Experimental Induction of Cerebral Aneurysms by Developmental Low Copper Diet

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Abstract

Optimal models are needed to understand the pathophysiology of human cerebral aneurysms (CA). We investigated the development of experimental CA by decreasing the activity of lysyl oxidases by dietary copper deficiency from the time of gestation and then augmenting vascular stress by angiotensin II infusion in adulthood. Rats were fed copper-free, low-copper, or normal diets at different time periods from gestation to adulthood. The incidences of CAs were evaluated and autopsy specimens performed to determine the coexistence of cardiovascular diseases. A copper-free diet from gestation was associated with high mortality rates (79.1%) resulting from rupture of ascending aorta aneurysms; a low-copper diet led to acceptable mortality rates (13.6%) and produced CAs and subarachnoid hemorrhage in 46.4% and 3.6% of animals, respectively. Higher proportions of CAs (up to 33.3%) in the rats primed for copper deficiency from gestation ruptured following angiotensin II infusion from adulthood. Gene expression array analyses of the CAs indicated that genes involving extracellular matrix and vascular remodeling were altered in this model. This model enables future research to understand the entire pathogenetic basis of CA development and rupture in association with systemic vasculopathies.

Key Words: Animal model, Angiotensin II, Aortic aneurysm, Cerebral aneurysm, Copper, Lysyl oxidase.

INTRODUCTION

Cerebral aneurysms (CA) occur in approximately 3% of the general population; their age distributions are bimodal (1, 2). The older-age peak suggests that old age and vascular risk factors may contribute to developing CA whereas the younger-age peak indicates that innate arterial wall fragility may be an operative mechanism (3–5). A substantial number of patients are prone to have multiple aneurysms (6) and to experience recurrent subarachnoid hemorrhage (SAH) because of aneurysm recurrence and de novo formation; the latter are more distinctive in pediatric and young adult patients (7). Therefore, genetic or developmental factors that induce intrinsic wall defects may be important pathogenetic mechanisms of aneurysm development.

Aneurysmal SAH is a life-threatening condition with high morbidity and mortality; therefore, surgical clipping or endovascular coiling is implemented in patients with asymptomatic CA to prevent rupture (8). Current therapeutic options are invasive and have potentially serious complications (9). In this regard, noninvasive pharmacological therapeutic options are necessary for CA; however, no therapy has been identified as yet, mainly because of a lack of knowledge about the mechanisms underlying aneurysm development, growth, and rupture. To identify potential pathophysiological targets, an optimal animal model that manifests cardinal features of human intracranial aneurysms is required. Several models using combinations of various pharmacological and surgical methods have been proposed (10). While these models show the rapid development of aneurysms and a high rate of rupture, there remains a need for more suitable models that exhibit spontaneous development with minimal manipulation and that allow stabilization through a healing process, thus permitting the development of multiple unruptured aneurysms with occasional rupture episodes, as are found in humans.

Although the key elements for developing aneurysms are unknown, growing aneurysms are more likely to occur with vessel wall fragility (11). Elastin and collagen fibers exhibit increased tensile strength that is essential for vessel wall integrity and function. Lysyl oxidases (LOX) are extracellular copper-containing enzymes that stabilize extracellular matrices by cross-linking elastin and collagen monomers into fibers (12). Copper is an essential micronutrient that is required for the activity of amine oxidases such as LOX (13). Copper

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deficiency often occurs in infants who consume diets of cow’s milk and formulas with low copper content and low copper bioavailability (14–16). Long-term copper deficiency during the developmental period can cause abnormalities of the vascular and skeletal systems. Thus, a specific type of copper deficiency from gestation might be a functional generator of CA. Intriguingly, most CA remain stable throughout the course of a lifetime, and only a small number of them evolve to being rupture-prone (9). Even less is known about the mechanisms that induce CA rupture, and only limited data exist on shear stress, inflammation, and hypertension as potential inducers of aneurysm rupture.

