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Association of Genetic Variants in Senataxin and Alzheimer's Disease in a Chinese Han Population in Taiwan

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Abstract

Development of Alzheimer's disease (AD) is characterized by progressive neuronal death and a decline in learning and memory. Mutations in human senataxin (SETX), an ortholog yeast protein of Sen1, have been identified to cause the syndrome of ataxia with oculomotor apraxia type 2 (AOA2) and juvenile amyotrophic lateral sclerosis (ALS4), two types of progressive motor neuron degeneration. However, the relationship between the *SETX* gene, which is involved in the regulation of RNA processing and DNA repair, and the predisposition for AD remains unclear. In this research, potential association of polymorphisms in the *SETX* gene with AD was investigated. A case-control study of a Chinese Han population in Taiwan was performed. Three single-nucleotide polymorphisms (SNPs), 3455T>G (rs3739922), 3576T>G (rs1185193) and 7759A>G (rs1056899) were studied. The experimental data showed that upon genotyping of the exonic polymorphism in the *SETX* gene, the T allele appeared at a lower rate than the G allele at position 3455 in AD patients compared with normal groups ($P < 0.05$, odds ratio (OR), 0.59, 95% confidence interval (CI), 0.40-0.89). Subjects with the GA genotype at position 7759 have higher incidences of AD development than with the AA genotype ($P < 0.05$, OR, 6.45, 95% CI, 1.24 to 33.70). Our results also showed that with six haplotypes (Hts) observed from the analyzed polymorphisms, distributions of the Ht4-GAA and Ht5-GCA haplotypes appeared to be significant 'risk' haplotypes between AD patients and controls (both $P < 0.05$, OR, 8.44, 95% CI, 1.07-66.60). These observations suggest that genetic variations in the *SETX* gene may contribute to AD pathogenesis in the Taiwanese Han population.

Key Words: Alzheimer's disease, DNA repair, polymorphism, senataxin, transcription

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Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disorder representing one of the commonest worldwide causes of dementia in the elderly. Patients with AD display several symptoms, including loss of neurons, cognitive dysfunction and trouble with language. The neuropathological features that appear in the brain of AD patients are two types of insoluble aggregates, namely senile plaques and neurofibrillary tangles, which are composed of amyloid peptides (Aβ) and hyperphosphorylated Tau proteins, respectively (8, 16). Mutations in genes coding for amyloidgenic processing proteins, including amyloid precursor protein (APP) and presenilin 1 and 2 (PSEN1, PSEN2), are predominantly associated with Aβ production and amyloid plaques deposition and likely to lead to early-onset AD (19, 20, 22). However, as most AD cases are sporadic or late-onset, and the causes remain largely unknown, additional genetic factors responsible for AD progression still need to be identified.

Human senataxin (SETX), a protein orthologous to the yeast Sen 1 protein, has known to be highly correlated with progressive motor neuron degeneration (5, 18). Genetic determinants identified that mutations in *SETX* cause the syndrome of ataxia with oculomotor apraxia type 2 (AOA2), and induce dominantly inherited juvenile amyotrophic lateral sclerosis (ALS4) as well as the tremor-ataxia syndrome (4, 5, 18). Both AOA2 and ALS4 are early-onset neurodegenerative diseases and usually occur before 25 years of age. However, the mechanisms of defects in SETX to induce neuronal death are still under exploration. A recent study has indicated that SETX localizes primarily in the nucleus and interacts with not only RNA polymerase II (RNAPII) core subunits RPB1 and RPB2 to promote RNAPII-dependent transcription termination, but also with partner proteins responsible for transcription elongation, replication, chromatin remodeling and DNA repair (27). In the *in vitro* cell tests, SETX is required for mitotic division of S/G2 states and potentially increasing the number of distinct nuclear foci in response to replication stress, transcription problems and DNA damage (27). SETX also functions as a putative RNA/DNA helicase (11) and may have the potential to resolve R loop, a transcriptional structure of RNA/DNA hybrids arising between the poly(A) site and a downstream transcription pause site (17), and further enhance transcription termination (21). These results provide the evidence for the role of human SETX, like its homolog yeast protein Sen1, in acting as a transcription regulator to mediate efficient RNA transcription and processing and to prevent further R loop induced-DNA damage (24). Interests in human SETX are heightened because mutations the *SETX* gene result in two genetic diseases, AOA2 and ALS4, caused by motor neuron degeneration (5, 18). However, the association of SETX and other neurodegenerative disorders is still unresolved. To explore the possible effects of genetic risks of the transcription regulator protein SETX with respect to susceptibility to AD pathophysiological processes, we performed a case and control study amongst the Chinese Han population in Taiwan. Our results showed associative relationship for the *SETX* gene with AD, which may have profound implications as a genetic risk marker in the Taiwanese population with AD progression.

