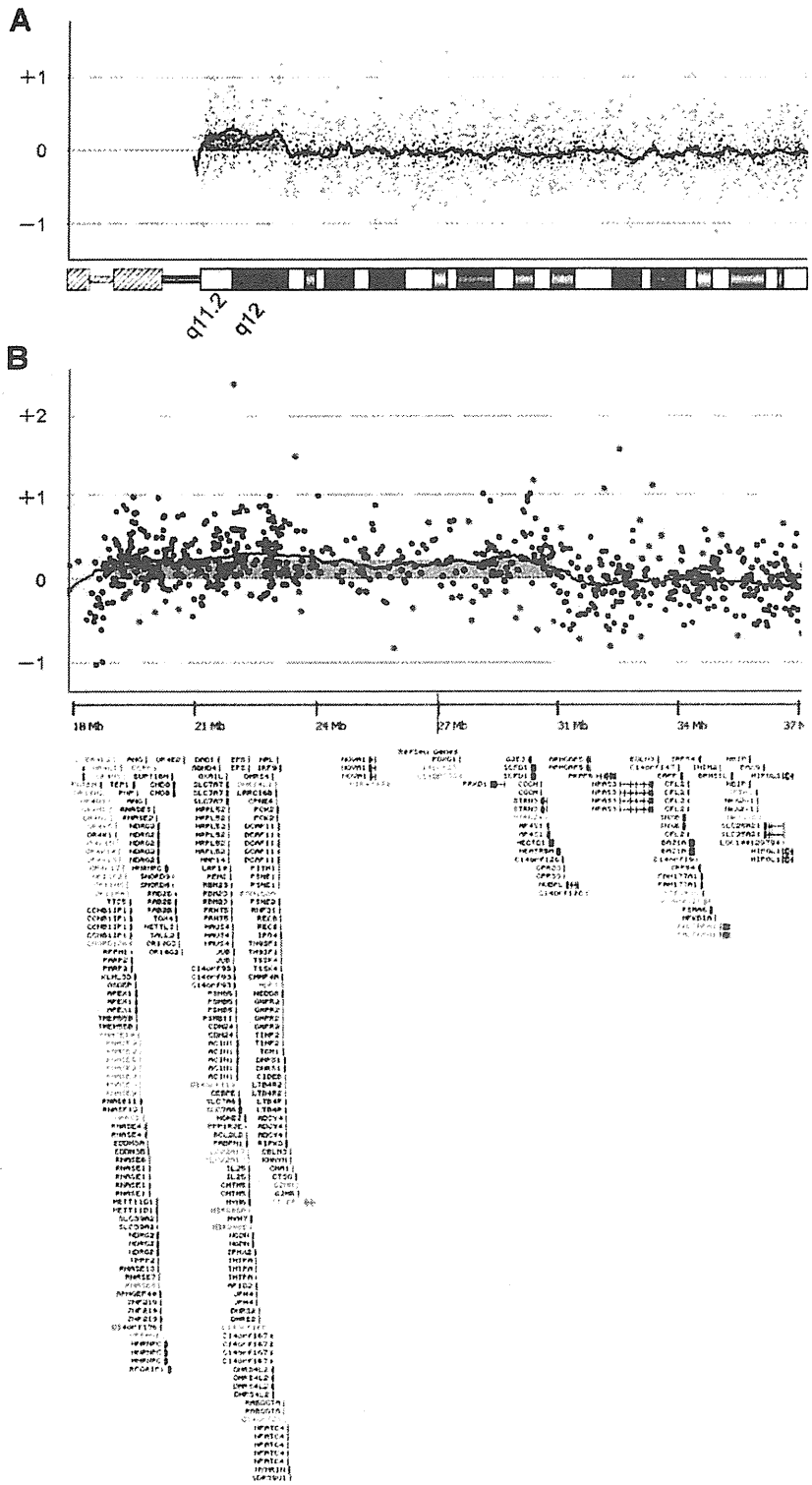


FIG. 2. Result of aCGH analysis for chromosome 14. A: Chromosome view indicating a genomic copy number gain of 14q11.2q12. The mean log2 ratio of this aberration region is 0.24, which indicates mosaicism of this marker chromosome. B: Aberration region expanded in gene view. The locations of the RefSeq Genes from the UCSC genome browser are shown under the gene view. [Color figure can be seen in the online version of this article, available at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552-4833].

SKY FISH analysis showed that the marker chromosome was derived from chromosome 14. Suspecting a relationship between the presence of marker chromosome 14 and upd(14)mat, we performed a DNA methylation test at *MEG3* in 14q32.2 [Hosoki et al., 2009], resulting in the abnormal hypomethylation of this gene (Fig. 1B). To confirm the origin of chromosome 14, microsatellite analysis using polymorphic markers on chromosome 14 was performed using ABI PRISM Linkage Mapping Set v2.5 (Applied Biosystems, Foster City, CA). Microsatellite polymorphism analysis indicated that both alleles of chromosome 14 were derived from the patient's mother and marker chromosome 14 was from her father (Fig. 1C). Fragment analysis at D14S275 showed a small peak of paternal inheritance, indicating the mosaic status of the marker of paternal origin (Fig. 1D). To further define the region of marker chromosome 14, microarray-based comparative genomic hybridization (aCGH) analysis performed using a 105K microarray kit (Agilent Technologies, Santa Clara, CA). The gain of genomic copy numbers was detected at 14q11.2-q12 indicating the molecular karyotype as arr14q11.2q12(19,761,035–30,941,609) × 1–1.5 (Fig. 2A,B). *FOXG1* was located in this region. Both parents had normal karyotypes.

## DISCUSSION

The present patient showed intrauterine growth retardation, feeding difficulty during the neonatal period, mild hypotonia, and postnatal growth retardation. These findings fit well with those of upd(14)mat. Chromosomal analysis revealed mosaicism of 47,XX, + mar(14)/46,XX. From the association of the clinical findings of this patient and the presence of small SMC 14, we suspected that her clinical symptoms were related to upd(14)mat and performed a DNA methylation test at *MEG3* in 14q32.2 and microsatellite polymorphism analysis. We successfully confirmed that her condition was upd(14)mat. Upd(14)mat is manifested in clinical features overlapping the Prader-Willi phenotype, particularly during infancy. Therefore, this syndrome is considered to be underestimated. Hosoki et al. [2009] recommended performing the *MEG3* methylation test for all undiagnosed infants with hypotonia.

Infantile spasms or seizures are uncommon complications of upd(14)mat. We postulated that an increased dosage of some genes in extra SMC could be responsible for West syndrome. To identify the affecting gene, we performed aCGH analysis. The analysis showed an increased dosage of 14q11.2-q12. These regions contain 124 RefSeq genes including *FOXG1*. Recently, *FOXG1* on 14q12 was reported to be a dose-sensitive gene, and duplication of this gene could cause severe epilepsy and developmental retardation [Yeung et al., 2009; Brunetti-Pierri et al., 2011]. Several patients with *FOXG1* haploinsufficiency have been associated with a Rett-like syndrome and epilepsy [Shoichet et al., 2005; Jacob et al., 2009]. Deletion of this gene could cause seizures, but not infantile spasms. On the other hand, duplication of this gene is reported to cause infantile spasms or seizures during infancy. Yeung et al. [2009] first reported a patient with 4.45 Mb microduplication in 14q12. This patient showed infantile spasms at 6 months. In addition, Brunetti-Pierri et al. [2011] studied six patients with duplication of the 14q12 region. In their series, the size of the duplication varied between ~3 and 14.5 Mb with the patient carrying the largest duplication showing a 14.5 Mb duplication in 14q11.2-q13.1. The shortest region of overlap for the duplicated regions in the six patients contained only three genes, including *FOXG1*. Three of the six patients showed infantile spasms. The authors concluded that *FOXG1* represented the most interesting candidate for explaining the abnormal neurodevelopment phenotypes [Brunetti-Pierri et al., 2011]. The present patient also showed infantile spasms. However, her seizures are not refractory and are well controlled by anti-epileptic drugs and ACTH therapy. Her developmental delay is also not so severe. The 14q11.2-q12 region involved in our patient was almost equal in size to the largest duplication in Brunetti-Pierri's series. *FOXG1* is a dose-sensitive gene, and the results of our patient strongly suggested that an increased dosage of a small amount of this gene might lead to a milder West syndrome and milder intellectual disability.

The West syndrome has a heterogeneous etiology. Recent molecular biological approaches have identified several causative genes. To date, *ARX*, *CDKL5*, *STXBP1*, and *SPTAN1* have been reported as being associated with West syndrome [Kato et al., 2006; Otsuka et al., 2010; Saitsu et al., 2010]. These previous reports state that haploinsufficiency or small mutations of these genes are related to their phenotypes. In addition, duplication of *FOXG1* was recently reported to cause severe epilepsy and developmental delay, including infantile spasms. Epilepsies associated with increasing gene dosage are rare [Ramocki et al., 2010; Brunetti-Pierri et al., 2011]. The results of the study of our patient will provide further evidence that not only duplication but also a small increasing dose of *FOXG1* could cause infantile spasms or seizure during early infancy. Of course, in our patient, the contribution of other genes in 14q11.2-q12 could not be excluded.

