

1 Title: Chimpanzee Down syndrome: A case study of trisomy 22 in a captive  
2 chimpanzee

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18 Abstract:

19 We report a case of chimpanzee trisomy 22 in a captive-born female. Because  
20 chromosome 22 in great apes is homologous to human chromosome 21, the present case  
21 is analogous to human trisomy 21, also called Down syndrome. The chimpanzee in the  
22 present case experienced retarded growth; infantile cataract and vision problems,  
23 including nystagmus, strabismus, and keratoconus; congenital atrial septal defect; and  
24 hypodontia. All of these symptoms are common in human Down syndrome. This case  
25 was the second reported case of trisomy 22 in the chimpanzee. The chimpanzee in our  
26 case became blind by 7 years old, making social life with other chimpanzees difficult,  
27 but opportunities to interact with other conspecific individuals have been offered  
28 routinely. We believe that providing her with the best care over the course of her life  
29 will be essential.

30

31 Keywords: chimpanzee, trisomy, chromosomal abnormality, Down syndrome, cataract,  
32 atrial septal defect

33

34 Introduction

35 Down syndrome in humans is a chromosome aberration caused by the presence of a  
36 third copy of chromosome 21 (HSA21), or trisomy 21 (Down 1866; Jacobs et al. 1959;  
37 Lejeune et al. 1959). Trisomy 21 is the most common chromosomal abnormality in  
38 humans, occurring in up to 1 in 600 live births, typically associated with retarded  
39 growth, cognitive delay, and physical disabilities (Antonarakis et al. 2004; Hernandez  
40 and Fisher 1996). McClure et al. (1969) reported the first case similar to Down  
41 syndrome in nonhuman animals. They described a female chimpanzee with trisomy 22.  
42 Her growth was retarded, and she had congenital heart disease. Two additional cases of  
43 trisomy 22 were later reported in two species of other great apes: gorilla and orangutan  
44 (Turleau et al. 1972; Andrieu et al. 1979). In contrast to the normal diploid number of 46  
45 in humans, the corresponding number of all of the great apes is 48, and chromosome 22  
46 in great apes is homologous to HSA21 (Dutrillaux 1979; Jauch et al. 1992; Richard and  
47 Dutrillaux 1998; Ried et al. 1993). Features of Down syndrome in humans have been  
48 associated with band q22.3 of chromosome 21, and the hybridization site for this band  
49 was found on the equivalent ape chromosome 22 in chimpanzees, gorillas, and  
50 orangutans (Luke et al. 1995).

51 In this report we describe a case of trisomy 22 in a captive-born female chimpanzee.  
52 The chimpanzee showed retarded growth and had infantile cataract, congenital heart  
53 disease, and hypodontia, features consistent with Down syndrome in humans.

54

55 Methods

56 A female chimpanzee named Kanako (GAIN No. 480, see Great Ape Information  
57 Network (GAIN) website for more information:

58 <https://shigen.nig.ac.jp/gain/ViewIndividualDetail.do?id=400>) was born on June 2,  
59 1992, at a facility in Japan owned at the time by a private company. The facility was  
60 transferred to Kyoto University in 2011 and has been renamed Kumamoto Sanctuary,  
61 Wildlife Research Center, Kyoto University. The history and mission of the  
62 organization have been described in Morimura et al. (2011).

63 Kanako's mother was named Kanae and her father was named Tarou. Both Kanae  
64 and Tarou were wild-captured individuals from Sierra Leone. Kanae's year of birth was  
65 estimated to be 1979. Tarou's year of birth was estimated to be 1977. Kanako, Kanae,  
66 and Tarou all belonged to the western subspecies *Pan troglodytes verus*.

