nature genetics

Mutations in the gene encoding the 3′-5′ DNA exonuclease TREX1 are associated with systemic lupus erythematosus

Min Ae Lee-Kirsch[1], Maolian Gong[2,14], Dipanjan Chowdhury[3,14], Lydia Senenko[1,14], Kerstin Engel[1,14], Young-Ae Lee[2,4,14], Udesh de Silva[5], Suzanna L Bailey[5], Torsten Witte[6], Timothy J Vyse[7], Juha Kere[8], Christiane Pfeiffer[9], Scott Harvey[10], Andrew Wong[7], Sari Koskenmies[11,12], Oliver Hummel[2], Klaus Rohde[2], Reinhold E Schmidt[6], Anna F Dominiczak[13], Manfred Gahr[1], Thomas Hollis[5], Fred W Perrino[10], Judy Lieberman[3] & Norbert Hübner[2]

TREX1 acts in concert with the SET complex in granzyme A–mediated apoptosis, and mutations in *TREX1* cause Aicardi-Goutières syndrome and familial chilblain lupus. Here, we report monoallelic frameshift or missense mutations and one 3′ UTR variant of *TREX1* present in 9/417 individuals with systemic lupus erythematosus but absent in 1,712 controls ($P = 4.1 \times 10^{-7}$). We demonstrate that two mutant *TREX1* alleles alter subcellular targeting. Our findings implicate TREX1 in the pathogenesis of SLE.

Homodimeric TREX1, the major mammalian intracellular DNase[1] and a component of the SET complex, associates with the endoplasmic reticulum and translocates to the nucleus in response to oxidative stress[2]. TREX1 causes single-stranded DNA damage during caspase-independent apoptosis activated by granzyme A[2]. Mutations in *TREX1* have recently been shown to cause Aicardi-Goutières syndrome (AGS1) (ref. 3), an autosomal recessive encephalopathy characterized by elevated interferon-α levels in cerebrospinal fluid, mimicking congenital viral infection, and autosomal dominant familial chilblain lupus (CHBL)[4], manifesting in early childhood with ulcerating acral skin lesions. Some individuals with AGS1 and CHBL develop antinuclear antibodies, suggesting that impaired DNA processing and caspase-independent apoptosis could trigger an autoimmune response. Moreover, *Trex1*$^{-/-}$ mice develop an autoimmune noninfectious inflammatory myocarditis[5].

Prompted by these observations, we investigated whether mutations in *TREX1* may have a role in common forms of SLE and Sjögren's syndrome. The hallmark of both disorders is the generation of antinuclear antibodies and the activation of interferon-α, which contributes to the autoimmune mediated inflammatory process. Symptoms in SLE may involve virtually any organ, whereas Sjögren's syndrome primarily affects tear and salivary glands and may present in connection with SLE (secondary Sjögren's syndrome).

We sequenced the coding region of *TREX1* in four cohorts from the UK, Germany and Finland (**Supplementary Methods** online). All participants gave informed consent, and the study was approved by ethics committees at each institution. We identified five heterozygous missense changes and one frameshift change in 6/218 individuals with SLE from the UK compared with 0/200 nonsynonymous changes in controls ($P = 3.1 \times 10^{-2}$) (**Table 1**). All missense changes affect highly conserved residues except one (E266G) (**Supplementary Fig. 1** online). In the German SLE cohort, we found four heterozygous missense changes, one frameshift and a single 3′ UTR variant in 6/199 affected individuals but only 2/1,512 controls ($P = 5.3 \times 10^{-5}$) (**Table 1**). Among these four nonsynonymous changes, two alter highly conserved residues, and two affected individuals carried the nonconserved variant E266G. Similarly, the only nonsynonymous change identified in two German controls was E266G. Moreover, we screened 169 German subjects with primary Sjögren's syndrome and identified one additional missense change in a completely conserved residue that was not observed in any of the control populations studied (**Supplementary Fig. 1**). Furthermore, we sequenced *TREX1* in 188 Finish nuclear family trios and did not detect any nonsynonymous change in these 564 individuals. The absence of nonsynonymous changes in this

Table 1 Frequency of *TREX1* mutations

Cohort	Number of mutations in affected individuals	Number of mutations in controls	*P* value
SLE combined	12/417	2/1,712	1.7×10^{-7}
UK	6/218	0/200	3.1×10^{-2}
Germany	6/199	2/1,512	5.3×10^{-5}

If we were to group only sequence variants not present in controls, the significance level in the combined data set would change to $P = 4.1 \times 10^{-7}$ (9/417 versus 0/1,712).

