Brain & Development 42 (2020) 705–712

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Original article

Meaningful word acquisition is associated with walking ability over 10 years in Rett syndrome

Tomoko Saikusa [a], Machiko Kawaguchi [b], Tetsuji Tanioka (Tetsu T) [c], Shin Nabatame (Shin N) [d], Satoru Takahashi [e], Kotaro Yuge [a], Shin-ichiro Nagamitsu [a], Tomoyuki Takahashi [a], Yushiro Yamashita [a], Yasuyuki Kobayashi [f], Chisato Hirayama [g], Tatsuyuki Kakuma [b], Toyojiro Matsuishi [h,*], Masayuki Itoh [i]

[a] *Department of Pediatrics and Child Health, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan*
[b] *Biostatistics Center, Kurume University, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan*
[c] *NPO Rett Syndrome Support Organization, 2-37-2 Tsudaminami-machi, Hirakata, Osaka, Japan*
[d] *Department of Pediatrics, Osaka University Graduate School of Medicine, Osaka 565-0871, Japan*
[e] *Department of Pediatrics, Asahikawa University, Asahikawa 078-8510, Japan*
[f] *Japan Rett Syndrome Association, 2-29-20-101 Kamiigusa, Suginami, Tokyo 167-002, Japan*
[g] *Sakuranbokai-Rett Syndrome, 63-2-101 Kawatsu, Iizuka, Fukuoka, Japan*
[h] *Research Center for Children, Research Center for Rett Syndrome, St. Mary's Hospital, Kurume, Fukuoka 830-8543, Japan*
[i] *Department of Mental Retardation and Birth Defect Research, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi-machi, Kodaira, Tokyo 187-8502, Japan*

Received 13 February 2020; received in revised form 29 May 2020; accepted 22 June 2020

Abstract

Purpose: To investigate walking ability in Japanese patients with Rett syndrome (RTT).

Methods: Walking ability was assessed in 100 female Japanese patients with RTT using univariate and multivariate analysis in all age groups, and in patients over 10 years of age. We analyzed walking ability and confounding factors including prenatal-perinatal histories, developmental milestones, somatic and head growth, anthropometric data, body mass index, age of loss of purposeful hand use, age at onset of stereotypic hand movement, history of autistic behavior, age at regression, presence or absence of seizures, and the results of *MECP2* genetic examination from the Japanese Rett syndrome database.

Results: Univariate analysis revealed that acquisition of walking in all age groups was significantly correlated with the acquisition of meaningful words, microcephaly, and crawling ($P < 0.0001$, $P = 0.005$, $P < 0.0001$, respectively). Univariate analysis revealed that walking ability over 10 years of age was significantly correlated with acquisition of meaningful words, microcephaly, and body mass index ($P < 0{,}0001$, $P = 0.005$, $P = 0.0018$, respectively). *MECP2* mutations R306C, R133C, and R294X were significantly associated with different acquisition of crawling ($P = 0.004$) and walking ($P = 0.01$). Multivariate analysis revealed that only acquisition of meaningful words was significantly correlated with walking ability over 10 years of age. This trend excluded the genetic effects of R306C, R133C, and R294X.

Conclusions: Meaningful word acquisition was robustly associated with walking ability over 10 years. Prognosis of walking ability may be predicted by the acquisition of meaningful words. This information is potentially useful for early intervention and the planning of comprehensive treatment for young children with RTT.

* Corresponding author.
E-mail address: toyojiro@st-mary-med.or.jp (T. Matsuishi).

https://doi.org/10.1016/j.braindev.2020.06.012
0387-7604/

Keywords: Rett syndrome; Univariate analysis; Multivariate analysis; Motor function; Ambulation; Methyl-CpG binding protein 2 mutation; Japanese database

1. Introduction

Rett syndrome (RTT) (OMIM #312750) is a neurodevelopmental disorder primarily affecting females. Most cases of RTT are caused by de novo mutations in the gene encoding methyl-CpG binding protein 2 (MECP2) [1]. Approximately 90%–95% of typical RTT cases exhibit loss-of-function mutations in the *MECP2* gene of the X-chromosome [2].

