

Ryuki Hirano · Hiroshi Takashima · Ryuichi Okubo ·
Keiko Tajima · Yuji Okamoto · Shimon Ishida ·
Kazuhiro Tsuruta · Takayo Arisato · Hitoshi Arata ·
Masanori Nakagawa · Mitsuhiro Osame ·
Kimiyo Arimura

Fine mapping of 16q-linked autosomal dominant cerebellar ataxia type III in Japanese families

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Abstract The autosomal dominant cerebellar ataxias (ADCAs) are a clinically and genetically heterogeneous group of disorders. To date, at least 11 genes and 13 additional loci have been identified in ADCAs. Despite phenotypic differences, spinocerebellar ataxia 4 (SCA4) and Japanese 16q-linked ADCA type III map to the same region of 16q22.1. We report four Japanese families with pure cerebellar ataxia and a disease locus at 16q22.1. Our families yielded a peak lod score of 6.01 at marker D16S3141. To refine the candidate region, we carried out genetic linkage studies in four pedigrees with a high density set of DNA markers from chromosome 16q22.1. Our linkage data suggest that the disease locus for 16q-ADCA type III is within the 1.25-Mb interval delineated by markers 17msm and CTTT01. We screened for mutations in 36 genes within the critical region. Our critical region lies within the linkage interval reported for SCA4 and for Japanese 16q-ADCA type III. These data suggest that the ADCA that we have characterized is allelic with SCA4 and Japanese 16q-linked ADCA type III.

Keywords Chromosome 16 · Positional cloning · 16q-ADCCA · Spinocerebellar ataxia 4 · Japan

Introduction

Autosomal dominant cerebellar ataxias (ADCAs) are clinically and genetically heterogeneous. ADCAs are classified into three subgroups according to their clinical features [1]. ADCA type I is characterized by ophthalmoplegia, optic atrophy, dementia, and extrapyramidal features. ADCA type II is characterized by retinopathy and extrapyramidal features. ADCA type III is characterized by isolated late-onset cerebellar ataxia.

To date, 11 genes and 13 additional loci have been associated with ADCAs. The causative mutations in 9 of these 11 genes are expansions of a repetitive element. Expansions of polyglutamine tracts account for spinocerebellar ataxia 1 (SCA1) [2], SCA2 [3,4], SCA3 [5], SCA6 [6], SCA7 [7], and SCA17 [8]. Expansion of a CTG repeat within a non-coding RNA is responsible for SCA8 [9], expansion of the pentanucleotide ATTCT repeat within intron 9 of the SCA10 gene causes SCA10 [10], and a CAG repeat expansion within the promoter of *PPP2R2B* causes SCA12 [11]. Recently, missense mutations of *PKCγ* have been identified as a cause of SCA14 [12], and a mutation of *FGF14* has been reported as a cause of SCA [13]. In addition to these identified disease genes, disease loci have been mapped for SCA4 [14], SCA5 [15], SCA11 [16], SCA13 [17], SCA15 [18], SCA16 [19], SCA18 [20], SCA19 [21], SCA20 [22], SCA21 [23], SCA22 [24], SCA24 [25], and SCA25 [26], although the responsible gene has not been identified yet.

SCA4 is an ADCA type I and is characterized by cerebellar ataxia with sensory axonal neuropathy. The SCA4 disease locus was originally mapped to 16q22.1 in a Utah family [14] and has subsequently been confirmed in SCA4 families from Germany [27].