67 Kanako was delivered after an apparently uncomplicated pregnancy. Kanae had given  
68 birth to a male 24 months before Kanako was born. The father had a total of seven  
69 offspring prior to Kanako. Besides Kanako, all of the Kanae's and Tarou's offspring were  
70 healthy and apparently normal except for one of Tarou's offspring who was born  
71 premature and died at 7 days. Kanae was 13 years old and Tarou was 15 years old when  
72 Kanako was born. Thus they were relatively young mother and father when Kanako was  
73 conceived. Based on the last day of maximal swelling of the mother, we estimated the  
74 pregnancy period to be 230 days, which is within the normal range for a chimpanzee  
75 pregnancy. Body weight was measured routinely, and the data were compared with those  
76 obtained from other individuals housed at the same institute (see Hamada et al. 1996 for  
77 details).

78 The heart disease was diagnosed using an echocardiogram (General Electric LOGIQ  
79 iM) in 2014 during a routine physical examination when Kanako was 22 years old.  
80 Under sedation with ketamine hydrochloride (10mg/kg), Kanako was put in a left lateral  
81 recumbant position, and a GE 3S sector transducer (1.5-3.6 MHz) was applied to the

82 thoracic area. Before diagnosis, an electrocardiogram and a physical examination were  
83 conducted under sedation with ketamine hydrochloride (10mg/kg) when she was 0, 1, 2,  
84 4, 6, 7, 8, 13, and 18 years old, and a chest X-ray was taken when she was 2, 13, and 18  
85 years old.

86 The echocardiogram results prompted us to conduct further chromosomal analysis. In  
87 2015, when Kanako was 22 years old, 10 mL of venous blood was collected with a  
88 heparinized syringe. Leucocytes obtained by erythrocytes lysis treatment from 1 ml of  
89 the whole blood were cultured for 70h to prepare metaphase chromosomes. The culture  
90 and chromosome preparations were conducted as previously described (Hirai et al.  
91 1998; 2003). Metaphase spreads prepared on slide glasses were provided for  
92 fluorescence in situ hybridization (FISH) with human paint probe (HSA21, Qbiogene:  
93 Total-chromosome paint 21 probe –Green, France).

94

## 95 Results

96

### 97 Birth and growth

98 At one day of age Kanako weighed 1940 g. The average weight for chimpanzee  
99 neonates is 1800 g (Gavan 1952). Staff noted that she was inactive, her arms and legs  
100 were limp, and she vocalized less frequently than other neonates in the same facility.  
101 When Kanako was 156 days old, her mother, Kanae, was anesthetized for a physical  
102 examination. As the anesthesia was wearing off, Kanae bit her own tongue. Kanako was  
103 then separated from Kanae for 4 days as Kanae recovered. When Kanako and her  
104 mother were reunited, the mother did not take care of Kanako. After that event, Kanako  
105 was hand-raised by human staff. During her first year she suffered from cough, snivel,

106 fever, diarrhea, and swelling around her right eye, but such symptoms are not  
107 uncommon in young chimpanzees. Although systematic investigation of behavioral  
108 development was not conducted, there were no notable abnormalities recorded in the  
109 daily care-taking notes, other than the features described above, until vision problems  
110 appeared at around 1 year of age (see below). Hypotonia was not formally investigated.  
111 Hyperflexibility of the joints was not quantitatively measured, but the flexibility of the  
112 joints appeared to be larger than normal. No problems were noted with her locomotor  
113 movement.

114 After age five, Kanako's growth was delayed compared to other individuals housed  
115 at the same facility (Fig. 1). In addition, she had hypodontia: only one maxillary  
116 premolar was present on each side and she did not have third molars.

117

#### 118 Cataract and vision problem

119 At the age of 305 days, staff noticed leukocoria in Kanako's left eye. At the age of  
120 352 days, leukocoria in her right eye was also noted. At the age of 354 days, staff  
121 observed that she searched for foods with her mouth, indicating clear decreased visual  
122 acuity. A funduscopy and slit-lamp examination confirmed the presence of cataracts.

