CHAPTER 9

THE CLINICOPATHOLOGIC SPECTRUM OF CEREBRAL HEREDITARY ANGIOPATHY WITH VASCULAR RETINOPATHY AND IMPAIRED ORGAN FUNCTION CAUSED BY *TREX1* MUTATIONS (CHARIOT). A REVIEW OF 78 MUTATION CARRIERS FROM 11 UNRELATED FAMILIES

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ABSTRACT

Background: We have shown that Cerebroretinal Vasculopathy (CRV), Hereditary Vascular Retinopathy (HVR), and Hereditary Endotheliopathy, Retinopathy, Nephropathy and Stroke (HERNS) are one disease caused by C-terminal frame-shift mutations in the DNA exonuclease *TREX1*. Here we define the clinicopathologic spectrum of this newly recognized and commonly misdiagnosed syndrome which we have renamed CHARIOT.

Methods: Standardized review of clinical, radiological, pathological and genetic findings in 11 unrelated families with CHARIOT.

Findings: We identified five distinct *TREX1* mutations in 78 subjects. Sixty-five mutation carriers had the characteristic clinical syndrome, similar across all mutations, of a vascular retinopathy followed by progressive focal neurological symptoms in association with white matter hyperintensities and contrast-enhancing frontoparietal mass lesions, often featuring massive edema. Mean (± SD) age at diagnosis was 42·9 ± 8·3 and at death 53·1 ± 9·6 years. Other cerebral manifestations were migraine (59%), cognitive decline (56%), psychiatric disturbances (42%) and seizures (17%). Non-cerebral involvement was typically mild and included anemia (74%), impaired liver (70%) and renal (50%) function, hypertension (60%) and Raynaud's phenomenon (40%). Pathological examination demonstrated a systemic vasculopathy with luminal narrowing and multi-laminated basement membranes. Presymptomatic mutation carriers (N=13; mean age: 35·1 ± 10·6 years) had Raynaud's phenomenon (54%), migraine (42%) and psychiatric symptoms (23%).

Interpretation: CHARIOT is an autosomal dominant, progressive, systemic small-vessel disease mainly characterized by progressive blindness due to vascular retinopathy, relentless neurological decline caused by cerebral mass and white matter lesions, and premature death. We propose diagnostic criteria to aid clinical recognition of this new disease.

INTRODUCTION

Cerebroretinal Vasculopathy (CRV),[1] Hereditary Vascular Retinopathy (HVR)[2,3] and Hereditary Endotheliopathy, Retinopathy, Nephropathy and Stroke (HERNS)[4] are autosomal dominant diseases initially described as independent entities. By concentrating on shared features of vascular retinopathy and brain lesions our international consortium mapped a common locus to chromosome 3p21·1-p21·3[5] and subsequently identified pathogenic heterozygous C-terminal frame-shift mutations in the *TREX1* gene. *TREX1* encodes a 3′-5′ DNA exonuclease involved in clearing cytosolic nucleic acids.[6] Thus, the three diseases were united into a single disorder and termed Retinal Vasculopathy with Cerebral Leukodystrophy.[6] Here we report a retrospective analysis of 78 *TREX1* mutation carriers from 11 unrelated families and provide the first comprehensive characterization of its genetic, clinical, neuro-radiological and pathological spectrum. The disease is commonly misdiagnosed as brain tumor, multiple sclerosis, or a central nervous system vasculitis. Prompted by the emerging clinical picture, pathogenesis, and absence of "leukodystrophy", we renamed the disease CHARIOT: Cerebral Hereditary Angiopathy with vascular Retinopathy and Impaired Organ function caused by *TREX1* mutations. To facilitate clinical recognition of CHARIOT, we formulated diagnostic criteria.

METHODS

We retrospectively evaluated medical records of 78 *TREX1* mutation carriers (35 females; 43 males) from 11 unrelated families from The Netherlands (3 families; n=37), USA (5 families; n=32), Australia (2 families; n=5), and Germany (1 family; n=4). Some genetic and clinical data from families 1-5 and 7-11 have been previously published in brief;[1-9] family 6 was recently identified and not reported before (Supplementary Table S1 and S2).

The institutional ethics committee at each participating institution approved the study and all living subjects provided written informed consent. Demographic and relevant clinical information was obtained from the medical records prior to September 1, 2009. All patients were personally interviewed and examined by one or more of the authors.

Vascular retinopathy was diagnosed from ophthalmologic reports of fundoscopic examination and fluorescein angiography. Cerebral lesions were identified by review of images on brain computed tomography (CT) or magnetic resonance imaging (MRI). Migraine,[10] liver and renal dysfunction, anemia, Raynaud's phenomenon[11] and hypertension were diagnosed according to established international criteria. Pathological findings were obtained from biopsy and autopsy reports in families 1, 2, 5 and 7-11. Neuropathologic data of families 1 and 7-9 have in part been previously published.[1, 4, 8, 9]

Descriptive statistics are based on the number of subjects for whom relevant data were available.

RESULTS

Study population

Five distinct C-terminal frame-shift *TREX1* mutations were identified (Chapter 8, Figure 1a). A clinical diagnosis of CHARIOT was made in 65/78 mutation carriers based on the presence of vascular retinopathy (n=64) or cerebral mass lesion with unknown retinopathy status (n=1) (Mutation carriers with symptomatic CHARIOT; MC+). Thirteen mutation carriers did not have vascular retinopathy or cerebral mass lesions at the time of inclusion in the study (MC-). Clinical, demographic, and neuro-radiological characteristics of living and deceased mutation carriers are summarized in Table 1. The clinical phenotype was similar across all mutations (Supplementary Table S1).

Mutation carriers with symptomatic CHARIOT (MC+)

Initial presentation and diagnosis (Table 1)

All 65 MC+ had developed a vascular retinopathy and/or cerebral mass lesion by middle age. Mean (± SD) age at clinical diagnosis was 42·9 ± 8·3 years (range 25-61). At the time of diagnosis, visual disturbances were present in 50/65 (77%) MC+; ten of them also had neurological symptoms. Asymptomatic retinopathy was discovered on screening because of neurological symptoms (n=4) or a family history of CHARIOT (n=5). For the remaining six MC+, documentation of initial symptoms (n=3) or visual function and retinal examinations were unavailable at the time of diagnosis (n=3). Thus, retinopathy was present at initial diagnosis in all MC+ with available data. Although the majority sought medical attention because of visual symptoms, a quarter did so because of neurological symptoms. A typical case of CHARIOT is described in the legend of Figure 1.

