doi:10.1006/geno.2002.6814, available online at http://www.idealibrary.com on IDEAL

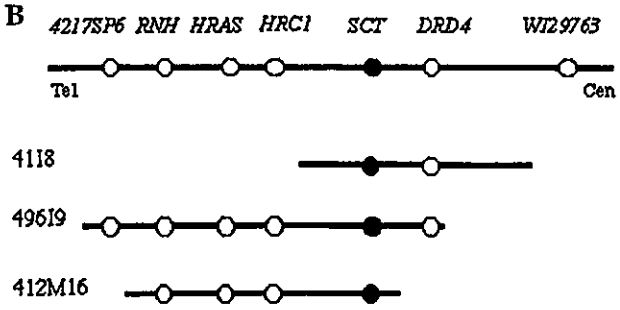


FIG. 2. Precise position of SCT and nearby genes. (A) Pulsed-field gel Southern blot analysis of genes from 11p15.5 in BAC clones. Probes for each of the five genes known to be present in the secretin-positive BACs were applied to Southern blots of BAC DNAs digested with three rare-cutting restriction enzymes (*MluI*, *NotI*, and *SalI*). Bands shared in common allow estimation of the distances between genes. (B) Gene and marker order of genes near secretin. Three BACs carrying secretin were assessed by PCR for the presence of nearby genes. The order provided is derived from these experiments and Southern blots in (A).

HRAS, *HRC1*, and *RNH* were positive in DNA from 412M16 and 496I9. The cosmid end sequence 4217SP6 [26] that is located more distal to these genes was present only on 496I9, and a more proximal marker, *WI-9763*, was negative for all three BAC clones. Southern analysis of large fragments generated by digestion with *MluI*, *NotI*, and *SalI* and fractionated by pulsed-field gel electrophoresis provided a more precise map (Fig. 2A). *SCT* was positioned between *DRD4* and *HRC1* (Fig. 2B) based on interpretation of restriction fragments shared among BACs. Draft sequence for BAC clone 496I9 has been developed by the Baylor College of Medicine Genome Sequencing Center under the accession number AC021663. While this sequence is incomplete, it supports the sequence and positional data developed in this study.

Additional sequence is available in the region (AC093486), but it is also incomplete. The BAC clone used to generate this sequence carries *RNH*, *HRAS*, and *SCT*, but not *DRD4*, which would support our order. Both NCBI (http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/maps.cgi?org=hum&chr=11) and Ensembl (http://www.ensembl.org/Homo_sapiens/mapview?chr=11) have attempted to assemble the sequence in this region and to reconcile it with radiation hybrid maps to provide an order. However, considering the incomplete nature of the sequences used for assembly, it is more likely that our order based on clone ends is correct. Moreover, our order agrees with one developed from large-scale restriction mapping by Higgins *et al.* [27], who found the order centromere–*DRD4*–*HRAS*–*RNH*–telomere. In any case, *SCT* is very close to *DRD4*, a finding supported by sequence data.

Methylation Analysis of *SCT* Promoter Region

Portions of the 11p15.5 region surrounding *SCT* have been shown to be subject to imprinting [28]. We investigated potential imprinting of *SCT* by analysis of methylation of its highly GC-rich promoter. RT-PCR revealed expression of *SCT* in lymphoblastoid cell lines (Fig. 3), and these were therefore used as a source of DNA for methylation studies. Southern analysis of genomic DNA from human blood cells and lymphoblastoid cell lines digested with *Hin*dIII and *Eag*I or *Hin*dIII and *Nar*I digestion revealed no evidence of partial methylation, because a single, completely digested fragment was observed in each sample (Fig. 4A).

SCT Mutation Analysis in Autism Patients

We screened a collection of patients diagnosed with autism or pervasive developmental disorders for sequence variation in *SCT* using several methods. Two groups of patients were studied. The first was a panel of 50 patients collected by the Autism Genetic Resource Exchange founded by the Cure Autism Now Foundation (http://www.agre.org/). This panel consists of individuals from multiplex autism families.

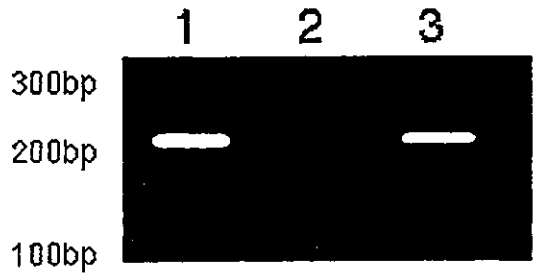


FIG. 3. RT-PCR analysis of secretin gene expression. Lymphoblasts (lane 1) and neuroblastoma cells (NT2, lane 3) express secretin mRNA, but fibroblasts (lane 2) express it only faintly.

A second group was 25 patients diagnosed with autism at Jichi Medical School in Japan. For both groups, a diagnosis of autism followed criteria of The Tenth Revision of the International Statistical Classification of Diseases and Related Health Problems (ICD10), The Diagnostic Statistical Manual (DSM) IIIR and IV.

We investigated gross-scale changes using PCR and Southern blot analyses. The VNTR was studied by PCR amplification. Table 1 provides allele frequency data for the VNTR in autistic and normal individuals. No significant difference in allele frequency existed between cases and controls. Southern analysis of restriction digests using *Hin*dIII and *Eag*I revealed the expected 1.5-and 3.6-kb fragments in each of the 75 autism samples (Fig. 4). Thus, no evidence for gross-scale changes in the gene or its associated VNTR could be found. Additionally, no alterations in methylation pattern were found (as measured by *Eag*I digestion).

We investigated small-scale changes in *SCT* using conformation-sensitive gel electrophoresis (CSGE), a method based on heteroduplex detection [29,30]. We identified five products with shifted bands using five PCR products representing the four *SCT* exons and promoter region. The sequences of the mismatched products showed the following nucleotide changes (Fig. 5).

A G residue at position 900 (Fig. 1) was a T in one Caucasian autism patient (HI0587) and a C in one Japanese patient (MRX125; Fig. 5A). This G is the last base of a predicted E-box transcription factor–binding sequence (CAGCTG) and disrupts a *Pvu*II site. Screening by PCR and *Pvu*II digestion in control samples revealed absence of the *Pvu*II site in 0/100 Caucasian control chromosomes, and in 2 of 112 chromosomes of Japanese origin (Fig. 5B).

A second alteration in the promoter sequence was a G→A change at position 962 (Fig. 1) in a predicted GC-box in a single autism patient of Caucasian origin (HI0304) (Fig. 5A). This base change destroys a *Nar*I site. Screening by PCR and digestion with *Nar*I demonstrated absence of the *Nar*I site in 1 of 100 Caucasian control chromosomes, and in none of the 112 Japanese control chromosomes (Fig. 5B).

A C→T transition was detected at position 1448 in exon 3 in one Japanese autism patient (MRX118; Fig. 5A). This alteration would predict a change in amino acid (Pro→Leu) at position 78 of the secretin precursor polypeptide. We designed a mismatch primer to create a *Hpa*II site to facilitate screening for this variant. Control chromosomes from 50 Caucasians and 56 Japanese were analyzed, and none was found to carry this alteration (Fig. 5B). DNA was available from this patient's unaffected mother and sister. Analysis of these samples demonstrated that each was heterozygous for the mutation (Fig. 5C).

A G→C substitution was detected in exon 4 at position 1743 in one Caucasian autism patient (HI0725; Fig. 5A). This alteration would predict an Arg→Thr change in the secretin precursor polypeptide at position 121. None of the 100 Caucasian and 112 Japanese control chromosomes carried this change as assayed by allele-specific oligonucleotide hybridization (Fig. 5B).

Finally, we detected a polymorphic insertion of a G residue at position 1139 (Fig. 1) in intron 1 in 10 of 50 Caucasian autistics and in 14 of 50 Caucasian controls (Fig. 5B).

Direct sequencing of PCR products from each of the 50 autism patients of Caucasian origin revealed one additional change that was missed by CSGE. This G→A transition at

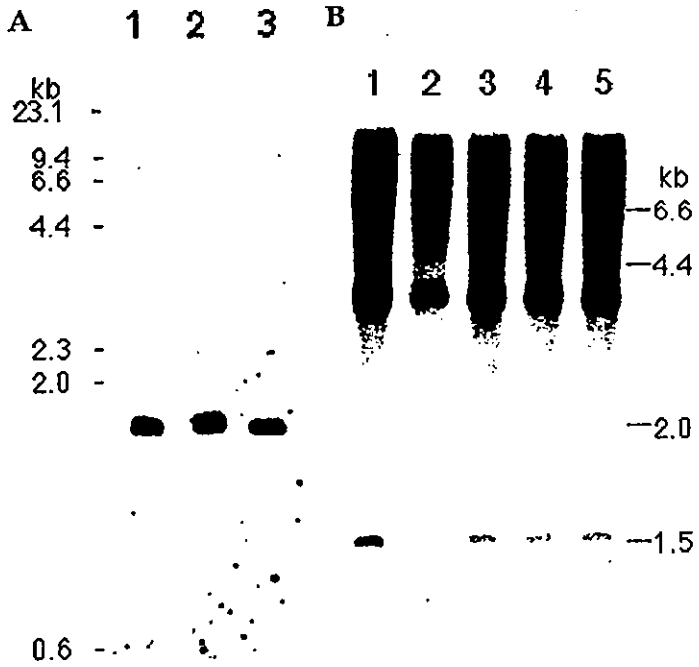
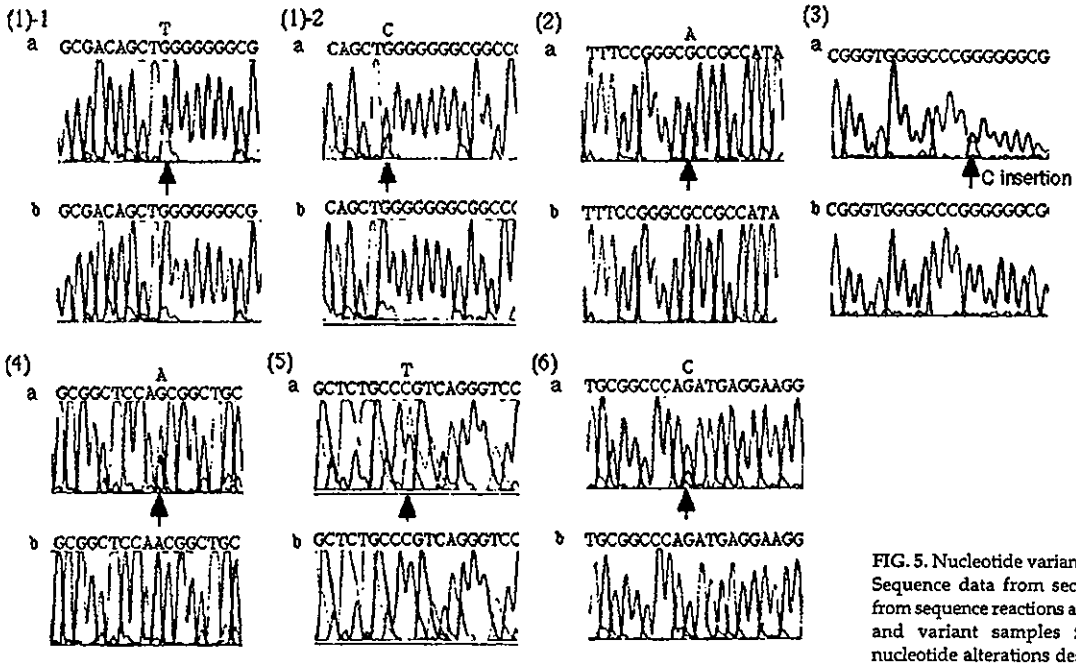


