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Research Letter

Split Hand Foot Malformation With Whorl-Like Pigmentary Pattern: Phenotypic Expression of Somatic Mosaicism for the *p63* Mutation

Rika Kosaki,[1] Yoko Naito,[2] Chiharu Torii,[2] Takao Takahashi,[2] Tatsuo Nakajima,[3] and Kenjiro Kosaki[2]*

[1]Department of Clinical Genetics and Molecular Medicine, National Center for Child Health and Development, Tokyo, Japan
[2]Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan
[3]Department of Plastic and Reconstructive Surgery, Keio University School of Medicine, Tokyo, Japan

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To the Editor:

In a recent issue of the *Journal*, Basel et al. [2006] published a research review entitled "The Expanding Panorama of Split Hand Foot Malformation." We congratulate the authors on their stimulating work and wish to present a family with split hand foot malformation (SHFM), hoping to expand a short stretch of the panorama. SHFM, also known as ectrodactyly, is characterized by the underdevelopment or absence of the central digital rays [Basel et al., 2006] and often represents a cardinal feature of the EEC syndrome, an autosomal dominant disorder defined by *e*ctrodactyly, *e*ctodermal dysplasia, and orofacial *c*lefts [Penchaszadeh and de Negrotti, 1976; Roelfsema and Cobben, 1996]. Most patients with EEC syndrome have heterozygous mutations in the *p63* gene, a cell cycle regulating transcription factor on chromosome 3q27 [Celli et al., 1999; van Bokhoven et al., 2001], whereas *p63* mutations are infrequent among SHFM patients without other features of the EEC syndrome [Ianakiev et al., 2000; van Bokhoven et al., 2001; de Mollerat et al., 2003]. Here, we report on a father with an apparent SHFM phenotype and his son with a classic EEC syndrome phenotype. A detailed physical examination and molecular studies demonstrated that the father had somatic mosaicism for a *p63* mutation.

The propositus was delivered at 35 weeks of gestation to a 32-year-old gravida 1, para 0 Japanese woman. The propositus' birth weight of 2,324 g was in the 50th centile, and the propositus' length of 43.5 cm was in the 10th centile. The propositus' head circumference of 32.5 cm was in the 50th centile. At birth, a diagnosis of EEC syndrome was made based on the central digital rays of both hands and feet; sparse scalp, eyebrow, and eyelash hair; thin and dry skin; and a bilateral cleft lip with cleft palate (Fig. 1).

The father of the propositus was a 44-year-old male with a normal developmental history. He had ectrodactyly of the hands without orofacial clefts (Fig. 2) and had been diagnosed as having nonsyndromic SHFM elsewhere. Upon his first visit to our genetic clinic accompanying his son (the propositus), he presented with several features that were atypical for nonsyndromic SHFM. He had gray hair on the right half of his scalp and brown thin hair on the left side. A hyperpigmented patch was present in the midfacial area. The skin of his upper extremities showed whorl-like and streaky patterns with mildly hyperpigmented patches and normal skin patches following the Blashko line (Fig. 2). The father, who likes outdoor activities, commented that some patches of his skin tanned when exposed to the sun, while others did not. The father had enamel hypoplasia with partial anodontia. His fourth fingernails were trapezoidal shaped. The father's parents and siblings were unaffected clinically.

After obtaining informed consent from the parents, the peripheral blood genomic DNA of the child was screened for mutation in the coding sequence of *p63* using polymerase chain reaction (PCR) sequencing,

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*Correspondence to: Kenjiro Kosaki, M.D., Department of Pediatrics, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail: kkosaki@sc.itc.keio.ac.jp
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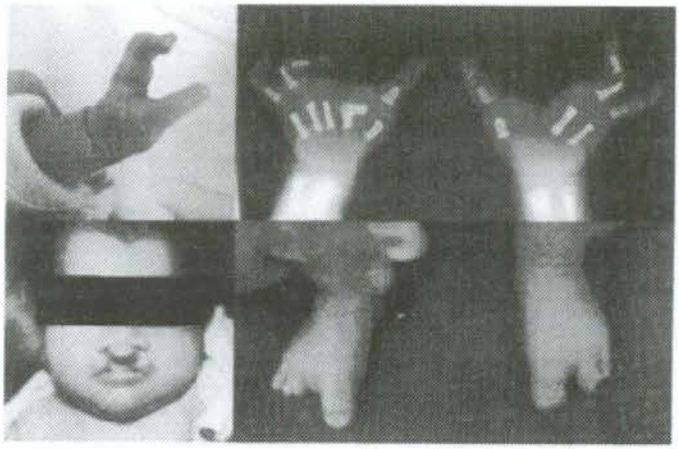

FIG. 1. The propositus at 1 month of age. Note split hand foot malformation and cleft lip and palate (bottom, left).

as described previously [Kosaki et al., 2001; Akahoshi et al., 2003]. A heterozygous cytosine to thymine transition at nucleotide 727 (727C > T), which leads to a R204W substitution within the DNA binding domain, was identified in genomic DNA extracted from the peripheral lymphocytes of the propositus and the father (Fig. 3). The substitution at R204 represents one of the most frequently mutated amino acids residues among previously reported EEC syndrome patients [Rinne et al., 2006].

We obtained two lines of molecular evidence that showed somatic mosaicism in the father: First, haplotype analysis of genomic DNA extracted from the peripheral blood [Martinez-Arias et al., 2002] yielded molecular evidence of mosaicism in the father, and systematic analysis of the SNPs flanking the C to T mutation revealed heterozygosity for the SNP (rs6790791: A/G), which was about 2 kb apart from the C to T mutation 727C > T. We performed PCR amplification of the father's peripheral blood genomic DNA across the rs6790791 SNP and the C to T mutation at nucleotide 727 and the PCR amplicons were subcloned into a plasmid vector using a standard method, followed by determination of the nucleotide sequences of the inserts. Analysis of 16 clones revealed three haplotypes: A-C, A-T and G-C. It was deduced that the C to T mutation at nucleotide 727 occurred on the wild-type chromosome harboring the A-C haplotype. Second, analysis of genomic DNA from hair root samples of the father revealed somatic mosaicism. Genomic DNA from

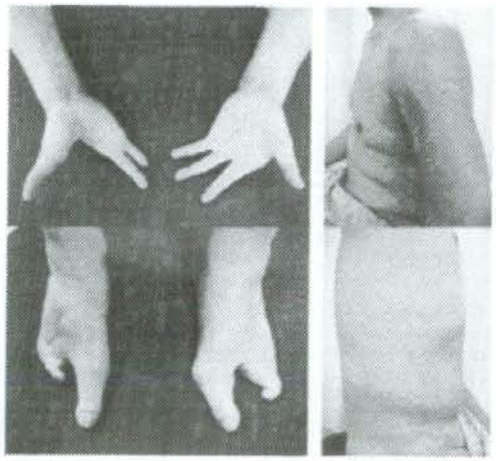

FIG. 2. The propositus' father at age 44 years. Note split hand foot malformation (left), and abnormal pigmentary pattern following the Blashko line (right).

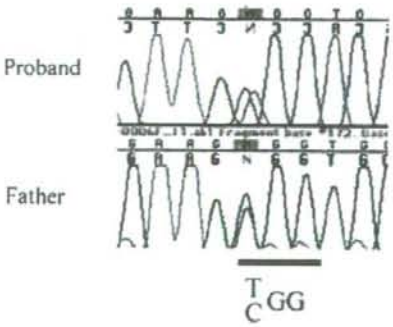

FIG. 3. A R204W mutation in the propositus (top) and his father (bottom). A heterozygous "C" to "T" transition at nucleotide 727 in exon 5, leading to an Arginine-to-Tryptophan substitution at amino acid 204 within the DNA-biding domain.

two hair roots of brown thin hair revealed a C to T mutation whereas genomic DNA from one hair root of gray hair revealed no mutation.

Since the son was fully affected without mosaicism, it is evident that the father had mutant germline cells in the gonad. In order to evaluate possible mosaic status of germline cells, haplotype analysis of the genomic DNA extracted from the father's semen was performed. Subcloning and sequencing analysis of the PCR products amplified from the father's semen genomic DNA across the rs6790791 SNP and the C to T mutation at nucleotide 727 revealed only two haplotypes: A-T and G-C. It was deduced that most of the diploid germline cells in the father's testes carried the *p63* mutation.

