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ORIGINAL ARTICLE

A functional analysis of *GABARAP* on 17p13.1 by knockdown zebrafish

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Array-based comparative genomic hybridization identified a 2.3-Mb microdeletion of 17p13.2p13.1 in a boy presenting with moderate mental retardation, intractable epilepsy and dysmorphic features. This deletion region was overlapped with the previously proposed shortest region overlapped for microdeletion of 17p13.1 in patients with mental retardation, microcephaly, microretrognathia and abnormal magnetic resonance imaging (MRI) findings of cerebral white matter, in which at least 17 known genes are included. Among them, *DLG4/PSD95*, *GPS2*, *GABARAP* and *KCTD11* have a function in neuronal development. Because of the functional importance, we paid attention to *DLG4/PSD95* and *GABARAP*, and analyzed zebrafish in which the zebrafish homolog of human *DLG4/PSD95* and *GABARAP* was knocked down and found that *gabarap* knockdown resulted in small head and hypoplastic mandible. This finding would be similar to the common findings of the patients with 17p13.1 deletions. Although there were no pathogenic mutations in *DLG4/PSD95* or *GABARAP* in a cohort study with 142 patients with idiopathic developmental delay with/without epilepsy, further studies would be required for genes included in this region.
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INTRODUCTION

Neurodevelopmental disorders are generally characterized by neurological symptoms that occur during stages of rapid development of the nervous system. These disorders encompass a variety of symptoms, including cognitive impairment, which can range from learning disabilities to mental retardation, neuromotor dysfunction and seizure disorders. In the post-genomic era, the availability of the human genome sequence for genome-wide analysis has revealed a high frequency of neurodevelopmental disorders that result not only from genomic sequence mutations but also from genomic copy-number aberrations.[1]

Recently, the use of whole-genome scanning with array-based comparative genome hybridization (aCGH) has enabled the more accurate delineation of subtle chromosomal rearrangements.[2–5] Many novel microdeletion and duplication syndromes have been characterized using this revolutionary cytogenetic testing;[6,7] for example, 1q41q42 deletion syndrome,[8] 15q13.3 deletion syndrome,[9] 16p11.2 deletion syndrome,[10,11] 17q12 deletion syndrome[12] and 17q21.31 deletion/duplication syndrome.[13–15] Despite its small size as compared with other chromosomes, chromosome 17 is associated with many newly characterized genomic syndromes. Chromosome 17 stands out among the human chromosomes in many respects. It is the largest human autosome with orthology to only a single mouse chromosome, mapping entirely to the distal half of mouse chromosome 11. Chromosome 17 is rich in protein-coding genes, having the second-highest gene density among the human chromosomes. It is also enriched in segmental duplications, ranking third in density among the autosomes. For these reasons, human chromosome 17 has been implicated in a wide range of human diseases.[16]

The proximal chromosome 17p arm, in which >23% of the genomic sequence consists of low-copy repeats, is associated with a wide variety of recurrent chromosomal aberrations resulting from nonallelic homologous recombination between low-copy repeats, including four well-known genomic disorders: Charcot–Marie–Tooth type1A disease; hereditary neuropathy with liability to pressure palsies, which results from reciprocal duplication or deletion of 17p12;[17] Smith–Magenis syndrome and the Potocki–Lupski syndrome, which is associated with deletion or duplication of 17p11.2.[18–21] Miller–Dieker syndrome characterized by lissencephaly and characteristic facial features is caused by subtelomeric deletion of 17p, in which *PAFAH1B/LIS1* is included,[22] and heterozygous duplication of *PAFAH1B/LIS1* has been reported to cause neurodevelopmental delays.[7,23]

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Recently, four mentally retarded patients with chromosomal microdeletions sharing an overlapping segment of 180 kb in 17p13.1 was reported.[24] Although the phenotypes of the patients were variable because of the variable deletion sizes, the common features were mental retardation, microcephaly and abnormal magnetic resonance imaging (MRI) findings of the cerebral white matter.[24] We identified a new patient with a microdeletion of 17p13.1, who shared common features. In the overlapping deletion region, some candidate genes for the phenotype are included. As little is known about their contribution to the phenotype, we analyzed *DLG4/PSD95* and *GABARAP* using zebrafish embryos with morpholino knockdown system as a model organism. We also performed a cohort study in which we looked for human mutations in *DLG4/PSD95* and *GABARAP*.

