FOXG1 mutations in Japanese patients with the congenital variant of Rett syndrome


Rett syndrome (RTT) is a severe neurodevelopmental disorder characterized by microcephaly, psychomotor regression, seizures and stereotypical hand movements. Recently, deletions and inactivating mutations in FOXG1, encoding a brain-specific transcription factor that is critical for forebrain development, have been found to be associated with the congenital variant of RTT. Here we report the clinical features and molecular characteristics of two cases of the congenital variant of RTT. We conducted mutation screenings of FOXG1 in a cohort of 15 Japanese patients with a clinical diagnosis of atypical RTT but without MECP2 and CDKL5 mutations. Two unrelated female patients had heterozygous mutations (c.256dupC, p.Gln86ProfsX35 and c.689G>A, p.Arg230His). Both showed neurological symptoms from the neonatal period, including hypotonia, irritability and severe microcephaly. Further, their psychomotor development was severely impaired, as indicated by their inability to sit unaided or acquire speech sounds, and they had a hyperkinetic movement disorder, because both displayed hand stereotypies and jerky movements of the upper limbs. Brain magnetic resonance imaging scans revealed delayed myelination with hypoplasia of the corpus callosum and frontal lobe. These cases confirm the involvement of FOXG1 in the molecular etiology of the congenital variant of RTT and show the characteristic features of FOXG1-related disorder.

Conflict of interest
The authors declare no conflicts of interest.
variant of RTT can be caused by point mutations as well as complete deletions in FOXG1.

At present, 17 point mutations in FOXG1 have been reported in patients with the congenital variant of RTT (13–20). Here, we report two FOXG1 mutations associated with the congenital variant, which confirms the involvement of FOXG1 in the pathophysiology of the congenital variant of RTT and helps to delineate the clinical features associated with FOXG1 mutations.

Materials and methods

Patients

Fifteen Japanese patients (13 females and 2 males) with a clinical diagnosis of atypical RTT based on the revised criteria for this disorder (2) were examined. Eight and seven patients were considered to have the congenital and early seizure variants, respectively. Direct sequencing of the entire coding sequences and exon–intron boundaries did not reveal MECP2 and CDKL5 mutations in these patients. The possibility of large rearrangements in these genes was excluded by using multiplex ligation-dependent probe amplification (MLPA) method (MRC-Holland, Amsterdam, The Netherlands).

Molecular analysis

Blood samples were collected from the patients and their parents after their written informed consent. Genomic DNA was extracted from peripheral blood leukocytes and used as the template for polymerase chain reaction (PCR). Appropriate primers were used to yield DNA fragments spanning the entire FOXG1 coding region (13). Mutation screenings were performed by direct sequencing of exon1-derived PCR products. Large rearrangements of FOXG1 DNA were detected by using the MLPA method according to the manufacturer’s instructions (MRC-Holland).

Results

Case report

Patient 1

This female patient, now aged 34 years, is the third child of healthy and non-consanguineous parents. She has two healthy brothers. She was born at term by spontaneous delivery after an uneventful pregnancy. Her birth weight was 2686 g and she had a relatively small head circumference (OFC of 31 cm; 10th percentile). During the neonatal period, she had strabismus, poor eye contact and inconsolable crying. She was referred to a clinical unit at 2 months of age. Physical examination revealed severe hypotonia and decelerated head growth. Microcephaly became more evident with time (OFCs of 38, 41, 43 and 44 cm at 6 months, 2, 5 and 7 years, respectively, all below the third percentile). The developmental milestones were severely delayed: she acquired head control at 7 months and turned over at 20 months. She displayed prominent hyperkinetic movement disorders with hand stereotypes, jerky movements of the upper limbs and frequent and inappropriate episodes of laughter. At 3 years of age, she had two episodes of hyperthermia-induced seizures. Although interictal EEG revealed focal spike discharges over the right parietal area, the seizures did not recur even without antiepileptic drug treatment. She remains incapable of sitting up unaided, as well as acquiring speech sounds and purposeful hand skills. Brain MRI scans performed at the age of 8 years showed delayed myelination in the frontal lobe with hypoplasia of the corpus callosum and frontal lobe (Fig. 1c,d).