In view of the finding that the father had the *p63* mutation in some of the peripheral blood cells, hair root cells and sperm, it is likely that the *p63* mutation occurred after fertilization. Because both somatic and germline tissues were affected, the father was considered to have gonosomal mosaicism [Siegel and Sybert, 2006].

It was not possible to obtain samples from other family members for the *p63* mutation analysis.

The father with SHFM and his son, who had SHFM as a partial feature of EEC syndrome, were both found to harbor a *p63* mutation. The father had a whorl-like and streaky pigmentary pattern following the Blashko line, indicating that he had mosaicism involving somatic as a result of a postzygotic mutation that had given rise to the clonal proliferation of two genetically distinct groups of cells [Happle, 1993; Siegel and Sybert, 2006]. The observation that the phenotypic expression of the father was milder, without orofacial clefting, supports the general notion that somatic mosaicism for an autosomal dominant disease usually leads to a mild phenotype: in mosaic individuals, only some cells express the mutant allele, while others express only normal alleles and any effects on morphogenesis are limited to the local environment of the cells in the tissues that express the mutant allele [Wallis et al., 1990].

In the molecular haplotyping of father's peripheral blood cells described above, among a total of 16 clones, 8 showed the G-C haplotype pattern, 4 showed the A-C haplotype pattern, and 4 showed the A-T haplotype pattern. Hence, it was deduced that approximately half of the diploid cell population harbored the C to T mutation, and that conversely, half of the diploid cell population had the wild type allele. The cell population without the *p63* mutation might have alleviated the father's phenotype to some extent.

It is interesting to note that the severity of the ectrodactyly of the father was comparable to that of the child, whereas the father did not express the orofacial clefting phenotype. Ectrodactyly represents a defect in the apical ectodermal ridge of the limb buds, whereas orofacial clefting represents a defect in the epithelium of the branchial arches [Mills et al., 1999; Yang et al., 1999]. Hence, the limb primordia might be more sensitive to the dose of the mutated p63 protein than the craniofacial primordia. A recent review on the phenotypic spectrum of the R204 p63 mutation (n = 27) demonstrating a striking difference between the incidence of orofacial clefting (26%) and limb malformations (63%) [Rinne et al., 2006] supports such a notion.

From a clinical standpoint, we suggest that an abnormal skin pigmentary pattern with alternating areas of affected and unaffected skin should be carefully searched for when giving genetic counseling to a person who apparently has an isolated SHFM. If such a pigmentary pattern is present, any offspring who inherits the mutated allele could manifest full-blown EEC syndrome with facial clefting.

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Clinical Report

Neocentromere Marker Chromosome of Distal 3q Mimicking dup(3q) Syndrome Phenotype

Kosuke Izumi,[1] Yukio Yamashita,[2] Michihiko Aramaki,[1] Rika Kosaki,[3] Noboru Hosokai,[4] Takao Takahashi,[1] and Kenjiro Kosaki[1]*

[1]Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan
[2]Department of Pediatrics, Kawasaki Municipal Kawasaki Hospital, Kawasaki, Japan
[3]Department of Clinical Genetics and Molecular Medicine, National Center for Child Health and Development, Tokyo, Japan
[4]Research and Development Division, Mitsubishi Kagaku Bio-Clinical Laboratory, Tokyo, Japan

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Supernumerary marker chromosomes (SMCs) lacking alpha-satellite sequences and possessing a newly derived functional centromere are referred to as neocentromere marker chromosomes (NMCs). Although the delineation of the chromosome content of these NMCs would be helpful for genetic counseling, such fine mapping has been difficult because of the limited sizes of the involved segments. We report on a female patient with mosaic NMC involving 3q26.3-3qter, the content of which was determined using an array CGH analysis. Our results support the validity of an array CGH-based approach to investigating the origins of SMCs. Further FISH analyses revealed that the NMC is characterized by an asymmetric inv-dup structure separated by a single-copy region. The present case had many manifestations of dup(3q) syndrome, the critical interval of which is considered to be 3q26.3-q27. Common features included mental and growth retardation, hirsutism, synophrys, a broad nasal root, anteverted nares, downturned corners of the mouth, and malformed ears. The observation gives further credence to the concept that the critical region responsible for the dup(3q) phenotype to 3q26.3-q27.

Key words: marker chromosome; supernumerary marker; neocentromere marker; analphoid marker; inverted duplication

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INTRODUCTION

Supernumerary marker chromosomes (SMCs) are identified in approximately 0.05% of liveborn children [Nielsen and Wohlert, 1991]. While most SMCs have a normal centromere with alpha-satellite sequences that can be detected using fluorescence in situ hybridization (FISH) [Callen et al., 1992], the remaining SMCs lack alpha-satellite sequences and possess a newly derived functional centromere, referred to as a neocentromere, that is formed outside the normal centromere domain [Slater et al., 1999; Amor and Choo, 2002]. These classes of SMCs can be categorized as neocentromere marker chromosomes (NMCs), or analphoid marker chromosomes. More than 60 cases with NMCs have been reported [Amor and Choo, 2002; Spiegel et al., 2003]. Many of these chromosomal abnormalities have been attributed to de novo inverted duplications of the distal segments of chromosomes [Rowe et al., 2000; Warburton et al., 2000; Spiegel et al., 2003]. Although the delineation of the chromosome content of these NMCs would be helpful for genetic counseling, such fine mapping has been difficult because of the limited sizes of the involved segments. Here, we report on a child with multiple anomalies and mosaic chromosome 3q NMC characterized by an asymmetric inv-dup structure separated by a single-copy region.

Grant sponsor: The Ministry of Health, Labour, and Welfare of Japan.
*Correspondence to: Kenjiro Kosaki, M.D., Department of Pediatrics, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail: kkosaki@sc.itc.keio.ac.jp
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CLINICAL REPORT

The female patient was born at 41 weeks to a non-consanguineous Japanese couple after an uneventful pregnancy without teratogen exposure. The weight, height and occipitofrontal circumference (OFC) at birth were 3,340 g (50th centile), 49.5 cm (50th centile), and 34.0 cm (50th centile), respectively. On the day of her birth, she presented with diastasis recti, an atrial septal defect and dysmorphic facies. Her development was delayed: she gained head control when she was 2–3 months old, sat without support when she was 10–11 months old, stood alone when she was 1.5 years old, and ran when she was 2 years old. Formal developmental testing when she was 3.5 years old revealed a developmental quotient of 38–39.

At 5 years and 2 months of age, the patient was referred to us for evaluation because of moderate to severe delays in psychomotor development. Her weight, height, and OFC were 16.6 kg (10–25th centile), 99.2 cm (below the 3rd centile), and 50.2 cm (50–75th centile), respectively. A physical examination revealed several dysmorphic features including (Fig. 1) synophrys, hirsutism, flat face with a low nasal bridge, anteverted nares, a thin upper lip, a long philtrum, and downturned corners of the mouth, and short fifth fingers on both hands. In addition to these features, generalized hyperpigmentation was noted. From the standpoint of gross motor development, she had an equivalent age of 37 months. She was able to jump with both feet and was able to step up and down stairs. From a cognitive standpoint, she had an equivalent age of 27 months. She was able to place seven blocks vertically and could copy circles and lines. However, she could not point out objects and had not said any meaningful words.

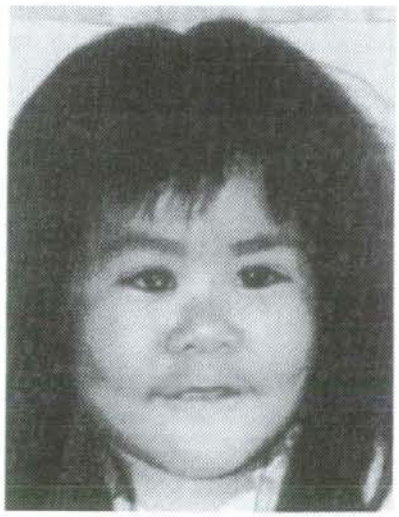

FIG. 1. The proposita at 5 years of age.