Clinical manifestations include microcephaly, loss of psychomotor abilities, intellectual disability (ID), autistic behaviors, and hand stereotypies. Recent large cross-sectional studies revealed substantial clinical variability in *MECP2* mutations [2]. It has been reported that girls and women with the mutations p.Arg270* (R270X) or p.Arg255* (R255X) present with more severe motor disability, whereas those with mutations p.Arg306Cys*, p.Arg133Cys*, and p.Arg294* (R306C, R133C, R294X, respectively), and C-terminal deletions exhibit a milder phenotype, and, in most cases, acquire the ability to walk [3–7]. However, it is difficult to estimate genotype-phenotype correlations because of the role of X inactivation. In the present study, we utilized the Japanese Rett syndrome database (JRSD), which was established in October 2012 and contains clinical data from over 102 RTT patients. Walking ability is important for family counselling and planning for the provision of care for young children with RTT. In the current study, we investigated factors related to ambulation ability in young children over 10 years of age with or without genetic effects, based on the JRSD.

2. Patients and methods

2.1. Japanese Rett syndrome database and subjects

The JRSD is operated by management officers consisting of child neurologists and RTT family associations, and is supported by the Japanese government. The registration document is completed with a combination of parents' questionnaire responses and doctor's examination data after children's diagnosis with RTT with clinical criteria and/or genetic abnormality [2]. A total of 102 female patients with RTT were registered from October 2012 to December 2015. For the current study, we obtained informed consent from all RTT parents and ethical approval from each institution. The JRSD registration document contains prenatal/perinatal history, developmental milestones, somatic and head growth, anthropometric data, growth status (body mass index [BMI]), age at regression, age at development of stereotypic hand movement, loss of purposeful hand function, history and age of onset of autistic behavior, eating and swallowing ability, vocalization/verbalization, periodic breathing, hand and foot temperature, dystonia, tremor, seizures, scoliosis, muscle tonus, dental and oral problems, and genetic examinations.

2.2. Gene testing

Total genomic DNA was prepared from peripheral blood leukocytes according to standard procedures. Participants in this study underwent complete *MECP2* mutation analysis, including exon 1 and evaluation for large DNA rearrangement, by Southern blotting or by multiple ligation-dependent probe amplification (MLPA) analysis. Whole exome sequencing (WES) was performed in some patients, as previously described [8].

2.3. Sentinel surveillance and statistical analyses

The Kaplan–Meier method was used to estimate survival curves and the log-rank test was used to compare estimated survival curves. In addition, univariate and multivariate logistic regression models were employed to test the relationships between walking ability over 10 years of age and other factors. All variables were visually inspected to assess their distribution. When variables were judged to be skewed and the normality assumption was not tenable, non-parametric tests, Spearman's rank correlations and Mann-Whitney U-tests were employed. χ^2 test and Fisher's exact test were used to examine the relationships between categorical variables. P-values less than 0.05 were considered to indicate significant differences. JMP Pro 13 (SAS institute, Cary, NC, USA) was used to perform all data analyses. The research protocol was approved by the Ethics Committees of National Center of Neurology and Psychiatry, and the Ethics Committees of each participating institution.

3. Results

Two patients were excluded from the study because of incomplete data for RTT diagnosis, according to recent clinical diagnostic criteria [2]. We analyzed registered data from 86 typical and 14 atypical RTT patients. All patients were female, and ages ranged from 1 year to 43 years of age (mean ± SD; 14.5 ± 11.2 years of age;

Table 1
Clinical distribution in Japanese Rett Syndrome Database.

Symptoms	Age at registration					
	1–5 yrs	6–10yrs	11–15yrs	16–20yrs	>21yrs	Total
Number of patients	19	25	18	6	32	100
Main criteria						
Partial/complete loss of acquired purposeful hand skills	19 (1 0 0)	23 (92.0)	14 (77.8)	5 (83.3)	25 (78.1)	86 (86)
Partial/complete loss of acquired spoken language	10 (52.6)	13 (52.0)	12 (66.7)	4 (66.7)	21 (68.8)	60 (60)
Gait abnormalities	17 (89.4)	22 (88.0)	17 (94.4)	6 (100)	30 (93.8)	92 (92)
Stereotypic hand movements	19 (100)	24 (96.0)	18 (100)	6 (100)	32 (100)	99 (99)
Supportive criteria						
Breathing disturbances when awake	11 (57.8)	17 (68.0)	13 (72.2)	6 (100)	18 (56.3)	65 (65)
Bruxism when awake	14 (73.6)	20 (80.0)	13 (72.2)	5 (83.3)	18 (56.3)	70 (70)
Impaired sleep pattern	11 (57.8)	14 (56.0)	8 (44.4)	6 (100)	22 (68.8)	61 (61)
Abnormal muscle tone	19 (100)	19 (76.0)	15 (83.3)	6 (100)	29 (90.6)	88 (88)
Peripheral vasomotor disturbances	18 (94.7)	24 (96.0)	18 (100)	6 (100)	28 (87.5)	94 (94)
Scoliosis/kyphosis	3 (15.7)	12 (48.0)	14 (77.8)	6 (100)	29 (90.6)	64 (64)
Inappropriate laughing/screaming spells	18 (94.7)	20 (80.0)	15 (83.3)	6 (100)	20 (62.5)	79 (79)
Autistic behavior with intense eye communication	16 (84.2)	16 (64.0)	13 (72.2)	6 (100)	24 (75.0)	75 (75)
Epilepsy	7 (36.8)	16 (64.0)	13 (83.3)	6 (100)	26 (81.3)	68 (68)

N = number of patients; yrs = years.