Vascular Retinopathy

Visual symptoms attributable to retinopathy included decreased acuity and field defects. Retinopathy could be visualized by fundoscopy, but was better appreciated with fluorescein angiography. Early stages were characterized by telangiectasias, micro-aneurysms and cotton wool spots (Fig. 2A, B) and, in the later stages, by perifoveal capillary obliteration and neovascularization (Fig. 2C, D). Histopathologic examination of the retina at autopsy (n=8) was consistent with scattered micro-infarcts. The retinal arteries had thickened hyalinized walls (Fig. 3A) and there were focal areas of disruption to the ganglion cells and inner nuclear layer of the retina, usually accompanied by vascular changes. In some, the pathologic process had progressed to retinal hemorrhage and neovascularization.

Cerebral Manifestations

All MC+ showed progressive focal neurological symptoms and/or cognitive impairment in association with an ever-increasing number and size of brain lesions (Table 1). Of the MC+ who were still alive at the time of the study, 12/30 (40%) had focal neurological symptoms and 11/29 (38%) had cognitive impairment, manifesting as bradyphrenia, apathy, irritability, and impaired memory and judgment. Of the MC+ who had already died, 28/29 (97%) had focal

Table 1. Manifestations of CHARIOT

	MC+		
	Living	**Deceased**	**MC-^**
Demographics			
Number of mutation carriers	30	35	13
Age at last follow-up or death			
Mean ± SD (yr)	47·3± 8·1	53·1± 9·6	35·1± 10·6
Range (yr)	34-62	32-72	18-58
Major signs/symptoms*			
Retinopathy	100 (30/30)	100 (34/34)	0 (0/12)
Age at diagnosis retinopathy			
Mean ± SD (yr)	41.2± 8.4	43.7± 7.7	N/A
Range (yr)	25-56	30-61	N/A
Cerebral			
Focal neurological	40 (12/30)	97 (28/29)	0 (0/13)
Cognitive decline	38 (11/29)	75 (21/28)	8 (1/13)
Migraine	48 (12/25)	75 (12/16)	42 (5/12)
Seizures	7 (2/27)	26 (7/27)	0 (0/12)
Psychiatric	31 (9/29)	52 (17/33)	23 (3/13)
Neuroradiology*			
White matter disease	95 (19/20[#])	100 (28/28)	33 (1/3)
Mass occupying lesions	75 (15/20)	91 (21/23^^)	0 (0/3)
White matter hyperintensities**	95 (18/19)	100 (16/16)	33 (1/3)
Calcifications***	71 (5/7)	45 (9/20)	Not Tested
Other organs involved*			
Liver[v]	65 (11/17)	74 (17/23)	Not Tested
Kidney[v]	50 (9/18)	50 (13/26)	0 (0/1)
Possible associations*			
Anemia	67 (8/12)	77 (17/22)	Not Tested
Hypertension	47 (9/19)	68 (21/31)	0 (0/2)
Raynaud's phenomenon	52 (14/27)	30 (10/33)	54 (7/13)
Gastrointestinal bleeding/telangiectasias	3 (1/30)	24 (8/34)	0 (0/13)

MC+: Mutation carriers with retinopathy or cerebral mass lesions; MC-: Mutation carriers without retinopathy or cerebral mass lesions. * Unless indicated otherwise, the disease manifestations presented in the table are shown as percentage of subjects followed by the number of subjects. The denominator varies according to the number of individuals with available data. [#] One subject with no evidence of white matter hyperintensities had an MRI done within 1 year of diagnosis with retinopathy. ^ Ten mutation carriers are from family 1 (mutation V235fs), 2 mutation carriers from family 11 (mutation L287fs), 1 from family 8 (mutationT249fs, this patient committed suicide at age 30). ^^ Five patients were excluded since the last neuroimaging available was more than 5 years before their death. ** Based on MRI scans only. *** Based on CT scans only. [v] Based on laboratory values. N/A:Not Applicable.