CYTOGENETIC AND MOLECULAR STUDIES

Cytogenetic analysis of the patient's peripheral blood lymphocytes showed mosaicism for an SMC: 47,XX,+mar[27]/46,XX[3] (Fig. 2A). A GTG-banded karyotype at a 500-band level did not show the chromosomal origin of the SMC. The parental karyotypes were normal. Spectral karyotyping (SKY) with SkyPaint probes (Applied Spectral Imaging, Inc., Carlsbad, CA) revealed the SMC to have been derived from chromosome 3 (data not shown).

To further analyze the content of the marker chromosome, an array CGH analysis [Cai et al., 2002] was performed using commercially available genomic DNA microarray slides (Human BAC Array 2500; Spectral Genomics, Houston, TX) containing 2,500 non-overlapping BAC and PAC clones with an average resolution of 1 Mb throughout the genome. Genomic DNA from the patient and a normal control subject were digested with *Eco*RI, labeled with Cy3 and Cy5, and hybridized according to the manufacturer's protocol. Hybridized microarray slides were scanned using a GenePix4000A scanner (Axon Instruments, Inc., Union City, CA). Analysis of the scanned images using Spectralware 2.0 software (Spectral Genomics) revealed an increased copy number of the long arm of chromosome 3 (Fig. 3). The patient-to-control fluorescence intensity ratio was elevated within the chromosomal region from 3q26 to 3qter. The elevation in the intensity ratio was augmented at the distal end of chromosome 3, within the chromosomal region from 3q29 to 3qter (Fig. 3).

The results of the array CGH analysis were confirmed using FISH and a series of single-copy BACs (Figs. 4 and 5). FISH with 3q subtelomeric probes RP11-778E2 (3q29, Fig. 4A) and TelVysion Multi-color FISH Probe Panel 3q probe (Abbott, Abbott Park, IL, data not shown) showed two signals for the 3q subtelomeric region at both ends of the SMC, indicating that the SMC had inverted duplication. Further FISH analysis revealed that the SMC in

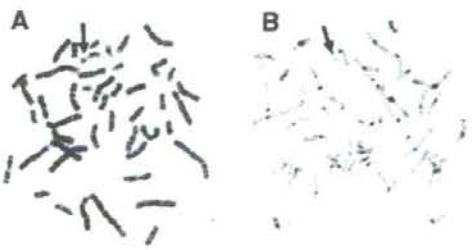

FIG. 2. G-banding (**A**) and C-banding (**B**) karyotype of the patient revealed the presence of the marker chromosome (arrows).

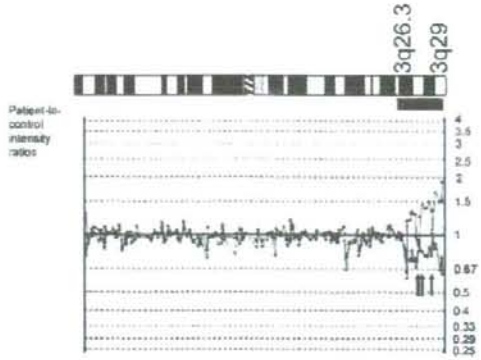

FIG. 3. Array CGH analysis. The longitudinal axis represents the patient-to-control fluorescence intensity ratios for each clone and the horizontal axis represents its relative location along chromosome 3. Blue plots represent the patient-to-control (Cy5-to-Cy3) fluorescence intensity ratios for each clone. Red plots represent the result of dye swap, which was used to reduce the possibility of dye-related artifacts. Gain of 3q from 3q26 to 3qter is demonstrated. Three clones that had not exhibited gain patterns in the array CGH analysis (arrows: RP11-102G2, RP11-63G1, and RP11-608P9) were present in one copy on the marker chromosome (see text). A gain of 3q from 3q26 to 3qter is demonstrated.

the present case might be asymmetric, containing one copy of the more proximally located BACs (RP11-114M1, RP11-682A21, RP11-245C23, RP11-89B3, RP11-45I24, RP11-102G2, RP11-63G1, BCL6, RP11-54L9, RP11-608P9, RP11-326J2, RP11-1129A7, RP11-16E21, and RP11-622P12), compared with two copies of the more-distal BAC (RP11-141C7, RP11-480A16, RP11-678H2, RP11-778E2, and RP11-313F11).

A metaphase FISH analysis using a 3q27.3 probe, BCL6, and a 3q29 probe, RP11-778E2 is shown in Figure 4C. The *BCL6* gene probe was selected because it is located approximately in the middle of the chromosomal region contained in the marker chromosome (i.e., 3q26.3 to 3qter). The RP11-778E2 probe produced a double signal on both ends of the marker chromosome, whereas the BCL6 probe produced a single signal on the marker chromosome between the two RP11-778E2 signals. If the marker chromosome had been a symmetric isochromosome, two BCL6 signals should have been observed. We concluded that the marker chromosome had an asymmetric structure, as shown in Figure 5.

No signal was observed on the SMC when the probe RP11-335L9 at 3q26.32 was used, a result indicating that this probe lay proximal to the breakpoint (data not shown). Thus, the two arms of the marker chromosome had asymmetric breakpoints with one located at or very close to 3q subtelomere, and the other one located at 3q26.3. Three clones that had not exhibited gain patterns in the array CGH analysis (Fig. 3, RP11-102G2, RP11-63G1, and RP11-608P9) were present in one copy on the marker chromosome.

C-banding (Fig. 2B) and a chromosome 3-specific alpha satellite probe (CEP3, SpectrumOrange, Abbott; Fig. 4A) failed to detect any centromeric

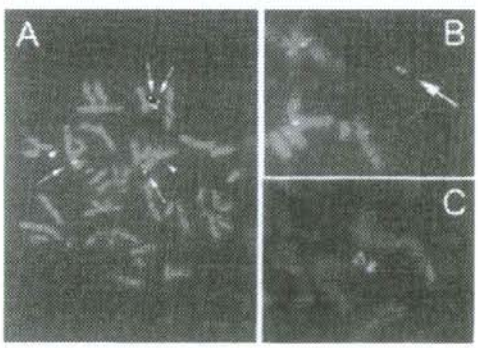

FIG. 4. Fluorescence in situ hybridization (FISH) analysis. **A**: Analphoid probe specific for the chromosomes 3 alpha satellite (red signals) did not produce any signal on the marker chromosome (arrowheads). A 3q29 probe RP11-778E2 (green signals) showed single signals on the normal homologous chromosomes 3 at distal 3q, and a double signal on the marker chromosome (arrows). **B**: Immunofluorescence of polyclonal goat antibodies generated to a centromeric antigen demonstrated CENP-E was present on the marker chromosome (arrow). **C**: A metaphase FISH analysis using a 3q27.3 probe, BCL6, and a 3q29 probe, RP11-778E2. The RP11-778E2 probe (green signals) produced a double signal at both ends of the marker chromosome, whereas the BCL6 probe produced a single signal between the two RP11-778E2 signals.

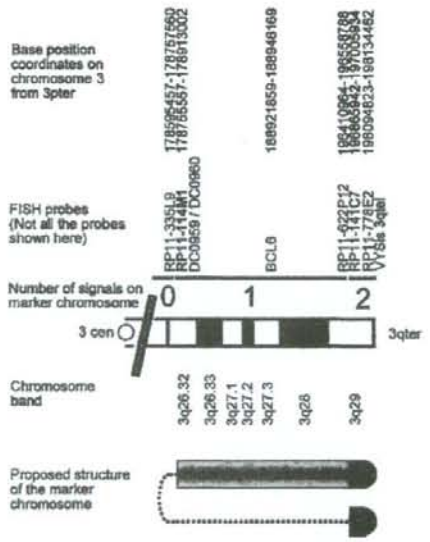

FIG. 5. Proposed structure of the marker chromosome. **Top**: Summary of the FISH experiments. An ideogram of the distal end of 3q is presented together with some of the FISH clones used in the present study and their base position coordinates on chromosome 3 from 3pter according to the March 2006 human reference sequence NCBI Build 36.1 assembled by UC Santa Cruz. DC0959 and DC060 represent the locations of the sequences that are homologous to the centromeric portion of chromosome 22, according to the Human Genome Segmental Duplication Database (see text). **Bottom**: The structure of the marker chromosome. The marker chromosome is characterized by an asymmetric inv-dup structure (black) separated by a single-copy region (gray).

or pericentromeric sequences on the marker chromosome. Immunofluorescence of polyclonal goat antibodies generated for a centromeric antigen demonstrated that Centromere-associated protein-E (CENP-E), a motor molecule that is important for chromosome movement [Weaver et al., 2003], was present on the marker chromosomes (Fig. 4B), indicating the presence of a neocentromere [Sullivan and Schwartz, 1995].

