

No association of *TDP-43* with sporadic frontotemporal dementia.

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Keywords: TDP-43; MASP2; frontotemporal dementia; genetic analysis; association study; amyotrophic

lateral sclerosis

Abstract

A hyperphosphorylated, ubiquitinated form of TDP-43, known as pathologic TDP-43, was shown to be a central component of ubiquitin-positive, tau-negative and alpha-synuclein-negative inclusions in frontotemporal lobar degeneration (FTLD-U) and amyotrophic lateral sclerosis (ALS). To investigate the role of the *TDP-43* gene in sporadic forms of frontotemporal dementia (FTD), we genotyped 10 single nucleotide polymorphisms covering the entire *TDP-43* genomic region, including the *MASP2* gene in 173 patients with sporadic FTD (including 7 patients that were diagnosed with FTD and ALS) and 184 matched controls from Germany. Although we could observe a weak trend towards a disease association in the FTD/ALS patients, no significant association with sporadic FTD could be demonstrated. There is no evidence, that common variants in *TDP-43* confer a strong risk to the development of sporadic FTD.

Introduction

Frontotemporal dementia (FTD; OMIM #600274) is a neurodegenerative disease associated with circumscribed degeneration of the prefrontal and anterior temporal cortex causing a wide spectrum of cognitive, behavioural and psychological symptoms [11]. Depending on the pathological phenotype, FTD can be classified into several categories, primarily I.) tauopathies (e.g. Pick's disease or corticobasal degeneration), II.) frontotemporal lobar degeneration (FTLD) with ubiquitinpositive tau-negative neuronal inclusions and III.) FTLD without tau-or ubiquitin-positive inclusions. FTD is the second most common cause of dementia accounting for up to 20% of studied cases with an average onset of dementia in the late 50s. FTD may represent, like many other neurodegenerative diseases, a genetically complex disorder where in addition to susceptibility genes that confer risk to the sporadic forms of the disease, mutations in specific genes are essential for disease development. Mutations are known for genes such as the microtubule-associated protein tau (*MAPT*, OMIM #157140; [6]), the endosomal ESCRTIII-complex subunit (*CHMP2B*, OMIM #609512; [15]), progranulin (*GRN*, OMIM #138945; [2]) and presenilin 1 (*PSEN1*, OMIM #104311; [4]).

Additionally, two recent studies demonstrated that abnormal TDP-43 variants are a major component of ubiquitinated cytoplasmic, nuclear and neuritic inclusions in frontotemporal lobar degeneration with ubiquitin-positive intracytoplasmic inclusions (FTLD-U; OMIM #607485) as well as amyotrophic lateral sclerosis (ALS; OMIM #105400) [1, 13]. The *TDP-43* (*TARDBP*; OMIM #605078) gene is ubiquitously expressed and functions in transcriptional repression and exon skipping. The TDP-43 product binds to the pyrimidine-rich motifs of TAR DNA of the human immunodeficiency virus 1 (HIV-1) long terminal repeat (LTR) region, which may contain critical regulatory elements that modulate HIV-1 gene expression. Although the physiological function of TDP-43 in brain cells has not yet been determined, it was found that the *TDP-43* gene generates at least 11 mRNA species by alternative

splicing [16]. TDP-43 is normally localized primarily to the nucleus, but under pathologic conditions in FTLD-U, TDP-43 is eliminated from nuclei of ubiquitinated inclusion-bearing neurons, a consequence of which may be a loss of TDP-43 nuclear functions.

Since specific pathological phenotypes and common genetic variants of genes causing familial forms of neurodegenerative diseases have been shown to exert risk to sporadic forms of the disease it is reasonable to investigate the role of *TDP-43* in patients with sporadic forms of FTD [8]. We therefore applied a whole gene approach and genotyped 10 single nucleotide polymorphisms (SNPs) covering the complete genetic region of *TDP-43* including the neighbouring mannan-binding lectin serine protease 2 gene (*MASP2*). For the statistical analysis, we determined the linkage disequilibrium (LD) structure, estimated D' and r^2 as implemented in Haploview [3], and explored single marker and haplotype associations. Using current diagnostic criteria [10], this was undertaken in thoroughly diagnosed, patients with sporadic FTD (n=194; mean age of onset 63.1 ± 10.3 years) and cognitively healthy (as assessed by the Mini Mental State Examination [5]), age-, gender-, and ethnicity-matched controls (n=184; 62.9 ± 11.6 years; for details see Supplementary material).