The first patient with upd(14)mat had a Robertsonian translocation (13;14) [Temple et al., 1991]. This syndrome was also reported in carriers of Robertsonian translocation involving chromosome 14 and in patients with normal karyotypes [Mitter et al., 2006]. Other chromosomal rearrangements frequently associated with upd are small SMCs [Starke et al., 2003; Liehr et al., 2004]. Mitter et al. [2006] reported 10 patients with upd(14)mat, two of whom had SMC 14. In our patient, we were also able to determine that the marker chromosome was derived from chromosome 14 by SKY FISH, microsatellite polymorphism analysis, and aCGH analysis. The coexisting of small marker chromosome 14 and upd(14)mat is likely to be originated in functional trisomic rescue or gamete complementation in the formation of the chromosome aberration in our patient [Kotzot, 2002].

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## REFERENCES

Brunetti-Pierri N, Paciorkowski AR, Ciccone R, Mina ED, Bonaglia MC, Borgatti R, Schaaf CP, Sutton VR, Xia Z, Jelluma N, Ruivenkamp C, Bertrand M, de Ravel TJL, Jayakar P, Belli S, Rocchetti K, Pantaleoni C, D'Arrigo S, Hughes J, Cheung SW, Zuffardi O, Stankiewicz P. 2011.

Duplications of *FOXG1* in 14q12 are associated with developmental epilepsy, mental retardation, and severe speech impairment. Eur J Hum Genet 19:102–107.

Hosoki K, Kagami M, Tanaka T, Kubota M, Kurosawa K, Kato M, Uetake K, Tohyama J, Ogata T, Saitoh S. 2009. Maternal uniparental disomy 14 syndrome demonstrates Prader-Willi syndrome-like phenotype. J Pediatr 155:900–903.

Jacob FD, Ramaswamy V, Andersen J, Bolduc FV. 2009. Atypical Rett syndrome with selective *FOXG1* deletion detected by comparative genomic hybridization: Case report and review of literature. Eur J Hum Genet 17:1577–1581.

Kato M. 2006. A new paradigm for West syndrome based on molecular and cell biology. Epilepsy Res 70:S87–S95.

Kotzot D. 2002. Supernumerary marker chromosomes (SMC) and uniparental disomy (UPD): Coincidence or consequence? J Med Genet 39:775–778.

Kotzot D, Utermann G. 2005. Uniparental disomy (UPD) other than 15: Phenotypes and bibliography updated. Am J Med Genet Part A 136A:287–305.

Liehr T, Claussen U, Starke H. 2004. Small supernumerary marker chromosomes (sSMC) in humans. Cytogenet Genome Res 107:55–67.

Mitter D, Buiting K, von Eggeling F, Kuechler A, Liehr T, Mau-Holzmann UA, Prott EC, Wieczorek D, Gillesen-Kaesbach G. 2006. Is there a higher incidence of maternal uniparental disomy 14 [upd(14)mat]? Detection of 10 new patients by methylation-specific PCR. Am J Med Genet Part A 140A:2039–2049.

Otsuka M, Oguni H, Liang JS, Ikeda H, Imai K, Hirasawa K, Imai K, Tachikawa E, Shimojima K, Osawa M, Yamamoto T. 2010. *STXBP1* mutations cause not only Ohtahara syndrome but also West syndrome—Result of Japanese cohort study. Epilepsia 51:2449–2452.

Ramocki MB, Tavyev YJ, Peters SU. 2010. The *MECP2* duplication syndrome. Am J Med Genet Part A 152A:1079–1088.

Saitsu H, Tohyama J, Kumada T, Egawa K, Hamada K, Okada I, Mizuguchi T, Osaka H, Miyata R, Furukawa T, Haginoya K, Hoshino H, Goto T, Hachiya Y, Yamagata T, Saitoh S, Nagai T, Nishiyama K, Nishimura A, Miyake N, Komada M, Hayashi K, Hirai S, Ogata K, Kato M, Fukuda A, Matsumoto N. 2010. Dominant-negative mutations in α-II spectrin cause West syndrome with severe cerebral hypomyelination, spastic quadriplegia, and developmental delay. Am J Hum Genet 86:881–891.

Shoichet SA, Kunde SA, Viertel P, Schell-Apacik C, von Voss H, Tommerup N, Ropers HH, Kalscheuer VM. 2005. Haploinsufficiency of novel *FOXG1B* variants in a patient with severe mental retardation, brain malformations and microcephaly. Hum Genet 117:536–544.

Starke H, Nietzel A, Weise A, Heller A, Mrasek K, Belitz B, Kelbova C, Volleth M, Albrecht B, Mitulla B, Trappe R, Bartels I, Adolph S, Dufke A, Singer S, Stumm M, Wegner RD, Seidel J, Schmidt A, Kuechler A, Schreyer I, Claussen U, von Eggeling F, Liehr T. 2003. Small supernumerary marker chromosome (SMCs): Genotype-phenotype correlation and classification. Hum Genet 114:51–67.

Temple IK, Cockwell A, Hassold T, Pettay D, Jacobs P. 1991. Maternal uniparental disomy for chromosome 14. J Med Genet 28:511–514.

Yeung A, Bruno D, Scheffer IE, Carranza D, Burgess T, Slater HR, Amour DJ. 2009. 4.45 Mb microduplication in chromosome band 14q12 including FOXG1 in a girl with refractory epilepsy and intellectual impairment. Eur J Med Genet 52:440–442.

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**CLINICAL RECORD**

# Successful cochlear implantation in a patient with mitochondrial hearing loss and m.625G>A transition

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## Abstract

*Objective*: We present a patient with mitochondrial hearing loss and a novel mitochondrial DNA transition, who underwent successful cochlear implantation.

*Case report*: An 11-year-old girl showed epilepsy and progressive hearing loss. Despite the use of hearing aids, she gradually lost her remaining hearing ability. Laboratory data revealed elevated lactate levels, indicating mitochondrial dysfunction. Magnetic resonance imaging showed diffuse, mild brain atrophy. Cochlear implantation was performed, and the patient's hearing ability was markedly improved. Whole mitochondrial DNA genome analysis revealed a novel heteroplasmic mitochondrial 625G>A transition in the transfer RNA gene for phenylalanine. This transition was not detected in blood DNA from the patient's mother and healthy controls. Mitochondrial respiratory chain activities in muscle were predominantly decreased in complex III.

*Conclusion*: This case indicates that cochlear implantation can be a valuable therapeutic option for patients with mitochondrial syndromic hearing loss.

**Key words:** Sensorineural Hearing Loss; Cochlear Implantation; Mitochondrial DNA

## Introduction

There have recently been many reported cases of sensorineural hearing loss of mitochondrial origin. In such patients, the effectiveness of cochlear implantation has been recognised in those with the m.1555A>G and m.3243A>G mutations.[1] However, the efficacy of such treatment for patients with other mitochondrial DNA mutations has not yet been defined.

Here, we present a patient with syndromic hearing loss, probably caused by a novel mitochondrial DNA mutation (m.625G>A), who gained excellent benefit from cochlear implantation.

## Case report

The patient, an 11-year-old girl, was the first child of healthy and nonconsanguineous Japanese parents. There was no family history of hearing loss or epilepsy, and the patient had had no perinatal problems. Her motor and cognitive development was normal, but she displayed an abnormally short stature for her age.

The patient's hearing difficulty had first been noticed by her mother at the age of six years. Two years later, the patient had been examined by an otolaryngologist for the first time, and bilateral hearing aids had been prescribed. However, her hearing ability continued to deteriorate. There had been no previous exposure to aminoglycoside antibiotics. In addition to hearing loss, at the age of eight years the patient had begun to suffer generalised tonic seizures, uncontrolled by valproic acid. At the age of 10 years, she had been referred to our institution, as her family had moved to the locality near our hospital.

On physical examination, the patient had a height of 119.0 cm (−3.0 standard deviations (SD)), a weight of 21.9 kg (−1.7 SD) and a head circumference of 53.6 cm (+0.9 SD). Cranial nerve and cerebellar functions were normal. Hypertrichosis was observed. Although her muscle force did not decrease, she was unable to exercise for extended periods of time. Deep tendon reflexes were normal, without spasticity. She was unable to communicate verbally, although her intelligence appeared normal as she could communicate in writing and could solve age-appropriate arithmetic problems. Otitis media was not found.

Laboratory data revealed mildly elevated blood lactate levels (24.0 mg/dl (normal range, <17 mg/dl), with a pyruvate level of 1.0 mg/dl (normal range, <0.9 mg/dl)), and noticeably elevated cerebrospinal fluid lactate levels (55.8 mg/dl, with a pyruvate level of 2.0 mg/dl).

Electroencephalography revealed no distinct epileptic discharge during waking and sleeping states.

Computed tomography showed no internal ear malformations. Magnetic resonance imaging (MRI) revealed mild brain atrophy without focal lesions (Figure 1a).

