Supplemental Data, patient 9), we reviewed the neuroimaging features of patients with *ACTA2* pathogenic variants across 4 tertiary pediatric hospitals.
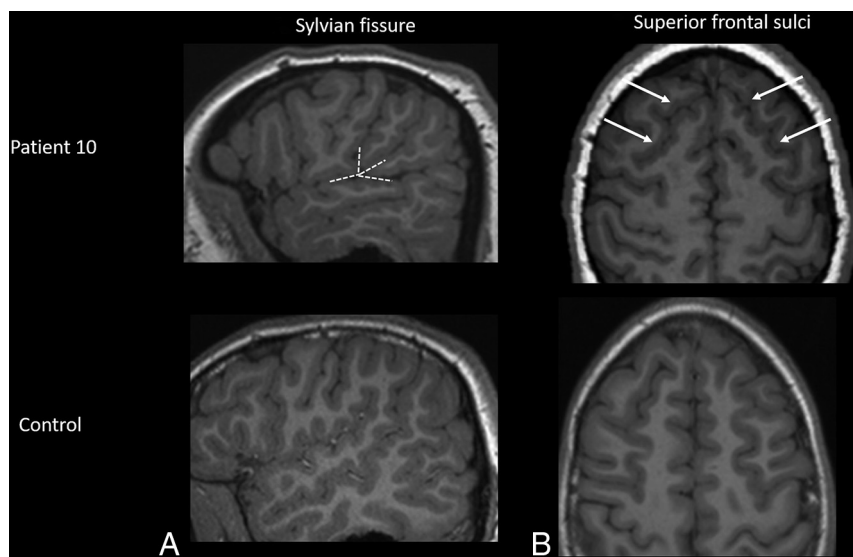
FIG 1. Peri-Sylvian and frontal dysgyria.

MATERIALS AND METHODS

Patient Population

This study included all patients with proved *ACTA2* pathogenic variants ($n = 13$) from 4 pediatric hospitals (UPMC Children's Hospital of Pittsburgh and Children's Hospital of Philadelphia; The Hospital for Sick Children, Toronto, Canada; and the Great Ormond Street Hospital, London, UK). Appropriate local research ethics board approval was obtained from each site. Patients were identified by a keyword ("*ACTA2*," "alpha actin," "MR imaging") search in their respective hospital electronic chart systems. Inclusion criteria were younger than 18 years of age and proved *ACTA2* pathogenic variants. Exclusion criteria were poor-quality imaging studies, including incomplete image acquisition and image degradation by artifacts. Patient demographics and clinical presentation were obtained from hospital electronic charts.

Image Interpretation

MR images of all patients were reviewed in consensus by 6 pediatric neuroradiologists (S.S, A.B, K.M, S.V.S, C.A.P.F.A., and F.A.) for the presence of MCDs, which were classified according to recently published international consensus recommendations.[6,7] In particular, dysgyria was diagnosed on T1-weighted and/or T2-weighted sequences if the cortex showed variable thickness and a smooth gray-white boundary but with an abnormal gyral pattern characterized by irregularities of sulcal depth and/or orientation. The location of the dysgyria was noted in each case. All patients also had MR angiograms that were used to subjectively note the presence or absence of a typical *ACTA2* arterial phenotype characterized by dilation of the proximal ICAs, abrupt caliber change with stenosis at the level of terminal ICAs, a straight course of the intracranial arteries, and absent Moyamoya-like collaterals.

RESULTS

Patient demographics and genetic and imaging findings are summarized in the Table and Online Supplemental Data. The cohort consisted of 9 females. The *ACTA2* pathogenic variants included the Arg179His mutation ($n = 11$) and the Arg179Cys mutation ($n = 2$).