In the present study, we sought to determine whether a certain type of copper deficiency and subsequent vascular insult induces aneurysm development and rupture relevant to human disease and how this phenotype is achieved.

**MATERIALS AND METHODS**

**Experimental Protocol**

To test whether timed copper deficiency during the developmental and growing periods can cause the formation of multiple CA, we designed a model with rodents fed special diets during specific time periods. The diets included normal diet ([ND]; AIN-93G Purified Rodent Diet with 6 mg/kg copper; Dyets, Inc., Bethlehem, PA); copper-free diet ([CFD]; modified AIN-93G Purified Rodent Diet without added copper; Dyets, Inc.); low-copper diet ([LCD]; Modified AIN-93G Purified Rodent Diet with 2 mg/kg copper; Dyets, Inc.); and copper-rich diet ([CRD]; Modified AIN-93G Purified Rodent Diet with 30 mg/kg copper; Dyets, Inc.). The time periods for the special diets extended from the gestation period to adulthood. The term from mid-pregnancy to birth is referred to as the prenatal period; the 4-week period when pups were kept together with their dam and littermates is referred to as infancy; the 2-month period after separation from the dam is referred to as youth; and the time period thereafter is considered adulthood. For this time schedule, we used pregnant Sprague-Dawley rats (mean gestational age, 12 days; n = 14; Daehan Bio, Seoul, South Korea) and their pups (n = 165). At P21-28, all pups were weaned and randomly housed, with 2 to 3 pups in each cage. The experimental model comprised 10 groups according to diet regimen and period. To inhibit the activity of LOX further, a LOX inhibitor, 0.12% -aminopropionitrile (BAPN; Sigma, St. Louis, MO), was added to some laboratory diets (17). Each group was designated as “A” or “B.” The “A” diet was administered during the prenatal period and infancy, and the “B” diet was administered during youth and adulthood. Accordingly, the groups were designated and fed as follows: ND-ND (n = 7); ND-CFD (n = 9); ND-CFD/BAPN (n = 15); ND-ND/BAPN (n = 13); CFD-ND (n = 12); CFD-CFD (n = 12); CFD-CFD/BAPN (n = 12); CFD-ND/BAPN (n = 13); LCD-CFD (n = 11); and LCD-CFD/BAPN (n = 33). To assess the effect of delayed copper supplementation, 2 additional groups, LCD-CFD/BAPN-ND (n = 15) and LCD-CFD/BAPN-CRD (n = 13), were used. These rats were administered ND or CRD for 2 months during adulthood after LCD-CFD/BAPN. The detailed timetable for the experimental schedule is shown in Figure 1.

For inducing CA rupture, we conducted additional experiments using 4 operations (n = 36): a flow-augmented common carotid artery (CCA) occlusion model (left CCA occlusion; n = 10), a doxycorticosterone acetate (DOCA)-salt hypertension model (n = 7), a left CCA occlusion + DOCA-salt hypertension model (n = 10), and an angiotensin II infusion model (n = 9). Rats fed with LCD-CFD/BAPN until adulthood (ND was administered afterward) were used, and the operations were applied for 2 months. For CCA occlusion, rats were anesthetized with isoflurane and the left CCA was dissected and ligated immediately below bifurcation area. DOCA (Sigma) 20 mg/kg was subcutaneously injected twice a week and 0.9% saline was supplied as drinking water during the experimental period. Angiotensin II (Sigma) 0.8 mg/kg/d was infused subcutaneously via osmotic pumps (model 1002, Alzet). For the rupture model, systolic blood pressure was measured before the treatment (baseline), and at 4 and 8 weeks after the induction using the tail cuff method (Kent Scientific Corporation, Torrington, CT). All procedures were performed with institutional approval and were accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

**Monitoring and Histopathology**

After separation from the mother, rat pups were raised under identical conditions except for diet. The littersmates were exposed to the same diet conditions and were thus assigned to the same group. The animals were monitored for survival for 5 months. The diets were prepared separately and the outcome measures were done independently of the breeder who prepared diet. The cumulative survival statistic was calculated by using Kaplan-Meier statistics. Log-rank p value was calculated to compare survival curves. Surviving rats were killed at 5 months and subjected to histopathological evaluation and molecular studies. In the rupture induction experiment, autopsies were performed on dead animals; the remaining animals were killed for gross pathological analysis 2 months after operations.