Interestingly, the Japanese ADCA families that have been mapped to 16q22.1 (16q-ADCA type III) belong to

R. Hirano · H. Takashima (✉) · R. Okubo · K. Tajima ·
Y. Okamoto · T. Arisato · H. Arata · M. Osame · K. Arimura
Department of Neurology and Geriatrics,
Kagoshima University School of Medicine,
8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan
e-mail: thiroshi@m3.kufm.kagoshima-u.ac.jp
Tel.: +81-99-2755332
Fax: +81-99-2657164

S. Ishida
First Department of Internal Medicine,
Osaka Medical College,
Daigaku-cho 2-7, Takatsuki, 569-8686 Osaka, Japan

K. Tsuruta
Department of Neurology,
Koga General Hospital,
Ikeuchi-cho, 1749-11 Sudaki Miyazaki, Japan

M. Nakagawa
Department of Neurology and Gerontology,
Kyoto Prefectural University of Medicine,
Kawaramachi Hirokoji, Kamikyo-ku, Kyoto 602, Japan

ADCA type III because those patients have pure cerebellar ataxia [28]. On this basis, therefore, others have speculated that these Japanese patients have an allelic disorder of SCA4. Currently, the candidate disease locus of SCA4 encompasses an approximately 3.69-cM interval between D16S3019 and D16S512 and overlaps the 3.8-Mb disease locus between GGAA05 and D16S3095 that was mapped in the Japanese families [27,29].

Using four families living in southern Japan and segregating ADCA type III, we linked ADCA in these families to the SCA4 disease locus and narrowed the interval for the Japanese families with 16q-ADCA type III from 3.8 Mb to 1.25 Mb. Also, by direct sequencing of characterized and predicted coding exons within this interval, we excluded coding mutations within genes annotated in this interval.

Materials and methods

Human subjects and clinical assessment

Four ADCA type III families were studied (families M01, M02, M03, and K01). All four families originate from southern Japan. Expert neurologists examined all family members. We defined affected individuals as only those with cerebellar signs.

In total, we examined 30 family members, of whom 20 were affected. We list the ages of unaffected family members in Fig. 1. All patients were referred by their primary physician or neurologist and signed informed consents approved by the Institutional Review Board of Kagoshima University.

Mutation analysis of known SCA genes

We isolated DNA from peripheral blood leukocytes of each patient. Prior to entry into our study, we screened each patient for all triplet repeat expansions associated with SCA (SCA1–3, 6–8, 12, 17, DRPLA) [2, 3, 4, 5, 6, 7, 8, 9, 11,30].

Linkage analysis

We performed linkage analyses of all family members using markers specific for identified SCA loci: chromosome 16q22.1 for SCA4 [14,27], chromosome 11p12-q12 for SCA5 [15], chromosome 22q13 for SCA10 [10], chromosome 15q14–21.3 for SCA11 [16], chromosome 19q13.3-q13.4 for SCA13 [17], chromosome 3p24.2–3pter for SCA15 [18,31], chromosome 8q23–24.1 for SCA16 [19], chromosome 7q22–q32 for SCA18 [20], chromosome 1p21–q21 for SCA19 [21], chromosome 7p21.3-p15.1 for SCA21 [23], and chromosome 1p21–q23 for SCA22 [24]. After screening these known loci by linkage analysis and identifying linkage to the SCA4 locus, we analyzed 18 markers including 3 new CA-repeat markers covering the 3.8-Mb 16q-ADCA type III region of the chromosome16q22.1 (TTCC01, D16S3086, GATA01, D16S421, CATG003, 17msm, D16S3107, D16S3085, 23msm, 25msm, D16S3025, TCTA01, CTTT01, D16S496, D16S3141, D16S3067, GT01, D16S3095). Marker information was obtained from the NCBI database and a previous report [29]. For the fine mapping, we designed 3 new CA-repeat markers (17msm, 23msm, 25msm). 17 ms m F/R (5'-ATGACCCCCTGGTCACTATG-3', 5'-CCATGTGCTTCAGGGAAGAT-3'); 23msm F/R (5'-CCAATCAAGGTAGGGTGTGC-3', 5'-ACCTAGGCCGGGTATGGT-3'); 25msm F/R (5'-CAACATCCAAGCCATCAGT-3', 5'-CCCATGTGTGTTCAGTCTTC-3')