Materials and Methods

Subjects

120 unrelated patients with late-onset of AD (mean age at onset, 74.9 ± 6.7 y.o., 54.2% female) and 90 normal control subjects (65.7 ± 8.7 y.o., 71.1% female) were enrolled in this study. Participants from a Taiwanese Chinese Han cohort were recruited from Chang-Hua Christian Hospital, Changhua, and China Medical University Hospital, Taichung, Taiwan. The institutional ethic committee of China Medical University approved this project, and informed consents were obtained from all subjects. Patients with AD were clinically diagnosed based on guidelines of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA). Dementia was diagnosed according to Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria.

Determination of SETX Gene Variants

Genomic DNA was extracted from peripheral blood samples with a genomic DNA extraction kit according to a standard manual (Qiagen, CA, USA). For analysis of *SETX* gene variants, three polymorphic sites were selected from the public dbSNP database for this study: 3455T>G (rs3739922), 3576T>G (rs1185193), and 7759A>G (rs1056899). The three single-nucleotide polymorphisms (SNPs) are nonsynonymous and are located in exon 10 (3455T>G and 3576T>G) and exon 26 (7759A>G), respectively. To identify the allele preference of these SNPs, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) techniques were used for genotyping. Specific primers and restriction enzymes for each PCR-RFLP reaction are shown in Table 1. Briefly, PCR amplification was performed in a total volume of 25 μl containing 5 ng genomic DNA and primer pairs. The PCR conditions were carried out at 95°C for 5 min (denaturation), followed by 40 cycles of 95°C for 30 sec (amplification),

Table 1. Primers of *SETX* gene polymorphisms performed by PCR-RFLP

Polymorphisms	Primers	Restriction Enzyme	Alleles	Allele size (bp)	ref SNP no.
3455T>G	F: 5'-CAAATGGTCAGGGTTGTACAGAT-3'	ApoI	G	303	rs3739922
(1152Phe>Cys)	R: 5'-AAACCGTAGTGGCTCTCTGAAT-3'		T	(99+204)	
3576T>G	F: 5'-GTTGAAGTTGGCTCAGGACACT-3'	BglII	T	439	rs1185193
(1192Asp>Glu)	R: 5'-GCATTGAAGAACACACAAGACC-3'		G	(168+271)	
7759A>G	F: 5'-ACCTCCTGTTCATGACCAACTT-3'	RsaI	A	435	rs1056899
(2587Ile>Val)	R: 5'-CTTTTCTTGGAACTGCTGTCCT-3'		G	(171+264)	

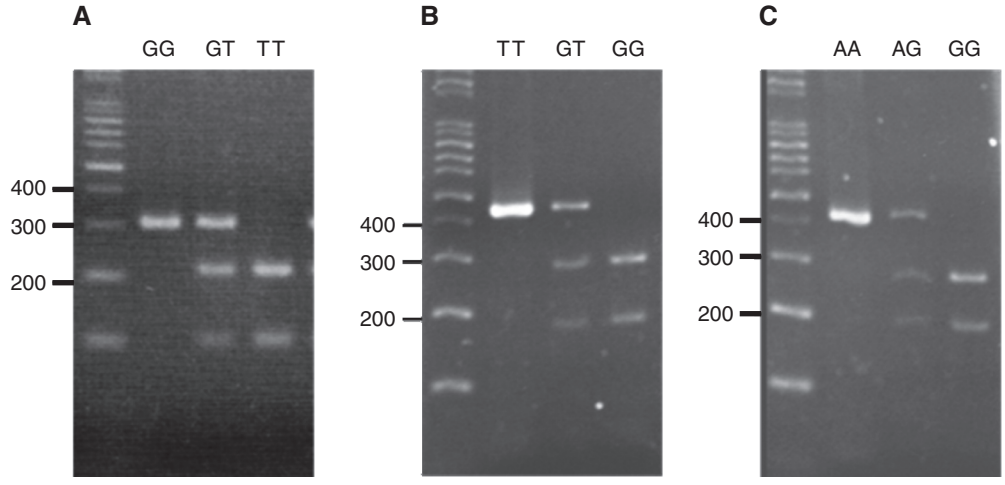

Fig. 1. PCR-RFLP patterns of *SETX* polymorphisms. Polymorphisms data were obtained after distinct restriction enzyme digestion (see Table 1). (a) 3455T>G (rs3739922) (b) 3576T>G (rs1185193) (c) 7759A>G (rs1056899).

