not only frequency MMN/MMF but also duration MMN/MMF to confirm our findings. Moreover, some electrophysiological studies have suggested abnormalities in temporal lobe auditory processing as reflected by N1 component in response to tones (e.g. Bruneau et al., 1999; Lincoln et al., 1995), although a direct comparison between N1 and MMN components should be cautioned as they reflect different aspects of sound processing. Some hemodynamic studies have also reported temporal lobe hypoperfusion in the resting state, which indicate overall dysfunction in the auditory cortex (Hashimoto et al., 2000; Ohnishi et al., 2000; Zilbovicius et al., 2000). Our design did not test the hypothesis that the auditory cortex dysfunction in autism is speech-sound specific, which should be tested in future studies.

The present study found a group difference in MMF latency, but not in MMF power. Previous findings from MMN/MMF studies in autism have been mixed. Three studies reported preserved MMN amplitude (Čeponienė et al., 2003; Gomot et al., 2002; Kemner et al., 1995), two studies reported reduced MMN/MMF amplitude/power (Seri et al., 1999; Tecchio et al., 2003), and one study reported enhanced MMN in autism (Ferri et al., 2003). As for latency, only Seri et al. (1999) reported prolonged latency of MMN in response to tones, whereas Gomot et al. (2002) reported shorter latency of tonal MMN in children with autism. The interpretation of Seri et al. study should be done with caution, since they tested subjects with tuberous sclerosis, which is not a typical case of autism. Gomot et al. explained their shorter tone-MMN latency by an overlap with an early P3a component. Moreover, Gomot et al. used frequency changes in pure tones, while our study used duration changes in pure tones and vowels and phoneme changes in vowels. This difference in the type of stimuli may partly explain the difference in results between the two studies. Two other studies reported intact MMN latency (Čeponienė et al., 2003; Ferri et al., 2003), and the remaining two provided no information on latency findings (Kemner et al., 1995; Tecchio et al., 2003). Čeponienė et al. (2003) found no differences between autistic and control children in MMN elicited by speech sound changes. The subjects of our study were adults, while the subjects of theirs were children. Delayed latency of MMN in response to speech sounds may be more evident in adults with autism, possibly due to a lack of normal development of specialization (or functional plasticity) for processing of speech sounds.

To our knowledge, the present study is the first to demonstrate a significant association between mismatch abnormalities and clinical symptoms in autism. However, due to a small sample size and a restricted range of CARS scores in our sample, the results should be regarded as tentative. Future studies should clarify how MMN/MMF abnormalities are related to specific cognitive profiles and social and communication problems, in individuals with autism.

Some other methodological issues in the current study need to be commented upon. First, a follow-up experiment employing children with autism should be conducted to generalize our findings. Secondly, the subject group was not restricted to high-functioning autism individuals to match IQ to healthy subjects, nor was there an IQ-matched (intellectually disabled) control group. However, since MMF indices were not significantly correlated with IQ, employing low-functioning subjects may not have produced marked confounds in the interpretation of our findings. However, since previous studies have shown lower amplitude of phonetic MMN in subjects with learning disabilities (Bradlow et al., 1999) and in those with intellectual disabilities (Kaga et al., 1999), future studies should employ individuals with learning and intellectual disabilities as a control group to clarify whether the present findings are specific to autism. Thirdly, although we found a significant negative correlation between dose of medication and some of the MMF latency indices, these correlations were not in a predicted direction. These results should not be considered to be definitive, however, since (1) the sample size is small and (2) uncorrected *P*s of 0.046 and 0.037 may not remain significant after a correction for multiple statistical comparisons.

In conclusion, this study, using a whole-head MEG, provides physiological evidence for delayed processing of change in speech sounds in the left auditory cortex in adults with autism. Our next goal will be to elucidate the relationship of this physiological abnormality of speech sounds at the basic level with higher-order communication deficits in autism.

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Polyhistidine tract expansions in HOXA1 result in intranuclear aggregation and increased cell death

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Abstract

HOXA1 gene is part of a cluster of homeotic selector genes that regulates the anteroposterior patterning of mammals during embryonic development. HOXA1 encodes two alternatively spliced mRNAs with two isoforms, A and B, the former contains the homeodomain and expressed in early embryonic development. HOXA1 contains a string of 10 histidine repeats. However, individuals heterozygous for 7, 9, 11, and 12 histidine repeat variants were present among the Japanese population, notably in some autism cases. To determine the biological implications of the different polyhistidine repeat lengths, we expressed these variants in COS-7 and a human neuroblastoma cell line (SK-N-SH). Expression of expanded variants of HOXA1 isoform A, containing 11 and 12 polyhistidine, resulted in early and great degree of protein aggregation in the nucleus. This aggregation resulted in accelerated cell death in cells expressing 11 and 12 expanded variants compared to those transfected with 7 and 10 polyhistidine variants. Furthermore, we showed that these aggregates were ubiquitinated and were inhibited by a histidine-modifying compound, DEPC. These data suggest that HOXA1 protein with polyhistidine tract expansions misfold, aggregate, and have a toxic effect on cell.

Keywords: HOXA1; Histidine repeat; Protein aggregation; Ubiquitin; Cell death; Diethylpyrocarbonate

Homeobox genes encode for transcription factors contributing to the regulation of embryonic patterning and organogenesis [1]. The clustered homeobox genes were highly conserved from *Drosophila* to human at the genomic level. In mammals, 39 of the Hox genes were identified and they encode to the class of proteins which share the evolutionary conserved homeodomain involved in the recognition of the target DNA sites [2]. HOXA1 is the first homeobox gene expressed in the developing hindbrain [3]. Its mRNA has two alternative splice variants; one containing the homeobox which encodes isoform A, and the shorter, isoform B, lacking the homeobox [4]. One of the noticeable features of HOXA1 isoform A is a stretch of 10 histidine repeats at amino acid positions 65–74. Although, its importance has not yet been established, it may possibly interact with Polycomb, a repressor of homeotic genes. The targeted disruption of the Hoxa1 gene in mice leads to numerous developmental defects, including hindbrain deficiencies and abnormal skull ossification and ultimately to neonatal death [5,6]. Ingram et al. [7] reported

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an association of the A218G polymorphism in HOXA1 gene and autism; however, a number of contradictory reports have also been made [8,9]. Racial differences of sample populations may be the reasons for these discrepancies.

In this study, we investigated HOXA1 gene variations in a Japanese population comprised of normal and autistic individuals, and found novel histidine repeat deletions and expansions. Expression of polyhistidine expanded forms of HOXA1 protein results in early intranuclear aggregation and increased cell death. Time-dependent protein aggregation and cell death could be an indication of apoptosis associated with the process of assigning segmental identity in the developing hindbrain. Moreover, expanded forms of HOXA1 isoform A, aggregated earlier and faster, suggest that longer histidine repeats may result in accelerated apoptosis.