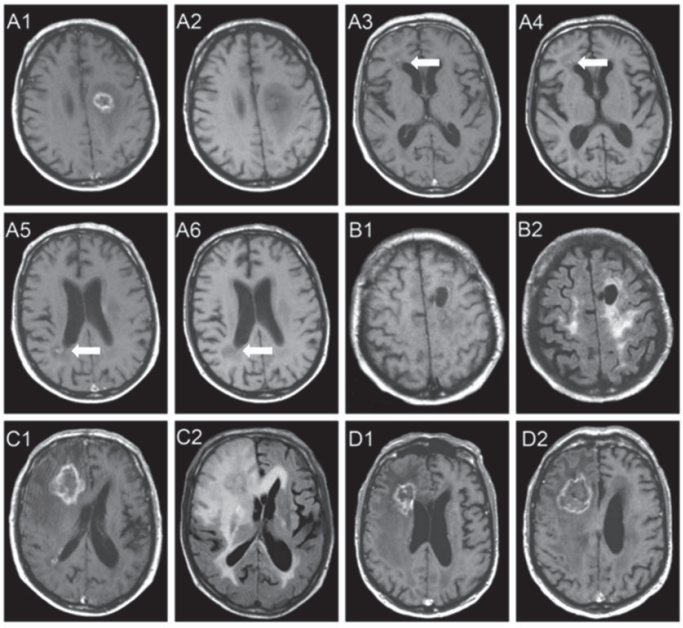

Figure 1. Presentation and progression of a typical case of CHARIOT.
At age 52, this man (family 2, V235fs mutation) reported progressive bilateral loss of vision. Ophthalmologic evaluation revealed a vascular retinopathy. At age 58 he developed a slowly progressive right-sided hemiparesis. He became intermittently irritable and passive and complained of headaches. His medical history revealed Raynaud's phenomenon and paroxysmal atrial fibrillation. In the left frontal white matter, there was a rim-enhancing lesion with mass-effect and surrounding edema on MRI (gadolinium enhanced T1-weighted in A1, non-enhanced T1-weighted in A2). This lesion demonstrated focal calcifications on CT (not shown). Two smaller rim-enhancing lesions were noted periventricularly in the right frontal (T1-weighted in A3, non-enhanced T1-weighted in A4) and parietal lobes (gadolinium enhanced T1-weighted in A5, non-enhanced T1-weighted in A6). A biopsy of the left fronto-parietal lesion revealed tissue necrosis. Dexamethasone (60 mg for 10 days) was started with slight improvement of the hemiparesis. Four months later, his headaches became worse and he developed word-finding difficulties and a wide-based gait in addition to his right-sided hemiparesis. Routine laboratory investigation showed a mild anemia and mildly impaired renal and liver function. There was a mild increase in cerebrospinal fluid protein with normal cell count and no oligoclonal bands. Antinuclear antibodies, extractable nuclear antigens, anticardiolipin IgG and IgM and anti-neutrophilic cytoplasmic antibodies were negative. On MRI, the left frontal lesion had diminished in size, showed now only minimal enhancement, although the surrounding edema and/or gliosis remained as a large zone of confluent T2 hyperintensities, with in this small nodular foci of faint enhancement (gadolinium enhanced T1-weighted in B1, FLAIR in B2).
Half a year later his condition worsened and he became easily agitated with emotional lability, disorientation, apathy and urinary incontinence. Additionally, he developed a left-sided hemiparesis with facial weakness and could walk only with assistance. MRI showed at the location of the pre-existing punctate enhancing white matter lesion now a large irregularly rim-enhancing lesion with central necrosis, and a large zone of surrounding edema extending in the corpus callosum, basal ganglia and parietal and temporal lobe, with some mass-effect of the right lateral ventricle; the pre-existing lesion adjacent to the parietal horn of the right lateral ventricle did not change significantly (gadolinium enhanced T1-weighted in C1, FLAIR in C2). A second biopsy showed mainly necrotic tissue. Corticosteroids provided temporary improvement of his gait. A repeat MRI two months later showed persistence of the right frontal lesion (gadolinium enhanced T1-weighted in D1 and D2). Open biopsy and partial debulking of the right frontal lesion was performed. Pathology showed largely necrotic tissue with scattered inflammatory cells, mainly around the vessel walls, which were thickened with adventitial fibrosis. In the following year, his condition deteriorated and he died of aspiration pneumonia at age 60. An autopsy was performed (data included in Figure 3).

neurological symptoms on examination prior to death and at least 21/28 (75%) had cognitive impairment. The single deceased MC+ without focal neurological symptoms or cognitive impairment died of heart disease two years after diagnosis of retinopathy.

Formal neuropsychological testing was performed in six MC+ with cognitive decline: five had dementia and one had mild cognitive impairment. In the five MC+ tested because of mild subjective complaints or scientific interest, no abnormalities were found.

Other brain symptoms included: migraine (24/41; 59%), seizures (9/54; 17%), and personality changes and psychiatric complaints, in particular depression and anxiety (26/62; 42%) (Table 1). In the 22 migraineurs in whom the subtype could be determined, six (27%) had migraine with aura and 16 (73%) had migraine without aura. In 14/17 (82%) migraineurs with a recorded age of migraine onset, the attacks had begun well before the visual or cerebral symptoms.

Premature death

Thirty-five MC+ were deceased at a mean age of 53·1 $\pm$ 9·6 years (range 32-72), primarily from complications of the neurological decline. One MC+ had died of heart disease. Mean survival time from symptom onset was 9·0 $\pm$ 6·7 years (range <1-26 years).

Neuroimaging

Neuroimaging was available for 48 MC+: both MRI and CT for 14, only MRI for 21, and only CT for 13. All lesions were restricted to the white matter with sparing of gray matter. Two types of lesions were regularly observed, often together: (i) focal, non-enhancing T2-hyperintense lesions scattered throughout the periventricular and deep white matter; and (ii) mass lesions that were T2-hyperintense and T1-hypointense, enhanced with gadolinium contrast, and usually were surrounded by extensive edema displacing adjacent structures leading to sulcal effacement (Fig. 1 and 4).

White matter hyperintensities typically were present early in the clinical course and were detected on MRI in all MC+ except for one who had been diagnosed with retinopathy for less than a year (34/35; 97%). Although non-specific, the lesions were excessive for the relatively young patient age and indicative of CHARIOT when found in combination with retinopathy and a family history of CHARIOT symptoms.

Mass lesions were observed in 36/43 (84%) MC+, sometimes at initial diagnosis but more frequently with advanced disease (Table 1). They were commonly associated with focal neurological symptoms (31/36; 86%). In all individuals with mass lesions, there was a lesion in the fronto-parietal lobe (26/26; 100%); additional lesions were sometimes seen in the cerebellum (1/26) and occipital lobe (1/26). The space occupying lesions usually grew with time, but also could remain stable or diminish in size (Fig. 4a).[12] Often they developed superimposed on pre-existing white matter hyperintensities. In five cases, restricted diffusion was observed, which was most pronounced at the center of the lesions and could persist for months. In 14/27 (52%) cases calcifications were seen on CT. Hemorrhage was not a typical feature (1/36).

Neuropathology

Neuropathologic examination was performed on 13 autopsy and 7 biopsy specimens from 20 MC+. The gross pathology at autopsy demonstrated minimal to marked involvement of the periventricular white matter, particularly the fronto-parietal lobes and occasionally the

