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Multiorgan manifestations of *COL4A1* and *COL4A2* variants and proposal for a clinical management protocol

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Abstract

COL4A1/2 variants are associated with highly variable multiorgan manifestations. Depicting the whole clinical spectrum of *COL4A1/2*-related manifestations is challenging, and there is no consensus on management and preventative strategies. Based on a systematic review of current evidence on *COL4A1/2*-related disease, we developed a clinical questionnaire that we administered to 43 individuals from 23 distinct families carrying pathogenic variants. In this cohort, we extended ophthalmological and cardiological examinations to asymptomatic individuals and those with only

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limited or mild, often nonspecific, clinical signs commonly occurring in the general population (i.e., oligosymptomatic). The most frequent clinical findings emerging from both the literature review and the questionnaire included stroke (203/685, 29.6%), seizures or epilepsy (199/685, 29.0%), intellectual disability or developmental delay (168/685, 24.5%), porencephaly/schizencephaly (168/685, 24.5%), motor impairment (162/685, 23.6%), cataract (124/685, 18.1%), hematuria (63/685, 9.2%), and retinal arterial tortuosity (58/685, 8.5%). In oligosymptomatic and asymptomatic carriers, ophthalmological investigations detected retinal vascular tortuosity (5/13, 38.5%), dysgenesis of the anterior segment (4/13, 30.8%), and cataract (2/13, 15.4%), while cardiological investigations were unremarkable except for mild ascending aortic ectasia in 1/8 (12.5%). Our multimodal approach confirms highly variable penetrance and expressivity in *COL4A1/2*-related conditions, even at the intrafamilial level with neurological involvement being the most frequent and severe finding in both children and adults. We propose a protocol for prevention and management based on individualized risk estimation and periodic multiorgan evaluations.

KEYWORDS
COL4A1, COL4A2, collagen, genotype–phenotype correlation, multiorgan

1 | INTRODUCTION

Type IV collagen is the main collagen component of the basement membrane. It is a unique member of the large collagen superfamily, which in vertebrates comprises 28 different types (Myllyharju & Kivirikko, 2004). It includes six genetically distinct α-chains designated α1 (IV) to α6(IV) (Myllyharju & Kivirikko, 2004; Oohashi et al., 1994; Sugimoto et al., 1994; Zhou et al., 1993). The genes *COL4A1* and *COL4A2*, both located on chromosome 13, are expressed in all tissues and encode the α1 and α2 chains, which are assembled intracellularly to form the α1α1α2 heterotrimer (Myllyharju & Kivirikko, 2004; Veit et al., 2006).

In the last two decades, several phenotypic features have been associated with *COL4A1/2* variants (Supplementary Figure 1). The first report of *COL4A1* pathogenic variants described two families with autosomal dominant porencephaly (Gould et al., 2005). Brain small vessel disease (BSVD) was associated with pathogenic variants in *COL4A1* in 2006 (Breedveld et al., 2006; Gould et al., 2006), and in *COL4A2* in 2012 (Verbeek et al., 2012; Yoneda et al., 2012). BSVD usually determines an increased vulnerability of small cerebral vessels that makes them prone to hemorrhage, even before birth or in relation to birth trauma (Breedveld et al., 2006; Gould et al., 2006). Clinical signs depend on the location and extent of the consequent parenchymal damage, and include neurodevelopmental delay, seizures, hemiplegia, and sometimes ocular anomalies such as the Axenfeld Rieger anomaly (Shields et al., 1985; Sibon et al., 2007). In 2007, *COL4A1* variants were also associated with autosomal dominant hereditary angiopathy with nephropathy, aneurysms, and muscle cramps (HANAC) syndrome (Plaisier et al., 2007). Unlike BSVD, HANAC syndrome involves medium and large caliber blood vessels in various regions of the body, also leading to aneurysm formation (Plaisier et al., 2007). Renal manifestations include microhematuria and kidney cysts, whereas neurological manifestations may include the consequences of leukoencephalopathic changes (Plaisier et al., 2007). Variants located in the miR-29 microRNA binding site within the 3′ untranslated region of *COL4A1* are associated with pontine microangiopathy and leukoencephalopathy (PADMAL) (Verdura et al., 2016), a form of cerebral small vessel disease (CSVD) characterized by recurrent ischemic strokes, especially in individuals between their 30s and 40s. Individuals with PADMAL experience a progressive decrease in cognitive and motor abilities, consistent with progressive multi-infarct dementia. Neuroimaging findings of PADMAL include widespread leukoencephalopathy and lacunar infarcts, mainly in the pons (Kikumoto et al., 2023; Verdura et al., 2016). *COL4A1/2* variants were identified as a susceptibility factor for adult-onset hemorrhagic stroke (Cho et al., 2022; Jeanne et al., 2012; Weng et al., 2012) and were associated with retinal arterial tortuosity (RATOR) (Zenteno et al., 2014), characterized by marked contortion of the second- and third-order retinal arteries with no impact on the first-order arteries and the venous system (Zenteno et al., 2014). Most individuals with this condition face episodes of transient vision impairment caused by retinal hemorrhage prompted by minor stress or trauma (Zenteno et al., 2014).

COL4A1/2-associated structural brain abnormalities include type 1 porencephaly, schizencephaly enlarged ventricles, intracranial calcifications, lacunar infarcts, leukoencephalopathy, and hemosiderin deposition (Cavallin et al., 2018; Yoneda et al., 2013).

The number of reported individuals with *COL4A1/2* variants has steadily increased over time and is difficult to quantify precisely. In 2015, a literature review identified 174 individuals (157 *COL4A1*, 17 *COL4A2*) (Meuwissen et al., 2015). As of August 21, 2023, the Human Gene Mutation Database (HGMD) (professional release

2023.2) listed 269 pathogenic or likely pathogenic variants in *COL4A1* and 42 in *COL4A2* (Stenson et al., 2003). Additionally, 64 variants of uncertain significance (VUS) have been identified in *COL4A1*, and 25 in *COL4A2*. Although reports describing pathological phenotypes associated with *COL4A1/2* abound, a comprehensive compilation of manifestations and variants is lacking. This gap is of significant concern since it limits an interdisciplinary approach to clinical care, thereby delaying guidance for optimal management and prevention strategies.

We addressed these shortcomings by aggregating literature data and clinical findings from a cohort of 43 individuals from 23 families with *COL4A1/2* pathogenic or likely pathogenic variants. After an extensive literature review, we devised a questionnaire for multi-organ investigation that we administered to individuals in our cohort. We extended prospective ophthalmological and cardiological examinations to oligosymptomatic and asymptomatic individuals in the cohort. Based on the multimodal results we obtained, we propose a protocol for management and prevention.

2 | MATERIALS AND METHODS

2.1 | A systematic literature review

The first step was a systematic literature review following the PRISMA guidelines (Page et al., 2021). Our aim was to make an inventory of all the reported phenotypic manifestations reasonably associated with *COL4A1/2* variants. The search was designed following the Population, Interventions, Comparators, Outcomes, and Study design (PICOS) criteria (Amir-Behghadami & Janati, 2020). We searched the Scopus, Pubmed, and HGMD databases for original articles published from 1984 (Smit et al., 1984) to August 3, 2023, using the following keywords: "(COL4A1 OR COL4A 1) OR (COL4A2 OR COL4A 2)." We included original articles, reviews, and case series written in English, which included original data on phenotypic manifestations of individuals carrying *COL4A1/2* variants classified as hot-VUS, likely pathogenic, or pathogenic (Ellard et al., 2020; Richards et al., 2015). We excluded reviews not containing original data, meta-analyses, as well as pre-clinical studies, non-relevant articles, and duplicates. The reasons for any exclusions were recorded, and the algorithm for study selection is illustrated in Figure 1, according to PRISMA guidelines (Richards et al., 2015). Article screening and data extraction were performed by two independent researchers (SG and LFS). We discussed any discrepancies until a consensus was reached. Disagreements were resolved by senior reviewers (SB and MM).

2.2 | Questionnaire design and retrospective clinical analysis

We used the information gathered through the systematic review to design a questionnaire that could provide a comprehensive phenotypical characterization. The questionnaires were filled out by individuals with pathogenic or likely pathogenic *COL4A1* or *COL4A2* variants or their caregivers, assisted by either the referring physician or a neurologist in our hospital. The questionnaire was in Italian and included ten sections. The first section included demographic data and family history. Sections two to ten investigated specific physiological functions or clinical conditions: neurological, cardiovascular, ophthalmological, renal, urogenital, oncological, orthopedic, prenatal issues, and other non-specific manifestations. Information collected in the questionnaires was then integrated with data extracted from clinical notes, including neuroimaging and other specific investigations. The study was approved by the Pediatric Ethics Committee of the Tuscany Region. Written informed consent was obtained from all individuals and/or their parents or legal guardians.

2.3 | Ophthalmological and cardiological investigations

The third part of our study concerned ophthalmological and cardiological investigations in all *COL4A1/2* variant carriers who, according to the questionnaire, were asymptomatic or manifested only a few or mild clinical symptoms, often nonspecific and commonly observed in the general population (i.e., oligosymptomatic), and had not undergone such investigations in the previous two years. This step aimed at a deeper phenotyping and detection of asymptomatic manifestations with the potential for progression or worsening.

Based on the prevalence and clinical relevance of ophthalmological abnormalities, as reported in the literature and emerging from our cohort, we assessed the best corrected visual acuity (BCVA) with age-appropriate visual acuity chart, IOP measurement, biomicroscopy of the anterior segment, and evaluation of the posterior segment with indirect ophthalmoscopy after pupil dilation with tropicamide 1% eye drops. In cooperative patients, we also performed spectral domain optical coherence tomography (SD-OCT) and anterior segment photography. We used the Heidelberg Spectralis SD-OCT platform (Heidelberg Engineering GmbH, Heidelberg, Germany) to measure retinal thickness and obtain morphological cross-sectional information using the built-in automated retinal layer segmentation software. In cooperative participants, we used the anterior segment OCT module to obtain qualitative data on the irido-corneal angle to search for subtle signs of goniodysgenesis and measure central corneal thickness (CCT).

Cardiological assessment in asymptomatic or oligosymptomatic individuals included a 12-lead electrocardiogram (ECG) and dynamic Holter ECG to search for any cardiac arrhythmias or conduction disturbances, and a transthoracic echocardiography to investigate functional and structural alterations (namely, valvular heart disease, aortic vessel aneurysms, and cardiac malformations).