PCR amplifications were performed as follows: aliquots (50 ng) of genomic DNA were used as templates with fluorescently labeled primers, HotStarTaq Polymerase (Qiagen), reaction buffer, and

dNTPs. After initial denaturation at 95°C for 15 min, amplification was performed for 40 cycles with denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min, and a 30-min final extension step at 72°C. PCR products and the size standard (TAMRA-GS500) were analyzed using an ABI Prism 377 Genetic Analyzer. The genotypes were determined using the GeneScan Analysis (version3.1.2) and Genotyper (version2.5) programs. Two-point linkage analysis was performed with the MLINK program of the FASTLINK (v 5.2) software package [32]. Autosomal dominant inheritance was assumed in these families. Based on our epidemiological study (data not shown), we set the disease allele frequency at 0.00001. Because the penetrance of this disease is a function of age, we assumed that the penetrance was 0 for persons aged 45 years and younger, that it was 1 for persons 65 years and older, and that for persons between 45 and 65 years it was 0.05 (age–45), which is the equation of the distribution function. Lod scores were calculated assuming equal allele frequencies.

Mutation screening

We screened for mutations in 21 known genes and 15 predicted genes defined by the NCBI annotation (<http://www.ncbi.nlm.nih.gov/RefSeq/key.html#query>). We screened each gene by sequencing the coding exons in affected patients. For published cDNAs, we identified coding exons by aligning the genomic and cDNA sequences; for predicted genes in the NCBI database, we accepted the predicted exons comprising the open reading frame. Using the Primer v3 program, we designed primers to amplify exons and intronic splice junctions. By PCR we amplified the coding exons of each gene from 50 ng of patient genomic DNA using these primers and hot start PCR method as defined for amplification of polymorphic markers. Using the pre-sequencing kit (USB), we purified patient PCR products and sequenced them with dye terminator chemistry using an ABI377 automated sequencer (Applied Bio-systems). We aligned the resulting sequences with the Sequencher sequence alignment program (Gene Codes). We numbered the nucleotides beginning with the adenine of the presumed initiating methionine and described mutations using standard nomenclature [33].

Results

Clinical features

The average age at onset was 56.7 years, and the existence of anticipation was unclear, because we could not obtain the accurate ages of onset for previous generations. Most of patients had a similar clinical phenotype: progressive ataxia, cerebellar signs, increased deep tendon reflexes, normal sensations, normal intelligence, and cerebellar atrophy on magnetic resonance imaging.

Mutation analysis and linkage of known SCA genes

We did not observe disease-associated triplet repeat expansions of SCA genes in any affected family members.

Fig. 1 Pedigrees of the four families with autosomal dominant cerebellar ataxia (ADCA) type III. *Filled symbols* indicate affected individuals and *open symbols* indicate healthy subjects. Family members less than 60 years and without clinical symptoms are indicated by *question marks*. The ages of unaffected family members are listed under the linkage data. *Oblique slashes* indicate deceased family members. *Squares* represent men and *circles* women. The conserved disease haplotype in each family is *boxed*. Furthermore, by linkage analysis, we excluded associa-