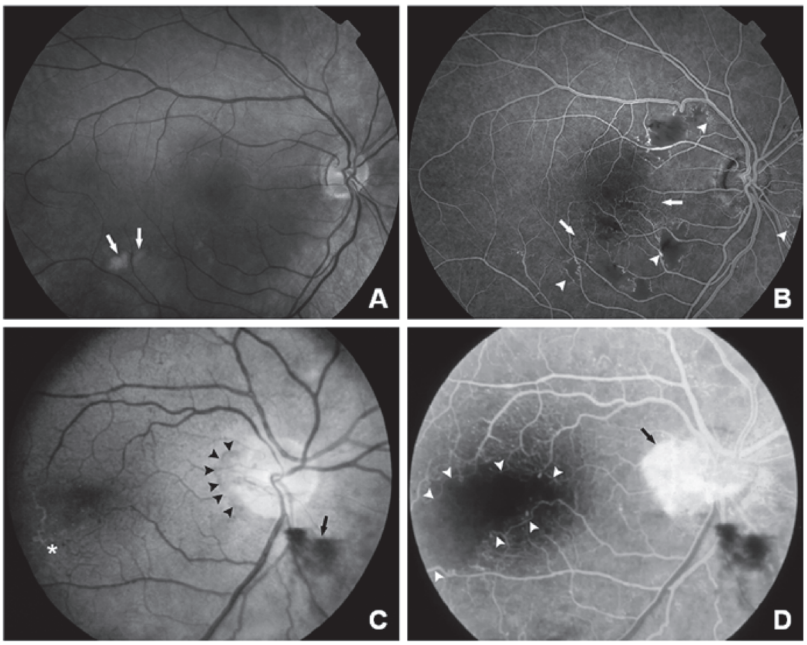

Figure 2. Fundoscopic (A and C) and fluorescein angiogram (B and D) images of the vascular retinopathy. Right eye of a 33-year-old man with cotton-wool spots (arrows, A), extensive areas of capillary obliteration with non-perfusion (arrows, B), and intraretinal microvascular abnormalities (arrowheads, B). Right eye of a 48-year-old woman with a neovascular membrane (arrowheads, C) and preretinal hemorrhage (arrow, C). Temporal to the macula, vascular sheathing and occlusion is present (asterix, C). The same eye shows profuse leakage from the membrane on the disc (arrow, D) and a large avascular region involving the fovea (arrowheads, D).

brainstem and cerebellum. Microscopic examination showed similar findings for both mass lesions and the smaller, scattered lesions seen on neuroimaging. Multiple, often confluent, foci of coagulation necrosis were identified in the white matter with sparing of the grey matter. The larger affected areas had extensive necrosis with focal calcification (Fig. 3B).

On microscopic evaluation a striking vasculopathy affecting the medium and small caliber arteries characterized these necrotic foci and adjacent white matter (Fig. 3B). Fibrinoid necrosis, adventitial fibrosis, luminal narrowing and mural hyalinization with collagenous material were hallmarks of the vasculopathy (Fig. 3B, C, D). Occasionally, vascular telangiectasias were observed. In some cases, a modest chronic inflammatory cell infiltrate, consisting predominantly of perivascular and parenchymal lymphocytes and plasma cells, was found near ischemic lesions. The cellular infiltrate was most consistent with a reaction to ischemic brain tissue without evidence of destruction or invasion of the vascular wall.

Focal calcifications and reactive astrocytosis were frequent findings. Myelin loss was substantial at autopsy. Neurofilament immunolocalization showed concomitant axon loss and frequently large numbers of swollen axonal spheroids, consistent with an ischemic process. Electron microscopy showed irregular thickening and splitting of the basement membranes in vessel walls (Fig. 3E), especially in the media with signs of smooth muscle cell and pericyte degeneration.

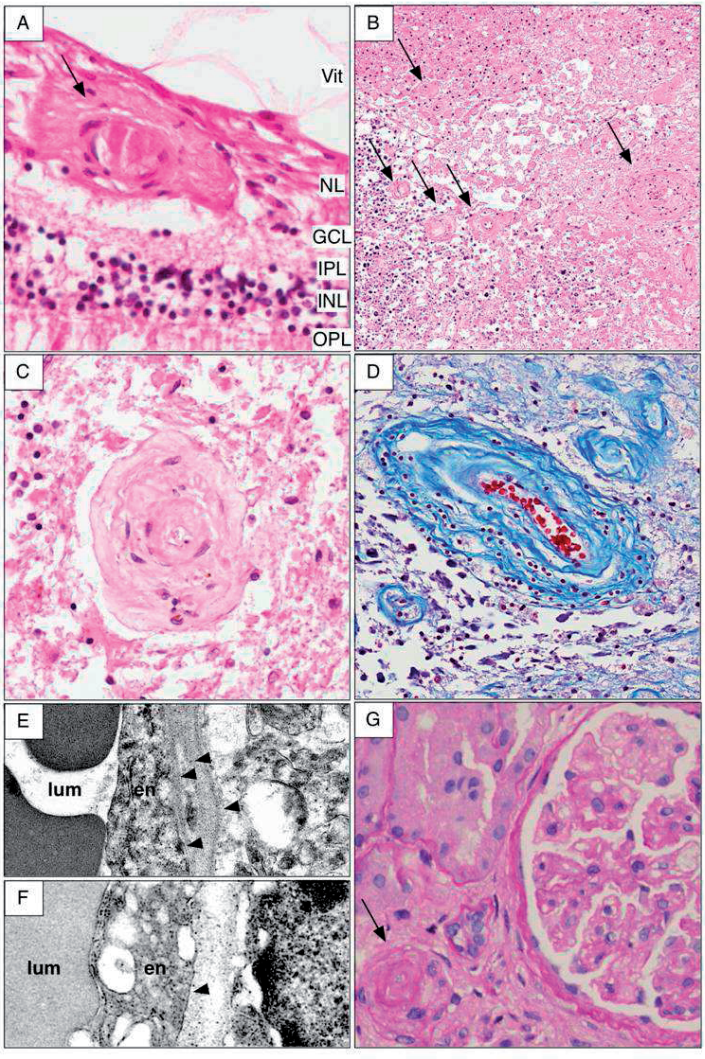

Figure 3. Representative histopathologic findings in the retina, brain and kidney. Microscopic examination of various organs shows a characteristic vasculopathy. The vessels of the inner layers of the retina often demonstrate damage to the walls with occasional deposition of amorphous material [arrow; panel A, hematoxylin and eosin (H&E) stain; vitreous (Vit); nerve fiber layer (NL); ganglion cell layer (GCL); inner plexiform layer (IPL); inner nuclear layer (INL); outer plexiform layer (OPL)] or thickened collagenous walls. The brain also shows a prominent vasculopathy in the white matter, most often adjacent to and in sites of coagulation necrosis. Small to medium sized vessels demonstrate vascular wall thickening with varying degrees of luminal narrowing (arrows; panel B; H&E; brain). In some cases, this progresses to a frank fibrinoid necrosis. In areas with extensive white matter ischemic damage, granular calcifications are particularly evident (dark blue staining in lower left of panel B). The vasculopathy may result in luminal obliteration leading to parenchymal necrosis (panel C; H&E; brain). There is concentric collagenous thickening of the vessel walls, mostly involving the medial layer of the vessels (panel D;Trichrome stain; brain). Ultrastructural examination of affected vessel walls in the brain demonstrates multilaminated basement membranes with duplication of the lamina densa [arrowheads; panel E; electron microscopy; lumen (lum); endothelial cell (en)] in contrast to that found in unaffected regions (arrowhead; panel F; electron microscopy). In the kidney, the vasculopathy is manifested by arteriosclerosis (arrow; panel G; H&E) and glomerulosclerosis.