Twelve of 13 patients (92%) had peri-Sylvian dysgyria, with abnormal bifurcation or trifurcation of the Sylvian fissure, which was appreciated rostrally on the sagittal sections (Fig 1*A* and Fig 2*B–E*). Ten of 13 patients had frontal dysgyria, with obliquely oriented superior frontal sulci, best appreciated in the axial plane (Fig 1*B* and Fig 2*C–E*). Two of 13 patients had generalized dysgyria in addition to the above. This was characterized by involvement of the entire cerebral cortex by an abnormal gyral pattern with variable sulcal depth and orientation (Fig 2*E*). In 10 patients, the dysgyria was associated with areas of undulated cortex that did not meet the criteria for PMG (Fig 3). Patients 3 and 13 developed seizures following a stroke, yet no other patients had epilepsy. All except 1 patient (patient 13) had the typical *ACTA2* arterial phenotype. This patient had normal intracranial imaging features (Fig 2*F*) except for a variable degree of arterial tortuosity.

DISCUSSION

We demonstrate previously under-recognized MCDs in most of our patients with the *ACTA2* pathogenic variants in the form of peri-Sylvian and frontal dysgyria. In addition, most of our patients with dysgyria had an undulating appearance of the cortex, which did not meet the criteria for PMG. We term this spectrum of malformations "*ACTA2*-related dysgyria." The typical *ACTA2* arterial phenotype was noted in all except 1 patient.

The vascular effects of *ACTA2* pathogenic variants are attributed to increased smooth-muscle cell proliferation in smaller-diameter muscular arteries and decreased contractility in larger elastic arteries.[4] Histologic specimens from patients with *ACTA2* pathogenic variants have shown large intracerebral arteries with marked intimal thickening and smooth-muscle cell proliferation; increased collagen and a relatively milder degree of smooth-muscle cell proliferation in the tunica media; as well as thickened, split, and fragmented internal elastic lamina, which showed less folding compared with that in controls.[4,8]