FIG. 4. Southern blot hybridization of SCT to detect possible gene alteration and methylation. (A) Southern blot hybridization of *SCT* to human genomic DNAs from blood, lymphoblastoid cell lines, and NT-2 cells. DNA was digested with *Hin*dIII and *Eag*I, and hybridized with a probe derived from the secretin coding sequence. No evidence for methylation of the *Eag*I site is observed. (B) Southern blot hybridization of secretin to human genomic DNAs from autistic patients. DNA was digested as in (A) and hybridized with a probe derived from *SCT*, including upstream sequences. No evidence of gross alteration is observed in these and an additional 70 samples from autistic patients.

doi:10.1006/geno.2002.6814, available online at http://www.idealibrary.com on IDEAL

A



B

	base change	position	aa change	autism Caucasian	autism Japanese	control Caucasian	control Japanese
(1)-1	900G to T	E-box		1/50	0/25	0/50	0/56
(1)-2	900G to C	E-box		0	1	0	2
(2)	962G to A	GC-box		1	0	1	-
(3)	1139G insertion	intron 1		10	-	14	-
(4)	1258G to A	exon 2	Gln47Gln	1	0	-	-
(5)	1448C to T	exon 3	Pro78Leu	0	1	0	0
(6)	1743G to C	exon 4	Arg121Thr	1	0	0	0

C

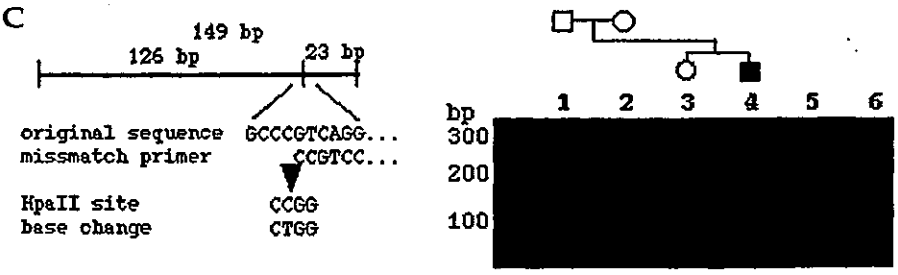


FIG. 5. Nucleotide variants in the SCT gene. (A) Sequence data from secretin variants. Traces from sequence reactions are provided for normal and variant samples for each of the six nucleotide alterations described in *SCT* (B). (B) Nucleotide variants in secretin. Each of the six variant positions is listed along with its position in the gene, potential coding sequence change, and frequencies in patient and control populations. (C) Exon 3 alteration in autistic patient and his normal relatives. PCR-based assay of this variant in the family of an autism patient from Japan. A PCR primer that allowed the creation of an *HpaII* site in the presence of the normal nucleotide was designed and used to assess the genotypes of members of the indicated pedigree in lanes 1–4. Mother, normal sister, and the patient are each heterozygous for this variant. Lanes 5 and 6 show results from control samples before and after the digestion, respectively.

position 1258 in exon 2 of patient HI0413 does not alter an amino acid.

We detected six base changes while screening *SCT* in the autism panels. Of these, two were promoter changes, one was a silent alteration of a codon, one was a common intronic insertion, and two were coding sequence changes that altered amino acid residues in the secretin precursor peptide. Both promoter mutations appear to be rare variants based on identification of these alterations in control samples. The amino acid-substituting alterations, however, were not found in control samples. We did not assess the conservative exon 2 change in control samples because it was not expected to alter gene function. Neither homozygous nor compound heterozyous individuals were found for any of the base changes.

Discussion

Autism is a heterogeneous disease, and it is likely to result from etiologies that are both genetic and environmental. From

currently available data, it is unlikely that any single genetic locus will account for the majority of autism cases, a conclusion that is underscored by the several reports of genetic linkage to numerous genomic regions. Linkage has been reported for 1p, 2q, 4q, 5p, 6q, 7q (7q31–q33), 10q, 13q, 15q11–q15, 16p, 18q, 19p, 22p, and X [5–9].

Considerable interest was focused on secretin after anecdotal reports of improved behavior in autistic patients after secretin injection [1]. Recently, more carefully controlled studies have not demonstrated an effect of secretin injection [2], and it appears unlikely that the majority of autism patients will benefit from this treatment. Nonetheless, we sought to determine whether mutations in *SCT* might be present in some autism patients. It is possible, for example, that the subset of patients who respond to secretin may harbor genetic variation in *SCT*.

To test this hypothesis, we isolated the human secretin gene and determined its chromosomal position and structure. The position of the gene at 11p15.5 between *HRAS1* and *DRD4* was intriguing because of prior reports of linkage and/or association to markers in this region in both autism and ADHD patients. In a genome-wide scan for autism, a weak association was found between autism and markers on 11p15 [8], and a positive association between autism and polymorphic markers in *HRAS* has also been reported [19,20]. ADHD has been associated with alleles at the DRD4 dopamine receptor by several studies [21,31], and ADHD is found in a fraction of autistic patients.

The human secretin gene is located at 11p15.5, a region characterized by chromosomal imprinting. The imprinted segment includes *IGF2*, *H19*, *HASH2*, and *CDKN1C*. These genes are relevant to Beckwith–Wiedemann syndrome and to Wilms tumor [32]. Imprinting of a gene involved in autism would complicate standard linkage and association studies, possibly explaining the absence of significant linkage in this region. However, no evidence for imprinting of *SCT* could be found in the small variety of tissues tested. While it is formally possible that *SCT* undergoes imprinting in a restricted set of tissues (as with *UBE3A* in the Prader–Willi/Angelman region of chromosome 15 [33]), the refined location of *SCT* between *HRAS1* and *DRD4*, both of which do not appear to be imprinted, supports the likelihood that it is not subject to imprinting.

We identified a VNTR proximal to the presumed promoter region of *SCT*. Many VNTRs have been characterized in the 11p15.5 region, some of which play a role in gene expression and genetic susceptibility to disease. *HRAS1* carries a 28-bp VNTR that ranges in length from 1000 to 2500 bp. This repeat is located ~1 kb distal to the polyadenylation signal for the HRAS1 transcript [34–37]. This minisatellite binds members of the rel/NF-kB transcriptional activator family, and the presence of this sequence can activate reporter gene constructs in some cell lines [35]. When enlarged, the *HRAS1* minisatellite appears to increase susceptibility to a variety of cancers, including breast, lung, ovary, colon, and bladder tumors as well as leukemia [34–37]. *DRD4* carries a 48-bp VNTR in exon 3 that exhibits length variation. The seven-repeat allele has been implicated in ADHD [21,31], while other alleles appear to be more common in individuals with risk-taking personalities [38,39]. Finally, the insulin gene's 14-bp VNTR, present in the gene's promoter region, has been shown to bind the transcription factor Pur-1 [40], and different lengths affect levels of transcription of both *INS* and neighboring *IGF2* [41]. Short lengths of this VNTR can be correlated with increased risk of juvenile-onset diabetes [40,42], while longer variants confer risk of polycystic ovary disease [43]. VNTRs are not unique to 11p15.5, but there is an unusual density found in this region. A VNTR in the cystatin B gene can expand to considerable lengths and cause progressive myoclonus epilepsy [44], and VNTRs have been described in a number of genes with suspected roles in neuropsychiatric disease, such as monoamine oxidase A [45], the serotonin transporter [46], and the dopamine transporter [47].

We observed only four alleles for *SCT* VNTR, and found no significant difference in their frequencies between autistic and control individuals. Sequencing of several copies of the 14- and 13-repeat alleles demonstrated each to be identical in sequence as well as in length. The 11- and 13-repeat VNTRs were found to lack the same repeat unit, and to demonstrate identical nucleotide substitutions, suggesting a common origin. While the frequencies of alleles differed between the North American and Japanese samples, these results were consistent with the reduced heterozygosity commonly found in the Japanese population. A 15-repeat allele was identified in the Japanese sample; this length was not found in the North American sample.

Both Southern and PCR-based analyses were carried out in the autism patients to define any alterations in the VNTR, but no abnormalities could be detected. The VNTR alleles in the autistic population were not significantly different from controls. It is therefore unlikely that deletion, duplication, or abnormal methylation of *SCT* and the immediately adjacent region plays a role in autism. The possibility of large heterozygous deletions including the entire gene and probe regions cannot be excluded.

By heteroduplex analysis and sequencing of *SCT* in normal and autistic individuals, we identified six separate base changes. Of the six nucleotide variants, only one was found to be a common polymorphism, and this was located in the first intron. Of the other changes, four have the potential to alter the function of *SCT*, while the fifth is a conservative coding substitution distant from splice junctions, unlikely to result in a functional change. We identified two promoter variants: a G residue of an E-box sequence is altered in two different patients, and a G in a GC-box is changed in one autistic individual. Each of these changes was observed at low frequencies in control samples, and the differences in frequency between cases and controls are not significant. The E-box change has been reported to alter activity of the rat secretin promoter [48]. Two nonconservative coding sequence changes were found in autistic individuals; neither could be demonstrated in the control populations. The exon 3 alter-

doi:10.1006/geno.2002.6814, available online at http://www.idealibrary.com on IDEAL

ation would predict a drastic amino acid change (Pro→Leu), and the exon 4 substitution would likewise alter the peptide considerably (Arg→Thr). Neither of these changes would be predicted to alter the processed hormone region of secretin, which is encoded in exon 2. However, it is conceivable that these alterations in the protein would alter processing of prosecretin to the mature polypeptide. It is also important to note that the exon 3 change was found in the unaffected mother and sister of an autistic male proband. Given the higher incidence of autism among males, it is possible that some genetic lesions have a higher penetrance in males than in females, and that this change does not confer a phenotype on females. However, the lack of phenotype in the proband's mother and sister suggests that this mutation is not solely responsible for the patient's autism. No convincing evidence of secretin mutation could be found in autistic patients, but the frequency of amino acid substitution and promoter variants appears somewhat high, especially considering the gene's small size.

Mutations in the methyl-CpG-binding protein-2 gene (*MECP2*) have recently been shown to result in Rett syndrome, and it has been suggested that control of gene expression through methylation and other chromatin changes is of particular importance in the CNS [49]. We could find no evidence for methylation of *SCT* in blood cells; however, the possibility that secretin undergoes brain-specific methylation that can be disturbed by sequence alterations cannot be ruled out. *SCT* lies in a region with genes that are subject to parental imprinting, and has a particularly CpG-rich promoter region. This scenario would be similar to the case of *UBE3A* in Angelman syndrome [50]. In support of this notion, Whitmore *et al.* [18] reported expression in most CNS tissues, with highest levels found in the medulla. Finally, it is possible that some of the base changes we have described might act to decrease secretin levels in the CNS or alter the pattern of gene methylation. These changes might be compounded by other genetic or environmental factors such as gender, secondary gene impairment, or exposure to pathogens that would increase the likelihood of autism. It is likely that further analysis of *SCT* will help to answer these questions. In particular, more patients should be analyzed for sequence variation, and study of gene mutations in the mouse may prove instructive.