Autopsies were performed immediately at the time of death. Causes of death were determined based on gross pathological changes. Because the autopsies showed thoracic hemorrhage or SAH, all rats that survived 5 months were subjected to histopathological studies of the heart, aorta, and brain. Rats were anesthetized and perfused with ice-cold saline, followed by perfusion with a bromophenol blue dye solution (2 mg/mL) dissolved in phosphate-buffered saline and gelatin mixture (20%); this dye-gelatin mixture was used to delineate pathological changes of the intracranial arteries. Thoracic cavity, aorta, and arteries of the circle of Willis were examined under a dissecting microscope and the incidences of aortopathy and intracranial aneurysm were assessed in each group. Aortopathy included the development of hemopericardium, hemothorax, ascending thoracic aorta aneurysm, or cardiac hypertrophy. Aneurysm indicated obvious outward bulging of the arterial wall compared to its parent artery in macroscopic examination. After examining external features of the major cerebral arteries, the brain tissues were fixed with 4% paraformaldehyde for

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24 hours and then cryopreserved as tissue blocks for cryostat sectioning at 30 μm with a cryostat (Leica CM 1900; Leica, Deerfield, IL). Coronal sections were taken at the level of the circle of Willis, which contained the bifurcation of major cerebral arteries. Sections were stained with hematoxylin and eosin to assess the damage to elastic lamina, inflammation, thrombosis, and endothelium, and were stained with Elastic van Gieson to confirm the disappearance of the internal elastic lamina in the macroscopically identified CA.

Gene Microarray Analyses
For molecular studies, animals were killed at 5 months after birth. As a normal control, age-matched Sprague-Dawley rats fed ND-ND were killed. Samples of the following 3 categories were then harvested: normal cerebral artery, aneurysm, and parent artery with aneurysm sac. Cerebral artery with aneurysm was carefully dissected free from the surrounding tissues under a dissecting microscope, and the entire aneurysm was then separated from the parent artery. Thrombosed aneurysms were not included for gene expression studies. The tissues immediately frozen in liquid nitrogen were stored at -70°C until RNA extraction. After extracting RNA by RNeasy Mini Kit (Qiagen, Valencia, CA), we pooled RNA to generate three test samples per category by combining 3 RNA samples from each category to decrease the influence of intersample variability. The concentration and quality of each RNA sample were determined using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA was labeled and hybridized to Agilent Rat GE v3 microarrays (Agilent Technologies), as described in the manufacturer’s technical manual. Microarrays were scanned with the Agilent DNA microarray scanner (Agilent Technologies), and the scanned images were analyzed using Feature Extraction software (Agilent Technologies) to obtain the signal intensity of the spots. Gene expression was normalized by median normalization methods and presented as the values of the aneurysm and parent artery samples divided by the values of the normal cerebral artery sample. The threshold for differentially regulated genes was set as a 2-fold increase or decrease to identify effector genes. The gene functions and associated pathways were evaluated by reviewing ENTREZ Gene (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene), Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/pathway.html), and the published literature.

