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C-terminal truncations in human 3′-5′ DNA exonuclease TREX1 cause autosomal dominant retinal vasculopathy with cerebral leukodystrophy

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Autosomal dominant retinal vasculopathy with cerebral leukodystrophy is a microvascular endotheliopathy with middle-age onset. In nine families, we identified heterozygous C-terminal frameshift mutations in *TREX1*, which encodes a 3′-5′ exonuclease. These truncated proteins retain exonuclease activity but lose normal perinuclear localization. These data have implications for the maintenance of vascular integrity in the degenerative cerebral microangiopathies leading to stroke and dementias.

We have previously described three families sharing common features of retinal and cerebral dysfunction. Visual loss, stroke and dementia begin in middle age, and death occurs in most families 5 to 10 years later. These diseases map to 3p21.1–p21.3 (ref. 1) and are called cerebroretinal vasculopathy (CRV)[2], hereditary vascular retinopathy (HVR)[3,4] and hereditary endotheliopathy, retinopathy, nephropathy and stroke (HERNS)[5]. We now designate these illnesses as autosomal dominant retinal vasculopathy with cerebral leukodystrophy (RVCL) (OMIM 192315). The neurovascular syndrome features a progressive loss of visual acuity secondary to retinal vasculopathy, in combination with a more variable neurological picture[1–7]. In a subset of affected individuals, systemic vascular involvement is evidenced by Raynaud's phenomenon and mild liver (micronodular cirrhosis)[2,5] and kidney (glomerular) dysfunction[5].

This retinal vasculopathy is characterized by telangiectasias, microaneurysms and retinal capillary obliteration starting in the macula. Diseased cerebral white matter has prominent small infarcts that often coalesce to pseudotumors. Neuroimaging studies demonstrate contrast-enhancing lesions in the white matter of the cerebrum and cerebellum. Histopathology shows ischemic necrosis with minimal inflammation and small blood vessels occluded with fibrin[5]. The white matter lesions resemble post-radiation vascular damage[2]. Ultrastructural studies of capillaries show a distinctive, multilamellar subendothelial basement membrane[5].

By combining haplotypes in the three RVCL families, we narrowed the disease gene to a 3-cM region between markers D3S1578 and D3S3564 that encompassed ~10 Mb, containing over 120 candidate genes[1]. We then sequenced the full coding region and intron-exon boundaries of 33 candidate genes within this region (**Supplementary Table 1** online).

Here we report the identification of mutations in *TREX1* (NM_033627), encoding DNA-specific 3′ to 5′ exonuclease DNase III. In the CRV[2] and HVR[3,4] pedigrees, a heterozygous 1-bp insertion (3688_3689insG) leads to V235fs and a consequent premature stop. In HERNS[5], a heterozygous 4-bp insertion (3727_3730dupGTCA) results in a frameshift at T249 (**Fig. 1a**,**b**).

Next, we examined six families with putative RVCL (**Supplementary Table 2** online)[2,6,7]. In each, we identified frameshift mutations affecting the C terminus of TREX1. In three, the alteration was V235fs, the same as that in the CRV and HVR pedigrees. Haplotype analysis suggests that they are not related (data not shown). We did not detect any of the mutations in panels of chromosomes matched by ancestry or location (**Supplementary Methods** online). In the CRV and

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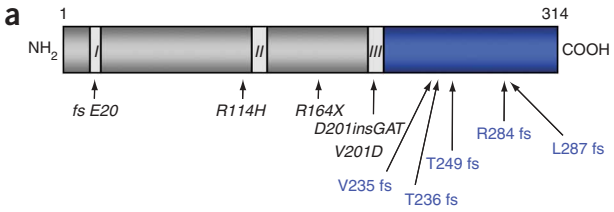

b

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                    240        250        260        270        280
TREX1  WT  PMYGVTASAR TKPRPSAVTT TAHLATTRNT SPSLGESRGT KDLPPVKDPG
V235   fs  PMYGGHSLC* .......... .......... .......... ..........
T236   fs  PMYGVNSLC* .......... .......... .......... ..........
T249   fs  PMYGVTASAR TKPRPSAVSH NHCTPGHNQE H*........ ..........
R284   fs  PMYGVTASAR TKPRPSAVTT TAHLATTRNT SPSLGESRGT KDLPPVKDPG
L287   fs  PMYGVTASAR TKPRPSAVTT TAHLATTRNT SPSLGESRGT KDLPPVKDPG

                    290        300        310        320
TREX1  WT  ALSREGLLAP LGLLAILTLA VATLYGLSLA TPGE*..... ....
V235   fs  .......... .......... .......... .......... ....
T236   fs  .......... .......... .......... .......... ....
T249   fs  .......... .......... .......... .......... ....
R284   fs  ALSKGGAAGP TGSAGHPDLG SSHTVWTIPG HTWGVGQEGK SDE*
L287   fs  ALSREGAAGP TGSAGHPDLG SSHTVWTIPG HTWGVGQEGK SDE*
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Figure 1 Diagram of TREX1 protein. (**a**) TREX1 has three exonuclease domains. Mutations in italics are associated with AGS[13], and those in boldface blue at the C terminus are associated with RVCL. (**b**) Comparison of the amino acid sequence of the C terminus of wild-type (WT) TREX1 with RVCL associated mutations. The abnormal sequence introduced by the frameshifts is depicted in blue.

HERNS families, all affected individuals over the age of 60 (but none of the unaffected individuals over the age of 60) carried a *TREX1* mutation (100% penetrance). In the HVR[3,4] family, 10 of the 11 mutation carriers over 60 years of age have retinopathy.