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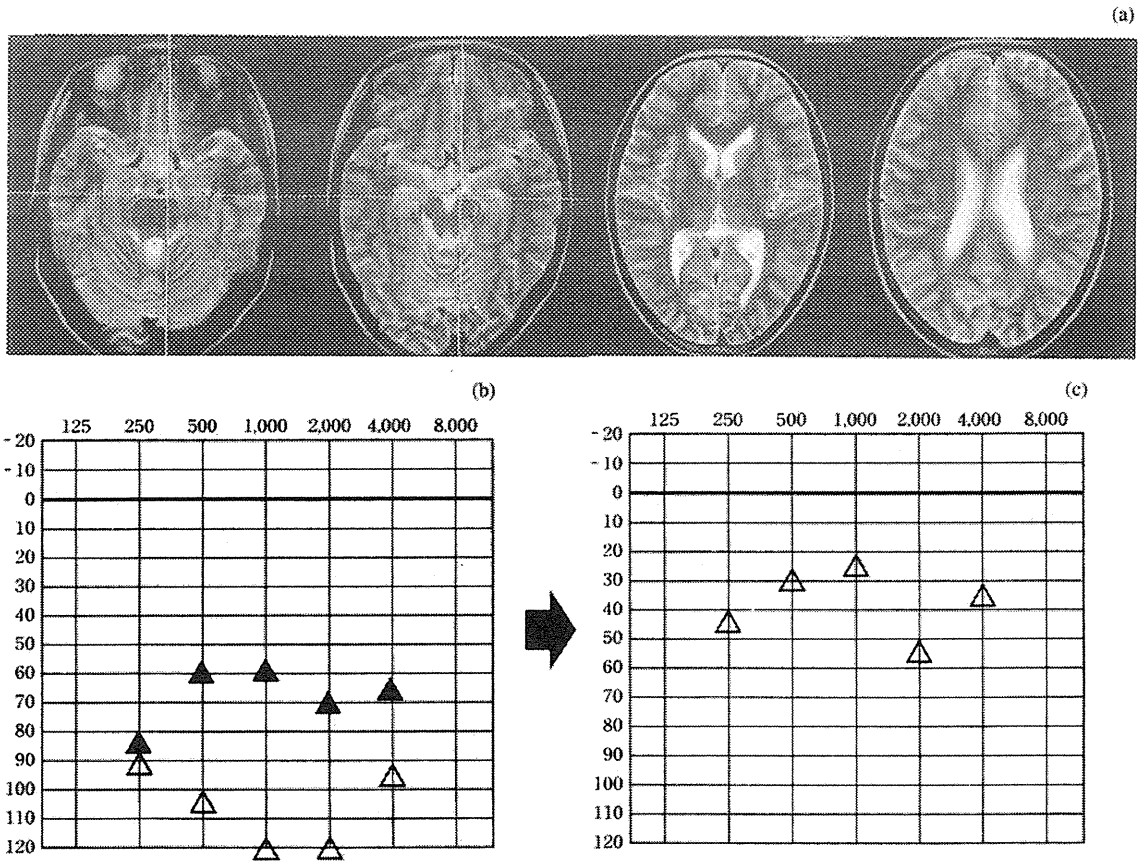


FIG. 1

(a) Axial magnetic resonance imaging brain scans, showing mild brain atrophy without focal lesions. (b) Left ear audiogram taken at 11 years, before cochlear implantation, following progression of hearing loss (hearing aids were no longer useful). (c) Left ear audiogram taken one month after implantation, showing significant improvement, with hearing thresholds of almost 25 – 45 dB. Δ = sound source 1 m away, without hearing aids; ▲ = with hearing aids in both ears

Formal pure tone audiography revealed hearing thresholds of between 90 and 120 dB at 250 through to 4 kHz. The patient's hearing aids only minimally improved her hearing thresholds (Figure 1b).

Auditory evoked potential testing showed a barely detectable auditory reaction at maximum intensity stimulation of 105 dB.

Therefore, the patient was considered to be a candidate for cochlear implantation.

Informed consent for participation in academic research was obtained from the patient and her parents.

During cochlear implantation, temporalis muscle and skin specimens were obtained.

Genomic DNA was extracted from blood, skin and muscle specimens. Sequencing of the whole mitochondrial genome was performed using the mitoSEQ resequencing system (Applied Biosystems, Foster City, California, USA). Polymerase chain reaction amplification was conducted, using forward mismatch primer (nucleotides 601–624, 5′-GCAATACACTGAAAATGTTTAGC-3′; where G = guanine, C = cytosine, A = adenine and T = thymine) and reverse primer (nucleotides 768–786, 5′-CGTTTTGAG CTGCATTGCT-3′). This enabled the m.625G>A sequence to be specifically recognised, and cut using the restriction enzyme BstOI (Promega, Madison, WI, USA). The proportion of heteroplasmy was approximately measured by using a mixture-template standard curve of wild type and mutant clones.

The activities of the mitochondrial respiratory chain complexes I, II, III and IV were assayed, using methods previously described.[2] We used the diagnostic criteria for respiratory chain disorders previously published by Bernier *et al.*[3]

*Cochlear implantation and clinical course*

The patient underwent left-sided cochlear implantation (using a CI24RCS device; cochlear LTD, Lane Cove, Australia) at the age of 11 years.

One month after implantation, she was able to use the telephone, clearly indicating improvement in her hearing function. Audiological data indicated a good response (Figure 1c). Her speech perception score increased to almost 100 per cent, from 0 per cent before surgery.

Twenty months after surgery, the patient and her parents were satisfied with her improved communication, and she continued to attend regular school classes. Her epileptic seizures were well controlled by carbamazepine and clonazepam. Her neurological signs and symptoms remained nonprogressive, possibly due to vitamin B1 supplementation.

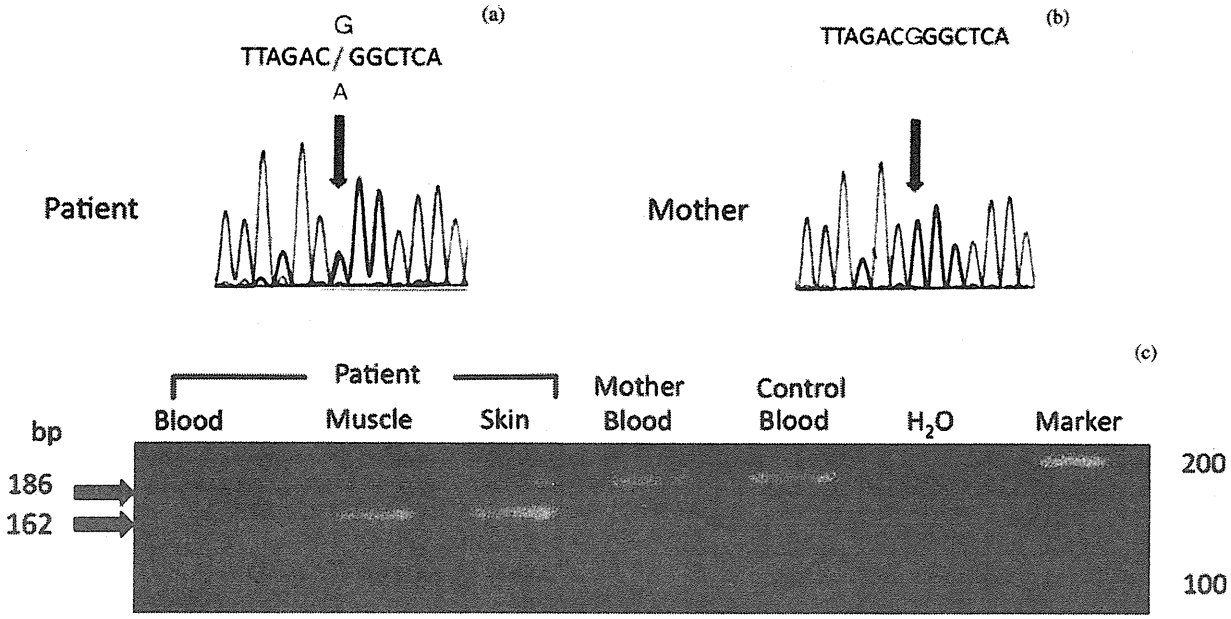


FIG. 2

Detection of the heteroplasmic m.625G>A transition. Diagrams represent screening of the patient's peripheral blood (a) and her mother's peripheral blood (b) for whole mitochondrial DNA genomes, and show the heteroplasmic m.625G>A transition in the patient's blood but not the mother's blood. (c) Electrophoretic strip showing that, in the presence of the m.625G>A mutation, the 186 base pair (bp) fragment was cleaved into 162 and 24 bp fragments (the latter not shown) by (BstOI is manufactured by Promega, Madison, WI, USA). This mutation was present in a heteroplasmic state in the patient's blood, muscle and skin, but was not detected in the mother's blood. Wild-type clones contained only the m.625G sequence.

*Histological analysis*

Unfortunately, many artifactual opaque fibers were observed in the temporalis muscle biopsy. Nevertheless, a few cytochrome c oxidase (COX) negative fibres were identified, although there were no ragged red fibres or strongly succinate dehydrogenase (SDH) reactive blood vessels (data not shown).

*Genetic analysis*

Whole mitochondrial DNA genome analysis, using peripheral blood DNA, detected two heteroplasmic base transitions: m.625G>A (Figure 2a) and m.5231G>A (data not shown).

The m.625G>A transition was present in a heteroplasmic state in the patient's blood, muscle and skin, but was not detected in her mother's blood (Figures 2b). The proportion of m.625G>A in muscle and skin was higher than that in blood (the approximate mutation load was 80 per cent in muscle and skin, and 70 per cent in blood) (Figure 2c). This transition was not present in 50 healthy controls.

The heteroplasmic m.5231G>A transition was present in both the patient's and her mother's blood.

*Biochemical analysis*

Respiratory chain enzyme assay showed that complex III activity was markedly decreased (30 per cent relative to citrate synthase, 17 per cent relative to complex II) while complex IV activity was slightly decreased (55 per cent relative to citrate synthase, 31 per cent relative to complex II).

## Discussion

Mitochondrial sensorineural hearing loss is divided into the nonsyndromic type associated with m.1555A>G and the syndromic type associated with m.3243A>G. The complex of mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (known as MELAS) is representative of the latter.[1]

We considered our case to be the syndromic type, because the patient had short stature and suffered from hypertrichosis and epilepsy. Moreover, she showed high lactate levels in her blood and cerebrospinal fluid, and mild brain atrophy on MRI.