Table 2
Distribution of *MECP2* mutations in Japanese Rett Syndrome Database.

	Number of patients per total
MECP2 mutation examination	92/102 (90.2%)
MECP2 mutations, identified	88/92 (95%)
Typical	86/102 (84%)
Genotype	N(%)
R168X	11 (11.8)
T158M	8 (8.6)
R255X	8 (6.4)
R270X	5 (5.4)
R294X	5 (5.4)
R133C	5 (5.4)
R306C	4 (4.3)
R306W	3 (3.2)

median; 11.4 years of age). Table 1 shows the age distribution and frequency of symptoms and signs. Of 100 RTT patients, 92 (92%) underwent genetic examination by Southern blotting, MLPA, or WES.

Of these patients, 88 (95%) exhibited various *MECP2* mutations (Table 2). *MECP2* mutations included R168X (11 patients) (12.5%), T158M (eight patients) (9%) and R255X (six patients) (6.8%) (Table 2). All patients developed head control (median; 4 months of age). Ninety-seven patients acquired the ability to roll over (median; 6 months), while three were never able to roll over (3%). Seventy-nine patients were able to sit unassisted (median; 8 months) (79%). Fifty-six patients acquired the ability to crawl (median; 11 months) (56%). Forty-nine patients did not acquire the ability to walk without support (median; 18 months) (49%). The age of acquisition of walking ranged from 11 months to 72 months (median: 18 months). Forty patients did not develop the ability to produce meaningful words (40%). Among the other patients, the age of first meaningful words ranged from 10 months to 72 months of age. Fifty-eight patients exhibited a severely small head circumference (56%).

3.1. Correlation of acquisition of walking ability, meaningful words and microcephaly

We hypothesized that walking ability over 10 years of age may be related to a range of factors highlighted in previous studies, as follows: acquisition of meaningful words, presence or absence of microcephaly, dystonia, abnormal muscle tone, breathing abnormalities, age of onset of stereotypic hand movement, age of regression of hand function, and presence or absence of scoliosis [9–12].

First, we analyzed all data related to motor function, then examined acquisition of walking ability in all age groups. The Kaplan–Meier method was used to assess the relationships between acquisition of head control, sitting alone, crawling, meaningful words, microcephaly and acquisition of walking. Fig. 1 shows the estimated mean proportion of patients who acquired walking at all ages, for 100 patients. The proportion of patients who acquired the ability to walk increased until 100 months, then tended to flatten. Patients who acquired meaningful words showed a significantly greater rate of acquiring walking than those who did not acquire meaningful words, for all age groups ($P < 0.001$). Patients with absence of microcephaly exhibited a significantly greater rate of acquiring of walking than those with microcephaly ($P = 0.005$).

