Identification of Chromosome 17 Trisomy in a Cynomolgus Monkey (*Macaca fascicularis*) by Multicolor FISH Techniques

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Keywords

Cynomolgus monkey (*Macaca fascicularis*) · Trisomy 17 · Patau syndrome · Multicolor FISH

Abstract

A female cynomolgus monkey (*Macaca fascicularis*) with facial features characteristic of Down syndrome showed abnormal behavior, unwariness toward humans, and poor concentration. The number of metaphase chromosomes in blood lymphocytes was examined and found to be 43, which indicated one extra chromosome to the normal diploid number ($2n = 42$). We then used Q-banding and multicolor FISH techniques to identify the extra chromosome. The results revealed an additional chromosome 17, with no other chromosomal rearrangements, such as translocations. Since no mosaicism or heterozygous variant chromosomes were observed, full trisomy 17 was assessed in this female cynomolgus monkey. Chromosome 17 corresponds to human chromosome 13, and human trisomy 13, known as Patau syndrome, results in severe clinical signs and, often, a short life span; however, this individual has reached an age of 10 years with only mild clinical signs. Although genomic differences exist between human and macaques, this individual's case could help to reveal the pathological and genetic mechanisms of Patau syndrome.

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Introduction

Cynomolgus monkeys (*Macaca fascicularis*) are widely used for safety evaluation in drug development because they are physiologically and anatomically close to humans. Over 25,000 cynomolgus monkeys are bred and reared under the Shin Nippon Biomedical Laboratories, Ltd. Group. The facility in Kagoshima, Japan, has a constant stock of approximately 2,800 cynomolgus monkeys. One individual, a female, showed abnormal behavior, unwariness toward humans, and poor concentration during rearing. It was noted that this individual had quite similar facial features to those associated with Down syndrome, namely, increased distance between the eyes, upward slanting eyes, and flattened nose (Fig. 1). Therefore, we examined metaphase chromosomes in blood lymphocytes and found that the number of chromosomes was not 42 but 43 per cell, suggesting a trisomy. To identify the genetic composition of the extra chromosome, we performed Q-banding analysis and multicolor FISH with human chromosome-specific painting probes. Here, we report the results of our investigation on this rare case.

Materials and Methods

Subject

The female cynomolgus monkey (ID number K141516) was born on June 18, 2010, at the Cambodia Laboratory of Shin Nippon Biomedical Laboratories, Ltd. (SNBL). Its dam and sire were feral animals in origin. After arriving at the facility in Kagoshima at 4 years old, the animal showed abnormal behavior: low wariness toward humans, slow actions, inability to grasp feed between the thumb and forefinger, and poor concentration. Anemia often occurs in this animal due to excessive menstrual bleeding, but this recovers spontaneously. This individual is being reared according to the standard procedures at the Kagoshima site of SNBL, Drug Safety Research Laboratories.

Cytogenetics

Peripheral blood was obtained from the femoral vein using a heparinized tube. Metaphase spreads from blood lymphocytes were prepared according to the standard protocol [Tanabe et al., 1994], with slight modification. In brief, 7.5% total blood was cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum and 2.5% phytohemagglutinin, M form (Gibco 10576015) for 70 h at 37°C. Colcemid solution (KaryoMAX™, Gibco 15210040) was applied at a final concentration of 0.04 µg/mL for the final 2 h of incubation. Harvested cells were suspended in a hypotonic 0.075 M KCl solution with sodium citrate (0.05%; w/v) for 25 min at 37°C and fixed with methanol:acetic acid (3:1) fixative. Metaphase spreads were obtained by using HANABI metaphase spreader (ADSTEC Corporation). The slides were stained with 2.5% Giemsa solution (Merck 109204) or 2% DAPI in 2× SSC, and then photographs were taken for counting the number of metaphase chromosomes. As a control, a normal female cynomolgus monkey of similar weight and age to the abnormal monkey was selected, and the number of chromosomes was determined. To identify the trisomic chromosome, we performed QFQ- or QFH-banding [Caspersson et al., 1970; Yoshida et al., 1975]. We assigned the macaque chromosome numbers according to the nomenclature [Rogers et al., 2006], which was slightly modified from the originally proposed idiogram of banded chromosomes in macaque species [Small et al., 1985; Wienberg et al., 1992].