Materials and Methods

Isolation of human secretin genomic DNA. A PCR primer pair, SECRETIN PIG1 (sense in exon 2) and SECRETIN PIG2 (antisense in exon 4) was synthesized according to the pig secretin sequence in a region that was highly homologous among the earlier described pig, mouse, and rat sequences, and used to amplify human genomic DNA. Amplified DNA was ligated into pCR-ScriptTM SK(+) plasmid vector using the pCR-ScriptTM SK(+) cloning kit (Stratagene) and transformed into XL1-blue competent cells. The insert sequence was determined using an automated DNA sequencer (ABI 373), and purified to use as a probe. The human male BAC library (RPCI 11) was screened by hybridization using the cloned PCR product as a probe. Positive BAC clones were digested with *Eco*RI, *Bam*HI, and *Hin*dIII, and shotgun-subcloned into the pBlueskript SK+ plasmid vector. Subclones were screened for *SCT* sequences by colony hybridization using the cloned PCR product. A positive *Hin*dIII subclone was further digested with *Eag*I and recloned. *Hin*dIII, *Bam*HI, and *Hin*dIII–*Eag*I subclones were subjected to sequence analysis using M13 forward and reverse primers and synthesized primers based on acquired sequences.

RT-PCR of secretin cDNA expression in cultured cells. Total RNA was extracted from cultured lymphoblasts, fibroblasts, and neuroblastoma cells (NT2) using Trizol Reagent (Gibco BRL), and cDNA was synthesized. Each cDNA was amplified by nested PCR using exo(-) *Pfu* polymerase. PCR primer pairs were SECRETIN 3 and SECRETIN PIG2 in a first PCR, and SECRETIN PIG1 and SEC EX4R in a second PCR. PCR products were extracted from the gel after electrophoresis, and were ligated into PCR ScriptTM SK(+) vector and transformed. Cloned cDNA were sequenced using M13 forward and reverse primers.

Mapping by fluorescence in situ hybridization. BAC clones were labeled with digoxigenin-11-dUTP (Boehringer, Mannheim) by nick translation, precipitated with sonicated salmon sperm DNA, and then resuspended in hybridization solution (65% formamide, 2× sodium saline citrate (SSC), 10% dextran sulfate) together with unlabeled Cot-1 DNA (Gibco) as a competitor. The DNA mixture was denatured at 70°C for 10 minutes, and then put onto the slides coated with denatured chromosomes. After overnight hybridization at 37°C, the slides were washed in 50% formamide–2× SSC at 43°C for 15 minutes, then washed again in 2× SSC at 43°C for 5 minutes followed by two washes by 4× SSC containing 0.1% Tween 20 at room temperature for 1 minute each. The slides were incubated with anti-digoxigenin–rhodamine, Fab fragments at 43°C for 30 minutes, then rinsed in 4× SSC containing 0.1% Tween 20 twice. The slides were counterstained with 500 ng/ml 4′,6-diamidino-2′-phenylindole dihydrochloride (DAPI; Boehringer Mannheim) and coverslipped. Photomicroscopy was conducted under a fluorescence microscope.

Radiation hybrid panel mapping. DNAs from the G3 Radiation Hybrid Panel (Research Genetics) were amplified by PCR (exon 1 primers) to detect the presence of the human secretin gene. Results (Vector:00000000000001000000000000000001100100000010000001000001000000101100000000000010000) were sent to the RH server (http://www-shgc.stanford.edu/RH/index.html), which returned the three markers that mapped most closely.

Fine-mapping of SCT. Several genes and markers from 11p15.5 were examined in the BAC clones positive for secretin by PCR with appropriate primers. BAC clone DNAs were digested with *Mlu*I, *Not*I, and *Sal*I, separated by pulsed-field gel electrophoresis, and transferred to nylon membrane. Pulsed-field gel electrophoresis was carried out in a CHEF mapper (BioRad) using the autoalgorithm for the molecular weight range of 7–70 kb. Nylon membranes were hybridized with probes for *DRD4*, *HRAS1*, *HRC1*, *RNH*, and *SCT*, respectively. Probes for *DRD4*, *HRAS1*, *HRC1*, and *RNH* were generated by PCR.

Methylation analysis of SCT promoter region. Genomic DNA was extracted from cultured human lymphoblasts and from blood lymphocytes. Genomic DNAs were digested with *Hin*dIII overnight. After ethanol precipitation, DNA was digested with *Eag*I overnight. DNAs were electrophoresed on 0.8% agarose gels, and transferred to nylon membranes. Nylon membranes were prehybridized and hybridized with a ^{32}P-labeled human secretin genomic DNA fragment consisting of exons 2–4. After overnight hybridization in a buffer containing 1.5× sodium chloride, sodium phosphate, EDTA buffer (SSPE) (1× SSPE, 0.15 M NaCl, 0.01 M NaH_2PO_4, 1 mM EDTA, pH 7.4), 1% sodium dodecyl sulfate (SDS), 0.5% fat-free milk, and 0.2 mg/ml sermon sperm at 65°C, the membranes were washed twice with 2× SSC, 0.1% SDS for 30 minutes at 65°C, followed by two washes with 0.5× SSC, 0.1% SDS for 30 minutes each at 65°C. The membranes were air-dried and autoradiographed at -80°C for 2 days.

Heteroduplex analysis of autism patients. We analyzed 50 Caucasian and 25 Japanese autism patients to identify base variation in *SCT* with heteroduplex analysis. Each exon and the promoter region was amplified by PCR. PCR reactions were done in 100 ng human genomic DNA, 1× exo(-) *Pfu* DNA polymerase reaction buffer, 1 μM each primer, 250 μM dNTPs, 8% dimethyl sulfoxide (DMSO), 20% betain, 1.25 U of exo(-) *Pfu* polymerase (Stratagene) in a 25-μl reaction. PCR conditions were an initial denaturation of 5 minutes at 98°C, then 30 cycles of 1 minute at 98°C, 1 minute at each annealing temperature, and 2 minutes at 75°C, followed by a final extension of 7 minutes at 75°C.

Primer pairs and annealing temperatures. We used the following primer pairs and annealing temperatures: promoter, *SEC* PRO F3, 5′-GCAGGTCCGAGGCTTCGCGGTG-3′, *SEC* PRO R1, 5′-AGGAGCTGAGCGCTGCGGCCAC-3′ (65°C); exon 1, 2448b F, 5′-AGCTGGGGGGGCGGCCCTGACCTT-3′, 5958B R, 5′-GTCGGGGTCAGGGCGCACTGGG-3′ (65°C); exon 2, SEC INT1 F2, 5′-CCACCCGCCCCCAGTGCGCCCTGAC-3′, SEC INT2 R2, 5′-CGTTGCTGCTGGGGCACCACGCC-3′ (57°C); exon 3, SEC INT2 F2, 5′-TGGTGCCCCAGCAGCAACGCGCGA-3′, SEC INT3 R2, 5′-GGGTCTGGGGTGGGGTCTGGAGT-3′ (63°C); exon 4, SEC INT3 F2, 5′-TGGAGGCCCAATTCCAGCAGCGGG-3′, SEC INT4 R1, 5′-ACTCCCAGCGTCTGGGTCTGAT-3′ (57°C).

PCR products were mixed with triple dye loading buffer, denatured 5 minutes at 98°C, and annealed 60 minutes at 68°C. Conformation-sensitive gel (10% acrylamide, 0.1% bis-acrolylpiperazine, 10% ethylene glycol, 15% deionized formamide, and 0.5× TTE buffer) electrophoresis (CSGE) with 0.25× TTE buffer as upper buffer and 1.0× TTE buffer as lower buffer, for 18 hours at 450 V. TTE buffer (1.2×) is 0.21 M Tris-HCl, pH 9.0, 0.07 M taurine, and 0.0012 M EDTA. After running the gel, bands were detected with ethidium bromide staining. PCR products that showed band shifts were sequenced directly.

Base change screening. For G900T/C in promoter region, the promoter region of *SCT* was amplified using the same primers and conditions as described earlier. PCR products were digested with *Pvu*II and electrophoresed on 1.5% agarose gel.

For C1448T in exon 3, a mismatch primer for the reverse sequence in exon 3 (E3Mut) was synthesized. This primer ends at the base adjacent to the mutated base and the second base from the 3′ end was changed from A to C to generate a *Hpa*II site (CCGG) in the normal sequence. Patient MRX118, members of his family, and normal DNA were amplified with this E3Mut primer and SEC INT2 F2 primer. PCR products were digested with *Hpa*II and electrophoresed on 2% agarose gel.

Allele-specific oligonucleotide hybridization. An aliquot of each PCR product was dotted onto a nylon membrane. The membrane was denatured by 0.4 M NaOH for 10 minutes and neutralized by 0.5 M Tris-HCl (pH 7.4), 2× SSC for 10 minutes. The membranes were prehybridized with 0.5 M Na_2HPO_4 (pH 7.2), 1 mM EDTA, 7% SDS at 65°C. Oligonucleotides with either the normal allele or the variant allele with eight bases on either side of the variant base were synthesized as probes. Each oligonucleotide was labeled with [^{32}Pγ]ATP with polynucleotide kinase. Labeled primer (15 μl) was mixed with 12 μl of salmon sperm DNA (10 mg/ml) and 4 μl of unlabeled alternative primer, heat-denatured, and mixed with the prehybridized membrane. The membrane was incubated at 65°C for 30 minutes, followed by cooling to 34°C. After an overnight incubation at 34°C, the membrane was washed with 5× SSC for 5 minutes twice at room temperature, followed by 2× SSC for 30 minutes at 34°C, and exposed to the X-ray film.

Acknowledgments

We thank the patients and their families for participation, and the Cure Autism Now Foundation for its efforts to provide samples from autism patients for study. We also thank Richard Gibbs and the BCM Human Genome Sequencing Center for ongoing support. This work was supported in part by grants from the National Institute of Child Health and Human Development of the United States National Institutes of Health, and from the Ministries of Education, Science and Culture, Japan.

RECEIVED FOR PUBLICATION FEBRUARY 5;
ACCEPTED MAY 25, 2002.