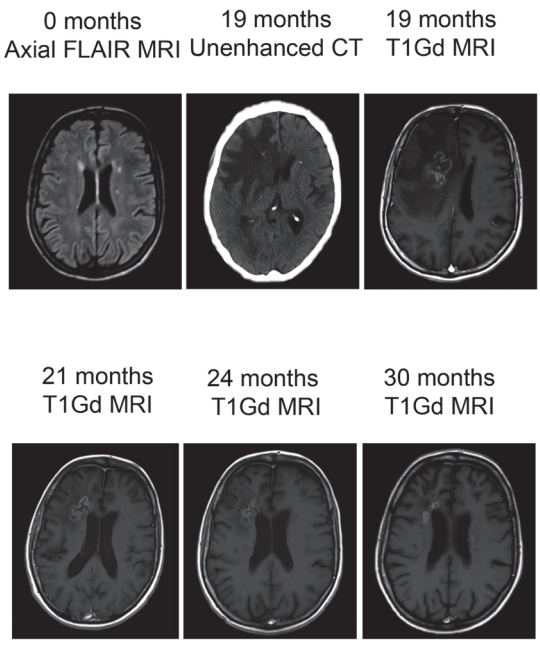

Figure 4a. Characteristic dynamic changes over time of contrast-enhancing cerebral mass lesions and white matter hyperintensities in a patient with CHARIOT. The first MRI shows punctate T2-hyperintense periventricular lesions (0 months, Axial FLAIR). Nineteen months later, a right frontal rim-enhancing lesion with associated calcification (unenhanced CT) and perifocal edema (gadolinium enhanced T1-weighted MRI) has developed on the location of a pre-existing punctate white matter hyperintensity. MRIs at two, five and eleven months after corticosteroid treatment for several weeks with clinical improvement show progressive reduction of the cerebral edema and contrast enhancement (gadolinium enhanced T1-weighted MRIs). Note the slight contrast-enhancement (at 19-30 months) and associated calcification also in the punctate T2 hyperintensities periventricularly on the left site.

Other affected organs

Liver

Liver-chemistry was abnormal in 28/40 (70%) MC+, typically showing modest elevations of alkaline phosphatase and gamma glutamyltransferase. Histologic examination of biopsy and autopsy specimens showed pathologic changes in 13 MC+ of whom three had normal laboratory parameters. The predominant finding was nodular regenerative hyperplasia (Supplementary Fig. S1). Other findings included micro- or macro-vesicular steatosis, periportal inflammation and portal or bridging fibrosis.

Kidney

In 22/44 (50%) MC+ renal disease was manifested clinically by a mild to moderate increase in serum creatinine (< 2·0 mg/dL) and/or proteinuria (< 2 g/24hr). Pathological findings on biopsy or autopsy specimens were noted in 18 MC+ including five who had normal laboratory values. The predominant lesions were arteriolosclerosis or arteriolonephrosclerosis and focal to diffuse global glomerulosclerosis (Fig. 3G). In one patient, renal disease was detected prior to retinopathy.

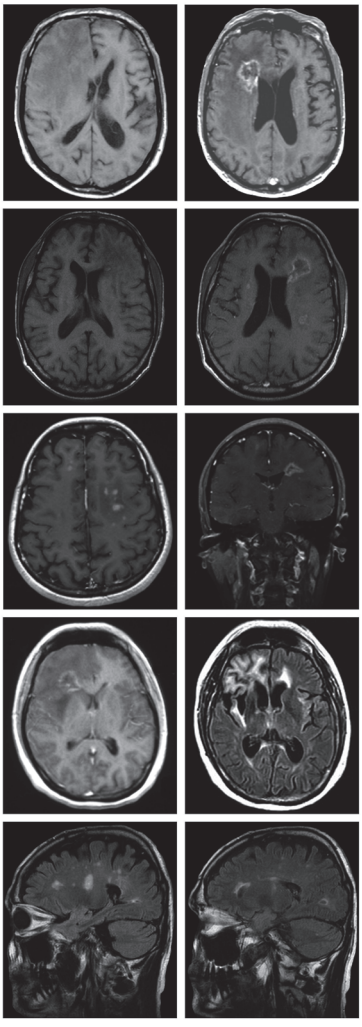

Figure 4b. Cerebral MRI scans of patients with different frame-shift mutations.
V235fs: Axial T1-weighted (left) and axial gadolinium enhanced T1-weighted (right) MRI images of a 59-year-old man (family 2) with a right frontal rim-enhancing lesion with mass-effect and surrounding edema.
T236fs: Axial T1-weighted (left) and axial gadolinium enhanced T1-weighted (right) images of a 40-year-old man (family 7) showing a left frontal rim-enhancing lesion, a smaller rim-enhancing lesion left parietal, and an enhancing punctateright frontal periventricular white matter hyperintensity.
T249fs: Axial (left) and coronal (right) gadolinium enhanced T1-weighted images (family 8) show a left frontal rim-enhancing lesion with mass-effect and some enhancing punctate T2 hyperintensities in the right frontal lobes.
R284fs: Axial T1-weighted (left) and FLAIR (right) images of a 32-year-old woman (family 10) showing a large right frontal rim-enhancing lesion and non-enhancing periventricular white matter hyperintensities frontal left.
L287fs: Saggital T1-weighted images of a 58-year-old man (family 11) reveal small and medium sized non-enhancing periventricular and subcortical white matter hyperintensities.

