Mutation analysis of the methyl-CpG-binding protein 2 gene (MECP2) in
Rett patients with preserved speech

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Abstract
Genomic DNAs from 35 Japanese sporadic patients with Rett syndrome (RTT) were screened for DNA mutations in the entire coding region and exon–intron boundaries of methyl-CpG-binding protein 2 (MECP2). We detected mutations in 30 (85.7%) of 35 patients. Among these 35 RTT patients, five patients (14%) had the preserved speech variant of this disease. Four respective mutations (R133C, R306C, R294X, 2 base pair (bp) deletion) were found in these five patients. Two patients had the same missense mutation, R133C. The patients with the R133C mutation and one with frameshift mutation presented the relatively mild clinical presentation, and the R133C mutation was not found in any other patient without preserved speech. We confirmed that the preserved speech variant is one of the clinical phenotypes of RTT and is also caused by MECP2 mutation. We speculated that the clinical phenotype of patients with the R133C missense mutation might be mild.

Keywords: Rett syndrome; Preserved speech variant; Methyl-CpG-binding protein 2; Mutation

1. Introduction
A diagnosis of Rett syndrome (RTT, MIM 312760) has been clinically determined using a battery of characteristic clinical criteria and a sequence of stages combined with differential diagnostic exclusions. Atypical Rett variants at the age of 10 or more years were defined by Hagberg and Skjeldal [1], however, such a diagnosis can be difficult when the information about childhood is limited.

Most patients with RTT cannot speak in words or phrases. Zappella (1992) described three Italian girls with preserved speech and clinical criteria characteristic of RTT [2]. One of the three girls had a sister with classical RTT. In 1998, 30 girls with the preserved speech variant from Sweden and Italy were reported [3]. All but one cases met the full symptom criteria for diagnostic and statistical manual of mental disorders (DSM-IV) autistic disorder. All met the required three out of six main criteria and 47% of the cases also met both these and the required five out of 11 supportive criteria for RTT variants as outlined by Hagberg and Skjeldal [1]. They could not determine whether or not these 30 girls had a variant of RTT, autistic disorder, a combination of the two, or a ‘new’ disorder.

The discovery of mutations in methyl-CpG-binding protein 2 (MECP2) by Amir et al. may provide us the tool for validation of suspected cases [4]. Recent studies indicate that 75–80% of classical RTT patients have mutations in MECP2 [4–6]; however, MECP2 mutations in atypical Rett variants, i.e. the preserved speech variant, were unknown [7].

The purpose of this study was to identify the existence of the MECP2 mutation, the characteristic mutation in RTT patients with preserved speech.

2. Patients and methods
Clinical diagnosis of RTT was made according to the Rett Syndrome Diagnostic Criteria Work Group [8].

2.1. Mutational analysis for the MECP2 gene
Genomic DNA was extracted from the peripheral blood of 35 unrelated Japanese patients with RTT who were followed at Kurume University Hospital and were screened for DNA mutations in the entire coding region and exon–intron boundaries of MECP2 by subjecting polymerase chain reaction (PCR) products to the denaturing gradient and gel electrophoresis method (DGGE) using the BIORAD Laboratories D GENETM system, and by the direct
sequencing technique [5]. Written informed consent was obtained from each patient’s parents.

3. Results

All the RTT patients screened in this study were sporadic and typical except for five patients with preserved speech variants. We detected DNA mutations in 30 of 35 patients (85.7%); 14 had missense mutations, nine had nonsense mutations, and four had mutations with a nucleotide deletion resulting in frameshift with a premature stop codon (Table 1). No mutations were found in five patients. Five patients with preserved speech revealed the mutations of R133C, R306C, R294X, 2 bp deletion. Two patients had the same mutation type; R133C. The patients with this mutation and the patient with DNA change, 543, 544 (TC) leading to 2 bp deletion, showed the mildest clinical presentation. The R133C mutation was not found in any other patient in whom speech had not been preserved.