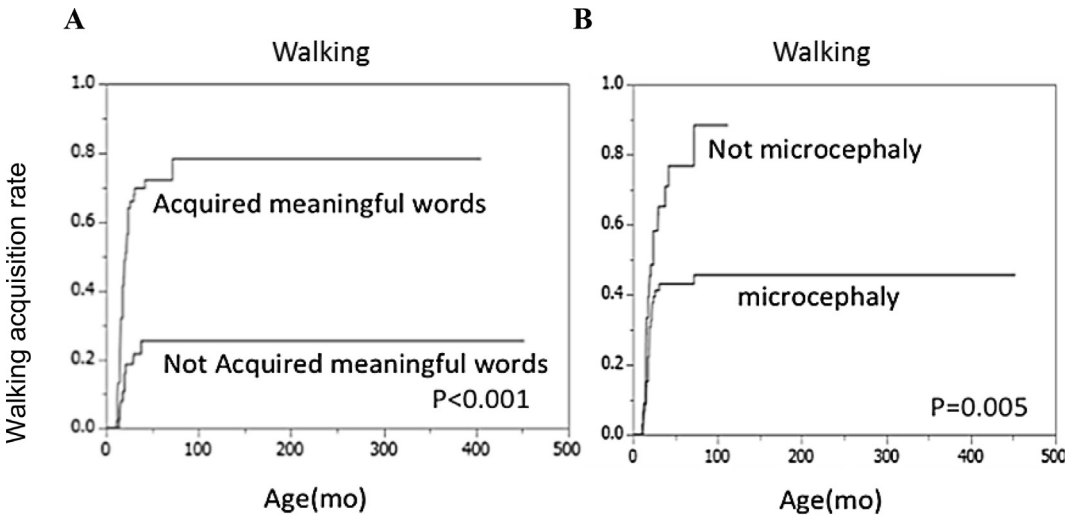

Fig. 1. Relationship between walking ability, acquisition of meaningful speech and microcephaly Walking ability was significantly different between patients who acquired meaningful words and those who did not acquire meaningful words, $P < 0.001$ (A). Walking ability was significantly different between patients with and without microcephaly, $P = 0.005$ (B), Kaplan–Meier method.

3.2. Acquisition of walking and effect of MECP2 mutation type (R306C, R133C, R294X)

Genotype *MECP2* mutation severity was categorized into mild (R306C, R133C, R294X) and other, based on previous reports [13]. To assess acquisition of walking, we also excluded R306C, R133C, R294X gene mutations, because an increasing number of patients with RTT did not undergo MECP2 gene testing in recent years. Fig. 2 shows an analysis of the acquisition of crawling (Fig. 2A) and walking (Fig. 2B) in relation to gene mutations, including R306C, R133C, R294X and other mutations. The rate of acquisition of crawling increased with age, until 30 months (Fig. 2A), and the number of patients that acquired walking increased until 40 months of age, then tended to flatten (Fig. 2B). Patients with mild phenotype mutations (R306C, R133C, R294X) exhibited significantly higher rates of acquiring crawling and walking ($P = 0.004$, $P = 0.01$, respectively).

3.3. Analysis of walking ability over 10 years of age

Age distribution was observed and analyzed in two age categories: over 10 years old, and all ages [12,13]. According to previous studies of motor symptoms

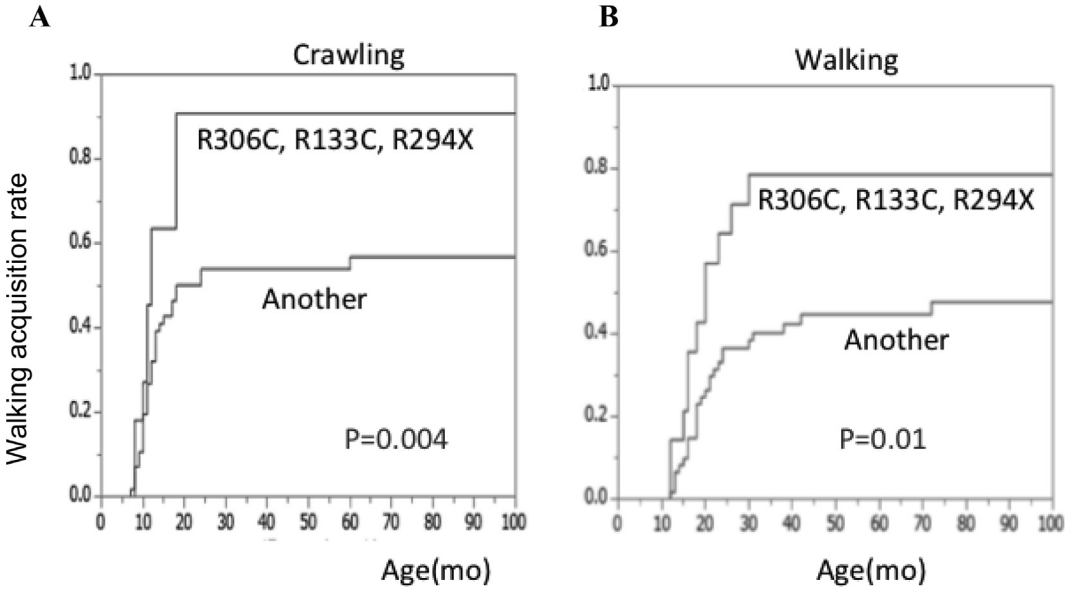

Fig. 2. Crawling and walking ability were significantly different between patients with *MECP2* mutations of R306C, R133C, R294X and other mutations Crawling ability and walking ability were significantly different between patients with *MECP2* mutations of R306C, R133C, R294X and other mutations. $P = 0.004$ (A), and $P = 0.01$ (B), respectively, Kaplan–Meier method.

