Anti-TRIB2 autoantibodies (Tribbles homolog 2)

Indications

- Narcolepsy and cataplexy (classical narcolepsy)
- Autoimmune uveitis

See also

- Autoantibodies in central neuropathies (narcolepsy)
- Laborinformation: Narkolepsie - Autoantikörper gegen Tribbles Homolog 2 (anti-TRIB2)

Autoantibodies against Tribbles homolog 2 (TRIB2) were detected for the first time in patients suffering from autoimmune uveitis by means of phage display technology using a cDNA library synthesized from human eye tissue (Zhang et al. 2005). Consecutive studies resulted in the demonstration of these antibodies (anti-TRIB2) also in patients suffering from narcolepsy and cataplexy (table 1).

Antigen

Tribbles homolog 2 (M_r 38,8 kDa; chromosome 2p4.3) pertains to one of the three members of the Tribbles family within the superfamily of protein kinases. Its amino acids sequence is similar to that of serine-threonine-kinases. Tribbles homolog 2 modulates numerous physiological and pathological processes of signal transduction. It is involved into apoptosis and regarded as a potent oncogene, the expression of which is augmented in certain subgroups of human myeloid leukemia (Hannon et al. 2012), it gains oncogenic properties in virtue of inactivation the transcription factor C/EBPα (CCAAT/enhancer-binding protein α) and may induce myelogenic leukemia in mice (Lohan und Keeshan 2013). Inter alia TRIB2 is increasingly expressed in hypocretin (orexin) producing neurons (HCRT neurons) of the dorsomedial, perifornical and lateral nuclei of hypothalamus (Cvetkovic-Lopes et al. 2010).

Table 1 Prevalence of autoantibodies against Tribbles homolog 2 in patients with narcolepsy, control groups and autoimmune uveitis.

Diseases and controls	n	anti-TRIB2 [%]	Authors
Narcolepsy and cataplexy *1	119	14,3 *2	Cvetkovic-Lopes et al. 2010
Narcolepsy without cataplexy	24	12,5 *2	
Idiopathic hypersomnia	23	0,0	
Multiple sclerosis	16	0,0	
Other inflammatory neuropathies	9	11,1	
Healthy controls	42	4,8	
Narcolepsy and cataplexy *1	90	21,1 *3	Kawashima et al. 2010
Narcolepsy without cataplexy	57	3,5	
Healthy controls	156	4,5	
Narcolepsy and cataplexy *1	88	26,1 *3	Toyoda et al. 2010
Narcolepsy without cataplexy	18	5,6	
Idiopathic hypersomnia	11	0,0	
Healthy controls	87	0,0	
Autoimmune uveitis	10	30,0	Zhang et al. 2005

n number of subjects studied

*1 HLA-DQB1*0602 positive patients.

*2 The percent quota of patients harboring antibodies refers to extinctions (Elisa) of ≥ 2 standard deviations (SD) above the mean of healthy controls. Setting cut off ≥ 1SD of healthy controls resulted in 39 % anti-TRIB2 positive patients with narcolepsy (56 of a total of 143).

*3 Cut off ≥ 2 SD above the mean of healthy controls.

Anti-TRIB2 autoantibodies (Tribbles homolog 2)

Prevalence

Autoantibodies to TRIB2 were detected for the first time in patients with autoimmune uveitis (Zhang et al. 2005) and later on by three working groups (Europe, USA, Japan) in patients with narcolepsy and cataplexy (table 1).

In narcolepsy anti-TRIB2 antibodies are present especially in the early phase of the disease. Cases with recent onset (≤ 2,3 years) were 7,4 times more likely to carry anti-TRIB2 than those with more distant onset (Cvetkovic-Lopes et al. 2010; Kawashima et al. 2010). Using this time-referenced breakpoint, 41 % of recent onset DQB1*0602 positive narcolepsy and cataplexy patients were anti-TRIB2 positive versus 4 % to 8 % in the other groups (Kawashima et al. 2010). Within a time course of about two years the antibody titers seem to decrease rapidly, but elevated titers (above 1 SD of the mean ELISA readings of healthy controls) could be measured as long as twenty years after disease onset (Cvetkovic-Lopes et al. 2010). As far as the prevalence of the antibodies in patients not manifesting cataleptic attacks is concerned, the reported data are inconsistent (table 1). Correlations between the antibody titers and the frequency of cataleptic attacks and the severity of sleepiness have been mentioned (Cvetkovic-Lopes et al. 2010) as well as correlations between manifestation of cataplexy and anti-TRIB2. No correlations were seen between low liquor hypocretin levels and antibody prevalence (Kawashima et al. 2010).