DISCUSSION

We report on a child with developmental delay, multiple anomalies, and mosaicism for an SMC derived from 3q26.3 to 3qter. Array CGH was effective in determining the origin of the SMC. Our results support the recent work published in this journal documenting the validity of an array CGH-based approach to investigating the origins of SMCs [Liehr et al., 2005].

The marker chromosome reported in the present case most likely had inverted duplication configuration because the probe RP11-778E2 (3q29) showed two signals for the 3q subtelomeric region on the SMC whereas more proximal probes (3q26.3-3q28) showed only one signal. Further FISH analyses revealed that the SMC was asymmetric and was characterized by an inv-dup structure separated by a single-copy region (Fig. 5). The observation from the array CGH experiments that the patient-to-control fluorescence intensity ratio was further elevated within the chromosomal region from 3q29 to 3qter is compatible with the notion that the marker chromosome has an asymmetric structure (Fig. 3).

A similar asymmetric marker chromosome structure has been proposed in chromosome 15 [Alonso et al., 2003; Ventura et al., 2003]. Heterozygous small paracentric inversions can trigger rearrangements that lead to an inv-dup structure that is separated by a single-copy region [Giglio et al., 2001]. A cross-over event within a small heterozygous paracentric inversion during meiosis can trigger a rearrangement characterized by an asymmetric inv-dup structure that is separated by a single-copy region when one of the parents has such a paracentric inversion [Giglio et al., 2001]. Unfortunately, we were unable to test whether one of the parents might have an inversion because they declined genetic testing.

The finding that an anti-centromere CENP-E antibody revealed positive signal(s) on the marker chromosome suggested that the chromosome probably harbors a functional kinetochore, even if a chromosome 3-specific alpha satellite probe did not detect the presence of any centromeric or pericentromeric alpha-satellite sequences on the marker chromosome. Based on these results, we concluded that the SMC represents an NMC derived from chromosome 3 with inverted duplication of the distal segments of the chromosome 3. The mechanism how neocentromere is formed is not well understood. Ohashi et al. [1994] suggested that an ancient centromere sequence might exist on the marker chromosome and might be activated through the chromosomal rearrangement. Two regions which are potentially relevant in terms of neocentromere formation were identified near the proximal end of the breakpoint (Fig. 5, top): a region known to function as the centromere in old world monkeys (RP11-114M1 at 3q26.32, base position coordinates on chromosome 3 from 3pter according to the March 2006 human reference sequence NCBI Build 36.1 assembled by UC Santa Cruz: chr3:178,755,557-178,913,002) [Ventura et al., 2004], and a region that is highly homologous to the centromeric region of chromosome 22 (DC0959 and DC0960 according to the Human Genome Segmental Duplication Database base position coordinates: chr3: chr3:180415171-180422188 and chr3:180437834-180445262) [Cheung et al., 2003].

Seven patients with a NMC of chromosome 3 and inverted duplication of the distal segments of the chromosome have been reported [Wandall et al., 1998; Portnoi et al., 1999; Cockwell et al., 2000; Gimelli et al., 2000; Teshima et al., 2000; Barbi et al., 2003]. Among the five patients who were relatively well characterized from a clinical standpoint, the phenotype varied significantly [Teshima et al., 2000]. This apparent lack of consistency with regard to the phenotype can be accounted for by two explanations. First, the breakpoints may have differed among the five cases. Second, the degree of mosaicism was not uniform among the five cases, leading to a variable degree of severity. Conversely, patients with a comparable degree of chromosomal extent and mosaicism would have a similar phenotype. As expected, one patient reported by Teshima et al. [2000] (Case 1) who had an NMC spanning a comparable region (i.e., 3q27.2 to 3qter) and a comparable degree of mosaicism (i.e., 71%) to the present case exhibited a phenotype including flat face with a low nasal bridge, anteverted nares, a thin upper lip, and a long philtrum that was quite similar to the present case. Re-analysis of the previously reported cases using molecular cytogenetic techniques and array CGH would be required to further refine the region with an increased copy number in each case.

Duplication or triplication of a specific autosomal segment tends to result in comparable phenotypes, regardless of the underlying mechanism responsible for the increased copy number [Schinzel, 1993]. As predicted, the present case, the patient with an NMC spanning 3q27.2 to 3qter patient [Teshima et al., 2000], and the patient with pure 3q duplication of 3q with 46,XY,der(3)dup(3)(qterq26.3) karyotype [Faas et al., 2002] had many common features including mental and growth retardation, hirsutism, synophrys, a flat nasal bridge, anteverted nares, and

downturned corners of the mouth. Indeed, those manifestations represent cardinal features of the dup(3q) syndrome, the critical interval of which is considered to be 3q26.3-27 [Aqua et al., 1995; Rizzu et al., 1997]. The observation that a patient with a non-mosaic NMC spanning 3q28-3qter lacked characteristic features of dup(3q) syndrome [Barbi et al., 2003] supports the concept that the critical region responsible for the dup(3q) phenotype maps to 3q26.3-27 [Aqua et al., 1995; Rizzu et al., 1997].

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CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

Case Report: Adult Phenotype of Mulvihill–Smith Syndrome

Tatsuhiko Yagihashi,[1,2] Motoichiro Kato,[3] Kosuke Izumi,[1,4] Rika Kosaki,[1,5] Kaori Yago,[6] Kazuo Tsubota,[7] Yuji Sato,[2] Minoru Okubo,[8,9] Goro Watanabe,[10] Takao Takahashi,[1] and Kenjiro Kosaki[1]*

[1]Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan

[2]Center for Clinical Research, Keio University School of Medicine, Tokyo, Japan

[3]Department of Neuropsychiatry, Keio University School of Medicine, Tokyo, Japan

[4]Department of Pediatrics, Rainbow Babies and Children's Hospital, Center for Human Genetics, University Hospitals Case Medical Center, Cleveland, Ohio

[5]Department of Clinical Genetics and Molecular Medicine, National Center for Child Health and Development, Tokyo, Japan

[6]Department of Dentistry and Oral Surgery, Keio University School of Medicine, Tokyo, Japan

[7]Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan

[8]Department of Endocrinology and Metabolism, Institute for Medical Research, Tokyo, Japan

[9]Okinaka Memorial Institute for Medical Research, Tokyo, Japan

[10]Department of Digestive Surgery, Toranomon Hospital, Tokyo, Japan

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Mulvihill–Smith syndrome (MSS) is characterized by premature aging, multiple pigmented nevi, decreased facial subcutaneous fat, microcephaly, short stature, mental retardation and recurrent infections, however the adult phenotype of MSS has yet to be delineated. We report a 28-year-old woman with Mulvihill–Smith syndrome, who had a solid pseudopapillary cystic tumor of her pancreas at age 17 years. Her distinctive sleep pattern includes severe insomnia with disappearance of sleep spindles and K-complexes, persisting muscle tone, and loss of slow wave sleep. The clinical and neurophysiological studies are compatible with agrypnia excitata, a sleep disorder attributable to a dysfunction of the thalamo-limbic system. Brain magnetic resonance imaging and single photon emission computed tomography revealed structural and functional deficits in the dorsomedial region of the thalamus and indicated that an alteration in the thalamo-limbic system may underlie the sleep disturbances in MSS. Furthermore, the rapid and severe decline in acquired cognitive function showed the distinct cognitive impairments resembling dementia, including intellectual deficits, memory disorder and executive dysfunction. We posit that an early onset tumor, sleep disorder and cognitive decline are adult manifestations of Mulvihill–Smith syndrome.

Key words: Mulvihill–Smith syndrome; solid pseudopapillary cystic tumor; sleep disorder; agrypnia excitata; dementia

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INTRODUCTION

Mulvihill–Smith syndrome (MSS) is characterized by premature aging, multiple pigmented nevi, lack of facial subcutaneous fat, microcephaly, short stature, and mental retardation [de Silva et al., 1997]. Immunodeficiency may also be a critical feature [Ohashi et al., 1993].