123 At 2 years old, a cataract surgery was conducted for intraocular lens implantation for  
124 both eyes at the same time. However, Kanako repeatedly rubbed her eyes after the  
125 surgery, leading to postoperative inflammation. This inflammation caused pupillary  
126 block, which led to glaucoma and later glaucosis. Four months later, trabeculectomy  
127 was conducted. However, her glaucoma had advanced. Strabismus and nystagmus were  
128 also noted (Fig. 2). By age seven, her left eye showed corneal opacity and keratoconus.  
129 The eye might have been able to sense strong light because it moved when a light was

130 shined on it. Staff repeatedly observed her fumbling and groping when she moved in a  
131 new environment or when she was searching for an object in front of her. Therefore, she  
132 was declared blind at 7 years of age. Her right eye had progressed to phthisis bulbi.

133

#### 134 Atrial septal defect

135 The echocardiogram with apical four-chamber view from the left thoracic wall  
136 revealed an atrial septal defect and right ventricular hypertrophy. Color Doppler  
137 imaging from the right parasternal area showed a large left-to-right shunt through the  
138 atrial septal defect (Fig. 3). Before detection of atrial septal defect via echocardiogram,  
139 cardiac murmur was not found. An electrocardiogram when Kanako was 18 years old  
140 revealed infrequent premature ventricular contractions. Enlargement of the right cardiac  
141 shadow was seen in a chest X-ray when she was 13 years old, but no clinical symptoms  
142 were detected.

143

#### 144 Chromosome and blood analysis

145 The results of the chromosomal analysis using FISH with HAS21 paint probes  
146 revealed that the metaphase spread of Kanako had diploid chromosome number 49 ( $2n$   
147 = 49) containing an extra chromosome. The extra chromosome was a member of three  
148 substances hybridized to HSA21 probes, being homologous to chromosome 22 of the  
149 chimpanzee. Kanako's karyotype was thus 49, XX, + 22 (Fig. 4). Almost all  
150 hematological and serum chemical values were within normal range and are listed in  
151 Table 1. The values for albumin and chloride were slightly outside the normal range,  
152 possibly because of a difference in measurement system or a measurement error.

153

154 Social interaction

155       Because Kanako is blind, she cannot safely escape aggressive interactions and  
156 therefore cannot stay with other chimpanzees. Nevertheless, chimpanzees are social  
157 creatures, and for Kanako's quality of life our goal was to provide an opportunity for her  
158 to stay together with a conspecific member. Because of her calm temperament, a wild-  
159 born female chimpanzee (named Roman) was selected to be an occasional partner of  
160 Kanako. Roman and Kanako were introduced in October 2010 when Kanako was 18  
161 years old. They were initially in two adjacent rooms separated by bars in the  
162 introductory session. Six months later, after three introductory sessions, they were  
163 allowed to be in the same space (an outdoor enclosure or indoor room, depending on  
164 weather and other conditions). Since then, these encounters have occurred about once  
165 per month (1.2 times per month on average) (Fig. 5). One session of their encounter  
166 lasts 30 to 60 minutes, with a staff member (EN) present to mediate their encounter.  
167 Roman was friendly to Kanako from the beginning of the introduction, and she  
168 occasionally tried to groom Kanako or invited her to play, but their interaction generally  
169 did not last long because Kanako did not move or react, or moved away. On some  
170 occasions Kanako approached Roman and Roman gently touched her, but Kanako  
171 rarely touched Roman. They typically simply sat near each other and spent time quietly.  
172 At the beginning of the encounter session, Kanako almost always emitted a vocalization  
173 specific to her, which was a mixture of chimpanzee play grunt and food grunt,  
174 indicating her positive reaction toward the encounter session.

175

176 Discussion



177 This report describes a second case of chimpanzee trisomy 22 (the first was reported  
178 by McClure et al. in 1969). Another case of a wild chimpanzee with abnormal  
179 behavioral development was reported by Matsumoto et al. (2015). The authors  
180 suspected Down syndrome, but chromosome abnormality was not tested. To the best of  
181 our knowledge, there is no other case where symptoms resembling Down syndrome  
182 have been noted in chimpanzees housed in Japan during the history of captive care. It is  
183 difficult to estimate the probability of a rare event using a small population, but given  
184 that around 500 chimpanzees have been born in captivity in Japan (Watanuki et al.  
185 2014), the probability of this autosomal trisomy in chimpanzees may be comparable to  
186 that of trisomy 21 in humans, which occurs in up to 1 in 600 births (Hernandez and  
187 Fisher 1996). The chimpanzee reported in the present case experienced stunted growth,  
188 infantile cataract, vision problems, congenital heart disease, and hypodontia. All of  
189 these symptoms are common in human Down syndrome (Down 1866; Bull 2011). The  
190 present case, along with the previously reported cases in apes, confirms that trisomy of  
191 great ape chromosome 22 results in a disorder similar to human Down syndrome  
192 (McClure et al. 1969; Turleau et al. 1972; Andrlé et al. 1979).