Materials and methods

Antibodies and reagents. Antibodies were purchased as follows, polyclonal anti-HOXA1 antibody (N-20) was from Santa Cruz, polyclonal anti-EGFP antibody was from Invitrogen, and polyclonal anti-ubiquitin antibody, Alexa fluor 546-conjugated anti-rabbit IgG antibody, and Alexa fluor 594-conjugated anti-goat IgG antibody were from Molecular Probes. Hoechst 33258 for nuclear staining was from Molecular Probes.

DNA sequencing of HOXA1 gene. Subjects consisted of 110 Japanese autistic patients, their parents ($n = 211$); and 336 normal controls. All autistic patients were diagnosed under DSM-IV criteria. This study was approved by the Ethical Committees of the Faculty of Medicine, University of Tokyo, and the Faculty of Medicine, Tottori University. Total DNA was extracted from lymphoblasts using a standard method. PCR primers were designed to amplify all exons of HOXA1, including flanking sequences. PCR products were subcloned in T-Vector (Promega) and sequenced.

HOXA1 plasmid constructions. Each HOXA1 isoform was amplified from lymphocyte mRNA of human normal controls and autistic individuals following the standard method. Since isoform A differs from isoform B primarily by the presence of the homeodomain region and expresses only in the developing embryo, isoform A constructs were generated partly from genomic DNA by PCR amplification and joined with the appropriate region from isoform B transcript. All PCR amplification steps were performed using Pfu Ultra DNA polymerase (Stratagene) and confirmed by sequencing. Each variant was ligated into pCMV-Script (Stratagene) and pEGFP-N1 (Invitrogen) to generate expression constructs.

Transfection and cell death assay. COS-7 and human neuroblastoma cell line (SK-N-SH) were maintained in DMEM (Sigma) with 10% fetal bovine serum (Gibco) and transfected with HOXA1 constructs using Fugene 6 transfection reagent (Roche) following manufacturer's protocol. After transfection, cells were maintained in DMEM supplemented with 10% serum. A mock transfection was also performed as a control. Floating dead cells from medium of transfected cells were harvested every 24 h for a period of 3 days, stained with Trypan blue (Gibco), and counted using a hemocytometer under a phase contrast microscope (Olympus IX-70). Lactate dehydrogenase (LDH) cytotoxicity assay (Wako, Tokyo) was also performed from the collected cell medium following the manufacturer's procedure.

Immunocytochemistry and fluorescence imaging. After transfection, cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 30 min and immunostained with anti-HOXA1 antibody and anti-ubiquitin antibody for 1 h at room temperature. Bounded antibodies were detected by incubation with Alexa fluor 595-conjugated anti goat IgG antibody for 1 h. For nuclear staining, cells were incubated with Hoechst 33258 for 30 min. All samples were mounted on glass slides and fluorescence images were obtained using Leica confocal microscope (TCS SP2).

Western blotting. Twenty-four hours after transfection, cells were harvested for total protein extraction. Briefly, cell samples were lysed and sonicated with 10 mM Tris–Cl (pH 7.4), 150 mM NaCl, 1 mM EDTA, and 1 mM EGTA plus protease inhibitor cocktail (Roche). Protein was quantified using Color-Producing Solution (Wako). Samples were run on 10% SDS–PAGE gels and transferred on a nylon membrane (Millipore) using a semi-dry transfer blotter (Bio-Rad). Membranes were incubated in either a polyclonal rabbit anti-EGFP antibody followed by incubation in a horseradish peroxidase-linked donkey anti-rabbit IgG antibody (Amersham), respectively, or in a polyclonal anti-HOXA1 antibody with a horseradish peroxidase linked sheep anti-goat antibody (Amersham). Detection was performed using ECL (Amersham–Pharmacia Biotech) and images were captured in X-ray film or a LAS-1000 plus imager (Fujifilm).

DEPC treatment. Twenty-four hours after transfection, cells were treated with diethylpyrocarbonate (DEPC) with a concentration of 20 mM for 30 min or 1 mM for 24 h. Then cells were fixed and mounted on the slides. The degree of protein accumulation within the cell nuclei of the transfected cells was counted. Cells were counted from 10 randomly selected microscope fields from each sample. The ratio of the number of aggregated cells over the total number of EGFP-positive cells was then computed. The same computation procedures were performed for cells processed after 18 and 42 h of incubation.

Results

Novel variants of histidine repeats in HOXA1 gene

All exons and flanking sequences of HOXA1 gene were subjected to direct sequencing. Using this approach, novel polyhistidine variants were detected. Families affected with autism and randomly selected normal individuals were screened for sequence of HOXA1 gene. Finally 7, 9, 11, and 12 repeat variants were detected in both normal and autism patients (Table 1). Nevertheless, we were interested in further expression study because some trinucleotide repeat expansions, such as polyglutamine and polyalanine, have already been implicated neurodegenerative and congenital diseases [10–12].

Intranuclear protein aggregations in polyhistidine variants of HOXA1

To investigate the cellular localization of the different variants of HOXA1, we generated expression constructs having the 7, 10, 11, and 12 polyhistidine repeats for the

Table 1
Allele frequencies of the polyhistidine variants in HOXA1 gene between autism patients (child), their parents, and normal individuals

Type	Child	Parent	Normal
7/10	2	4	11
9/10	0	0	1
10/10	105	200	312
11/10	3	6	12
12/10	0	1	0
Total	110	211	336

The length of histidine repeats in HOXA1 gene was assessed by sequencing of genomic DNA and was classified under five types of allelic variants.

two HOXA1 isoforms fused with and without the N-terminus of GFP (Fig. 1A). COS-7 and SK-N-SH cells that were transfected with the wild-type (10 histidine repeats) HOXA1 isoform A and immunostained with anti-HOXA1 antibody revealed that HOXA1 protein is diffusely localized in the nucleus (Fig. 1B). In contrast, 11 and 12 histidine variants of HOXA1 isoform A resulted in noticeable protein aggregations in the nucleus. On the other hand, deleted form (7 his-