including walking, we divided patients into four groups, as follows: those currently able to walk, those who were previously able to walk then lost walking ability, those unable to walk at over 10 years of age, and those with unknown walking status (Fig. 3). Of 56 patients over 10 years old, 31 were currently able to walk, 17 patients never learned to walk, six patients had lost the ability to walk, and two patients had unknown walking status. We divided participants into two groups depending on walking prognosis, as follows: participants who were still walking after 10 years of age, and a group of participants who were able to walk previously then lost the ability to walk, or who had never walked (Figs. 3, 4). The Kaplan–Meier method was used to assess the relationships between acquisition of crawling, acquisition of meaningful words, and walking ability over 10 years of age. The results shown in the figures indicate that walking ability was related to the acquisition of crawling (Fig. 4A), and the acquisition of meaningful words (Fig. 4B) over 10 years of age. As shown in Fig. 4, the proportion of patients with the ability to walk over 10 years of age was significantly related to the acquisition of crawling, and the acquisition of meaningful words ($P < 0.001$, $P < 0.001$, respectively).

Univariate analysis and multivariate analysis were used to assess the relationships between walking ability and early clinical signs. Univariate analysis revealed that walking ability was significantly correlated with meaningful word acquisition, microcephaly and BMI ($P < 0.0001$, $P = 0.005$, $P = 0.0018$, respectively). Lower BMI was negatively correlated with walking ability (Table 3). Walking ability was not related to dystonia, abnormal muscle tone, presence of breathing abnormalities, stereotypic hand movement, regression of hand function, or scoliosis. Multivariate analysis revealed that walking ability was only significantly correlated with meaningful words ($P = 0.0003$; odds ratio = 15.872). Even when we excluded gene effects, acquisition of meaningful words was the only robustly significant factor associated with walking ability over 10 years of age. Microcephaly, BMI, dystonia, scoliosis, and crawling were not correlated with walking ability over 10 years of age (Table 4).

4. Discussion

RTT, a neurodevelopmental disorder predominantly affecting females, has been characterized by apparently normal initial development followed by frank regression of fine motor and communication skills, typically between 6 and 18 months of age [13]. Our univariate analysis revealed that walking ability was correlated with crawling, meaningful word acquisition, microcephaly, *MECP2* mutation type, and BMI. Multivariate analysis revealed that only meaningful word acquisition was robustly related to walking ability when patients were over 10 years old. A previous study reported that walking was delayed in 30/38 RTT patients (79%); of these, 18 patients could not crawl until 4 years of age [14]. Furthermore, 55% of patients began to walk without having acquired the ability to crawl [14]. Another study reported that patients that had obtained meaningful words began crawling and walking significantly earlier than those without meaningful word acquisition, using univariate analysis [15]. Never acquiring the ability to walk is reported to be related to the early loss of language [11]. The abnormal acquisition of early skills is reported to be in accord with a marked decrease in head size beginning in early postnatal life [16], and the current data support these previous findings. In the current study, acquisition of meaningful words was a robust factor related to various symptoms or functions,

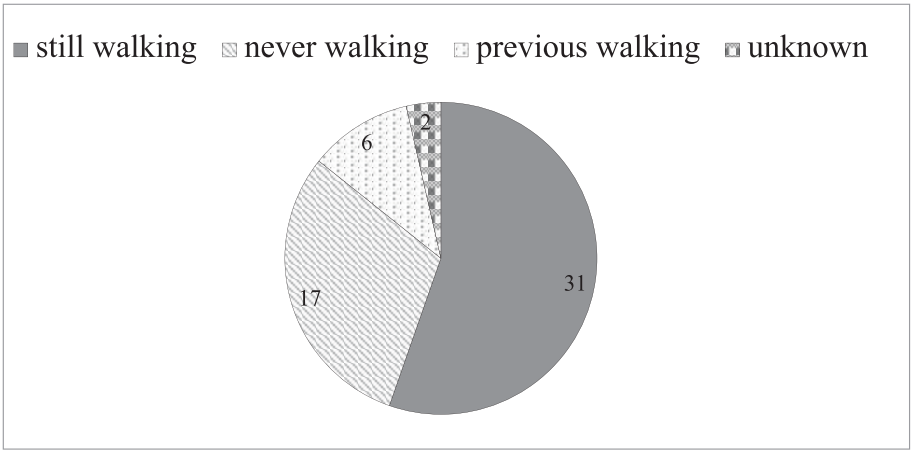

Fig. 3. Division of patients into four groups based on acquired and sustained walking abilities over 10 years of age We divided patients into four groups based on acquired and sustained walking abilities over 10 years of age: currently able to walk, previously able to walk but cannot walk at present, never able to walk, and unknown. Of 56 patients over 10 years old, 31 were currently able to walk, 17 patients never learned to walk, six patients had lost the ability to walk, and two patients had unknown walking status.