3 | RESULTS

3.1 | Systematic review

Of the 1469 studies initially selected, 182 met the eligibility criteria (Figure 1 and Supplementary Table 1) and included 647 individuals

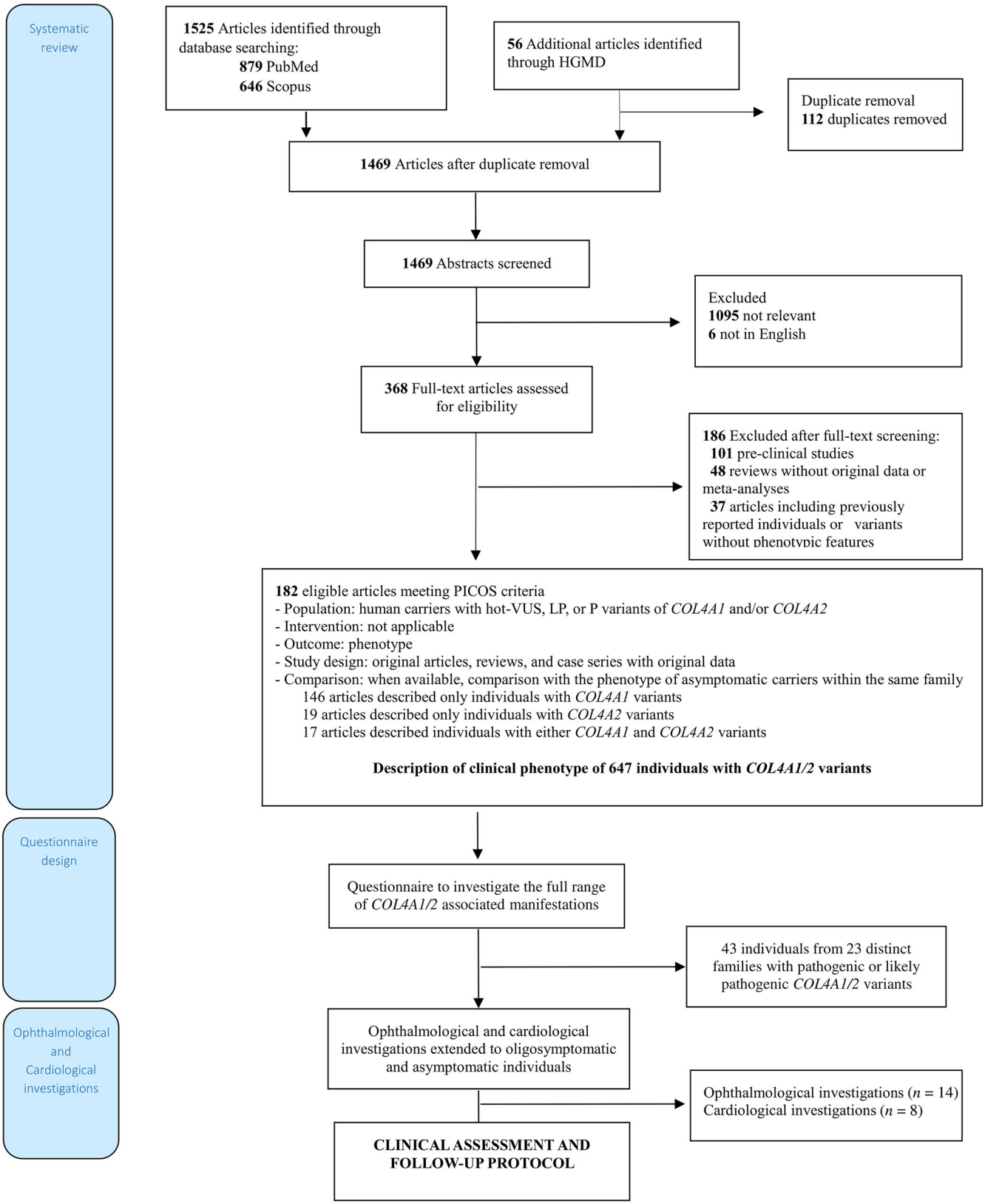

FIGURE 1 Study design flow chart according to the PRISMA guidelines (Page et al., 2021). HGMD, human genome mutation database; P, pathogenic; LP, likely pathogenic; VUS, variant of unknown significance.

from 485 families with pathogenic, likely pathogenic, or hot-VUS variants.

We found 546 individuals from 430 families with *COL4A1* variants. This group included 208 children (81 males, 64 females, and 63 of unspecified gender) and 177 adults (91 females, 84 males, and 2 of unspecified gender); in the remaining 161 individuals, age was not specified.

We identified 93 individuals from 53 families with *COL4A2* variants. This group included 29 adults (16 females, 12 males, and 1 individual of unspecified gender), 44 children (19 males, 10 females, and 15 of unspecified gender), and 20 additional individuals whose age was not specified.

Finally, eight individuals from four different families had a duplication involving both *COL4A1* and *COL4A2*; this group was composed of one child (male), and seven adults (5 females and 2 males).

Overall, a total of 405 *COL4A1* and 52 *COL4A2* variants were included. All the variants were heterozygous, except four (2 *COL4A1* and 2 *COL4A2* homozygous variants). Variants arose de novo in 148 individuals (136 *COL4A1* and 12 *COL4A2*) and were inherited in 82 (66 *COL4A1* and 16 *COL4A2*). For variants with familial inheritance, segregation and associated phenotypes were described over two generations in 41 families (39 *COL4A1*, 2 *COL4A2*), three generations in 17 families (14 *COL4A1*, 3 *COL4A2*), four generations in two families (both *COL4A1*), and five generations in one family (*COL4A1*).

The complete list of clinical manifestations is presented in Tables 1 and 2 (see Discussion, Section 4).

3.1.1 | Neurological manifestations

Neurological manifestations were identified in 507/647 (78.4%) individuals (430 *COL4A1*, 70 *COL4A2*, and 7 with a duplication involving both genes). Common findings were structural brain abnormalities in 338/647 (52.2%), stroke in 195/647 (30.1%), seizures or epilepsy in 183/647 (28.3%), intellectual disability and/or developmental delay in 154/647 (23.8%), and motor deficits in 152/647 (23.5%).

Abnormal white matter signal was the most frequent structural brain abnormality reported in 167/647 (25.8%) and was defined as non-specific in 96/647 (14.8%) (84 *COL4A1*, 5 *COL4A2*, 7 with a duplication involving both *COL4A1* and *COL4A2*) or as leukoencephalopathy in 71/647 (11.0%) (66 *COL4A1*, 5 *COL4A2*). This distinction may however be arbitrary and rater dependent.

Porencephaly was the second most frequent structural brain abnormality, being reported in 124/647 (19.2%) individuals (98 *COL4A1*, 26 *COL4A2*). The size of porencephaly ranged from small cavities to extensive cystic scars. Depending on size and location, carriers were asymptomatic or manifested mainly motor deficits, seizures, or both (Vahedi et al., 2007; Zagaglia et al., 2018). Porencephaly was either isolated or associated with other structural brain abnormalities and cortical malformations such as schizencephaly and polymicrogyria.

Schizencephaly was the most common cortical malformation in 38/647 (5.9%) individuals (35 *COL4A1* and 3 *COL4A2*). Other malformations of cortical development included polymicrogyria in 11/647 (1.7%) individuals (9 *COL4A1* and 2 *COL4A2*), focal cortical dysplasia in 6/647 (0.9%) (5 *COL4A1* and 1 *COL4A2*), and lissencephaly in 1/647 (0.2%) (*COL4A1*).

Other brain abnormalities included intracranial calcifications in 36/647 (5.6%) (34 *COL4A1* and 2 *COL4A2*), abnormal cerebral ventricles in 35/647 (5.4%) (35 *COL4A1*), and basal ganglia abnormalities in 20/647 (3.1%) (19 *COL4A1* and 1 *COL4A2*). *COL4A1* variants can be associated with a pattern of intracranial calcifications in the subependymal region and around the porencephalic areas (Livingston et al., 2011; Tonduti et al., 2012). Central nervous system abnormalities also included intracranial aneurysms involving the internal carotid artery in 11/647 (1.7%) (10 *COL4A1* and 1 *COL4A2*), cerebellar involvement in 11/647 (1.7%) (10 *COL4A1* and 1 *COL4A2*), hydrocephalus in 9/647 (1.4%) (8 *COL4A1* and 1 *COL4A2*), hydranencephaly in 3/647 (0,5%) (all *COL4A1*), Dandy–Walker malformation in 1/647 (0.2%) individuals (*COL4A1*), and cerebral atrophy in 1/647 (0.2%) (*COL4A1*).

Stroke occurred in 195/647 (30.1%) individuals and was reported in 170/546 (31.1%) individuals with *COL4A1* variants, 18/93 (19.4%) of those with *COL4A2* variants, and 7/8 (87.5%) with a duplication involving both genes. In 89/195 (45.6%) individuals (82 *COL4A1* and 7 *COL4A2*), the stroke occurred prenatally and involved mostly the frontal lobes and basal ganglia (George et al., 2023; Itai et al., 2021). Of the 89 subjects with prenatal strokes, 83/89 (93.2%) had a solely hemorrhagic event (76 *COL4A1* and 7 *COL4A2*) and 2/85 (2.4%) had both hemorrhagic and ischemic damage (both *COL4A1*); in the remaining 4 patients, the type was unknown. In 8/195 (4.1%) individuals (6 *COL4A1* and 2 *COL4A2*), the stroke occurred at pediatric age and was hemorrhagic in 6/8 (75.0%) (4 *COL4A1* and 2 *COL4A2*) and ischemic in 2/8 (25.0%) (both *COL4A1*). One individual with a *COL4A1* missense variant experienced five intracerebral hemorrhages between the ages of 17 and 22, all of which occurred during physical activity (Vahedi et al., 2007). In 66/195 (33.8%) individuals (54 *COL4A1*, 6 *COL4A2*, and 6 with a duplication involving both genes), the stroke occurred in adult age and was ischemic in 31/66 (47%) individuals (25 *COL4A1* and 6 with a duplication involving both genes) and hemorrhagic in 28/66 (42.4%) individuals (26 *COL4A1* and 2 *COL4A2*); in seven individuals, the type of stroke was not specified. Recurrent strokes in adulthood were reported in 29/66 individuals (43.9%) (27 *COL4A1* and 2 *COL4A2*), most of them (25/29) occurring in nine PADMAL families (Li et al., 2022; Siitonen et al., 2017; Verdura et al., 2016; Zhao et al., 2019). Other 4/29 individuals (2 *COL4A1* and 2 *COL4A2*) from four different families had recurrent hemorrhagic strokes in adulthood (Campo-Caballero et al., 2020; Gunda et al., 2014; John et al., 2015; McHugh & Esenwa, 2020). In 32/195 (16.4%) individuals (28 *COL4A1*, 3 *COL4A2*, and one with a duplication involving both genes), the age at first stroke was unknown. The consequences of stroke varied from mild to severe, including minor strokes that caused temporary or minimal impairment as well as more severely debilitating events leading to long-term disability (Campo-Caballero et al., 2020; Gould et al., 2006; Meuwissen et al., 2015; Verdura et al., 2016).

TABLE 1 Spectrum of the main clinical manifestations in *COL4A1/COL4A2* carriers.