Results & Discussion

The LD structure based on all 10 SNPs shows that the markers of the *TDP-43-MASP2* region forms one block of relatively high D' values (Supplementary material Fig. 1). In contrast, high r^2 values are only observed sporadically across both genes; generally a complex pattern of low and high r^2 values could be observed, indicating an evolutionary (mutational) history of fragmentation. No significant association between *TDP-43* and *MASP2* single markers and FTD was observed in this German sample (Supplementary material Table 1). Similarly, a haplotype analysis revealed no significant associations for all haplotypes (Supplementary material Table 2). Interestingly, a weak association for the two

markers rs2273348 ($p=0.05$) and rs12711521 ($p=0.06$) could be observed in the 7 individuals with FTD/ALS. Additionally, we analysed 3 sequences (rs12072993, rs7536030 and rs3737613) within the TDP-43 region that are categorized as SNPs in the major SNP databases (Entrez SNP and HapMap), however, these sequences turned out to be monomorph in the German sample.

Although several new candidate genes for familial forms of FTD could be identified in the last years, the identification of genetic risk factors in patients with sporadic forms of FTD struggles behind the genetic research in other neurodegenerative diseases. Recent findings that TDP-43 is a component of some FTLD and ALS cases, led us to examine if genomic *TDP-43* variants may also confer risk to sporadic forms of FTD [1, 13]. Using a carefully ascertained case-control sample we performed a whole gene approach [9] by genotyping of 10 densely spaced SNPs (average distance 3.2 kb) which covered the entire genomic region of *TDP-43* and the neighbouring downstream gene, *MASP2* and determined the LD structure. After adjustment for multiple testing single marker and haplotype analysis did not reveal evidence of a significant association. Since all markers at the *TDP-43* region form one single LD block spanning the entire gene, it appears rather unlikely that we missed a possible association due to an untyped variant. Likewise with statistical power, as a power analysis revealed that, at a significance level of $\alpha=0.05$, we had a power of 88 % to detect a risk allele of 25% frequency mediating a relative risk of 2.0.

Since it was shown that abnormal TDP-43 protein variations in FTD are also involved in sporadic cases of ALS and increasing evidence supports a possible connection of some FTD types with ALS [1, 7, 13, 14], we analysed a sub-group of patients that suffered from FTD and ALS. Given the small sample size, we observed a weak trend towards a possible association which clearly needs to be further explored using larger FTD/ALS cohorts. It is likely that the known FTD/ALS types associated with TDP-43 inclusions are a separate clinicopathological subtype of FTD that can be classified as a proteinopathy of

TDP-43 [1]. Similarly, sporadic FTD cases may represent a different class of a clinicopathological spectrum compared to sporadic and familial FTLD-U [13].

In summary, single marker as well as haplotype analysis did not reveal evidence of a significant association of common *TDP-43* and *MASP2* variants with sporadic FTD in this German sample.

Acknowledgement

We wish to thank all individuals who participated in this study for their contribution and would also like to thank both dementia outpatient unit employees for their help in collecting and processing of samples. This work was funded by the German National Genome Network (NGFN) and the German Ministry for Education and Research; Grant Number 01GS0465.

Disclosure Statement: No authors have actual or potential conflicts of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted that could inappropriately influence this work.

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Supplementary material

Methods

Patients with FTD (N=173) were recruited at the memory outpatient units with a special focus on FTD at the TU-Munich and the University of Regensburg and thoroughly diagnosed according to current diagnostic criteria [12]. The patient sample had a male to female ratio of 1.2, showed an age at onset of 63.1 ± 10.3 years (mean \pm SD) and consisted of individuals with the diagnosis of FTD (N=123), FTD/ALS (FTD with amyotrophic lateral sclerosis (ALS), N=7), PPA (primary progressive aphasia, N=20), and SD (semantic dementia, N=23). The control group (N=184) was matched for age, geographical location and ethnicity and consisted of cognitively healthy elderly subjects (62.9 ± 11.8 years) who were recruited from the memory clinic and community based geriatric day-care units. The cognitive status of individuals in the control group was assessed using the Mini Mental State Examination (MMSE; [5]) and individuals who scored under 28 were excluded. Blood samples of each subject were taken after informed consent had been obtained. The study protocol was approved by the institutional review boards of both universities.

Information for all SNPs were derived from public databases, the average intermarker distance of all SNPs was 3.2 kb. Genotyping was performed through a PCR based primer extension reaction and detection of the allele-specific extension products by matrix-associated laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (Sequenom, San Diego, CA) at the Dept. of Psychiatry, Munich. The average call rate for all SNPs was above 98% and the genotype distributions of all SNPs were in Hardy-Weinberg equilibrium. LD was estimated by D' and r^2 as implemented in Haploview [3]. Allelic/genotypic associations with FTD were tested by logistic regression analyses using age and sex as covariates. Haplotypic associations were calculated using χ^2 tests as implemented in Haploview.