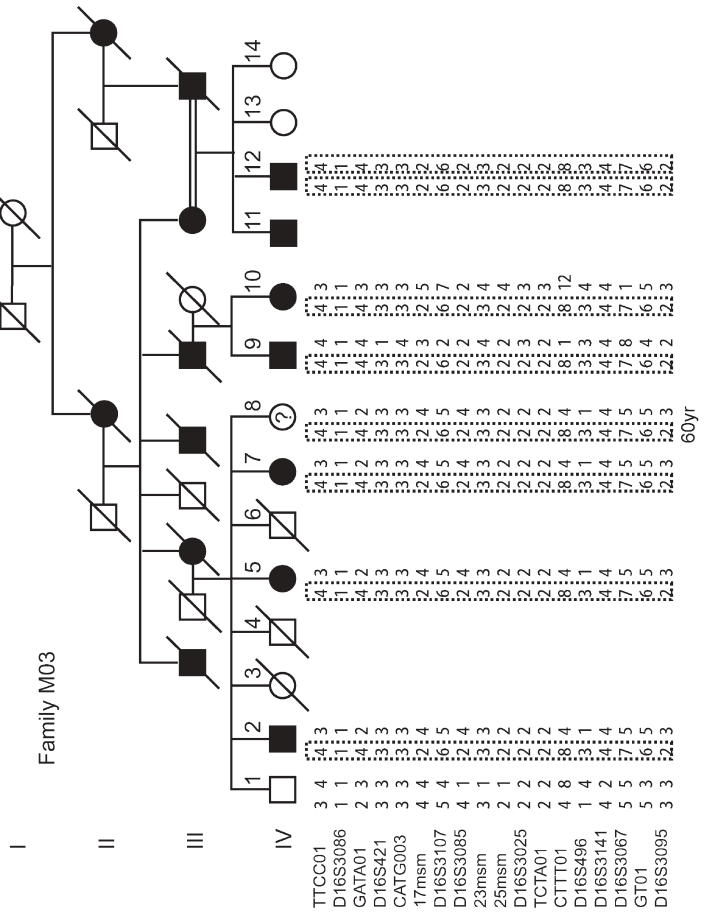
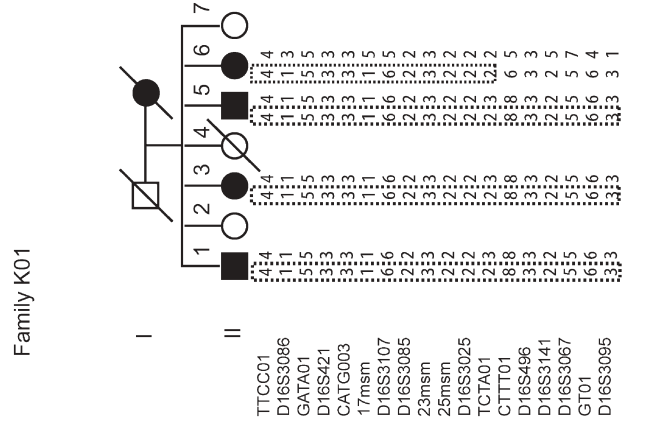
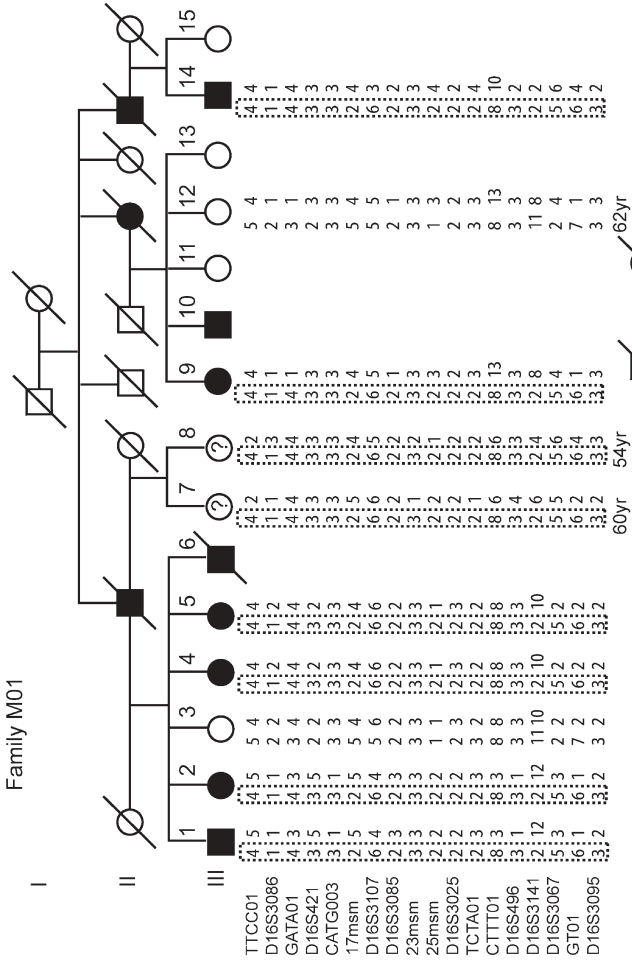
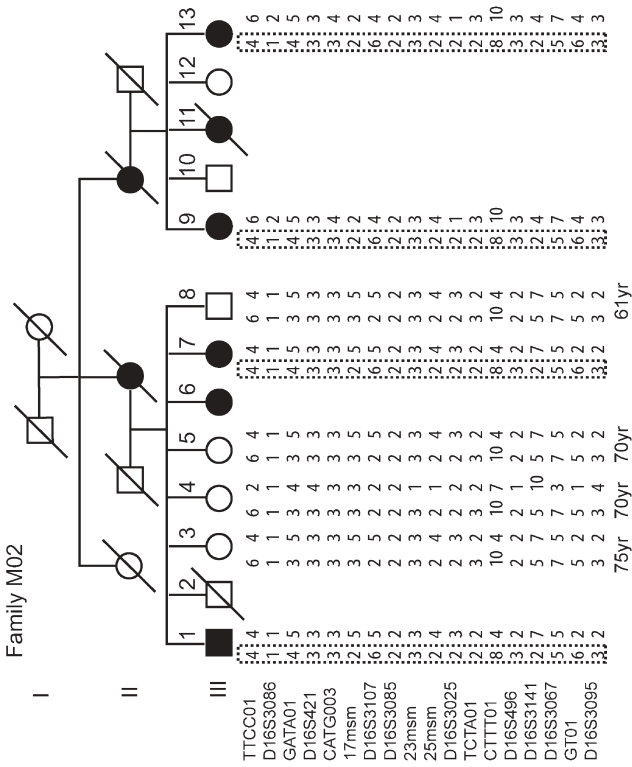