	Individuals from systematic review + study cohort *COL4A1*, *n* (%)	Individuals from systematic review *COL4A1*, *n* (%)	Individuals from study cohort *COL4A1*, *n* (%)	Individuals from systematic review + study cohort *COL4A2*, *n* (%)	Individuals from systematic review *COL4A2*, *n* (%)	Individuals from study cohort *COL4A2*, *n* (%)	Individuals from systematic review *COL4A1* & *COL4A2*, *n* (%)
Disease	575 (546 + 29 new)	546	34	102 (93 + 9 new)	93	9	8
Neurological manifestations	449 (78.1)	430 (78.8)	21 (61.8)	76 (74.5)	70 (75.3)	6 (66.7)	7 (87.5)
Brain structural abnormalities	306 (53.2)	285 (52.2)	21 (61.8)	51 (50.0)	46 (49.5)	5 (55.6)	7 (87.5)
Abnormal white matter signal	161 (28.0)	150 (27.5)	11 (32.4)	12 (11.8)	10 (10.8)	2 (22.2)	7 (87.5)
Non-specific abnormalities	95 (16.5)	84 (15.4)	11 (32.4)	7 (6.9)	5 (5.4)	2 (22.2)	7 (87.5)
Leukoencephalopathy	66 (11.5)	66 (12.1)	0 (0.0)	5 (4.9)	5 (5.4)	0 (0.0)	0 (0.0)
Porencephaly	103 (17.9)	98 (17.9)	5 (14.7)	27 (26.5)	26 (28.0)	1 (11.1)	0 (0.0)
Cerebral ventricles abnormalities	38 (6.6)	35 (6.4)	3 (8.8)	2 (2.0)	0 (0.0)	2 (22.2)	0 (0.0)
Schizencephaly	38 (6.6)	35 (6.4)	3 (8.8)	3 (2.9)	3 (3.2)	0 (0.0)	0 (0.0)
Intracranial calcifications	36 (6.3)	34 (6.2)	3 (8.8)	2 (2.0)	2 (2.2)	0 (0.0)	0 (0.0)
Basal ganglia abnormalities	24 (4.2)	19 (3.5)	5 (14.7)	1 (1.0)	1 (1.1)	0 (0.0)	0 (0.0)
Polymicrogyria	12 (2.1)	9 (1.6)	3 (8.8)	2 (2.0)	2 (2.2)	0 (0.0)	0 (0.0)
Cerebellar abnormalities	11 (1.9)	10 (1.8)	1 (2.9)	3 (2.9)	1 (1.1)	2 (22.2)	0 (0.0)
Hydrocephalus	10 (1.7)	8 (1.5)	2 (5.9)	2 (2.0)	1 (1.1)	1 (11.1)	0 (0.0)
Intracranial aneurysms	10 (1.7)	10 (1.8)	0 (0.0)	1 (1.0)	1 (1.1)	0 (0.0)	0 (0.0)
FCD	6 (1.0)	5 (0.9)	1 (2.9)	1 (1.0)	1 (1.1)	0 (0.0)	0 (0.0)
Hydranencephaly	3 (0.5)	3 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nodular heterotopia	1 (0.2)	1 (0.2)	0 (0.0)	4 (3.9)	3 (3.2)	1 (11.1)	0 (0.0)
Lissencephaly	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Dandy–Walker malformation	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Stroke	177 (30.8)	170 (31.1)	8 (23.5)	19 (18.6)	18 (19.4)	1 (11.1)	7 (87.5)
Hemorrhagic stroke	127 (22.1)	121 (22.1)	6 (17.6)	12 (11.8)	11 (11.8)	1 (11.1)	1 (12.5)
ICH	113 (20.0)	108 (19.8)	5 (14.7)	8 (7.8)	8 (8.6)	0 (0.0)	0 (0.0)
IVH	12 (2.1)	11 (2.0)	1 (2.9)	4 (3.9)	3 (3.2)	1 (11.1)	0 (0.0)
Ischemic stroke	35 (6.1)	35 (6.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (75.0)
Hemorrhagic and ischemic stroke	3 (0.5)	2 (0.4)	1 (2.9)	1 (1.0)	1 (1.1)	0 (0.0)	0 (0.0)
Seizures or epilepsy	176 (30.6)	164 (30.0)	14 (41.2)	23 (22.5)	19 (20.4)	4 (44.4)	0 (0.0)
Intellectual impairment	149 (25.9)	137 (25.1)	14 (41.2)	19 (18.6)	17 (18.3)	2 (22.2)	0 (0.0)
Intellectual disability	108 (18.8)	100 (18.3)	9 (26.5)	10 (9.8)	9 (9.7)	1 (11.1)	0 (0.0)
Developmental delay	45 (7.8)	41 (7.5)	5 (14.7)	4 (3.9)	3 (3.2)	1 (11.1)	0 (0.0)
Motor deficits	141 (24.5)	131 (24.0)	11 (32.4)	21 (20.6)	21 (22.6)	0 (0.0)	0 (0.0)
Quadriplegia	51 (8.9)	46 (8.4)	5 (14.7)	3 (2.9)	3 (3.2)	0 (0.0)	0 (0.0)
Hemiparesis	41 (7.1)	38 (7.0)	4 (11.8)	7 (6.9)	7 (7.5)	0 (0.0)	0 (0.0)
Hemiplegia	19 (3.3)	19 (3.5)	0 (0.0)	5 (4.9)	5 (5.4)	0 (0.0)	0 (0.0)

TABLE 1 (Continued)

	Individuals from systematic review + study cohort *COL4A1*, *n* (%)	Individuals from systematic review *COL4A1*, *n* (%)	Individuals from study cohort *COL4A1*, *n* (%)	Individuals from systematic review + study cohort *COL4A2*, *n* (%)	Individuals from systematic review *COL4A2*, *n* (%)	Individuals from study cohort *COL4A2*, *n* (%)	Individuals from systematic review *COL4A1* & *COL4A2*, *n* (%)
Migraine	18 (3.1)	16 (2.9)	2 (5.9)	1 (1.0)	0 (0.0)	1 (11.1)	0 (0.0)
Ophthalmological manifestations	215 (37.4)	189 (34.6)	26 (76.5)	14 (13.7)	10 (10.8)	4 (44.4)	1 (12.5)
Cataract	120 (20.9)	111 (20.3)	9 (26.5)	3 (2.9)	3 (3.2)	0 (0.0)	1 (12.5)
Retinal artery tortuosity	56 (9.7)	51 (9.3)	5 (14.7)	2 (2.0)	2 (2.2)	0 (0.0)	0 (0.0)
Hemorrhagic retinal lesions	17 (3.0)	16 (2.9)	1 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Corneal abnormalities	26 (4.5)	26 (4.8)	0 (0.0)	1 (1.0)	0 (0.0)	1 (11.1)	0 (0.0)
Microcornea	15 (2.6)	15 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Megalocornea	1 (0.2)	1 (0.2)	0 (0.0)	1 (1.0)	0 (0.0)	1 (11.1)	0 (0.0)
Sclerocornea	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Anterior segment abnormalities	21 (3.7)	18 (3.3)	3 (8.8)	1 (1.0)	0 (0.0)	1 (11.1)	0 (0.0)
Axenfeld Rieger anomaly	10 (1.7)	9 (1.6)	1 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Embryotoxon	8 (1.4)	7 (1.3)	1 (2.9)	1 (1.0)	0 (0.0)	1 (11.1)	0 (0.0)
Synechiae	5 (0.9)	5 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Iris abnormalities	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Optic disc abnormalities	19 (3.3)	12 (2.2)	7 (20.6)	3 (2.9)	2 (2.2)	1 (11.1)	0 (0.0)
Microphthalmia	18 (3.1)	18 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Glaucoma	5 (0.9)	5 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Retinal detachment	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Microspherophakia	1 (0.2)	1 (0,2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Renal manifestations	76 (13.2)	71 (13.0)	5 (14.7)	5 (4.9)	5 (5.4)	0 (0.0)	0 (0.0)
Hematuria	59 (10.3)	56 (10.3)	3 (8.8)	4 (3.9)	4 (4.3)	0 (0.0)	0 (0.0)
Microhematuria	31 (5.4)	30 (5.5)	1 (2.9)	1 (1.0)	1 (1.1)	0 (0.0)	0 (0.0)
Renal cyst	29 (5.0)	29 (5.3)	0 (0.0)	2 (2.0)	1 (1.1)	1 (11.1)	0 (0.0)
eGFR < 90 mL/min/1.73 m^2	21 (3.7)	21 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Proteinuria	17 (3.0)	17 (3.1)	0 (0.0)	1 (1.0)	1 (1.1)	0 (0.0)	0 (0.0)
Congenital abnormality of the urinary tract	14 (2.4)	14 (2.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Kidney atrophy or hypoplasia	6 (1.0)	6 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hydronephrosis	5 (0.9)	5 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Renal failure	2 (0.3)	2 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Chronic glomerulonephritis	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cardiovascular manifestations	36 (6.3)	31 (5.7)	5 (14.7)	9 (8.8)	8 (8.6)	1 (11.1)	0 (0.0)
CHD	12 (2.1)	11 (2.0)	1 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cardiac arrhythmias	12 (2.1)	8 (1.5)	4 (11.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Arterial hypertension	9 (1.6)	8 (1.5)	1 (2.9)	1 (1.0)	0 (0.0)	1 (11.1)	0 (0.0)

(Continues)

TABLE 1 (Continued)

	Individuals from systematic review + study cohort *COL4A1*, *n* (%)	Individuals from systematic review *COL4A1*, *n* (%)	Individuals from study cohort *COL4A1*, *n* (%)	Individuals from systematic review + study cohort *COL4A2*, *n* (%)	Individuals from systematic review *COL4A2*, *n* (%)	Individuals from study cohort *COL4A2*, *n* (%)	Individuals from systematic review *COL4A1* & *COL4A2*, *n* (%)
Thoracic or visceral aneurysms	4 (0.7)	4 (0.7)	0 (0.0)	8 (7.8)	7 (7.5)	1 (11.1)	0 (0.0)
Acute coronary syndrome	2 (0.3)	0 (0.0)	2 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
SCAD	3 (0.5)	3 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Aortic sclerosis	2 (0.3)	0 (0.0)	2 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mitral valve prolapse	2 (0.3)	2 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Aortic dissection	1 (0.2)	1 (0.2)	0 (0.0)	1 (1.0)	1 (1.1)	0 (0.0)	0 (0.0)
Muscular manifestations	85 (14.8)	80 (14.7)	5 (14.7)	1 (1.0)	1 (1.1)	0 (0.0)	0 (0.0)
Elevated CK	54 (9.4)	50 (9.2)	4 (11.8)	1 (1.0)	1 (1.1)	0 (0.0)	0 (0.0)
Muscular cramps	26 (4.5)	21 (3.8)	5 (14.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Muscular atrophy	6 (1.0)	6 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Muscular dystrophy	3 (0.5)	2 (0.4)	1 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Others							
Hemolytic anemia	25 (4.3)	25 (4.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Varus or valgus foot	6 (1.0)	2 (0.4)	4 (11.8)	2 (2.0)	0 (0.0)	2 (22.2)	0 (0.0)
Raynaud phenomenon	6 (1.0)	6 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)
Scoliosis	1 (0.2)	1 (0.2)	0 (0.0)	2 (2.0)	0 (0.0)	2 (22.2)	0 (0.0)
Osteoporosis	2 (0.3)	0 (0.0)	2 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Glioblastoma	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Digestive bleeding	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pseudointestinal obstruction	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pulmonary hemorrhage	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Vertebral artery dolichoectasia	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Note: Five individuals from two families had already been previously reported (25) and were therefore not included in the general group count. However, their ophthalmological and cardiological data from the analyses are still documented as they were not reported in the publication.

Abbreviations: ASD, anterior segment dysgenesis; CHD, congenital heart disease; CK, creatine kinase; eGFR, estimation of the glomerular filtration rate; FCD, focal cortical dysplasia; ICH, intracranial hemorrhage; IVH, intraventricular hemorrhage; RATOR, retinal artery tortuosity; SCAD, spontaneous coronary artery dissection.

Seizure or epilepsy occurred in 183/647 (28.3%) individuals (164 *COL4A1* and 19 *COL4A2*). Among them, 53/183 (29%) had focal onset seizures, 30/183 (16.4%) epileptic spasms, and 10/183 (5.5%) generalized onset seizures. In the remaining 90 patients, seizure or epilepsy type were not specified. In 114/183 (62.3%) patients, epilepsy onset was in the pediatric age (102 *COL4A1* and 12 *COL4A2*), in 14/183 (7.7%) in adulthood (13 *COL4A1* and 1 *COL4A2*), while in the remaining 53/183 (29.0%) age of onset was unknown. Despite the high prevalence of epilepsy in COL4A1/2-related conditions, a comprehensive characterization was only available in two studies (Wang et al., 2020; Zagaglia et al., 2018). Zagaglia et al. (2018) reported 38 new patients with epilepsy. The most frequent epilepsy phenotype was childhood-onset focal seizures in 28/38 (73.7%) patients, often resistant to antiseizure medications and associated with episodes of status epilepticus. It was not specified whether status episodes were related to acute cerebrovascular events or part of the uncontrolled epilepsy. In 13/28 patients with focal seizures (46.4%), the presumed seizure onset zone was located near a porencephalic cyst or a malformation of cortical development; while in the remaining 15/28 (53.6%) patients with focal seizures without porencephaly, the authors found diffuse abnormalities on brain magnetic resonance imaging (MRI), including ventricular enlargement and

TABLE 2 Spectrum of clinical presentations of *COL4A1*/*COL4A2*-associated disease.