MATERIALS AND METHODS

Subjects

The indicated patient was diagnosed as having a chromosomal deletion by our cohort study for genomic copy-number aberrations. After obtaining informed consent from patients' families based on the permission from the ethical committee of the institution, total of 300 peripheral blood samples were obtained from patients with idiopathic mental retardation and/or epilepsy whose etiology were unknown and were used to investigate genomic copy-number aberrations using aCGH. Then, the samples with no genomic copy-number aberrations were used to screen for candidate gene mutations. A total of 142 samples derived from patients with neurological symptoms including developmental delay with/without epilepsy were used for the second screening. Genomic DNA was extracted from peripheral blood using a QIAquick DNA extraction kit (Qiagen, Hilden, Germany).

aCGH analysis

aCGH analysis was performed using a Human Genome CGH Microarray 244 chip (Agilent Technologies, Santa Clara, CA, USA).[25] Genomic copy numbers were visualized using CGH Analytics version 3.5 software (Agilent Technologies).

Fluorescence *in situ* hybridization analysis

Metaphase or prometaphase chromosomes were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes according to the standard technique and used for fluorescence *in situ* hybridization (FISH) as described elsewhere.[25] Fifteen BAC clones, RP11-104O19, RP11-1038H1, RP11-198F11, RP11-305G1, RP11-46I8, RP11-457I18, RP11-104N2, RP11-61B20, CTD-2015K4, RP11-542C16, RP11-104H15, RP11-1081A10, RP11-89D11, RP11-1099M24 and RP11-1D5, all of which mapped around 7p13.2p13.1, and RP11-153A23, which mapped on 17q25.3 and used as a marker, were selected from the UCSC Human genome browser, March 2006 (http://genome.ucsc.edu/) (Table 1).

Microsatellite marker analyses

Microsatellite marker analyses were performed to determine the origin of the deletion using six microsatellite markers, D17S1832, D17S938, D17S796, GATA158H04, D17S1881 and D17S578 (Table 1), which were selected from the web site of Marshfield Clinic Research Foundation (http://www.marshfieldclinic.org/chg/pages/default.aspx). The information of the markers primers was obtained from the UCSC Human genome browser, March 2006 (http://genome.ucsc.edu/). Polymerase chain reaction (PCR) amplification was performed according to the standard method. The amplicons were visualized by ethidium bromide staining after separation by electrophoresis on a 12% acrylamide gel.[25]

Table 1 Summary of the molecular and cytogenetic analyses

Band	*Name of BAC/STS/microarray probe*	*Start*	*End*	*Result*	
	RP11-104O19	4 001 079	4 169 913	No deletion	
	RP11-1038H1	4 298 665	4 492 230	No deletion	
	RP11-198F11	4 517 318	4 671 880	No deletion	
	RP11-305G1	4 728 799	4 904 789	No deletion	
	RP11-46I8	4 851 185	5 025 099	No deletion	
	A_16_P20572628[a]	4 931 447	4 931 506	No deletion	
	A_14_P136153[a]	4 937 065	4 937 124	Deletion	
17p13.2	RP11-457I18	5 093 185	5 301 848	Deletion	
	D17S1832	5 913 299	5 913 667	NI	
	RP11-104N2	6 106 313	6 307 675	Deletion	
	D17S938	6 189 992	6 190 259	NI	
	D17S796	6 192 246	6 192 499	NI	Deletion indicated by aCGH
	GATA158H04	6 273 795	6 273 980	NI	
	D17S1881	6 468 416	6 468 811	Deletion pat	
	D17S578	6 764 604	6 764 878	NI	
	RP11-61B20	6 780 969	6 943 107	Deletion	
	CTD-2015K4	7 069 964	7 234 617	Deletion	
	RP11-542C16	7 122 619	7 223 586	Deletion	
	A_16_P03208860[a]	7 203 140	7 203 194	Deletion	
17p13.1	A_16_P03208864[a]	7 209 558	7 209 612	No deletion	
	RP11-104H15	7 193 987	7 378 309	No deletion	
	RP11-1081A10	7 360 964	7 530 114	No deletion	
	RP11-89D11	7 436 436	7 603 767	No deletion	
	RP11-1099M24	7 680 807	7 868 524	No deletion	
	RP11-1D5	7 918 567	8 082 208	No deletion	
17q25.3	RP11-153A23	73 516 547	73 694 284	Positive marker	

Abbreviations: aCGH, array-based comparative genome hybridization; deletion pat, deletion of paternally derived allele; NI, not informative.
Genome location corresponds to the March 2006 human reference sequence (NCBI Build 36).
[a]The names of microarray probe neighboring the both breakpoints were listed.