In the syndromic type of mitochondrial hearing loss, the retrocochlear auditory pathways require investigation, specifically to establish whether the auditory peripheral nerve and central nervous system (CNS) are intact or not. However, successful cochlear implantation has been reported in patients with the mitochondrial encephalopathy, lactic acidosis and stroke-like episode complex.[4–6] Sue *et al.* have reported successful cochlear implantation in such a patient, who had profound, bilateral hearing loss.[6]

Our case, too, underwent successful cochlear implantation, despite possible CNS disorders. In patients with many types of mitochondrial, profound, sensorineural hearing loss, we speculate that cochlear implantation may represent a promising treatment, because hearing loss associated with mitochondrial disorders is more likely to be caused by cochlear dysfunction than retrocochlear abnormalities.[1,6,7] Results from a guinea pig cochlear model also suggest that chronic mitochondrial dysfunction may most predominantly affect the stria vascularis and supporting cells.[8] Therefore, we believe that cochlear implantation should be considered in patients with progressive sensorineural hearing loss associated with a mitochondrial disease, regardless of whether their hearing loss is syndromic or nonsyndromic.

Of course, this treatment option should be reviewed for the potential complications; it may develop contraindications on the MRI scan (unless the magnet in the receiver-stimulator has been moved), or adverse events such as post-implant meningitis due to bacterial cellulitis.[9]

In our patient, whole mitochondrial DNA genome analysis detected two different heteroplasmic, one-base substitutions: m.625G>A and m.5231G>A.

Although heteroplasmic single nucleotide polymorphisms are rare, the m.5231G>A transition is unlikely to be pathogenic, because it has been listed as a single nucleotide polymorphism in the Mitomap database,[10] and because it was carried by our patient's healthy mother.

On the other hand, the m.625G>A transition (which involves the transfer RNA gene for phenylalanine) has not previously been reported in association with disease. This transition lies in close vicinity to the site of the m.622G>A mutation, which has been reported to be present in mild mitochondrial disease with hearing impairment.[11] Moreover, other mutations in the same transfer RNA gene for phenylalanine (e.g. m.582T>C, m.583G>A, m.606A>G, m.608A>G, m.611G>A, m.618T>C, m.636A>G and m.642T>C) have been recognised and listed in Mitomap, with deafness frequently mentioned as a clinical symptom.[12–14] In our patient, respiratory enzyme studies revealed a significant defect in complex III and a possible slight defect in complex IV, relative to citrate synthase and complex II. These results resembled those for other mutations of the same mitochondrial transfer RNA gene for phenylalanine, such as m.622G>A and m.618T>C.[11,15] Moreover, m.625G>A was not identified in our patient's mother's peripheral blood DNA, implying a *de novo* origin of the mutation, although this is not conclusive because only blood DNA was available from the mother. Such sporadic mutations have been reported in other patients with the same mitochondrial transfer RNA phenylalanine gene mutation.[12,16]

- **This report describes the case of a girl with mitochondrial sensorineural hearing loss**
- **Cochlear implantation was effective, and improved the patient's quality of life**
- **The mitochondrial DNA 625G>A mutation may be pathogenic for syndromic hearing loss**

Accordingly, we conclude that the m.625G>A transition may cause mitochondrial respiratory dysfunction and syndromic hearing loss. Another standard muscle biopsy and cybrid study would clarify the pathogenicity of the m.625G>A transition.

## Conclusion

We report a sporadic case of progressive sensorineural hearing loss and epilepsy due to a mitochondrial disorder, successfully treated with cochlear implantation. The novel, heteroplasmic m.625G>A transition in the mitochondrial transfer RNA gene for phenylalanine may have been the pathogenic mutation in this case.

Cochlear implantation should be considered for patients with progressive, profound, bilateral, sensorineural hearing loss due to mitochondrial disease other than that due to the m.3243A>G mutation of the transfer RNA (tRNA(leu)) gene, or the m.1555A>G mutation of the 12s rivosomal RNA.

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### References

1 Sinnathuray AR, Raut V, Awa A, Magee A, Toner JG. A review of cochlear implantation in mitochondrial sensorineural hearing loss. *Otol Neurotol* 2003;**24**:418–26
2 Rahman S, Blok RB, Dahl H-HM, Danks DM, Kirby DM, Chow CW *et al.* Leigh syndrome: clinical features and biochemical and DNA abnormalities. *Ann Neurol* 1996;**39**:345–51
3 Bernier FP, Boneh A, Dennett X, Chow CW, Cleary MA, Thorburn DR. Diagnostic criteria for respiratory chain disorders in adults and children. *Neurology* 2002;**59**:1406–11
4 Rosenthal EL, Kileny PR, Boerst A, Telian SA. Successful cochlear implantation in a patient with MELAS syndrome. *Am J Otol* 1999;**20**:187–91
5 Karkos PD, Anari S, Johnson IJ. Cochlear implantation in patients with MELAS syndrome. *Eur Arch Otorhinolaryngol* 2005;**262**:322–4
6 Sue CM, Lipsett LJ, Crimmins DS, Tsang CS, Boyages SC, Presgrave CM. Cochlear origin of hearing loss in MELAS syndrome. *Ann Neurol* 1998;**43**:350–9
7 Yamasoba T, Tsukuda K, Oka Y, Kobayashi T, Kaga K. Cochlear histopathology associated with mitochondrial transfer $RNA^{Leu(UUR)}$ gene mutation. *Neurology* 1999;**52**:1705–7
8 Yamasoba T, Goto Y-I, Komaki H, Mimaki M, Sudo A, Suzuki M. Cochlear damage due to germanium-induced mitochondrial dysfunction in guinea pigs. *Neurosci Lett* 2006;**395**:18–22
9 Papsin BC, Gordon KA. Cochlear implants for children with severe-to-profound hearing loss. *N Engl J Med* 2007;**357**:2380–7
10 mtDNA Coding Region Sequence Polymorphism. In: http://www.mitomap.org/bin/view/MITOMAP/PolymorphismsCoding [6 August 2011]
11 Deschauer M, Swalwell H, Strauss M, Zierz S, Taylor RW. Novel mitochondrial transfer RNA (Phe) gene mutation associated with late-onset neuromuscular disease. *Arch Neurol* 2006;**63**:902–5
12 Mancuso M, Filosto M, Mootha VK, Rocchi A, Pistolesi S, Murri L. A novel mitochondrial tRNAPhe mutation causes MERRF syndrome. *Neurology* 2004;**62**:2119–21
13 Konings A, Van Camp G, Goethals A, Eyken EV, Vandevelde A, Azza JB *et al.* Mutation analysis of mitochondrial DNA 12SrRNA and tRNASer(UCN) genes in non-syndromic hearing loss patients. *Mitochondrion* 2008;**8**:377–82
14 Valente L, Piga D, Lamantea E, Carrara F, Uziel G, Cudia P *et al.* Identification of novel mutations in five patients with mitochondrial encephalomyopathy. *Biochim Biophys Acta* 2009;**1787**:491–501
15 Kleinle S, Schneider V, Moosmann P, Brandner S, Krähenbühl S, Liechti-Gallati S. A novel mitochondrial $tRNA^{Phe}$ mutation inhibiting anticodon stem formation associated with a muscle disease. *Biochem Biophys Res Commun* 1998;**247**:112–15
16 Darin N, Kollberg G, Moslemi AR, Tulinius M, Holme E, Grönlund MA *et al.* Mitochondrial myopathy with exercise intolerance and retinal dystrophy in a sporadic patient with a G583A mutation in the mt tRNA(phe)gene. *Neuromuscul Disord* 2006;**16**:504–6

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CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

# Clinical and Genomic Characterization of Siblings With a Distal Duplication of Chromosome 9q (9q34.1-qter)

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We report herein on two female siblings exhibiting mild intellectual disability, hypotonia in infancy, postnatal growth retardation, characteristic appearance of the face, fingers, and toes. Their healthy mother had a translocation between 9q34.1 and the 13pter. FISH and array CGH analysis demonstrated that the two children had an additional 8.5 Mb segment of the 9q34.1-qter at 13pter. The clinical features of the present cases were similar to those of previously reported 9q34 duplication cases; however, the present cases did not exhibit other abnormal behaviors, such as autistic features or attention deficit disorders, those are reportedly associated with 9q34 duplications. A 3.0 Mb region (9q34.1-q34.3) within 9q34 duplication in our patients are overlapped with duplication region of previously reported cases and is proposed to be critical for the presentation of several phenotypes associated with 9q34 duplications. © 2011 Wiley-Liss, Inc.

**Key words:** 9q34 duplication; intellectual disability; array CGH; dysmorphism

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## INTRODUCTION

Duplications of a distal region of the long arm of chromosome 9 (9q34) are rare and few cases have been reported. The first association between 9q34 duplications and phenotypic abnormalities were demonstrated in seven cases in a large pedigree [Allderdice et al., 1983]. The patients had low birth weight, initial poor feeding and thriving, slight psychomotor retardation, characteristic appearance of the face, fingers, and toes. Hyperactive behavior, heart murmur, and ptosis and strabismus were also noted. In another case, a girl of 3 years and 2 months carried a 9q34 duplication and a deletion of 3p26-pter due to a balanced translocation in her mother [Hodou et al., 1987]. This patient presented with dolichocephaly, characteristic facial appearance, and long thin fingers and toes, all of which are phenotypes noted in previous cases of 9q34 duplication; she also exhibited features associated with 3p terminal monosomy. In addition, duplication of 9q34-qter and monosomy of a small region on 12p13.3 in a male infant was described by Spinner et al. [1993]. The same patient was followed up at 18 years of age, and the duplicated and deleted regions were determined in detail by array-based comparative genomic hybridization (array CGH) analysis [Youngs et al., 2010]. The patient exhibited autistic features, hyperactivity, and attention deficit disorder in addition to the features associated with 9q34 duplications reported previously. Gawlik-Kuklinska et al. [2007] reported the case of a 17-year-old girl with an interstitial 7.4 Mb duplication of 9q34.1-q34.3 determined by array CGH analysis and compared the clinical features of the patient with those of previous cases. This patient exhibited the features common to patients with 9q34 duplications and three additional phenotypes of food-seeking behavior, obesity, and secondary amenorrhea.