	Isolated presentation (COL4A1)	Syndromic presentation (COL4A1)	Isolated presentation (COL4A2)	Syndromic presentation (COL4A2)	Isolated presentation (COL4A1 & COL4A2)	Syndromic presentation (COL4A1 & COL4A2)
Neurological manifestations						
Brain structural abnormalities	✓	✓	✓	✓	✓	✓
Abnormal white matter signal	✓	✓	✓	✓	✓	✓
Aspecific abnormalities	✓	✓	✓	X	/	/
Leukoencephalopathy	✓	✓	✓	✓	/	/
Porencephaly	✓	✓	✓	✓	/	/
Cerebral ventricles abnormalities	✓	✓	/	/	/	/
Schizencephaly	✓	✓	✓	✓	/	/
Intracranial calcifications	✓	✓	✓	✓	/	/
Basal ganglia abnormalities	✓	✓	✓	X	/	/
Polymicrogyria	✓	✓	X	✓	/	/
Cerebellar abnormalities	X	✓	X	✓	/	/
Hydrocephalus	✓	✓	✓	✓	/	/
Intracranial aneurysms	X	✓	✓	X	/	/
FCD	✓	✓	✓	X	/	/
Hydranencephaly	✓	✓	/	/	/	/
Nodular heterotopia	X	✓	✓	✓	/	/
Lissencephaly	X	✓	/	/	/	/
Dandy–Walker malformation	X	✓	/	/	/	/
Stroke	✓	✓	✓	✓	✓	✓
Hemorrhagic stroke	✓	✓	✓	✓	✓	X
ICH	✓	✓	✓	✓	/	/
IVH	✓	✓	✓	X	/	/
Ischemic stroke	✓	✓	X	X	✓	✓
Hemorrhagic and ischemic stroke	X	✓	✓	X	/	/
Seizures or epilepsy	✓	✓	✓	✓	/	/
Intellectual impairment	✓	✓	✓	✓	/	/
Intellectual disability	X	✓	X	✓	/	/
Developmental delay	✓	✓	✓	✓	/	/
Motor function deficit	X	✓	X	✓	/	/
Quadriplegia	X	✓	X	✓	/	/
Hemiparesis	X	✓	X	✓	/	/
Hemiplegia	X	✓	X	✓	/	/
Migraine	X	✓	X	✓	/	/
Ophthalmological manifestations						
Cataract	✓	✓	X	✓	X	✓
	X	✓	X	✓	/	/

(Continues)

TABLE 2 (Continued)

	Isolated presentation (COL4A1)	Syndromic presentation (COL4A1)	Isolated presentation (COL4A2)	Syndromic presentation (COL4A2)	Isolated presentation (COL4A1 & COL4A2)	Syndromic presentation (COL4A1 & COL4A2)
Retinal artery tortuosity						
Hemorrhagic retinal lesions	X	✓	/	/	/	/
Corneal abnormalities	X	✓	X	✓	/	/
Microcornea	X	✓	/	/	/	/
Megalocornea	X	✓	X	✓	/	/
Sclerocornea	X	✓	/	/	/	/
Anterior segment defect	✓	✓	X	✓	/	/
Embryotoxon	X	✓	X	✓	/	/
Axenfeld Rieger anomaly	✓	✓	/	/	/	/
Synechiae	X	✓	/	/	/	/
Iris abnormalities	X	✓	/	/	/	/
Optic disc abnormalities	X	✓	X	✓	/	/
Microphthalmia	✓	✓	/	/	/	/
Glaucoma	✓	✓	/	/	/	/
Retinal detachment	X	✓	/	/	/	/
Microspherophakia	X	✓	/	/	/	/
Renal manifestations						
Hematuria	✓	✓	X	✓	/	/
Microhematuria	✓	✓	X	✓	/	/
Renal cyst	✓	✓	X	✓	/	/
eGFR < 90 mL/min/1.73 m^2	✓	✓	X	X	/	/
Proteinuria	✓	✓	X	✓	/	/
Congenital abnormality of urinary tract	✓	✓	/	/	/	/
Kidney atrophy or hypoplasia	✓	✓	/	/	/	/
Hydronephrosis	✓	X	/	/	/	/
Renal failure	✓	X	/	/	/	/
Chronic glomerulonephritis	X	✓	/	/	/	/
Cardiovascular manifestations						
CHD	X	✓	/	/	/	/
Cardiac arrhythmias	X	✓	/	/	/	/
Arterial hypertension	X	✓	X	✓	/	/
Thoracic or visceral aneurysms	✓	X	/	/	/	/
Acute coronary syndrome	✓*	✓	/	/	/	/
SCAD	✓	✓	/	/	/	/
Aortic sclerosis	X	✓	/	/	/	/

TABLE 2 (Continued)

	Isolated presentation (COL4A1)	Syndromic presentation (COL4A1)	Isolated presentation (COL4A2)	Syndromic presentation (COL4A2)	Isolated presentation (COL4A1 & COL4A2)	Syndromic presentation (COL4A1 & COL4A2)
Mitral valve prolapse	X	✓	/	/	/	/
Aortic dissection	✓	X	✓	X	/	/
Muscular manifestations						
Elevated CK	✓	✓	X	✓	/	/
Muscular cramps	✓	✓	/	/	/	/
Muscular atrophy	X	✓	/	/	/	/
Muscular dystrophy	X	✓	/	/	/	/
Others						
Hemolytic anemia	X	✓	/	/	/	/
Varus or valgus foot	X	✓	X	✓	/	/
Raynaud phenomenon	X	✓	/	/	X	✓
Scoliosis	X	✓	X	✓	/	/
Osteoporosis	X	✓	X	✓	/	/
Glioblastoma	X	✓	/	/	/	/
Digestive bleeding	X	✓	/	/	/	/
Pseudointestinal obstruction	X	✓	/	/	/	/
Pulmonary hemorrhage	X	✓	/	/	/	/
Vertebral artery dolichoectasia	X	✓	/	/	/	/

Note: Isolated presentation indicates no other organ involvement. Syndromic presentation indicates the presence of multiorgan involvement. COL4A1& COL4A2 indicate the individuals with duplication involving COL4A1 and COL4A2. ✓ indicates that isolated presentation refers to the individual described in our study cohort.

Abbreviations: ASD, anterior segment dysgenesis; CHD, congenital heart disease; CK, creatine kinase; eGFR, estimation of the glomerular filtration rate; FCD, focal cortical dysplasia; ICH, intracranial hemorrhage; IVH, intraventricular hemorrhage; RATOR, retinal artery tortuosity; SCAD, spontaneous coronary artery dissection.

asymmetry or periventricular leukoencephalopathy and extensive white matter loss (Zagaglia et al., 2018).

Motor deficits were reported in 152/647 individuals (23.5%) (131 *COL4A1* and 21 *COL4A2*) and usually followed prenatal stroke co-occurring with anatomic (porencephaly) and clinical (delayed milestones) impairment (Meuwissen et al., 2015; Zagaglia et al., 2018). These motor deficits occurred in 84/152 (55.3%) children (68 *COL4A1* and 16 *COL4A2*), and in 10/152 (6.6%) adults (5 *COL4A1* and 5 *COL4A2*); in the remaining 58 individuals, the age at which they were observed was not specified.

Intellectual disability or developmental delay were reported in 154/647 (23.8%) individuals (137 *COL4A1* and 17 *COL4A2*). This group comprises 70/154 (45.5%) individuals of pediatric age (59 *COL4A1* and 11 *COL4A2*), and 29/154 (18.8%) adults (27 *COL4A1* and 2 *COL4A2*). In the remaining 55 individuals, age at onset was not specified but the nature of these manifestations leads them back to an early age. Some studies identified *COL4A1/2* variants in individuals where developmental delay was the initial clinical concern (Deciphering Developmental Disorders Study, 2017; Turner et al., 2019).

Of the 70 children with intellectual disability and/or developmental delay, 61/70 (87.1%) had porencephaly or other structural abnormalities. All 29 adults with intellectual disability had a history of typical BSVD with hemorrhagic stroke. Additionally, in 19/29 (65.5%) adults, neuroimaging was consistent with PADMAL with white matter hyperintensity and pontine or hemispheric lacunae (Siitonen et al., 2017; Verdura et al., 2016). Among individuals of unspecified age, 7/55 (12.7%) had a diagnosis of developmental delay (all *COL4A1*) and 42/55 (76.4%) had moderate or severe intellectual disability (38 *COL4A1* and 4 *COL4A2*).

Prenatal abnormalities mainly included ventriculomegaly and posterior fossa malformations (George et al., 2023; Maurice et al., 2021) or intrauterine growth restriction.

3.1.2 | Ophthalmological manifestations

Ophthalmological manifestations were reported in 200/647 (30.9%) individuals (189 *COL4A1*, 10 *COL4A2*, and 1 with a duplication involving both *COL4AA1* and *COL4A2*). The most common manifestation was cataract (18%), followed by RATOR (8.2%), corneal abnormalities (4.0%), anterior segment abnormalities (2.8%), microphthalmia (2.8%), and optic disc abnormalities (2.2%).

COL4A1-related cataract included 65/111 (58.6%) children and 39/111 (35.1%) adults; in the remaining 7 individuals, age was not specified. Systemic comorbidities are common in children with cataract and are not necessarily known before cataract is diagnosed (Kessel et al., 2021; Rechsteiner et al., 2021). Cataract was an isolated feature in 20/111 (18.0%) of *COL4A1* variant carriers, 16 of whom were from one large Chinese family (Xia et al., 2014). Syndromic cataract remained asymptomatic in individuals with mild systemic features (Rechsteiner et al., 2021), in which case it likely remains underdiagnosed. One study focusing on the genetic causes of bilateral congenital cataract using whole exome sequencing found a monogenic etiology in 20/27 families, including two novel variants in *COL4A1*, accounting for 10% of the families (2/20) (Rechsteiner et al., 2021). A study investigating the genetic causes of bilateral cataracts in 211 Danish children showed *COL4A1* variants to account for 1% (Kessel et al., 2021).

RATOR was detected in 53/647 individuals (51 *COL4A1*and 2 *COL4A2*; adults: 41 *COL4A1* and 2 *COL4A2*; 4 children, all *COL4A1*; 6 individuals whose age was not specified). Hemorrhagic retinal lesions were reported in 16/647 (2.5%) (16 *COL4A1* variants).

Corneal abnormalities were reported in 26/647 (4.0%) individuals (26 *COL4A1*) and included microcornea, corneal opacity, and megalocornea (Slavotinek et al., 2015).

Other findings, occurring either in isolation or combined with other systemic manifestations (Dahl et al., 2020), were anterior segment abnormalities in 18/647 (2.8%) individuals (18 *COL4A1*), microphthalmia in 18/647 (2.8%) individuals (18 *COL4A1*), and optic disc abnormalities in 14/647 (2.2%) (12 *COL4A1*, 2 *COL4A2*). A *COL4A1* variant was also identified in an individual with unilateral microphthalmia and an anterior segment dysgenesis known as Peter's anomaly (Deml et al., 2014; Kylat, 2022). Posterior embryotoxon, consisting in a thickened and anteriorly displaced Schwalbe's line, is usually seen in Axenfeld–Rieger spectrum and was reported in nine unrelated *COL4A1* individuals.

