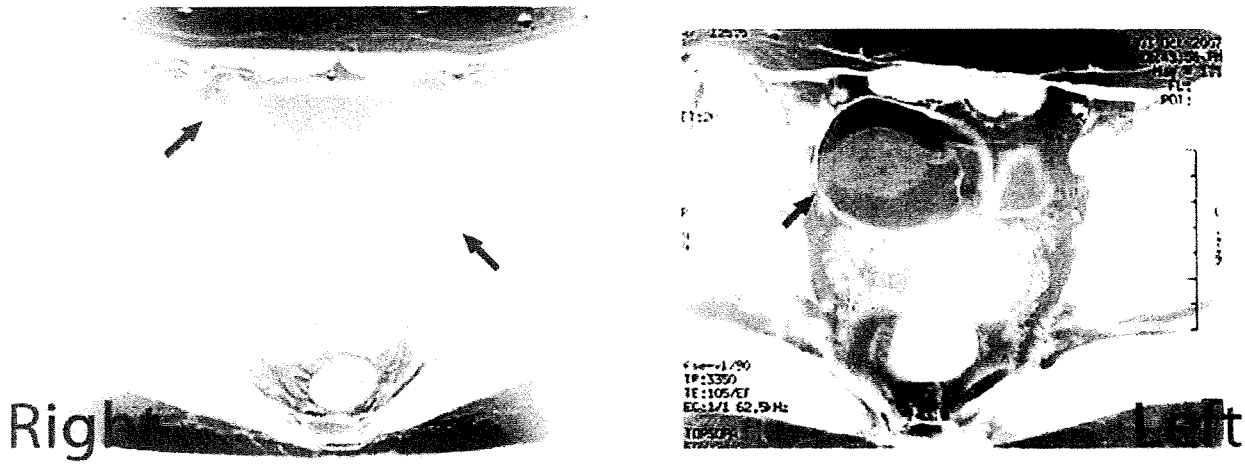

Fig. 1. Ovarian teratomas demonstrated on pelvic MRI. **A** Axial T_2-weighted fat suppression image of patient 1 one year after discharge shows bilateral ovarian cystic tumors (arrows) with the presence of fat. **B** Axial T_2-weighted image of patient 2 five years after discharge shows a right ovarian cystic tumor with the presence of a fluid level. These tumors are suspected of mature ovarian teratoma.

trast, Iizuka et al. [7] reported that patients without tumor resection had recovered though the duration of ventilatory support had been significantly longer than that of patients with tumor resection. Seki et al. [8] reported that early removal of the ovarian teratoma had resulted in prompt neurological response. Both of our patients had ovarian teratoma(s), but they finally showed excellent recovery without resection. The tumors did not change in size for year(s). These consequences suggest that tumor resection may not always be necessary though recovery may be late.

Acute onset of encephalitis after prodromal infection symptoms and spontaneous recovery associated with disappearance of the NMDAR antibodies despite the long-standing existence of ovarian teratoma(s) in the present cases and those reported by Iizuka et al. [7] remain to be clarified. Abnormal improvement reaction may be triggered by acute viral infection or other events, and autoantibodies against NMDAR may be produced temporally in these patients.

We reported 2 cases of NMDAR-related encephalitis with ovarian teratoma. They recovered without tumor resection. The timing and indication of tumor resection remain to be clarified.

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BRIEF RESEARCH COMMUNICATION

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Neuropsychiatric Genetics

TRPM7 Is Not Associated With Amyotrophic Lateral Sclerosis-Parkinsonism Dementia Complex in the Kii Peninsula of Japan