References

1. Horvath, K., *et al.* (1998). Improved social and language skills after secretin administration in patients with autistic spectrum disorders. *J. Assoc. Acad. Minor. Phys.* 9: 9–15.
2. Sandler, A. D., *et al.* (1999). Lack of benefit of a single dose of synthetic human secretin in the treatment of autism and pervasive developmental disorder. *N. Engl. J. Med.* 341: 1801–1806.
3. Rapin, I., and Katzman, R. (1998). Neurobiology of autism. *Ann. Neurol.* 43: 7–14.
4. Spiker, D., *et al.* (1994). Genetics of autism: characteristics of affected and unaffected children from 37 multiplex families. *Am. J. Med. Genet.* 54: 27–35.
5. Hallmayer, J., *et al.* (1996). Autism and the X chromosome. Multipoint sib-pair analysis. *Arch. Gen. Psychiatry* 53: 985–989.
6. Consortium, I.M.G.S.o.A. (1998). A full genome screen for autism with evidence for linkage to a region on chromosome 7q. *Hum. Mol. Genet.* 7: 571–578.
7. Barrett, S., *et al.* (1999). An autosomal genomic screen for autism. Collaborative linkage study of autism. *Am. J. Med. Genet.* 88: 609–615.
8. Philippe, A., *et al.* (1999). Genome-wide scan for autism susceptibility genes. Paris Autism Research International Sibpair Study. *Hum. Mol. Genet.* 8: 805–812; erratum: 8: 1353 (1999).
9. Auranen, M., *et al.* (2000). Analysis of autism susceptibility gene loci on chromosomes 1p, 4p, 6q, 7q, 13q, 15q, 16p, 17q, 19q and 22q in Finnish multiplex families. *Mol. Psychiatry* 5: 320–322.
10. Bayliss, W., and Starling, E. (1902). The mechanism of pancreatic secretion. *J. Physiol.* 28: 325–352.
11. Gafvelin, G., Jornvall, H., and Mutt, V. (1990). Processing of prosecretin: isolation of a secretin precursor from porcine intestine. *Proc. Natl. Acad. Sci. USA* 87: 6781–6785.
12. Kopin, A., *et al.* (1991). The secretin gene: evolutionary history, alternative splicing, and developmental regulation. *Proc. Natl. Acad. Sci. USA* 88: 5335–5339.
13. Itoh, N., Furuya, T., Ozaki, K., Ohta, M., and Kawasaki, T. (1991). The secretin precursor gene. Structure of the coding region and expression in the brain. *J. Biol. Chem.* 266: 12595–12598.
14. Ohta, M., Funakoshi, S., Kawasaki, T., and Itoh, N. (1992). Tissue-specific expression of the rat secretin precursor gene. *Biochem. Biophys. Res. Commun.* 183: 390–395.
15. Lan, M., Kajiyama, W., Donadel, G., Lu, J., and Notkins, A. (1994). cDNA sequence and genomic organization of mouse secretin. *Biochem. Biophys. Res. Commun.* 200: 1066–1071.
16. Bonetto, V., Jornvall, H., Mutt, V., and Sillard, R. (1995). Two alternative processing pathways for a preprohormone: a bioactive form of secretin. *Proc. Natl. Acad. Sci. USA* 92: 11985–11989.
17. Kopin, A., Wheeler, M., and Leiter, A. (1990). Secretin: structure of the precursor and tissue distribution of the mRNA. *Proc. Natl. Acad. Sci. USA* 87: 2299–2303.
18. Whitmore, T. E., *et al.* (2000). Human secretin (SCT): gene structure, chromosome location, and distribution of mRNA. *Cytogenet. Cell Genet.* 90: 47–52.
19. Herault, J., *et al.* (1995). Autism and genetics: clinical approach and association study with two markers of HRAS gene. *Am. J. Med. Genet.* 60: 276–281.
20. Comings, D., Wu, S., Chiu, C., Muhleman, D., and Sverd, J. (1996). Studies of the c-Harvey-Ras gene in psychiatric disorders. *Psychiatry Res.* 63: 25–32.
21. Holmes, J., *et al.* (2002). Association of DRD4 in children with ADHD and comorbid conduct problems. *Am. J. Med. Genet.* 114: 150–153.
22. Osoegawa, K., *et al.* (1998). An improved approach for construction of bacterial artificial chromosome libraries. *Genomics* 52: 1–8.
23. Carlquist, M. H. J., Forssmann, W., Thulin, L., Johansson, C., and Mutt, V. (1985). Human secretin is not identical to the porcine/bovine hormone. *IRCS Med. Sci.* 13: 217–218.
24. Schneider, R., *et al.* (1992). The human ribonuclease/angiogenin inhibitor is encoded by a gene mapped to chromosome 11p15.5, within 90 kb of the HRAS protooncogene. *Cytogenet. Cell Genet.* 59: 264–267.
25. Petronis, A., Van Tol, H., Lichter, J., Livak, K., and Kennedy, J. (1993). The D4 dopamine receptor gene maps on 11p proximal to HRAS. *Genomics* 18: 161–163.
26. Russell, M., *et al.* (1996). A 500-kb physical map and contig from the Harvey *ras-1* gene to the 11p telomere. *Genomics* 35: 353–360.
27. Higgins, M. J., *et al.* (1994). An ordered *Not*I fragment map of human chromosome band 11p15. *Genomics* 23: 211–222.
28. Reik, W., and Maher, E. R. (1997). Imprinting in clusters: lessons from Beckwith-Wiedemann syndrome. *Trends Genet.* 13: 330–334.
29. Ganguly, A., Rock, M. J., and Prockop, D. J. (1993). Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: evidence for solvent-induced bends in DNA heteroduplexes. *Proc. Natl. Acad. Sci. USA* 90: 10325–10329; erratum: 91: 5217 (1994).
30. Alonso, A., Martin, P., Albarran, C., Garcia, O., and Sancho, M. (1996). Rapid detection of sequence polymorphisms in the human mitochondrial DNA control region by polymerase chain reaction and single-strand conformation analysis in mutation detection enhancement gels. *Electrophoresis* 17: 1299–1301.
31. Comings, D., *et al.* (1999). Studies of the 48 bp repeat polymorphism of the *DRD4* gene in impulsive, compulsive, addictive behaviors: Tourette syndrome, ADHD, pathological gambling, and substance abuse. *Am. J. Med. Genet.* 88: 358–368.
32. Richard, C. D., *et al.* (1993). A radiation hybrid map of the distal short arm of human chromosome 11, containing the Beckwith-Wiedemann and associated embryonal tumor disease loci. *Am. J. Hum. Genet.* 52: 915–921.
33. Vu, T. H., and Hoffman, A. R. (1997). Imprinting of the Angelman syndrome gene, *UBE3A*, is restricted to brain. *Nat. Genet.* 17: 12–13.
34. Ding, S., Larson, G., Foldenauer, K., Zhang, G., and Krontiris, T. (1999). Distinct mutation patterns of breast cancer-associated alleles of the *HRAS1* minisatellite locus. *Hum. Mol. Genet.* 8: 515–521.
35. Trepicchio, W., and Krontiris, T. (1992). Members of the rel/NF-κB family of transcriptional regulatory proteins bind the *HRAS1* minisatellite DNA sequence. *Nucleic Acids Res.* 20: 2427–2434.
36. Lindstedt, B. A., Ryberg, D., Zienolddiny, S., Khan, H., and Haugen, A. (1999). *Hms1* VNTR alleles as susceptibility markers for lung cancer: relationship to microsatellite instability in tumors. *Anticancer Res.* 19: 5523–5527.

37. Weitzel, J. N., *et al.* (2000). The *HRAS1* minisatellite locus and risk of ovarian cancer. *Cancer Res.* 60: 259–261.
38. Benjamin, J., Li, L., Patterson, C., Greenberg, B. D., Murphy, D. L., and Hamer, D. H. (1996). Population and familial association between the D4 dopamine receptor gene and measures of novelty seeking. *Nat. Genet.* 12: 81–84.
39. Benjamin, J., *et al.* (2000). Association between tridimensional personality questionnaire (TPQ) traits and three functional polymorphisms: dopamine receptor D4 (DRD4), serotonin transporter promoter region (5-HTTLPR) and catechol *O*-methyltransferase (COMT). *Mol. Psychiatry* 5: 96–100.
40. Kennedy, G., German, M., and Rutter, W. (1995). The minisatellite in the diabetes susceptibility locus *IDDM2* regulates insulin transcription. *Nat. Genet.* 9: 293–298.
41. Paquette, J., Giannoukakis, N., Polychronakos, C., Vafiadis, P., and Deal, C. (1998). The INS 5′ variable number of tandem repeats is associated with IGF2 expression in humans. *J. Biol. Chem.* 273: 14158–14164.
42. Bennett, S., *et al.* (1995). Susceptibility to human type 1 diabetes at *IDDM2* is determined by tandem repeat variation at the insulin gene minisatellite locus. *Nat. Genet.* 9: 284–292.
43. Waterworth, D., *et al.* (1997). Linkage and association of insulin gene VNTR regulatory polymorphism with polycystic ovary syndrome. *Lancet* 349: 986–990.
44. Lalioti, M. D., *et al.* (1997). Dodecamer repeat expansion in cystatin B gene in progressive myoclonus epilepsy. *Nature* 386: 847–851.
45. Kunugi, H., *et al.* (1999). A functional polymorphism in the promoter region of monoamine oxidase-A gene and mood disorders. *Mol. Psychiatry* 4: 393–395.
46. Fiskerstrand, C., Lovejoy, E., and Quinn, J. (1999). An intronic polymorphic domain often associated with susceptibility to affective disorders has allele dependent differential enhancer activity in embryonic stem cells. *FEBS Lett.* 458: 171–174.
47. Kang, A., Palmatier, M., and Kidd, K. (1999). Global variation of a 40-bp VNTR in the 3′-untranslated region of the dopamine transporter gene. *Biol. Psychiatry* 46: 151–160.
48. Mutoh, H., *et al.* (1997). The basic helix–loop–helix transcription factor BETA2/NeuroD is expressed in mammalian enteroendocrine cells and activates secretin gene expression. *Proc. Natl. Acad. Sci. USA* 94: 3560–3564.
49. Amir, R., *et al.* (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat. Genet.* 23: 185–188.
50. Matsuura, T., *et al.* (1997). *De novo* truncating mutations in E6-AP ubiquitin-protein ligase gene (*UBE3A*) in Angelman syndrome. *Nat. Genet.* 15: 74–77.

J Hum Genet (2002) 47:262–265

SHORT COMMUNICATION

Hong Li · Takanori Yamagata · Masato Mori
Mariko Y. Momoi

Association of autism in two patients with hereditary multiple exostoses caused by novel deletion mutations of *EXT1*

Received: December 20, 2001 / Accepted: February 18, 2002

Abstract Two boys from separate families presented with hereditary multiple exostoses (EXT) and autism associated with mental retardation. Their fathers both expressed a clinical phenotype of hereditary multiple exostoses milder than those of the patients and without the associated mental disorder. The *EXT1* and *EXT2* genes from lymphocytes of the affected individuals were analyzed by using denaturing high-performance liquid chromatography and direct sequencing. A novel deletion mutation, 1742delTGT-G in exon 9 of *EXT1*, causing a frameshift was detected in one boy and his father. Another novel deletion mutation, 2093delTT in exon 11 of *EXT1*, causing transcription termination was detected in the other affected boy and his father. *EXT1* is expressed in the brain, and both EXT1 and EXT2 proteins are associated with glycosyltransferase activities required for the biosynthesis of heparan sulfate, which also has activity in the brain. The coincidental association of mental disorders in the boys was not completely excluded. However, these results suggest the involvement of EXT1 in the development of mental disorders, including mental retardation and autism.