Maintenance of zebrafish

Adult zebrafish (*Danio rerio*) were maintained at 28.5 °C under 14-h light/10-h dark cycle conditions. Fertilized eggs from natural crosses were collected a few minutes after spawning and cultured at 28.5 °C in water containing 0.006% NaCl, 0.00025% methylene blue and 0.003% *N*-phenylthiourea. Embryos were staged according to morphology and hours post-fertilization (hpf).[26]

Sequence analyses of *dlg4* and *gabarap* in zebrafish

The 5′ untranslated region (UTR) of *dlg4* in our zebrafish strain was cloned using the 5′ RACE method with primers (Supplementary Table 1) that were designed according to the reported *dlg4* sequence (NM_214728). The partial fragment of zebrafish *gabarap* that contains incomplete 5′ and 3′ UTRs and a complete open reading frame was also amplified by reverse transcribed (RT)-PCR with primers (Supplementary Table 1) that was designed according to the reported zebrafish *gabarap* (Accession #BC065894). This PCR product was cloned into the pGEM-T vector (Promega, Madison, WI, USA), and the resulting plasmid, pGEM-*gabarap*, was served as a template for RNA probe synthesis. Total RNA was extracted from 24 hpf embryos using the RNeasy Mini kit (Qiagen). cDNA was synthesized using an Omniscript RT kit (Qiagen). All PCR reactions were performed with KOD plus DNA polymerase (Toyobo, Osaka, Japan). For 5′ RACE, the 5′-Full RACE Core Set (TAKARA, Otsu, Japan) was used.

Whole-mount *in situ* hybridization

Whole-mount *in situ* hybridization was performed as described.[27] The antisense RNA probe for *gabarap* was synthesized using T7 RNA polymerase (TAKARA, Otsu, Japan), and the pGEM-*gabarap* clone described above was used as the template.

Morpholinos and microinjection

Morpholino antisense oligos (MOs) were purchased from GENE TOOLS (Philomath, OR, USA) (Supplementary Table 1). Storage and microinjection were performed as described.[27]

Preparation of zebrafish head sections

Zebrafish embryos at appropriate stages were fixed with 4% paraformaldehyde in PBS at 4 °C overnight. The fixed embryos were gradually dehydrated with ethanol, soaked in xylene, transferred to Paraplast Plus embedding medium (McCormick Scientific, St Louis, MO, USA), and embedded in Paraplast Plus under microscopic observation. Specimens were cut into serial sections (7 μm) and stained with Mayer's hematoxylin and eosin solution.

Sequence analyses of *DLG4/PSD95* and *GABARAP*

All *DLG4/PSD95* and *GABARAP* exons from the indicated patient and 142 patients with neurological symptoms were amplified with PCR using originally designed primers located in both neighboring intronic sequences (Supplementary Table 2) according to the standard method. All amplicons were subjected to direct sequencing using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster city, CA, USA). Sequencing reactions were analyzed with a 3130×l Genetic Analyzer (Applied Biosystems).

RESULTS

Molecular cytogenetic analysis

We identified a 2.3-Mb microdeletion spanning from 17p13.2 to p13.1 in a patient with developmental delay and epilepsy (Figures 1a and b; Table 1). Two-color FISH analysis using BAC clones confirmed the deletion region (Figure 2a). There was no deletion in his parents' DNA, indicating a *de novo* deletion in the patient. According to the March 2006 human reference sequence (NCBI Build 36.1), the final karyotype of this patient was arr 17p13.2p13.1 (4 937 065–7 203 194)×1.ich (RP11–104O19+, RP11–1038H1+, RP11–198F11+, RP11–305G1+, RP11–46I8+, RP11–457I18−, RP11–104N2−, RP11–61B20−, CTD–2015K4−, RP11–542C16−, RP11–104H15+, RP11–1081A10+, RP11–89D11+, RP11–1099M24+, RP11–1D5+, RP11–153A23+) dn (Table 1). Among the six microsatellite markers used, only D17S1881 was informative, and showed that this patient had bands in common only with his mother (Figure 2b; Table 1). Thus, we deduced that the paternally derived allele at this locus was deleted in the patient.

Patient report

A 5-year-old boy was born spontaneously at a gestational age of 39 weeks and 1 day after an uneventful pregnancy. At his birth, his father was 33 years old and his mother was 31 years old. Both parents were healthy and nonconsanguineous. A 9-month-old younger sister was healthy. The proband's birth weight was 2480 g (<10th percentile), his birth length was 49.0 cm (50th percentile), and his head circumference was 33 cm (50th percentile). The amniotic fluid was contaminated by meconium, and amniotic fluid aspiration by tracheal incubation was performed. Because of the asphyxic respiration, he was admitted to the neonatal intensive care unit. Electrocardiography and ultrasound examination showed no abnormality of the heart or abdominal organs, except for a slightly dilated uteropelvic region. Measurement of the auditory brainstem response revealed no abnormality.

His cryptorchidism was surgically repaired at the age of 16 months. He showed gross motor delay, establishing head control at 5 months, turning over at 8 months, sitting alone at 11 months, walking alone at 2 years and speaking his first word at 14 months. Electroencephalography was performed at 17 months because of the developmental delay, and no obvious abnormality was observed at this time.

