Clinical, molecular and histopathological features of short stature syndrome with novel *CUL7* mutation in Yakuts: new population isolate in Asia

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Background: In total, 43 patients having short stature syndrome in 37 Yakut families with autosomal recessive prenatal and postnatal nonprogressive growth failure and facial dysmorphism but with normal intelligence have been identified.

Methods: Because Yakuts are considered as a population isolate and the disease is rare in other populations, genomewide homozygosity mapping was performed using 763 microsatellite markers and candidate gene approach in the critical region to identify the causative gene for the short stature syndrome in Yakut.

Results: All families shared an identical haplotype in the same region as the identical loci responsible for 3-M and gloomy face syndromes and a novel homozygous 4582insT mutation in Cullin 7 (*CUL7*) was found, which resulted in a frameshift mutation and the formation of a subsequent premature stop codon at 1553 (Q1553X). Yakut patients with short stature syndrome have unique features such as a high frequency of neonatal respiratory distress and few bone abnormalities, whereas the clinical features of the other Yakut patients were similar to those of 3-M syndrome. Furthermore, abnormal vascularisation was present in the fetal placenta and an abnormal development of cartilage tissue in the bronchus of a fetus with *CUL7* mutation.

Conclusion: These findings may provide a new understanding of the clinical diversity and pathogenesis of short stature syndrome with *CUL7* mutation.

Population isolates that have experienced marked decline in population size and have strikingly different demographic histories, have been used with great success in mapping and cloning mendelian disease genes.¹ Yakuts are an East Asian population, and live in the northeastern part of Siberia in the Republic of Sakha of the Russian Federation. Yakuts emigrated from southern to northern Siberia in the 13th or 14th century AD and their population has expanded rapidly to >440 000 people in a particular restricted area.² Y-chromosome haplotype analysis of Yakuts has found that at some point they experienced a serious decline in their population size, which is known as the bottleneck effect.^{2–3} In addition, Yakuts exhibit high frequencies of some mendelian disorders.³ Taken together, these findings suggest that Yakuts are a population isolate that can provide a unique model for genetic studies.¹

Yakuts have several hereditary diseases, with short stature syndrome being one of the main conditions.⁴ We have identified short stature syndrome in Yakut families with facial dysmorphism and with a relatively large head but with normal intelligence. These patients do not show apparent abnormalities in bone structure and hormone production, but some of them experienced respiratory distress at birth. The clinical spectrum of short stature syndrome in Yakuts resembles that of 3-M syndrome (OMIM 273750), which is a rare autosomal recessive disorder characterised by severe prenatal and postnatal growth retardation and facial dysmorphism but with normal intelligence.⁵ Although the characteristic radiological findings of slender long bones and tall vertebral bodies have often been reported in patients with 3-M syndrome, these radiological findings have not been commonly observed in short stature syndrome in Yakuts.⁵ Gloomy face syndrome is another similar type of short stature disorder; it has been reported that patients with gloomy face syndrome do not show any radiological abnormalities.⁶ Recently, it has been shown that both 3-M and

gloomy face syndromes are both caused by a mutation of Cullin 7 (*CUL7*) and should be grouped together.⁷ However, <50 patients with 3-M syndrome and <10 patients with gloomy face syndrome have been reported to date, therefore the clinical diversity of these disorders has not yet been fully documented.^{5–15} We describe the clinical and histopathological features of short stature syndrome in Yakuts with a new nonsense mutation in *CUL7*.

METHODS

Subjects

In the regional Department of Medical Genetical Consultation, Republican Hospital No 1, National Medical Centre, Yakutsk, Russia, we recruited 43 patients with hereditary short stature syndrome and their 39 unaffected parents and relatives from 37 Yakut families. Affected siblings were recruited from six families. There were 25 (58%) female and 18 (42%) male patients. We retrospectively reviewed the medical records of these patients. Birth size and subsequent lengths/heights were expressed as standard deviation scores (SDS) according to the Yakut standards. In addition, we used 104 DNA samples from healthy Yakut people as normal controls. Blood samples were obtained from all the patients, their parents, and controls after obtaining informed consent. Genomic DNA was extracted from peripheral blood leukocytes using a standard protocol. This project was approved by the ethics committee of Niigata University.