specific melting temperature for each primer pair for 30 sec (annealing), and 72°C for 40 sec (extension), with a final elongation step at 72°C for 7 min. Five microliters of PCR amplicons were digested overnight by various restriction enzymes as described in Table 1 (New England Biolabs, MA, USA) at 37°C in a total volume of 20 μl. The PCR products and digestion fragments were separated by agarose gel electrophoresis and ethidium bromide staining.

Statistical Analysis

The distributions of allele and genotype frequencies for each SNP were determined by χ^2 test using 2×2 and 2×3 contingency tables. The odds ratios (OR) and 95% confidence interval (CI) were calculated using SPSS version 10.0 software (Chicago, IL, USA) according to the presence of the reference allele and genotype frequencies. Adherence to Hardy-Weinberg equilibrium (HWE) was performed using Pearson's χ^2 test with one degree of freedom. The haplotype test for all subjects was carried out with Phase v2.1 software program using Bayesian algorithm (23). The statistical analysis was determined by comparing a given haplotype with a combination of all other haplotypes. $P < 0.05$ was considered statistically significant.

Results

A total of 210 subjects of Taiwanese Han Chinese comprised this study cohort. One hundred and twenty subjects were diagnosed as having AD, and 90 subjects served as healthy controls. Three exonic gene variants in the *SETX* gene were selected for this study: 3455T>G (rs3739922), 3576T>G (rs1185193) and 7759A>G (rs1056899), and these gene variations will cause amino acid substitutions at positions 1152Phe>Cys, 1192Asp>Glu and 2587Ile>Val, respectively. Changes of amino acid residues might cause alteration of protein activities. Genetic variants of each subject were identified in the presence of distinct restriction enzymes (Fig. 1); the distribution of genotypes and allele frequencies for the three polymorphisms are shown in Table 2. The numbers of AD patients and normal controls did not match the total numbers of the recruited participants, because some samples with poor quality of genomic DNA could not be successfully examined.

Table 2. Genotype and allele frequencies of *SETX* gene polymorphisms among AD patients and healthy controls

SNPs	Genotype/allele	Cases (%)	HWE[a]	Controls (%)	HWE	*P*-value*	OR (95% CI)
3455T>G	TT	26 (22.4)	0.024	38 (42.7)	0.925	0.0080	0.38 (0.15-0.92)
	TG	70 (60.3)		40 (44.9)			0.96 (0.42-2.21)
	GG	20 (17.2)		11 (12.4)			1
	T	122 (52.6)		116 (65.2)		0.0105	0.59 (0.40-0.89)
	G	110 (47.4)		62 (34.8)			1
3576T>G	TT	37 (32.7)	2.0×10^{-5}	43 (50.0)	0.189	0.0146[b]	1.72 (0.30-9.94)
	TG	74 (65.5)		39 (45.3)			3.79 (0.67-21.65)
	GG	2 (1.8)		4 (4.7)			1
	T	148 (65.5)		125 (72.7)		0.1259	0.71 (0.46-1.10)
	G	78 (34.5)		47 (27.3)			1
7759A>G	GG	44 (37.6)	2.1×10^{-5}	48 (55.2)	0.919	0.0026[b]	2.75 (0.53-14.34)
	GA	71 (60.7)		33 (37.9)			6.45 (1.24-33.70)
	AA	2 (1.7)		6 (6.9)			1
	G	159 (67.9)		129 (74.1)		0.1748	0.74 (0.48-1.14)
	A	75 (32.1)		45 (25.9)			1

[a]HWE, *P*-values of deviation from Hardy-Weinberg equilibrium constant. *P*-values were compared by χ^2 test* and Fisher's exact test[b]. OR, odds ratio; 95% CI, 95% confidence interval.