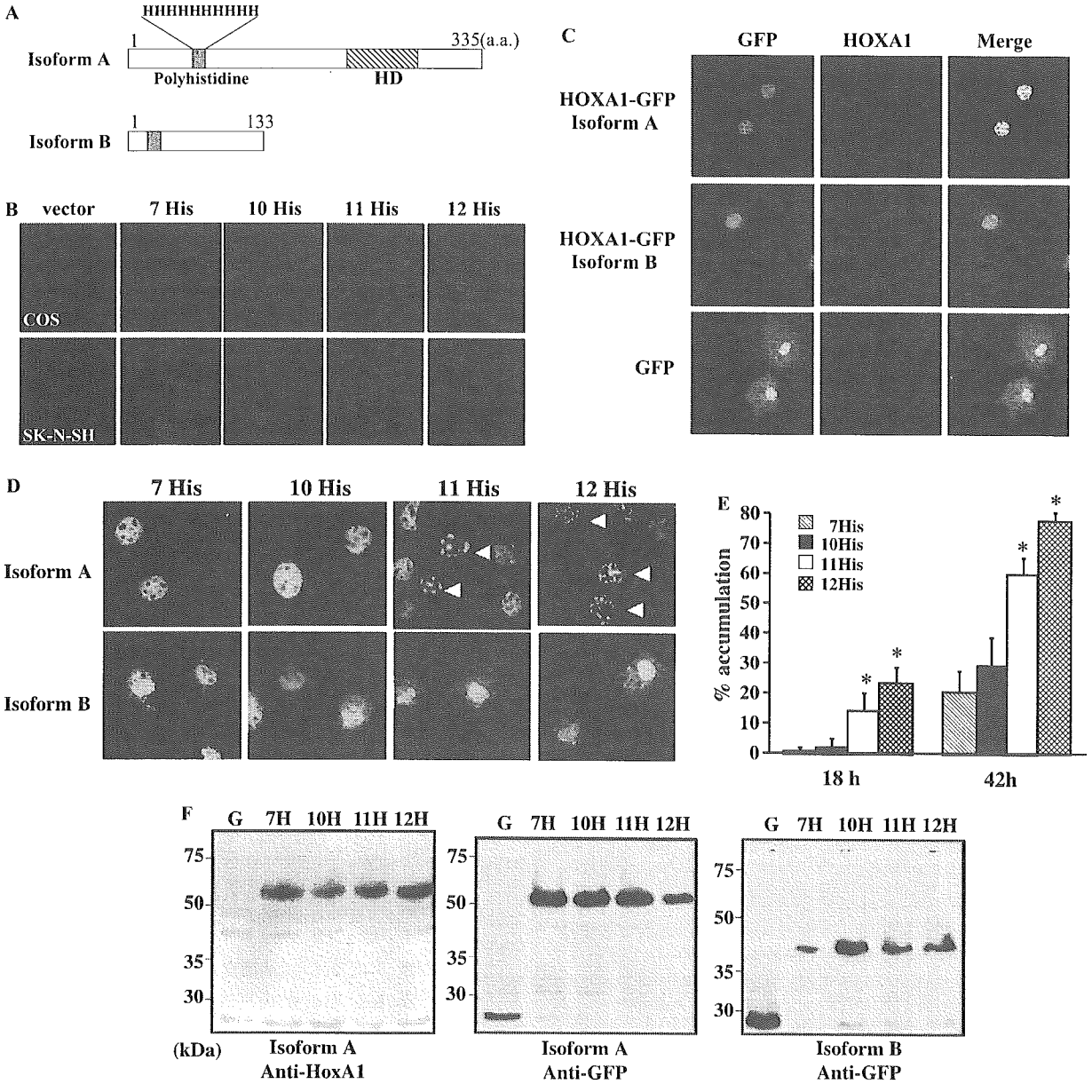

Fig. 1. Expression of 11- and 12-polyhistidine repeats of HOXA1-GFP protein results in intranuclear aggregations. (A) HOXA1 expression constructs with two isoforms A and B. Isoform A contains homeodomain (HD). Variants of the same gene were constructed containing 7, 11 or 12 polyhistidine repeats. (B) In cellular localization of HOXA1 isoform A protein. COS-7 cells and SK-N-SH cells were transiently transfected with HOXA1 isoform A with polyhistidine variants and stained with the anti-HOXA1 antibody. Nuclear aggregation of 11 and 12 histidine variants of HOXA1 protein was noticeable in both cells. (C) COS-7 cells transfected with HOXA1-GFP isoforms A and B, and stained with the anti-HOXA1 antibody. Mock GFP vector expression used as a control. (D) COS-7 cells transfected with HOXA1-EGFP fusion construct. Fluorescence images were taken 24 h after transfection. Arrows indicate HOXA1-EGFP protein aggregations in the nucleus. (E) The number of cells with protein aggregations was scored at 18 and 42 h after transfection. Values were expressed as the ratio of nuclei exhibiting aggregation over the total number of HOXA1-EGFP fluorescent cells. Each bar represents the standard error of the mean (SEM) from three independent experiments. $^{*}P < 0.05$ significantly different from the values of 7- and 10-polyhistidine repeats. (F) Western blot analysis for HOXA1-GFP fusion protein. Protein extracts from COS-7 cells were prepared 24 h after transfection with HOXA1-GFP constructs. Western blotting was performed using anti-HOXA1 and/or anti-GFP antibodies. HOXA1 isoform A, 63 kDa; isoform B, 41 kDa; GFP, 27 kDa (G, GFP; H, histidine repeats).

tidine repeats) has the same pattern as the wild-type protein in both cells (Fig. 1B). There was little or no detectable signal from mock vector transfected cells. When cells were expressed with wild-type HOXA1 isoform A and GFP fusion protein, HOXA1-GFP was found localized in the nucleus and this was also detected by the anti-HOXA1 antibody (Fig. 1C). Since anti-HOXA1 antibody only detected isoform A, HOXA1-GFP isoform B was not stained. For the GFP fluorescence analysis, expression of 11 and 12 histidine variants of HOXA1-GFP isoform A resulted in protein aggregations in the nucleus but not in wild-type (10 histidine repeats) and deleted form (7 histidine repeats) in both COS-7 and SK-N-SH cells (Fig. 1D). This is also consistent with the untagged HOXA1 protein localization as shown in Fig. 1B. There is a significant difference in the percentage of scored cells with protein aggregations in variants with 11 and 12 histidine repeats after 18 and 42 h of transfection, although protein aggregations were also found in wild-type and 7 histidine repeat variant after 42 h (Fig. 1E). While protein aggregates were noticeable in expanded forms of isoform A, no significant aggregation was observed in expanded forms of isoform B. (Fig. 1D). The same result was also found in SK-N-SH cells (data not shown). Expression of HOXA1-GFP fusion protein was also confirmed by Western blotting with anti-GFP and anti-HOXA1 antibodies (Fig. 1F).