Genomewide homozygosity mapping

We performed a genomewide homozygosity mapping of the 37 families having short stature syndrome using 763 microsatellite markers (ABI Prism Linkage Mapping Set HD-5; Applied

Abbreviations: OMIM, Online Mendelian Inheritance in Man; RFLP, restriction fragment length polymorphism; SDS, standard deviation scores

Table 1 Haplotype analysis of patients with 3-M syndrome in 37 Yakut families

Pedigree	Patient	D6S1552	M4265	M4298	CUL7	D6S1582	D6S282	D6S271	D6S1604	D6S451
1	654	7	3	2	4582insT	4	5	5	2	4
2	3015	7	3	2	4582insT	4	5	5	2	4
3	3352	7	3	2	4582insT	4	5	5	2	4
4	AA763	7	3	2	4582insT	4	5	5	2	4
5	4087	7	3	2	4582insT	4	5	5	2	4
6	AA398	7	3	2	4582insT	4	5	5	2	4
7	434	7/10	3	2	4582insT	4	5	5	2	4
	AA1471	4/7	3	2	4582insT	4	5	5	3	4
8	634	7	3	2	4582insT	4	5	5	2	1/4
9	402	7	3	2	4582insT	4	5	5	2	4
10	AA2366	7	3	2	4582insT	4	5	5	2	4
11	557	5/7	3	2	4582insT	4	5	5	2	3/4
	558	5/7	3	2	4582insT	4	5	5	2	3/4
12	AA3982	7	3	2	4582insT	4	5	5	2	4
13	AA566	7	3	2	4582insT	4	5	5	2	4
14	386	7	3	2	4582insT	4	5	5	2	4
15	AA205	7	3	2	4582insT	4	5	4/5	2/3	3/4
16	595	5/7	2/3	2/4	4582insT	4	5	5	2	4
17	AA609	6/10	3	2	4582insT	4	5	5	2	4
	AA610	6/10	3	2	4582insT	4	5	5	2	4
18	AA101	7	3	2	4582insT	4	5	5	2	3/4
19	1282	7	3	2	4582insT	4	5	5	2	4
20	AA810	7/8	3	2	4582insT	4	5	5	2	4
21	513	7	3	2	4582insT	4	5	5	2	3/4
22	1807	7	3	2	4582insT	4	5	5	2	4
23	4113	6/7	3	2	4582insT	4	5	5/6	2/8	3/4
	AA91	6/7	3	2	4582insT	4	5	5/6	2/8	3/4
24	AA134	7	3	2	4582insT	4	5	5	2	4
25	130	7	3	2	4582insT	4	5	5	2	4
	132	7	3	2	4582insT	4	5	5	2	4
26	870	6/7	3	2	4582insT	4	5	5	2	4
27	AA820	6/7	3	2	4582insT	4	5	5	2	4
28	AA562	7	3	2	4582insT	4	5	5	2	4
29	694	7	3	2	4582insT	4	5	5	2	4
30	1119	7	3	2	4582insT	4	5	5	2	4
31	AA3405	7	3	2	4582insT	4	5	5	2	4
32	AA545	7	3	2	4582insT	4	5	5	2	4
33	AA806	2/7	1/3	2	4582insT	4	5	5	2	4
34	AA939	7	3	2	4582insT	4	5	5	2	4
35	AA2436	6/7	3	2	4582insT	4/5	5	5	2	4
	AA2447	6/7	3	2	4582insT	4/5	5	5	2	4
36	AA3367	6/7	3	2	4582insT	4	5	5	2	4
37	AA456	6/7	3	2	4582insT	4	5	5	2	4
Patient's major alleles		7	3	2	4582insT	4	5	5	2	4
Allele frequency in general Yakut population (%)		15	19	0		28	22	7	20	19

Two alleles, separated by slash (/) are shown for cases in which phase could not be determined.