[1]Klinik für Kinder- und Jugendmedizin, Technische Universität Dresden, 01307 Dresden, Germany. [2]Max Delbrück Center for Molecular Medicine (MDC), 13125 Berlin-Buch, Germany. [3]CBR Institute for Biomedical Research, Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02125, USA. [4]Charité - Universitätsmedizin Berlin, Pediatric Pneumology and Immunology, Campus Virchow-Klinikum, 13353 Berlin, Germany. [5]Center for Structural Biology, Department of Biochemistry, Wake Forest University Health Sciences, Winston-Salem, North Carolina 27157, USA. [6]Medizinische Hochschule Hannover, Klinische Immunologie, 30625 Hannover, Germany. [7]Imperial College, Faculty of Medicine, Section of Rheumatology and Molecular Genetics, Hammersmith Hospital, London W12 0NN, UK. [8]Karolinska Institute, Department of Biosciences and Nutrition, and Clinical Research Centre, 14157 Huddinge, Sweden. [9]Klinik für Dermatologie, Technische Universität Dresden, 01307 Dresden, Germany. [10]Department of Biochemistry, Wake Forest University Health Sciences, Winston-Salem, North Carolina 27157, USA. [11]University of Helsinki, Department of Medical Genetics and [12]Department of Dermatology, 00014 Helsinki, Finland. [13]BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow G12 8TA, UK. [14]These authors contributed equally to this work. Correspondence should be addressed to M.A.L.-K. (minae.lee-kirsch@uniklinikum-dresden.de) or N.H. (nhuebner@mdc-berlin.de).

Received 20 February; accepted 29 May; published online 29 July 2007; doi:10.1038/ng2091

cohort may be explained by the different demographic history of the Finnish population[6].

In addition, we identified 13 synonymous variants in *TREX1* in the entire data set (**Supplementary Table 1** online). All variants, except one, occurred at a very low allele frequency (between 0.01% and 1.9%) and were found at similar frequencies among affected individuals and controls across the different populations tested.

Altogether, we observed one 3′ UTR variant and 11 nonsynonymous changes in 417 individuals with SLE (2.64%) but only two nonsynonymous changes in 1,712 controls ($P = 1.7 \times 10^{-7}$). Our combined data identify a strongly increased relative risk (RR) for development of SLE in individuals that carry *TREX1* variants (RR = 25.3; 95% confidence interval = 5.6–232.4). The phenotypic features of individuals with SLE carrying *TREX1* mutations encompass a broad clinical spectrum of the disease, including symptoms of Sjögren's syndrome (**Supplementary Table 2** online). This observation prompted us to investigate *TREX1* in a cohort of individuals with primary Sjögren's syndrome. We found one nonsynonymous mutation in 169 individuals with primary Sjögren's syndrome that was absent in 1,712 controls, raising the possibility of a common underlying genetic risk for these systemic autoimmune diseases (**Supplementary Table 2**).

Five missense changes found in affected individuals are located within the C terminus outside the catalytic domains, and the two frameshift mutations, P212fs and D272fs, result in C terminally truncated proteins (**Supplementary Fig. 1** and **Fig. 1**). Among the unique features of TREX1 not present in the homologous TREX2 is a highly conserved C-terminal extension of 79 residues with no known homology[1] (**Fig. 1**). Computational analysis predicted a single transmembrane helix involving amino acid residues 286–309 (**Supplementary Fig. 2** online). As a truncated version of TREX1 (residues 1–242) is nearly fully capable of catalysis (F.W.P., unpublished observation), we predicted that these mutations would not greatly affect enzyme activity. The exonuclease activities of recombinant D272fs-D272fs homodimers and D272fs–wild-type heterodimers were 56% and 107%, respectively, of the activity of wild-type homodimers (**Supplementary Fig. 3** online). Our *in vitro* findings suggest that no major enzyme deficiency is caused by D272fs.