Table 3. Haplotype frequencies of *SETX* gene polymorphisms between AD patients and controls

Haplotypes[a]		Cases (%)	Controls (%)	*P*-value*	OR (95% CI)
Ht1	GAG	79 (35.6)	61 (33.9)	0.7225	1.08 (0.71-1.63)
Ht2	TAG	67 (30.2)	69 (38.3)	0.0858	0.70 (0.46-1.05)
Ht3	TCA	52 (23.4)	43 (23.9)	0.9130	0.97 (0.61-1.55)
Ht4	GAA	10 (4.5)	1 (0.6)	0.0158	8.44 (1.07-66.60)
Ht5	GCA	10 (4.5)	1 (0.6)	0.0158	8.44 (1.07-66.60)
Ht6	TCG	4 (1.8)	5 (2.8)	0.5107	0.64 (0.17-2.43)

[a]Haplotypes are shown in sequence: rs3739922T>G, rs1185193T>G, and rs1056899A>G. **P*-value was compared by χ^2 test. OR, odds ratio; 95% CI, 95% confidence interval.

Our data indicated that in the analysis of Hardy-Weinberg equilibrium (HWE) for the three genetics variants, the genotype distributions of healthy controls were in the HWE (all $P > 0.1$), but in the whole cohort of patients, there appeared a significant deviation of HWE ($P = 0.024$, 2.0×10^{-5} and 2.1×10^{-5}, respectively, Table 2). After analyzing allele frequency, the 3455T>G polymorphism attained a statistically significant association with AD (Table 2). The 3455T>G variant was T:G 52.6%:47.4% in AD patients, compared with 65.2%:34.8% in controls (Table 2). The OR of the T allele indicated a 0.59-fold lower risk than with the G allele (95% CI, 0.40 to 0.89), suggesting that the G allele at 3455T>G is a risk factor that correlates with AD. The distribution of genotype frequency of 3455T>G polymorphism also presented a statistical difference ($P = 0.008$). In this SNP, the TT:GG ratio was 22.4%:17.2% in the subjects with AD and 42.7%:12.4% in controls. The OR of the TT genotype indicated a protective effect than with the GG genotype (OR = 0.38, 95% CI, 0.15 to 0.92). On genotyping the SNP 7759A>G, we found that this variant exhibited statistical difference at the 0.05 level in genotype frequency ($P = 0.0026$) (Table 2). The GA:AA ratio was 60.7%:1.7% in the subjects with AD compared with 37.9%:6.9% in controls. The OR of the GA genotype indicated a 6.45-fold higher risk than with the AA genotype (95% CI, 1.24 to 33.70), suggesting that the subjects with the GA genotype at 7759A>G have higher incidences of AD development.

We also analyzed the haplotype distributions association between patients and controls. The haplotype frequencies of the *SETX* gene at the three polymorphic loci are shown in Table 3, and the six haplotypes were observed in both AD patients and controls. The frequency of the most common haplotype (Ht1-GAG) in the patients was 35.6%, compared with 33.9% in the controls. After haplotype-specific analysis, both

Ht4-GAA and Ht5-GCA haplotypes appeared to be significant 'risk' haplotypes (both $P = 0.0158$, OR = 8.44, 95% CI, 1.07-66.60) compared with either the non-Ht4 or non-Ht5 haplotype in AD patients and control groups. Similar haplotype-specific analyses showed no significant differences between the two groups of subjects for the four other haplotypes, Ht1-GAG, Ht2-TAG, Ht3-TCA and Ht6-TCG. These results may be interpreted as a subtle hint that individuals carrying the Ht4-GAA and Ht5-GCA haplotypes have a higher risk of developing AD.

Discussion

Excessive Aβ peptides demonstrably increase the risk of synaptic loss, which may disturb brain functions, before neuronal death (12, 26). Most cases of AD are sporadic, and factors important in disease development are still unclear, which motivated us to attempt to identify additional genetic factors that are also responsible for AD progression. Many reports had shown the relation between genetic variations and neural degenerative disease (13, 15). For example, it had been proved that polymorphism in the cystatin C (CST3) gene was associated with AD and vascular dementia in a Chinese Han Population in Taiwan (15). We performed a case-control study and found genetic variations of the *SETX* gene. The gene product of which is involved in regulation of transcription termination and DNA repair, and found that such genetic variations in *SETX* were highly associated with etiology of AD in Taiwanese population.