Glaucoma has been reported in three adults and two children with *COL4A1* variants. A study on 257 glaucoma individuals identified 27 likely pathogenic variants, which make *COL4A1* a potential risk factor for primary open angle glaucoma (Huang et al., 2015).

3.1.3 | Renal manifestations

Renal manifestations were reported in 76/647 (11.7%) individuals (71 *COL4A1* and 5 *COL4A2*). Hematuria was the most common abnormality, occurring in 60/647 (9.3%) individuals (56 *COL4A1* and 4 *COL4A2*), especially as microhematuria (30 *COL4A1* and 1 *COL4A2*). Other renal manifestations associated with *COL4A1* variants were renal cysts (5.3%) and proteinuria (3.1%) occurring in both children and adults, always in association with more severe neurological conditions and ocular abnormalities.

A *COL4A1* variant was detected in 20 individuals from a Cypriot family whose phenotype was primarily renal (with manifestations ranging from microhematuria to end-stage renal disease), although two individuals had additional vascular events (i.e., one stroke and one aortic dissection) (Gale et al., 2016).

Kitzler et al. (2019) described congenital anomalies of the kidney and urinary tract, particularly vescico-ureteral reflux, among 14 individuals with *COL4A1* variants from 12 different families.

Cardiovascular manifestations

Cardiovascular manifestations were reported in 39/647 (6.0%) individuals (31 *COL4A1* and 8 *COL4A2*). Congenital heart defects (CHD) were detected in 11 children with *COL4A1* variants. The most frequent CHD was patent foramen ovale (PFO) (4/11). The spectrum of the other CHDs included cor triatriatum, hypertrophic cardiomyopathy (Petrovski et al., 2019; Slavotinek et al., 2015), severe tricuspid regurgitation, and valve dysplasia (Meuwissen et al., 2015). Two additional individuals were described, one having small to moderate patent ductus arteriosus and the other with atrial and ventricular septal defects with mild to moderate hypoplastic left ventricle. Mitral prolapse was reported in two adults with *COL4A1* variants (Meuwissen et al., 2015). Cardiac arrhythmias were identified in 8/647 (1.2%) individuals (all *COL4A1*), the most frequent being supraventricular arrhythmias (5/8) (Meuwissen et al., 2015; Plaisier et al., 2010) with no further details available for the three remaining individuals (Itai et al., 2021; Zagaglia et al., 2018). A *COL4A1* variant was described in four individuals from the same family with Marfan syndrome and aneurysms of the thoracic aorta (Aubart et al., 2018). A *COL4A2* missense variant was described in seven related individuals with at least one visceral aneurysm (mainly involving carotid, renal, and splenic arteries), but no other clinical manifestations (Donner et al., 2022). Three individuals with spontaneous coronary artery dissection (SCAD) harbored disruptive, causative de novo variants of *COL4A1* (Tarr et al., 2022; Zekavat et al., 2022). Arterial hypertension was reported in 8/647 (1.2%) individuals as a secondary finding.

3.1.4 | Muscular manifestations

Muscular manifestations were described in 81/647 (12.5%) individuals (80 *COL4A1* and 1 *COL4A2*). A usually mild increase in serum creatine kinase (CK) was reported in 51/647 (7.9%) individuals (50 *COL4A1* and 1 *COL4A2*). Elevations in plasma CK levels were associated with muscle cramps in 21/51 (41.2%) individuals or, more rarely, with myopathy and muscle atrophy in 6/647 (0.9%) (six *COL4A1*) or muscular spasms in 2/647 (0.3%) individuals (2 *COL4A1*). This type of manifestation occurred either in isolation or in combination with neurological, renal, and ophthalmological alterations, namely, in the context of the HANAC syndrome. In one family with HANAC syndrome, muscle cramps occurred as abrupt, childhood onset severe pain episodes involving various muscles (rarely in the calf) and were not linked to specific electromyographic abnormalities (Haga et al., 2023). A congenital muscular dystrophy phenotype was observed in 2/647 (0.3%) infants with *COL4A1* variants. One of them was diagnosed with

Walker–Warburg syndrome (WWS) and died at age 6 months due to severe respiratory failure; the other (aged around 1 year at the time of publication) was diagnosed with the WWS and muscle–eye–brain (MEB) disease spectrum and died prematurely. In these patients, no other causative variants in genes currently known to underlie MEB/WWS were identified (Labelle-Dumais et al., 2011).

3.1.5 | Other systemic findings

Hemolytic anemia was reported in 25/647 (3.9%) individuals (25 *COL4A1*), of whom 19/25 (76.0%) during the neonatal period (19/25). Raynaud phenomenon was reported in 6/647 (0.9%) individuals (all *COL4A1*). A boy with schizencephaly, retinal arteriosclerosis, and renovascular hypertension experienced severe and repetitive alveolar hemorrhage at age 9 years (Abe et al., 2017).

3.2 | Questionnaire results

We analyzed the sensitivity of our questionnaire by comparing the inclusion of clinical manifestations for each physiological system (i.e., neurological, cardiovascular, ophthalmological, renal, urogenital, and orthopedic) for oncological, prenatal issues, and other non-specific manifestations, with the data reported in the open section where additional clinical manifestations could be added if not covered by the multiple choice part. We found that some manifestations were not covered in the cardiology section in 2/6 (33.3%) individuals (i.e., coronary heart disease, pulmonary embolism, CHD consisting of single atrium, and double superior vena cava), in the orthopedics section in 2/12 (16.7%) individuals (i.e., fibrous dysplasia of the femur and osteoporosis), in the neurology section in 4/27 (14.8%) individuals (i.e., attention-deficit hyperactivity disorder, pontine Wallerian degeneration, turricephaly, and venous angioma), and in the ophthalmology section in 1/25 (4%) individuals (i.e., hemorrhagic cataract). In all remaining sections, all clinical manifestations were covered by the multiple-choice questionnaire.

A total of 43 individuals from 23 families (18 *COL4A1* and 5 *COL4A2*) completed the questionnaire (23 children—20 *COL4A1* and 3 *COL4A2*; 20 adults—14 *COL4A1* and 6 *COL4A2*).

Neurological manifestations were reported in 27/43 (62.8%) individuals (21 *COL4A1* and 6 *COL4A2*). The most common clinical manifestation was epilepsy, occurring in 18/43 (41.9%) (14 *COL4A1* and 4 *COL4A2*), always with childhood onset (within the first year of life in 7 individuals). At onset, 12/18 (66.7%) had focal onset seizures, 4/18 (22.2%) epileptic spasms, and 2/18 (11.1%) generalized seizures. Among individuals with epilepsy, 15/18 were children (13 *COL4A1* and 2 *COL4A2*) and 3/18 were adults (1 *COL4A1* and 2 *COL4A2*). All these individuals had brain MRI abnormalities ranging from isolated porencephaly or intracranial calcifications to malformations of cortical development and extensive white matter damage. EEGs showed abnormalities ranging from focal paroxysmal activities to hypsarrhythmia. Two unrelated individuals with *COL4A1* variants initially presented with West syndrome which then evolved into Lennox–Gastaut syndrome. Developmental delay/intellectual disability was reported in 16/43 (37.2%) individuals (14 *CO4LA1* and 2 *COL4A2*). A total of 10/23 (43.5%) pediatric individuals (9 *COL4A1* and 1 *COL4A2*) had developmental delay/intellectual disability, while 6/20 (30.0%) of the adults (5 *COL4A1* and 1 *COL4A2*) had a history of language delay and/or intellectual disability. Various motor deficits were reported in 11/43 (25.6%) individuals (all *COL4A1*), all pediatric, with 4 having hemiparesis and 5 quadriparesis, typically co-occurring with a hemorrhagic stroke or epileptic encephalopathy. Stroke was reported in 9/43 (20.9%) individuals (8 *COL4A1* and 1 *COL4A2*). Overall, 7 individuals had hemorrhagic strokes in childhood, 1 had both hemorrhagic and ischemic strokes in adulthood, and 1 had a stroke of unknown type. Among the 7 hemorrhagic strokes, 2 were classified as intraventricular hemorrhage (IVH). Structural brain abnormalities were found in 26/43 (60.5%) individuals (21 *COL4A1* and 5 *COL4A2*), ranging from mild white matter abnormalities and intracranial calcifications to malformations of cortical development and extensive brain damage secondary to hemorrhagic events.

Ophthalmological manifestations were identified in 26/43 (60.5%) individuals (22 *COL4A1* and 4 *COL4A2*). These were either common like myopia in 8/43 (18.6%) and astigmatism in 8/43 (18.6%) or rarer such as cataract in 7/43 (16.3%) and optic disc abnormalities in 4/43 (9.3%). Cataract and optic disc atrophy never co-occurred. Cataract was diagnosed in childhood in 5/7 (75%) individuals and optic disc abnormalities were diagnosed in childhood in all individuals.

Cardiac manifestations were identified in 8/43 (18.6%) individuals (7 *COL4A1* and 1 *COL4A2*), of whom 7 were adults and 1 was a child. Among the adults, 2/43 (4.7%) had acute coronary syndrome (ACS) that occurred at age 31 in one of them. Other findings were arterial hypertension in 2/43 (4.7%) and atrial fibrillation in 2/43 (4.7%) individuals. Two male twins aged 22 years, carrying a *COL4A1* variant, also carried a *SCN5A* pathogenic variant and had Brugada syndrome type II. One child with CHD requiring cardiac surgery was also reported.

Orthopedic issues were reported in 12/43 (27.9%) individuals (8 *COL4A1* and 4 *COL4A2*), with varus or valgus foot deformities (14.0%) and scoliosis (4.7%) being the most common ones. All foot deformities were diagnosed at a young age, while scoliosis was diagnosed in adulthood in one individual.

Renal abnormalities were documented in 5/43 (11.6%) individuals (all *COL4A1*), the most common being hematuria (7%), reported in three children.

Muscular cramps and myalgia with onset at pediatric age were reported in 5/43 (11.6%) individuals (all *COL4A1*) from three different families. In 4/5 of these individuals, increased CK serum levels were also reported since childhood and were accompanied by a mild increase of lactate dehydrogenase (LDH) in two of them. Two individuals with muscular cramps underwent a muscle biopsy, which documented mild myopathy in one and a reduction of muscular dystrophin and alpha-dystroglycan in the other. In this latter patient, the analysis of the respiratory chain assay showed reduced activity of mitochondrial complexes, including succinate dehydrogenase, and a slight

increase in the activity of the enzyme citrate synthase. Additional miscellaneous findings are reported in Supplementary Table 2.

3.3 | Ophthalmological investigations

Deep ophthalmological evaluation revealed only minor abnormalities with minimal to absent clinical impact on visual function among 13 participants. Anterior segment dysgenesis, including Axenfeld–Rieger anomaly, occurred in 4/13 (30.8%) individuals; strabismus and cataract in 2/13 (15.4%). Funduscopic examination revealed RATOR in 5/13 (38.5%) individuals (Supplementary Table 3). The peripapillary retinal nerve fiber layer (pRNFL) was unremarkable in all but five individuals from two different families, where a sectorial reduction of pRNFL thickness was detected (Supplementary Table 4).

3.4 | Cardiological investigations

Among the 10 recruited individuals, 9 agreed to undergo cardiac investigations while 1 was excluded due to a previously established diagnosis of atrial fibrillation (AF). The dynamic Holter ECG was normal for all participants. The echocardiographic examination revealed aortic sclerosis in one individual, associated with mild mitral regurgitation. In a third individual, mild ectasia of the ascending aorta was detected. The remaining echocardiographic assessments yielded normal results (Supplementary Table 5).