We next examined whether the frameshift mutations that eliminate the putative transmembrane domain might alter TREX1 subcellular localization using N-terminal green fluorescent protein (GFP)-TREX1-fusion constructs. In HeLa cells, wild-type GFP-TREX1 associated with the endoplasmic reticulum, as shown by colocalization

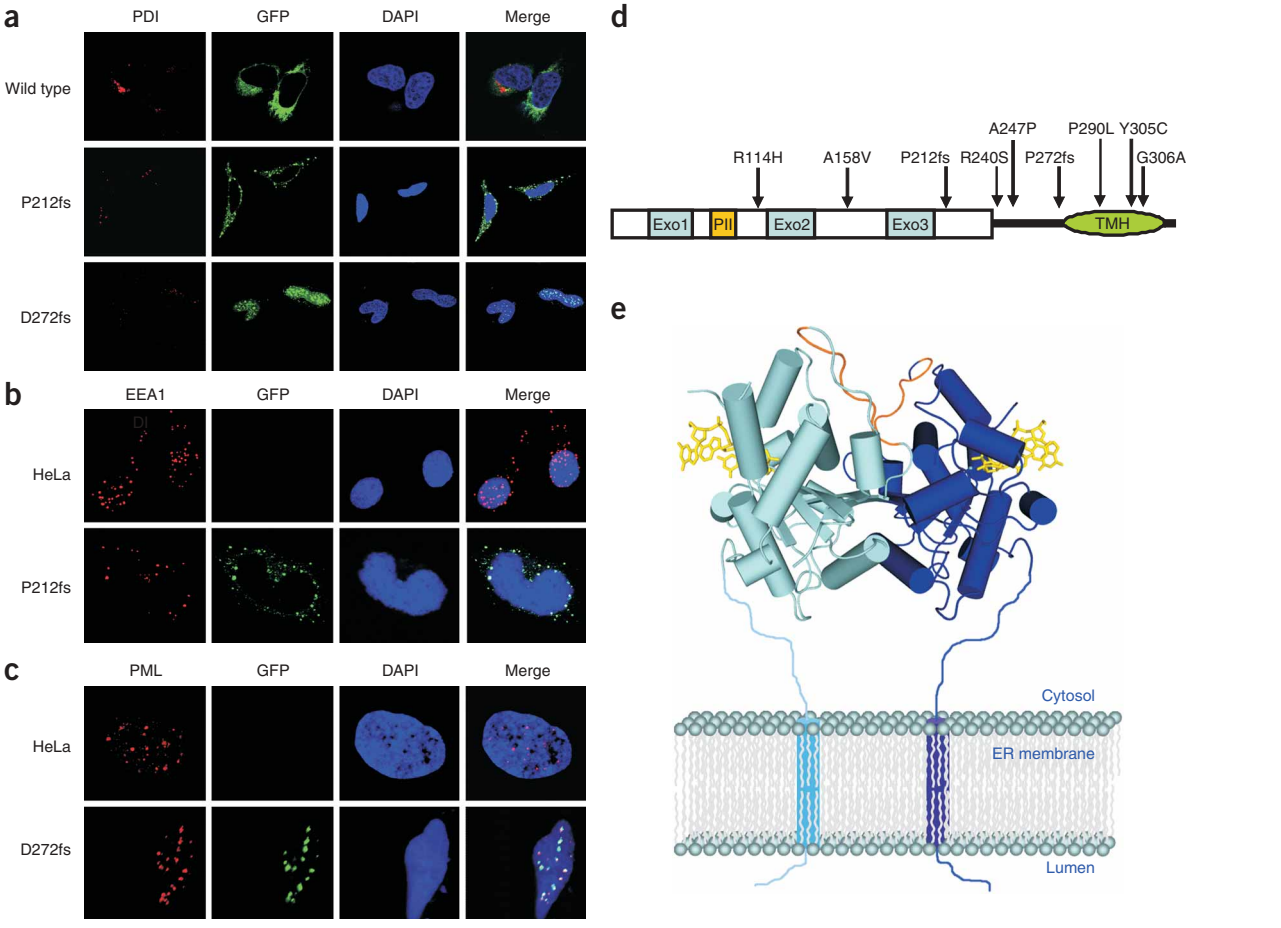

Figure 1 *TREX1* mutations alter subcellular targeting. (**a**) Subcellular localization (green) of GFP-TREX1-fusion proteins (wild-type, P212fs and D272fs) in HeLa cells. The endoplasmic reticulum (ER) was labeled with antibody to protein disulfide isomerase PDI; red, and nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI; blue). (**b**) HeLa cells stained with antibody to early endosome antigen 1 (EEA1; red). GFP-TREX1-P212fs colocalizes with endosomal EEA1. (**c**) HeLa cells stained with antibody to PML (red). Nuclear GFP-TREX1-D272fs colocalizes with PML nuclear bodies. (**d**) Localization of *TREX1* mutations. Indicated are the exonuclease domains (Exo1–Exo3), the polyproline II motif (PII) and the transmembrane helix (TMH). (**e**) Hypothetical model of the TREX1 dimer (monomers in cyan and blue) containing the two active sites bound to DNA (yellow). The dimer is connected through a C-terminal linker to the transmembrane region, which anchors TREX1 in the ER.

with protein disulfide isomerase (**Fig. 1**). GFP-TREX1-P212fs, in which the last 103 amino acid residues are replaced by a 64-residue aberrant peptide, was distributed in a punctate pattern throughout the cytoplasm. Costaining with early endosome antigen 1 confirmed uptake into endosomal vesicles (**Fig. 1**). In contrast, GFP-TREX1-D272fs, which lacks the final 42 residues, was almost exclusively localized within the nucleus (**Fig. 1**) and was distributed in a speckled pattern within the interchromatin space, suggesting association with subnuclear organelles (**Fig. 1**). Promyelocytic leukemia protein (PML) is a major component of PML nuclear bodies, dynamic structures that act as sensors of DNA damage and recruit single-stranded DNA