Biosystems, Foster City, California, USA) covering the entire autosome with an average interval of 4.6 cM. We carried out PCR using various MapPair microsatellite markers and analysed PCR products using an ABI Prism 3100 genetic analyser (Applied Biosystems). We determined all allele sizes using GeneScan V.3.1.2 and GENEMAPPER programs (both Applied Biosystems). The genetic distance between adjacent markers was determined using the Marshfield sex-averaged linkage map. In addition, we developed two dinucleotide polymorphic markers between D6S1552 and D6S1582, based on simple repeat information from the UCSC Human Genome Browser (May 2004; <http://genome.ucsc.edu/index.html>). The primer sequences for each new marker were: 5'-AAAAATTAGCTGGG CATGGTG-3' and GCAAGAGATCAAGACTTCAAG-3' (M4265 forward and reverse primers, respectively), and 5'-ACCCACTC TACAAGAAAGAG-3'; reverse primer, ATGCTCTCAGCAGTCAA GAG-3' (M4298 forward and reverse primers, respectively).

The allele frequencies of the markers were determined by analysing 50 Yakut control subjects.

Mutational analysis of *CUL7*

A series of 25 intronic primers for amplifying the 25 coding exons of *CUL7* was designed. Each exon was amplified by PCR

and the amplified products were purified with ExoSAP-IT (Amersham Biosciences, Amersham, Buckinghamshire, UK) in a cycle sequencing reaction using BigDye Terminator V.3.0 (Applied Biosystems). We purified the reaction products using a NucleoSEQ kit (Maclerey-Nagel, Duren, Germany) and analysed the products using an ABI 3100 DNA sequencer (Applied Biosystems).

PCR-restriction fragment length polymorphism analysis

The exon 25 of *CUL7* was amplified using the primers 5'-AGCAAAAGGATATACCAGGAG-3' (forward) and 5'-TCCGTCT CTTCTCCAAGTTC-3' (reverse). The 240 bp fragment was digested with *HinfI* endonuclease, resulting in 115 and 125 bp fragments in the control individuals, whereas an undigested 240 bp fragment was obtained in the patients.

Histopathological analysis

We analysed the lungs and placentas from fetuses of 26 weeks' gestation with and without 3-M syndrome. For molecular genetics, PCR-restriction fragment length polymorphism analysis was performed to identify the homozygous 4582insT mutation. The tissues were fixed in 10% formaldehyde solution, embedded in paraffin wax, and sections stained with H&E.

Figure 1 Mutational analysis of *CUL7*. (A) Direct sequencing (see Methods) revealed a homozygous T insertion at position 4582 of *CUL7*. The patient (P) shows an undigested 240 bp fragment, whereas one of the patient's siblings (S1) and three controls (N1, N2, and N3) show fragments of 115 and 125 bp. The patient's heterozygous father (F), heterozygous mother (M) and another sibling (S2) all show both fragment types. Ma, standard DNA size marker.

RESULTS

Identification of novel *CUL7* mutation in short stature syndrome in Yakuts

Because Yakuts are considered as a population isolate and short stature syndrome is a rare recessive inherited disease commonly found in Yakutia, we applied the homozygosity mapping approach.¹⁻¹⁶ A genome-wide homozygosity mapping revealed that 23 of the 37 families shared a 7-5-5 haplotype at *D6S1552*, *D6S282* and *D6S271* in the homozygous state (table 1). Therefore, we carried out fine genotyping using additional polymorphic markers in this region (ie, *M4265*, *M4298*, *D6S1582*, *D6S1604* and *D6S451*) (table 1). Loss of homozygosity at the telomere site was found at *M4298* in patient 595 (pedigree 16), and loss in the centromere site at *D6S271* in patients AA205 (pedigree 15), 4113 (pedigree 23) and AA91 (pedigree 23) (table 1). Although loss of homozygosity was observed at *D6S1582* in patients AA2436 and AA2447 (pedigree 35), the patients shared the 5-5-2-4 haplotype, which was identical to those of the other patients at *D6S282*, *D6S271*, *D6S1604* and *D6S451* in the homozygous state. Therefore, this could be accounted for by replication slippage with the marker *D6S1582*. Notably, at *M4298*, all the patients except patient 595 were homozygous for allele 2, whereas none of the Yakut controls had allele 2 (table 1). Taken together, the results indicated that the causative gene of short stature syndrome in Yakuts is located in a 623 kb segment between *M4298* and *D6S271* (table 1).