Fluorescence in situ Hybridization

FISH was conducted as described in Tanabe et al. [1996] and Omori et al. [2017], with some modifications. Human chromosome-specific and chromosome arm-specific painting probes were kindly provided by Prof. Dr. Thomas Cremer (LMU, Munich, Germany). We used 13q, 16, 17, 18, 19, 21q-specific DNA probes, and probe labeling was performed by DOP-PCR [Telenius et al., 1992] in the presence of biotin-16-dUTP (Roche 11093711103), digoxigenin-11-dUTP (Roche 11093088910), or dinitrophenol-11-dUTP (PerkinElmer NEL551). Combinatorially labeled probes were mixed with human Cot-1 DNA, subjected to ethanol precipitation, and resuspended in hybridization solution (50% formamide and 10% dextran sulfate in 2× SSC). After hybridization for approximately 24–36 h, the slides were washed twice in 2× SSC, 3 times for 5 min in 0.1× SSC at 62.5°C, and for 2 min in 4× SSC. The metaphase cells were then blocked in 4× SSC containing 5% BSA and 0.2% Tween-20 for 20 min at 37°C prior to probe detection. Probe detection was performed according to the standard procedure us-

ing avidin-Cy5 (Jackson ImmunoResearch), rabbit anti-DNP antibody (Sigma), and mouse anti-digoxigenin antibody (Sigma) in the first layer, and goat anti-rabbit Alexa 488-conjugated antibody (Molecular Probes) and sheep anti-mouse Cy3-conjugated antibody (Jackson ImmunoResearch) in the second layer. The slides were counterstained with DAPI, and mounted in Vectashield Antifade (Vector). For multiplex FISH analysis [Speicher et al., 1996], 24XCyte human mFISH probes (MetaSystems), chromosome-specific painting probes combinatorially labeled with 5 fluorochromes, were used according to the manufacturer's protocol. FISH images were captured and analyzed using a Leica DM5000B fluorescent microscope equipped with a CCD camera and CW4000 image analysis software (Leica Microsystems).

Results

Counting the number of chromosomes derived from female monkey K141516 showed that the incidence of 43 chromosomes was high, with a frequency of 95.9% (116/121). Among the 121 metaphases observed in total, 116 metaphases had 43 chromosomes, and the number of chromosomes in the remaining 5 metaphases ranged from 37 to 42. One of the causes for these lower numbers was considered to be the loss of chromosomes during preparation of the metaphase spreads. The diploid chromosome number of macaque species has been reported as $2n = 42$ [Fernandez-Donoso et al., 1970]; therefore, the obtained counting results suggested that female monkey K141516 has full trisomy, possessing one extra chromosome without mosaicism. To identify the trisomic chromosome, QFQ- or QFH-banding was performed, and the extra chromosome was found to be a small submetacentric chromosomes. The candidate chromosomes were 16, 17, 18, 19, and 20, which are homologous to human chromosomes 17, 13, 18, 19, and 16, respectively. Therefore, we performed multicolor FISH with human chromosome-specific painting probes to precisely identify the trisomic chromosome. Using combinatorially labeled painting probes specific for human chromosomes 13q, 16, 17, 18, and 19, three signals were only detected with the chromosome 13q probe. No split signals were observed with the painting probes; specifically, no chromosomal rearrangements, such as translocations, were observed. In addition, we used human chromosome 13, 18, and 21 probes in multicolor FISH because these 3 chromosomes are common human trisomic chromosomes and because we had noted facial features similar to those in humans with Down syndrome, trisomy 21 (Fig. 1). The results showed that 2 chromosomes each were painted by the chromosome 18 and 21q probes, while the chromosome 13q probe labeled 3 chromosomes (Fig. 2). In addition, we

Fig. 1. Facial features of cynomolgus monkeys. **a** Normal female monkey at the age of 6 years. **b** Female trisomy monkey K141516 with abnormal appearance at 6 years old.