RESULTS
High Early Mortality With CFD During the Developmental Period
To determine the diet regimen and time point to induce an optimal spontaneous aneurysm, we tested various diet regimens during different time periods (Table). Copper deprivation throughout the developmental period and youth produced highly lethal disease, with a 58.3% mortality rate for rats fed CFD-CFD and 100% mortality rate for rats fed CFD-CFD/BAPN. When we administered the CFD separately at various...
time points over the course of a lifetime, maternal transfer from CFD during the prenatal period and infancy had the greatest impact on mortality. Most rats fed CFD-CFD or CFD-CFD/BAPN had very rapid disease progression and died within 10 weeks (Fig. 2). However, LCD during the perinatal period resulted in relatively low mortality rates (LCD-CFD, 9%; LCD-CFD/BAPN, 18%). Although autopsy results demonstrated pericardial and/or thoracic hemorrhage in most rats with little interlitter variability (Fig. 3A-D), some additional deaths after 10 weeks were determined to have resulted from SAH (Fig. 4D). Thoracic hemorrhage and SAH were thought to occur because of ascending thoracic aorta aneurysm rupture and intracranial aneurysm rupture, respectively.

Long-Term Vascular Outcome With Copper-Deficient Diet

We defined 2 forms of copper deficiency-related vascular outcomes: aortopathy and CA. The incidences of aortopathy and CA according to exposure degree and timing of copper deficiency were evaluated at 5 months after birth (Table). Aortopathy developed in virtually all rats fed CFD during their lifetime and highly lethal aortopathy occurred very early, whereas cardiac hypertrophy was detected late in some surviving rats (Fig. 3). The incidence of CA could not be determined because of the high mortality rate. Cerebral aneurysms were induced frequently in 32% of rats fed LCD-CFD and in 46% of rats fed LCD-CFD/BAPN, resulting in modest aortopathy rates (8% and 21%, respectively). However, ND during the perinatal period and later copper deficiency showed no pathological changes in the heart, aorta, and thoracic cavity but was associated with CA in 8% of rats fed ND-CFD/BAPN, in 11% of rats fed ND-CFD, and in 33% of rats fed ND-CFD/BAPN.

Effect of Late Supplementation of CRD on Aneurysm Development

Next we investigated whether the delayed copper supplementation could attenuate the development of CA. Copper was supplemented with ND (6 mg/kg copper) or CRD (30 mg/kg copper) for 2 months after 3 months of LCD-CFD/BAPN. Although mortality was not found, aortopathy was noted in 13.3% and 15.3% of rats fed LCD-CFD/BAPN-ND and LCD-CFD/BAPN-CRD, respectively. As compared with the continued CFD/BAPN group, the delayed copper supplementation failed to alter the disease progression (Table).

Morphological Features of Cerebral Aneurysm

LCD during the perinatal period and CFD/BAPN during the youth and adulthood periods induced CA in 46.4%. The majority of those aneurysms were stable and unruptured over the course of 5 months and the incidence of SAH was as low as 3.6%. Cerebral aneurysms were distributed uniformly in the arterial bifurcation area of the circle of Willis as single, double, or even triple and some were associated with internal carotid artery ectasia and basilar artery dolichoectasia, suggesting overall intracranial vascular wall deformity (Supplementary Fig. 1). Histological evaluations showed a breakdown of continuous endothel-
lial cell layer, faint smooth muscle layer, and disorganized elastic lamina (Fig. 4). BAPN treatment in combination with CFD changed the disease phenotype from a benign to a severe form, such as rupture or large aneurysm. The mean aneurysm size in LCD-CFD/BAPN rats ($915.4 \pm 137.3\ \mu m$) was significantly larger than that in LCD-CFD rats ($713.7 \pm 108.2\ \mu m$; $p < 0.001$).