During this work, Huber *et al* reported identification of *CUL7* as the causative gene of 3-M and gloomy face syndromes. *CUL7* is present in this critical region (table 1). Because the clinical presentations of short stature syndrome in Yakuts were similar to those of 3-M syndrome except for neonatal respiratory distress and low frequencies of slender tubular bones and tall vertebral bodies, we considered *CUL7* a good candidate gene for short stature syndrome in Yakuts. Consequently, we performed sequence analysis of *CUL7* in the patients using a series of 25 intronic primers to amplify the 25 coding exons of *CUL7* and found a novel homozygous insertion T at position 4582 in exon 25 (fig 1A). This type of mutation is a frameshift mutation with the development of a subsequent premature stop codon at position 1553 (Q1553X). All Yakut patients were homozygous for this frameshift mutation. To confirm the frequency of this substitution mutation in a normal population, we performed PCR-RFLP analysis. The 4582insT mutation changes the recognition site of the *HinfI* endonuclease, causing the production of an undigested fragment (fig 1B). This insertion was observed, as a heterozygous mutation, in one of the 200 control chromosomes studied.

Demographics and clinical features of 43 patients with Q1553X *CUL7* mutation

The length of gestation varied from 35 to 42 (median 37) weeks, and 5 of 35 (14%) babies were delivered by caesarean section. The mean birth length was 42.0 (−6.2 SDS; range −12.2 to −1.8) cm, and mean birth weight was 2330 (−3.04 SDS; range −1.06 to −5.06) kg. We evaluated the SDSs of height by age from birth to 45 years (fig 2). The mean (SD) SDS was −5.6 (1.8) in females, −5.3 (1.5) in males and −5.5 (1.7 in total). There was no significant difference between the sexes ($p = 0.18$). The mean SDS of height for each age was from −4.23 to −6.57 and there was no significant correlation between age and SDS ($r = 0.13$, $p = 0.07$). The mean occipito-frontal head circumference was 36.3 cm (range 32 to 37 cm), which was comparable with the gestational age.

In total, 18 (41.9%) infants had severe asphyxia and respiratory distress at birth and 11 (25.6%) infants required mechanical ventilation. In five (11.6%) families, newborns who were diagnosed clinically as having the same disease died immediately after birth of unknown causes. The respiratory failure observed was not due to respiratory distress syndrome caused by the lack of a lung surfactant or a lung infection.

Figure 2 Body length SDS by age in 43 Yakut patients with 3-M syndrome. We evaluated body length SDS in 25 female (open circles) and 18 male (closed squares) patients of different ages. In all, 23 female and 16 male patients of different ages were evaluated >2 times. Red bars represent mean body length SDS.

Figure 3 Clinical features of Yakut patients of different ages with 3-M syndrome from different families. (A) Newborn patient with 3-M syndrome; note the large hydrocephaloid skull, relatively short stature, short neck, short and wide thorax, enlarged abdomen (arrowheads), and prominent heels (arrow). (B) A 5-year-old girl with 3-M syndrome; note the short stature, short neck, enlarged abdomen (arrowhead), hyperlordosis (left arrow) and prominent heels (arrow). The lines behind the patient denote body length (cm). (C) A 39-year-old man with 3-M syndrome; note the short stature, short neck, short and wide thorax, micromelia, and deformed sternum (arrows). The lines behind the patient denote body length (cm). (D) Siblings with 3-M syndrome: 9-year-old girl (left) and 8-year-old boy (right). Note short stature, hydrocephaloid skull, short neck, deformed sternum, and micromelia. (E) Siblings with 3-M syndrome: 1-year-old boy (left) and 4-year-old girl (right). Note short stature, short neck, deformed sternum, micromelia and enlarged abdomen (arrow). (F) Radiograph of the thoracolumbar spine of a 17-year-old patient with 3-M syndrome, showing hypoplasia of the 12th ribs. (G) Radiograph of the arms of a 13-year-old boy with 3-M syndrome shows flared metaphyses (arrow) and slender bones. Parental/guardian informed consent was obtained for publication of this figure.