Table 1 Two-point lod scores between TTCC01 and D16S3095

Markers	Lod scores, Z at theta						
	0	0.05	0.1	0.15	0.2	0.3	0.4
TTCC01	3.23	2.81	2.38	1.97	1.56	0.8	0.23
D16S3086	1.72	1.53	1.33	1.11	0.89	0.47	0.14
GATA1	3.69	3.27	2.83	2.39	1.94	1.06	0.34
D16S421	2.99	2.64	2.29	1.94	1.59	0.91	0.3
CATG003	1.46	1.22	1	0.79	0.6	0.28	0.08
17msm	4.94	4.37	3.78	3.18	2.6	1.42	0.47
D16S3107	6	5.42	4.81	4.16	3.48	2.07	0.73
D16S3085	2.48	2.17	1.86	1.54	1.24	0.67	0.22
23msm	1.93	1.64	1.37	1.1	0.85	0.42	0.12
25msm	2.73	2.4	2.06	1.72	1.39	0.76	0.23
D16S3025	0.9	0.75	0.6	0.47	0.35	0.16	0.04
TCTA01	2.3	1.99	1.68	1.37	1.07	0.53	0.15
CTTT01	-0.97	2.31	2.17	1.91	1.58	0.89	0.29
D16S496	3.73	3.34	2.93	2.51	2.07	1.2	0.41
D16S3141	6.01	5.43	4.8	4.15	3.46	2.04	0.71
D16S3067	5.57	5.01	4.42	3.79	3.14	1.82	0.63
GT01	5.79	5.24	4.65	4.02	3.36	2	0.7
D16S3095	1.51	1.34	1.15	0.96	0.76	0.4	0.13

	M01	M02	M03	K01
TTCC01	4	4	4	4
D16S3086	1	1	1	1
GATA01	4	4	4	5
D16S421	3	3	3	3
CATG003	3	3	3	3
17msm	2	2	2	1
D16S3107	6	6	6	6
D16S3085	2	2	2	2
23msm	3	3	3	3
25msm	2	2	2	2
D16S3025	2	2	2	2
TCTA01	2	2	2	2
CTTT01	8	8	8	-
D16S496	3	3	3	3
D16S3141	2	2	4	2
D16S3067	5	5	7	5
GT01	6	6	6	6
D16S3095	3	3	2	3