193 In the first reported case of chimpanzee trisomy 22, researchers evaluated behavioral  
194 development in the affected chimpanzee and showed that development of sitting and  
195 standing postures were delayed (McClure et al. 1969). Conclusions about retardation of  
196 behavioral development cannot be made in Kanako's case because systematic  
197 investigation in this regard was not conducted. Furthermore, data for retrospective  
198 assessment, such as video recordings, are not available. However, the lack of  
199 abnormalities noted in daily caretaking before the age of one, except for neonatal  
200 inactivity and limp limbs, suggests that there was no severe retardation in behavioral

201 development. Kanako's infantile cataract that began to emerge at around 1 year of age  
202 and her eventual blindness prevented us from evaluating her behavioral development  
203 afterwards, because behavioral abnormalities are difficult to distinguish from visual  
204 problems. The trisomic chimpanzee reported by McClure et al. (1969) died before  
205 reaching 2 years of age. Kanako has survived until adulthood and is alive at the time of  
206 writing the present report. Our goal has been to provide Kanako with the best care and  
207 quality of life. One critical component of this effort is giving her an opportunity to  
208 interact with another chimpanzee (see Miyabe-Nishiwaki 2010, Hayashi et al. 2013, and  
209 Sakuraba et al. 2016 for another case of care of a disabled chimpanzee; see also  
210 Matsuzawa, 2016). A detailed and thorough pathological examination of Kanako,  
211 including autopsy imaging, will be conducted after her natural term.

212

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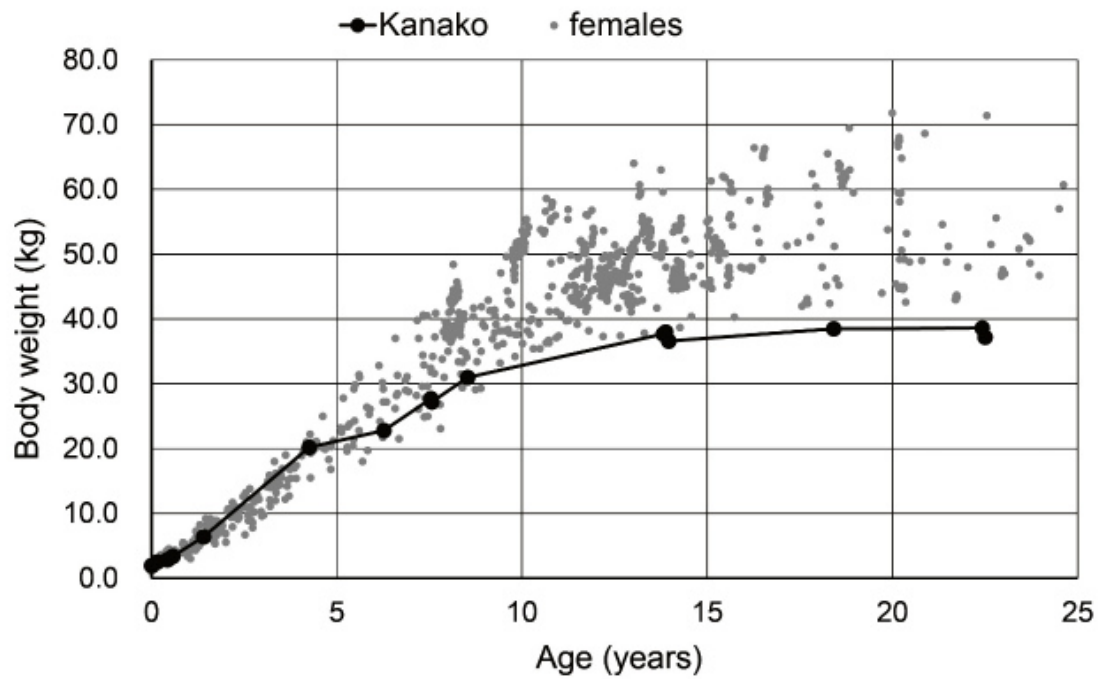
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290 Figures:

291

292 Fig. 1. Weight gain of Kanako (black line) and other females housed at the same facility

293 (grey dots)



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297 Fig. 2. Strabismus was noted after trabeculectomy at 3 years of age.



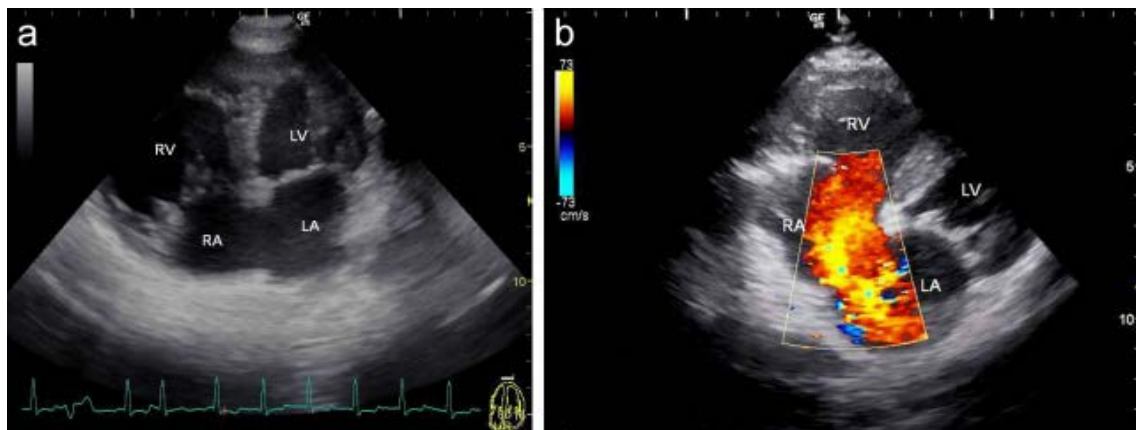
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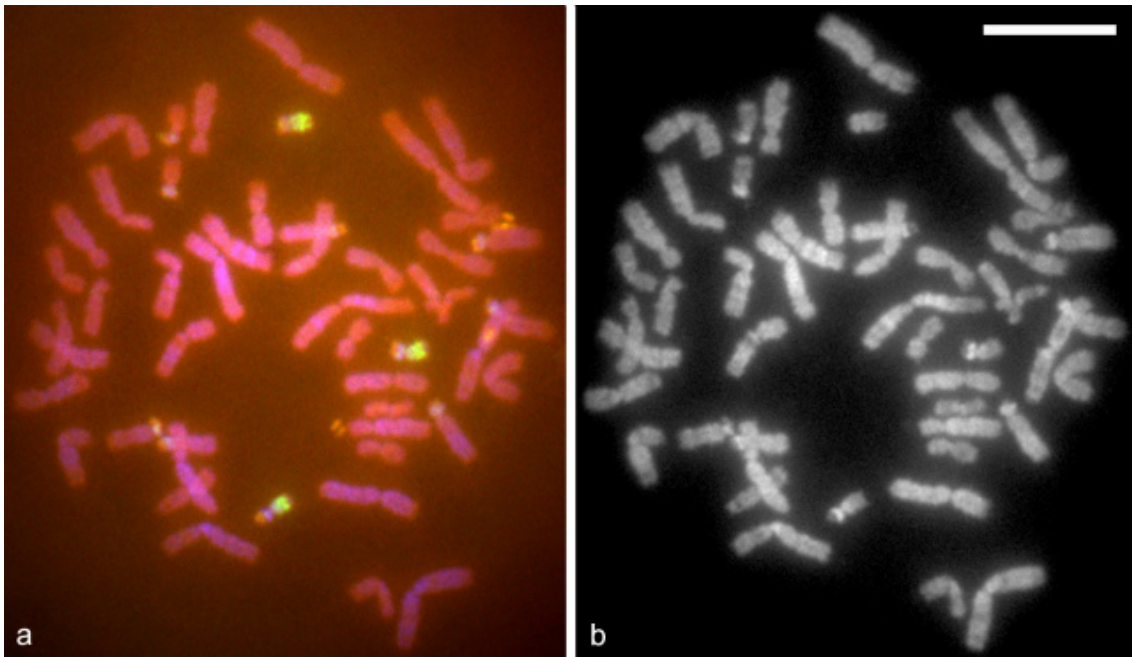
302 Fig. 3 Heart defect analysis: (a) apical four chamber view showing the atrial septal  
303 defect; (b) Doppler image from the right parasternal area showing a large left-to-right  
304 interatrial shunt. RV=right ventricle, LV=left ventricle, RA=right atrium, LA=left  
305 atrium