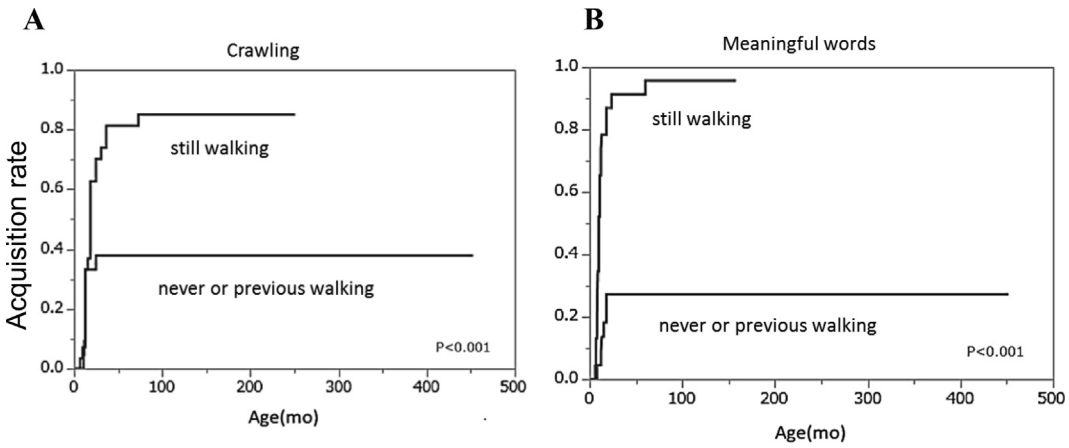

Fig. 4. Relationships between walking ability, crawling ability, and acquisition of meaningful words over 10 years of age For patients over 10 years old, walking ability was significantly different between those who acquired crawling and those who did not acquire crawling ($P < 0.001$) (A). Walking ability was significantly different between patients who acquired meaningful words and those who did not acquire meaningful words ($P < 0.001$) (B), Kaplan–Meier method. We divided participants into two groups depending on walking prognosis, as follows: participants who were still walking after 10 years of age, and participants who were either able to walk then lost walking ability or who were never able to walk (Fig. 3).

Table 3
Univariate analysis of walking ability and other factors over 10 years of age.

Factors	All patients with RTT		Excluding the patients with R306C, R133C, R294X variants	
	Odds ratio	P-value	Odds ratio	P-value
Meaningful words	7.125	<0.0001	7.959	0.0001
Microcephaly	0.265	0.005	0.261	0.0071
Dystonia	0.833	0.7357	0.831	0.7617
Scoliosis	0.778	0.6572	0.786	0.6905
BMI	1.279	0.0018	1.372	0.0019

Walking ability was significantly correlated with the acquisition of meaningful words, microcephaly and BMI; $P < 0.0001$, $P = 0005$, $P = 0.0018$, respectively. Lower BMI was negatively correlated with walking ability. BMI: Body Mass Index

Table 4
Multivariate analysis of walking ability and other factors over 10 years of age.

Factor	All patients with RTT		Excluding the patients with R306C, R133C, R294X variants	
	Odds ratio	P-value	Odds ratio	P-value
Meaningful words	15.872	0.0003	16.506	0.0015
Microcephaly	1.366	0.7132	1.706	0.5877
Dystonia	0.526	0.4024	0.685	0.6623
Scoliosis	0.758	0.7357	0.918	0.921
BMI	1.192	0.1186	1.257	0.1452

Walking ability was significantly correlated with the acquisition of meaningful words ($P = 0.0003$; odds ratio = 15.872). Although we excluded the effects of genes, meaningful word acquisition was significantly correlated with walking ability.

including cortical ability (microcephaly), growth/nutrition (BMI), motor ability (crawling), and genotype.