Other possible clinical associations

Other findings observed in MC+ more frequently than in the general population included anemia, hypertension, and Raynaud's phenomenon (Table 1). Normocytic and normochromic anemia (25/34; 74%) was typically mild to moderate (hematocrit 27-36%). Of those with anemia, six had documented microscopic gastrointestinal bleeding or telangiectasias. Hypertension was present in 30/50 (60%) MC+, often with concomitant renal disease (18/26; 69%). Raynaud's phenomenon found in 24/60 (40%) MC+ was mild, without ischemic injury or pulp infarcts, and did not require treatment. Autoimmune markers were positive in 3/18 (17%) MC+. Anti-nuclear antibodies were detected by immunofluorescence on Hep2 substrate in two subjects (speckled pattern with a titer of 1:80 and 1:640 respectively) and anti-cardiolipin IgG by ELISA in a third.

Mutation carriers without retinopathy or cerebral lesions (MC-)

Thirteen *TREX1* mutation carriers had no evidence of retinopathy or cerebral lesions at the time of their last examination. Their mean age at last follow-up was 35·1 $\pm$ 10·6 years (range 18-58). The most common clinical symptoms noted were Raynaud's phenomenon (7/13; 54%), migraine (5/12; 42%), and psychiatric complaints (3/13; 23%). One MC- committed suicide in his twenties. Three MC- underwent MRI scans, which showed only mild white matter hyperintensities in one at 45 years of age.

DISCUSSION

We present here the genetic and clinicopathologic spectrum of CHARIOT, a newly recognized autosomal dominant, systemic small vessel disease caused by C-terminal frame-shift mutations in *TREX1*. We identified 5 different mutations in 78 subjects from 11 unrelated families and found a strikingly similar disease profile. CHARIOT should be considered in individuals in middle age with a vascular retinopathy and neuropsychiatric symptoms, particularly if similar features are present in family members. Brain imaging will often reveal contrast-enhancing mass lesions, white matter hyperintensities and focal calcifications. Genetic testing of *TREX1* can confirm the diagnosis or identify pre-symptomatic patients. Proposed diagnostic criteria and other supporting features for CHARIOT are summarized in Table 2.

Based on our extensive clinical experience of over 20 years with CHARIOT, once patients develop retinopathy, all will suffer progressive neurological decline and most will die within ten years, usually from pneumonia or sepsis in the setting of severe general debilitation. To date, no mutation carrier has lived a normal lifespan without developing CHARIOT, thus suggesting 100% penetrance and premature mortality. The 13 MC- were on average ten years younger than the MC+ and thus may still develop the full CHARIOT syndrome.

Many patients with CHARIOT also have migraine and Raynaud's phenomenon, typically preceding the onset of retinopathy and cerebral lesions. Compared to the general population, migraine prevalence among CHARIOT patients was five times higher in males and nearly three times greater in females, using the same diagnostic criteria.[13] The prevalence of Raynaud's phenomenon was approximately two times higher in both sexes.[14] These statistics suggest that

Table 2. Proposed diagnostic criteria for CHARIOT

Major Diagnostic Criteria
Vascular retinopathy (with in the early phase hemorrhages, intraretinal microvascular abnormalities and/or cotton wool spots)
Signs and symptoms of progressive focal and/or global brain dysfunction with neuroimaging showing contrast-enhancing cerebral mass lesions and/or cerebral white matter hyperintensities
Family history of autosomal dominant inheritance with middle-age onset of disease manifestations#
C-terminal frame-shift mutation in *TREX1*
Supportive features
Microvascular liver disease (nodular regenerative hyperplasia)
Microvascular kidney disease (arterio- or arteriolonephrosclerosis, glomerulosclerosis)
Possibly associated features
Migraine
Raynaud's phenomenon
Anemia consistent with blood loss and/or chronic disease
Hypertension
Microscopic gastrointestinal bleeding

De novo mutations may be possible although none have been reported to date.

TREX1 mutations have a causal relationship to migraine and Raynaud's phenomenon as has previously been reported in a genetic study of family 2.[15] As both migraine and Raynaud's phenomenon are common in the general population, their presence alone cannot be used for a definitive clinical diagnosis of CHARIOT. The same applies to other CHARIOT-associated symptoms such as nephropathy and hepatic dysfunction. Although typically occurring later, they may represent the initial manifestation of CHARIOT, but are too non-specific for establishing the diagnosis.

Not surprisingly, many patients were initially misdiagnosed. The vascular retinopathy was commonly confused with diabetic retinopathy but the differential diagnosis also includes branch retinal vein occlusion, hypertension, sickle cell disease, collagen vascular disease and radiation, or idiopathic retinopathy.[1,16] The early white matter lesions in CHARIOT were often confused with multiple sclerosis, multi-infarct dementia, vasculitis, or other hereditary white matter diseases.[17,18] Ring-enhancing mass lesions with a necrotic central core and surrounding edema were commonly mistaken for neoplasms or tumefactive multiple sclerosis in several patients leading to multiple brain biopsies and surgical resection. Some patients with rapid progression of neurological symptoms were diagnosed with acute ischemic stroke, but diffusion restriction corresponding to a vascular territory was not seen. Interestingly, in some of our patients, diffusion restriction indicative of cytotoxic edema was observed in the center of a mass lesion.

The clinical and histopathologic findings are consistent with the hypothesis that the systemic small vessel vasculopathy of CHARIOT is caused by an endotheliopathy which

disrupts the vascular basal membrane and leads to progressive loss of microvascular blood flow. The histopathologic findings in the brain are reminiscent of delayed radiation necrosis, a condition which is believed to be secondary to endothelial cell-dysfunction.[19] Electron microscopy showed multi-laminated capillary basement membranes in the brain, kidney, stomach, appendix, omentum, and skin.[4] Capillary occlusion is suggested by retinal and cerebral vessel wall thickening and lumen obliteration, as well as a number of other pathologies. These include nodular regenerative liver hyperplasia, which is likely due to diminished hepatic blood flow,[20] and renal arteriosclerosis, arteriolosclerosis, and glomerulosclerosis, which, like microscopic gastrointestinal bleeding, are probable manifestations of small vessel disease.