The clinical features of the patients are summarised in table 2. In all, 95% of the patients had a characteristic triangular face with a hypoplastic midface; a broad, frontal-bossing, low nasal bridge; a depressed nasal root; a long philtrum; prominent mouth and prominent, full lips; and a pointed chin (fig 3A–E). A short neck, short and wide thorax, brachydactyly, micromelia of the hands and feet and prominent heels were observed in >95% of the patients in all generations, whereas a hydrocephaloid skull, sternum deformity, accentuated lumbar lordosis, muscle hypotonia and a large abdomen became less obvious in adult patients. All the patients showed normal intelligence. Hypoplasia of the 12th rib (4 cases), polydactyly (3), and syndactyly (1) were also observed in some patients (fig 3F). Male hypogonadism was not observed. Hypospadias was observed in one case. The sizes of the uterus and ovary of the female patients were normal, except in two cases. Two men and one woman with the syndrome have children of normal height. Notably, the characteristic radiological findings for 3-M syndrome (slender tubular bones or tall vertebral bodies) were observed in few patients (fig 3G). Bone aging of the patients' hands was delayed in the first decade, but normalised after 16 years of age. The patients did not have a predisposition to malignancy. Three patients were > 40 years old with proportionally short stature <130–140 cm. The facial dysmorphism is not prominent in these three patients.

Histopathology of lung and placenta of patients with *CUL7* mutation

The histopathological analysis in mice lacking *p185*, the mouse homologue of *CUL7*, shows a defect in the differentiation of trophoblasts, including an abnormal vascular structure in their placenta and the insufficient inflation of alveolar spaces in the

lungs.¹⁷ To determine whether these abnormalities are present in humans, we histopathologically examined the lungs and placenta from fetuses of 26 weeks' gestation with and without a homozygous 4582insT mutation in *CUL7*. In the lungs of the fetus with *CUL7* mutation, the amount of cartilaginous lamina of the medium and large bronchi was small and the cartilaginous lamina insufficiently developed (fig 4A), whereas there were no obvious abnormalities in the alveolar tissues, including vascularisation and cellular infiltration of these tissues (fig 4B). In the placenta of the fetus with *CUL7* mutation, a marked increase in the number of chorionic villi with numerous syncytial knots and intervillous lacuna constriction was observed (fig 4C), whereas the number of trophoblast giant cells was normal (fig 4D).

DISCUSSION

In this study, using the homozygosity mapping approach, we have mapped the candidate region for short stature syndrome in Yakuts to the identical locus as that for 3-M syndrome, and found a new frameshift mutation in *CUL7*. In addition, we report the detailed clinical features of 43 patients with an identical mutation, 4582insT, and show the abnormal development of the cartilaginous lamina of the bronchus and abnormal vascularisation of the placenta of a fetus with *CUL7* mutation.

The prevalence rate of short stature syndrome with *CUL7* mutation in Yakut is ~0.01% (at least 43 patients among 440 000 people); the carrier frequency of the 4582insT mutation of *CUL7* is approximately 2% in the Yakut population. The results indicate that Yakuts have a founder chromosome responsible for the *CUL7* mutation. The result also indicates that Yakuts are a population isolate. Genetic analysis in population isolates makes it easier to find causative genes for

Table 2 Clinical features at the first time of diagnosis in 43 Yakut patients with 3-M syndrome