Immunopathology

The classical narcolepsy (narcolepsy with cataplexy) is characterized by excessive daytime sleepiness, attacks of muscle atony triggered by strong emotions (cataplexy) and REM sleep occurring early at sleep onset. The prevalence of the disease accounts for about 5 x 10^{-4} (Longstreth et al. 2007). Morphological signs are a nearly complete loss (< 90 %) of hypocretin secreting neurons (HCRT neurons) in the dorsomedial, perifornical and lateral hypothalamic nodes (Peyron et al. 2000; Thannickal et al. 2000), the cause of which is also supposed in pathological autoimmune processes (overview: Overeem et al. 2009). The tight association of the disease with HLA allele DQB1*0602 in more than 95 % of the afflicted individuals (Mignot et al. 2001), the association with polymorphisms of the T-cell receptor α (Hallmayer et al. 2009) and the demonstration of autoantibodies against the Tribbles homolog 2, which is also expressed by HCRT neurons gave a new boost to these considerations. However, the causes leading to the formation of anti-TRIB2 autoantibodies are not known so far. It is unclear whether anti-TRIB2, generated by an yet unknown primary autoimmune process, is causing HCRT cell death, or whether anti-TRIB2, generated as by-product of a non-immunological co-occurring damage of HCRT cells, is sustaining a chronic neuronal inflammation or last not least whether anti-TRIB2 constitutes a mere epiphenomenon. However, after intra-ventricular injection of anti-TRIB2 positive patient sera in mice, not only morphological lesions were observed within the lateral hypothalamus (loss of NeuN, synaptophysin, HCRT neurons) but also manifestations of narcolepsy like symptoms (Katzav et al. 2013). These experimental studies and the amelioration of the clinical course following IVIG therapy in some patients (Cvetkovic et al. 2010) point to the possibility of sharing immune-pathological processes.

Test methods

Determination of antibodies was done by Elisa using a recombinant gluthation-S-transferase fusion peptide of 28 C-terminal amino acids of TRIB2 (Cvetkovik et al. 2010) or by means of radioimmunoprecipitation of a recombinant *in vitro* transcribed and translated ^{35}S-methionin labeled complete TRIB2 protein (Kawashima et al. 2010; Toyoda et al. 2010). The latter method seems to be more sensitive than the Elisa (table 1). Materials used for antibody determination were serum and/or CSF.

Anti-TRIB2 autoantibodies (Tribbles homolog 2)

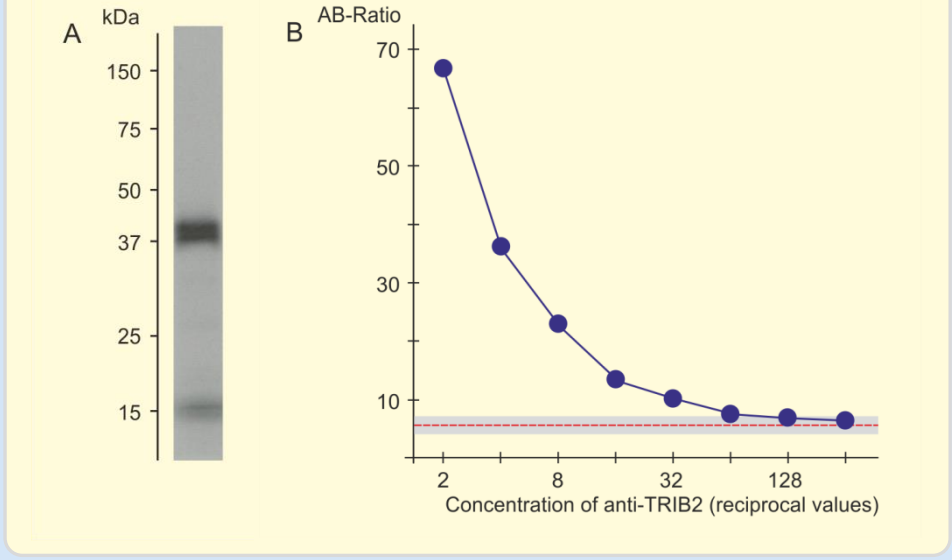

Figure 1 Demonstration of autoantibodies against TRIB2 by means of radioimmunoprecipitation assay (in collaboration with Dipl.-Leb. Chem O. Bauer).

A Autoradiogram of SDS-PAGE separated, *in vitro* transcribed and translated ^{35}S-methionine labeled full length TRIB2 protein (343 amino acids, M, 38,8 kDa) after chromatography on Sephadex G25 to eliminate non - incorporated ^{35}S-methionine. The cDNA used for synthesis of the template was obtained from a human cerebellum cDNA library by amplification using appropriate primers (100 % accordance with the consensus sequence NP_06675.1; Q92519) cloned into an modified pCITE-4a vector (Novagene). The radiolabeled antigen is nearly free of contaminations and shows the expected molecular weight.