At the age of 2 years, he developed cataplectic attack seizures that sometimes produced unconsciousness. He received valproic acid at 2 years 5 months of age, but his seizures were not controlled with this treatment. An electroencephalography revealed hypsarrhythmia with diffuse polyspikes and waves burst (Supplementary Figure 1). He was admitted to the hospital for adrenocorticotropic hormone therapy to control his epileptic attacks at the age of 2 years and 11 months.[28] At this time, his height was 87.8 cm (10th percentile), his weight was 11 kg (3rd percentile) and his head circumference was 48.1 cm (<25th percentile). Dysmorphic features including, a broad nasal root, a high arched palate, low-set ears, rough hair, telecanthus, microretrognathia (Figure 3), and flat feet were noted. His development quotient was 46 according to the Enjoji Scale of Psychological Development.[29] Blood cell counts, glucose, urinalysis, amino acid studies, liver and thyroid function studies and cerebrospinal fluid were normal. After adrenocorticotropic hormone therapy, the incidence of the epileptic attacks was reduced. G-banded chromosomal analysis showed a normal male karyotype of 46,XY. To survey the etiology of developmental of this patient, the patient's blood sample was supplied for this study. Brain MRI at his age of 5 years showed mild white matter volume reduction (Supplementary Figure 2).

Injection of *dlg4*–MO shows no distinct defects in brain development

In the deleted region of chromosome 17p13.1 of this patient, two genes, *DLG4/PSD954*[30] and *GABARAP*[31] may be responsible for this patient's developmental delays because the earlier studies showed the involvement of these two genes in neurological functions.

Zebrafish *dlg4* is expressed in developing and adult brain, in a manner similar to the expression of its homolog in both human and mouse,[32–34] suggesting that zebrafish can be used as a model animal to examine this gene. Therefore, we first focused on the function of *dlg4* during cerebral development in zebrafish. Although a zebrafish homolog of *dlg4* has been reported (NM_214728), sequence information of the 5′ UTR in our zebrafish strain was needed for functional