### Increased Efficacies of Aneurysm Development and Rupture through Flow Augmentation, Hypertension Induction, and/or Angiotensin II Infusion

For inducing rupture of CA, flow augmentation by CCA occlusion, hypertension induction by DOCA-salt model or angiotensin II infusion were used. Groups of dietary DOCA-salt, CCA occlusion + DOCA-salt, and angiotensin II infusion increased the systolic blood pressure over the induction period of 2 months, without significant differences among the three groups (Supplementary Table S1). Seven (70%) of 10 in the CCA occlusion group showed CA, including 1 ruptured aneurysm at the junction of basilar artery and vertebral artery. Five (71.4%) of 7 in the DOCA-salt model group had CA including 1 SAH. The CCA occlusion + DOCA-salt model resulted in highest proportion (90%) of rats with CA, but rupture was not identified. Seven (77.8%) of 9 in the angiotensin II infusion group had CA, including 3 ruptured aneurysms. Rupture was apparently more prevalent with the angiotensin II infusion compared with the other operations (Table).

### Molecular Signature of Intracranial Aneurysms

To obtain a global view of the changes in gene expression in the CA, we performed gene expression arrays in the CA and its parent artery samples and normal artery ($n = 3$ per group). Each sample was pooled from ND-ND, LCD-CFD, and LCD-CFD/BAPN groups of rats at age 5 months. Of approximately 35,000 genes, we analyzed differentially upregulated and downregulated genes of the aneurysm and parent artery compared with the normal artery. There were small proportions of differentially regulated genes in the aneurysm compared to the normal artery (3,659 genes). Remarkably, both aneurysm and parent artery expressed similar patterns of differentially upregulated genes compared with normal artery (Fig. 5A). When the ontology of those genes was determined, significant genes involved extracellular matrix, immune and inflammatory responses, neural crest cell differentiation, and blood vessel remodeling (Fig. 5B; Supplementary Tables S2–S4). In the category of significantly downregulated genes, the largest changes were for genes involved in extracellular matrix signaling, including collagen type 1, collagen type 3, collagen type 6, elastin, fibrillin 1, fibrillin 2, LOX, LOXL1, and LOXL2 (Fig. 5C).

### DISCUSSION

The most significant finding of this study is that copper deficiency during the developmental period induces a variety of vascular wall abnormalities involving thoracic aorta aneurysms and CA. The disease phenotype and severity were dependent on the degree and timing of copper deficiency. Moreover, later angiotensin II infusion to rats primed for copper deficiency during developmental periods exhibited apparently higher proportion of CA with more prominent vascular change and rupture, which suggests that angiotensin II may further mediate the rupture process in the stable aneurysm. This is the first preclinical study to show the entire pathogenetic basis of CA development and rupture, and to provide a great deal of information to enable future research to access its therapeutic target.
LOX has a critical role in the matrix assembly (12). LOX requires 1 tightly bound copper at its active site (13), and its activity in growing animals is influenced directly by the amount of dietary copper (17). Because adequate copper status and functional LOX are essential in early stages of vascular development (18, 19), we created our model by feeding the dams a copper-deficient diet starting from pregnancy and by feeding the offspring the same diet for 4 weeks after birth. In some rats, BAPN was added to further accentuate the decreased LOX activity by copper deficiency (20). Phenotypes of our model varied according to the intensity and duration of the deficiency. Severely copper-deficient rats died of hemopericardium and hemothorax during youth or died of cardiac failure during adulthood. Ascending thoracic aortas were dilated with distortion and depletion of elastic fibers, and the hearts were hypertrophied. Because copper also operates in other essential enzyme systems as a cofactor, however, mortality may have been the result of the differential impact on development of multiple organs (21). The clinical relevance of our model relates to the fact that cow’s milk and some homemade infant formulas have insufficient copper levels and are unfavorable for bioavailability and utilization of copper (14–16). Moreover, several lines of evidence suggest the average daily intake of copper in Western diets is lower than what is recommended to be adequate (22, 23). More individuals than expected may experience long-term suboptimal copper status starting from the early developmental period and, perhaps, are at risk for development of aortopathy and CA.