In this report, we present two female siblings with 9q34.1-qter duplications and compare the clinical features and 9q34 duplication region of these patients with those of two previously reported cases using array CGH analysis. We also discuss the loci potentially responsible for the several phenotypes associated with a specific segment of 9q34.

Additional supporting information may be found in the online version of this article.

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## CLINICAL REPORTS

*Patient 1.* The patient was a 4-year-old girl and the first child of healthy, non-consanguineous Japanese parents. The family history was unremarkable. She was born at 40 weeks of gestation weighing 2,564 g and measuring 47.3 cm in length with an occipitofrontal circumference (OFC) of 33 cm, all within the standard range (10th–90th centile) for female Japanese neonates. The child was first evaluated at a cardiology clinic to investigate a heart murmur in the neonatal period. She was diagnosed with Ebstein anomaly, which was surgically repaired when she was 2-month old. At the age of 4 months, she was referred to our hospital due to generalized hypotonia and developmental delay. She rolled over at 12 months and sat up at 18 months. She stood with support at 24 months and started to walk unaided at 2.5 years. At 3 years of age, her height was 84 cm (−2.2 SD), body weight was 12.4 kg (−0.7 SD), and OFC was 49 cm (−0.2 SD). She could speak several meaningful words and understand simple sentences. Her developmental quotient (DQ) was 67, indicating mild intellectual disability. She was a sociable and friendly girl.

Clinical examination revealed that she had a characteristic facial appearance, including a round face, hypertelorism, almond-shaped palpebral fissures, telecanthus, depressed nasal bridge, short nose, microstomia, microretrognathia, short philtrum, and Cupid's bow upper lip (Fig. 1A). Her fingers were slender but not tapered (Fig. 1C). Neurological examination revealed that the cranial nerves were intact except for strabismus. Ocular fundi were normal. She walked slowly, but no ataxia was evident. Muscle tonus of the extremities was normal. Tendon reflexes of extremities were normal, and pathological reflex was absent. There was no evidence of epilepsy. Routine laboratory investigations were normal.

*Patient 2.* The patient was a 3-year-old girl and was the second child of the parents of Patient 1. She was born at 40 weeks of gestation weighing 2,874 g, measuring 49 cm in length with an OFC of 34.3 cm (all normal values for female Japanese neonates). She exhibited generalized hypotonia, but no feeding problems were observed during the neonatal period. She was referred to our hospital at the age of 19 months due to developmental delay. She exhibited head control at the age of 4 months. She rolled over at 9 months, sat at 10 months, and cruised between 11 and 12 months. She started to walk unaided at 18 months. Her height at 3 years was 88 cm (−2.4 SD), body weight was 10.1 kg (−2.7 SD), and OFC was 47 cm (−0.7 SD). DQ at the age of 3 was 72, indicating mild intellectual disability. She routinely exhibited affectionate and sociable behavior. She also had a round face with full cheeks, hypertelorism, almond-shaped palpebral fissures, telecanthus, depressed nasal bridge, short nose, microstomia, microretrognathia, short philtrum, and Cupid's bow upper lip (Fig. 1B). Ultrasonography of the abdomen showed no urogenital defects. No ophthalmic anomalies other than strabismus were found on routine evaluation. Neurological examination was not remarkable except strabismus. No epileptic seizures were observed. Routine laboratory investigations were normal. The clinical features of both patients and two previously reported cases of 9q34 duplication are summarized in Table I.

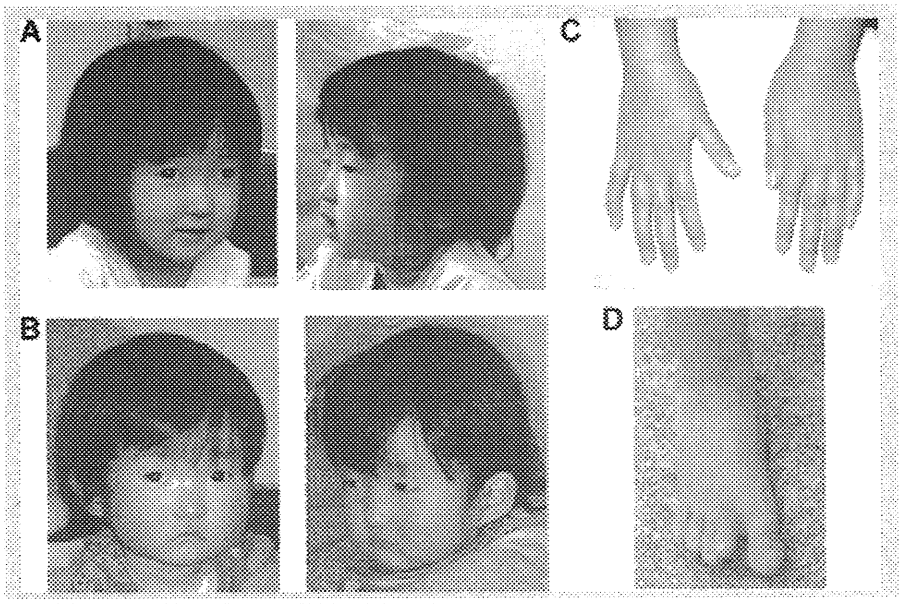


**FIG. 1. A: Frontal and lateral views of Patient 1 at 3 years of age. Phenotypes include round face, hypertelorism, telecanthus, short nose, depressed nasal bridge, microstomia, microretrognathia, short philtrum, and Cupid's bow upper lip. B: Frontal and oblique view of Patient 2 at 2 years of age. Phenotypes include round face, hypertelorism, almond-shaped palpebral fissures with telecanthus, short nose, depressed nasal bridge, microstomia, microretrognathia, short philtrum, and Cupid's bow upper lip. C: Hands of Patient 1 with long and thin fingers. D: The right foot of Patient 1. She has long toes with increased space between the first and second toes.**

TABLE I. Clinical Features of Patients With a 9q34.1-qter Duplication

| Phenotypic features | Gawlik-Kuklinska et al. [2007] | Youngs et al. [2010] | Patient 1 | Patient 2 |
|---|---|---|---|---|
| General | | | | |
| Hypotonia | + | + | + | + |
| Failure to thrive | + | − | − | − |
| Intellectual disability | Mild | Mild | Mild | Mild |
| Cardiac anomalies | − | + | + | − |
| Overweight/obesity | + | + | − | − |
| Scoliosis | + | − | − | − |
| Facial characteristics | | | | |
| Dolichcephaly | + | + | − | − |
| Facial asymmetry | + | + | − | − |
| Narrow horizontal palpebral fissures | + | + | − | − |
| Deep-set eyes | + | + | − | − |
| Long nose | + | + | − | − |
| Prominent chin | + | + | − | − |
| Microstomia | + | + | + | + |
| Microretrognathia | + | + | + | + |
| Short philtrum | + | − | + | + |
| Round face | − | − | + | + |
| Hypertelorism | − | − | + | + |
| Depressed nasal bridge | − | − | + | + |
| Almond-shape palpebral fissures | − | − | + | + |
| Telecanthus | − | − | + | + |
| Short nose | − | − | + | + |
| Extremities | | | | |
| Long and thin fingers | + | + | + | + |
| Increased space between first and second toes | + | + | + | + |

+, present; −, absent.

## MATERIALS AND METHODS

### Cytogenetic Analysis

Cultured lymphoblastoid cells isolated from each patient were treated with colchicine (Sigma–Aldrich, St. Louis, MO) for 1 hr at a concentration of 20 ng/ml in culture medium, and then incubated in a hypotonic solution of 75 mM KCl at 37°C for 30 min. After incubation, cells were fixed with Carnoy's fixative (3:1 mixture of methanol and acetic acid), spread on glass slides in a humid atmosphere and air-dried. Chromosomal analysis was carried out on GTG banded chromosomes at a resolution of 400–550 bands. Fluorescence in situ hybridization (FISH) was performed on metaphase chromosome spreads from each patient. Commercial probes covering subtelomeric regions were used according to the manufacturer's protocols (ToTelVysion, Abbott Laboratories. Abbott Park, IL) [Flint et al., 1995]. In order to confirm the chromosomal rearrangement in detail, additional FISH analysis was carried out from the patients and their parents using a series of bacterial artificial chromosome (BAC) clones (Clontech Laboratories, Inc., Mountain View, CA) that map to chromosome regions 9q34 and 13q31.

### Array CGH Analysis

Genomic DNA was isolated from peripheral blood lymphocytes of the two patients, their parents, and three normal controls by phenol/chloroform extraction. Array CGH analysis was performed using the Agilent Human Genome CGH 244K microarray platform (Agilent Technologies, Santa Clara, CA) according to standard protocols provided by the manufacturer. This array spans the entire human genome at a median resolution of approximately 8.9 kb. Genomic copy numbers were analyzed with Genomic Workbench (Standard Edition 5.0.14; Agilent Technologies).