In the series published by D'Arco et al,[5] the abnormally oriented frontal lobe gyri were, at the time, aptly termed "abnormal radial gyration." On review of 6 cases from the article by D'Arco et al, with the addition of a further 7 cases in our series, we have termed the abnormal gyration "dysgyria," based on the latest 2020 consensus guidelines,[6,7] with this feature not only limited to the frontal lobes but also involving peri-Sylvian regions in most of our patients and generalized distribution in a minority. The peri-Sylvian dysgyria in our series was characterized by an abnormal bifurcation or trifurcation of the Sylvian fissure best appreciated on the sagittal plane. As mentioned earlier, the undulating appearance of the cortex in regions of dysgyria in our study is distinct from the appearance of PMG in that these cases lack the
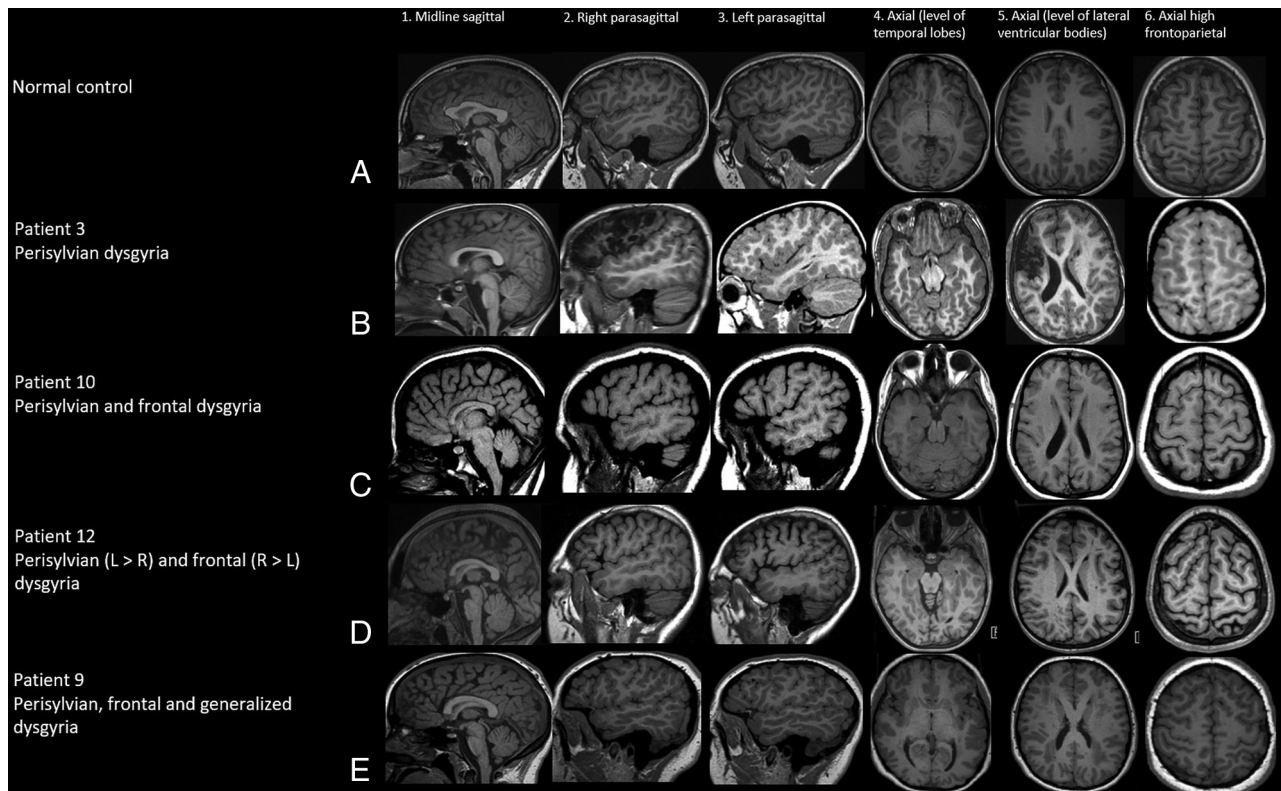
FIG 2. T1-weighted images showing the spectrum of dysgyria in *ACTA2* pathogenic variants. L indicates left; R, right.
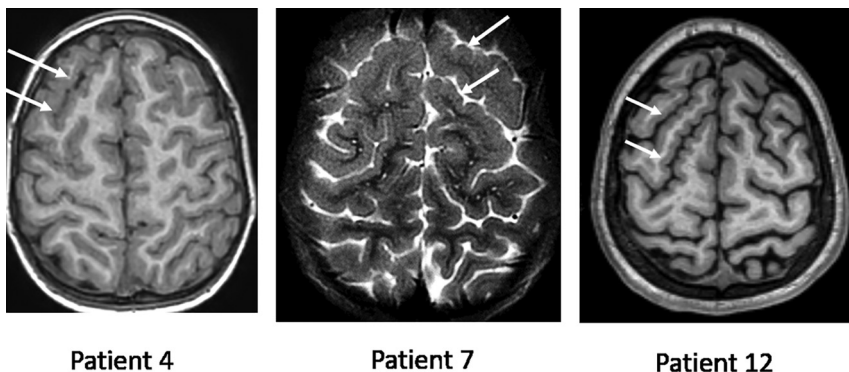

FIG 3. Undulating cortex in regions of dysgyria (*arrows*).

small microgyri at chaotic angles that have been described in classic PMG. We, therefore, use the term *ACTA2*-related dysgyria to describe our spectrum of findings.

The structural brain malformations in patients with an *ACTA2* pathogenic variant were initially attributed to either a mechanical effect from "rigid" vessels or abnormal cross-regulation between different isoforms. Before recent evidence confirming otherwise,[9-12] it was presumed that α-actin was not expressed in the brain parenchyma; therefore, it would be unlikely for *ACTA2* pathogenic variants to directly influence brain development.[5]

While rigid vessels may explain some of the features seen in *ACTA2* pathogenic variants, they do not explain the spectrum of dysgyria that we have described in this study. For instance, histologic

specimens show intimal and medial thickening with a relatively spared adventitia,[4,8] explaining the luminal stenosis, but they do not explain malformations of structures extrinsic to the vascular wall. Recent studies demonstrating α-actin[9-11] and, more specifically, *ACTA2*[12] expression in the brain parenchyma shed light on potential alternate hypotheses for the presence of MCDs in these patients. In 2017, Moradi et al[11] described differing roles of α-, β-, and γ-actin in axon growth, guidance, arborization, and synaptogenesis and demonstrated that all 3 actin isoforms were expressed in motor neurons, with their respective messenger RNAs localizing within axons. Alpha-actin, in particular, was highly expressed in the axonal compartment and axonal branch points, with depletion of α-actin associated with altered filopodia dynamics, reduced filopodia length, and diminished ability to form axonal collateral branches. Another recent study demonstrated that *ACTA2* was expressed in neural stem cells of embryonic C57BL/6 mice and played an essential role in neural stem cell migration.[12]