Since its recognition by Mulvihill and Smith [1975], eight patients have been reported [Shepard, 1971; Elliott, 1975; Wong et al., 1979; Baraitser et al., 1988; Ohashi et al., 1993; Bartsch et al., 1994; de Silva et al., 1997; Ferri et al., 2005]. Because both male and female patients have been described, as well as a patient born to a consanguineous couple has been reported [Ohashi et al., 1993], the mode of inheritance is likely to be autosomal recessive. The causative gene has not been identified so far.

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*Correspondence to:
Kenjiro Kosaki, M. D., Department of Pediatrics, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.
E-mail: kkosaki@sc.itc.keio.ac.jp
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The adult phenotype of MSS has yet to be delineated. Two adult MSS patients presented with tumors, and one patient exhibited cognitive decline and sleep disorder [Bartsch et al., 1999; Ferri et al., 2005]. Whether or not these conditions represent characteristic adult features of MSS has not been clarified. We report a patient with MSS who developed tumors, a sleep disorder with severe insomnia and cognitive decline and suggest that these features are indeed unique adult manifestations of MSS.

CLINICAL REPORT

A Japanese girl was born by a spontaneous vaginal delivery at 38 weeks gestation after an unremarkable pregnancy. The parents were nonconsanguineous and phenotypically normal. The birth weight was 2,570 g (10–25th centile), the head circumference (OFC) 31.5 cm (10–25th centile), and the crown heel length 45.6 cm (10–25th centile). She was hospitalized for a month because of feeding difficulties.

During infancy and early childhood, she had multiple episodes of infections, including recurrent otitis media and a severe varicella infection that required hospitalization for 2 weeks. At age 1 year, multiple pigmented nevi became noticeable on the trunk. The number of nevi increased with age, and when the patient was 3 years old, a dermatologist diagnosed her as having LEOPARD syndrome because of multiple pigmented nevi, short stature, and mild hearing loss. She also had delayed motor development. She exhibited tonic postures of the upper limbs at age 3 months. With physical therapy, she was able to walk at age 1 year. She also started to speak meaningfully around the same time, and her speech development has been age-appropriate since then. She attended elementary school from age 6 years and achieved average grades. At age 13 years after entering the seventh grade, she developed bilateral sensory neural hearing impairment. Then her social interaction including personal contacts with her peers became poor and her scholastic achievement also declined.

At age 17 years, Werner syndrome was suspected because of a premature senile appearance; however, a Western blot for WRN protein showed a normal pattern. Her G-banded karyotype was normal. At age 20 years, she developed diabetes mellitus and started oral hypoglycemics. At age 24 years, band keratopathy and cataract developed; she had bilateral corneal transplantations at age 26 years and an intraocular lens placement at age 27 years.

At age 25 years, she was referred to our genetics clinic. She weighed 24.5 kg and was 138.4 cm tall; her OFC was 50.0 cm (<3rd centile), medians for 7 6/12, 10 0/12, and 4 3/12 years, respectively. She had a triangular face, a lack of facial subcutaneous fat, multiple pigmented nevi, a low posterior hairline, alopecia, bifid uvula, and a high pitched-voice (Fig. 1). Her external genitalia were normal. A bone radiograph showed brachydactyly with a shortening of the distal phalanges. The results of immunological studies including IgG, IgA, and IgM levels, PHA stimulation test, and lymphocyte subpopulation analysis were unremarkable. The patient's specific features (the progeria-like appearance, short stature, microcephaly, diffuse pigmented nevi, and metacarpophalangeal pattern [Bartsch et al., 1994]) allowed us to diagnose MSS.

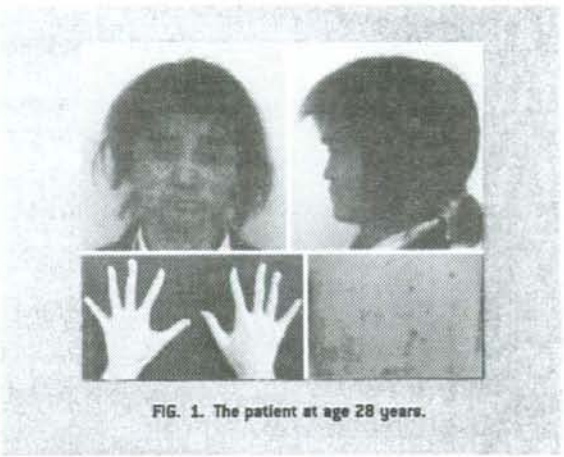

FIG. 1. The patient at age 28 years.

Development of Tumors

At age 17 years, the number of pigmented nevi increased. Abdominal ultrasonography revealed an asymptomatic pancreatic mass, which was resected surgically. The post-operative diagnosis was a solid pseudopapillary cystic tumor of the pancreas.

At age 25 years, she developed paresis and hyperesthesia of the right thumb and index finger. A head MRI showed nothing that could account for the findings; however, a 2.0 cm mass was incidentally identified in the right cerebellum. A re-evaluation performed 3 years later revealed that the size of the mass was unchanged, so the lesion was considered to be a benign tumor or cyst.

At age 27 years, she underwent surgical removal of a tongue tumor; the histopathologic diagnosis was an ulcer of the tongue with chronic inflammation, in which no evidence of malignancy was disclosed.

Sleep Disturbances and Neurophysiological Examinations

At age 26 years, she developed excessive daytime sleepiness and nighttime insomnia with hypnagogic hallucinations. However, these symptoms had been mild and had not interfered with her normal activities until age 28 years, when she became emotionally unstable and irritable. She had paresis and hyperesthesia of the right thumb and index finger and a slightly ataxic gait with myoclonic jerks. Her insomnia and emotional disturbances gradually deteriorated, and she began to experience visual hallucinations in the daytime.

The patient underwent polysomnography, comprising electrooculography, electroencephalography (EEG), electromyogram of the submentalis and the tibialis anterior muscles, ECG and nasal airflow. The EEG background findings with the eyes closed showed a posterior dominant rhythm of 9–10 Hz with intermittent 3–6 Hz slow waves. Sleep recordings revealed a complete loss of sleep spindles and K-complexes, which indicated an alteration of the physiological transitional process from being awake to falling

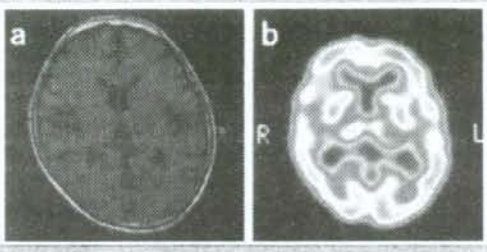

FIG. 2. Brain imaging at age 28 years. a: MRI. Note the bilateral atrophy of the dorsomedial nucleus of the thalamus and pulvinar, and the mild enlargement of the cortical sulci. b: Brain single photon emission computed tomography [^{99m}ECD-SPECT]. Note the focal reduction of regional cerebral blood flow in the left thalamus (b).

asleep, and further demonstrated a remarkable decrease in slow-wave sleep stages characterized by 1–2 Hz slow-waves, low chin muscle tone and the absence of motor activity in the four limbs. Furthermore, these polysomnographic recordings documented an absence of typical REM sleep episodes, with a low-voltage fast background and hypotonia. During sleep, intermittent myoclonic jerks often appeared with the persistence of chin muscle tone. The overall polysomnographic recordings were characterized by the loss of sleep spindles and K-complexes, sleep fragmentation as a result of increased arousals and persisting muscle tone, and an especially marked loss of slow wave sleep.

Neuropsychological and Neuroimaging Examinations

Clinical neuropsychological tests were performed at age 28 years. The patient's full-scale intelligence quotient (FIQ) on the Wechsler Adult Intelligence Scale III was 51 (verbal IQ = 55; performance IQ = 54), indicating global intellectual impairment. The Wechsler Memory Scale-Revised (WMS-R) revealed that she had severe memory deficits (general memory index 53, visual memory index 74, verbal memory index 54). On executive function, she showed poor performances on the Trail-making Test parts A and B, the Wisconsin Card Sorting Test and the Word Fluency Test. Taken together, the neuropsychological assessments demonstrated her cognitive deficits including intellectual disability, memory disorder and executive dysfunction.

Brain MRI at age 28 years revealed bilateral atrophy of the dorsomedial nucleus of the thalamus and pulvinar, and mild enlargement of the cortical sulci (Fig. 2a). Brain single photon emission computed tomography (^{99m}ECD-SPECT) demonstrated a focal reduction of regional cerebral blood flow in the left thalamus (Fig. 2b).