Fig. 2 Haplotype analysis of 16q22.1 in four families. The disease-associated haplotypes are shown. Common alleles are indicated by dotted lines

Fig. 3 Physical map of 16q22. This map shows the critical region of SCA4/16q-ADCA type III and is based on the human genome sequence NT_010498.14. This figure shows the distance of microsatellite markers. The three bars indicate the critical regions in the study [14, 27, 28,29]. There are 36 candidate genes in our critical region

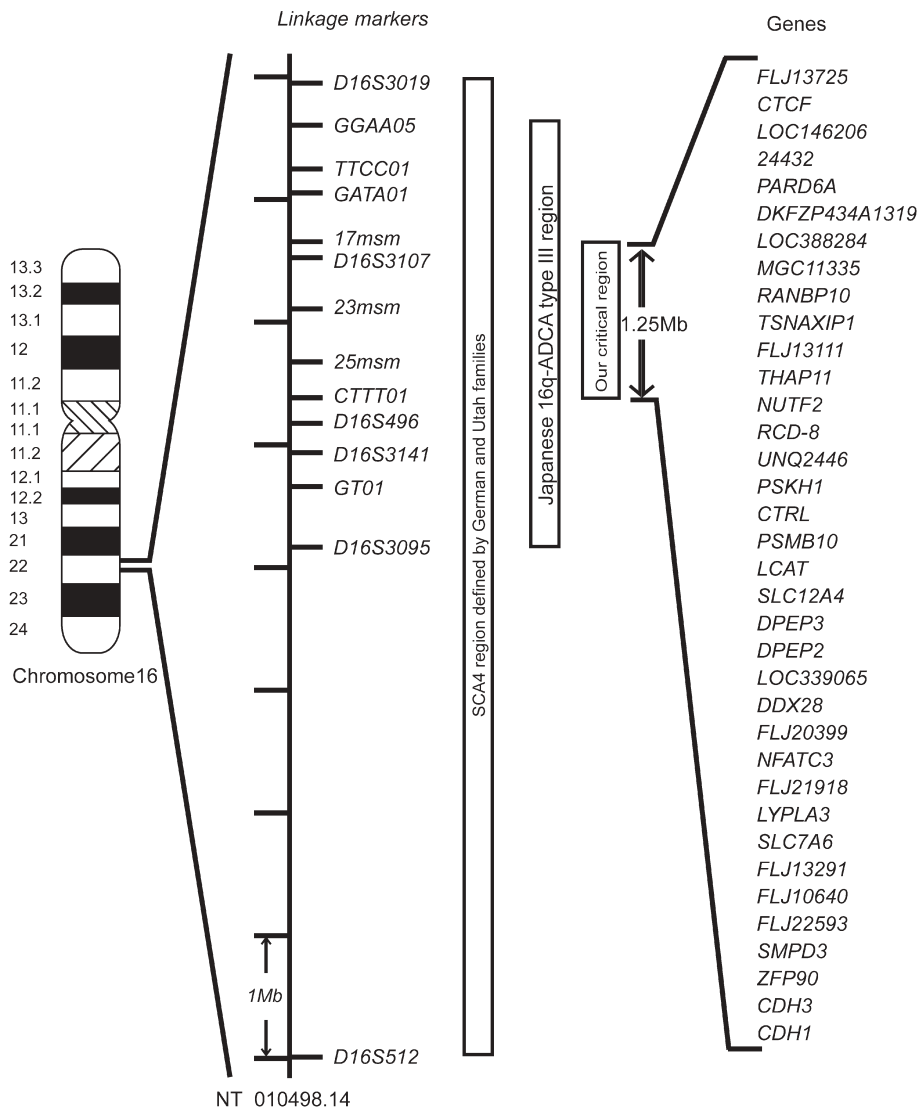


Table 2 The analyzed candidate genes and the sequence variants (*homo* homozygous mutation, *hetero* heterozygous mutation)