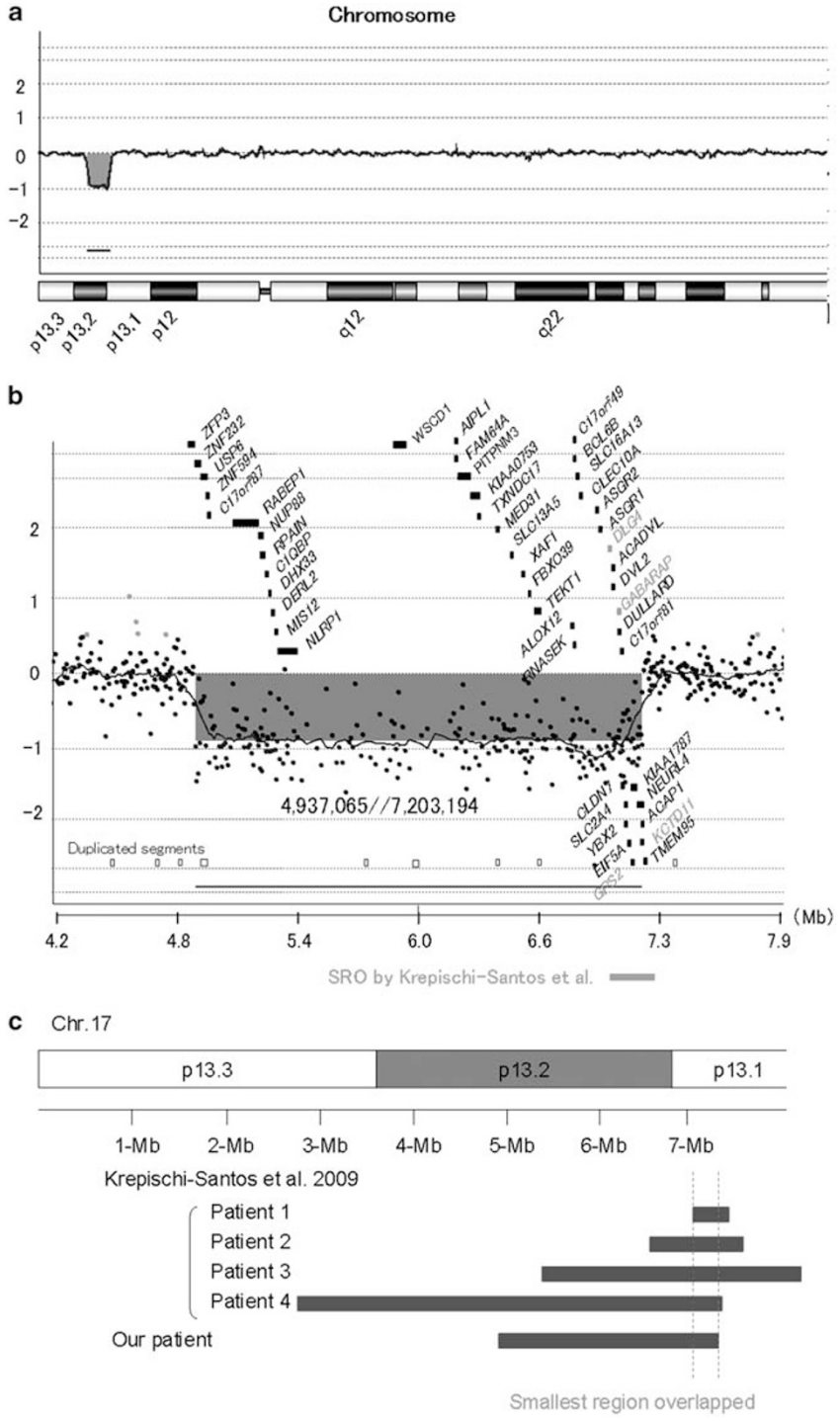

Figure 1 Physical map of the short arm of chromosome 17 and the deletion regions observed in the patients. (**a**, **b**) aCGH profiles of chromosome 17 as shown by CGH analytics in chromosome view (**a**) and gene view (**b**). Vertical axis indicates the $\log_2$ ratio of the genomic copy number. The blue rectangle indicates the region of copy-number aberration with a size of 2.3 Mb. The aberration area is expanded in gene view (**b**). Dots indicate the locations and the corresponding $\log_2$ ratios of the probes. Black rectangles on gene view indicate locations of the known genes in this region (shown in italics). Candidate genes, *DLG4*/*PSD95*, *GPS2*, *GABARAP* and *KCTD11*, are emphasized by red. Open rectangles indicate the locations of segmental duplications and copy-number aberrations. SRO is depicted below gene view.[24] (**c**) The ranges of the deletions are depicted by blue bars. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

analysis with gene knockdown using MOs. Thus, we performed 5′ RACE and obtained a 12-nucleotide fragment that corresponds to the upstream region of the translation initiation site, which was found to be identical to the reported sequence (data not shown). Then, to determine whether deletion of the *DLG4*/*PSD954* locus resulted in brain growth impairment, we performed gene knockdown of *dlg4* in zebrafish. Microinjection of a translation-blocking type morpholino against *dlg4* (*dlg4*-MO), however, resulted in various types of defects

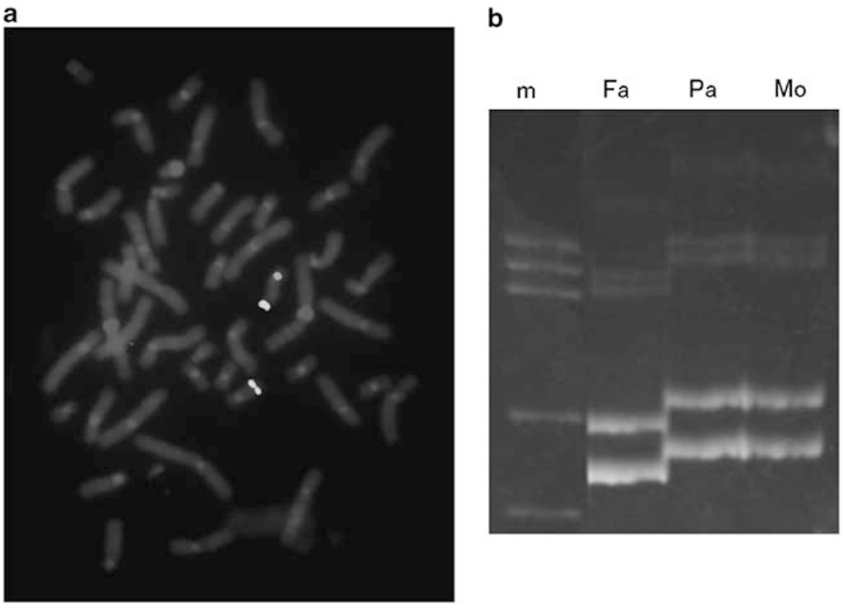

Figure 2 Molecular cytogenetic analyses. (**a**) Two-color FISH analysis using a combination of the BAC clone CTD-2015K4 (red) as the target and RP11-153A23 (green) as markers of chromosome 17. There is only one red signal, which indicates deletion of this region in one allele. (**b**) Microsatellite marker analyses using D17S1881. PCR products of D17S1881 were separated on an acrylamide gel and visualized with ethidium bromide. m, molecular size marker; Fa, father; Pa, patient; Mo, mother. The bands of the present patient are common only with that of the mother. This indicates that the deletion is derived from the paternally derived chromosome. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