Key words Hereditary multiple exostoses · Mental retardation · Autism · *EXT1* · *EXT2*

Introduction

Hereditary multiple exostoses (EXT; MIM 133700) is an autosomal dominant inherited bone disorder that is characterized by the formation of cartilage-capped exostoses, short stature, and skeletal deformities. Various mutations in *EXT1* on 8q24.1 and *EXT2* on 11p11–12 have been reported in EXT families (Phillippe et al. 1997; Wells et al. 1997; Wuyts et al. 1998; Dobson-Stone et al. 2000; Francannet et al. 2001; Seki et al. 2001). *EXT1* and *EXT2* are thought to be responsible for EXT in more than 70% of EXT patients.

Although the association of developmental disorders with EXT has not been reported to date, tricho-rhino-phalangeal syndrome (TRPS) type II (MIM 150230) is associated with exostoses and mental disorder. TRPS that is characterized by a dysmorphic appearance involving the face, scalp, hair, and skeletal system is assigned to the *TRPS1* gene (Lüdecke et al. 2001), which resides on 8q24.12. Unlike TRPSI and TRPSIII, TRPSII presents with mental retardation (MR) in most cases and a deletion mutation including 8q24.11–q24.13 involving *TRPS1* and *EXT1* has been detected in TRPSII. These findings suggest that some gene other than *TRPS1* in the deletion area is the likely cause of MR, and *EXT1* is one candidate. Ishikawa-Brush et al. (1997) reported a patient who presented with autism, MR, and EXT with a balanced translocation 46,X,t(X;8)(p22.13;q22.1). Two genes at the breaking point were identified, and neither were responsible for the EXT or autism. No information was presented concerning EXT1 in this patient, but the association of exostoses and developmental disorders in this patient suggested that EXT-related genes can play an important role in the developing brain. Here, we describe two Japanese families with a deletion mutation of *EXT1*, wherein the probands showed developmental disorders.

Subjects and methods

Patient 1. Patient 1 was a 5-year-old boy. He began to develop multiple bony tumors in early infancy and was diagnosed as having EXT. He was also diagnosed as having autism, according to DSM-IV criteria (American Psychiatric Association 1994), associated with moderate MR. No dysmorphic phenotype of TRPS was observed. His

H. Li · T. Yamagata (✉) · M. Mori · M.Y. Momoi
Department of Pediatrics, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi, Tochigi 329-0498, Japan
Tel. +81-285-58-7366; Fax +81-285-44-6123
e-mail: takanori@jichi.ac.jp

developmental quotient was approximately 40. His father had developed multiple exostoses in his teens, but the father's condition was much milder than that of his son. The father's intelligence was within the normal range. The patient's mother and his brother were unaffected. The paternal grandfather and two brothers had EXT, but detailed clinical information was not available. None of the relatives showed mental disorder.

Patient 2. Patient 2 was a 5-year-old boy. Within a month after birth, he was found to have bony tumors which led to a diagnosis of EXT. He was also diagnosed as having autism associated with mild MR according to the DSM-IV criteria. No dysmorphic phenotype of TRPS was observed. His developmental quotient was 65. His father developed multiple exostoses in his second decade and had normal intelligence. His mother and a sister were unaffected. His father's sister developed EXT in her teens. There was no information about his paternal grandparents. No relatives showed mental disorder.

Denaturing high-performance liquid chromatography (DHPLC) screening. Lymphocytes were obtained from the patients and their parents with the informed consent of the parents, and genomic DNA was extracted. Each exon of the *EXT1* and *EXT2* genes was amplified by polymerase chain reaction (PCR) by using primer sets described by Wells et al. (1997) and Philippe et al. (1997), respectively. Heteroduplex formation was induced by heat denaturation of PCR products at 94°C for 5 min, followed by gradual reannealing from 94° to 25°C over 45 min. DHPLC analysis was performed with the WAVE DNA-fragment analysis system (Transgenomic, Omaha, NB, USA). PCR products were eluted at a flow rate of 0.9 ml/min with a linear acetonitrile gradient. Heterozygous profiles were detected as distinct elution peaks from homozygous wild-type peaks. PCR products shown to be heteroduplexes were subjected to direct sequencing analysis.

Results

We screened all exons of *EXT1* and *EXT2* of two patients by DHPLC. In Patient 1, exon 3 and exon 9 of *EXT1* and the 3′-untranslated region (UTR) of *EXT2* showed heteroduplexes. Direct sequencing showed 1742delTGT-G in exon 9 of *EXT1* (Figure 1B), which caused a frameshift from 581 valine and resulted in a stop codon after five amino acids. A similar DHPLC pattern in exon 9 was detected in the boy's father but not in his mother (Figure 1A), and the 1742delTGT-G deletion was also detected in his father. Direct sequencing of the boy's sample also detected a base substitution, C1065T, in exon 3 of *EXT1*, which has already been reported as a polymorphism, and another, G2210A, in the 3′-UTR of *EXT2*. Both base substitutions were inherited from his mother. We screened for G2210A in the 3′-UTR of *EXT2* in 23 normal controls and detected it in six subjects (data not shown). Patient 2 showed a heteroduplex in exon 11 of *EXT1*, and direct sequencing detected a two-base deletion, 2093delTT (Figure 2B). This deletion caused a frameshift from 698 phenylalanine resulting in a stop codon after 31 amino acids. A similar DHPLC pattern in exon 11 was detected in his father but not in his mother or in a normal control (Figure 2A), and this same 2093delTT deletion was detected in his father, but not in his mother or sister.

Discussion

A novel two-base deletion, 1742delTGT-G in exon 9 of *EXT1*, detected in Patient 1, and another novel two-base deletion, 2093delTT in exon 11 of *EXT1*, detected in Patient 2, were associated with the expression of exostoses in each family. Both two-base deletions induced a frameshift predicted to result in premature termination of the

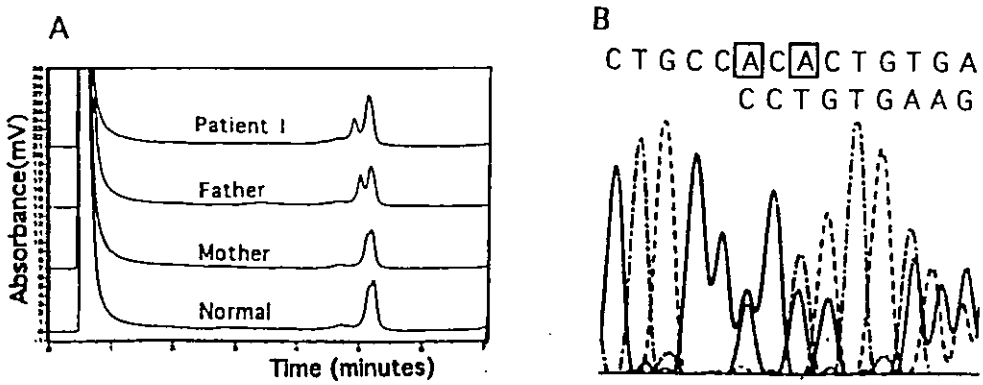


Fig. 1. A The denaturing high-performance liquid chromatography (DHPLC) elution profiles of exon 9 of *EXT1*. Profiles of *Patient 1*, his *parents*, and a *normal control* are shown. B The results of direct sequencing of exon 9 of *EXT1* at the deleted site detected in Patient 1. The deleted bases are enclosed in *boxes*. The reverse sequence is shown because the deleted site was too close to the primer-setting region to read in a forward direction. The reverse sequence shows 1742delACA-C. The *lower sequence* is that of the mutated allele

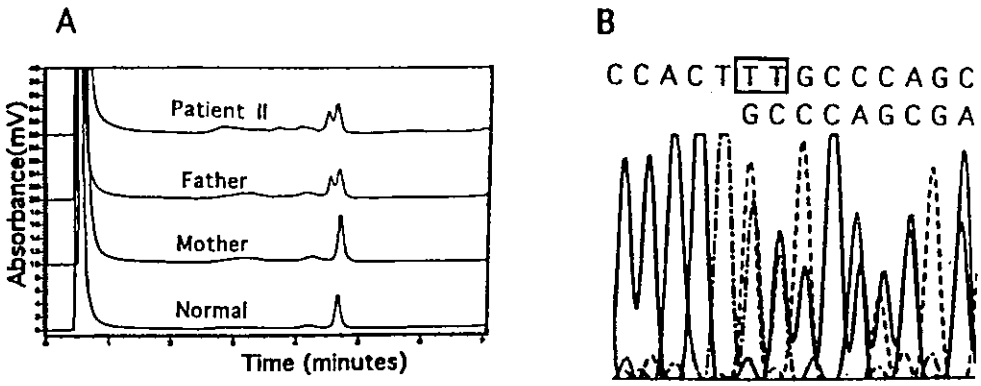


Fig. 2. A The DHPLC elution profiles of exon 11 of *Patient 2*, his *parents*, and a *normal control*. B The direct-sequencing data of Patient 2 showed a 2093delTT deletion causing a frameshift mutation (forward sequences are shown). The *box* indicates the deleted bases. The *lower sequence* is that of the mutated allele

translation of *EXT1*. Both exon 9 and exon 11 are in the C-terminal region of *EXT1*. The majority of mutations in *EXT1* have been reported in exon 1, which harbors a membrane-binding region and catalytic domain of the EXT protein, and fewer mutations have been reported in exons in the C-terminal region. In exon 9, five mutations have been reported to date. Despite the fact that the majority of missense mutations have been reported in the N-terminal side of the central region, the H627del mutation in exon 9 has been shown to abolish heparan sulfate synthesis (Cheung et al. 2001). Cheung et al. (2001) suggested that another important functional domain may reside within this region. 1742delTGT-G was at the 581st amino acid, which results in termination before this possibly active site is reached. In exon 11, only one nonsense mutation, C2101T(R701X), has been reported in a Japanese patient (Seki et al. 2001). The 2093delTT deletion was eight bases upstream from this nonsense mutation. The existence of patients with EXT having a mutation in this region suggests that this C-terminal region has important functions, such as in protein–protein interactions (Cheung et al. 2001).

The coincidental association of these mutations with a developmental disorder and EXT cannot be excluded in light of the general susceptibility of males to developmental disorders. The general prevalence of autism is approximately 5/10,000 people, while that of EXT is 1–2/100,000 (Ishikawa-Brush et al. 1997). Thus, the likelihood of a coincidental association of EXT and autism in these two patients is quite low.

EXT1 is expressed ubiquitously in adult tissues, including the brain (data not shown). Despite this ubiquitous nature, functional activity of EXT1 has not been detected except in the actively growing bone. *EXT1* is a putative tumor suppressor gene. The exostoses can be explained by the dysfunction of this tumor suppressor function, which is likely caused by the loss of function of one allele. Both the EXT1 and EXT2 proteins are associated with glycosyltransferase activities required for the biosynthesis of heparan sulfate (McCormick et al. 2000). Heparan sulfate is an important side chain of the proteoglycans, which receives signaling molecules, and modulates a wide spectrum of regulation processes not only in the bony tissues but also in the developing brain (Bandtlow and Zimmermann 2000). The gene homologous to *EXT1* in *Drosophila melanogaster* has been linked to hedgehog signaling (Bellaiche et al. 1998). Therefore, accumulating data suggest that EXT1 is functioning in the developing tissues during embryogenesis. Thus, the association of autism and MR in our two boys with deletion mutations in *EXT1* suggests that the *EXT1* gene is involved in the development of these conditions.