Witt-Engerström et al. reported that, on the basis of acquired and sustained walking ability, patients with RTT aged 22–44 years could be divided into three groups: currently walking, previously walking, and never able to walk [12]. Dystonic signs were most common among patients who were previously able to walk then became unable to walk. Early progression of scoliosis and weakness were most prevalent among patients who were never able to walk [12]. Extrapyramidal signs, including stereotypic hand movements, gait disturbance, bruxism, bradykinesia, hypomimia, scoliosis, rigidity and dystonia, have been observed in almost all patients with RTT, affecting the daily lives and walking ability of patients [17,18]. We divided patients over 10 years of age into four groups (Fig. 3). Walking ability was related to meaningful words, but dystonic signs and scoliosis were not related to walking ability. The discrepancy between the current findings and the previous report may have arisen because the diagnosis of dystonia was not reported in our study [12]. Data regarding dystonia were

collected in 67 of 100 cases (67%). Among these 67 cases, the presence of dystonia in patients with RTT was reported in 20 cases (29.9%). Over 10 years, data regarding dystonia were collected in 42 of 56 cases (75%). Among these 42 cases, dystonia was reported in 14 cases (33.3%). It is possible that the diagnosis of dystonia is difficult in some cases, and our database did not include the detail of the neurological examination of extrapyramidal signs. Unfortunately, we also did not evaluate the severity of scoliosis in the current study.

Our study confirmed the importance of genotypes associated with severe and mild phenotypes [3–5]. Tarquine et al. reported that typical RTT was associated with more severely affected growth (height, weight, head circumference, and BMI) than atypical RTT. Decreased growth, including body weight, height and microcephaly, was associated with more impaired development, higher disease severity, and specific MECP2 mutations (pre-C-terminal truncation, large deletion, T158M, R168X, R255X, and R270X) [16]. In previous reports, the mutations T158M, R255X and R168X, and R270X have generally been associated with more severe phenotypes, while R306C, R133C, R294X and 3′ truncations have been associated with less severe disease [3–5]. Patients with R306C, R133C, R294X and 3′ truncations are reported to acquire more gross motor skills and lose fewer skills, particularly in fine motor and expressive language abilities [6,7]. However specific mutations may not be the only determinant of severity within specific individuals due to the existence of other factors, such as X-chromosome inactivation, genetic background (the interplay of other genetic variations), and distribution of abnormal genes in specific brain regions [3,5,19.20]. Mutation type has some effect on the phenotypic manifestation of RTT, and the pattern of X inactivation is thought to determine phenotypic severity [21]. MECP2 interacts with a wide variety of cofactors. The intrinsically disordered nature of MECP2 permits a high degree of structural flexibility, allowing MECP2 to interact with many diverse protein partners. MECP2 utilizes a variety of mechanisms to regulate gene expression, which is dependent on the proteins with which it interacts at any given time. The clinical variability of these mutation suggests that it plays a major role in the function of MECP2 protein [22].

The database used in this study included information provided by parents and caregivers of RTT patients, with confirmation by a pediatric neurologist. Furthermore, 92 of 100 (92%) RTT participants underwent gene testing. Using a Japanese database, our results revealed that acquisition of meaningful words was the only factor that was robustly and significantly correlated with walking ability over 10 years. However, microcephaly, dystonia, scoliosis and BMI were not correlated with walking abilities in the multivariate analysis.

5. Limitations of this study

Several limitations of this study may should be considered in interpreting the results. The principal limitation was the relatively small sample size of the study, limiting the generalizability of the results. Our study also used a cross-sectional design. However, our study also had several unique features, including collaborative study of parents or caregivers, and direct examination by a pediatric neurologist. In most previous studies, data were derived from questionnaires without direct assessment of participants by clinicians experienced in the diagnosis of RTT. In addition, we performed multivariate analysis, because many factors may be tightly linked with other factors.

In conclusion, our findings may be useful for informing the development of early intervention methods, and the planning of comprehensive treatment for young infants with RTT. The acquisition of meaningful words was only significantly correlated with walking ability over 10 years of age among patients with RTT.

Author contributions

All authors have been involved in drafting or revising the manuscript, have given final approval, and agree to be accountable for all aspects of the work involved. Each author's individual participation is outlined below. TS, Shin N, ST, TM, and MI did the conceptualization and design of the study and acquisition, analysis, and interpretation of the data. MK and TK did the statistical analysis of the data. Tetsu T, KY, SN, TT, YY, YK, and CH performed the follow-up examinations and interpretation of the data.

Sources of funding

This work was partially supported by a Grant-in-Aid for Research from the Japan Agency for Medical Research and Development (AMED) and a grant for research on Rare and Intractable Disease from the Ministry of Health, Labour and Welfare, Japan.

This work was partly supported by Grants-in-Aid for Scientific Research (No. 18K07893 to TM) from the Ministry of Education, Culture, Sports, Science and Technology, MEXT, and also partly supported by JSPS KAKENHI Grant Number 16H01880 to TM).