DISCUSSION

The patient reported herein exhibited all of the shared features of previously reported MSS patients: short stature, senile appearance, and pigmented nevi [de Silva et al., 1997; Ferri et al., 2005]. In addition, she had many of the common features of MSS: a high-pitched voice, alopecia, chronic and recurrent infections, hearing loss, cataract, mental retardation and a distinctive metacarpophalangeal pattern (Table I). Based on this recognizable phenotype, we diagnosed the patient as having MSS.

As this syndrome is characterized by premature aging, the development of tumors at an unusually young age is significant. Solid-pseudopapillary tumor of the pancreas, which developed in the present patient at age 17 years, is a relatively rare low-grade malignant tumor that seldom metastasizes. The tumor is commonly found in women of child-bearing age. Thus, the onset age of the solid-pseudopapillary tumor in the present patient was substantially lower than average [Papavramidis and Papavramidis, 2005]. Two previously reported MSS patients also exhibited the early onsets of tumors: signet ring cell carcinoma of the stomach in a 23-year-old patient [Bartsch et al., 1999] and squamous cell carcinoma of the tongue in a 20-year-old patient [Ferri et al., 2005]. We suspect that early onset tumors may represent an important adult MSS phenotype that needs attention. Since two of the three tumors arose in the epithelial cells of the gastrointestinal tract, MSS patients may be susceptible to specific type(s) of tumors of the gastrointestinal tract. The development of an abnormal mass in the tongue of the present patient is also noteworthy. Although pathological examination did not reveal tumor cells in the abnormal tongue mass, the report by Ferri et al. [2005] of squamous cell carcinoma of the tongue may indicate that the tongue or oral mucosa of patients with MSS are susceptible to tumors. The significance of the cerebellar mass in the patient reported herein remains undetermined.

We posit that cognitive deterioration in adults, in addition to the developmental delay, is an underappreciated feature of MSS. The rapid and severe cognitive decline observed in our patient cannot be accounted for by neural alterations arising from simple premature aging. The patient herein reported started the decline in acquired cognitive function around age 26 years, and showed the distinct cognitive impairments resembling dementia, including intellectual deficits, memory disorder and executive dysfunction at age 28 years. A similar clinical course suggesting a progressive decline in cognitive function was also described in a patient with MSS who exhibited mental retardation (IQ56) at age 25 years [Ferri et al., 2005].

The cognitive decline in the patient was further aggravated by a distinctive sleep pattern abnormality resembling agrypnia excitata, which is ascribed to a dysfunction of the thalamo-limbic system [Lugaresi and Provini, 2001; Montagna and Lugaresi, 2002]. Agrypnia excitata is observed in patients with fatal familial insomnia, Morvan fibrillary chorea, and delirium tremens, and is characterized by peculiar polysomnographic findings, including the absence of sleep spindles and K-complexes, the complete loss of slow-wave sleep, and abnormal REM sleep with lack of muscle atonia. The distinctive features of the sleep pattern in the patient also include severe insomnia with marked disappearance of sleep spindles and K-complexes, persisting muscle tone, and loss of slow wave sleep. Since the same sleep pattern abnormality has been reported in another adult MSS patient [Ferri et al., 2005], agrypnia excitata could be a feature of MMS. The fact that brain MRI and SPECT studies in the present patient revealed structural and

TABLE I. Clinical and Laboratory in Nine Cases of Mulvihill–Smith Syndrome

	Mulvihill and Smith [1975]	Shepard [1971] and Elliott [1975]	Wong et al. [1979]	Baraitser et al. [1988]	Ohashi et al. [1993]	Bartsch et al. [1994, 1999]	de Silva et al. [1997]	Ferri et al. [2005]	Present case
Sex	M	M	F	M	F	M	M	F	F
Age	17	3, 4	14	7	30	20, 23	4	25	28
Low birth weight	+	+	+	+	−	−	−	NR	−
Short stature	+	+	+	+	+	+	−	+	+
Microcephaly	+	+	+	+	+	+	+	+	+
High pitched voice	+	NR	NR	+	−	+	+	+	+
Lower facial hypoplasia	+	+	+	+	+	+	+	+	+
Hypertelorism	NR	+	+	−	+	+	−	−	−
Pigmented nevi	+	+	+	+	+	+	+	+	+
Facial fat reduced	+	NR	+	+	+	+	+	+	+
Alopecia	+	+	NR	NR	−	−	−	−	+
Deafness	+	+	+	+	+	+	−	+	+
Cataract	−	−	−	NR	−	+	−	+	+
Brachydactyly	+	+	NR	NR	+	+	−	NR	+
Diabetes	+	−	−	−	−	−	−	−	+
Recurrent infections	+	+	+	−	+	+	+	+	+
T cell dysfunction	NR	NR	NR	NR	+	+	+	NR	−
Abnormal Ig levels	+	NR	−	+	+	+	+	+	−
Development of tumor	−	−	−	−	−	Gastric	−	Tongue	Pancreas tongue cerebellum?
Mental retardation	Borderline	Moderate	−	Mild	Severe	Mild	−	Mild	Mild
Psychological findings	NR	NR	NR	NR	NR	Depression	NR	Depression hallucination	Depression hallucination
Sleep disorder	NR	NR	NR	NR	NR	NR	NR	+	+

NR, not recorded; Ig, immunoglobulin.

functional deficits in the dorsomedial region of the thalamus further support the notion that the alterations in the thalamo-limbic system may underlie sleep disturbances with MSS, because the thalamus is a structure involved in the regulation of sleep.

In summary, we suggest that early onset tumors, cognitive deterioration, and severe insomnia accompanied by agrypnia excitata may represent an emerging phenotype of adults with MSS.

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Current Topics

Hiraoka, S. et al.：Nature Med., 13：1363-1367, 2007

糖ヌクレオチド輸送体 SLC35D1 は骨格の形成に必須である

—糖鎖科学，発生生物学と骨・関節疾患研究の新たな接点

池川志郎，古関明彦，古市達哉，平岡秀一

小胞体の糖ヌクレオチド輸送体 SLC35D1 のノックアウトマウスは重度の骨格形成異常を呈した．われわれはマウスとヒトの表現型の解析から，*SLC35D1* が蝸牛様骨盤異形成症（Schneckenbecken dysplasia）の原因遺伝子であることを発見した．SLC35D1 は軟骨のコンドロイチン硫酸鎖の合成に必須で，その機能不全は軟骨マトリックスの形成障害を生じる．

脊椎動物の骨格は，内軟骨骨化（enchondral ossification）とよばれる一連の過程を経て形成される．この過程では，未分化間葉系細胞がまず将来の骨格の形に凝集し軟骨細胞へ分化する．軟骨細胞の急速な増殖や細胞外マトリックス合成に伴って，軟骨組織は成長・拡大していく．分化・増殖した軟骨は肥大化し，石灰化・血管侵入などの過程を経て，次第に骨に置き換わっていく[1]．この過程に異常が起こると，骨系統疾患と総称される骨格の形成異常を特徴とする一群の疾患を引き起こす[1)2)]．現在，400近い骨系統疾患が知られており，このうち約160の疾患で原因遺伝子が見つかっている[3]．また，内軟骨骨化に関係する遺伝子の異常は，変形性関節症，椎間板ヘルニアなど，一般の人々が非常に高い確率で罹患する骨・関節疾患の原因になる[4)~6)]．このため，内軟骨骨化の機構の解明は，生物学だけでなく医学上の大きな課題になっている．

軟骨には，細胞外マトリックスに糖タンパク質の一種であるプロテオグリカンが豊富に存在し，軟骨組織が受ける圧力を吸収するなど重要な役割を担っている．プロテオグリカンは，タンパク質（コアプロテイン）にグルコサミノグリカンという糖鎖が多数結合した構造をもつ分子の総称である．プロテオグリカンを構成する糖鎖は，軟骨細胞の小胞体やゴルジ体などの細胞内小器官の中で，活性化された糖ヌクレオチドをもとに合成される．その際，細胞質でつくられた活性化糖ヌクレオチドを，膜を越えて細胞内小器官内へ運び込む必要がある．その役割を担っているのが，糖ヌクレオチド輸送体で，ヒトでは17個の糖ヌクレオチド輸送体遺伝子が知られている[7]．