TREX1 (DNase III) is a DNA-specific 3′ to 5′ exonuclease ubiquitously expressed in mammalian cells[8–10]. It is thought to function as a homodimer, with a preference for single-stranded DNA and mispaired 3′ termini[8]. TREX1 is a part of the SET complex[11] that normally resides in the cytoplasm but translocates to the nucleus in response to oxidative DNA damage[12].

Recently, homozygous mutations in *TREX1* have been reported to cause Aicardi-Goutière syndrome (AGS)[13]. AGS is a rare, familial, early-onset progressive encephalopathy featuring basal ganglia calcifications and cerebrospinal fluid lymphocytosis, mimicking congenital viral encephalitis[14]. Notably, mutations associated with AGS disrupt the enzymatic sites in TREX1. This loss of exonuclease function[13] (**Fig. 1**) is hypothesized to cause the accumulation of altered DNA that triggers a destructive autoimmune response[13]. No phenotype was reported for the heterozygous carriers of these mutations; however, a heterozygous mutation in *TREX1* causing familial chilblain lupus has been reported recently[15].

The distinctive clinical course and pathology of RVCL compared with AGS suggests separate disease mechanisms. The frameshift mutations observed in RVCL are downstream of the regions encoding the catalytic domains, whereas in AGS, homozygous mutations occur that alter exonuclease function. The heterozygous mutations observed in RVCL did not impair the enzymatic activity of TREX1 (**Fig. 2a**), in comparison with the R114H substitution in AGS[13].

To investigate how the RVCL TREX1 proteins differ from the wild type, we performed expression studies using confocal microscopy on cells transfected with TREX1 tagged with a fluorescent protein (**Fig. 2b** and **Supplementary Fig. 1** online). The wild-type TREX1 labeled with fluorescent protein (FP-TREX1) localized to the perinuclear region. In contrast, the TREX1 proteins FP-V235fs and FP-T249fs were diffusely distributed in the cytoplasm and the nucleus, as was the case for the fluorescent protein alone (**Fig. 2b** and **Supplementary Videos 1–4** online). Protein blotting confirmed that the expressed proteins were of the correct size (**Fig. 2c**). These results suggest a perinuclear targeting signal within the C terminus of TREX1. Consequently, we generated a construct containing the C-terminal 106 amino acid residues of TREX1 (FP-C-106). This protein showed a perinuclear localization pattern identical to that of the wild-type TREX1 protein (**Fig. 2b**). The TREX1 protein containing amino acid change R114H, found in AGS, also had the same pattern as the wild-type protein. In contrast, the protein with the alteration closest to the C terminus of TREX1, FP-287fs, was diffusely distributed, like the other two truncated proteins (data not shown).

The TREX1 proteins found in individuals with RVCL lack part of the C terminus. In haploinsufficient individuals, this may prevent an interaction with the SET proteins and therefore may prevent formation of the SET complex. The SET complex is hypothesized to target DNA repair factors, including TREX1, to damaged DNA under conditions of oxidative stress[11,12]. Lack of sufficient TREX1 associated with the SET complex may result in failure of granzyme A–mediated cell death[12]. Alternatively, the dissemination of untethered TREX1 in the nucleus and cytoplasm may have detrimental effects, especially on endothelial cells.

The clinical syndromes in these families and the study of their mutations should deepen our understanding of exonuclease function, homeostasis of the endothelium and events leading to premature vascular aging. RVCL and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) represent two examples of monogenic disease featuring a cerebral microangiopathy for which the genetic defects are now

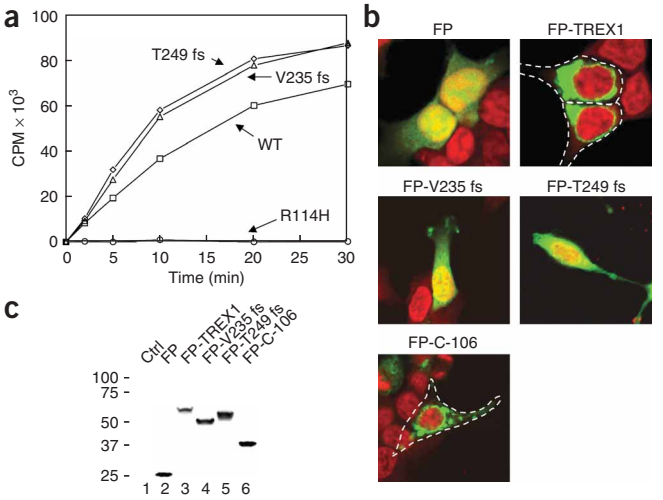

Figure 2 Functional consequences of RVCL associated *TREX1* mutations. (**a**) Assessment of 3′-5′ exonuclease activity using equivalent amounts of purified recombinant proteins expressed in *E. coli*. (**b**) Confocal microscopy of HEK293T cells showing transiently expressed fluorescent protein (FP)-tagged TREX1 proteins (green), TOPRO3 staining of nuclei (red) and overlay (yellow). Similar expression patterns were obtained for wild-type protein and for proteins derived from constructs containing mutations associated with AGS and RVCL in CHO, HL-60 and HeLa cells (data not shown). (**c**) Protein blot analysis of untransfected cells (1) and cells transfected with enhanced yellow fluorescent protein (eYFP) (2), wild-type TREX1 (3), TREX1 mutants (4,5) and the C-terminal 106 amino acids (6), all linked to eYFP.

known and from which we can gain new insights into the origin of strokes and dementia.

We obtained consent from all participants in this study, and the study was approved by the Office for Protection of Research Subjects at UCLA and the Human Research Protection Office at Washington University School of Medicine.

Note: Supplementary information is available on the Nature Genetics website.

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COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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