Polyhistidine tract expansions of HOXA1 result in increased cell death

Since there was no difference in the subcellular localization between the untagged HOXA1 and HOXA1-GFP protein, GFP-fused constructs were used for the following experiments. To test whether the expression of expanded histidine repeats of HOXA1-GFP causes an increase in cell death, we examined the rate of cell death in transfected COS cells by scoring dead cells and also using the LDH release assay. After 48–72 h of transfection, a significant increase in the percentage of dead cells was noted in cells transfected with 11 and 12 polyhistidine variants (Fig. 2A). This increase in cell death may have been a result of increased protein aggregation. These also correlated with the results from LDH cytotoxicity assay wherein the amount of LDH released into the medium was higher in cells transfected with 11 and 12 polyhistidine variants as compared to the control, 7 and 10 polyhistidine repeat variants, 48–72 h after transfection (Fig. 2B). By staining with Hoechst33258, nuclear fragmentation was observed in cells expressed with HoxA1 containing the 11 and 12 histidine repeats, which colocalized with GFP fusion fluorescence but not with wild type and 7 histidine repeat variants (Fig. 2C).

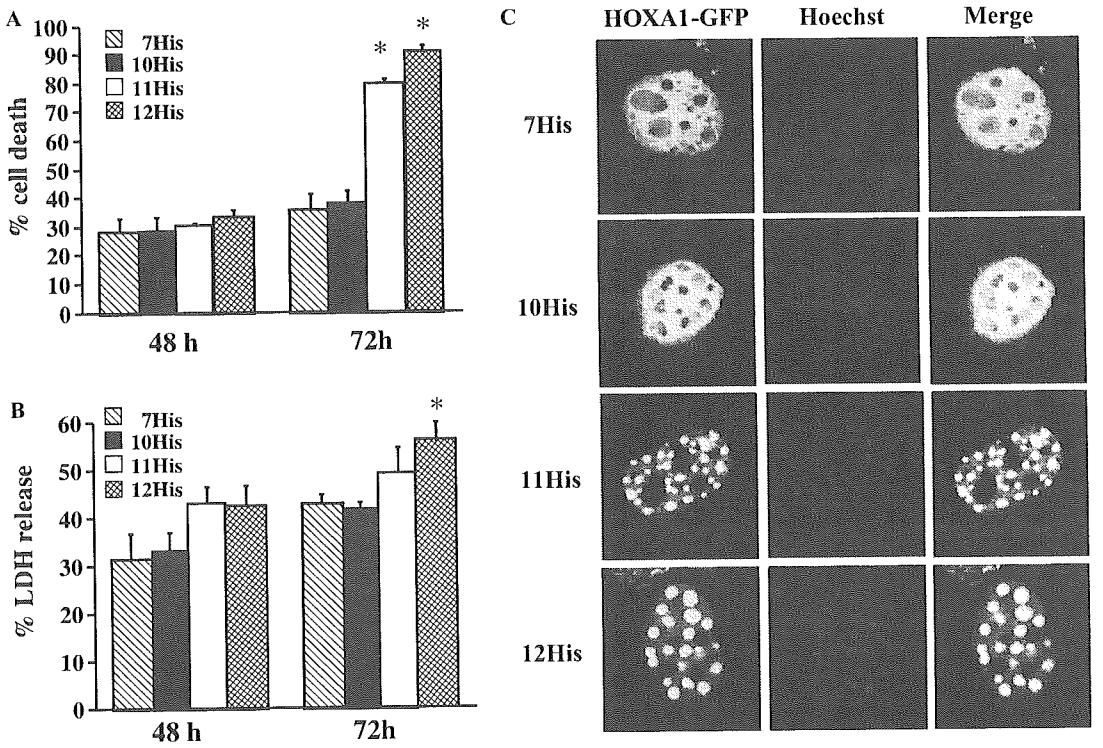

Fig. 2. Polyhistidine expansions of HOXA1-GFP results in increased cell death in COS-7 cells. COS-7 cells were transfected with HOXA1-GFP plasmid constructs. Assays were performed every 24 h for 3 consecutive days. (A) Floating dead cells from the medium were collected and stained with trypan blue and scored. Ratio of the number of dead cells over the total number of cells was computed. Values for percent cell death are expressed as means (SEM) from four independent experiments. (B) Lactate dehydrogenase (LDH) release assay. Each bar represents the mean (SEM) from three independent experiments. $*P < 0.05$ significantly different from the value of control. (C) COS-7 cells were fixed and stained with Hoechst after transfection. The fragmented nucleus was seen in cells with 11 and 12 histidine repeats of HOXA1-GFP.

Nuclear aggregations of HOXA1 are ubiquitinated and inhibited by DEPC

Improper folding is believed to cause aggregation of cellular proteins and often cause cell death as observed in polyglutamine diseases [12]. We examined the transfected cells by immunofluorescence staining with anti-ubiquitin antibody. More than 50% of aggregated cells exhibited ubiquitin immunoreactivity in both 11- and 12-polyhistidine variants 18–24 h after transfection (Fig. 3). Although the image background was diffusely labeled for ubiquitin, immunoreactivity was concentrated at the site of aggregation. Cells transfected with 7- and 10-polyhistidine variants did not exhibit conspicuous aggregation prior to 18 h post-transfection and did not show any significant increase in ubiquitin immunoreactivity (Fig. 3).

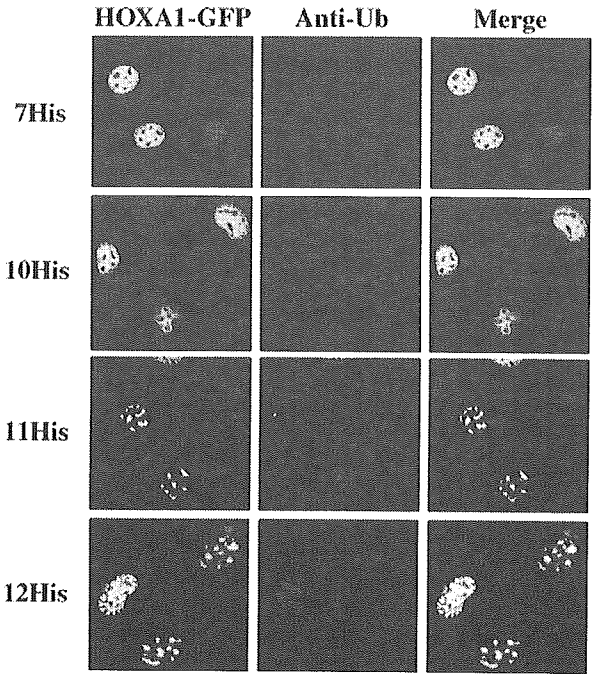

Fig. 3. Aggregations of HOXA1-GFP protein are ubiquitinated. COS-7 cells were transfected with 10, 11, and 12 polyhistidine constructs of HOXA1-GFP isoform A and fixed 24 h after transfection. Immunostaining revealed that nuclear aggregations were positive for ubiquitin in 11 and 12 histidine repeat transfectants.