Fig. 2. Multicolor FISH on a metaphase plate of cynomolgus monkey K141516 using painting probes for human chromosomes 13q, 18, and 21q. **a** Metaphase counterstained with DAPI. **b** Three-color FISH image. Two green signals represent a pair of homologous regions of cynomolgus monkey chromosome 3 corresponding to human chromosome 21. Two light blue signals indicate cynomolgus monkey chromosome pair 18 corresponding to human chromosome 18. Three magenta signals label trisomic cynomolgus monkey chromosomes 17, corresponding to human chromosome 13. Asterisks indicate the trisomic chromosomes.

conducted multiplex FISH using 24XCyte probes and confirmed that only the chromosome 13 probe marked 3 chromosomes (Fig. 3). All other probes painted only 2 chromosomes, indicating disomy. These results suggested that monkey K141516 has full trisomy of chromosome 17, which is homologous to human chromosome 13.

Discussion and Conclusion

Human trisomy 13 is the third most common autosomal numerical abnormality at birth, after trisomy 21 and trisomy 18. Human trisomy 13 is known as Patau syndrome [Patau et al., 1960]. It occurs at an incidence of approximately 1:5,000–7,000 live births in humans [Magenis et al., 1968; Baty et al., 1994]. Common signs of trisomy 13 may include holoprosencephaly (failure of the forebrain to di-

vide properly), midline anomalies, structural eye defects (microphthalmia and retinal dysplasia), heart defects, and severe intellectual disability. Facial anomalies such as cleft lip, cleft palate, and auricular morphological aberration are also common. Approximately 80% of patients have severe congenital cardiovascular anomalies and high juvenile mortality (death by 1 year of age). Monkey K141516 with trisomy 17 in the present study did not show morphological facial or cardiovascular anomalies typical of Patau syndrome in humans, but did show minor structural abnormalities in the eyes. Follow-up research revealed a partially cloudy macula and larger than normal optic disc cupping (coloboma). In functional status, poor use of the thumb, slow actions, and a deficit in learning ability were also observed. Although high juvenile mortality has been reported in humans with Patau syndrome, monkey K141516 has just reached an age of 10 years without severe clinical signs. This

Fig. 3. Multiplex FISH (M-FISH) on a metaphase of cynomolgus monkey K141516. **a** Metaphase counterstained with DAPI. **b** M-FISH image after hybridization with 24XCyte (MetaSystems) painting probes. Three painting signals represent the trisomic chromosomes 17 (asterisks), which were detected by the human chromosome 13 probe.

Table 1. Summary of reported cases with trisomies in primates

Human trisomies	Primate trisomies	Physical and/or behavioral features	References
HSA21	PTR22 PTR22 PPY22	Clinically and behaviorally like Down syndrome Growth delay, hypodontia, strabismus, trabeculectomy Clinically and behaviorally abnormal	McClure et al., 1969 Hirata et al., 2017 Andrle et al., 1979
HSA18	PPA17 PHA18 AAB × ALG18 MNE18 MMU18	Multiple congenital abnormalities Intrauterine growth retardation, congenital joint deformities Interspecific hybrids, no abnormalities Inappropriate social and motor behavior Hydrocephalic affliction, motor deficiencies, delay of social behavior	Lear et al., 2001 Howell et al., 2006 Hirai et al., 2017 Swartz and Sackett, 1994 de Waal et al., 1996
HSA13	PHA17 MFA16 MNE16 MNE16	Polydactyly of the forelimbs, patent foramen ovale, pylectasis Slow action, flatter face, repetitive behavior Mosaic karyotype, 75.7% trisomy, 11.2% disomy, developmental delay, mental retardation, cleft lip, situs inversus of the heart Deficits in learning and cognitive tasks, abnormal social behavior and motor control, congenital defect	Moore et al., 2007 Ward et al., 1994 Vigfusson et al., 1986 Ruppenthal et al., 2004

HSA, *Homo sapiens*; PTR, *Pan troglodytes*; PPA, *Pan paniscus*; PPY, *Pongo pygmaeus*; PHA, *Papio hamadryas*; AAB, *Aotus azarae boliviensis*; ALG, *Aotus lemurinus griseimembra*; MMU, *Macaca mulatta*; MNE, *Macaca nemestrina*; MFA, *Macaca fascicularis*.

long life is probably not only due to an absence of severe abnormalities in morphology and systematic function, but also the differences in genomic content between human chromosome 13 and cynomolgus monkey chromosome 17.