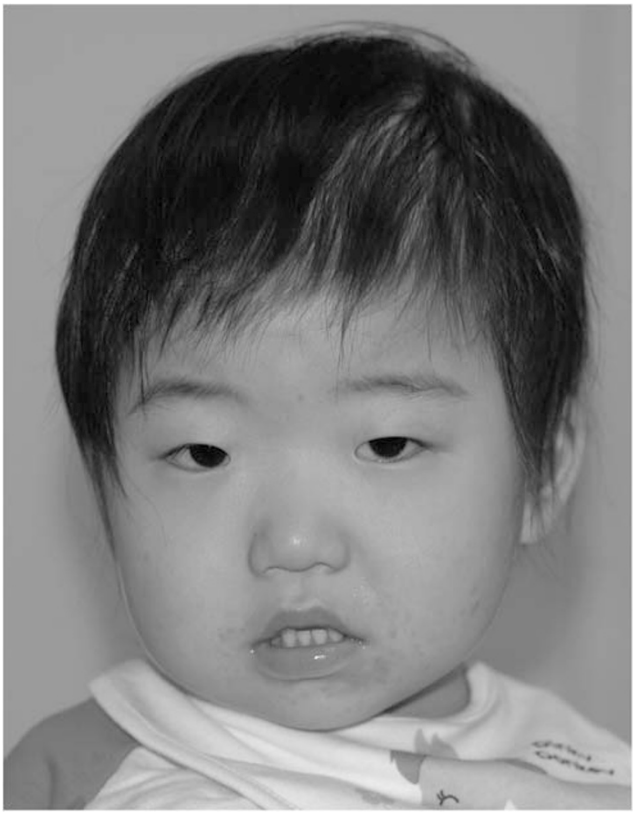

Figure 3 The patient at the age of 2 years and 11 months. Dysmorphic features including broad nasal root, high arched palate, low-set ears, rough hair and telecanthus are shown.

that differed from one individual to the next with no obvious tendency (Supplementary Figure 3). As we could not confirm a possible relationship between the phenotype of our patient and deletion of the *DLG4/PSD954* locus, we abandoned further investigation of the participation of *dlg4* in zebrafish brain development.

gabarap is involved in cerebral development

Using the zebrafish, we next investigated whether *gabarap*, another gene that could be responsible for the symptoms of this patient, has any functions in brain development, because this could be responsible for the symptoms of 17p13.1 microdeletion syndrome. First, the gene expression pattern of *gabarap* during development in zebrafish was determined. At 24 hpf, *gabarap* was expressed in the head, especially in the telencephalon, hindbrain and rhombomere (Figure 4a). Later in development, at 72 hpf, expression was observed in the ventral central nervous system through the forebrain to the hindbrain (Figure 4b). As these expression patterns of *gabarap* in the developing head indicated possible functions of this gene in brain development, we performed gene knockdown of *gabarap*. When 3 ng of *gabarap*-MO1, a translation-blocking MO, was injected into each embryo, the most of morphants (88%, $n=150$) showed slight dwarfism of the entire body with a severely hypoplastic head and mandible (Figures 4g and h). Although generalized growth retardation may be involved, it is also possible that these prominently hypoplastic head and mandible indicate the microcephaly and microretrognathia seen in the previously reported patients with 17p13.1 microdeletion syndrome.[24] To confirm whether this defect was a specific effect of the gene knockdown of *gabarap*, *gabarap*-MO2, another translation-blocking MO with different sequence, was used. When 3 ng of *gabarap*-MO2 was injected, approximately two thirds of the morphants (63%, $n=90$) of *gabarap*-MO2 showed a similar phenotype as did those of *gabarap*-

