Intracerebral Hemorrhage and *COL4A1* and *COL4A2* Mutations, from Fetal Life into Adulthood

Cerebral small vessel disease (SVD) is an important cause of stroke, and an increasing number of single-gene disorders causing SVD have recently been identified. Of these, CADASIL (cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is most common; others such as mutations in the HtrA serine protease 1 (*HTRA1*) gene and the *TREX1* gene have also been identified.[1–3]

In this issue of *Annals of Neurology*, Weng and colleagues[4] investigated the potential role of *COL4A1* mutations in patients with sporadic late onset intracerebral hemorrhage (ICH).

ICH, accounting for 10 to 15% of all strokes, is associated with the highest rate of mortality, with up to 50% of individuals dying within the first year following their ICH. Mutations in collagen IV gene *COL4A1* have recently been reported to underlie a spectrum of cerebrovascular diseases, including ICH. Weng and colleagues[4] were able to find 2 novel putative *COL4A1* mutations in 2 of 96 patients diagnosed with sporadic cerebral amyloid angiopathy, or presumed hypertension-related ICH. To test the biosynthetic consequences of these putative mutations, they developed and validated a cell culture-based functional assay using nonpathogenic polymorphisms and previously confirmed disease-causing mutations. They were able to show that COL4A1 proteins containing a known mutation, or the 2 putative mutations identified in this study, impair secretion of COL4A1 and lead to protein accumulation within cells. The findings presented herein raise the possibility that *COL4A1* mutations may underlie a significant proportion of new cases of ICH every year in the United States and in other parts of the world, and that therapies aimed at promoting protein folding might be effective in preventing hemorrhagic stroke in some patients.

COL4A1 dominant mutations are responsible for a broad range of clinical phenotypes presenting from the fetal period until late adulthood. *COL4A1* is expressed in the basement membranes of blood vessels and many organs, and mutations are the cause of a SVD affecting the brain, eyes, muscle, and kidneys. With more cases reported, it is becoming increasingly clear that a wider spectrum of cerebrovascular disease may occur than initially considered.

Several case reports have illustrated that onset can occur antenatally, during the second trimester of pregnancy, resulting in severe antenatal destructive changes resembling hydranencephaly, often associated with involvement of the cerebellum.[5–7] A diagnosis can also be made in the neonatal period, showing porencephaly, present at birth, due to the occurrence of an antenatal parenchymal haemorrhage.[7,8] Hemiplegia and leukoencephalopathy, sometimes associated with intracranial calcifications, have been reported in children presenting in infancy or early childhood.[9–12] Finally, a diagnosis can also be made following presentation with adult stroke, SVD, and cerebral aneurysms (Figure).[13,14] Trauma, exercise, and anticoagulation have been recognized as precipitating factors of intracerebral hemorrhage in affected individuals. It was suggested by Gould et al[13] that vaginal delivery could be a trauma leading to a parenchymal hemorrhage with subsequent porencephaly, and it was recommended that this potential trauma should be avoided in a family where a child is known to be affected by a *COL4A1* mutation. Recent case series have, however, shown that affected individuals are more often already developing their hemorrhage many weeks before their delivery.[6–8] It is not yet known how often a parenchymal hemorrhage in a preterm infant occurs in the context of a *COL4A1* mutation. Although 5 to 7% of extremely preterm infants do develop a parenchymal hemorrhage in the neonatal period, an associated *COL4A1* mutation has only been reported once in a set of twins.[15]

In the familial cases, there is a wide variability in age of onset and phenotype between the affected members, including both cerebrovascular and extracerebral manifestations. Many asymptomatic mutation carriers are known; however, at magnetic resonance imaging screening SVD or aneurysms are often identified.[9,10] Other genetic and environmental factors probably contribute to the variability.

COL4A1 mutations may also be associated with extracerebral clinical features, such as cataracts, microcornea, Axenfeld–Rieger anomaly, and retinal hemorrhage.