To show that the observed protein aggregation was related to the presence of histidine repeats, we treated HOXA1 transfected cells with diethylpyrocarbonate (DEPC), a known histidine-modifying compound [13]. The modification of histidine repeats resulted in a significant decrease of HOXA1 protein aggregation in the nucleus for cells transfected with 11 and 12 histidine variants (Figs. 4A and B). Formation of aggregates was also observed 24 h after 1 mM treatment of DEPC in 11- and 12-poly-H variants, however, the inhibition of aggregation was temporary. Twenty-four hours later, the formation of aggregates slowed down and cells exhibited signs of apoptosis. Taken together, these results suggest a possible involvement of histidine repeat expansions in protein aggregation.

Discussion

Homopolymeric amino acid tracts exist in many human proteins, including a variety of transcription factors [14,15]. In this study, we have identified novel variants of HOXA1 gene encoding various polyhistidine repeat lengths. Since these variants can be observed in both affected and unaffected individuals, their contribution to autism pathophysiology remains controversial. At present, no report

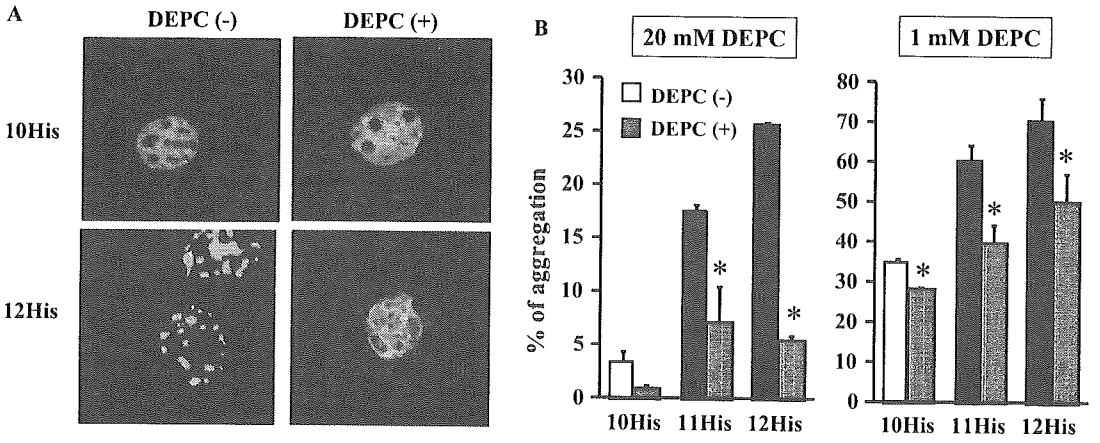

Fig. 4. DEPC delayed nuclear aggregations. (A) Fluorescent images for COS-7 transfected with 11 and 12 polyhistidine variants of HOXA1-GFP, treated with and without diethylpyrocarbonate (DEPC). Twenty-four hours after transfection, cells were treated with 20 mM DEPC for 30 min. Treatment with DEPC reduces HOXA1-GFP protein aggregates. (B) Formation of aggregates delayed after 1 mM treatment of DEPC for 24 h or 20 mM for 30 min. After treatment with DEPC, the percentage of protein accumulation over the number of transfected fluorescent cells for 11, 12, and also 10- polyhistidine variants were significantly reduced as compared to untreated cells. Each bar represents the mean (SEM) from three independent experiments. $^{*}P < 0.05$ significantly different from untreated control.

has ever been made about polyhistidine repeat expansion and a possible link with neurodegenerative disease. An increasing number of human neurological diseases have been linked to the triplet expansion of normal tracts of single amino acid repeats such as polyglutamine repeats [10–12]. More recently, expansions of alanine tracts have been shown to cause at least nine human diseases, including mental retardation and malformations of the brain [16,17]. Repeat expansion diseases share a number of similar characteristics, including formation of ubiquitinated protein aggregations, neuronal dysfunction, and cell death [18,19]. Here, we showed that histidine repeat expansion resulted in protein aggregation in the nucleus and that this aggregation was positively correlated with time after transfection and increasing repeat length (Fig. 1E). These aggregations were highly ubiquitinated and resulted in early cell death with the fragmented nuclei (Figs. 2 and 3); however, it still remains unclear whether this cell death is accompanied by caspase activations as seen in the polyglutamine-induced cell death [20]. DEPC is known as a histidine-modifying compound. Loomans et al. [13] reported that polyhistidine activity for an antimicrobial protein, calprotectin, was reversed by addition of DEPC. DEPC decreased and delayed protein aggregations in HOXA1 transfected cells (Fig. 4), suggesting that increasing histidine repeat length is correlated with HOXA1 protein aggregation and improper protein folding caused by polyhistidine expansion. It is generally believed that an altered, aggregation prone, conformation of the mutant protein confers the loss of a normal function, the gain of a toxic new function, or both [10–12]. In HOXA1 protein, polyhistidine is thought to be the site to interact with other proteins [4] and the variation in histidine repeat lengths may influence the function of the proteins that bind to HOXA1. Alteration of protein partner binding interaction is already described to confer neuronal damage in polyglutamine diseases, such as HIP-1, HAP, and GAPDH (as well as many others) for huntingtin [21–23], LANP and GAPDH for ataxin-1 [24], and A2BP for ataxin-2 [24,25]. To analyze the interaction between HOXA1 and its cofactors, HOXB1 and EphA1, may give us more physiological relevance. Moreover, in our study, protein aggregation of HOXA1 was noticeable in cells expressing variants of isoform A, while no significant aggregation was observed for cells transfected with isoform B variants (Fig. 1D). These findings further suggest that the two isoforms of HOXA1 may have different roles in patterning during the development. Furthermore, the degree of protein aggregation correlated positively with increasing length of histidine repeats. We hypothesize that this may be a mechanism for apoptosis associated with the process of assigning segmental identity in the developing brain. In vivo studies will be necessary for the future work to identify a novel function of HOXA1 protein with histidine tract expansions in the brain.