Features	Frequency: patients who had each clinical feature, n (%)				
	Age at first examination (years)				
	0–5	6–10	11–15	≥16	Total
Number of patients	25	8	5	5	43
Head and face					
Hydrocephaloid skull	25 (100)	7 (88)	3 (60)	0	35 (81)
Characteristic face*	24 (96)	7 (88)	5 (100)	5 (100)	41 (95)
Hypertelorism	9 (36)	4 (50)	1 (20)	5 (100)	19 (44)
Epicanthus	14 (56)	1 (12)	2 (40)	0	17 (40)
Thick and wide eyebrows	18 (72)	4 (50)	4 (80)	2 (40)	28 (65)
Dysplasia of ears	11 (40)	4 (50)	5 (100)	0	20 (47)
Body					
Short proportional stature	25 (100)	8 (100)	5 (100)	5 (100)	43 (100)
Short neck	23 (92)	8 (100)	5 (100)	5 (100)	41 (95)
Short and wide thorax	25 (100)	8 (100)	5 (100)	5 (100)	43 (100)
Deformed sternum	24 (96)	3 (38)	3 (60)	0	30 (70)
Accentuated lumbar lordosis	25 (100)	7 (88)	5 (100)	0	37 (86)
Muscular hypotonia	25 (100)	7 (88)	5 (100)	0	37 (86)
Brachydactyly	25 (100)	8 (100)	5 (100)	5 (100)	100 (43)
Micromelia of hands and feet	25 (100)	8 (100)	5 (100)	5 (100)	100 (43)
Simian crease	14 (56)	6 (75)	2 (40)	0	22 (51)
Clinodactyly of the fifth fingers	3 (12)	0	1 (20)	0	4 (9)
Large abdomen	25 (100)	0	0	0	25 (58)
Prominent heels	25 (100)	8 (100)	5 (100)	5 (100)	43 (100)
Birth heart defects	4 (16)	0	0	0	4 (9)
Hepatomegaly	5 (20)	0	0	0	5 (12)
Platypodia	9 (36)	3 (38)	1 (20)	0	13 (30)
Radiological findings					
Scoliosis	1 (4)	3 (38)	3 (60)	0	7 (16)
Slender tubular bones	0	0	1 (20)	0	1 (2)
Flared metaphyses	2 (8)	1 (12)	3 (60)	5 (100)	11 (26)
Tall vertebral bodies†	2 (8)	0	2 (40)	0	4 (9)
Spina bifida occulta	2 (8)	1 (12)	3 (60)	3 (60)	9 (21)
Abnormal bone age‡	25 (100)	7 (88)	2 (40)	2 (40)	39 (91)

*Triangular face, hypoplastic midface, broad, frontal bossing, low nasal bridge, depressed nasal root, long philtrum, full lips, prominent mouth and lips, pointed chin.
†High vertebral bodies with reduced anterior–posterior and transverse diameter in lumbar region.
‡Bone age of the hands did not correspondence with chronological age.

mendelian inherited diseases and susceptibility genes for complex diseases.^{1–18} Although many population isolates have been reported among Caucasians, Yakuts are considered the first population isolate in Asia, thus, they have an important

role in the identification of susceptibility genes for complex diseases, particularly in people with an Asian background.¹⁸

3-M and gloomy face syndromes are both characterised by growth retardation and facial dysmorphism. Although they can

Figure 4 Histopathological analysis of lungs and placenta of a normal fetus and a fetus with 3-M syndrome at 26 weeks of gestation, stained with H&E. (A) The lung of the fetus with *CUL7* mutation has a few small islands of cartilaginous tissue (arrows) in medium and large bronchi; (B) the lung of the normal fetus shows well-developed cartilaginous tissues (arrows). (C) The placenta of the fetus with *CUL7* mutation has dissociative growth of the chorionic villi with numerous syncytial knots (dashed arrows) and intervillous lacuna constriction (arrows); (D) placenta of fetus without mutation is normal. Scale bar, 100 μm.

be clinically distinguished by bone abnormalities, slender tubular bones and tall vertebral bodies, in childhood, Huber *et al* proposed that both conditions should be grouped together. Huber *et al* found similar bone abnormalities in an elderly patient with gloomy face syndrome and one with 3-M syndrome, and recently identified *CUL7* as the causative gene of both syndromes.⁷ Although Yakut patients with short stature syndrome have similar cardinal clinical features to those of patients with 3-M syndrome (namely, severe growth retardation and characteristic face), they rarely show bone abnormalities even in the elderly.^{5 8 9 14 15} These findings suggest that these bone abnormalities are not crucial clinical features of short stature syndrome with *CUL7* mutation.