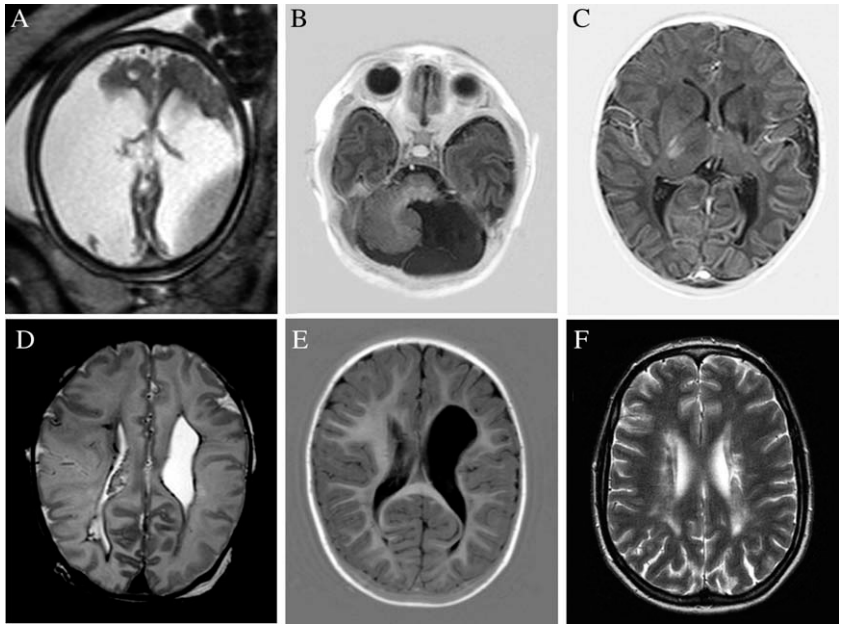

FIGURE: Figure Composite magnetic resonance imaging figure of 5 cases with *COL4A1* mutation, diagnosed from the fetal period until adulthood: (A) T2 weighted spinecho sequence (T2SE) performed at 37 weeks gestation, showing extensive bilateral infarction; (B, C) neonatal inversion recovery of a child diagnosed in utero, showing absence of the left cerebellar hemisphere, associated with a wedge-shaped lesion in the left basal ganglia, associated with asymmetry of myelination of the posterior limb of internal capsule and subsequent development of hemiplegia; (D) T2SE performed in the neonatal period while subject was receiving hypothermia for neonatal encephalopathy, showing left-sided porencephaly and periventricular low- and high-signal intensity lesions, confirmed to be calcification on postmortem; (E) inversion recovery at 9 months in a child presenting with familial hemiplegia; and (F) adult woman diagnosed at the age of 37 years because of motor deterioration and history of congenital cataract. T2-weighted image shows hyperintense lesions in the periventricular white matter.

Renal cysts have been described in newborns and in adults with HANAC (hereditary angiopathy, nephropathy, aneurysms, and cramps) syndrome.[6,16] *COL4A1* has been associated with Walker–Warburg syndrome, with severe cortical malformation.[17] Pathogenicity of the latter mutations was demonstrated by the same in vitro test presented by Weng et al in this issue.

The relevance of the paper by Weng et al[4] is in the systematic study of *COL4A1* mutations in sporadic ICH. Among the circa 75 patients reported up to 2011, besides the familial cases, sporadic, de novo *COL4A1* mutations were previously described in infants with severe prenatal hemorrhage, resembling hydranencephaly,[6] and also in a 25-year-old man with recurrent ICH.[14] Most of the reported *COL4A1* mutations are missense changes in the highly conserved Gly-Xaa-Yaa repeat of the triple helix domain, which gives stability to the protein chain and promotes assembly of the triple helix; in the noncollagenous (NC1) domain,[8,15] important for extracellular matrix interactions and turnover; or in the start codon,[9] or are splice site mutations.[6] COL4A1 and COL4A2 assemble to form a heterotrimeric triple helix.

In mice, intracerebral and ocular hemorrhages are associated with *Col4a2* mutations, and recently human *COL4A2* mutations have been found in familial and sporadic porencephaly, ICH, and SVD.[18–20] Additionally, variants at the *COL4A*1 and *COL4A2* locus on chromosome 13q34 have also been associated with susceptibility to coronary artery disease, underscoring the role of these genes in vascular diseases.[21]

Mutation analysis should no longer be restricted to infants presenting with infantile hemiplegia and porencephaly on neuroimaging, but should also be considered when neuroimaging shows a pattern of periventricular leukomalacia associated with mild periventricular calcification without any family members showing porencephaly, which is sometimes associated with extracerebral findings.[11] The data from Weng and colleagues[4] do suggest that sporadic late onset intracerebral hemorrhage is another indication for *COL4A1* testing and certainly in recurrent ICH.[14] The identification of *COL4A2* mutations in both sporadic and familial ICH suggests that the mutational screening should be performed in parallel for both *COL4A1* and *COL4A2*. If confirmed, *COL4A1* and *COL4A2* could be included in the screening of genes for Walker–Warburg syndrome.

Two major issues limit the applicability of mutation analysis for *COL4A1* and *COL4A2* in genetic counseling: the lack of a genotype–phenotype correlation and the reduced penetrance. The presence of asymptomatic carriers is particularly problematic in families with severely affected children, limiting the predictive value of genetic tests.[8,17] Unless novel therapies, such as chemical chaperons or other methods to prevent protein misfolding, are

developed, genetic testing also has limited value in prenatal diagnosis.

Potential Conflicts of Interest

L.S.d.V.: honoraria, Imperial College London; royalties, An Atlas of Amplitude-Integrated EEGs in the Newborn (Informa Health), An Atlas of Neonatal Brain Sonography (Mac Keith Press); travel expenses, Newborn Brain Symposium (St Louis, 2011), Advances in Neonatal Medicine (Würzberg, 2011), Imaging Advances in Newborn Brain Injury (San Francisco, 2011).

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DOI: 10.1002/ana.23544