after exposure of cells to ultraviolet light[7]. Moreover, PML is induced by interferons, and its expression is required for interferon-induced apoptosis and antiviral host defense[8]. Costaining for PML showed that GFP-TREX1-D272fs colocalized with PML nuclear bodies (**Fig. 1**). Thus, both frameshift mutations alter the subcellular distribution of TREX1 and might thereby interfere with its function. The crystal structure of TREX1 (residues 1–242) identified a polyproline II helix (**Fig. 1**)[9], a known site of protein-protein interactions. It is possible that the identified missense mutations might interfere with exonuclease activity, subcellular targeting or interactions with other proteins. Moreover, variation in the 3′ UTR could result in altered transcript levels[10]. This will require further study.

Our *in vitro* assays suggest that the C terminus is required for cellular compartmentalization of TREX1. Based on these findings, we propose that each TREX1 monomer is anchored in the endoplasmic reticulum membrane via the C-terminal transmembrane domain (**Fig. 1**). The connecting linker region that contains multiple predicted sites of serine phosphorylation (**Supplementary Fig. 1**) may be subject to post-translational modification in response to oxidative stress, allowing nuclear translocation of TREX1. In this model, the active sites of each monomer are readily accessible to single-stranded DNA[9].

Viral infection has been implicated as an environmental factor in the pathogenesis of SLE and Sjögren's syndrome[11]. Viral infection triggers immune responses via recognition of viral DNA through toll-like receptor 9 (TLR9) in endosomes. Yet cytosolic DNA can induce a TLR9-independent type I interferon immune response, suggesting the existence of a distinct cytosolic DNA sensor[12]. By virtue of its high affinity for single-stranded DNA and cytosolic localization, TREX1 may be a plausible candidate for this role. Further studies looking at induction of interferon responsive genes in cells in which TREX1 is silenced are necessary to investigate this hypothesis.