Corrections for multiple testing were considered if mandatory.

Tables and Figures

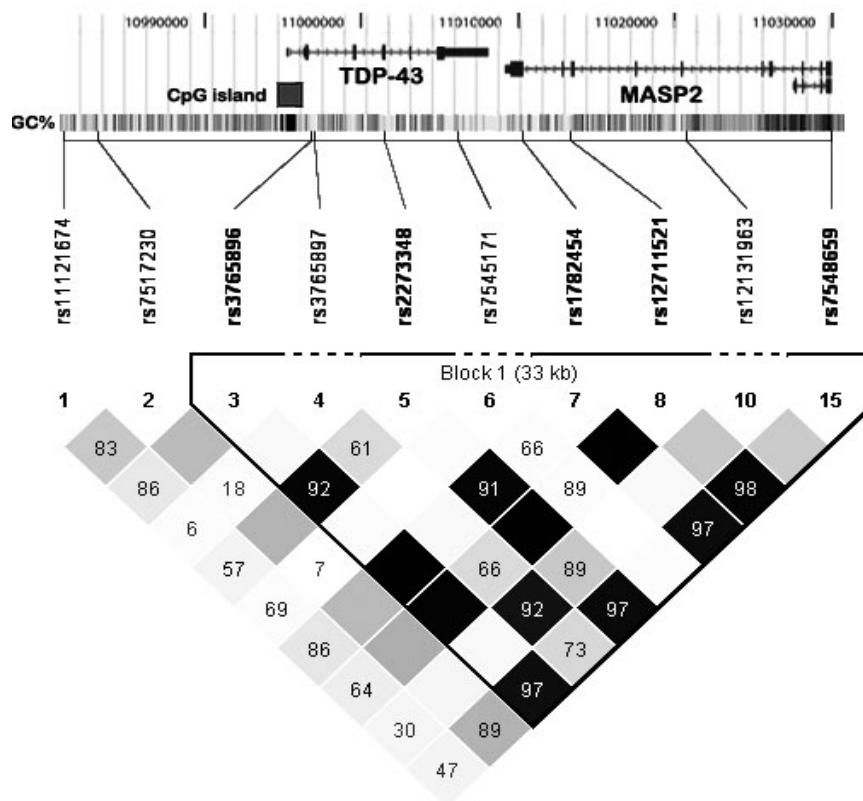
Table 1:

SNP	Position (hg17)	Gene(s)	Role	Alleles	Major allele frequencies (Cases/Controls)	p-values*
rs11121674	10992854	TDP-43	Upstream	A/G	0.835 / 0.820	0.582
rs7517230	10995065	TDP-43	Promoter	T/A	0.341 / 0.339	0.950
rs3765896	11008686	TDP-43	Intron 2	A/G	0.844 / 0.828	0.563
rs3765897	11008848	TDP-43	Intron 2	A/G	0.974 / 0.973	0.904
rs2273348	11013343	TDP-43	Intron 3	A/G	0.176 / 0.175	0.960
rs7545171	11017984	TDP-43	Exon 6	A/C	0.009 / 0.003	0.292
rs1782454	11022101	TDP-43	3'-UTR	C/T	0.838 / 0.824	0.620
rs12711521	11025182	MASP2	Exon 8	T/G	0.817 / 0.806	0.712
rs12131963	11032536	MASP2	Intron 5	C/T	0.023 / 0.016	0.512
rs7548659	11041705	MASP2	Promoter	A/C	0.792 / 0.766	0.415

Table 2:

Haplotype ID	Sequence	Frequency (Cases/Controls)	p value
Block 1			
1.1	AGCTA	0.789 / 0765	0.438
1.2	GATGC	0.147 / 0.156	0.757
1.3	AGCTC	0.026 / 0.041	0.263
1.4	AACGC	0.023 / 0.014	0.346

Figure 1:



Legends to the Tables and Figure:**Table 1: SNP description, allele distribution and association with FTD.**

The locations of the SNPs on Chromosome 1 and the location of the *TDP-43* and *MASP2* genes according to the UCSC map (hg17) are demonstrated. The distribution of the alleles in cases and controls and the association result of a regression analysis with p-values are shown. * = uncorrected.

Table 2: Common haplotypes, frequencies and associations with FTD.

All haplotypes with a frequency > 1% within the LD block of the *TDP-43/MASP2* genes (10 SNPs) in the German sample were tested for association with FTD using χ^2 statistics.

Figure 1:

Linkage disequilibrium structure of the *TDP-43* and *MASP2* gene region. Pair-wise r^2 values are intensity-coded: black = high r^2 values, white = low r^2 values. The haplotype block indicated by high D' values is superimposed. The genomic region is shown on top.