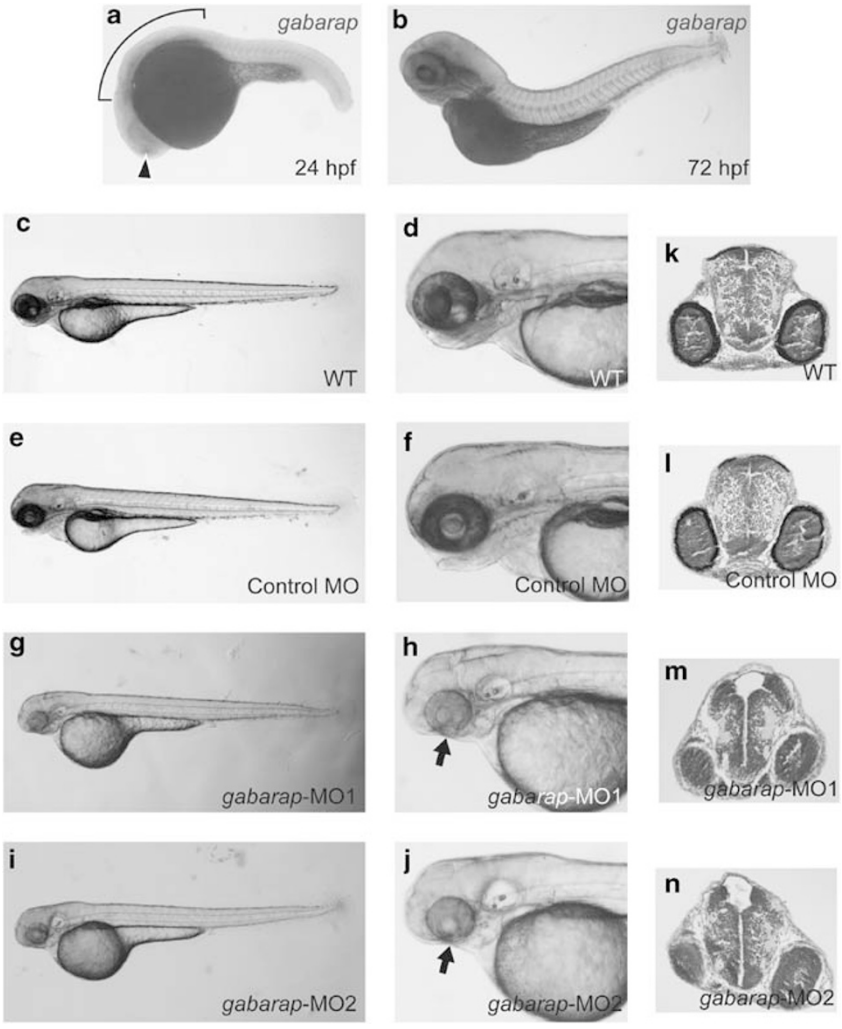

Figure 4 Functional analyses of *gabarap* using zebrafish. Spatial expression of *gabarap* in wild-type embryos at 24 hpf (**a**) and 72 hpf (**b**). These specimens were fixed with 4% paraformaldehyde, dehydrated and rehydrated gradually. According to these steps, the shapes of fixed specimens are distinct from that of live embryos (compare with **c**–**j**). The arrowhead and the bracket (**a**) indicate expression of *gabarap* in the telencephalon and in the hindbrain to the rhombomeres, respectively. Gene knockdown of *gabarap* was analyzed using zebrafish at 72 hpf (**c**–**n**). Hypoplastic head and mandible (arrows) are shown in *gabarap* morphants (**g**–**j**). Cross-sections containing forebrain of zebrafish at 72 hpf (**k**–**n**). Ventricular dilatations were observed in *gabarap*-morphants (**m**, **n**). Dorsal is to the top. (**a**–**k**) Rostral is to the left.

MO1 (Figures 4i and j). This phenotypes was hardly observed in wildtype (1.3%, n=300) or control-MO injected (6.7%, n=90) embryo (Figures 4c–f). These results indicated that the hypoplastic head and mandible were caused by the gene knockdown of *gabarap*, and that *gabarap* is required for normal development of anterior structures. Although we tried the mRNA rescue experiment using mRNA of *GABARAP*, we could not obtain compelling consistent results (data not shown).

To examine whether *gabarap*-MO morphants show histological abnormalities of the brain, cross-sections of the head were prepared. On the basis of the expression pattern of *gabarap*, we focused on sections that included the forebrain. In normal cerebral development of the zebrafish, the brain ventricle narrows as development progresses and almost fills up at 72 hpf (Figures 4k and l). Compared to that, the ventricle in both *gabarap* morphants was clearly retained in sections from the same relative positions of both wild-type and control morphants (Figures 4m and n). Overall, these results strongly suggest that *gabarap* has important functions in normal brain development in zebrafish.

Mutation screening for *DLG4/PSD95* and *GABARAP*

No disease-causing mutations in *DLG4/PSD95* and *GABARAP* were found in the remaining allele of the indicated patient and other 142 patients with neurodevelopmental disorders.

DISCUSSION

A microdeletion of 17p13.2p13.1 was identified in our patient who showed characteristic dysmorphic features, developmental delay and intractable epilepsy. This deletion was overlapped with previously reported microdeletions of 17p13.1 (Figure 1).[24] The reported four patients showed common clinical manifestations including mental retardation, microcephaly, abnormal brain MRI findings with delayed myelination and some dysmorphic findings including epicanthic folds, low nasal bridge and microretrognathia, despite the variability of deletion size. As the deletion end breakpoint identified in our patient was on the most telomeric region compared with the previously reported deletions, this narrowed the shortest region overlapped (SRO) to chr17 (7 054 400–7 203 194), from 178.3 to 148.8 kb (Table 2; Figure 1).[24] The overlapping clinical manifestation between