Cerebral aneurysms are frequently subjected to therapeutic challenges because of the low risk of rupture and invasiveness of current therapies (24); therefore, the most practical steps in CA research are to define a high-risk aneurysm and to identify a therapeutic target for noninvasive treatment. Our knowledge of the mechanisms leading to aneurysm development, progression, and rupture is greatly limited because we observe only the final phenotype in humans. Therefore, investigators have made an effort to develop animal models to examine the entire course of CA. Oral chemical agents to induce wall fragility slowly were initially tried in rats and primates, but the incidence of intracranial aneurysms was low, the size was disproportionally small, and the histological changes were subtle and without any arterial wall bulging (20, 25–27). These observations are in line with our data indicating that BAPN-only treatment during adulthood produced CA in only 7.7%. The next attempt used multiple chemical or mechanical methods to injure healthy arteries, and these trials were associated with a high rate of mortality and rupture rate within a very short time period (28, 29). However, the applied methods and outcomes were quite different from what occurs in humans, and it was difficult to assess effects of pharmacological treatment on the phenotype because the efficacy was dependent on whether the drug was specific to each injury component rather than overall pathophysiology.

FIGURE 3. Aortopathy development. (A-F) Photos of rats fed a copper-free diet starting from the gestation period showing various phenotypes of aortopathy. For each rat, including dead and living rats, the chest wall was opened and examined for pericardial effusion (A), pericardial hematoma (B), hemothorax (C), ascending thoracic aorta aneurysm (D, arrowheads), and cardiomegaly (E, F). The main causes of early mortality were thought to be pericardial tamponade, mediastinum compression, and hemodynamic shock by ascending thoracic aorta rupture. The heart was sliced coronally at the level of the interventricular septum to examine the myocardium hypertrophy. Comparing with normal heart (E), cardiomegaly and myocardium hypertrophy were frequently combined with aortopathy in the copper-free diet model (F). Bar represents 5 mm.
Because CA result mainly from a mismatch in the matrix assembly of the tunica media and hemodynamic stress (9, 30), we attempted to weaken the integrity of the vascular wall by a long-term sublethal copper-deficient diet starting from the pregnancy and lactation periods. Given that copper deprivation from the time of gestation seems to be indispensable to aneurysm formation, our model may mimic particular kinds of CA accompanied by congenital connective tissue diseases such as Ehlers-Danlos and Marfan syndromes. However, copper deprivation from youth following the developmental normal diet also developed CA, while a normal diet following the developmental copper deprivation did not result in the development of CA. These findings indicate that induced CA in our model are not entirely based on congenital fragility of extracellular matrix in the vascular wall, but also on the matura-
tion status of extracellular matrix in youth and early adulthood. To clarify the critical period of copper deprivation on developing CA, we performed the additional experiments with later copper supplementation from adulthood that failed to attenuate the development of CA. Taken together, the developmental and youth periods seem to be critical for the effects of copper deprivation on inducing CA.

Cerebral aneurysms were multiple, macroscopically distinguishable, and detected in 45% of the experimental group. The proportions could be significantly increased to 70%-90% by flow augmentation, hypertension induction, or angiotensin II infusion, which indicates that normal-looking arteries also have intrinsic arteriopathy susceptible to aneurysmal change under a stress condition. Histopathological features included a discontinuation of elastic lamina, loss of cellular components, and disorganization of muscle fiber structure. Although most aneurysms were stabilized as unruptured aneurysms, in some aneurysms that were induced by later angiotensin II, the vascular wall was further weakened, eventually resulting in aneurysmal rupture. Meanwhile, flow augmentation and induced hypertension with DOCA-salt did not provoke aneurysm rupture. These findings suggest that the inflammation is essential to degenerative changes of the CA wall leading to rup-