Furthermore, several studies have shown that knockout of specific actin isoforms leads to compensatory upregulation of other isoforms so that the overall levels of actin are maintained.[11,13-16] Also, notably, no actin isoform differs from another by more than 7% at the primary amino acid level;[1] therefore,

cross-regulation between actin isoforms, especially when there is depletion of one isoform and compensatory up-regulation of other isoforms, may also play a role in *ACTA2* pathogenic variants. These observations may, therefore, help classify *ACTA2*-related dysgyria as a migrational or postmigrational disorder involving the processes of neuronal migration and organization, axonogenesis, and dendritogenesis.

The consistent distribution of dysgyria in the peri-Sylvian regions and frontal lobes is intriguing. One possible explanation may be that developing vessels perform scaffolding and a paracrine signaling function for developing neural cell populations.[17] Indeed, whole-brain MR angiography in our index patient (patient 9) revealed the presence of arterial branches coursing along the abnormally oriented sulci (Fig 4). Recent genetic insights have shown that the embryogenesis of the vascular and nervous systems are closely interlinked and that the axon growth cone and endothelial tip cell respond to growth and guidance cues in similar ways.[18-22] In addition, there is cross-talk between neural and vascular cells for the purpose of normal development, and signals from both neural and vascular tissue can influence the branching of one another.[17,23] For instance, neuronal activity has been found to influence cerebrovascular density, vessel branching, and maturation via molecular cues as well as direct cell-to-cell contact of neural cell types with endothelial cells.[17,24]

Similarly, CNS vasculature patterning plays a crucial role in preventing mispositioning of neuronal precursor cells and in providing a scaffold for neuronal migration, axonal projections, and cell soma arrangements in the developing brain.[25-27] Thus, stiff and noncompliant *ACTA2*-mutant arteries may present a rigid scaffold to the developing brain, resulting in abnormal gyral patterning. Another factor that may contribute to dysgyria includes aberrant tension-induced growth of white matter, influencing folding patterns in a viscoelastic model of the brain, as described in an excellent recent review of the mechanics of cortical folding.[28] Therefore, an interplay of various factors during the
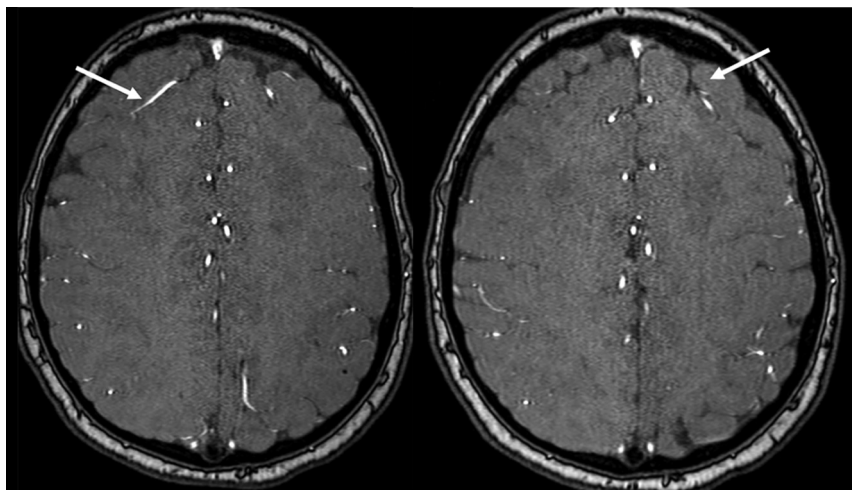

FIG 4. Anterior cerebral artery branches follow abnormal sulci (*arrows*).