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Amyotrophic lateral sclerosis-parkinsonism dementia complex (ALS/PDC) is a distinct neurodegenerative disorder characterized by ALS pathology with neurofibrillary tangles (NFTs) in the spinal cord and brain. Recent clinical studies have revealed a high incidence and a high familial occurrence of ALS/PDC in both Guam and the Kii peninsula of Japan, suggesting a strong genetic predisposition to this disorder. The T1482I variant (rs8042919) of *TRPM7* gene which is suggested to play roles in regulating the cellular homeostasis of Ca^{2+}, Mg^{2+}, and trace metals, has recently been reported to be associated with Guamanian patients with ALS/PDC. To investigate whether *TRPM7* is associated with Kii ALS/PDC, we conducted parametric linkage analyses of the *TRPM7* locus in a large extended family with ALS/PDC. Linkage analysis did not reveal any evidence supporting the linkage to the *TRPM7* locus. Resequencing of the entire coding region of *TRPM7* did not reveal any pathogenic mutations in an affected individual in this family. The allele frequencies of the T1482I in affected individuals in this family or in those from other families are not significantly different from those in regional controls or those in HapMap-JPT samples. These results indicate that *TRPM7* is not associated with ALS/PDC in the Kii peninsula of Japan. © 2009 Wiley-Liss, Inc.

Key words: ALS/PDC; extended family; *TRPM7* gene; linkage analysis; resequencing

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INTRODUCTION

Amyotrophic lateral sclerosis and parkinsonism-dementia complex (ALS/PDC) is a unique form of ALS highly prevalent in the island of Guam, southern West New Guinea, and the Kii peninsula of Japan [Kimura, 1961; Elizan et al., 1966; Gajdusek and Salazar, 1982]. Both Guamanian and Japanese patients with ALS/PDC are pathologically characterized by neurofibrillary tangles (NFTs) in the brain and spinal cord in addition to the ALS pathology affecting the upper and lower motor neurons [Hirano et al., 1961]. High incidences of ALS in Guam and the Kii peninsula of Japan have been reported since the 1950s [Kurland and Mulder, 1954] and 1960s [Kimura, 1961], respectively. Recent studies have indicated that the PDC type is still common, but also that the incidence of the ALS type is decreasing in both Guam and the Kii peninsula of Japan [Plato et al., 1969; Kuzuhara, 2007]. Because the disease focus occurs in a restricted area among three genetically different populations, genetic and/or environmental factors have been proposed as the etiologies of this disorder. A recent epidemiological study of Kii ALS/PDC has revealed that approximately 70% of patients have a family history of ALS/PDC [Kuzuhara et al., 2001;

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Kuzuhara, 2007]. Furthermore, families with multiple cases of ALS/PDC are common in Guam [Kurland and Mulder, 1955; Morris et al., 2004]. These observations made in both Guam and the Kii peninsula of Japan strongly suggest the involvement of genetic factors in ALS/PDC.

A comprehensive mutational analysis of 19 candidate genes including ALS/FTLD-related genes (*SOD2*, *SOD3*, *ALS2/alsin*, *SMN1*, *PGRN*, *ANG*, *VEGF*, *VCP*, *VAPB*, *DCTN1*, *CHMP2B*, and *TARDBP/TDP-43*), the tauopathy-related gene (*GSK3β*), and parkinsonism-related genes (*α-synuclein*, *LRRK2*, *parkin*, *DJ-1*, *PINK1*, and *ATP13A2*) did not reveal any mutations in these genes in the patients with ALS/PDC [Tomiyama et al., 2008]. The T1482I variant of transient receptor potential melastatin 7 gene (*TRPM7*) has recently been reported to be associated with five Guamanian ALS/PDC patients [Hermosura et al., 2005]. *TRPM7* is a member of the TRP superfamily of ion channels that has been suggested to play roles in the homeostatic regulation of Ca^{2+} and Mg^{2+}. The association of the *TRPM7* variant with ALS/PDC may support the environmental factor hypothesis that prolonged exposure to low level of Ca^{2+} and Mg^{2+} contributes to the high incidence of development of ALS/PDC [Garruto, 1991]. To explore the implication of *TRPM7* in Kii ALS/PDC, we have conducted parametric linkage analyses of the *TRPM7* locus, and resequenced the entire coding region of *TRPM7* of an affected individual using a large extended family with ALS/PDC in the Kii peninsula of Japan. We further compared the frequencies of T14821 in the affected individuals in this family or in those from other families with those in regional controls or HapMap-JPT samples to investigate the potential association of T1482I with ALS/PDC in the Kii penisula.