Three gene variants in the *SETX* selected in this study were 3455T>G, 3576T>G, and 7759A>G. *SETX* encodes a protein with 2,677 amino acids. The N-terminal domain (1-667 amino acids) of SETX is critical for the mitotic progression to enter S/G2 phases and the C terminus contains a helicase domain and a nuclear localization sequence, suggesting that SETX functions as a putative RNA/DNA helicase (2, 27). Several missense mutations located in the N- and C-terminal domains of SETX have been identified in patients with ALS4 and AOA2 (2). Although the three *SETX* SNPs chosen for the present study are not located at the N- and C-termini, our data showed that genetic variations in these three SNPs were associated with significantly higher susceptibility to AD, suggesting that disturbance of SETX functions might cause abnormal neuronal activities, eventually giving rise to neuronal death. In this study, we found that the distributions of genotype frequencies of the three tested SNPs in the AD cases, but not in normal controls, significantly deviated from HWE. Departure from HWE may result from failure in the requisite assumptions of HWE (*e.g.* selection, non-random mating, genetic drift, mutation) (9). Each of these factors would affect the inheritance pattern of the SNPs involved (3). The other theory to explain this result is that more complex models (*i.e.* non-Mendelian inheritance) might lead to this result. For example, epistasis, which could not be used to analyze the data in this study, may explain this association (6, 14).

In yeast, Sen1 was first identified as an RNA/DNA helicase and mediated efficient transcription termination (10). Human SETX has been known to interact with RNAPII subunits and is involved in the RNAPII-dependent transcription termination process. Hence, defects in SETX could cause disturbances in protein expression, giving rise to abnormal neuronal activities. This might be a reason why that mutations in the *SETX* gene are associated with two motor neuron degenerative diseases, AOA2 and ALS4.

In biochemical studies of yeast, loss of Sen1 to accumulate RNA/DNA hybrids (R loops) has been reported to exhibit higher incidence of genomic mutation or recombination during transcription, presumably because forming R loop exposes single-strand nontemplate DNA and elicits transcription-associated homologous recombination to repair ssDNA damage (17). In the cell analysis, human SETX also interacts with proteins required for DNA repair including MRE11 and RAD50, suggesting that SETX is likely to serve a purpose to repair DNA damage (27). Taken together, these data show that SETX helicase plays a pivotal role in the R loop resolution and in the prevention of genome instability raised from R loop-mediated DNA damage.

Mounting evidence suggests that at least two molecular mechanisms are contributed to the pathogenesis of AD. One is Aβ hypothesis. Overproduction of Aβ and their assembly into aggregated forms may appear to cause potent neurotoxicity and lead to the disturbance of neurotransmission and even advanced cognitive behavior, contributing to the unique AD etiology (11, 24). Another cause of AD is aberrant mitotic signal altering neuronal phenotype that plays early roles in the pathogenesis of AD (25).

Progression of DNA replication slows down while replication forks collide with DNA lesions, R loops or transcription units and these conditions might cause toxicity to cells due to higher rates of recombination and chromosomal instability (1, 7). Since SETX acts to prevent from forming the R loop barriers and facilitates the fork progression, therefore suppressing SETX function could affect RNA biosynthesis and manifest higher levels of DNA damage and genomic rearrangements. Although, to get a risk marker tested, a long time follow-up experiment from the normal people without AD remains to be performed, our observations might provide some evidence that failure to regulate DNA replication and RNA transcription by deficient SETX could contribute, in part, to the

pathogenesis of AD.

Our studies is the first report which focuses on the SNPs in motor neuron degenerative disease related gene "*SETX*" and their effects on AD risk in the Taiwanese Han population. Although these three SNPs located in coding sequence are not on the N-terminal protein-protein interaction domain or C-terminal nuclear localization signal (NLS) and DNA/RNA helicase domain, these SNPs are missense substitutions resuiting in a single amino acid change may cause the conformational change to affect protein functions. It seems that *SETX* gene plays a interesting role for advanced neuropathology research, because its mutations and SNPs are associated with three neurodegenerative diseases, AOA2, ALS4 and AD. The T allele of SETX 3455T>G, GA genotype of SETX 7759A>G, Ht4-GAA and Ht5-GCA haplotypes may be developed into potential biomarkers for early detection and prediction. Because Alzheimer's is a multi-factor disease. Hence, our findings can also provide a new insights in AD molecular pathogenic process.

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