3.5 | Genotype–phenotype correlations

Missense variants involving glycine substitutions are among the most frequently reported *COL4A1/2* pathogenic or likely pathogenic variants (Jeanne & Gould, 2017; Zagaglia et al., 2018) (Supplementary Figure 2). The specific amino acid substituting glycine does not seem to affect disease severity in COL4A1/2-related conditions (Jeanne & Gould, 2017). However, variants closer to the amino terminus of *COL4A1* tend to be associated with milder pathology, while those nearer to the carboxyl end of the triple helical domain have been linked with more severe cerebrovascular disease (Jeanne & Gould, 2017). Additionally, variants clustered around integrin-binding domains are more likely to be associated with myopathy and nephropathy (Jeanne & Gould, 2017). Finally, all causative variants of PADMAL syndrome are located at the microRNA-29 binding site in the 3′ untranslated region (UTR) of *COL4A1* and lead to increased *COL4A1* expression. This upregulation is believed to be a key pathogenic mechanism, resulting in fibrosis and a reduction of smooth muscle cells in the small cerebral arteries, potentially leading to cerebrovascular accidents in adulthood (Verdura et al., 2016).

In our cohort, glycine substitutions account for 2/5 of the variants in *COL4A2* and 14/18 of *COL4A1*. Of the total 23 variants (18 in *COL4A1* and 5 in *COL4A2*) we report, 19 have not previously been reported (15 in *COL4A1* and 4 in *COL4A2*), 3 *COL4A1* variants were described in a previous study (Zagaglia et al., 2018), and 1 *COL4A2* variant (homozygous) was reported in an 18-year-old male with autism spectrum disorder, hyposmia, and multiple pituitary hormone deficiency (Dahl et al., 2020). The latter variant was also identified in a 9-year-old boy in our series, where it was heterozygous and maternally inherited. The boy had focal epilepsy and cerebellar abnormalities on MRI (cerebellar tonsillar herniation), while his carrier mother exhibited localized posterior embryotoxon.

An intergenerational phenotype exacerbation occurred in 9/11 families. For instance, in the family carrying the *COL4A1* c.2798G>C/p.(Gly933Ala) variant, the proband had perinatal intraventricular hemorrhage, optic nerve atrophy, focal epilepsy, and developmental delay, whereas his mother was asymptomatic. In another family with the *COL4A2* c.3766C>T/p.(Arg1256Ter) variant, the proband had generalized epilepsy, intellectual disability, turricephaly, and white matter abnormalities on brain MRI, whereas his mother was asymptomatic. Conversely, in a family with the *COL4A1* c.1585G>A/p.(Glu529Lys) variant, all three carriers (two twins and their mother) exhibited muscular cramps and elevated CK with homogeneous inter- and intragenerational phenotypes.

From both HGMD database (professional release 2023.2) and our cohort, most pathogenic missense and in frame duplication/insertion variants of *COL4A1/2* occurred in the central part of the protein, spanning from amino acid 600 to 1050 (Figure 2). Furthermore, most *COL4A2* variants are associated with single organ manifestations, while *COL4A1* variants are associated with either multiorgan or single organ manifestations. From our study, patients harboring VUS variants did not show any main clinical differences compared to patients harboring likely pathogenic or pathogenic variants. This lack of significant clinical differentiation can be attributed to the pleiotropic nature of COL4A1/2-related diseases and their potential to render vulnerable anatomical areas that are also subject to other potentially pathogenic complex genetic influences. The most telling example might be the risk of small-vessel cerebrovascular disease in a hypertensive subject compared to a normotensive one. Finally, we observed no definitive genotype–phenotype correlation except for the variants located in the 3′UTR of *COL4A1* associated with PADMAL syndrome.

4 | DISCUSSION

Integrating a literature review with multidisciplinary investigations enhances the comprehensiveness of cataloging the potential multiorgan consequences associated with *COL4A1/2* variants. However, the mechanisms driving multiorgan involvement remain only partially understood. While some hypotheses have been formulated and experimentally supported (Figure 3), only certain manifestations recur frequently enough to confidently attribute them to COL4A1/2-disease (Kuo et al., 2012). It is important to consider ascertainment bias as well, given that clinical and molecular investigations often focus on more severe phenotypes observed in probands, while milder manifestations and those prevalent in the general population may not prompt extensive scrutiny.

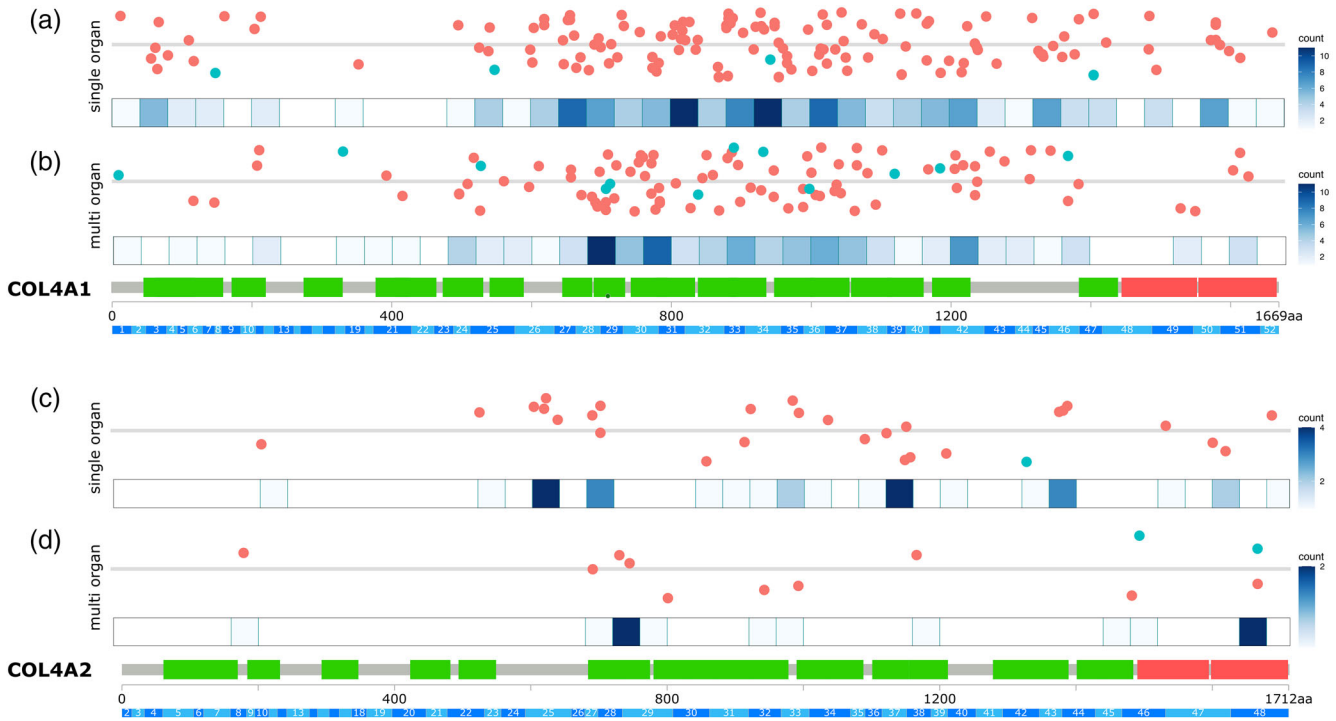

FIGURE 2 Schematic representation of *COL4A1/2* pathogenic or likely pathogenic variants and associated phenotype. The COL4A1/2 proteins are shown, consisting of exon boundaries with their relative protein-coding regions and an amino acid scale. The collagen triple helix domain (light green region) and the non-collagenous domain of type IV collagen (light red region) are represented as rectangle boxes. The squares presented on the *x*-axis represent sliding windows of 40 residues/protein positions. Missense and in-frame duplication/insertion *COL4A1/2* variants with either single-organ or multi-organ manifestations are represented via jitter plots. Genetic variants reported from HGMD (professional release 2023.2) are depicted in red; genetic variants reported from this study are depicted in cyan. The intensity scale of variant distribution is located on the right.

Neurological complications are frequent and severe, as observed both in our cohort and in the literature, particularly stroke and epilepsy (Itai et al., 2021; Maurice et al., 2021; Meuwissen et al., 2015; Zagaglia et al., 2018), and are present with greater severity in children with de novo variants (Itai et al., 2021; Maurice et al., 2021; Zagaglia et al., 2018). However, variants situated within the miR-29 binding site of *COL4A1* are associated with PADMAL syndrome, characterized by recurrent ischemic strokes with onset in adulthood (Verdura et al., 2016).

Our cohort and the literature indicate that most strokes are hemorrhagic and occur mostly prenatally or in adulthood (Gould et al., 2006; Verdura et al., 2016; Weng et al., 2012). An investigation encompassing 450,000 individuals aged 40–69 years revealed that missense variants in the triple helix region of *COL4A1/2* are correlated with a heightened risk of intracerebral hemorrhage but did not carry a significant risk for ischemic stroke (Cho et al., 2022).The severity of stroke can vary but motor impairment and/or intellectual disability are frequent consequences in children.

Our series and literature reports include a wide spectrum of neuroimaging abnormalities, that is, porencephaly, malformations of cortical development, leukoencephalopathy, basal ganglia abnormalities, and intracranial calcifications. Most structural abnormalities are related to ischemic or hemorrhagic insults occurring during embryonic development. In fact, prenatal hemorrhages dating before the 26th week of gestation are associated with schizencephaly or polymicrogyria (Kirkham et al., 2018). Periventricular or intraventricular hemorrhages occurring between the 22nd and 34th week can lead to porencephaly, with or without abnormal neuronal migration (Kirkham et al., 2018). Cerebral hemorrhages or ischemic damage occurring later during pregnancy are more likely associated with periventricular leukoencephalopathy (Kirkham et al., 2018). Conversely, as for PADMAL syndrome, it has been hypothesized that substantial fibrosis and diminution of vascular smooth muscle cells in the small cerebral arteries may over time precipitate cerebrovascular accidents in adulthood (Verdura et al., 2016).

Epilepsy is often secondary to stroke or cortical malformations and is usually focal, as previously reported (Zagaglia et al., 2018) and confirmed by us. Epilepsy can also be an isolated manifestation and its severity varies from isolated focal seizures to severe epileptic encephalopathy (Wang et al., 2020; Zagaglia et al., 2018). We acknowledge that in our case series, epilepsy might have been overrepresented (41.9% vs 28.3%) due to a selection bias as we are a tertiary reference center for epilepsy.