Table 2 The deletion sizes of the reported patients

	Genomic position of the deletion		
Patient	*Start*	*End*	*Size of the deletion*
Krepischi-Santos *et al.*[24]			
Patient 1	7 054 400	7 341 000	286 600
Patient 2	6 507 600	7 567 600	1 060 000
Patient 3	5 405 100	8 133 500	2 728 400
Patient 4	2 796 600	7 232 700	4 436 100
Our patient	4 937 065	7 203 194	2 266 129
SRO	7 054 400	7 203 194	148 794

Abbreviation: SRO, smallest region overlapped.
Genome location corresponds to the March 2006 human reference sequence (NCBI Build 36).

our patient and the previously reported patients are mental retardation, MRI abnormalities and distinctive facial features including epicanthic folds, low nasal bridge and microretrognathia (Supplementary Table 3). Thus, the responsible genes for these manifestations would be included in the narrowed SRO. Compared with the clinical phenotypes of four patients,[24] our patient is unique with intractable epilepsy but no microcephaly.

Mapping genomic rearrangements in patients with multiple congenital anomalies has increased our understanding of the etiology of diseases and has led to the identification of a number of disease-causing genes, including *CHD7*[35] and *STXBP1*.[36] The narrowed SRO of 17p13.1 is small with the size 148.8 kb but is gene rich and includes 17 genes (known protein-coding genes taken from mRNA reference sequences, UCSC Human Genome Browser March 2006; Figure 1b, Supplementary Table 4). The genes in this SRO were analyzed, and picked up four genes, *DLG4*/*PSD95*, *GPS2*, *GABARAP* and *KCTD11* as the candidates for causing mental retardation.[24] *DLG4*/*PSD95* encodes a member of the membrane-associated guanylate kinase family, which is highly expressed in excitatory glutamatergic synapses in the brain.[37] *DLG4*/*PSD95* is important for coupling the *N*-methyl-D-aspartate receptor to pathways that control bidirectional synaptic plasticity and learning.[38] However, several studies have reported that *DLG4*/*PSD95* knockout mice do not show an essential function in synaptic development for *DLG4*/*PSD95*.[39] Likewise, our knockdown zebrafish showed no morphological abnormalities. A possible function for this protein in neurodevelopment cannot, however, be ruled out without further examination. *KCTD11* is a suppressor of the Sonic Hedgehog signaling, which is known to be deregulated in medulloblastoma.[40] Hedgehog signaling has been described as having a critical function in brain morphogenesis by regulating the ventral patterning of the neural tube.[41] *GPS2* interacts with regulatory factor X4 variant 3 (RFX4_v3) to modulate transactivation of genes involved in brain morphogenesis and mutagenesis in mice shows that Rfx4_v3 is crucial for normal brain development.[42] From these reasons, haploinsufficiency of these genes may also contribute to the phenotype of this patient.

Regarding candidate genes for brain developmental delay, we paid attention to *GABARAP* in the deletion region. GABA(A) receptors are ligand-gated chloride channels that mediate inhibitory neurotransmission. By screening a cDNA library using GABA(A) receptor γ-2S subunit as a bait, *GABARAP* was identified as a potential linker between GABA(A) receptors and microtubules.[31,43] Currently, a more general function is emerging for *GABARAP*, including transport of GABA(A) receptors to and from the cell surface, organization of GABA(A) receptors into post-synaptic clusters, and regulation of steady-state receptor density.[43] We analyzed expression patterns of the genes included in the SRO by use of *in silico* library (UCSC genome browser), and *GABARAP* showed higher expression levels in the brain. As little is known about the functions of *GABARAP*, we paid attention to that as a potential candidate gene for neuronal development. To confirm the contribution of this to the phenotype of the patient, we used the gene knockdown technique in zebrafish. As the results showed hypoplastic brain and mandible in *gabarap* knockdown zebrafish that correlate with common phenotypes of the patient with 17p13.1 deletion, human *GABARAP* is likely to contribute to the development of anterior structure. Among the patients with 17p13.1 deletion, only our patient showed intractable epilepsy. As there may be an unmasked mutation of *GABARAP*, we analyzed the sequence of *GABARAP* in the remaining allele of our patient, but there was no pathogenic mutation. Thus, the reason of the phenotypic difference among the patients with 17p13.1 deletion is unknown. We analyzed 142 DNA samples derived from patients with mental retardation with/without epilepsy, and there were no pathogenic nucleotide alterations in patients with neurodevelopmental disorders. It is, however, still possible that nucleotide mutations in *DLG4*/*PSD95* and *GABARAP* are the pathogenic cause of neurodevelopmental delay and epilepsy.

As there are other powerful candidate genes, including *GPS2*, and *KCTD11*, for neurodevelopmental delay and epilepsy in this region, further studies for the genes including in this region would be required.

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