All patients with preserved speech fulfilled five out of six main criteria (except microcephaly) in the inclusion criteria and three to five supportive criteria [1]. They spoke 20–50 words or expressed two-word sentences. Three had echolalia similar to that of patients with autistic disorder. This patient group showed fewer examples of microcephaly, growth failure, epilepsy, scoliosis, and respiratory abnormality than did patients with classical RTT, while their motor disturbance was much milder. All could walk unsupported (two could even run) and their hand functions were relatively preserved. All were severely mentally retarded and in stage III. The details of development for each patient are described as follows.

3.1. Patient 1 (missense mutation: R133C) current age: 9 years

Maternal age at delivery was 39 years. Pregnancy and delivery were uneventful. Her development up to 36 months was normal. She spoke her first word and walked alone at around 12 months. She sometimes clapped her hands at 12–24 months. After 36 months, it gradually became difficult for her to use hands to take off the clothes and holding a pencil. She said that she tried using a pencil, but consistently failed. She became emotionally withdrawn at 4 years of age and her gross motor function gradually deteriorated. She became more interested in people again from 5 years of age. Hand-washing-like stereotypy started at 6 years of age. At 7 years of age, she could run and in-line skate, but her general motor skills were clumsy. She could communicate with short sentences but echolalia was noted. She could eat with a spoon but could use neither scissors nor pencil. Her growth was normal. She recently experienced afebrile seizures twice and abnormal breathing behavior (breath-holding). Her intelligence quotient (IQ) was 23 (Tanaka-Binet) at 7 years of age, which might be underestimated because of her poor performance skills and social interaction. A cranial magnetic resonance imaging (MRI) showed normal; however, her cerebral blood flow in the bi-frontal lesion was decreased as determined by ethyl cysteinate dimer (ECD-SPECT) study.

3.2. Patient 2 (missense mutation: R306C) current age: 10 years

Pregnancy and delivery were normal. She achieved head control at 3 months, sitting alone at 8 months, and rolling over at 9 months. She walked alone at 15 months and spoke her
first word at 18 months of age. She had gained eight words by 24 months of age. Thereafter, her eye contact with people became poor. Hand function deteriorated from 3 years of age and hand-washing-like stereotypy started at 4 years of age. Cranial MRI at 4 years of age was normal. She can now speak 40–50 words, sing a short song, and communicate with two-word sentences. She can walk for 20 min and takes walks with her dog. Her IQ at 5 years of age was 38 (Tanaka-Binet). She showed no microcephalus, seizure, breathing irregularities, scoliosis, or autonomic dysfunction. Her electroencephalogram (EEG) showed right frontal spikes.

3.3. Patient 3 (missense mutation: R133C) current age: 9 years

Due to breech presentation, the patient was delivered by Caesarian-section. Motor milestones revealed that head control was achieved at 4 months, and rolling over at 8–9 months. She seldom crawled. She walked alone at 15 months, and spoke four to five words at 18 months. She had gained 20 words by 24 months. Her developmental quotient (DQ) at 38 months of age was 47 and she was diagnosed as mentally retarded. Her hand function was delayed. She started hand wringing at 5 years. She experienced breathing irregularities from around 6 years. Beginning at 7 years of age, she developed epileptic seizures. She started toe-walking and walked on her knees upon awaking. She has echolalia and speaks three to four words now. She repeats the same word, ‘Urusai’ (‘shut-up’ in English). Her DQ at 7 years 8 months of age was 21.