Anticipation has been observed in some families with EXT (Francannet et al. 2001), although the underlying mechanism is not known. Our two families also showed anticipation, with earlier onset in patients than in their fathers. The difference that the patients had autism and mental retardation but their fathers did not may result from some unknown mechanism of *EXT1* or from a combination with some other gene's effect.

To our knowledge, this is the first report of patients who developed developmental disorders in association with EXT. There are several possible explanations for this association. First, the association may be coincidental, although the likelihood is low, as discussed above. Second, in most cases of exostoses, a mild developmental abnormality might be missed due to the lack of follow up by a neurologist or pediatric neurologist. Third, a mutation in a gene other than *EXT1* and *EXT2* may have been involved in the development of the mental disorders. Autism is considered to be a polygene disease. Therefore, EXT1 may be one gene relating to autism pathogenesis. Further studies of the function of EXT proteins in the developing brain and additional pediatric patients with EXT and mental disorders may allow a novel function of EXT proteins to be identified.

References

American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders, 4th edn. American Psychiatric Association, Washington, DC

Bandtlow CE, Zimmermann DR (2000) Proteoglycans in the developing brain: new conceptual insights for old proteins. Physiol Rev 80:1267–1290

Bellaiche Y, The I, Perrimon N (1998) Tout-velu is a *Drosophila* homologue of the putative tumour suppressor EXT-1 and is needed for Hh diffusion. Nature 394:85–88

Cheung PK, McCormick C, Crawford BE, Esko JD, Tufaro F, Duncan G (2001) Etiological point mutations in the hereditary multiple exostoses gene EXT1: a functional analysis of heparan sulfate polymerase activity. Am J Hum Genet 69:55–66

Dobson-Stone C, Cox RD, Lonie L, Southam L, Fraser M, Wise C, Bernier F, Hodgson S, Porter DE, Simpson AH, Monaco AP (2000) Comparison of fluorescent single-strand conformation polymorphism analysis and denaturing high-performance liquid chromatography for detection of EXT1 and EXT2 mutations in hereditary multiple exostoses. Eur J Hum Genet 8:24–32

Francannet C, Cohen-Tanugi A, Le Merrer M, Munnich A, Bonaventure J, Legeai-Mallet L (2001) Genotype–phenotype correlation in hereditary multiple exostoses. J Med Genet 38:430–434

Ishikawa-Brush Y, Powell JF, Bolton P, Miller AP, Francis F, Willard HF, Lehrach H, Monaco AP (1997) Autism and multiple exostoses associated with an X,8 translocation occurring within the GRPR gene and 3′ to the SDC2 gene. Hum Mol Genet 6:1241–1250

Lüdecke HJ, Schaper J, Meinecke P, Momeni P, Gross S, von Holtum D, Hirche H, Abramowicz MJ, Albrecht B, Apacik C, Christen HJ, Claussen U, Devriendt K, Fastnacht E, Forderer A, Friedrich U, Goodship TH, Greiwe M, Hamm H, Hennekam RC, Hinkel GK, Hoeltzenbein M, Kayserili H, Majewski F, Mathieu M, McLeod R, Midro AT, Moog U, Nagai T, Niikawa N, Ørstavik KH, Plöchl E, Seitz C, Schmidtke J, Tranebjærg L, Tsukahara M, Wittwer B, Zabel B, Gillessen-Kaesbach G, Horsthemke B (2001) Genotypic and phenotypic spectrum in Tricho-Rhino-Phalangeal syndrome types I and III. Am J Hum Genet 68:81–91

McCormick C, Duncan G, Goutsos KT, Tufaro F (2000) The putative tumor suppressors EXT1 and EXT2 form a stable complex that accumulates in the Golgi apparatus and catalyzes the synthesis of heparan sulfate. Proc Natl Acad Sci U S A 297:668–673

Philippe C, Porter DE, Emerton ME, Wells DE, Simpson AH, Monaco AP (1997) Mutation screening of the EXT1 and EXT2 genes in patients with hereditary exostoses. Am J Hum Genet 61:520–528

Seki H, Kubota T, Ikegawa S, Haga N, Fujioka F, Ohzeki S, Wakui K, Yoshikawa H, Takaoka K, Fukushima Y (2001) Mutation frequencies of EXT1 and EXT2 in 43 Japanese families with hereditary multiple exostoses. Am J Med Genet 99:59–62

Wells DE, Hill A, Lin X, Ahn J, Brown N, Wagner JE (1997) Identification of novel mutations in the human EXT1 tumor suppressor gene. Hum Genet 99:612–615

Wuyts W, Van HW, De Boulle K, Hendrickx J, Bakker E, Vanhoenacker F, Mollica F, Lüdecke HJ, Sayli BS, Pazzaglia UE, Mortier G, Hamel B, Conrad EU, Matsushita M, Raskind WH, Willems PJ (1998) Mutations in the EXT1 and EXT2 genes in hereditary multiple exostoses. Am J Hum Genet 62:346–354

Neuropathology 2002; **22**, 294–298

Case Report

Co-existence of nemaline and cytoplasmic bodies in muscle of an infant with nemaline myopathy

Kiyotaka Suwa,[1] Masashi Mizuguchi,[1] Mariko Y. Momoi,[1] Minako Nakamura,[2] Kunimasa Arima,[2,3] Hirofumi Komaki,[3] Ikuya Nonaka[3]

[1]Department of Pediatrics, Jichi Medical School, Minamikawachi, Tochigi, [2]Department of Ultrastructure and Histochemistry, Tokyo Institute of Psychiatry, Tokyo, and [3]Departments of Laboratory Medicine and Ultrastructural Research, National Center of Neurology and Psychiatry, Kodaira, Japan

A sporadic case of congenital myopathy had severe muscle weakness of neonatal onset. Nemaline and cytoplasmic bodies were detected in muscle biopsies taken at 4 months of age. These findings were consistent with a diagnosis of nemaline myopathy (severe neonatal form). The simultaneous and abundant presence of these two types of sarcoplasmic inclusion has been found in only a few cases. However, these cases suggest that the sarcoplasmic inclusions may be formed, at least partially, by common mechanisms.

Key words: Congenital myopathy, cytoplasmic body, nemaline body, Z-line.

INTRODUCTION

A subpopulation of patients with benign congenital myopathy has skeletal muscles containing abnormal, rod-like structures called nemaline bodies.[1] The definition of nemaline myopathy has since been expanded to include more severe cases, and is presently divided into three classes (severe neonatal, moderate congenital, and adult-onset forms), depending upon the age at onset, clinical symptoms and disease course.[2] Nemaline myopathy is genetically heterogenous and is classified into autosomal dominant (NEM1) and recessive (NEM2) forms. Genetic studies have recently identified three genes underlying this disorder: α-tropomyosin$_{slow}$ on chromosome 1q22-q23,[3] nebulin on chromosome 2q22,[4] and skeletal muscle α-actin on chromosome 1q42.[5] There are also several other candidate genes, such as α-actinin.[6] A mutation in any of these genes can result in clinically significant nemaline myopathy.

Cytoplasmic bodies are sarcoplasmic inclusions of skeletal myofibers that were first described by Engel in muscles with myotonic dystrophy and in denervated muscles.[7] Although a small number of the inclusions may occur non-specifically in a variety of neuromuscular diseases, they are found in abundance in a small sub-population of patients with congenital myopathy termed cytoplasmic body myopathy.[8,9] The genetic basis of this disorder remains unknown.

Although both nemaline and cytoplasmic bodies derive from the Z-line materials, there have been only a few cases in which both bodies could be found in abundance.[9–14] We report here an unusual case of congenital myopathy in which these inclusions were found in the same muscle.

CLINICAL SUMMARY

Our patient was a Japanese boy born to non-consanguineous, healthy parents who had no family history of neuromuscular diseases. After an uneventful pregnancy, he was delivered at 42 weeks' gestation by Caesarean section. He weighed 3230 g and appeared healthy immediately after birth. However, several minutes later, his crying became feeble and he became generally cyanotic. He was then transferred to a neonatal intensive care unit. He was dyspneic and his activity was poor. He could neither close his mouth nor excrete in the oropharynx and larynx. He had a high-arched palate, but no contracture of the joints. His muscles, including those of his face, were atrophied and weak. His deep tendon reflexes were reduced. His ocular movements were full and smooth. Creatine kinase serum level was normal. Genetic studies were not performed. He

Correspondence: Kiyotaka Suwa, Department of Pediatrics, Jichi Medical School, Minamikawachi-machi, Kawachi-gun, Tochigi, Japan. E-mail: kiyotaka@jichi.ac.jp

Received 28 June 2002; revised and accepted 23 July 2002.

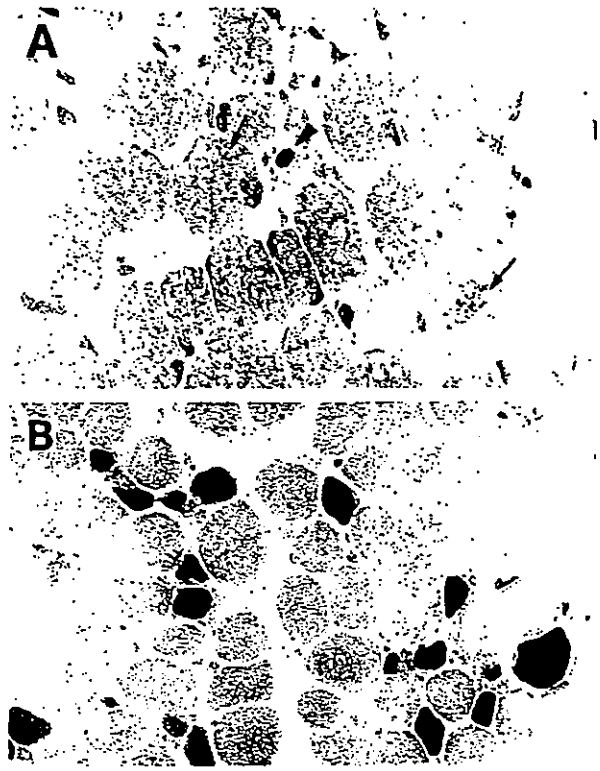


Fig. 1 Light microscopic findings of the biopsied muscle: (a) muscle fibers show a moderate variation in fiber size. Some fibers contained nemaline bodies (arrow) and others contain cytoplasmic bodies (arrowhead). Modified Gomori trichrome staining; (b) type 1 fibers account for as much as 65% of total fibers. Many of them are atrophic. Routine ATPase staining.