SLC35D1（solute carrier 35D1）は，プロテオグリカンの糖鎖の一種であるコンドロイチン硫酸鎖（CS鎖）の合成に用いられる糖ヌクレオチドを輸送することが知られていた[8)9)]．しかし，その生体内での機能は全く不明だった．

Nucleotide-sugar transporter SLC35D1 is critical to chondroitin sulfate synthesis in cartilage and skeletal development

Shiro Ikegawa/Haruhiro Koseki/Tatsuya Furuichi/Shuichi Hiraoka：Laboratory of Bone and Joint Diseases, SNP research Center and Laboratory for Developmental Genetics, Research Center for Allergy and Immunology, RIKEN（理化学研究所・遺伝子多型研究センター・変形性関節症関連遺伝子研究チーム/免疫アレルギー科学総合研究センター・免疫器官形成研究グループ）

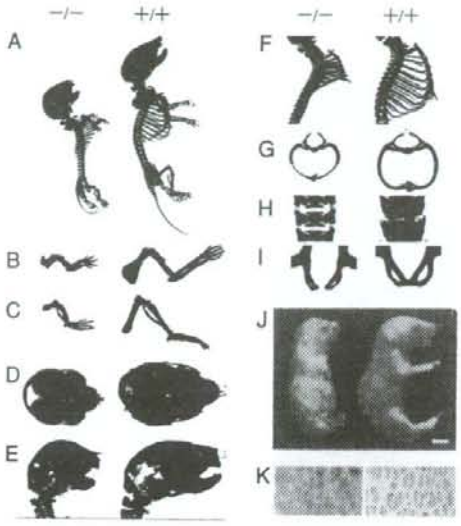

図1 *Slc35d1*遺伝子欠損マウスの表現型
軟骨の形成障害による重度の骨軟骨異形成症．A）骨格全身像．B）上肢．C）下肢．D）頭蓋前後像．E）頭蓋側面像．F）脊椎と肋骨．G）胸郭．H）腰椎正面像．I）骨盤．J）外観．スケールバー＝5mm．K）成長軟骨（増殖層）の病理組織像．＋/＋：野生型，−/−：*Slc35d1*遺伝子欠損マウス

*Slc35d1*遺伝子欠損マウス

われわれは，ES細胞を用いた相同組換え法によって，*Slc35d1*遺伝子を欠損したマウスを作製した[9]．このマウスは顔，四肢，背骨の形成の異常を示し，出生直後に呼吸不全により死亡した（図1）．マウスの軟骨を組織学的に調べたところ，軟骨細胞の形態異常，細胞外マトリックスの著しい減少，およびプロテオグリカン凝集体の減少があった．この結果からSlc35d1が，マウスの骨格形成，軟骨マトリックスの代謝に不可欠な分子であることがわかった．

軟骨プロテオグリカン凝集体の主成分は，アグリカンである．アグリカンのコアタンパク質には100本以上のCS鎖が結合している．酵母細胞を使った試験管内の実験で，SLC35D1はCS鎖の合成に用いられる糖ヌクレオチドを輸送することが知られていた．そこで，*Slc35d1*欠損マウスの軟骨組織中のCS鎖を測定したところ，含量は約1/4に，糖鎖長は半分に低下しており，短いCS鎖が付加された異常なアグリカンが形成されていた．この結果から，SLC35D1によって輸送される糖ヌクレオチドは，軟骨のCS鎖の合成に用いられており，正常な含量と正常な糖鎖長のCS鎖がマウスの骨格形成に必須であることがわかった．

蝸牛様骨盤異形成症

*Slc35d1*欠損マウスの骨格異常，軟骨の病理組織像を詳細に検討したところ，ヒトの致死性の骨系統疾患である蝸牛様骨盤異形成症（Schneckenbecken dysplasia：SBD）の病像と非常によく似ていることがわかった（図2 A）．SBDは，妊娠後期から新生児早期までの周産期に死亡する骨系統疾患で，常染色体劣性の遺伝形式をとる．Schneckenbeckenとはドイツ語で，蝸牛（かたつむり）のような骨盤（Schneck＝蝸牛，Becken＝骨盤）という意味で，骨盤を形成する腸骨という骨が，特徴的な形をしていることに由来する（図2 C）[1]．SBDの*SLC35D1*遺伝子の変異を調べた結果，2例でナンセンス変異を見つけた．1例は血族結婚による創始者変異のホモ接合体，もう1例は複合ヘテロ接合体であった．これらの変異タンパク質は，糖ヌクレオチド輸送機能を完全に欠損していた．すなわち，マウスと同様にヒトでも，SLC35D1の機能の欠損は重度の骨格の形成異常を引き起こすことがわかった（図2 D～F）．

おわりに

周産期に死亡する重症の骨系統疾患の多くでは，原因遺伝子が見つかっていないため，予後の推定，治療方針の決定，再発リスクの評価，遺伝カウンセリングなど，周産期医療，産科・小児科診療のさまざまな局面に大きな問題を与えている．今回の原因遺伝子の発見は，SBDの遺伝子診断，保因者診断を可能とし，この分野の医療を一歩前進させることになる．原因遺伝子の同定により，SBDの病態，発症のメカニズムの解明が進み，将来的には治療につながることが期待できる．また，骨系統疾患では，表現型の類似した疾患では，多くの場合，類似した遺伝子に異常が見つかって

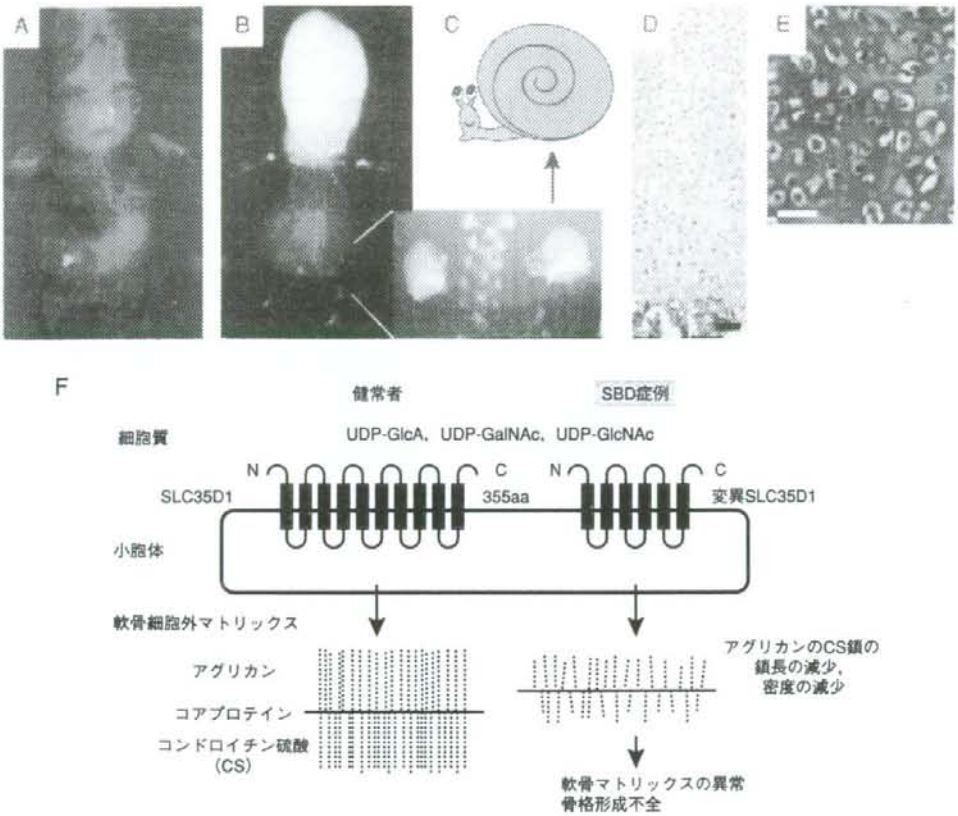

図2　蝸牛様骨盤異形成症（Schneckenbecken dysplasia：SBD）の表現型
A）外見．B）レントゲン像（全身正面像）．C）骨盤正面像．「蝸牛／かたつむり（Schneck）のような骨盤（Becken）」と称されるユニークな形態異常．D）成長軟骨．柱状配列が消失している．スケールバー＝100 μm．E）増殖層の軟骨細胞．細胞は丸く細胞外基質に乏しい．スケールバー＝25 μm．F）SLC35D1の機能とSBD発症のメカニズム．変異SLC35D1はCS鎖の合成のための基質を小胞体内に輸送できないので，軟骨マトリックスの形成が障害される