### Southern Blot Analysis

Genomic DNA samples (10 μg) from the patients, their parents, and the normal controls were digested with *Hin*dIII, separated on a 0.9% agarose gel, and transferred by the alkaline method to a nylon membrane (Hybond-N+; GE Healthcare, Tokyo, Japan). The membrane was sequentially hybridized with [$\alpha$-$^{32}$P]dCTP-labeled *ABCA6* (exons 17–19) and *SP2* (exons 4–7) cDNA. A 301 bp *ABCA6* or a 798 bp *SP2* cDNA probe was prepared by amplifying the cDNA library of human lymphoblastoid cells with AmpliTaq-

Gold (Applied Biosystems, Foster City, CA) using specific primer pairs for *ABCA6* (sense: 5′-ATCTTTTCAGTGATCTGGATAAG-3′; antisense: 5′-AGGGTCAATAACACTTTAGTTT-3′), and for *SP2* (sense: 5′-GTCTACATCCGCACGCCTTC-3′; antisense: 5′-CCGCCGCAGTTGGCCTTA-3′), respectively. The PCR products were subcloned into pGEM-T easy vector (Promega, Madison, WI), and the nucleotide sequence of the probes was confirmed. Hybridization was performed in hybridization solution containing 5× standard saline citrate (SSC), 5× Denhardt's solution, and 0.5% SDS at 66°C overnight. The membrane was washed three times with 2× SSC containing 0.1% SDS at 37°C for 20 min and once with 0.1× SSC containing 0.1% SDS at 55°C for 10 min, and then radioactivity was quantified with a BAS 1800 image analyzer (FUJIFILM, Tokyo, Japan). The radioactivity of *ABCA6* versus *SP2* was determined for both patients and their parents (RP1, RP2, RF, RM) relative to the mean of the three normal controls (RC).

## RESULTS

### Additional 9q Subtelomeric Signal

The G-banding pattern of the both patients showed a 46,XX normal female karyotype. FISH with probes for subtelomeric regions revealed an additional 9q subtelomeric signal on the short arm of a D-group chromosome (chromosome 13, 14, or 15) in both patients (data not shown).

### 9q34 Duplication

To assess the chromosomal rearrangements in more detail, FISH analysis was performed in both patients and their parents with three BAC clones (RP11-40A7 and RP11-81N19) from chromosome 9q34 and RP11-524C15 from chromosome 13q31. The result indicated that the mother had a translocation; a 9q34.1-qter segment from one chromosome 9 was translocated to the terminus of chromosome 13p (Fig. 2, lower panel, indicated by a yellow arrow). Both patients had two normal chromosomes 9 and the derivative chromosome 13, which had an additional 9q34.1-qter segment at the p-terminal (Fig. 2, lower panels, indicated by yellow arrows). The father did not show any abnormalities (data not shown). These results indicate that the additional 9q34.1-qter segment at the p-terminal of chromosome 13 was of maternal origin (Fig. 2). The breakpoint of the translocation fell between two BAC clones at RP11-81N19 (129.2 Mb from the 9p terminus) and RP11-40A7 (133.4 Mb). Detailed mapping of the 13p breakpoint is not necessary because 13p does not code any genes. Thus, the duplicated segment was estimated to be 6.8–11.0 Mb derived from the 9q-terminus at position 140.2 Mb [46,XX.ish der(13)t(9;13)-(q34.1;pter)mat] (Fig. 2).

### 8.5 Mb Duplication of 9q34.1-qter

We performed array CGH using genomic DNA from each patient to determine the precise size of the additional 9q34 segment and

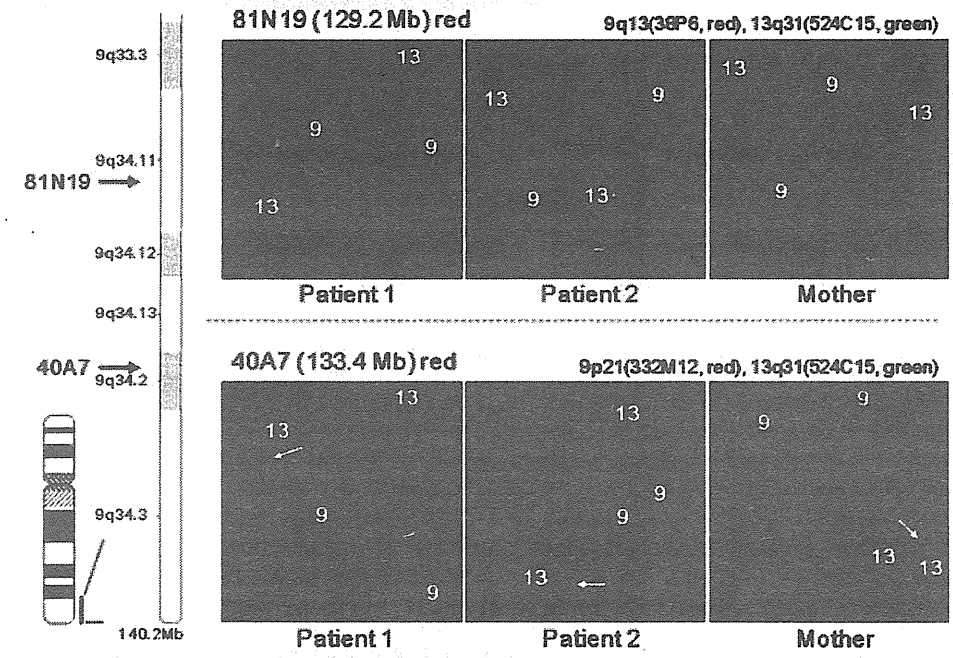


**FIG. 2. Partial metaphases of FISH with BAC clone RP11-81N19 probe show two red signals on both 9q terminal regions of the mother and each patient (upper panel) and no signal on chromosome 13. Partial metaphases of FISH with BAC clone RP11-40A7 probe show a red signal on one 9q terminal region and the short arm of derivative chromosome 13 (yellow arrow) in the mother and three signals in both patients; two red signals on both 9q terminal regions and an additional signal on the short arm of derivative chromosome 13 (yellow arrow) (lower panel). RP11-38P6 (red), RP11-332M12 (red), and RP11-524C15 (green) are used as markers for 9q13, 9p21, and 13q31, respectively.**

identify any other genomic abnormalities. Array CGH analysis of samples from Patients 1 and 2 demonstrated that the genomic copy number of 9q34.1-qter was 1.5-fold higher than the normal region (Fig. 3A,B). The size of the 9q34.1-qter duplication in both patients was approximately 8.5 Mb, from positions 131.7 to 140.2 Mb of chromosome 9 (Fig. 3). The breakpoint (position 131.7 Mb) of the 9q34 duplication in both patients was located in *FNBP1*, which encodes formin-binding protein 1. Analyses of Patients 1 and 2 revealed 12 and 15 copy number variations (CNVs), respectively (data not shown). CNVs are generally defined as the copy number differences of genomic DNA larger than 1 kb that vary in copy number between individuals. Patients 1 and 2 both had a 0.5-fold decrease in the genomic copy number of *ABCA6*, which encodes ATP-binding cassette, sub-family A, member 6; this is not recognized as a CNV (MIM 612504; Supplemental Fig. A and B).

### *ABCA6* Deletion in Both Patients and Their Mother

To confirm whether *ABCA6* was deleted in both patients and their parents, we performed Southern blot analysis using two cDNA probes against *ABCA6* (exons 17–19) and *SP2* (exons 4–7). *SP2* maps to 17q21, approximately 21 Mb proximal to *ABCA6*, and was not deleted in either patient based on the array CGH analysis. Southern blot analysis showed a decreased radioactive signal from *ABCA6* in family members (Supplemental Fig. C). When the mean ratio of *ABCA6* signal to *SP2* signal of the three normal controls was defined as 1.0, the ratio of *ABCA6* signal to *SP2* signal of the patients and their mother was approximately 0.5 and their father was 0.85 (Supplemental Fig. D). Thus, the both patients and their mother were heterozygous for an *ABCA6* deletion.

## DISCUSSION

Duplications of 9q34 cause intellectual disability and multiple congenital anomalies. Reported cases presented with a variety of clinical features depending on the size of the duplication and the presence of other chromosomal abnormalities [Allderdice et al., 1983; Hodou et al., 1987; Spinner et al., 1993; Gawlik-Kuklinska et al., 2007; Youngs et al., 2010]. Our patients had a 9q34.1-qter duplication and partial 13p monosomy due to a translocation between 9q34.1 and 13pter in their healthy mother. Array CGH and Southern blot analyses confirmed that these patients had a 9q34.1-qter duplication and a heterozygous deletion of *ABCA6* (17q24). Because 13p does not code for any genes and the heterozygous deletion of *ABCA6* did not cause any phenotypic abnormalities in the mother, the present patients exhibited "pure" 9q34.1-qter duplications without any other chromosomal abnormalities involving coding genes.