In summary, we discovered polyhistidine repeat variants in HOXA1 protein from a Japanese population comprised of normal and autistic individuals. Certain individuals were heterozygous for 7-, 9-, 11-, and 12-histidine repeats. No homozygous case has been found for any of these variants. In vitro expression of expanded variants resulted in early nuclear protein aggregation and an increase in cell death. We speculated that polyhistidine tract expansions may have an important role for correct folding of the HOXA1 protein and function.

Acknowledgments

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CATATONIA IN INDIVIDUALS WITH AUTISM SPECTRUM DISORDERS IN ADOLESCENCE AND EARLY ADULTHOOD: A LONG-TERM PROSPECTIVE STUDY

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The objective is to cast light on diagnosis and catastasis, course, and comorbidity as concerned with catatonia in patients with autism spectrum disorders (ASDs) with respect to long-term prospective follow-up. Eleven patients (all male) were enrolled. The mean age and the mean follow-up duration were 27.6 years (standard deviation (SD) 5.5) and 18.7 years (SD 8.7), respectively. The mean IQ was 27 (SD 16.4). Information was garnered from medical case records; current examination and observation of patients, interview of parents, and questionnaires completed by parents or other caretakers. Informed consent was obtained from the parents. Criteria for catatonia in this study were: (1) abrupt stop of movements and maintenance of immobility or bizarre posture beginning in adolescence and early adult life, (2) such a cataleptic state had continued for at least several minutes and appeared many times a day to the point of interfering with

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daily activities. We described two typical catatonic cases of ASDs. The average onset age was 19 years (SD 6). In all cases, our diagnostic criteria of catatonia evaluating at worse are fully compatible with those of Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV). In 8 out of 11, the onset of catatonia was clearly preceded by the appearance of slowness in movements accompanying the exacerbation of obsessive-compulsive symptoms. Catatonia was also found to have some connection with Tourette syndrome (3 cases), adjustment disorders ($N = 1$), and depressive mood disorders ($N = 1$). In one case, the manifestations of catatonia had to be distinguished from parkinsonism caused by antipsychotics.

Catatonia in ASDs seems to be a chronic condition in most cases. However, there were also a few cases in which catatonia repeatedly aggravated over short spans of time. Catatonia in ASDs may be considered an epiphenomenon of ASDs or a manifestation of comorbidity in adolescence or early adulthood.

I. Introduction

The concept of catatonia has broadened in recent years. It is no more restricted to schizophrenia but is also thought to occur in mood and organic disorders (Fink and Taylor, 2003). Realmuto and August (1991) reported three catatonic adolescents with autism and other psychiatric conditions. Among patients with autism in adolescence and early adulthood, motoric immobility is occasionally observed along with delayed action and repeated conduct. Wing (1996) defined "catatonia" as this sort of immobility. Although catatonia in autism spectrum disorders (ASDs) has been recognized among clinicians and researchers, the nature of catatonia in ASDs is yet to be clarified. The nature and treatment of this psychomotor syndrome as well as its scope and course remain virtually unidentified. Few published studies are available on the long-term course of catatonia in patients with ASDs.

We previously reported about eight ASD patients with catatonia, aged 20 or over, who had regularly visited the outpatient clinic at Department of Neuropsychiatry of Tokyo University Hospital (Ohta *et al.*, 1999). With an elapse of almost 6 years, "*M*," a child psychiatrist, seeing most of these ASD patients with catatonia, moved to the "*Z*" center. Many of the patients also did so later. In the meantime, there appeared new patients suspected of having catatonia.

This study is primarily designed to cast light on diagnosis and catastasis, onset and course, and association with complications as concerned with catatonia in patients with ASDs in terms of the long term prospective study. A few suggestions for treatment of catatonia in ASDs are made.

II. Subjects

The subjects came up to a total of 11, including 8 cases reported at the 40th Congress of the Japanese Society for Child and Adolescent Psychiatry (Ohta *et al.*, 1999). The three new cases were identified among ASDs patients, aged 20 or over, who regularly visited the "ℤ" Center from June to December in 2003, who fit into the criteria for catatonia, and who had symptoms that considerably hampered their everyday lives or had had them in the past. All the authors of this chapter were seeing at least one of those patients as the attending doctors or the clinical psychologist. The 11 patients fulfilled the criteria of autistic disorder in DSM-IV-Text Revision (TR) (APA, 2000). The average age at the time of initial diagnosis was 8.7 years (standard deviation (SD) 6.4) with the mean follow-up duration at 18.7 years (SD 8.7). All of them were male. The ages at the time of our investigation averaged 27.6 years (SD 5.5, age range: 21–40). Mean IQ was 17 on the Tanaka–Binet scale of intelligence (within the range of 13–70). As evaluated according to the Ohta Staging, which is an evaluation system of cognitive development in autistic children devised and standardized by Ohta *et al.* (Mutoh *et al.*, 2003; Ohta, 1987; Ohta *et al.*, 1989), three came on Stage II, four on III-1, two on III-2, and one on IV.

At the time of investigation, 8 of the 11 patients were visiting the doctor on a regular basis. The whereabouts of two cases reported in 1999 were unknown, and information about conditions of another case could be secured from the mother by telephone.

III. Methods

Regarding the course of the illness, data could be obtained from the statements made by the parents and the descriptions in the medical charts, as well as psychiatric interviews of each visit. The parents consented to our publishing the results of the study.

The term "catatonia," once solely attributed to schizophrenia, is broadly employed today as a behavioral syndrome for other disorders. Psychiatrically, we took catatonia as a failure to manifest spontaneous will and defined it as follows, while referring to descriptions about catatonic disorders in the Catatonic Disorder Due to a General Medical Condition and the Catatonic Features Specifier in the 4th edition of the DSM-IV-TR (APA, 2000), and those both in the Guideline (WHO, 1992) on Organic Catatonic Disorders in International Classification of Diseases, 10th revision (ICD-10) and Diagnostic Criteria for Research (DCR) (WHO, 1993), and those by Wing (Wing, 1996; Wing and Shah, 2000).

A. Criteria For Catatonia in this Study

Catatonia is a behavioral syndrome, and the severity changes during the span of a day and according to the mode of life, so that it would be difficult to come to grips with the loss of voluntary will. Therefore, we focused on movement that comes out and then stops halfway, in situations where the conditions may be accurately grasped by physicians at the outpatient clinic or the parents and other persons in their everyday lives. We picked up only the cases that fell under the category of this condition at the worst time.

1. In adolescence and early adult life they had abruptly stopped their movements and gotten locked into immobility or maintained bizarre posture.
2. Such a cataleptic state had continued for at least several minutes and appeared many times a day.
3. The disturbance caused clinically significant impairment in social, occupational, or other important areas of functioning, and continued for 3 months or more.
4. Clear drug-induced parkinsonism or cases in which the immobility could be explained by an inner state of absorption should be excluded.