きた．今後，SBDの類縁疾患，ならびに，SLC35D1以外の糖ヌクレオチド輸送体，またはCS鎖の合成にかかわる遺伝子の変異を調べることによって，新たな骨系統疾患の原因遺伝子の発見が期待できる．

近年，変形性関節症や椎間板ヘルニアなどの骨・関節疾患と，グルコサミンやコンドロイチンなど糖鎖との関係が巷間の注目を浴びているが，科学的に未解明な部分が多い．今回の研究成果は，SLC35D1を中心としたヌクレオチド輸送体やCS鎖合成に携わる分子が，軟骨代謝，骨格形成に密接に関与していることを示している．骨格形成，軟骨代謝における糖鎖の機能への糖鎖生物学的観点からのアプローチは，骨・関節のcommon diseaseの病態の解明に不可欠で，高齢化社会の最大の課題であるこれらの疾患の画期的な治療薬・治療法の開発につながる可能性をもっている．

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● 筆頭著者プロフィール ●

池川志郎：1976年，大阪府立茨木高校，'83年，東京大学医学部医学科卒業．13年間の整形外科医としての勤務の後，癌研究会癌研究所・生化学部（中村祐輔部長）の研究生として，医科学研究の道に入る．2000年より現職（遺伝子多型研究センター・変形性関節症関連遺伝子研究チーム・チームリーダー），ゲノム医科学を武器に，骨・関節疾患の原因遺伝子の発見，分子病態の解明に挑んでいる．目下の主な研究テーマは，椎間板ヘルニア，変形性関節症，骨粗鬆症などの骨・関節の"common disease"の疾患感受性遺伝子の同定と治療法の開発．

骨系統疾患と遺伝子異常

―蝸牛様骨盤異形成症の原因遺伝子 *SLC35D1* の発見―

池川志郎[*1*2]　古市達哉[*1]　西村　玄[*2*3]

要　旨

骨系統疾患とは，骨・軟骨など骨格を形成する組織の成長・発達・分化の障害により骨格異常を来す疾患の総称である．ノックアウトマウスの解析を起点としたヒトとマウスの融合遺伝学的アプローチにより，周産期に死亡する重篤な骨系統疾患である蝸牛様骨盤異形成症（Schneckenbecken dysplasia）の原因遺伝子 *SLC35D1* を発見した．これは，小胞体での糖ヌクレオチドの輸送および軟骨でのコンドロイチン硫酸鎖の合成に関与する，全く新しいタイプの骨系統疾患の原因遺伝子であった．

はじめに
―骨系統疾患とは―

骨系統疾患（skeletal dysplasia）とは，骨・軟骨・腱・靭帯など骨格を形成する組織の成長・発達・分化の障害により，骨格の異常を来す疾患の総称である[1)2)]．2005 年に，全世界の主要な骨系統疾患研究者で構成される International Skeletal Dysplasia Society（ISDS）の Nosology group で制定された最新の分類[3)]では，複数の家系が確認され疾患概念が明確なものだけでも 372 疾患がこのカテゴリーに属している．1家系のみ，もしくは少数の散発例のみの報告など疾患としての概念が定まらないものを含めると，その数はゆうに 1,000 を超す．目下の分類は，代表的疾患を中心としたX線所見の共通性をもとに，骨系統疾患を 37 の「グループ」に分類している（表1）[3)]．

*1 理化学研究所ゲノム医科学研究センター
骨関節疾患研究チーム　*1 同 チームリーダー
*2 骨系統疾患コンソーシウム
*3 東京都立清瀬小児病院 放射線科 部長

キーワード：骨系統疾患，蝸牛様骨盤異形成症，糖ヌクレオチド輸送体，*SLC35D1*，コンドロイチン硫酸

骨系統疾患と遺伝子

骨系統疾患はほとんどが遺伝性の疾患（単一遺伝子病）である．近年の分子遺伝学，DNA 解析技術の急速な進歩を背景に，骨系統疾患の遺伝子解析は目覚ましい進展を遂げた[2)]．これは，近年のゲノム科学の進展によるところが大きい．1992 年の時点では，ムコ多糖症（mucopolysaccharidosis）を除くと同定されていた疾患遺伝子はわずか 9 であったが，目下の分類では 146 の疾患遺伝子が記載され[3)]，しかも毎月のように新しい遺伝子が見つかっている．我々のグループもこれ

表1 骨系統疾患（skeletal dysplasia）の分類

1. FGFR3 group：FGFR3 グループ
2. Type Ⅱ collagen group：Ⅱ型コラーゲングループ
3. Type Ⅺ collagen group：Ⅺ型コラーゲングループ
4. Sulphation disorders group：硫酸化障害グループ
5. Perlecan group：Perlecan グループ
6. Filamin group：Filamin グループ
7. Short-rib dysplasia（with or without polydactyly）group：短肋骨（多指）異形成症グループ
8. Multiple epiphyseal dysplasias and pseudoachondroplasia group：
多発性骨端異形成症および偽性軟骨無形成症グループ
9. Metaphyseal dysplasias：骨幹端異形成症
10. Spondylometaphyseal dysplasias（SMD）：脊椎骨幹端異形成症
11. Spondylo-epi（-meta）physeal dysplasias［SE(M)D］：脊椎・骨端（・骨幹端）異形成症
12. Severe spondylodysplastic dysplasias：重症脊椎異形成症
    - Achondrogenesis type 1A（ACG1A）：軟骨無発生症 1A
    - Spondylometaphyseal dysplasia, Sedaghatian type：脊椎・骨幹端異形成症（Sedaghatian 型）
    - Opsismodysplasia：成熟遅延骨異形成症
    - Fibrochondrogenesis：繊維性軟骨発生症
    - Schneckenbecken dysplasia：蝸牛様骨盤異形成症
13. Moderate spondylodysplastic dysplasias（brachyolmias）：中等症脊椎異形成症（短体幹症）
14. Acromelic dysplasias：遠位肢異形成症
15. Acromesomelic dysplasias：遠位中間肢異形成症
16. Mesomelic and rhizo-mesomelic dysplasias：中間肢・近位肢中間肢異形成症
17. Bent bones dysplasias：弯曲骨異形成症
18. Slender bone dysplasia group：狭細骨異形成症グループ
19. Dysplasias with multiple joint dislocations：多発性脱臼を伴う骨異形成症
20. Chondrodysplasia punctata（CDP）group：点状軟骨異形成症（CDP）グループ
21. Neonatal osteosclerotic dysplasias：新生児骨硬化性異形成症
22. Increased bone density group（without modification of bone shape）：
骨変形を伴わない骨硬化性疾患グループ
23. Increased bone density group with metaphyseal and/or diaphyseal involvement：
骨幹端・骨幹罹患を伴う骨硬化性疾患
24. Decreased bone density group：骨密度低下を示すグループ
25. Defective mineralization group：骨石灰化障害を示すグループ
26. Lysosomal storage diseases with skeletal involvement（Dysostosis multiplex group）：
骨変化（多発性異骨症）を伴うリソソーム蓄積症
27. Osteolysis group：骨溶解症グループ
28. Disorganized development of skeletal components group：骨軟骨組織の過誤腫性骨異形成症グループ
29. Cleidocranial dysplasia group：鎖骨頭蓋異形成症グループ
30. Craniosynostosis syndromes and other cranial ossification disorders：
頭蓋縫合早期癒合症候群と他の頭蓋骨骨化異常症
31. Dysostoses with predominant craniofacial involvement：主に頭蓋顔面を侵す異骨症
32. Dysostoses with predominant vertebral and costal involvement：主に脊椎肋骨を侵す異骨症
33. Patellar dysostoses：膝蓋骨異骨症
34. Brachydactylies（with or without extraskeletal manifestations）：短指趾症
35. Limb hypoplasia - reduction defects group：四肢低形成 - 欠失グループ
36. Polydactyly - Syndactyly - Triphalangism group：多指 - 合指 - 3 分節母指グループ
37. Defects in joint formation and synostoses group：関節形成不全・骨癒合症グループ