Several studies support a role for *TREX1* in immunity. Absence of functional *TREX1* results in accumulation of single-stranded DNA in cells and multi-organ inflammation in knock-out mice.[21-23] In humans, mutations that abolish *TREX1* exonuclease activity are associated with three different autoimmune diseases: autosomal recessive Aicardi-Goutières syndrome (AGS), autosomal dominant familial chilblain lupus (FCL), and systemic lupus erythematosus (SLE). AGS is associated with complete absence of exonuclease activity and mimics an *in utero* viral encephalopathy, possibly secondary to activation of the immune system by host DNA.[24] Heterozygous *TREX1* mutations are found in FCL, an autoimmune disease that primarily affects the skin.[25,26] Rare variants in *TREX1* have also been associated with SLE in some patients.[27] Further support for a role in innate immunity comes from studies demonstrating that *TREX1* degrades HIV-1 DNA generated during infection, thereby preventing an interferon-mediated immune response.[28]

In contrast to the mutations seen in other *TREX1* diseases, CHARIOT *TREX1* frame-shift mutations result in a mislocalized but functional exonuclease and are likely associated with a gain-of-function or toxic effect. Unlike the role for immunity in AGS, FCL and SLE, the cerebral pathology of CHARIOT does not resemble an autoimmune disease as features of vasculitis are missing. However, auto-immunity may play a role, especially in the derailment of mass lesions at a certain age.

Since the initial description of the disease in 1988,[1] we have identified ten additional families, expanded and better delineated the clinical phenotype and discovered the causal gene. However, treatment to prevent, reverse or halt the disease is still lacking. Immunosuppressive agents, including cyclophosphamide, were given to a few individuals without benefit (Atkinson, unpublished data). Intra-vitreal bevacizumab was effective for the proliferative retinopathy in a single patient.[29] Corticosteroids can reduce cerebral vasogenic edema, but do not improve the underlying lesions. Further research is needed to determine the pathogenesis of CHARIOT and develop effective treatments for this devastating disorder.

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AUTHORS' CONTRIBUTIONS

The study was designed and coordinated, the data were analysed, and the first drafts and various revisions of the manuscript were written by AHS, PHK, GMT, JPA and MDF under supervision by JPA, GMT, JH and MDF who also take overall responsibility. Figures were designed by AHS, JPA, PTVMJ, GRK. All authors were involved in the data collection, literature search, data interpretation, vouch for the completeness and accuracy of their data, contributed to drafting and the final report, and participated in the decision to submit the findings for publication.

ROLE OF THE FUNDING SOURCE

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author (GMT), AHS, PHK, JPA and MDF had full access to all the data in the study and had final responsibility for the decision to submit for publication.

CONFLICT OF INTEREST

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SUPPLEMENTARY MATERIAL

Table S1. Manifestation of CHARIOT for clinically affected and unaffected individuals subdivided by family and mutation status

	MC-^	**MC+**		
Mutation	**V235fs; T249fs; L287fs**	**V235fs**		
Origin	**Dutch; US**	**Dutch; US; Australia**		
Original disease name	***HVR; HERNS***		***CRV; US***	***HVR; Dutch***
Family number	**2, 8, 11**	**1, 2, 3, 4, 5, 6**	***1***	***2***
Demographics				
Number of mutation carriers	13	43	18	20
Age at last follow-up or death				
Mean ± SD (yr)	35·1 ± 10·6	52·6 ± 8·3	51·0 ± 5·9	55·4 ± 9·7
Range (yr)	18-58	35-72	41-62	35-72
Major signs/symptoms*				
Retinopathy	0 (0/12)	100 (42/42)	100 (17/17)	100 (20/20)
Age at diagnosis retinopathy				
Mean ± SD (yr)	N/A	45·1 ± 6·9	45·0 ± 4·7	45·5 ± 8·0
Range (yr)	N/A	35-61	40-55	35-61
Cerebral				
Focal neurological	0 (0/13)	66 (25/38)	94 (15/16)	41 (7/17)
Cognitive decline	8 (1/13)	62 (23/37)	88 (15/17)	38 (6/16)
Migraine	42 (5/12)	54 (13/24)	50 (1/2)	60 (12/20)
Psychiatric	23 (3/13)	45 (19/42)	56 (10/18)	40 (8/20)
Seizure	0 (0/12)	14 (6/43)	28 (5/18)	5 (1/20)
Neuroradiology*				
White matter disease	33 (1/3)	97 (30/31[#])	100 (14/14)	92 (11/12[#])
Mass occupying lesions	0 (0/3)	65 (20/31)	79 (11/14)	33 (4/12)
White matter hyperintensities**	33 (1/3)	96 (23/24)	100 (8/8)	91 (10/11)
Calcifications***	Not Tested	56 (9/16)	56 (5/9)	57 (4/7)
Other organs involved*				
Liver[V]	Not Tested	65 (20/31)	56 (9/16)	90 (9/10)
Kidney[V]	0 (0/1)	34 (10/29)	36 (5/14)	27 (3/11)
Possible associations*				
Anemia	Not tested	70 (21/30)	80 (12/15)	50 (6/12)
Hypertension	0 (0/2)	53 (19/36)	56 (10/18)	40 (6/15)
Raynaud's phenomenon	54 (7/13)	49 (20/41)	6 (1/18)	85 (17/20)
Gastrointestinal bleeding/ telangiectasias	0 (0/13)	16 (7/43)	33 (6/18)	5 (1/20)

MC+: Mutation carriers with retinopathy or cerebral mass lesions; MC-: Mutation carriers without retinopathy or cerebral mass lesions. * Unless indicated otherwise, the disease manifestations presented in the table are shown as percentage of subjects followed by the number of subjects. The denominator varies according to the number of individuals with available data. ^ Ten mutation carriers from family 1 (mutation V235fs), 2 mutation carriers from family 11 (mutation L287fs), 1 from family 8 (mutationT249fs, this patient committed suicide at age 30).