B. Severities of Catatonia

The severities of catatonia were classified into "none," "mild," "moderate," and "severe," and the degree of severity was judged depending on the social impairment caused by compulsions referring to "interference due to compulsive behaviors" on the Yale-Brown obsessive-compulsive scale (Y-BOCS) (Goodman *et al.*, 1989a,b). "Mild" represents slight impairment in social and vocational activities without hampering efficiency as a whole; "moderate," some degrees of impairment evidently existent in those activities; and "severe," the degree at which the patients and their families feel it measurably difficult to cope with.

IV. Presentation of Cases

Case 4: 27-year-old male; IQ 40, Ohta Stage III-2

This is a typical case of catatonia as described by Wing (1996), which continued for about 10 years.

There was nothing noteworthy about him in the prenatal, perinatal, and infantile periods. At 12 months of age he started toddling. At about 18 months, he appeared to lag far behind in language development and was markedly

TABLE I

DESCRIPTION OF 11 CASES WITH ASDs AND CATATONIA

Case	1	2	3	4	5	6
Sex	m	m	m	m	m	m
Outcome	Visiting	Visiting	Dropout	Visiting	Visiting	Dropout
Current age	31	30	40	27	25	31
Ohta Stage[a]	II	III-2	III-1	III-2	I-3	IV
Age of first visit (yy:mm)	9:06	15:03	7:11	3:05	5:06	23:05
Duration of follow-up (yy:mm) (Oct 2003)	18:00	14:08	32:07	23:10	19:11	5:06
IQ	17	30	22	40	13	70
Preceding slowness (age)	15	No	No	14	15	20
Preceding symptoms such as obsessive–compulsive symptoms (OCS)		Bad feeling, negativism, self-injurious, aggression		Excitement, OCS↑, manifestation of TS[b]	Aggression↑ ordering↑	
History of antipsychotics	Yes			Yes		
Age of manifestation of catatonia	23	15	19	19	18	21
Social situation at onset	Workshop	Special school	Workshop	Competitive job	Workshop	Workshop
Severity at worst	Mild	Moderate	Moderate	Severe	Moderate	Severe
Course and outcome of catatonia (yrs)	Suddenly developed and lasted for 7 months After that no catatonia	1st lasted few months 2nd occurred along with TS	Suddenly occurred with slowness and lasted for less than 1 year. After that time no catatonia	Has lasted in a mild form, but no TS symptoms	Difficulty in initiation has lasted	Subacutely occurred and lasted till the time of dropout
Second phase (yr)		19				
Epilepsy			Yes	Yes		
Psychiatric comorbidity		TS[b]		TS[b]		
Family history						

(*Continued*)

TABLE I (*Continued*)

Case	7	8	9	10	11
Sex	m	m	m	m	m
Outcome	Visiting	Dropout	Visiting	Visiting	Visiting
Current age	27	28	23	21	21
Ohta Stage[a]	III-1	III-1	III-2	III-1	II
Age of first visit (yy:mm)	15:11	3:11	3:05	3:08	4:03
Duration of follow-up (yy:mm) (Oct 2003)	11:07	23:03	20:01	17:10	18:01
IQ	19	14	32	27	13
Preceding slowness (age)	No	19	20	17	20
Preceding symptoms such as obsessive–compulsive symptoms (OCS)	Repetitive movement↑	Touching compulsion	Ordering	Sleep disturbance, ritual behavior	Touching compulsion, excitement
History of antipsychotics	Yes (2nd time)				Yes
Age of manifestation of catatonia	15	21	21	17	20
Social situation at onset	Special school	Workshop	Workshop	Workshop	Workshop
Severity at worst	Moderate	Severe	Moderate	Severe	Moderate
Course and outcome of catatonia (yrs)	1st suddenly occurred and lasted for 1 years. 2nd (21yr) occurred followed by eyes rolling and lasted for 8 months. After that time no catatonia.	Lasted for 2years, mitigated at the time of dropout	Separating from his sib, he entered a group home. Soon after disappeared	Lasted for less than 2 years	Lasted for more than 1year, but touching compulsion has lasted with the same intensity
Second phase (yr)	21				
Epilepsy				Yes	
Psychiatric comorbidity	Parkinsonism?		Adjustment disorder	Sleep disturbance	TS[b]
Family history			Sib: schizophrenia	Father: depression	

[a]Ohta Stage: Levels of cognitive development in autistic children devised and standardized by Ohta *et al* (1989).
[b]TS: Tourette syndrome.

hyperactive. He did not respond when his name was called out. When he turned 2-year old, he was diagnosed as having autism.

After the fourth birthday, he began having severe tantrums when he saw the very slightly disorganized tableware on the dinner table.

While in special classes at the elementary school (6-year old), his hyperactivity remained unabated. He threw in a fit of temper when he was not allowed to do familiar routines or when his schedule was upset. With the administration of haloperidol, his condition turned better. As a fifth grader, he had an epileptic seizure in autumn. Although EEG showed nothing abnormal, treatment with valproic acid commenced.

In January, of the sixth year at elementary school, Tourette syndrome (TS) appeared abruptly. The major symptoms he exhibited included facial grimacing, hiccupping, and rapid jerking of the body concurrent with the utterance of bizarre sounds. These well-defined forms of tics had disappeared when he moved up to the second year of junior high school. Several months later, however, self-injurious behavior emerged. There were sudden outbursts during which he banged his head against the table violently.

In October, he took to hanging his clothes on the hanger and taking them off again and again. He also repeatedly said "itadaki-masu," a short prayer of thanks before a meal in Japan. These repetitive actions lasted from 5 min to more than 10 min. Toward the end of the year, increased slowness affecting movements became conspicuous. In May, of the third year at junior high school, he again started loosing his temper easily. Daily doses of pimozide were increased to 4 mg and these temper tantrums diminished as a result.

After he entered senior high school for mentally handicapped children, he persisted to the characteristic pattern of behavior in everyday life without relapse of temper tantrums.

He finished senior high school and found a job at 18. It seemed that he somehow managed to do his own part, although it was said that he had poor ability of concentration. He rarely lost his temper. It seemed that all things were going well with him at home and work.

Repetitive actions increased in October, of the year, when he turned 19-year old. In January of the next year, he became slow moving. In April, he began exhibiting such symptoms as the repetition of bizarre behaviors, and freezing in postures during activity such as making tea. The manifestations of these abnormalities of behavior and posture lasted several minutes. In May, he became unusually concerned with keeping the tableware on the dinner table in perfect order or arranged exactly. Repetition of such words and phrases as "I'm home!", "Good night," and "itadaki-masu" increased in frequency. Also, repetition of a word or phrase just spoken by another person increased remarkably.