Notably, 41.9% of the Yakut patients with 3-M syndrome had asphyxia and respiratory distress at birth and 25.6% of them required mechanical ventilation. Although respiratory distress at birth has not been investigated in 3-M or gloomy face syndromes, mice lacking *CUL7* die immediately after birth of respiratory distress.¹⁷ Histopathological examination of the lungs of fetal and neonatal mice lacking *CUL7* revealed that their lungs failed to inflate and showed markedly reduced alveolar space. In contrast, histopathological examination of the lungs of a Yakut fetus with a *CUL7* fetus showed development of small cartilaginous tissues in medium and large bronchi; however, we did not detect any marked abnormality in the alveolar space of the lungs. *CUL7* may affect the development of cartilaginous tissues in the lungs and may also cause respiratory distress at birth in humans. To clarify how the mutation of *CUL7* impairs its function and whether this mutation is related to the development of respiratory distress, further investigation is required. The clinical features of short stature syndrome also resemble those of Mulibrey nanism (OMIM 253250) and Silver–Russell syndrome (OMIM 180860).^{19 20} However, the average height SDS of Yakuts with short stature syndrome is shorter than those of these two syndromes.^{19 20} In addition, short stature syndrome does not progressive during the lifespan of <40 years and does not induce any other physical, vascular and neurological problems. Therefore, we speculate that *CUL7* may only affect prenatal and postnatal body size and not head size.

The precise mechanism of prenatal and postnatal growth retardation in 3-M syndrome remains unclear. From the analysis of mice lacking *CUL7*, it could be speculated that *CUL7* affects vasculogenesis or angiogenesis, and intracranial aneurysm has been reported in patients with 3-M syndrome.⁷ However, we have never found any vascular malformation or aneurysm in our patients. To clarify the histopathological effects of the *CUL7* mutation further, we performed histopathology on the lungs and placenta of a fetus with and one without *CUL7* mutation. The number of villi in the placenta was markedly increased in the fetus with the mutation. This finding is consistent with the finding that mice lacking *CUL7* show marked increases in the number of fetal blood vessels in labyrinths.¹⁷ Thus, the imbalance in the number of fetal and maternal blood vessels may underlie the mechanism of prenatal growth retardation in 3-M syndrome.

The molecular mechanism of *CUL7* mutations remains to be elucidated. *CUL7* assembles an E3 ubiquitin ligase complex containing S-phase kinase-associated protein 1A (Skp1), F-box-only protein 29 (Fbx29) and RBX1 ring-box 1 (ROC1), and also promotes ubiquitination.²¹ Moreover, it has been shown that *CUL7* with nonsense (R1445X) and missense (H1464P) mutations in the Cullin domain hampers the ability of *CUL7* to interact with ROC1, resulting in the failure to assemble polyubiquitin chains.⁷ Although the Q1533X (4582insT) mutation that we found in patients in Yakutia is

located outside the Cullin domain, this mutation may also alter the ability of *CUL7* bind to ROC1.⁷ Another possibility is that the 4582insT mutation might induce nonsense-mediated mRNA decay. The 4582insT mutation causes premature termination at nucleotide 4582, which is 447 nucleotides upstream of the last exon–exon junction in *CUL7*. Recent molecular genetics have revealed that the premature stop codon terminates >50–55 nucleotides upstream of the last exon–exon junction and elicits nonsense-mediated mRNA decay, which reduces the mRNA level to ~5–25% that of normal controls.²² This mechanism might also affect the pathogenesis of the 4582insT mutation.

Here, we show that short stature syndrome in Yakuts is caused by the 4582insT mutation of *CUL7*. This information may be useful for genetic counselling for Yakuts. This is the second report on patients with *CUL7* mutation and the first comprehensive study of the clinical features of patients and histopathological findings of the lungs and placenta of patients with *CUL7* mutation. These results may provide new insights for understanding the clinical diversity and pathogenesis of this rare hereditary short stature syndrome.

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