Demographics, neuroimaging findings, and clinical presentation

Patient	Age	Sex	Mutation	Dysgyria			Undulating Cortex in Regions of Dysgyria	Presenting Symptom	Seizures	Typical *ACTA2* Vascular Phenotype
				Peri-Sylvian	Frontal	Generalized				
1	5 yr	M	Arg179His	+	+	+	No	Cardiac arrest in early life	No	Yes
2	4 yr	F	Arg179His	+	+	−	No	Strokelike episodes	No	Yes
3	6 yr	F	Arg179His	+	−	−	Yes	Stroke	Yes	Yes
4	13 wk	M	Arg179His	+	−	−	Yes	Aniridia	No	Yes
5	3 yr	F	Arg179His	+	+	−	Yes	Cardiac symptoms	No	Yes
6	4 yr	F	Arg179His	+	+	−	Yes	Left hemiparesis	No	Yes
7	2 mo	F	Arg179Cys	+	+	−	Yes	Anisocoria and cataract	No	Yes
8	4 yr	M	Arg179His	+	+	−	No	Congenital mydriasis	No	Yes
9	11 yr	F	Arg179His	+	+	+	Yes	Congenital mydriasis	No	Yes
10	17 yr	F	Arg179His	+	+	−	Yes	Chronic headaches	No	Yes
11	8 yr	F	Arg179His	+	+	−	Yes	Spastic quadriplegic cerebral palsy, headaches	No	Yes
12	11 yr	F	Arg179His	+	+	−	Yes	Stroke	Yes	Yes
13	7 yr	M	Arg179Cys	−	−	−	NA	None (screened as part of family)	No	No

Note:— + indicates present; −, absent; NA, not applicable.

stages of neuronal migration, axonogenesis, and vasculogenesis may each contribute to the patterns of dysgyria that we have described in this article.

CONCLUSIONS

Dysgyria is common in patients with *ACTA2* pathogenic variants and has a frontal and peri-Sylvian predominance. Although the underlying mechanisms are yet to be elucidated, recent insights suggest the potential roles of mutant α-actin in neuronal migration and axonogenesis, abnormal cross-regulation between actin isoforms, aberrant neurovascular cross-talk, and an abnormal vascular scaffold, resulting in these malformations.

Disclosure forms provided by the authors are available with the full text and PDF of this article at www.ajnr.org.

