Chapter 6

TREX1 mutations are not associated with sporadic inclusion body myositis

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Abstract

Sporadic inclusion body myositis (IBM) is the most frequent acquired myopathy above the age of fifty. The exact mechanism causing this disease is not known, but immune-mediated features are prominent and are probably to play a role in its pathogenesis. *TREX1* gene mutations are associated with a large range of auto-immune diseases, such as systemic lupus erythematosus. We investigated whether mutations in the *TREX1* gene were associated with sporadic IBM. Fifty-four patients with sporadic IBM were tested for *TREX1* mutations by direct sequencing. All 54 patients tested negative for pathogenic mutations in the *TREX1* gene. One presumed non-pathogenic polymorphism was found in 42 out of 54 patients. We conclude that *TREX1* mutations do not play a role in the pathogenesis of sporadic IBM.

Introduction

Sporadic inclusion body myositis (IBM) is an acquired myopathy presenting mostly above the age of fifty. Patients usually present with painless weakness of the quadriceps muscles, finger flexors or with dysphagia. Hallmarks of sporadic IBM are changes in the muscle with an inflammatory as well as degenerative character. A cause-effect relationship between these features is at present lacking and the pathogenesis is unclear. One theory supporting the idea that sporadic IBM is primarily immune-mediated, is based on the initiation of an immune response by an unknown cause leading to clonal expansion of CD8+ T-cells and invasion of non-necrotic muscle fibers and cytokine release. In this view, the degenerative muscle fiber changes are secondary.[1] The strong association with the autoimmune-prone 5.1 HLA haplotype and high incidence of other autoimmune diseases in patients with sporadic IBM support an autoimmune pathogenesis. However, the degenerative muscle changes and lack of effect of immune modulating therapies in sporadic IBM are different from many other immune-mediated disorders.

We hypothesized that TREX1 might play a role in sporadic IBM. Mutations in the *TREX1* gene are linked to a spectrum of autoimmune diseases, i.e. Aicardi-Goutière, childblain lupus and systemic lupus erythematosus.[2] Futhermore, Trex1 knockout mice were reported to spontaneously develop an inflammatory myocarditis.[3] TREX1 is the major 3'-5' exonuclease in mammalian cells and acts preferentially on single-stranded DNA (ssDNA). It is localized in the cytoplasm but can be mobilized to the nucleus in case of DNA damage. A study in mouse embryo fibroblasts with a *Trex1* -/- phenotype showed that Trex1 deficiency lead to a constitutive activation of DNA damage checkpoint and extranuclear accumulation of endogenous ssDNA.[4] These changes may lead to autoimmunity, but the exact mechanism of action has not yet been clarified. TREX1 deficiency and the consequent accumulation of ssDNA in the cytoplasmic compartment are thought to trigger an antiviral-like immune response through activation of the innate immune response. In sporadic IBM abnormal expression of an unidentified ssDNA binding protein in skeletal muscle fibers has been demonstrated,[5] as well as accumulation of TAR DNA binding protein-43 (TDP-43).[6] ssDNA accumulation could trigger the activation of the innate immune response in sporadic IBM patients. Accumulation of ssDNA results in elevated levels of type I interferons in the absence of an infection.[4, 7] TREX1 has also been opted to be a negative stimulator of the interferon-stimulatory DNA response, which causes increased immune activity.[8] Alternatively, TREX1 deficiency leads to activation of NK and T-cells, thus causing inflammation,[7, 9]

which may be related to the T-cell invasion of non-necrotic muscle fibers in sporadic IBM.

In this study we explored whether *TREX1* mutations are associated with sporadic IBM.

Methods

The present study comprised 54 patients diagnosed with sporadic IBM, selected from a national cohort of 86 patients with sporadic IBM. All patients gave informed consent. The study was approved by the Ethics committee of the Leiden University Medical Center. All patients fulfilled the ENMC criteria[10] for definite (n=49) or probable (n=5) sporadic IBM. The *TREX1* gene was screened for mutations by direct sequencing. The coding exon with the surrounding intronic regions was divided in three fragments and amplified by PCR. The primers used were: TREX1_Ex2AF5'gaatgtgctggtcccactaa gg3', TREX1_Ex2AR 5'aaggctaggagcaggttggc3', TREX1_Ex2BF 5'ctctccctgtgtgtggctcc3', TREX1_Ex2BR 5'ttgtgacagcagatggtcttgg3', TREX1_Ex2CF 5'ctaggcagcatctacactcgcc3', TREX1_Ex2CR 5'atcctgctagggaaagtgaggg3'. PCR products were analysed on an ABI3730 sequencer (Applied Biosystems, Foster City, CA, USA) and genotypes were assigned using SeqScape software (Applied Biosystems). The reference sequence NM_016381 (*TREX1* isoform A) was used and sequence variants were described according to the HGVS nomenclature recommendations.

Results

The patients were 50-87 years of age (median 69) and 69% were male. The duration of symptoms varied between one and 29 years (median 11). Thirty-two patients (59%) presented with weakness of the quadriceps, 9 patients (17%) with finger flexor weakness and 6 (11%) with dysphagia. All muscle biopies showed endomysial lymphocytic infiltrates, invasion of non-necrotic muscle fibers and rimmed vacuoles. Nineteen patients (35%) had another auto-immune disease, such as rheumatoid arthritis, type I diabetes or sarcoidosis.

All 54 patients tested negative for pathogenic mutations in the *TREX*1 gene. We did detect one single polymorphism (c.696C>T p.Tyr232Tyr) in 42 of the 54 patients. This polymorphism is frequently found in the Caucasian population and is considered to be non-pathogenic.

Discussion

The results of this genetic screening of well-defined sporadic IBM patients did not reveal a role for *TREX1* mutations in sporadic IBM. This does not rule out a *TREX1* mutation in a rare case of sporadic IBM, but given the relatively large group of patients an important pathological role of *TREX1* can be considered very unlikely. Whether TREX1 accumulates in the sporadic IBM muscle as a secondary effect and therefore may still have a role in the pathogenesis of sporadic IBM, remains to be investigated.

References

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