CEREBRAL SMALL-VEssel DISEASE ASSOCIATED WITH COL4A1 AND COL4A2 GENE DUPLICATIONS

A nonsmoking woman, aged 44 years, presented with transient right-sided hemiparesis. CT showed leukoencephalopathy without infarction (figure). Blood pressure was normal. Blood count, plasma glucose levels, C-reactive protein, renal/liver function tests, cardiac enzymes, atrial fibrillation factor, HIV/syphilis/hepatitis B and C serology, and lactate acid levels were normal. Low-density lipoprotein (LDL) cholesterol levels were slightly elevated (1.25 g/L). Screening tests for prothrombotic disorders (serum fibrinogen, D-dimer, fibrin degradation products, antithrombin III level, protein C and S level, factor V Leiden, prothrombin gene mutation, antiphospholipid antibodies) were normal. Carotid duplex scanning, transvaginal echocardiography, and 24-hour Holter ECG were normal. Acetylsalicylic acid 160 mg once daily was started.

At age 56, she presented with right-sided leg weakness. MRI (figure) showed 4 infarction zones, 6 microbleeds, leukoencephalopathy, and vertebral dolichoectasia. LDL cholesterol level was 1.4 g/L. Thoraco-abdominal-pelvis CT, eye fundus, and lumbar puncture were normal. Atorvastatin 40 mg, indapamide 2.5 mg once daily were added. Blood 

1.3-galactosidase activity level was normal.

At age 59, she presented with left-sided hemiplegia. MRI showed right-sided subcortical fronto-middle cerebral artery infarction (figure).

At age 61, MRI showed progressive leukoencephalopathy (figure). Vertebrobasilar dolichoectasia and microbleeds were stable compared with imaging performed at age 56.

During the observation period between 44 and 61 years, the patient developed neither hypertension nor diabetes, and carotid duplex scanning and cardiac examinations (performed after each new neurologic event) remained normal.

Sequencing of the 52 exons of COL4A1 was negative but quantitative multiplex PCR of short fluorescent fragments analysis showed complete gene duplication (figure), confirmed with microsatellites spanning the COL4A1/COL4A2 locus. Three microsatellites—D13S1265, D13S1315, and AFM07swE5—showed 2 different allele peaks, with each one being twice as high as the other one. Microsatellite D13S285 showed 3 different alleles (figure). Comparative genomic hybridization performed with 180K Agilent Technologies (Santa Clara, CA) microarray showed that this 8.1-Mb-long 13q33.2q34 duplication encompasses 45 genes, including COL4A1 and COL4A2 (figure). The duplication of none of these genes has been previously reported in vascular leukoencephalopathy. The centromeric breakpoint of the duplication was not located within a known gene.

Fluorescence in situ hybridization analysis showed subtelomeric trisomy of the long arm of chromosome 13 and monosomy of the short arm of chromosome 15p11.2. The trisomic 13q33.2q34 region was translocated on the centromere of one of the two chromosomes 15, as follows: 46,XX.ish der(15) t (13;15)(q33.2q34;p11.2) (wcp13,+13qter+;13qter+, D15Z1+, wcp15+).arr 13q33.2q34(106,991,469-115,096,466)x3.

The deletion of region NOR 15p is considered as a chromosomal nondeleterious variant.

The patient had 2 brothers and 1 daughter, all asymptomatic. Both patient’s parents were deceased (aged 67 and 85, without history of brain vascular disease). Genetic analysis of the patient’s daughter and of one of her brothers did not detect the 13q33.2q34 duplication.

Discussion. COL4A1 point mutations have been recently identified as a cause of autosomal dominant hereditary cerebrovascular disease, often associated with systemic injury. 2,3 Reported cerebral manifestations include porencephaly, leukoencephalopathy, hemorrhage, TIA, brain infarction, dilated perivascular spaces, microbleeds, mental retardation, epilepsy, migrane, dolichoed tomotic siphons, and aneurysms. 5 Most COL4A1 and COL4A2 mutations reported so far are missense point mutations. 5,6 Both dominant-negative mechanism and haploinsufficiency have been proposed as pathogenic mechanisms. 4 Very rare cases of partial 13q33.2q34 region trisomy involving COL4A1/COL4A2 genes have been reported, mostly with duplications larger than in our patient, causing various fetal malformations including diaphragmatic hernia, corpus callosum agenesis, and cerebellar malformations leading to pregnancy interruption. 5,6
Radiologic and genetic data