**FIGURE 4. Cerebral aneurysm development.** (A-D) Rats were killed by cardiac perfusion with bromophenol blue dye plus gelatin to delineate the artery structure. Low-copper diet or copper-free diet during the developmental period induced multiple intracranial aneurysms (A, black arrowheads). Cerebral aneurysms occurred mainly in a fusiform or sidewall aneurysm (B, C). Some rats with induction operations in adulthood were identified to have subarachnoid hemorrhage in an elective autopsy or after a sudden death (D). (E-J) Hematoxylin and eosin staining showed a single, continuous layer of endothelial cells and two to three layers of smooth muscle cells in the normal cerebral artery (E, F). In aneurysm (H, I), disrupted endothelial cell layers and disorganized smooth muscle layers were noted. Elastic van Gieson staining shows one layer of elastic lamina (white arrowheads) in a normal artery (G); a profoundly disorganized elastic lamina is seen in an aneurysm (J). Bars represent 300 μm (E, H) and 50 μm (F, G, I, J).
Because of the simplicity of the model, large aneurysms, high incidence of aneurysms, and acceptable mortality, this sequential model with copper deficiency and angiotensin II would be suitable for high-throughput studies to determine critical pathways participating in the pathophysiology of CA.

Some aneurysm populations display odd features, including multiplicity, large size, and rupture presentation as small aneurysms (3, 31). It is speculated that these features are partly related to developmental abnormalities of the artery wall. Some hints about the concept of innate wall deformity are obtainable from our model, which is characterized by the concurrent development of aortopathy and intracranial aneurysm. The cervicocephalic muscular arteries are derived from the neural crest, which also participates in cardiac development and outflow valves (32). Thus, an abnormality of the neural crest may be the common pathogenetic factor in ascending thoracic aorta aneurysm and intracranial aneurysm (33, 34). The relatively weak association between abdominal aortic aneurysm and intracranial aneurysm (35) further supports the theory of neurocristopathy because the abdominal aorta is clearly different from the thoracic aorta and cervicocephalic artery in origin, structure, and function of the vascular wall (36). The ultimate duties of neural crest-derived mesenchymal cells are to compose and stabilize the tunica media of arteries via matrix production and assembly (36). Thus, it appears reasonable that the alteration in matrix assembly induces the pathology in the ascending thoracic aorta and intracranial artery walls, as found in our model.

Although the exact underlying mechanisms are not fully known, several factors including extracellular matrix integrity,
inflammation, and endothelial maintenance have been suggested to be involved in the formation and/or rupture of intracranial aneurysm (37). Although copper deficiency was primarily intended to alter the LOX activity in our study, various changes in cell motility and migration, cell signaling, and transcriptional regulation were also anticipated because LOX affects intracellular dynamics in an extracellular matrix–independent manner (11, 12). As expected, our microarray analysis of aneurysms identified expression changes for various genes involved in endothelial matrix formation and blood vessel remodeling, several of which (i.e., COL1A2, COL3A1, COL5A2, elastin, fibrillin 1, and fibrillin 2) have been implicated as effectors of aneurysm development (37, 38). This model may allow the study of the pathogenesis of aneurysms over a longer period regarding the epigenetic machinery surrounding aneurysm development, growth, and rupture.

Our model may have limitations to understanding the actual pathophysiology of human CA. Many of phenotypes in our model were fusiform and sidewall aneurysms that differ from the majority of human CA. Most human CA arise at the arterial bifurcation and show a saccular form and the traditional rat CA model in which CA are induced by the ligation of unilateral CCA and high salt diet may be superior to the model. However, there is no clinical evidence that CA are more prevalent in patients with proximal carotid disease and hypertension. The traditional model and our model may be mutually complementary to understanding the pathophysiology of human CA. Furthermore, a recent pathological study showed that the majority of human aneurysms have abundant amount of fresh collagen, suggesting that aneurysms do not remain unchanged for decades but undergo dynamic collagen remodeling (39). Thus, the majority of human CA may not have an innate weakness but respond to a milieu that is hostile to vascular integrity. Nevertheless, our model has great merit for the study of the pathophysiology of CA in association with systemic arteropathies.

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