MATERIALS AND METHODS

Samples

Genomic DNA was extracted from peripheral leukocytes according to standard protocol after obtaining informed written consent from patients. The clinical and pathological evaluations of the family members are described elsewhere [Tomiyama et al., 2008]. The research project was approved by the ethics committee of Niigata University, Mie University School of Medicine, and the University of Tokyo.

Linkage Analysis of the TRPM7 Locus on Chromosome 15q21.2

Parametric pair-wise linkage analysis of the TRPM7 locus was performed using the Superlink program (http://bioinfo.cs.technion.ac.il/superlink/) [Fishelson and Geiger, 2002] with the 23 family members including the 8 affected individuals (Supplementary Figure, A family). The pedigree information was updated based on information obtained after publication of our previous study [Tomiyama et al., 2008]. Pair-wise lod scores at D15S978 and D15S1016 flanking the TRPM7 locus at 1.6 Mb upstream and 2.6 Mb downstream, respectively, were obtained using autosomal dominant (AD) and autosomal recessive (AR) models. A disease gene frequency of 0.01 and penetrance rates of 0.9 and 1.0 were used.

Resequencing of Entire Coding Regions of TRPM7

Coding regions of *TRPM7* were amplified by polymerase chain reaction (PCR) using TaKaRa LA Taq (TaKaRa, Tokyo, Japan) for one patient (V-10) clinically diagnosed with ALS/PDC. The primers were designed using ExonPrimer (http://ihg2.helmholtz-muenchen.de/ihg/ExonPrimer.html; see Supplementary Data). The PCR products were purified using ExoSAP-IT (USB), and subjected to direct nucleotide sequence analysis using a BigDye terminator Cycle Sequencing kit v3.1 and an ABI3100 sequencer (Applied Biosystems, Foster City, CA). The obtained sequence data were analyzed by Variant Reporter™ Software v1.0 (Applied Biosystems).

Association Analysis of T1482I

The allele frequencies of T1482I was investigated in other 7 patients with ALS/PDC included in the linkage study (VI-2, VI-7, VI-9, VI-17, VI-18, VI-21, and VII-4) and another 1 recently diagnosed patient (VI-20) in the A family (Supplementary Figure), 16 Kii-ALS/PDC index patients from other families (6 multiplex families and 10 apparently sporadic patients), and 27 control subjects living in the same region by resequencing exon 28 in *TRPM7* using a primer pair of 5′-TGGTGTCCAGGTAGAATAAAG-3′ and 5′-TTCACTGCTCATGTGTTTGAC-3′, or that described in the Supplementary Table (Exon28F and Exon28R).

Association analysis of T1482I variant was conducted with χ^2 analysis of the allele frequencies of 9 patients in family A, or of 16 index patients in other families, and those in 27 regional controls, or HapMap-JPT samples.

RESULTS

Pair-wise LOD scores were −infinity at both D15S978 and D15S1016 for AD and AR models with complete penetrance. We obtained LOD scores of 0.64/−0.62 and 0.51/−1.65 for AD and AR

TABLE I. Pair-Wise LOD Scores at D15S978 and D15S1016

Mode of inheritance	AD model		AR model	
Penetrance	0.9	1.0	0.9	1.0
D15S978	0.64	−infinity	0.51	−infinity
D15S1016	−0.62	−infinity	−1.65	−infinity

TABLE II. Comparison of Allele Frequencies of T1482I in Patients With Those in Controls