In April of the following year, he became fussier and manifested motoric immobility more frequently regardless of mood, which varied greatly from

day to day. In September, he maintained a rigid posture while in standing position for several hours a day. Prompted to move, he wouldn't budge an inch. When forced to move, he returned to where he stood and continued to stand as stiff as a stature. This symptomatology caused disruption of his occupational functioning.

When the patient was additionally dosed with bromazepam (BZP) 4 mg, these symptoms tended to abate, but were not ameliorated completely. At the end of the third year, he resigned from his job and moved to a sheltered workshop in April. Through 1 year, after he became 21, motoric immobility occurred every day. Once it occurred, it lasted for 10 min to several hours.

When he was 22, the frequency of the psychomotor disturbance decreased to three times a week. Three years later, when he was 25 , his condition improved considerably. The maintenance of a rigid posture lasted only several minutes. A few months later he moved to a residential welfare institution. As immobility had almost disappeared, the dosage of BZP 5 mg was tapered off.

Soon after that, he had an epileptic seizure after an absence of over 5 years. Once the seizure returned, it occurred once or twice a month. With an increase in the dosage of an antiepileptic drug, the occurrence rate was on the decline. Relapse of the seizure did not change the residual catatonic symptoms. Epileptic seizures occurred infrequently for the 2 years that followed and have not occurred for more than 1 year now. There are no MRI abnormalities. ECG examinations revealed only a slight degree of paroxysmal abnormalities.

Case 7: 27-year-old male; IQ 19, Ohta Stage III-1

This is the case in which catatonic symptoms appeared twice and disappeared quickly.

No abnormalities were observed during the prenatal and perinatal periods.

At age 1, he started walking by himself. It was not long before he exhibited signs of hyperkinesia, mutism, and apathy. From infancy, he had a strong inclination to adhere to a pattern of behavior in everyday life. Even now, he has been occupied in doing a ritualistic custom at mealtime and has a mania for collecting plastic models of monsters.

In July, when he was a first grade junior high school student, he began to act rudely or take a defiant attitude. When he got angry, he slapped or pinched his opponent's hand.

At the age of 15 months, he started walking up and down or extending his hands compulsively. It was around that time that motoric immobility emerged. He rejected any approach when he was prompted to move. However, it disappeared in a year or so.

When he was 16-year old, he visited us, as he could not shake off the self-injurious behaviors. With the use of an antipsychotic agent, self-injurious and aggressive behaviors were reduced notably.

His condition remained in remission till he reached 21 years and 6 months of age, when eye-rolling suddenly occurred. This symptom responded to treatment with anticholinergic medication.

Two months later, he began to show bizarre behavior. He stood motionless with one leg raised. He also began to threaten to scratch his family members with his nails. After his dose of antipsychotic medication was increased, these abnormalities gradually faded away.

At present, he is not aggressive and freezing has disappeared. He still follows his characteristic pattern of behavior in every day life. He entered the 2005 Special Olympics World Winter Games and won three Gold medals.

V. Results

Average age at onset and frequency in ASDs: The average age at onset of catatonia was 19 years (SD 6, age range: 15–23). Out of 69 cases, who were 20 years or over and visited outpatient clinic of "乙" center, 8 (11.6%) had current symptoms of catatonia or had a past history of catatonia. As for the remaining three cases, who had been followed-up till 3 years ago; the whereabouts of two were unknown, whereas the third one was under treatment in another hospital (See Table I).

A. Symptoms and Severity of Catatonia

In DSM-IV-TR (APA, 2000), five symptoms characterize catatonia or catatonic disorder due to a general medical condition—that is, the maintenance of imposed postures (catalepsy), including waxy flexibility, or the absence of movements

TABLE II
Distribution of DSM-IV Criteria for Catatonia in 13 Catatonic Episodes in Patients with ASD

Case	1	2		3	4	5	6	7	8	9	10	11	12
Times of catatonic phase	1	1	2	1	1	1	1	1	2	1	1	1	1
Motoric immobility	1	1	1	1	1	1	1	1	1	1	1	1	1
Excessive motor activity	0	1	1	0	0	1	0	0	0	0	0	0	1
Extreme negativism or mutism	0	1	1	0	1	1	1	1	1	1	1	1	1
Peculiarities of voluntary movement	1	1	1	1	1	1	1	1	1	1	1	1	1
Echolalia or echopraxia	0	1	1	1	1	0	1	0	0	0	0	0	0
Number of positive items	2	5	5	3	4	4	4	3	3	3	3	3	4

1: Present.
0: Not present.

(akinesia) with the manifestation of stupor; hyperactivity; extreme negativism (evidently nonmotivated resistance or the maintenance of a stiff posture against attempts to be moved), mutism; stereotypy, significant mannerism or the strangeness of volitional movements demonstrated by a significant grimace; and echolalia or echopraxia. Should there be two or more such symptoms, it can be argued that catatonia coexists. After "*M*" had selected probable catatonic cases on the basis of the worst state, "*K*" independently evaluated them, and confirmed the cases to have catatonia by the existence of more than two of the five symptoms. Four cases were considered severe, six cases moderate, and one mild (See Table II).

B. Preceding Conditions

Before the manifestation of a typical catatonic symptom in which movements come to a halt in a strange posture, eight cases had prodromal symptoms, typically a gradually emerging sluggishness with compulsive behaviors lasting for more than 1 year. In the other three cases, onset of catatonia was abrupt with no preceding prodromal phase.

C. Psychiatric Complication, Family History, and Medication

Three cases were diagnosed with TS. At present, one of them (Case 11) still has it (see Table I).

The obsessive-compulsive symptoms in Case 9 seemed to increase in periods of greater family turmoil and conflict with his schizophrenic sibling. Catatonia may have been precipitated by increased stress.

Case 10 had a family history of mood disorder. Complications with epilepsy were observed in three cases, and in one of them, epileptic seizures recurred twice or so a month after the alleviation of catatonia (Case 4). In Case 10, the onset of epilepsy came for the first time at the age of 20 after the alleviation of catatonia. Only three cases were on antipsychotics before onset of catatonia. Of the remaining 8 cases, Case 7 took antipsychotics when he had catatonia for the second time. For Case 7, above all, a discreet differentiation was required between catatonia and antipsychotic-induced parkinsonism (see the case presentation).

D. Courses

Catatonic symptoms showed considerable fluctuations during the span of a day, in all cases. Those changes could be observed even at the worst time. The alleviation of symptoms did not signify full improvements in the attitude of refusal