9q34 duplication has been analyzed in detail using array CGH in only two other patients. Gawlik-Kuklinska et al. [2007] reported the case of the female with a 7.4 Mb (RP11-269P11 to RP11-295G24; 127.3–134.7 Mb) duplication of 9q34.1-q34.3 (Fig. 4) and compared the patient's clinical features to those of previously reported 9q34 duplication cases [Spinner et al., 1993], including a male patient later shown to have a 13.8 Mb (126.4–140.2 Mb) duplication of 9q33.3-qter [Youngs et al., 2010] (Fig. 4). The following

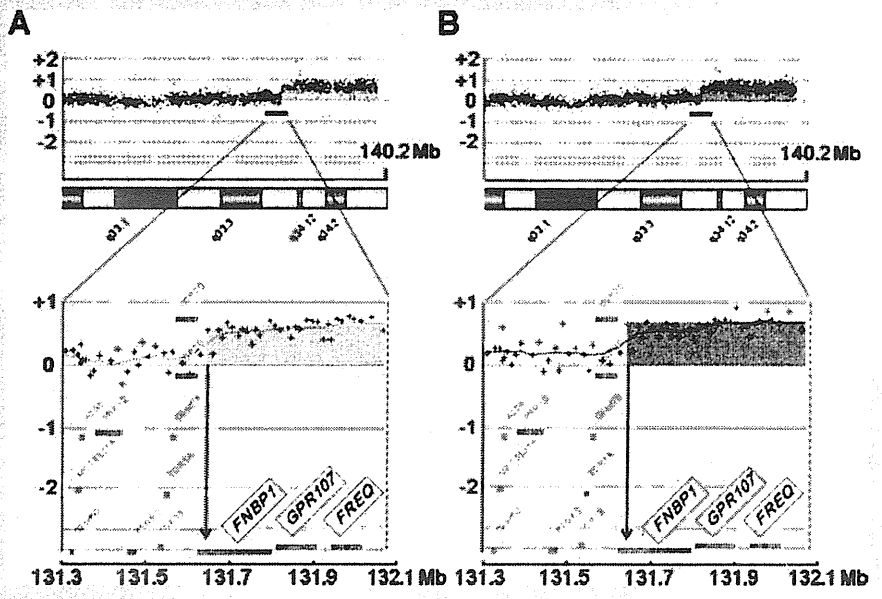


**FIG. 3. A: Graphical representation of the results of the array CGH analysis (Agilent 244K oligonucleotide array) from Patient 1 shows the duplication of distal 9q34.1-qter (upper panel). The x- and y-axis denote genomic position and $\log_2$ ratio, respectively. B: Graphical representation of the results of the array CGH analysis from Patient 2 also shows the duplication of distal 9q34.1-qter (upper panel). The breakpoint in 9q34 was located in the *FNBP1* gene (131.7 Mb) in both patients (lower panels of A and B), which indicated that the size of the duplication was approximately 8.5 Mb (131.7–140.2 Mb) according to NCBI human genome build 36.3.**

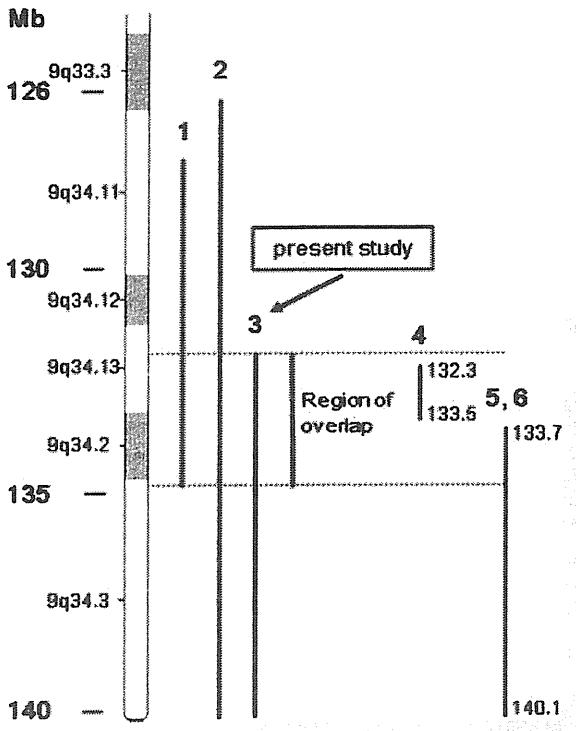


**FIG. 4. A schematic illustration based on NCBI human genome build 36.3 of 9q34 duplications from two previously reported cases, the present patients, and three patients from DECIPHER. The duplications in the previously reported patients and our patients are denoted as 1 (127.3–134.7 Mb) [Gawlik-Kuklinska et al., 2007], 2 (126.4–140.2 Mb) [Youngs et al., 2010], and 3 (131.7–140.2 Mb) [present study]. The 3.0 Mb (131.7–134.7 Mb) overlapping region of all three 9q34 duplications is denoted as "Region of overlap." The duplications in the patients from DECIPHER are denoted as 4 (P253579; age 17, 46,XX) (132.3–133.5 Mb), 5 (P254131; age 2, 46,XX) (133.7–140.1 Mb), and 6 (P255167; age 2, 46,XY) (133.7–140.1 Mb).**

features were common to both patients in these reports: hypotonia, intellectual disability, developmental delay, characteristic head and facial features associated with dolichocephaly, facial asymmetry, narrow palpebral fissures, deep-set eyes, long nose, prominent chin, microstomia, microretrognathia, and characteristic features of the extremities, including long thin fingers and toes and camptodactyly (Table I). Gawlik-Kuklinska et al. [2007] concluded a 7.4 Mb (127.3–134.7 Mb) duplicated region in their patient was critical for the phenotypes they observed (Fig. 4). Like these two previously reported cases, our patients also exhibited hypotonia, mild intellectual disability, developmental delay, microstomia, microretrognathia, and long thin fingers and toes. Thus, the 3.0 Mb region (131.7–134.7 Mb) of 9q34.13-q34.3 that overlapped in the cases reported by previous studies [Gawlik-Kuklinska et al., 2007; Youngs et al., 2010], and in our patients is most likely associated with the manifestation of the phenotypes observed in all four patients (Fig. 4, Table I). Unlike the other patients, our patients did not have dolichocephaly, facial asymmetry, narrow palpebral fissures, deep-set eyes, or long nose. The locus or loci associated with these phenotypes may be located in a region (127.3–131.7 Mb) that is proximal to the overlapping region (Fig. 4, Table I). Our patients exhibited other characteristic facial features, such as round faces, hypertelorism, almond-shaped palpebral fissures, telecanthus, and short nose; those were not observed in the previously reported cases (Table I). The distal-most segment of 9q34 (134.7–140.2 Mb) in our patients is the strongest candidate for the origin of these phenotypes (Fig. 4). However, these phenotypes were not observed in Patient 2 [Youngs et al., 2010], who had the same 9qter duplication. Therefore, the duplication of the proximal segment (127.3–131.7 Mb) of the overlapping region may have more impact on facial appearance than the duplication of the distal segment of the overlapping region. Clinical analyses of more patients with 9qter duplication (134.7–140.2 Mb) are necessary to determine the phenotypes caused by duplication of this region. It should be noted that DECIPHER (Database of Chromosomal Imbalance and Phenotype in Human using Ensembl Resources) includes two patients (P254131 and P255167) with the same 9q34.2-qter duplication (133.7–140.1 Mb) and heterozygous deletion of 17pter (0.01–0.41 Mb) (Fig. 4, numbers 5, 6). These patients exhibited hypotonia (non-myopathic), intellectual disability, developmental delay, patchy café au lait pigmentation spots on the skin, and speech delay. The heterozygous 17pter 0.4 Mb deletion has not been reported to cause any diseases, including intellectual disability. Another patient (P253579) presenting with facial abnormality, intellectual disability, and developmental delay had a 9q34.1-q34.2 duplication (132.3–133.5 Mb) in the 3.0 Mb overlapping region (Fig. 4, number 4). Notably, these two duplicated regions are included in the duplicated region in our patients, but they do not overlap with each other. These findings suggest the following correlations between duplicated chromosomal segments of 9q34 and phenotypes: (1) two duplicated segments (133.7–140.1 and 132.3–133.5 Mb) in 9q34 are associated with intellectual disability and developmental delay; and (2) the locus or loci associated with characteristic facial appearance may be within a duplicated region of 1.2 Mb (132.3–133.5 Mb), even though the detailed clinical features of P253579 are not available. Of the 18 genes that map to this 1.2 Mb region, individual duplications of 12 genes are reported in the Database of Genomic Variants (DGV; found in normal population). Thus, increased copy number of one or more of the other six genes (*FUBP3*, *EXOSC2*, *ABL1*, *NUP214*, *FAM78A*, and *PPAPDC3*) in this region could be the cause of the intellectual disability, developmental delay, and characteristic facial appearance observed in our patients and P253579.

Chromosomal rearrangements, arising from unequal recombination between repeated sequences, are found in a subset of patients with autism spectrum disorder [Marshall et al., 2008]. Abnormal behaviors, including hyperactive behavior [Allderdice et al., 1983], food-seeking behavior [Gawlik-Kuklinska et al., 2007], hyperactivity, attention deficit disorders, and atypical autism [Youngs et al., 2010], were also reported in some patients with 9q34 duplication. Unlike these patients, our patients exhibited friendly and affectionate social behaviors and did not exhibit autistic features or attention deficit disorder. It is important to repeatedly monitor the behaviors

of our patients to determine whether the 9q34.1-qter duplication is associated with abnormal behaviors. In summary, our findings indicate that the duplication of 9q34 is a heterogeneous clinical condition and duplications of different segments of 9q34 are associated with a variety of symptoms. Genomic and clinical analyses of more patients carrying 9q34 duplications are necessary to better characterize the correlation between clinical phenotypes and specific 9q34 loci.

## ACKNOWLEDGMENTS

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## REFERENCES

Allderdice PW, Eales B, Onyett H, Sprague W, Henderson K, Lefeuvre PA, Pal G. 1983. Duplication 9q34 syndrome. Am J Hum Genet 35:1005–1019.

Flint J, Wilkie AO, Buckle VJ, Winter RM, Holland AJ, McDermid HE. 1995. The detection of subtelomeric chromosomal rearrangements in idiopathic mental retardation. Nat Genet 9:132–140.