	No. of subjects (frequency)			
	Affected in A family	Affected in other families	Control in the region	HapMap-JPT
Genotype				
T/T	0 (0.0%)	1 (6.3%)	0 (0.0%)	2 (4.4%)
C/T	2 (22.2%)	4 (25.0%)	11 (40.7%)	16 (35.6%)
C/C	7 (77.8%)	11 (68.8%)	16 (59.3%)	27 (60.0%)
	No. of alleles (frequency)			
	Affected in A family	Affected in other families	Control in the region	HapMap-JPT
Allele				
T	2 (11.1%)	6 (18.8%)	11 (20.4%)	20 (22.2%)
C	16 (88.9%)	26 (81.3%)	43 (79.6%)	70 (77.8%)
P-value	0.38[a] [0.29][b]	0.86[a] [0.68][b]		

[a] *P* values of χ^2 tests obtained by comparison of allele frequencies of T1482I between affected individuals and the regional controls are shown.
[b] *P* values of χ^2 tests obtained by comparison of allele frequencies of T1482I between affected individuals and HapMap-JPT samples are shown in parenthesis. There was no significant difference in the allele frequencies of T1482I between the regional controls and HapMap-JPT samples ($P = 0.79$).

models with incomplete penetrance (0.9), respectively (Table I). These results suggest that the linkage of Kii ALS/PDC to the *TRPM7* locus is unlikely in this family.

Resequencing of the entire coding regions of TRPM7 of a patient (V-10) from family A revealed two homozygous SNPs in introns (IVS3-26G>C [rs2063011] and IVS22-41T>A [rs675011]). Because both are present in the majority of the HapMap samples, they are unlikely to be pathogenic for Kii ALS/PDC.

Allele frequencies of T1482I variant (rs8042919) in the nine affected individuals in the A family are similar to those in regional controls ($P = 0.38$; Table II). We further extended the analysis to the 16 index patients from other families, but we did not observe any significant association of T1482I with ALS/PDC ($P = 0.86$). Allele frequencies of T1482I in the regional controls are similar to those in the HapMap-JPT samples ($P = 0.79$; dbSNP: http://www.ncbi.nlm.nih.gov/SNP/). Resequencing of exon 28 of TRPM7 revealed a previously described polymorphism (IVS28+15 C/T, rs3109894), which also did not show any significant association with ALS/PDC. Taken together, we conclude that the T1482I variant is not associated with Kii ALS/PDC.

DISCUSSION

In this study, we did not obtain any supportive evidence for the genetic linkage of Kii ALS/PDC to the *TRPM7* locus and resequencing analysis of the entire *TRPM7* of the affected individual did not reveal any causative mutations. Furthermore, the allele frequencies of T1482I in the affected individuals are not significantly different from those in regional controls or those in HapMap-JPT samples. Thus, it is unlikely that T1482I or *TRPM7* is associated with the Kii-ALS/PDC.

The structure of a large extended family with ALS/PDC in the Kii of Japan is complex for explicitly determining the inheritance mode [Tomiyama et al., 2008]. There are three consanguineous marriages in this pedigree, suggesting an AR pattern, whereas the disease occurs partially in the successive generations, suggesting an AD pattern with reduced penetrance. The complexity of the inheritance pattern has also been discussed with regard to ALS/PDC families in Guam. Formal segregation analysis of Guamanian ALS/PDC families rejected both dominant and recessive models, but were consistent with a 2-allele major locus model [Bailey-Wilson et al., 1993]. Indeed, a genome-wide association study of Guamanian ALS/PDC using 834 microsatellite markers did not provide any associated markers with a genome-wide significant level ($P < 0.0001$). Furthermore, pair-wise linkage analysis of 17 microsatellite markers in which they determined the threshold for further study ($P < 0.015$) has shown some interesting loci such as D3S2406 (LOD score = 0.78) and D20S103 (LOD score = 1.82) but failed to identify a convincing single locus [Morris et al., 2004]. These results suggest that familial ALS/PDC is not caused by a mutation of a single gene but is a complex disease involving genetic and environmental factors. Further extended association and linkage studies employing high-density single nucleotide polymorphisms (SNPs) and a larger sample size may be useful to identify susceptibility genes for the Kii ALS/PDC.

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