T236fs	**T249fs**		**R284fs**	**L287fs**
German	**US**		**Australia**	**Dutch**
		HERNS; US		
7	**8, 9**	**8**	**10**	**11**
4	11	10	3	4
41^^	46·4 ± 9·1	44·4 ± 7·8	40·7 ± 5·0	49·0 ± 17·1
N/A	32-60	32-53	36-46	34-68
100 (4/4)	100 (11/11)	100 (10/10)	100 (3/3)	100 (4/4)
39·8 ± 6·9	33·4 ± 2·6	33·4 ± 2·6	34·0 ± 8·5	44·3 ± 11·9
30-46	30-37	30-37	25-42	33-56
75 (3/4)	82 (9/11)	80 (8/10)	67 (2/3)	33 (1/3)
67 (2/3)	36 (4/11)	30 (3/10)	67 (2/3)	33 (1/3)
0 (0/2)	88 (7/8)	88 (7/8)	100 (3/3)	25 (1/4)
33 (1/3)	27 (3/11)	30 (3/10)	100 (3/3)	0 (0/3)
0 (0/4)	100 (1/1)	Unknown	67 (2/3)	0 (0/3)
100 (2/2)	100 (11/11)	100 (10/10)	100 (3/3)	100 (1/1)
100 (2/2)	100 (11/11)	100 (10/10)	67 (2/3)	100 (1/1)
100 (2/2)	100 (6/6)	100 (5/5)	100 (2/2)	100 (1/1)
Not Tested	38 (3/8)	29 (2/7)	67 (2/3)	Not Tested
100 (4/4)	100 (1/1)	Not Tested	67 (2/3)	100 (1/1)
50 (1/2)	78 (7/9)	75 (6/8)	100 (3/3)	100 (1/1)
Not Tested	100 (1/1)	Not Tested	100 (3/3)	Not Tested
Not Tested	78 (7/9)	75 (6/8)	67 (2/3)	100 (2/2)
0 (0/2)	0 (0/11)	0 (0/10)	0 (0/2)	100 (4/4)
50 (2/4)	0 (0/11)	0 (0/10)	0 (0/3)	0 (0/3)

^^ Data on one patient only. N/A: Not Applicable. [#] One subject with no evidence of white matter hyperintensities had an MRI done within 1 year of diagnosis with retinopathy. ** Based on MRI scans only. *** Based on CT scans. [V] Based on laboratory values. The five patients that were excluded from the neuro-imaging section in the deceased category of Table 1 (because the last scan was made more than 5 years before death) are included in this table.

Table S2. The 11 CHARIOT families with five different mutations in the *TREX1* gene

Family	Origin	Mutation (DNA)	Mutation (protein)	Number of mutation carriers	Reference
1	North America (European)	3688_3689insG	V235fs	18	1
2	Netherlands	3688_3689insG	V235fs	30	2, 3
3	North America (Ashkenazi-Jewish)	3688_3689insG	V235fs	1	1
4	North America	3688_3689insG	V235fs	1	6
5	Australia	3688_3689insG	V235fs	2	6
6	Netherlands	3688_3689insG	V235fs	1	Unpublished
7	Germany	3691_3692insA	T236fs	4	8
8	North America (Chinese)	3727_3730dupGTCA	T249fs	11	4
9	North America	3727_3730dupGTCA	T249fs	1	9
10	Australia	3835_3836insA	R284fs	3	7
11	Netherlands	3843_3844insG	L287fs	6	6

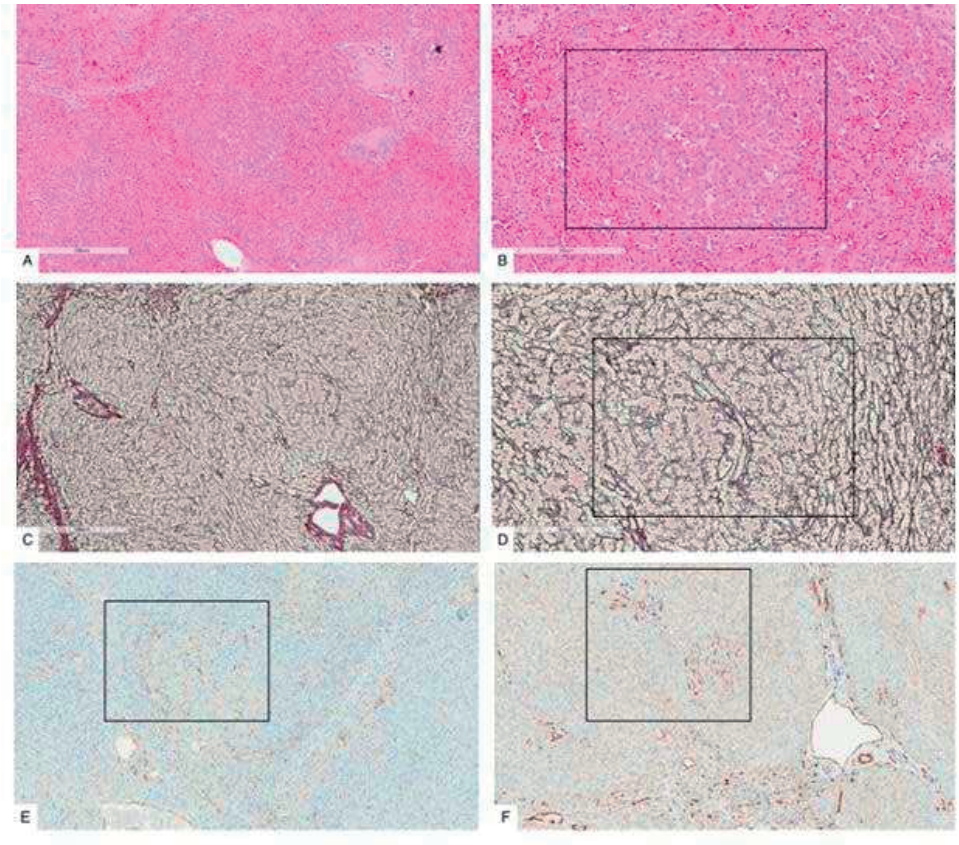

Figure S1. Regenerative hyperplasia and capillarization of the liver. The liver shows an abnormal parenchymal architecture (Panel A and B; hematoxylin and eosin (H&E) stains and panel C and D, reticulin stains) In A, the portal tracts (seen on the right) and terminal hepatic venules are unevenly spaced; in addition, a vague nodule is present centrally. Within the boxed area in B, the nodule is comprised of hepatic cords delineated from surrounding parenchyma by blood-filled sinusoids. The cords in the latter regions are atrophic (see panel C) compared to the more hypertrophic cords in the center (boxed area in panel D). None of the cords, however, are greater than 2 nuclei broad. Panels E and F are immunohistochemical stains highlighting aberrant activation of hepatic stellate cells (boxed area in E, anti-smooth muscle actin) and altered sinusoidal endothelial cells with expression of anti-CD34 (boxed area in F). The latter signifies a process of closure of sinusoidal endothelial fenestrae and presence of basement membrane. These are features of "capillarization" of the sinusoids.