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310 Fig. 4. Chromosome paint analysis with HSA21 probes: (a) chromosomes stained by  
311 DAPI (4',6-diamidino-2-phenylindole) (b) metaphase spread of the chimpanzee Kanako.  
312 The probe highlighted three substances of chromosome 22 (green) on chromosomes  
313 stained by rhodamine (red), showing trisomy 22. Scale bar = 10  $\mu$ m.



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318 Fig. 5. Kanako (right) and Roman (left) staying in the same space



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322 Table 1. Results of hematological and serum chemical examination

Item		Kanako	Average and range of normal chimpanzees <sup>1)</sup>
Erythrocytes	(10 <sup>4</sup> /μL)	549	510 (420 - 600)
Hemoglobin	(g/dL)	14	13.6 (11.5 - 15.7)
Hematocrit	(%)	46.4	42.0 (35.4 - 48.6)
Thrombocytes	(10 <sup>4</sup> /μL)	11.4	23.0 (9.7 - 36.3)
Leucocytes	(/μL)	13600	9100 (2,900 - 15,400)
C-reactive protein	(mg/dL)	0.33	N/A
Total protein	(g/dL)	7.6	7.5 (6.5-8.5)
Albumin	(g/dL)	2.8	3.7 (3.0-4.5)
A/G (albumin/globulin ratio)		0.6	1.0 (0.6-1.4)
total Bilirubin	(mg/dL)	0.2	N/A
ALP (alkaline phosphatase)	(U/L)	152	114.3 (33.0-269.8)
AST (aspartate transaminase)	(U/L)	20	18.1 (5.1-31.2)
ALT (alanine transaminase)	(U/L)	41	30.8 (10.5-51.1)
LDH (lactate dehydrogenase)	(U/L)	269	320.8 (175.0-768.4)
GGT (γ-glutamyltransferase)	(U/L)	30	28.5 (6.0-72.4)
CK (creatinine kinase)	(U/L)	121	229.0 (19.0-660.3)
Cholinesterase	(U/L)	317	N/A
total Cholesterol	(mg/dL)	214	212.2 (129.1-295.4)
Triglycerides	(mg/dL)	65	109.2 (4.6-213.7)
BUN (blood urea nitrogen)	(mg/dL)	11.3	11.5 (3.7-19.3)
Creatinine	(mg/dL)	0.81	1.0 (0.4-2.2)
Amylase	(IU/L)	93	N/A
Glucose	(mg/dL)	69	83.6 (52.8-114.5)
Sodium	(mEq/L)	136	138.4 (133.0-143.7)
Potassium	(mEq/L)	3.8	3.8 (3.0-4.6)
Chloride	(mEq/L)	90	101.1 (90.8-111.3)
Calcium	(mEq/L)	9.1	9.1 (8.3-10.0)

323 1) Howell et al. (2003)