	Genes	GenBank accession no.	Status	No. of coding exons	Sequence variants	NCBI SNP No.
1	FLJ13725	NM_024519	Validated	21	–	
2	CTCF	NM_006565	Provisional	10	–	
3	LOC146206	XM_085383	Model	34	–	
4	24432	NM_022914	Predicted	12	–	
5	PAR6A	NM_016948	Provisional	3	–	
6	DKFZP434A1319	NM_032140	Predicted	7	–	
7	LOC388284	XM_370984	Model	4	–	
8	MGC11335	NM_030819	Predicted	2	–	
9	RANBP10	NM_020850	Provisional	14	–	
10	TSNAXIP1	NM_018430	Provisional	8	–	
11	FLJ13111	NM_025082	Predicted	8	–	
12	THAP11	NM_020457	Reviewed	1	367–369delCAG (homo)	rs3982383
13	NUTF2	NM_005796	Reviewed	4	–	
14	RCD-8	NM_014329	Provisional	27	–	
15	UNQ2446	NM_198443	Predicted	3	–	
16	PSKH1	NM_006742	Provisional	2	–	
17	CTRL	NM_001907	Provisional	7	–	
18	PSMB10	NM_002801	Reviewed	8	–	
19	LCAT	NM_000229	Reviewed	6	–	
20	SLC12A4	NM_005072	Provisional	24	–	
21	DPEP3	NM_022357	Provisional	10	1502C>T (homo)	unknown
22	DPEP2	NM_022355	Provisional	10	–	
23	LOC339065	XM_294794	Model	2	–	
24	DDX28	NM_018380	Reviewed	1	–	
25	FLJ20399	NM_017803	Predicted	15	810C>T (hetero)	unknown
26	NFATC3	NM_173165	Reviewed	13	–	
27	FLJ21918	NM_024939	Predicted	15	–	
28	LYPLA3	NM_012320	Reviewed	6	1224G>C (hetero)	unknown
29	SLC7A6	XM_375362	Model	9	–	
30	FLJ13291	NM_032178	Predicted	5	–	
31	FLJ10640	NM_019023	Predicted	17	1797C>T (homo)	rs4783622
32	FLJ22593	NM_024703	Predicted	1	–	
33	SMPD3	NM_018667	Provisional	7	–	
34	ZFP90	XM_085375	Model	9	509G>C (homo) 571C>T (homo) 822insG (homo)	rs1728770 ss10822241 unknown
35	CDH3	NM_001793	Reviewed	16	–	
36	CDH1	NM_004360	Reviewed	16	2076C>T (hetero)	rs1801552

All gene names and accession numbers were referred to NCBI. Status indicates the possibility of reality in each gene, this information is from NCBI, and each meaning of status is displayed in NCBI homepage (<http://www.ncbi.nlm.nih.gov/>)

tion with the SCA disease loci for SCA5, SCA10, SCA11, SCA13, SCA15, SCA16, SCA18, SCA19, SCA21, and SCA22 (data not shown). However, we obtained a positive two-point lod score at D16S515 for SCA4/16q-ADCA type III. To verify this association, we screened 18 additional linkage markers located in the SCA4/16q-ADCA type III candidate region between D16S3043 and D16S3095. By two-point analysis, the highest lod score was obtained at D16S3141 ($Z=6.01$ at $\theta=0$) and the lod scores of 8 markers exceeded 3.0 (Table 1). On haplotype analysis, all affected members in families M01, M02, and M03 showed the same haplotype from the marker TTCC01 to D16S3095. Genetic cross-over was detected between TCTA01 and CTTT01 in family K01.