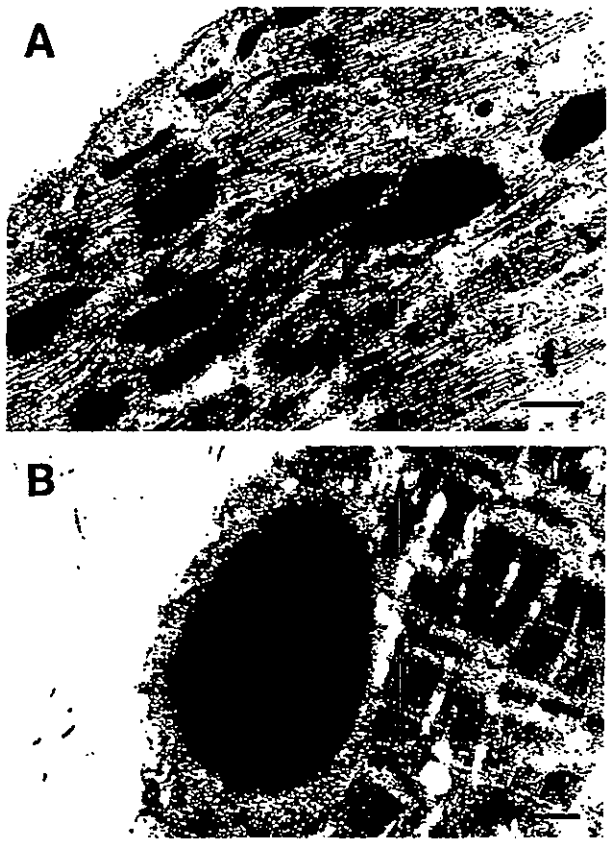


Fig. 2 Electron microscopic findings of biopsied muscle: (a) typical nemaline bodies appear as rod-like structures with electron densities almost identical to those of the Z-lines. Nemaline bodies tend to line up as extension of the Z-line; (b) typical cytoplasmic body having an electron-dense core and a surrounding halo. Core consists of granular materials and halo contains thin filaments. Bars = 0.5 μm.

was intubated, and has been mechanically ventilated ever since.

During the following 3 years, he remained hypotonic, dependent on a respirator, and immobile showing a frog leg position. He could not lift his arms and legs against gravity. Despite his severe motor disability, his mental development was comparatively normal. He could pursue objects with his eyes and distinguish people.

PATHOLOGICAL FINDINGS

A biopsy of the patient's left biceps brachii muscle was performed when he was 4 months of age with the informed consent of his parents. Histological examination of the biopsy revealed a moderate variation in muscle fiber size, which measured 5–30 μm in diameter. The number of centrally placed nuclei was normal. Neither necrotic nor regenerating fibers were present. Fibrous tissue in the interstitium was mildly increased. Gomori trichrome staining revealed nemaline bodies in approximately 2% of the fibers, and cytoplasmic bodies in approximately 3% of the fibers (Fig. 1a). There were no ragged-red fibers. Peripheral nerve bundles were well myelinated. Intermyofibrillar networks were well organized. ATPase staining indicated type 1 predominance and type 2B deficiency. The proportions of type 1, 2A, 2B and 2C fibers were 65%, 30%, 0% and 5%, respectively (Fig. 1b).

Electron microscopy revealed ultrastructural features typical of nemaline and cytoplasmic bodies. Both inclusions had electron densities similar to those of the Z-lines. Nemaline bodies (Fig. 2a) were rod-like sarcoplasmic inclusions, approximately 0.35×0.35×1.6 μm in size. They were abundant in the peripheral regions of the muscle fibers and often lined up along continuations of the Z-lines. At higher magnification, they appeared to have a lattice-like structure. Cytoplasmic bodies (Fig. 2b) were oval structures with diameters ranging 2.3–6.5 μm, having a dense core surrounded by a halo. Images at higher mag-

nification showed that the core and halo consisted of granular materials and thin filaments, respectively. The number and morphology of mitochondria appeared to be normal.

DISCUSSION

The clinical picture of the present case was dominated by severe, generalized muscle weakness of neonatal onset. The non-progressive clinical course, as well as the myopathologic features such as type 1 fiber predominance, was typical of a congenital myopathy. The diagnosis of nemaline myopathy was made based on the presence of nemaline bodies.

A small number of nemaline bodies may be found in other conditions, such as central core disease, mitochondrial disorders, and myopathies associated with hemodialysis and with HIV infection. Regardless of etiology, nemaline bodies show electron densities identical to those of the Z-lines, and often appear as extensions of these lines. Furthermore, they are immunoreactive for α-actinin, a major component of the Z-line.[15]

The patient's muscle had many cytoplasmic bodies, a pathologic structure that is less disease-specific than nemaline bodies. Cytoplasmic bodies may occur in association with numerous disorders such as cytoplasmic body myopathy, inflammatory myopathies, chronic muscular dystrophies, granulomatous myopathy, myotonic dystrophy, periodic paresis, hypothyroidism and chronic ingestion of senna and emetine. The immunopathologic properties of cytoplasmic bodies vary considerably among these disorders, suggesting that they have different developmental mechanisms.[16] Despite such heterogeneity, cytoplasmic bodies develop in close association with the Z-line. They contain actin, have a core with electron density similar to that of the Z-line, and occasionally are structurally continuous with that line.[16,17]

To our knowledge, simultaneous coexistence of a substantial number of nemaline and cytoplasmic bodies (present in more than 1% of myofibers) has previously been documented in only seven cases (Table 1).[6,9–14] Two of these (Cases 6 and 7) had myopathy caused by extrinsic (infectious, toxic or mechanical) factors. These two cases also had pathologic changes that primarily involved mitochondria (increase in number and paracrystalline inclusion).[13,14] These mitochondrial abnormalities were absent in the present case. Another case (Case 5) had cytoplasmic body myopathy of adult onset.[8] The remaining four patients (Cases 1–4) had definite or possible nemaline myopathy. The clinical presentation of Case 1 is quite similar to that of the present case,[10] but the other three cases show a later onset and slower progression of disease.[6,11,12] Given such varied clinical pictures, it is unlikely that the simple coexistence of the two inclusions predicts the

Table 1 Previously reported cases showing coexistence of nemaline and cytoplasmic bodies on muscle biopsy

Case/Sex	Age at onset/biopsy (years)	Course	Severity	Other important myopathologic changes	Possible etiologic factors	Reference
1/M	0/0	Non-progressive	Severe	Marked variation in fiber size	Genetic? (sporadic case)	Itakura *et al.* (1998)[10]
2/?	Childhood	?	?	?	Genetic (α-topomyosin$_{slow}$ mutation)	Corbett *et al.* (2001)[6]
3/M	19/22	Slowly progressive	Mild	Type 1 fiber predominance	Genetic? (sporadic case)	Mori *et al.* (1996)[11]
4/F	40/60	Slowly progressive	Mild	Marked variation in fiber size	Genetic? (sporadic case)	Gerard *et al.* (1991)[12]
5/F	22/28	Slowly progressive	Severe	Variation in fiber size, central nuclei	Genetic? (sporadic case)	Jerusalem *et al.* (1979)[9]
6/M	?/43	Slowly progressive	?	Inflammation, increased mitochondria, paracrystalline inclusion	HIV infection, zidovudine	Pezeshkpour *et al.* (1991)[13]
7/M	63/63	Acute	Severe	Increased mitochondria, paracrystalline inclusion	Muscle relaxant, corticosteroid	Matsubara *et al.* (1994)[14]

course of myopathy. Similarly, previous studies on nemaline myopathy have shown no correlation between the number of nemaline bodies and the age at onset or severity of disease.[18,19]

Recently, a mouse model of nemaline myopathy (NEM1) was established by introducing a dominant-negative mutant of the α-tropomyosin$_{slow}$ gene.[6] Skeletal muscle of this transgenic mouse shows hypertrophy of type 2B fibers. The most prominent finding is the presence of many nemaline and cytoplasmic bodies. This suggests that dysfunction of α-tropomyosin$_{slow}$ directly leads to formation of these two derivatives of the Z-line materials. Myopathology in this mouse is very similar to that of the present case, although the status of the α-tropomyosin$_{slow}$ gene in our patient remains unknown. Alternatively, nemaline and cytoplasmic bodies can be experimentally produced by chronic administration of emetine to rats. Interestingly, these pathologic changes are reversible, disappearing gradually after withdrawal of emetine.[20] Thus, these data suggest that the two inclusions may be simultaneously formed by common extrinsic factors.

In this paper, we report a sporadic case of nemaline myopathy in which both nemaline and cytoplasmic bodies were present in the same muscle. We also carried out a brief review of the literature about similar pathologic findings in human patients with nemaline and other myopathies, and in animal models of these disorders. Despite its rarity, simultaneous existence of the two Z-line derivatives may be due to common mechanisms of formation of these inclusion bodies.

REFERENCES

1. Shy GM, Engel WK, Somers JE, Wanko T. Nemaline myopathy: a new congenital myopathy. *Brain* 1963; **86**: 793–810.
2. Shimomura C, Nonaka I. Nemaline myopathy. Comparative muscle histochemistry in the severe neonatal, moderate congenital, and adult-onset forms. *Pediatric Neurol* 1989; **5**: 25–31.
3. Laing NG, Wilton SD, Akkari PA *et al.* A mutation in the alpha tropomyosin gene TPM3 associated with autosomal dominant nemaline myopathy NEM1. *Nature Genet* 1995; **9**: 75–79.
4. Pelin K, Hilpela P, Donner K *et al.* Mutations in the nebulin gene associated with autosomal recessive nemaline myopathy. *Proc Natl Acad Sci USA* 1999; **96**: 2305–2310.
5. Nowak KJ, Wattanasirichaigoon D, Goebel HH *et al.* Mutations in the skeletal muscle alpha actin gene in patients with actin myopathy and nemaline myopathy. *Nature Genet* 1999; **23**: 208–212.
6. Corbett MA, Robinson CS, Dunglison GF *et al.* A mutation in α-tropomyosin$_{slow}$ affects muscle strength, maturation and hypertrophy in a mouse model for nemaline myopathy. *Hum Molec Genet* 2001; **10**: 317–328.
7. Engel WK. The essentiality of histo- and cytochemical studies of skeletal muscle in the investigation of neuromuscular disease. *Neurology* 1962; **12**: 778–794.
8. Mizuno Y, Nakamura Y, Komiya K. The spectrum of cytoplasmic body myopathy: report of a congenital severe case. *Brain Dev* 1989; **11**: 20–25.
9. Jerusalem F, Ludin H, Bischoff A, Hartmann G. Cytoplasmic body neuromyopathy presenting as respiratory failure and weight loss. *J Neurol Sci* 1979; **41**: 1–9.
10. Itakura Y, Ogawa Y, Murakami N, Nonaka I. Severe infantile congenital myopathy with nemaline and cytoplasmic bodies: a case report. *Brain Dev* 1998; **20**: 112–115.
11. Mori H, Sato K, Hirasawa E, Kondo T, Mizuno Y. A 22-year-old man with long-standing weakness and atrophy predominantly in the lower extremities (in Japanese). *No Shinkei* 1996; **48**: 763–771.
12. Gerard JM, Telerman-Toppet N, Borenstein S, Flament-Durand J. Sporadic Z disk myopathy with accumulation of rods and cytoplasmic bodies (in French). *Rev Neurol (Paris)* 1991; **147**: 144–147.
13. Pezeshkpour G, Illa I, Dalakas MC. Ultrastructural characteristics and DNA immunocytochemistry in human immunodeficiency virus and zidovudine-associated myopathies. *Hum Pathol* 1991; **22**: 1281–1288.
14. Matsubara S, Okada T, Yoshida M. Mitochondrial changes in acute myopathy after treatment of respiratory failure with mechanical ventilation (acute relaxant-steroid myopathy). *Acta Neuropathol (Berl)* 1994; **88**: 475–478.
15. Jockusch BM, Veldman H, Griffiths GW, van Oost BA, Jennekens FG. Immunofluorescence microscopy of a myopathy. α-Actinin is a major constituent of nemaline rods. *Exp Cell Res* 1980; **127**: 409–420.
16. Caron A, Viader F, Lechevalier B, Chapon F. Cytoplasmic body myopathy. familial cases with accumulation of desmin and dystrophin. An immunohistochemical, immunoelectron microscopic and biochemical study. *Acta Neuropathol (Berl)* 1995; **90**: 150–157.
17. Fukuhara N, Inoue Y, Yoshimura N, Sugita H, Li K. Histochemical, immunohistochemical and electron microscopic study on cytoplasmic body (in Japanese). *Rinsho Shinkeigaku* 1987; **27**: 1713.
18. Nonaka I, Tojo M, Sugita H. Fetal muscle characteristics in nemaline myopathy. *Neuropediatrics* 1983; **14**: 47–52.