(A) At age 44, CT shows diffuse periventricular leukoencephalopathy (a and b). MRI at age 56 shows 3 of the 4 foci of hyperintensity on diffusion-weighted imaging (DWI), 1 in the left-sided subcortical prerolandic area (corresponding to the watershed territory between the middle and anterior cerebral artery territory, explaining the right leg weakness) (c) and 2 periventricular on the right side (d); several infratentorial and supratentorial microbleeds (i.e., a total of 6 microbleeds, including 1 right-sided mesial temporal, 3 cerebellar microbleeds (e) is shown in e), 1 left-sided putaminal (f), and 1 right-sided cortical parietal) on gradient echo–weighted imaging (e and f); diffuse posterior predominant leukoencephalopathy on fluid-attenuated inversion recovery (FLAIR) sequences (g and h); and severe vertebrobasilar dolichoectasia on time-of-flight imaging (i). At age 59, MRI DWI shows an acute right-sided frontal periventricular infarction (j). The most recent MRI (k and l) at age 61 reveals slowly progressive leukoencephalopathy on FLAIR sequences, together with the earlier brain infarctions embedded in the coexisting leukoencephalopathy. (B) Quantitative multiplex PCR of short fluorescent fragments analysis of 5 out of the 52 COL4A1 exons including the last exon and exons from 2 control genes (PBGD and CACNA1A).

Each peak corresponds to the amplification of an exon. Blue represents the amplification of control DNA and red the amplification of the patient’s DNA. The heights of the 2 control genes are overlaid. COL4A1 amplicons’ heights are 1.5-fold higher in the patient’s DNA compared to the control DNA. All 52 COL4A2 exons showed the same profile. This is the profile typically observed in case of duplication. (C) D13S285 microsatellite analysis shows the presence of 3 different alleles (1 arrow on each allele) in the patient and 2 normal heterozygous alleles in the control. (D) Results of array comparative genomic hybridization analysis with view of chromosome 13 and focus on the duplicated region. On the left (whole chromosome view), the duplicated region of chromosome 13 (corresponding to the 13qter region) can be seen in the lower part. In the enlarged picture on the right, the duplicated region is represented by the vertical line. Control DNA is labeled in green and the patient’s DNA is labeled in red. When control and patient DNA are in equal amount, the log ratio of the fluorescence intensity is zero (green and red dots are overlaid), and when the patient’s DNA is duplicated, the log ratio of fluorescence intensity is 0.5. The positions of the first and last duplicated probes are indicated (positions 106,991,469 and 115,096,466) as well as the size of the duplication (8,104,997 bp). (E) fluorescence in situ hybridization results with subtelomeric probes specific for chromosomes 13qter and 15qter. Two normal hybridization signals of subtelomere 13q (red) are seen on both chromosomes 13 and an additional signal of subtelomere 13q (red) is seen on derivative chromosome 15 (green).
One adult patient with leukoencephalopathy, epilepsy, and one acute brain infarction associated with partial trisomy 13q22q34 has also been reported. However, multiple additional clinical features (all lacking in our patient) were present in this patient.7

To our knowledge, COL4A1/COL4A2 gene duplications have never been described in patients with vascular leukoencephalopathy associated with recurrent brain infarctions. Our patient has a duplication spanning 45 genes, including both COL4A1 and COL4A2 genes. We suspected small-vessel disease as the underlying mechanism because of the presence of lacunar infarctions, microbleeds, and leukoencephalopathy.

Although we suspect that this duplication might be involved in the phenotype of our patient, the causality cannot be formally established at present. A strong argument for causality would be the identification of additional patients and families with either de novo duplications of these genes or familial cosegregation. Skin biopsy analyses in these patients could possibly give more insight into the underlying pathophysiology provoked by these mutations.

We emphasize the need for both point mutations and copy number analyses of COL4A1 and COL4A2 genes in patients in whom mutations of these genes are suspected. The identification of additional patients with overlapping duplications would help to better understand the underlying mechanisms leading to the vascular brain disease.

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