All four of our families had a common disease-associated haplotype 6–2–3–2–2–2 for markers D16S3107, D16S3085, 23msm, 25msm, D16S3025, and TCTA01, respectively (Fig. 2). These data suggest a single founder and place the disease locus between markers 17msm and

CTTT01, a distance of 1.25 Mb. Consanguineous marriage was present in family M03 and consistent with this, IV-12 had a homozygous disease-associated haplotype in the critical region.

Mutation screening of the genes within the critical region

The physical map of the region of 16q22 on which our analyses focused is shown in Fig. 3. The critical region we identified within this locus contains 21 known genes and 15 predicted genes from the NCBI annotation. The DNA sequencing of the coding exons and 50 bases of the flanking introns were performed in at least two patients from each family. We found 9 sequence variants (two insertion/deletion mutations and 7 single nucleotide changes) in the coding exons of 7 of 36 genes (Table 2). The heterozygous mutation 822insG in *ZFP90* causes a frameshift mutation leading to premature termination, but

it does not co-segregate with the disease allele in these families and was also detected in control DNA. *THAP11* contains CAG repeats interrupted by CAA in the coding exon, which encodes 29 polyglutamines. We found one deletion of 29 polyglutamines in our patients, but this variant did not co-segregate with the disease. We confirmed the un-expanded PCR products of *THAP11* in our patients with a homozygous disease-associated haplotype to rule out the possibility of a large CAG expansion. Four novel sequence variants were detected in 4 genes (Table 2); however, these variants did not co-segregate with the disease in our families.

Discussion

The SCA4 locus was first mapped to 16q22.1 in a large Utah family in 1996 [14] and was refined to a 3.69-cM region between D16S3019 and D16S512 in German and Utah families in 2003 [27]. Japanese ADCA families also mapped within the SCA4 candidate region, and the disease was reported as "16q-linked ADCA type III" [28,29]. The clinical phenotype of the Japanese patients is clearly distinguishable from SCA4, and it is unclear whether or not they are allelic disorders. However, our critical region resides within the newly reported intervals of SCA4 and 16q-ADCA type III; this again suggests that SCA4 and 16q-ADCA type III could be allelic disorders.

We report four new Japanese families with ADCA with disease linkage to chromosome 16q22.1. All family members are from the southern part of Japan. According to the clinical classification of ADCAs, our families belong to ADCA type III, and now we have shown that they also have 16q-ADCA type III.

Comparing the molecular data from our families with that of the reported Japanese families with 16q-ADCA type III, we observed that both showed the same-sized PCR products for five DNA markers [GT01 (189 bp), CTTT01 (242 bp), TCTA01 (219 bp), CATG003 (194 bp), GATA01 (158 bp)] and different-sized PCR product for TTCC01 [26]. Based on the previous report, DNA samples were collected from a wider area of Japan. The similarities of the clinical phenotypes between our families and the previously reported Japanese 16q-ADCA type III families and the occurrence of the same haplotype in five adjacent markers suggest that most Japanese families segregating 16q-ADCA type III originated from a single founder. Therefore, we utilized the founder haplotype for our mapping and this narrowed the candidate region (Figs. 2 and 3). Finally, we reduced the critical interval to 1.25 Mb. Although there were at least 96 candidate genes for the minimum interval from previous studies [29], our linkage study reduced the candidate genes to 36.

Based on our fine mapping data, we screened the coding exons of the 36 candidate genes (21 known genes and 15 predicted genes). We identified 5 single nucleotide polymorphisms and 4 novel variants. Assuming a common founder mutation, we also excluded deletions of any exons by PCR amplification of each exon from a patient

homozygous for the disease-associated allele (data not shown); our method would have detected any homozygous deletion greater than 20 base pairs. Our observations suggest that the disease-causing mutation could arise from a genomic rearrangement or be in the promotor region, intron, or a non-coding exon of a gene that we screened or in an unidentified gene. In summary, this report provides new linkage information and thereby will facilitate the identification of the genetic basis of SCA4/16q-ADCA type III.

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