Ophthalmological involvement includes a broad set of manifestations ranging from refraction disorders (e.g., myopia, hyperopia, and astigmatism) to congenital abnormalities (i.e., bilateral congenital

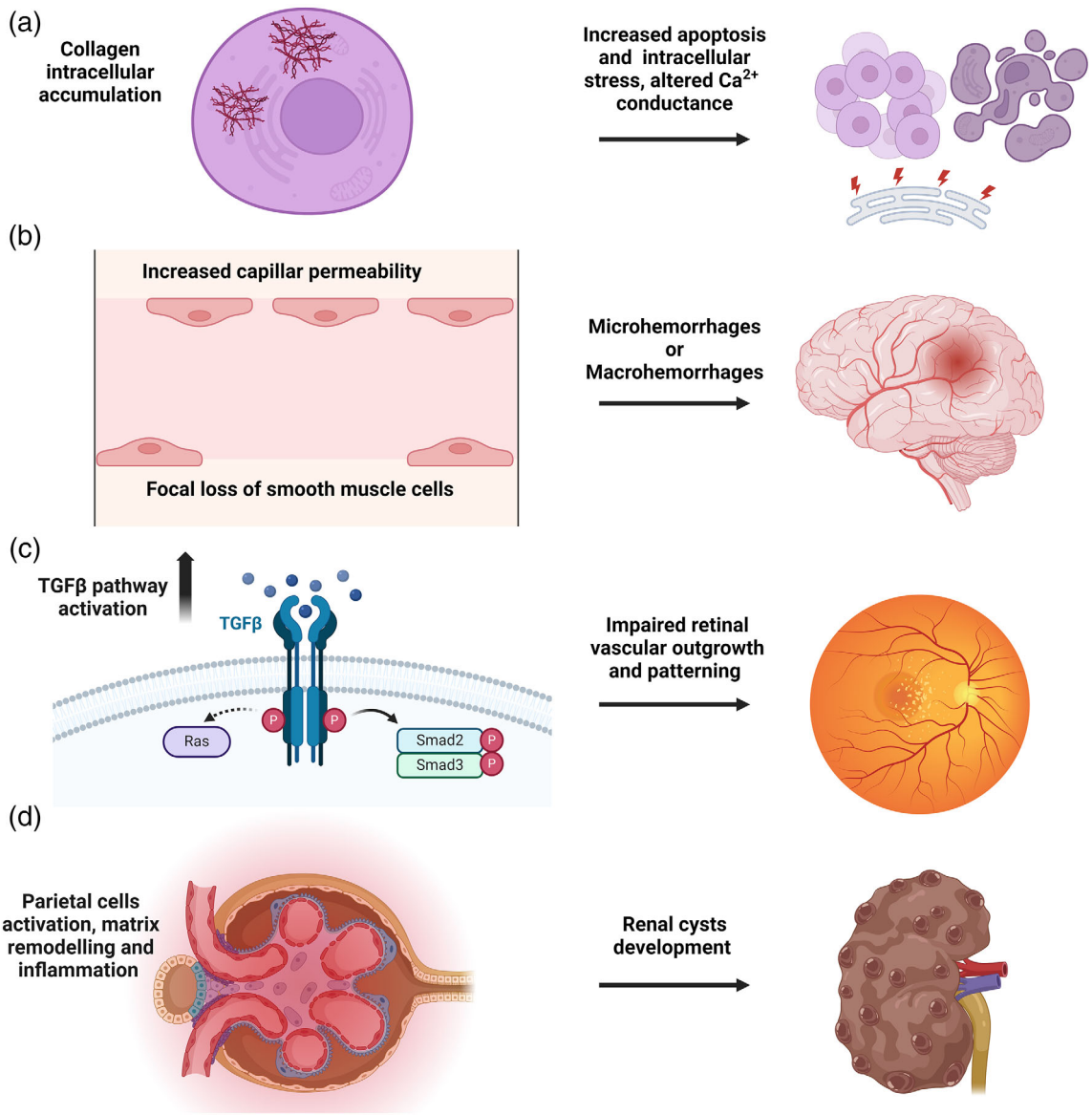

FIGURE 3 Mechanisms proposed for COL4A1/2-related disease manifestations. (a) Intracellular accumulation of COL4A1 or COL4A2 mutant proteins can lead to increased intracellular stress, reduced cellular proliferation, and apoptosis. This alters the arterial wall extracellular matrix membrane and Ca^{2+} conductance (Labelle-Dumais et al., 2019; Lemmens et al., 2013; Murray et al., 2014; Ruigrok et al., 2006; Yamasaki et al., 2023) (b) Transient generalized increase in capillaries permeability of the blood–brain barrier can lead to microhemorrhages, whereas focal loss of smooth muscle cells is associated with macrohemorrhages (Ratelade et al., 2018) (c) Augmented TGFβ signaling due to *COL4A1* variants impairs the retinal vascular outgrowth and patterning (Mao et al., 2022). (d) Renal cyst development, and eventually chronic kidney disease, result from molecular alteration of the collagenous network in Bowman's capsule and activation of parietal epithelial cells, matrix remodeling, and inflammation (Chen et al., 2016).

cataracts, primary congenital glaucoma, microphthalmia, and anophthalmia), and alterations of the retina or optic disc (Rechsteiner et al., 2021; Slavotinek et al., 2015). The most severe ocular alterations include RATOR and anterior segment dysgenesis (ASD), which have the potential to evolve from the asymptomatic or oligosymptomatic stage to severe complications. Alterations in the retinal artery increase the risk of rupture and bleeding with temporary loss of vision or even retinal detachments with permanent vision loss (Zenteno et al., 2014). ASD is frequently associated with posterior embryotoxon and increased level of intraocular pressure (IOP) (Shields et al., 1985; Sibon et al., 2007). Acute or chronic IOP elevation can lead to glaucoma causing progressive optic neuropathy with irreversible loss of vision as a consequence. Labelle-Dumais et al. reported the histological alteration of inner limiting membrane in *COL4A1* knockout mice as a possible explanation for the optic nerve hypoplasia observed in *COL4A1* phenotypes (Labelle-Dumais et al., 2011). In our series, study of inner and outer retinal layers with structural SD-OCT did not reveal significant abnormalities in retinal segmentation or of retinal pigment

epithelium-Bruch membrane (RPE-BM) complex. A family carrying the *COL4A1* c.4105G>C/p.(Gly1369Arg) variant showed diffuse, bilateral pRNFL alteration, a sign of subclinical optic neuropathy. Since optic neuropathy can be an expression of glaucoma, we checked IOP and CCT as main variables for glaucoma diagnosis in all members of this family. IOP resulted within normal values, thus suggesting that optic neuropathy could be a subtle *COL4A1* manifestation. Although we obtained only cross-sectional data, it is still possible that some *COL4A1* variants alter the development of retinal ganglion cell layer, which can only be evaluated with OCT. Our limited sample is insufficient to define a possible ocular biomarker but confirms literature findings on retinal vessel tortuosity and minimal, asymptomatic, changes such as cataract (not clinically relevant), posterior embryotoxon, and Axenfeld–Rieger anomaly. Nevertheless, our findings highlight the diagnostic value of searching for subtle ocular manifestations, especially to validate variants of uncertain significance. Larger studies with modern OCT technology (i.e., swept source OCT, OCT-angiography) will shed light on structural peculiarities of the retina and optic nerve in this population.

The spectrum of cardiovascular manifestations associated with *COL4A1/2* variants includes CHDs, namely, PFO and atrial septal defects, and arterial aneurysms or dissection (Aubart et al., 2018; Meuwissen et al., 2015; Zagaglia et al., 2018). One individual with a de novo *COL4A1* variant in our series had CHD with a single atrium, associated with a double superior vena cava. CHD remains a relatively rare presenting clinical condition of *COL4A1* variants and has not been reported in association with *COL4A2* variants at all. The prevalence of CHDs among individuals with *COL4A1* variants in our study is 11/228 (4.8%), which is higher than its prevalence in the general pediatric population (0.8%–1.2%) (Wu et al., 2020). *COL4A1/2* variants have been reported as causative of visceral artery aneurysm and, in one study, two missense variants of *COL4A1* were associated with spontaneous coronary artery dissection (SCAD) (Zekavat et al., 2022) as either an isolated cardiovascular phenotype or syndromic presentation (Zekavat et al., 2022). In our cohort, a woman experienced ACS at the age of 31, without any other identifiable risk factor. ACSs are frequently caused by the rupture of unstable atherosclerotic plaques (Steffensen & Rasmussen, 2018) that contain extracellular matrix membrane proteins, including collagen (Yang et al., 2016). Single nucleotide polymorphisms in *COL4A1/2* genes have indeed been found to play a role in plaque dysregulation (Nadkarni et al., 2009; Yang et al., 2016), *COL4A1* is also studied as a potential cause of left ventricular noncompaction cardiomyopathy (Hirono et al., 2020). Supraventricular tachyarrhythmia has also been reported in some individuals with COL4A1-related HANAC syndrome (Plaisier et al., 2010). The prevalence of cardiac arrhythmias in individuals with *COL4A1* variants (2.1%) does not differ from that of the general population (1.5%–5%) (Desai & Hajouli, 2023).

In our cohort, only mild renal abnormalities emerged, consisting mainly of hematuria and isolated renal cysts (Meuwissen et al., 2015; Plaisier et al., 2010). These findings are in line with previous observations, although more severe conditions such as chronic kidney disease and urinary tract malformations have also been reported (Gale et al., 2016; Kitzler et al., 2019; Plaisier et al., 2010).

In our cohort, 4/5 individuals with muscle involvement presented a mild phenotype consisting in slight elevation of CK and muscular cramps. However, one child carrying a *COL4A1* variant underwent muscular biopsy that identified dystrophic features. In the literature, increased serum CK and muscular cramps have been reported as either an isolated finding or in association with multiorgan manifestations. In addition, two patients with WWS have been described with a causative *COL4A1* variant (Labelle-Dumais et al., 2011).

Our study confirms the considerable heterogeneity of the manifestations linked to *COL4A1/2* variants, their expression in various organ systems and inter and intrafamilial variability (Giorgio et al., 2015) (Figure 4). Pleiotropy, where a single genetic variant impacts multiple, seemingly unrelated phenotypic traits, appears to be a predominant theme in COL4A1/2-related conditions. The broad range of clinical features observed in patients supports the idea that pleiotropic effects, rather than distinct, separate syndromes, are at play. However, it is noteworthy that certain syndromes such as HANAC and PADMAL exhibit a more distinct clinical profile with less overlap of other phenotypic features, suggesting that these may represent more specific entities within the broader spectrum of COL4A1/2-related disorders. This complexity highlights the need for further research to unravel the interplay between genetic variants, environmental factors, and additional genetic influences to better understand and predict the phenotypic outcomes of *COL4A1/2* variants.

Some *COL4A1/2*-related clinical manifestations and anatomic abnormalities, such as stroke, cerebrovascular or visceral aneurysms, epilepsy, CHDs, ASD, tortuosity of retinal vessels, and renal and urinary tract abnormalities, have the potential to lead to more severe complications if not adequately monitored and treated. Given the lack of a clear genotype–phenotype correlation for most variants, establishing guidelines for follow-up and prevention is challenging. Nonetheless, based on insights gained from this study, there seems to be sufficient information to propose an initial framework for management and preventative strategies. Although *COL4A2* variants seem to imply a lower risk of extra-neurological manifestations compared with *COL4A1*, we devised a shared protocol for individuals with variants in either gene in view of the heightened risk of cerebrovascular events, and the overall increased fragility of mutation carriers.

4.1 | Diagnostic framework

COL4A1/2 variants exhibit a broad spectrum of associated pathologies, which can manifest either in isolation or with multi-organ syndromic manifestations (see Table 1 and Table 2). Since the same manifestations can also be present in individuals who do not carry pathogenic variants in these genes, clinical judgment guiding genetic testing may be challenging and should always include a comprehensive evaluation of the proband and family members.