B Standard curve of the radioimmunoprecipitated ^{35}S-methionine-TRIB2 using a polyvalent rabbit anti-TRIB2 antibody (TRIB2 (H-53); Santa Cruz Biotechnology). The hatched line depicts the mean of five negative controls, the grey area matches ± 1SD. The calculations were done according to Frey et al. 1998.

Literature

Cvetkovic-Lopes V, Bayer L, Dorsaz S, Maret S, Pradervand S, Dauvilliers Y, Lecendreux M, Lammers GJ, Donjacour CE, Du Pasquier RA, Pfister C, Petit B, Hor H, Mühlethaler M, Tafti M: Elevated Tribbles homolog 2-specific antibody levels in narcolepsy patients. J Clin Invest (2010); 120(3): 713 - 719 (PMID: 20160349).

Grandinetti KB, Stevens TA, Ha S, Salamone RJ, Walker JR, Zhang J, Agarwalla S, Tenen DG, Peters EC, Reddy VA: Overexpression of TRIB2 in human lung cancers contributes to tumorigenesis through downregulation of C/EBPα. Oncogene (2011); 30(30): 3.328 - 3.335 (PMID: 21399661).

Hallmayer J, Faraco J, Lin L, Hesselson S, Winkelmann J, Kawashima M, Mayer G, Plazzi G, Nevsimalova S, Bourgin P, Hong SC, Honda Y, Honda M, Högl B, Longstreth WT Jr, Montplaisir J, Kemlink D, Einen M, Chen J, Musone SL, Akana M, Miyagawa T, Duan J, Desautels A, Erhardt C, Hesla PE, Poli F, Frauscher B, Jeong JH, Lee SP, Ton TG, Kvale M, Kolesar L, Dobrovolná M, Nepom GT, Salomon D, Wichmann HE, Rouleau GA, Gieger C, Levinson DF, Gejman PV, Meitinger T, Young T, Peppard P, Tokunaga K, Kwok PY, Risch N, Mignot E: Narcolepsy is strongly associated with the T-cell receptor alpha locus. Nat Genet (2009); 41(6): 708 - 711 (PMID: 194121769).

Anti-TRIB2 autoantibodies (Tribbles homolog 2)

Hannon MM, Lohan F, Erbilgin Y, Sayitoglu M, O'Hagan K, Mills K, Ozbek U, Keeshan K: Elevated TRIB2 with NOTCH1 activation in paediatric/adult T-ALL. Br J Haematol (2012); 158(5): 626 - 634 (PMID: 22775572).

Katzav A, Arango MT, Kivity S, Tanaka S, Givaty G, Agmon-Levin N, Honda M, Anaya JM, Chapman J, Shoenfeld Y: Passive transfer of narcolepsy: anti-TRIB2 autoantibody positive patient IgG causes hypothalamic orexin neuron loss and sleep attacks in mice. J Autoimmun (2013); 45: 24 - 30 (PMID: 23834844).

Kawashima M, Lin L, Tanaka S, Jennum P, Knudsen S, Nevsimalova S, Plazzi G, Mignot E: Anti-Tribbles homolog 2 (TRIB2) autoantibodies in narcolepsy are associated with recent onset of cataplexy. Sleep (2010); 33(7): 869 - 874 (PMID: 20614846).

Longstreth WT Jr, Koepsell TD, Ton TG, Hendrickson AF, van Belle G: The epidemiology of narcolepsy. Sleep (2007); 30(1): 13 - 26 (PMID: 17310860).

Mignot E, Lin L, Rogers W, Honda Y, Qiu X, Lin X, Okun M, Hohjoh H, Miki T, Hsu S, Leffell M, Grumet F, Fernandez-Vina M, Honda M, Risch N: Complex HLA-DR and -DQ interactions confer risk of narcolepsy-cataplexy in three ethnic groups. Am J Hum Genet (2001); 68(3): 686 - 699 (PMID: 11179016).

Overeem S, Black JL 3rd, Lammers GJ: Narcolepsy: immunological aspects. Sleep Med Rev (2008); 12(2): 95 - 107 (PMID: 18291691).

Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, Li R, Hungs M, Pedrazzoli M, Padigaru M, Kucherlapati M, Fan J, Maki R, Lammers GJ, Bouras C, Kucherlapati R, Nishino S, Mignot E: A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med (2000); 6(9): 991 - 997 (PMID: 10973318).

Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM: Reduced number of hypocretin neurons in human narcolepsy. Neuron (2000); 27(3): 469 - 474 (PMID: 11055430).

Toyoda H, Tanaka S, Miyagawa T, Honda Y, Tokunaga K, Honda M: Anti-Tribbles homolog 2 autoantibodies in Japanese patients with narcolepsy. Sleep (2010); 33(7): 875 - 878 (PMID: 20614847).

Zhang Y, Davis JL, Li W: Identification of tribbles homolog 2 as an autoantigen in autoimmune uveitis by phage display. Mol Immunol (2005); 42(11): 1.275 - 1.281 (PMID: 15950723).