REFERENCES

1. Cheever TR, Ervasti JM. **Actin isoforms in neuronal development and function.** *Int Rev Cell Mol Biol* 2013;301:157–213 CrossRef Medline
2. Gabbiani G, Schmid E, Winter S, et al. **Vascular smooth muscle cells differ from other smooth muscle cells: predominance of vimentin filaments and a specific alpha-type actin.** *Proc Natl Acad Sci U S A* 1981;78:298–302 CrossRef Medline
3. Glukhova MA, Kabakov AE, Frid MG, et al. **Modulation of human aorta smooth muscle cell phenotype: a study of muscle-specific variants of vinculin, caldesmon, and actin expression.** *Proc Natl Acad Sci U S A* 1988;85:9542–46 CrossRef Medline
4. Munot P, Saunders DE, Milewicz DM, et al. **A novel distinctive cerebrovascular phenotype is associated with heterozygous Arg179 ACTA2 mutations.** *Brain* 2012;135:2506–14 CrossRef Medline
5. D'Arco F, Alves CA, Raybaud C, et al. **Expanding the distinctive neuroimaging phenotype of ACTA2 mutations**. *AJNR Am J Neuroradiol* 2018;39:2126–31 CrossRef Medline
6. Oegema R, Barakat TS, Wilke M, et al. **International consensus recommendations on the diagnostic work-up for malformations of cortical development.** *Nat Rev Neurol* 2020;16:618–35 CrossRef Medline
7. Severino M, Geraldo AF, Utz N, et al. **Definitions and classification of malformations of cortical development: practical guidelines.** *Brain* 2020;143:2874–94 CrossRef Medline
8. Georgescu MM, da Cunha Pinho M, Richardson TE, et al. **The defining pathology of the new clinical and histopathologic entity ACTA2-related cerebrovascular disease.** *Acta Neuropathol Commun* 2015;3:81 CrossRef Medline
9. McHugh KM, Crawford K, Lessard JL. **A comprehensive analysis of the developmental and tissue-specific expression of the isoactin multigene family in the rat.** *Dev Biol* 1991;148:442–58 CrossRef Medline
10. Tondeleir D, Vandamme D, Vandekerckhove J, et al. **Actin isoform expression patterns during mammalian development and in pathology: insights from mouse models.** *Cell Motil Cytoskeleton* 2009;66:798–815 CrossRef Medline
11. Moradi M, Sivadasan R, Saal L, et al. **Differential roles of α-, β-, and γ-actin in axon growth and collateral branch formation in motoneurons.** *J Cell Biol* 2017;216:793–814 CrossRef Medline
12. Zhang J, Jiang X, Zhang C, et al. **Actin Alpha 2 (ACTA2) downregulation inhibits neural stem cell migration through rho GTPase activation.** *Stem Cells Int* 2020;2020:4764012 CrossRef Medline
13. Crawford K, Flick R, Close L, et al. **Mice lacking skeletal muscle actin show reduced muscle strength and growth deficits and die during the neonatal period.** *Mol Cell Biol* 2002;22:5887–96 CrossRef Medline
14. Schildmeyer LA, Braun R, Taffet G, et al. **Impaired vascular contractility and blood pressure homeostasis in the smooth muscle alpha-actin null mouse.** *FASEB J* 2000;14:2213–20 CrossRef Medline
15. Perrin BJ, Ervasti JM. **The actin gene family: function follows isoform.** *Cytoskeleton (Hoboken)* 2010;67:630–34 CrossRef Medline
16. Cheever TR, Li B, Ervasti JM. **Restricted morphological and behavioral abnormalities following ablation of β-Actin in the brain.** *PLoS One* 2012;7:e32970 CrossRef Medline
17. Paredes I, Himmels P, Ruiz de Almodóvar C. **Neurovascular communication during CNS development.** *Dev Cell* 2018;45:10–32 CrossRef Medline
18. Adams RH, Eichmann A. **Axon guidance molecules in vascular patterning.** *Cold Spring Harb Perspect Biol* 2010;2:a001875 CrossRef Medline
19. Quaegebeur A, Lange C, Carmeliet P. **The neurovascular link in health and disease: molecular mechanisms and therapeutic implications.** *Neuron* 2011;71:406–24 CrossRef Medline
20. Eichmann A, Thomas JL. **Molecular parallels between neural and vascular development.** *Cold Spring Harb Perspect Med* 2013;3:a006551 CrossRef Medline
21. Tam SJ, Watts RJ. **Connecting vascular and nervous system development: angiogenesis and the blood-brain barrier.** *Annu Rev Neurosci* 2010;33:379–408 CrossRef Medline
22. Wälchli T, Wacker A, Frei K, et al. **Wiring the vascular network with neural cues: a CNS perspective.** *Neuron* 2015;87:271–96 CrossRef Medline
23. Carmeliet P. **Blood vessels and nerves: common signals, pathways and diseases.** *Nat Rev Genet* 2003;4:710–20 CrossRef Medline
24. Lacoste B, Comin CH, Ben-Zvi A, et al. **Sensory-related neural activity regulates the structure of vascular networks in the cerebral cortex.** *Neuron* 2014;83:1117–30 CrossRef Medline
25. Javaherian A, Kriegstein A. **A stem cell niche for intermediate progenitor cells of the embryonic cortex.** *Cerebral Cortex* 2009;19:i70–77 CrossRef Medline
26. Li S, Haigh K, Haigh JJ, et al. **Endothelial VEGF sculpts cortical cytoarchitecture.** *J Neurosci* 2013;33:14809–15 CrossRef Medline
27. Paredes MF, James D, Gil-Perotin S, et al. **Extensive migration of young neurons into the infant human frontal lobe.** *Science* 2016;354:aaf7073 CrossRef Medline
28. Garcia KE, Kroenke CD, Bayly PV. **Mechanics of cortical folding: stress, growth and stability.** *Philos Trans R Soc Lond B Biol Sci* 2018;373:2017321 CrossRef Medline