3.4. Patient 4 (nonsense mutation: R294X) current age: 20 years

Pregnancy was normal. She was born via vacuum delivery due to weak labor. Her motor and speech developments were normal up to 24 months, e.g. head control at 3 months, rolling over at 6 months, sitting alone at 7 months, and pincer grasp at 10 months. She spoke her first word at 12 months, walked alone at 15 months; however, her speech development stagnated after 3 years of age. She became autistic and development stagnated. She kept saying ‘Baka’ (‘stupid’ in English) to other people. She started fine hand wringing behavior at 4 years, much like that seen in autistic children, and she was hyperactive. Her parents joined local RTT parents association when she was 8 years old; however, they felt that their daughter was different from other RTT girls because of preserved gross/fine motor and speech abilities. She gradually lost her ability to speak by the age of 12 years. Scoliosis and gaitapraxia became prominent at around 19 years of age; thus the parents returned to our hospital for re-evaluation. She was severely retarded, but showed no microcephalus, seizures, or breathing abnormalities.

3.5. Patient 5 (two nucleotide mutation at 543, 544 TC of MECP2) current age: 21 years

Pregnancy and delivery were uneventful. She achieved head control at 3 months, rolling over at 5 months, sitting alone at 7–8 months, and walking alone at 13 months. She spoke three words at 18 months and spoke two-word sentences at 24 months. Her eye contact became poor at 24 months. At the age of 40 months, she was diagnosed as having autistic disorder. Her motor development was normal; however, she began hand wringing at 36 months, which became prominent after 60 months. She showed mild breathing abnormalities and scoliosis but had no microcephaly. Her cranial computerized tomography (CT) was normal, but EEG was abnormal with right centrotemporal spikes at 60 months of age. She developed epilepsy at 17 years of age. There was no deterioration in speech and she can still run.

4. Discussion

We confirmed that the preserved speech variant is one of the clinical phenotypes of RTT and is also caused by the MECP2 mutation. Two patients (patients 4 and 5) were initially diagnosed as having autistic disorder. The diagnosis of RTT delayed because clinical expressions such as gross motor and hand functions, and speech were preserved. The psychological stress and anxiety for these families before the diagnosis was made were tremendous. The discovery of the MECP2 gene mutation in RTT may allow an earlier diagnosis of RTT in patients with atypical variants; i.e. those with preserved speech variant and forme fruste.

We experienced two patients with R133C missense mutation in the preserved speech variant of RTT. The R133C mutation was not found in any other patient in whom speech had not been preserved. We speculate that the clinical phenotype of the R133C missense mutation might be milder. Another patient with preserved speech and the R133C mutation has been reported by Huppke et al. [6]. Nielsen has also reported a 30-year-old Danish patient with the R133C mutation [9]. She was suspected as having RTT at 18 years. She speaks short sentences and is still ambulatory and uses her hands for eating. Analysis of 50 Danish patients revealed 40 patients having the MECP2 mutation; two had R133C missense mutation, but it was not found in any of their classical cases. One of them was preserved speech variant and the other was the forme fruste type. Nielsen also speculated that R133C might lead to a milder phenotype. One of two Japanese patients with the R133C missense mutation in Okayama was also found to be preserved speech type (Dr Iori Oumori, personal communication). The reason why the clinical phenotype of patients with the R133C missense mutation was mild is unknown. One possibility of pheno-
otypic variability may be associated with the type of MECP2 mutation. Cheadle et al. have reported that significantly milder expressions of the disease were noted in patients carrying the missense mutation as compared with those with truncating mutations [10]. The varying pattern of X inactivation and/or some modifier gene might also be related to disease severity. X inactivation studies in our patients are in progress.

One patient with preserved speech had a novel two-nucleotide deletion mutation at 543, 544 TC of MECP2, leading to the replacement of amino acids at 183–233 codons and causing a premature termination at 233 codon in 486 amino acids of the normal MECP2 product. The putative protein has a normal methyl-binding domain (MBD), but is missing a transcriptional repression domain (TRD). The disease manifestation in this patient is even milder than that in R133C missense mutation patients in regard to speech and motor function. De Bona et al. reported MECP2 mutation in three preserved speech variants of the disease, with two patients showing deletions of 41 and 44 bp each, which were similar to the deletions observed in classical patients [7].

Further studies of the MECP2 mutation type and pattern of X inactivation in a large number of patients with preserved speech are necessary to confirm genotype–pheno-type correlations.

References