In non-human primates, several cases of autosomal trisomies corresponding to human trisomies 21, 18, and 13 have been reported, such as chimpanzee trisomy 22 [McClure et al., 1969; Hirata et al., 2017], bonobo trisomy 17 [Lear et al., 2001], orangutan trisomy 22 [Andrle et al., 1979], baboon trisomies 17 and 18 [Howell et al., 2006; Moore et al., 2007], and night monkey trisomy 18 [Hirai et al., 2017]. In macaques, 2 cases homologous to human trisomy 18 [Swartz and Sackett, 1994; de Waal et al., 1996] and 3 cases homologous to human trisomy 13 [Vigfusson

et al., 1986; Ward et al., 1994; Ruppenthal et al., 2004] have been reported. Cases of trisomy 17 in *Macaca nemestrina* as well as trisomy 18 in *Macaca mulatta* are likely to result in severe signs and multiple anomalies during development, even in mosaic individuals; however, cases of trisomy 17 in *M. fascicularis* do not show the severe trisomy 13 features seen in humans. This characteristic phenotype can be associated with the genomic structure of cynomolgus monkey chromosome 17, which is unique among macaque species (<https://genome.ucsc.edu/cgi-bin/hgGateway>). These cases are summarized in Table 1. According to extensive comparative BAC-FISH analysis of all chromosomes between macaques and humans, the present centromere on macaque chromosome 17, which corre-

sponds to the human chromosome 13q21.31 region, is derived from the neocentromere that formed during the course of primate evolution [Cardone et al., 2006; Ventura et al., 2007; Stanyon et al., 2008; Sangpakdee et al., 2018]. This suggests that the present human chromosome 13 conserved the syntenic organization of the primate ancestral chromosome and that deletion of rDNA loci, centromere inactivation, and neocentromere formation (centromere repositioning) with the duplication of approximately 427 kb corresponding to human 13q21.31 (61,282,357–61,709,544) surrounding the neocentromere have successively occurred in the Old World monkey branches [Cardone et al., 2006; Sangpakdee et al., 2018]. During these events, loss of chromatin involving the NOR region and/or the appearance of neocentromeres with the additional 427 kb could have affected the total status of gene expression in the macaque chromosome 17. Within the duplicated 427-kb segment there are no coding genes, but several long noncoding RNAs are present. Therefore, a possible implication would be that these ncRNAs contribute to decrease the severe clinical signs, especially in *M. fascicularis*; however, it is still unknown why severe clinical signs are retained in *M. nemestrina* (Table 1). Probably 3D organization of the chromatin with regard to dynamic alteration of topologically associating domains and compartments near the neocentromeric DNA region at 13q21.31 could affect the status of gene expression on the macaque chromosome 17 [Cardone et al., 2006; Lazar et al., 2018]. Thus, detailed analyses of the genetic differences between *M. fascicularis* and *M. nemestrina* would be important for understanding the mechanisms attenuating the clinical signs and the genetic basis of cynomolgus monkey trisomy 17 without Patau syndrome lethality.

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Statement of Ethics

This animal research was conducted according to ethical standards in Shin Nippon Biomedical Laboratories, Ltd., Drug Safety Research Laboratories, which is accredited by AAALAC International.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.K. made contributions to performing cytogenetic and FISH analyses, and drafting the manuscript. S.Y. made contributions to finding the K141516 individual and promoting the conception and design of the study. F.C. participated in drafting the manuscript and revising it critically for important intellectual content. H.T. made contributions to performing cytogenetics and FISH analyses, and drafting the manuscript for the final version, ensuring that the experimental works are appropriately investigated.

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