Identification of FOXG1 mutations

We identified heterozygous FOXG1 mutations in both patients (Fig. 2). Patient 1 showed duplication of cytosine at nucleotides 256 (c.256dupC, p.Gln86ProfsX35), resulting in the loss of the three main functional domains of FOXG1 (Fig. 2a). This frameshift mutation has also been identified in an unrelated patient (20). Patient 2 showed a novel missense mutation (c.689G>A, p.Arg230His) within the DNA-binding forkhead domain, which affects a residue highly conserved in different species (Fig. 2b). Testing of their parents confirmed that both the mutations were de novo. We did not find deletions in FOXG1 in our cohort.
**Discussion**

We identified two heterozygous FOXG1 mutations in two patients with the congenital variant of RTT. These new cases provide additional support for delineating the clinical features of the FOXG1-related phenotypes. Both the patients had hypotonia and irritability in the neonatal period. Deceleration of head growth, leading to severe microcephaly, was recognized soon afterwards. They also had strabismus and poor eye contact. Their motor development was severely impaired and voluntary hand use was absent. They were unable to sit unaided and did not acquire speech sounds. They showed a prominent hyperkinetic movement disorder, with hand stereotypies and jerky movements of the upper limbs. These clinical features are similar to those previously described in patients with the congenital variant of RTT (13–20).

Large-scale molecular screenings of FOXG1 have been conducted mainly in female individuals with typical and atypical RTT (14–16). The preponderance of female patients with FOXG1 mutations may be because of this bias. Recent studies have shown point mutations and deletions in 14q12 in male individuals with the congenital variant of RTT as well (17, 20).

Given that FOXG1 is an autosomal gene, FOXG1 mutations may be responsible for the clinical features in female and male individuals with this form of RTT. The c.256dupC mutation has been identified in a male patient who presented similar clinical features to those observed in our female patient (20). This recurrent mutation caused duplication of cytosine after seven subsequent cytosines in FOXG1, suggesting that this cytosine stretch may be prone to replication errors and present a mutation hotspot in FOXG1.

FOXG1 is a DNA-binding transcription factor with a forkhead domain that represses target genes. It recruits transcriptional co-repressor proteins via two protein-binding domains (JARID1B and Groucho-binding domains). Interaction between FOXG1 and its co-repressor proteins is critical for early brain development (21). Missense mutations in the functional domains of FOXG1 or late truncating mutations possibly cause a milder phenotype, because the resulting proteins may retain some functions (16). However, Patient 2, who had a missense mutation of the DNA-binding forkhead domain, presented with a severe phenotype similar to that of Patient 1, who harbored a frameshift mutation that resulted in the loss of the three main
functional domains of FOXG1. The missense mutation (p.Arg230His) appeared to affect the affinity of FOXG1 for DNA. Our findings support the idea that a missense mutation in the forkhead domain impairs its target recognition and causes mislocalization of the protein in the nucleus (19). Thus, missense mutations within the DNA-binding domain, as well as clear loss-of-function mutations, can have a severe impact on FOXG1 function. Our data, taken together with previous findings, indicate that the genotype does not predict the severity of the phenotype. Indeed, a late truncating mutation (p.Tyr416X) that affects the C-terminal part of FOXG1 but maintains the three known functional domains reportedly causes the most severe phenotype (14).

FOXG1 plays an important role in forebrain development (10). Brain MRI scans of our two patients showed poor development of the frontal lobe and hypoplasia of the corpus callosum, which are similar to the findings of previous FOXG1 mutation reports (14, 15, 20). FOXG1 mutant mice are an interesting animal model for investigating how FOXG1 haploinsufficiency affects brain development and neuronal function. Although FOXG1 homozygous-mutant mice die shortly after birth with severe brain defects (9), the heterozygous mutants have less severe brain defects but still exhibit a reduction in the volume of the neocortex, hippocampus and striatum and a thin cortex because of reduced thickness of the superficial cortical layers (12). Furthermore, FOXG1 heterozygous-mutant mice exhibit learning deficits in fear-condition behavioral tests (11). These animal data are consistent with the findings that humans with FOXG1 haploinsufficiency have poor forebrain development as well as cognitive and motor defects.

In conclusion, we identified a novel mutation and a recurrent mutation in FOXG1 in two patients with the congenital variant of RTT. Our data support the involvement of FOXG1 in the molecular etiology of this form of RTT. We suggest that FOXG1 mutation analysis should be performed in female and male patients with developmental features suggestive of the congenital variant and brain malformations including poor frontal lobe development and hypoplasia of the corpus callosum.

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References