TREX1 is not the first DNase found to be associated with SLE. Mutations in the gene encoding DNase I, the most abundant extracellular DNase, cause SLE in humans and mice[13,14], and *Dnase2a*$^{-/-}$ mice develop a rheumatoid arthritis–like syndrome[15]. A dual role of TREX1 as DNA-degrading enzyme in granzyme A–mediated apoptosis and potentially as cytosolic DNA sensor may explain how impaired TREX1 function could induce an environment in which autoimmunity is perpetually stimulated and sustained.

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

ACKNOWLEDGMENTS

We thank all subjects for participation in this study. We thank W.L. Gross, E. Gromnica-Ihle, H.-H. Peter, S. Schnarr and M. Linné for clinical assistance. We acknowledge technical assistance of C. Flachmeier, M. Schwarz and A. Müller. We thank M. Weber at the Technische Universität Dresden confocal microscopy core facility. We thank M.T. Pisabarro, K. Köhler, M.C. Cardoso and F.C. Luft for helpful discussions and comments. This work was supported by a Marie Curie Development Host Fellowship from the European Commission to M.A.L.-K., a grant from the German Ministry of Science and Education (BMBF Kompetenznetz Rheuma C2.12) to T.W., US National Institutes of Health (NIH) grant AI45587 to J.L., NIH grant GM069962 to F.W.P. and a grant-in-aid from the National Genome Research Network (NGFN2) of the German Ministry of Science and Education (BMBF) to Y.-A.L. and N.H.

AUTHOR CONTRIBUTIONS

M.A.L.-K. developed the project. M.A.L.-K. and N.H. codirected the project. M.G., K.E., L.S., Y.-A.L. and O.H. performed mutation screening. F.W.P., U.d.S., S.L.B., S.H. and T.H. generated bacterial expression constructs, performed biochemical analysis of recombinant TREX1 and generated the structural model of TREX1. L.S. and K.E. generated mammalian expression constructs and performed transfection and immune histochemistry. D.C. and J.L. performed studies on granzyme A–mediated apoptosis. T.J.V., A.W., J.K., S.K., T.W., R.E.S., A.F.D., M.G. and C.P. contributed individuals with SLE or controls. K.R. performed statistical analysis. M.A.L.-K. wrote the paper with J.L. and N.H.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

Published online at http://www.nature.com/naturegenetics
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions

1. Mazur, D.J. & Perrino, F.W. *J. Biol. Chem.* **274**, 19655–19660 (1999).
2. Chowdhury, D. *et al. Mol. Cell* **23**, 133–142 (2006).
3. Crow, Y.J. *et al. Nat. Genet.* **38**, 917–920 (2006).
4. Rice, G. *et al. Am. J. Hum. Genet.* **80**, 811–815 (2007).
5. Morita, M. *et al. Mol. Cell. Biol.* **24**, 6719–6727 (2004).
6. Laan, M. & Paabo, S. *Nat. Genet.* **17**, 435–438 (1997).
7. Boe, S.O. *et al. J. Cell Sci.* **119**, 3284–3295 (2006).
8. Regad, T. & Chelbi-Alix, M.K. *Oncogene* **20**, 7274–7286 (2001).
9. de Silva, U. *et al. J. Biol. Chem.* **282**, 10537–10543 (2007).
10. Mendell, J.T. & Dietz, H.C. *Cell* **107**, 411–414 (2001).
11. James, J.A., Harley, J.B. & Scofield, R.H. *Curr. Opin. Rheumatol.* **13**, 370–376 (2001).
12. Stetson, D.B. & Medzhitov, R. *Immunity* **24**, 93–103 (2006).
13. Napirei, M. *et al. Nat. Genet.* **25**, 177–181 (2000).
14. Yasutomo, K. *et al. Nat. Genet.* **28**, 313–314 (2001).
15. Kawane, K. *et al. Nature* **443**, 998–1002 (2006).