19. Nienhuis AW, Coleman RF, Brown WJ, Munsat TL, Pearson CW. Nemaline myopathy. A histopathologic and histochemical study. *Am J Clin Pathol* 1967; **48**: 1–13.

20. Hopf NJ, Goebel HH. Experimental emetine myopathy: enzyme histochemical, electron microscopic, and immunomorphological studies. *Acta Neuropathol (Berl)* 1993; **85**: 414–418.

臨床編 II

ミトコンドリア病(狭義) 診断学

ミトコンドリア病を疑わせる臨床症状・検査成績
臨床症状概論

Clinical symptoms and signs of mitochondrial disorder

山形崇倫　桃井真里子

Key words: ミトコンドリア異常症，臨床症状

1. 概念・定義

ミトコンドリア異常症は，ミトコンドリアの機能低下に起因する疾患の総称である．ミトコンドリアは，生体のエネルギー産生の主要な部位で，赤血球以外の全身の組織，細胞に存在する．ミトコンドリアの障害によりエネルギー産生の低下を来し，なかでも，エネルギー依存度の高い，中枢神経系，骨格筋と心筋に障害が出やすく，中心的な症状となるが，全身の種々の臓器の障害を起こし，多彩な症状を示す[1-4]．

2. 分　　類

KSS[5]，MERRF[6]，MELAS[7]の3大病型のほか，多様な疾患に分類される．これらの疾患概念は，独立した，特有の症状を有するが，互いに共通する症状も有している．各疾患の症状を表1に示す[8]．

以下に，ミトコンドリア異常を疑わせる所見，症候について述べるが，これらの疾患概念に完全に合致しない例もあり，また，発症初期には症状がそろっていないこともあるので，幾つかの症状が存在する場合，他の症候の存在の有無を調べ，ミトコンドリア異常症を疑い検査を進めるべきである．

3. ミトコンドリア異常を疑う臨床症候

a. 家族歴

ヒトのミトコンドリア(mtDNA)は，すべて母親由来であるため，ミトコンドリア異常症は母系遺伝となる．よって，母系遺伝が明らかな疾患は，ミトコンドリア異常症の可能性が考えられる．しかし，ミトコンドリア異常症には，異常ミトコンドリアの比率が一定以上にならないと発症しない閾値効果があるため，母が発症しておらず，子供たちに発症する，一見常染色体劣性遺伝形式にみえることもある．

b. 発症年齢

発症年齢は多彩で，病状の進行速度も多様である．乳児期早期に発症して，早期に死亡するLeigh症候群もあれば，成人になってから発症する疾患も多い．同一疾患概念の中では好発年齢があるが，発症年齢，進行速度に幅がある疾患も多い．

c. 各疾患に共通する症候

低身長，精神遅滞または退行，けいれん，筋力低下は各疾患に共通し，高頻度にみられる症候である．

d. 各組織において出現する症状[1-4]

以下は，ミトコンドリア異常症として出現する可能性のある症候である．

Takanori Yamagata, Mariko Y. Momoi: Department of Pediatrics, Jichi Medical School　自治医科大学小児科

表1 主要なミトコンドリア異常症の各病型における臨床的特徴

	KSS	CPEO	Pearson 症候群	Leber病	MERRF	MELAS	Leigh 脳症	NARP
家族歴	−	±	−	+	+	+	+	+
発症年齢	幼児期-20歳	幼児期-30歳	0歳	8-60歳 (20歳代が多い)	2-20歳 (10歳前後)	1-20歳 (15歳未満70%)	0歳	0-60歳 (10歳前後が多い)
低身長	+	±	±	−	+	+	+	±
知能障害	+	±	±*	−	⊕	⊕	⊕	⊕
けいれん	−	−	−	−	⊕	+	⊕	+
小脳失調	+	±	−	−	⊕	±	+	⊕
ミオクローヌス	−	−	−	−	⊕	±	+	+
頭痛・嘔吐発作	−	−	−	−	−	⊕	−	−
脳卒中様発作	−	−	−	−	±	⊕	−	±
視神経萎縮	−	−	−	⊕	±	±	±	−
外眼筋麻痺	⊕	⊕	−	−	−	−	±	−
網膜色素変性	⊕	±	−	−	−	−	±	⊕
心伝導障害	⊕	±	−	−	−	−	−	−
心筋症	±	−	±	−	±	±	±	−
筋力低下	+	+	−	−	+	+	+	⊕
難　聴	+	−	−	−	+	+	±	±
尿細管障害	±	−	+	−	±	±	±	±
貧　血	−	−	⊕	−	−	−	−	−
末梢神経障害	±	±	−	−	+	±	±	⊕
高乳酸血症	+	±	+	−	+	+	+	±
髄液蛋白増加	+	±	?	−	−	−	−	−
RRF	+	+	?	−	+	+	−	−
mtDNA変異	欠失	欠失	欠失	点変異	点変異	点変異	点変異	点変異

−：ほとんどみられない，±：ときにみられる，+：しばしば認める，○：主要症状.
*幼児期にKSS移行例あり.

1) 中枢神経系

(1) 精神遅滞または知的退行：高頻度に出現する．精神遅滞として，乳幼児期から明らかな場合から，成人後に発症してくるものまである．

(2) けいれん：全般強直間代けいれん，部分発作，ミオクローヌス発作など，多彩な発作が出現する．

(3) 小脳失調：MERRF，KSSなど，一部の疾患で出現する．小脳失調を呈する患者の中で，ミトコンドリア異常が占める割り合いは比較的高いと推定される．

(4) 頭痛：MELASなどでしつこい頭痛を反復することがある．片頭痛を起こすこともある．

(5) ミオクローヌス：MERRFなどで出現する．

(6) 脳卒中様発作：MELASに特徴的であるが，他の疾患でもまれにみられることがある．血管の支配領域と一致しない梗塞巣が特徴的である．

(7) 中枢性呼吸障害：Leigh症候群やβ酸化異常を有する疾患で，脳幹の機能障害などから呼吸停止を来すこともある．乳児突然死症候群(SIDS)の鑑別にも必要である．

2) 骨 格 筋

緩徐進行性の筋力低下，筋萎縮，易疲労性がみられる．

3) 外 眼 筋

眼瞼下垂，外眼筋麻痺による眼球運動制限が，CPEO，KSSでみられる．

4) 眼[9]

(1) 網膜色素変性：KSS，NARPを中心に出現する．

(2) 視神経萎縮：Leber病で特異的に出現する．

(3) 視野異常：MELASで後頭葉での梗塞様発作後に，同名半盲や皮質盲が残る．

5) 難　聴

多くの疾患で出現し，ミトコンドリア異常を疑わせる主要徴候の一つである．

6) 心　臓

(1) 心筋症：発症頻度は高くないが，多くの疾患で出現し得る症状である．肥大型心筋症が多いが，拡張型もある．心筋症単独で発症する例もあるので，心筋症をみたら，ミトコンドリア異常症も念頭に置く必要がある．

(2) 心伝導障害：KSSに特異的である．

7) 膵　臓

(1) 糖尿病：KSSやMELASの3243変異で好発するが，他の点変異でも発症する．糖尿病のみの家系もある．インスリン依存性のことも，インスリン非依存性糖尿病となることもある．

(2) Pearson症候群など，膵外分泌不全を伴う疾患もある．

8) 肝　臓

Leigh症候群などで，乳児期に高度の肝障害を来す例がある．軽度の肝機能障害は，各疾患で高頻度にみられるが，肝不全を起こすような高度の肝障害に至ることはまれである．

9) 腎　臓

尿細管機能障害は，各疾患でみられる．各疾患で進行すると腎障害が出現し腎不全に至ることもあるが，病初期から腎障害が中心になることはまれである．

10) 消 化 管

腹痛の訴えは多く，慢性の便秘の頻度は高い．逆に，慢性下痢を起こすこともある．病状の進行とともにイレウスに至ることもある．

11) 血　液

著明な汎血球減少がPearson症候群の特徴的症状である．軽度の汎血球減少あるいは貧血は，他の疾患でもみられる．

12) 内 分 泌

副甲状腺機能低下症がKSSなどで起こることがある．

13) 末梢神経障害

感覚性neuropathyがNARPに特徴的である．MERRFや他の疾患でも軽度の末梢神経障害は好発し，ミトコンドリア異常を疑う所見の一つである．

14) 奇　形

ミトコンドリア異常症での奇形は一般的ではないが，胎児期から高度の代謝障害にさらされていた例では，小頭症や脳の形成異常，顔貌の異常が報告されており，奇形があることがミトコンドリア異常症の否定にはならない．

おわりに

ミトコンドリア異常症は，多様な疾患で，各疾患の中でも多様性がある．知的障害，小脳症状，けいれんなどの神経症状や筋症状とともに，低身長，難聴，網膜色素変性症などの多彩な症状を示す場合は，ミトコンドリア異常症を疑う必要がある．

■文　献

1) 後藤雄一：診断へのアプローチ．ミトコンドリア病(埜中征哉，後藤雄一編)，p77-79，医学書院，1997.
2) Larsson NG, Clayton A: Molecular genetics aspects of human mitochondrial disorders. Annu Rev Genet 29: 151-178, 1995.
3) Munnich A, Rustin P: Clinical spectrum and diagnosis of mitochondrial disorders. Am J Med Genet 106: 4-17, 2001.
4) Rachel A, et al: Mitochondrial dysfunction and neuromuscular disease. Muscle Nerve 24: 170-191, 2001.
5) Kearns TP, Sayre GP: Retinitis pigmentosa, external ophthalmoplegia and complete heart block. Arch Ophthalmol 60: 280-289, 1958.
6) Fukuhara N, et al: Myoclonus epilepsy associated with ragged-red fibers (mitochondrial abnormali-

ties): disease entity or a syndrome? Light- and electron-microscopic studies of two cases and review of literature. J Neurol Sci 47: 117-133, 1980.
7) Pavlaskis SG, et al: Mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes: a distinctive clinical syndrome. Ann Neurol 16: 481-488, 1984.
8) 斉藤茂子, 桃井真里子：ミトコンドリア異常症の臨床. —どんな時にミトコンドリア異常症を疑い, どんな検査を行うか—. 小児内科 30: 1131-1134, 1998.
9) Biousse V, Newman NJ: Neuro-ophthalmology of mitochondrial diseases. Semin Neurol 21: 275-291, 2001.