Conflict of interest

None of the authors have conflicts of interest to declare.

References

[1] Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 1999;23:185–8.

[2] Neul JL, Kaufmann WE, Glaze DG, Christodoulou J, Clarke AJ, Bahl-Buisson N, et al. Rett syndrome: revised diagnostic criteria and nomenclature. Ann Neurol 2010;68:944–50.

[3] Neul JL, Fang P, Barrish J, Lane J, Caeg EB, Smith EO, et al. Specific mutations in methyl-CpG-binding protein 2 confer different severity in Rett syndrome. Neurology 2008;70:1313–21.

[4] Smeets EE, Chenault M, Curfs LM, Schrander-Stumpel CT, Frijns JP. Rett syndrome and long term disorder profile. Am J Med Genet Part A 2009;149A:199–205.

[5] Bebbington A, Anderson A, Ravine D, Fyfe S, Pineda M, de Klerk N, et al. Investigation genotype-phenotype relationships in Rett syndrome using an international data set. Neurology 2008;70:868–75.

[6] Robertson L, Hall SE, Jacoby P, Ellaway C, de Klerk N, Leonard H. The association between behavior and genotype in Rett syndrome using the Australian Rett Syndrome Database. Am J Med Genet B Neuropsychiatr Genet 2006;141:177–83.

[7] Lane JB, Lee HS, Smith LW, Cheng P, Percy AK, Glaze DG, et al. Clinical severity and quality of life in children and adolescents with Rett syndrome. Neurology 2011;77:1812–8.

[8] Iwama K, Mizuguchi T, Takeshita E, Nakagawa E, Okazaki T, Nomura Y, et al. Genetic landscape of Rett syndrome-like phenotypes revealed by whole exome sequencing. J Med Genet 2019;56:396–407.

[9] Kerr AM, Prescott RJ. Predictive value of the early clinical signs in Rett disorder. Brain Dev 2005:S20–4.

[10] Killan JT, Lane JB, Lee HS, Skinner SA, Kaufmann WE, Glaze DG, et al. Scoliosis in Rett syndrome: progression, comorbidities, and predictors. Pediatr neurol 2017;70:20–5.

[11] Huppke P, Held M, Laccone F, Hanefeld F. The spectrum of phenotypes in females with Rett syndrome. Brain Dev 2003;25:346–51.

[12] Witt-Engerström I, Hagberg B. The Rett syndrome: gross motor disability and neural impairment in adults. Brain Dev 1990;12:23–6.

[13] Neul JL, Lane JB, Lee H-S, Geet S, Barrish JO, Annese F, et al. Developmental delay in Rett syndrome: data from the natural history study. J Neurodev Disord 2014;6:20.

[14] Segawa M. Early motor disturbances in Rett syndrome and its pathophysiological importance. Brain Dev 2005;27:S54–8.

[15] Segawa M. Pathophysiology of Rett syndrome from the standpoint of clinical characteristics. Brain Dev 2001;23:S94–8.

[16] Tarquino DC, Motil KJ, Hou W, Lee H-S, Glaze DG, Skinner SA, et al. Growth failure and outcome in Rett syndrome. Neurology 2012;79:1653–61.

[17] FitzGerald PM, Jankovic J, Glaze DG, Schultz R, Percy AK. Extrapyramidal involvement in Rett's syndrome. Neurology 1990;40:293–5.

[18] Temudo T, Ramos E, Dias K, Barbot C, Vieira JP, Moreira A, et al. Movement disorders in Rett syndrome: an analysis of 60 patients with detected MECP2 mutation and correlation with mutation type. Mov Disord 2008;23:1384–90.

[19] Schanen C, Houwink EJ, Dorrani N, Lane J, Everett R, Feng A, et al. Phenotypic manifestations of MECP2 mutations in classical and atypical Rett syndrome. Am J Med Genet 2004;126A:129–40.

[20] Cuddapah VA, Pillai RB, Shekar KV, Lane JB, Motil KJ, Skinner SA, et al. Methyl-CpG-binding protein 2 (MECP2) mutation type is associated with disease severity in Rett syndrome. J Med Genet 2014;51:152–8.

[21] Downs J, Leonard H, Wong K, Newton N, Hill K. Quantification of walking-based physical activity and sedentary time in individuals with Rett syndrome. Dev Med Child Neurol 2017;59:605–11.

[22] Leonard H, Cobb S, Downs J. Clinical and biological progress over 50 years in Rett syndrome. Nat Rev Neurol 2017;13:37–51.