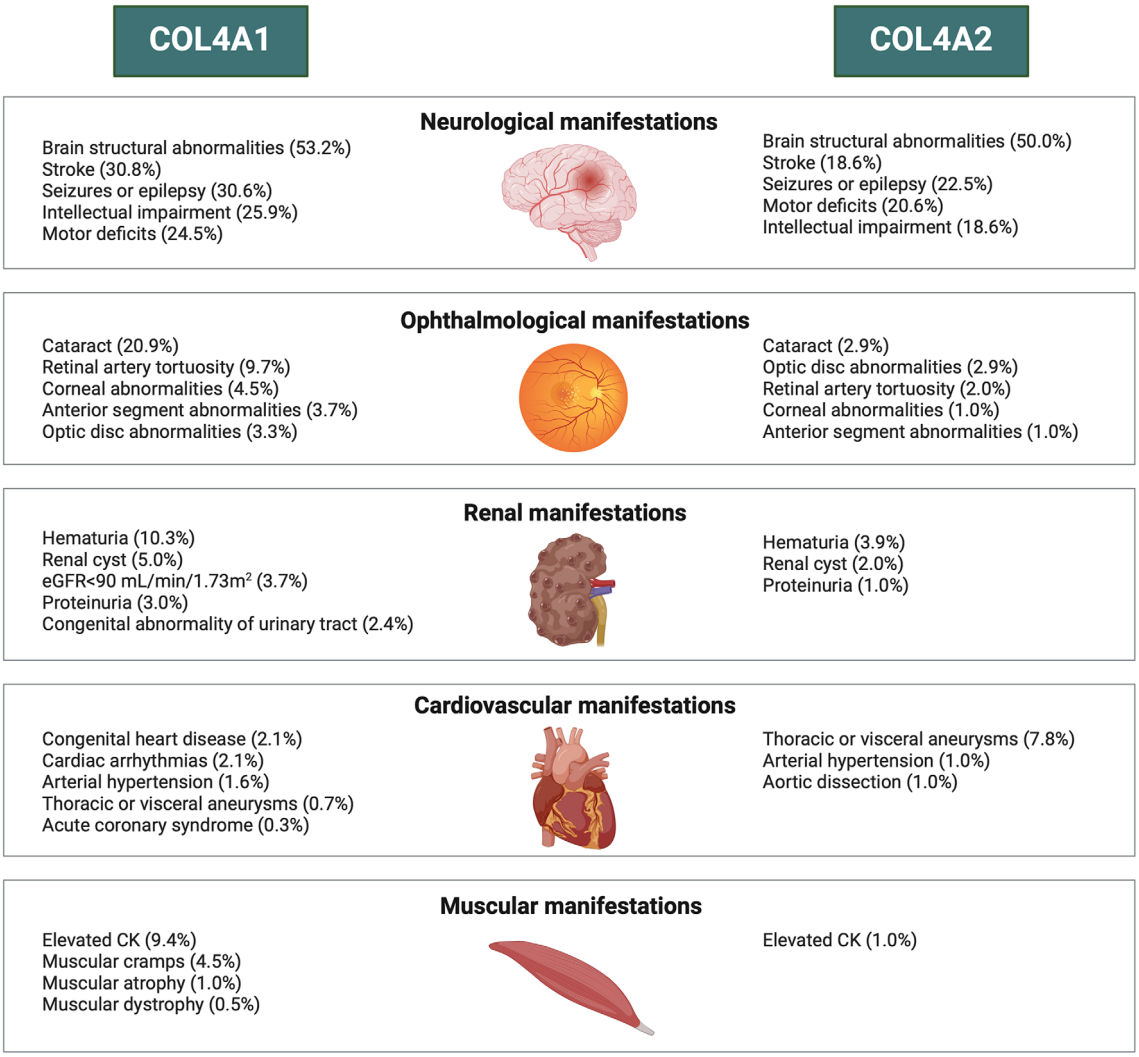

FIGURE 4 Summary of the incidence of the commonest features in COL4A1/2-related disease.

We recommend *COL4A1/2* sequencing analysis in individuals with the following conditions:

1. Identifiable manifestations in the prenatal period

- Ventriculomegaly and fetal growth restriction (Itai et al., 2021)
- Fetal intraparenchymal brain hemorrhage (George et al., 2023; Maurice et al., 2021)
- Schizencephaly (Itai et al., 2021; Zagaglia et al., 2018)

2) Identifiable manifestations in the postnatal period

- Porencephaly (Gould et al., 2006)
- Epilepsy caused by brain structural abnormalities suggestive of COL4A1/2-disorder (Plaisier et al., 2009; Zagaglia et al., 2018)
- Congenital cataract (Coupry et al., 2010; Rechsteiner et al., 2021)
- Clinical picture of cerebral palsy with brain structural abnormalities suggestive of COL4A1/2 disorder (van Eyk et al., 2023)
- Retinal artery tortuosity (Sibon et al., 2007)
- Anterior segment dysgenesis (Deml et al., 2014; Kylat, 2022)
- Elevated CK with recurrent muscular cramps (Plaisier et al., 2010)
- Asymptomatic microhematuria or renal cysts with an apparently genetic cause (Meuwissen et al., 2015; Plaisier et al., 2010)
- Congenital abnormalities of the kidney and/or urinary tract (Gale et al., 2016; Kitzler et al., 2019)

If sequencing analysis is negative, a search for copy number variants (CNV) including *COL4A1/2* should be performed (Saskin et al., 2018).

4.2 | Proposal for a clinical management protocol

Management of individuals with *COL4A1/2* variants should be shared across a multidisciplinary team of specialists who are well-trained to assess the complex phenotypic variability. Clinical management should also be coordinated between specialists and the general practitioner/pediatrician to avoid redundancy of examinations and personalize

management to reach an appropriate balance between the invasiveness of the proposed investigations and their potential benefits. We suggest an initial comprehensive multi-organ assessment after a genetic diagnosis. The timing of follow-up should then be determined by each specialist, considering the potential progression and multi-organ risks inherent in these individuals and their comorbidity profile.

4.2.1 | Neurology

A neurological assessment should be performed in all individuals carrying *COL4A1/2* variants, including an accurate clinical history and physical examination searching for signs of past strokes, seizures, and impairment of motor, speech, and cognitive abilities.

Brain MRI, including angiographic sequences, should be performed in all carriers to search for possible structural and/or vascular brain abnormalities, such as major intracranial aneurysms. Follow-up investigations should be guided by imaging findings or symptoms.

If the individual has epilepsy and is on antiseizure medication, it is important to evaluate the adherence to and efficacy of treatment.

4.2.2 | Ophthalmology

An ophthalmological clinical evaluation of carriers is crucial for monitoring possible ocular manifestations and complications. We recommend at least an initial evaluation with fundoscopic and slit-lamp examinations. Longitudinal evaluation of pRNFL with OCT should be performed based on the severity of the initial clinical presentation. Intraocular pressure measurements should be performed in all individuals with ASD to detect a possible association with glaucoma. The frequency of this examination should be decided based on the clinical severity of ASD.

4.2.3 | Cardiology

We recommend an initial cardiological evaluation, including an ECG and echocardiography. Holter ECG should be considered based on the individual's symptoms, risk factors, and family history. If no alterations are observed, periodic ECG and echocardiography monitoring every 3–4 years is recommended. If morphological or functional alterations are detected, or if any clinical signs or symptoms occur, closer follow-up and evaluation by an expert cardiologist should be arranged.

4.2.4 | Nephrology

We recommend at least an initial evaluation with a blood pressure check, measurement of serum creatinine concentration and estimation of the glomerular filtration rate (eGFR), urine sediment analysis, and abdominal magnetic resonance. If a magnetic resonance is not feasible, an abdominal ultrasound is indicated to detect possible malformation or vascular abnormalities. If no alterations are observed at the first evaluation, we advise monitoring of urine sediment analysis and eGFR every 2 years in both adults and children, together with renal ultrasound.

Detection of isolated microscopic hematuria in individuals without renal or urinary tract abnormalities should prompt yearly monitoring of blood pressure, urine sediment analysis, and eGFR.

If renal/urinary tract abnormalities, decreased eGFR or proteinuria exceeding 0.3 g/day or a urinary protein-to-creatinine ratio >0.3 are detected during the initial diagnostic work-up, an evaluation by an expert nephrologist is advised.

4.2.5 | Blood tests

A baseline blood cell count and measurement of serum CK concentration may be helpful for screening muscle alterations and hemolytic anemia. Given the increased susceptibility to hemorrhagic stroke, assessment of coagulation indices is advised.

4.2.6 | Prenatal monitoring

Prenatal ultrasound should be performed according to an individualized screening protocol during pregnancy in women who are carrier of the variant; fetal MRI after the 20th week of gestation should be performed to exclude brain malformations, vascular events in the fetus, and multiorgan malformations.

4.2.7 | Genetic counseling

Consultation with a clinical geneticist or genetic counselor is advised to explain the risks and implications related to the inheritance pattern and possible complications during pregnancy.

4.2.8 | Treatment

In individuals with *COL4A1/2*-related small vessel disease, anticoagulant or antiplatelet therapy is not recommended (Mancuso et al., 2020). If their use is unavoidable, it should be carefully considered based on the patient's clinical history and bleeding risk, for both children (van Eyk et al., 2023) and adults. Furthermore, in *COL4A1/2*-associated cerebral small vessel disease, intravenous thrombolysis is not recommended in the acute treatment of ischemic stroke (Mancuso et al., 2020). However, decisions on reperfusion or recanalization therapy following a prior intracerebral hemorrhage should be made on a case-by-case basis as stated in the American Heart Association Guidelines (Greenberg et al., 2022). Recurrent lacunar strokes have been reported in PADMAL despite antiplatelet therapy (Li et al., 2022). Antiplatelet therapy is therefore not indicated without a prior ischemic stroke, similar to other inherited microangiopathies such as autosomal

dominant cerebral arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (Mancuso et al., 2020; Meschia et al., 2023). There is no evidence to suggest an elective response to any specific antiseizure medication, the choice of which should be based on clinical and EEG findings. It has been suggested that in individuals with collagen diseases, fluoroquinolones should not be used, unless no other options are available (Yu et al., 2019). No evidence has emerged for an increased risk of surgical bleeding. Therefore, no recommendations are made regarding the avoidance of surgical procedures or the use of therapies aimed at reducing the risk of bleeding.

4.2.9 | Prevention

Due to the increased risk of hemorrhagic stroke, smoking should be avoided and hypertension should be adequately treated. A target blood pressure value ≤130/80 mmHg is recommended for all individuals with *COL4A1/2* variants, especially if there ia a history of hemorrhagic events or renal disease, as recommended by the American Heart Association Guidelines (Whelton et al., 2018). Sustained head pressure during birth or postnatal physical activities that may cause head trauma should be avoided (Gould et al., 2006). Psychological support should be considered for the family and may be useful in improving the quality of life of affected individuals and their family members.

AUTHOR CONTRIBUTIONS

Simone Gasparini: Conceptualization; patient enrollment; data acquisition and analysis; drafting of the manuscript. **Simona Balestrini**: Conceptualization; patient enrollment; data acquisition and analysis; critical review. **Luigi Francesco Saccaro**: Conceptualization; patient enrollment; data acquisition; critical review. **Giacomo Bacci**: Patient enrollment; data acquisition and analysis. **Giorgia Panichella**: Data acquisition; drafting of the manuscript. **Martino Montomoli**: Conceptualization; patient enrollment. **Gaetano Cantalupo**: Patient enrollment; data acquisition. **Stefania Bigoni**: Patient enrollment; data acquisition. **Giorgia Mancano**: Patient enrollment; data acquisition. **Simona Pellacani**: Patient enrollment; data acquisition. **Vincenzo Leuzzi**: Patient enrollment; data acquisition. **Nila Volpi**: Patient enrollment; data acquisition. **Francesco Mari**: Patient enrollment; data acquisition. **Federico Melani**: Patient enrollment; data acquisition. **Mara Cavallin**: Patient enrollment; data acquisition. **Tiziana Pisano**: Patient enrollment; data acquisition. **Giulio Porcedda**: Patient enrollment; data acquisition. **Augusto Vaglio**: Data analysis; critical review. **Davide Mei**: Drafting of the manuscript; data analysis; critical review. **Carmen Barba**: Patient enrollment; data acquisition. **Elena Parrini**: Drafting of the manuscript; data analysis; critical review. **Renzo Guerrini**: Conceptualization; patient enrollment; multiple revisions; critical review.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

PATIENT CONSENT STATEMENT

Written informed consent was obtained from all individuals and/or their parents or legal guardians.

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