Gawlik-Kuklinska K, Iliszko M, Wozniak A, Debiec-Rychter M, Kardas I, Wierzba J, Limon J. 2007. A girl with duplication 9q34 syndrome. Am J Med Genet Part A 143A:2019–2023.

Hodou S, Yorifugi T, Tsuruta S, Hashida K, Ohta S, Ieshima A. 1987. Distal 9q trisomy syndrome: Report of the first oriental case and literature review. Acta Neonatal Jpn 23:347–352.

Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, Shago M, Moessner R, Pinto D, Ren Y, Thiruvahindrapduram B, Fiebig A, Schreiber S, Friedman J, Ketelaars CE, Vos YJ, Ficicioglu C, Kirkpatrick S, Nicolson R, Sloman L, Summers A, Gibbons CA, Teebi A, Chitayat D, Weksberg R, Thompson A, Vardy C, Crosbie V, Luscombe S, Baatjes R, Zwaigenbaum L, Roberts W, Fernandez B, Szatmari P, Scherer SW. 2008. Structural variation of chromosomes in autism spectrum disorder. Am J Hum Genet 82:477–488.

Spinner NB, Lucas JN, Poggensee M, Jacquette M, Schneider A. 1993. Duplication 9q34→qter identified by chromosome painting. Am J Med Genet 45:609–613.

Youngs EL, McCord T, Hellings JA, Spinner NB, Schneider A, Butler MG. 2010. An 18-year follow-up report on an infant with a duplication of 9q34. Am J Med Genet Part A 152A:230–233.

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FULL-LENGTH ORIGINAL RESEARCH

# *CDKL5* alterations lead to early epileptic encephalopathy in both genders

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## Summary

**Purpose: Genetic mutations of the cyclin-dependent kinase-like 5 gene (*CDKL5*) have been reported in patients with epileptic encephalopathy, which is characterized by intractable seizures and severe-to-profound developmental delay. We investigated the clinical relevance of *CDKL5* alterations in both genders.**

**Methods: A total of 125 patients with epileptic encephalopathy were examined for genomic copy number aberrations, and 119 patients with no such aberrations were further examined for *CDKL5* mutations. Five patients with Rett syndrome, who did not show methyl CpG-binding protein 2 gene (*MECP2*) mutations, were also examined for *CDKL5* mutations.**

**Key Findings: One male and three female patients showed submicroscopic deletions including *CDKL5*, and two male and six female patients showed *CDKL5* nucleotide alterations. Development of early onset seizure was a characteristic clinical feature for the patients with *CDKL5* alterations in both genders despite polymorphous seizure types, including myoclonic seizures, tonic seizures, and spasms. Severe developmental delays and mild frontal lobe atrophies revealed by brain magnetic resonance imaging (MRI) were observed in almost all patients, and there was no gender difference in phenotypic features.**

**Significance: We observed that 5% of the male patients and 14% of the female patients with epileptic encephalopathy had *CDKL5* alterations. These findings indicate that alterations in *CDKL5* are associated with early epileptic encephalopathy in both female and male patients.**

**KEY WORDS: *CDKL5*, Epileptic encephalopathy, Genomic copy number aberration, Mutation, Gender.**

Epileptic encephalopathies are a group of conditions in which neurologic deterioration results mainly from epileptic activity. The clinical and electroencephalography (EEG) characteristics depend on the age of onset and may change over time (Zupanc, 2009). An underlying genetic background has been suggested in patients with epileptic encephalopathy (Nabbout & Dulac, 2008). An X-linked gene coding for cyclin-dependent kinase-like 5 gene (*CDKL5*; MIM #300203) is one of the genes responsible for epileptic encephalopathy. Kalscheuer et al. (2003) identified de novo balanced X autosome translocations in two female patients with infantile spasms, in whom *CDKL5* was disrupted. Since then, the phenotypic spectrum of *CDLK5* abnormalities has expanded to include features resembling Rett syndrome (RTT; MIM #312750) with early onset seizures (Evans et al., 2005; Mari et al., 2005). Now, phenotypic features of *CDLK5* abnormalities are widely recognized as early infantile epileptic encephalopathy-2 (EIEE-2; MIM #30062) and are characterized as severe epileptic encephalopathy associated with early onset and refractory seizures (Archer et al., 2006; Pintaudi et al., 2008).

Although the consequence of *CDKL5* alterations has also been attributed to X-linked dominant infantile spasm syndrome-2 (ISSX2), mutations have been identified not only in female patients but also in some male patients with severe mental retardation and early onset intractable seizures (Elia et al., 2008; Fichou et al., 2009; Sartori et al., 2009).

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Therefore, we performed a comprehensive analysis for *CDKL5* in both female and male patients with epileptic encephalopathy.

## Methods

### Patients

After obtaining approval of the study protocol by the ethics committee of the institution and informed consent from the families of the patients, peripheral blood samples of 125 patients (59 male and 66 female) with epileptic encephalopathy of unknown etiology were collected, together with their clinical information, including neuroimaging findings. Epileptic encephalopathies are defined as disorders in which there is a temporal relationship between deterioration in cognitive, sensory, and motor function and epileptic activity, which includes frequent seizures and/or extremely frequent interictal paroxysmal activity (Nabbout & Dulac, 2003). Five female patients with RTT who did not show methyl CpG-binding protein 2 gene (*MECP2*) mutations (which are often associated with RTT) were also included in the cohort study for *CDKL5* mutations.

### Microarray-based comparative genomic hybridization (aCGH) analysis

The genomic copy numbers of the patients with epileptic encephalopathies were determined using the Human Genome CGH Microarray 105K (Agilent Technologies, Santa Clara, CA, U.S.A.) as described previously (Shimojima et al., 2010).

### Validation of the genomic copy number aberrations

Fluorescent in situ hybridization (FISH) analysis was performed for the large chromosomal deletion by using bacterial artificial chromosome (BAC) clones as probes, RP11-106N3 and CTD-2335C24 including *CDKL5* as a target, and RP11-1051J20 as a marker (Fig. 1, Table S1). The deletion identified in Patient 1 was too small to be detected by a BAC clone; therefore, multiplex polymerase chain reaction (PCR) analysis was used for validation. Two DNA fragments, exon 1B (421 bp) and exon 2 (350 bp) of *CDKL5*, were amplified in the same PCR reaction tube, separated by agarose gel electrophoresis, and visualized by ethidium bromide staining.

### Cohort study for *CDKL5*

Samples from 119 patients (58 male and 61 female) that showed no genomic copy number aberrations at the first screening by microarray-based comparative genomic hybridization (aCGH) in this study were included in the second cohort. Five samples obtained from female patients with RTT who did not show *MECP2* mutations were also included. The genomic sequences of all 23 exons of *CDKL5* were analyzed by the standard PCR direct-sequencing method using primers listed in Table S2. A recently identified exon 16B, which if included in the mature mRNA produces as a new *CDKL5* isoform, was also analyzed in this study (Fichou et al., 2010). When nucleotide changes were identified in samples for which parental samples were available, trio analyses were performed to test whether the mutation was de novo or familial. DNA samples collected from 100 healthy Japanese volunteers (50 male and 50 female) comprised the control cohort.

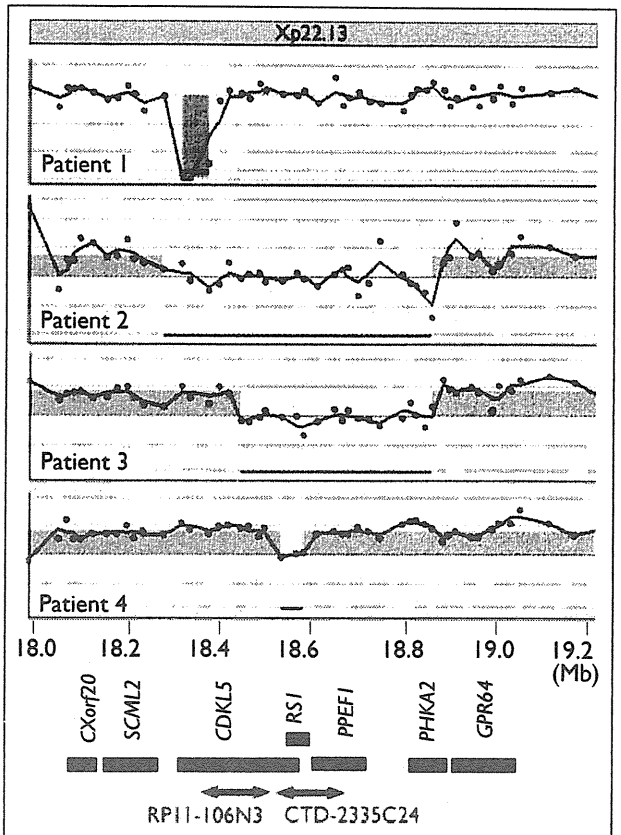


**Figure 1.**
Results of aCGH. The *CDKL5* region was expanded in Gene View with Agilent Genomic Workbench software (Agilent Technologies). The *x* and *y* axes indicate chromosomal positions and $\log_2$ ratios of intensity, respectively. Moving averages are shown in blue and red lines in male and female patients, respectively. Dots indicate the positions and $\log_2$ ratio of the probes. Rectangles and bars with arrows in both edges indicate the locations of the genes and BAC clones used for FISH, respectively. All patients show genomic copy number aberrations in the *CDKL5* region.


## Results

### Genomic copy number aberrations

In Patient 1, an aberration was identified at Xp22.13, indicating a nullisomy of this region (Fig. 1, Table S3). This region corresponds to exon 1 of *CDKL5*. Subsequent

multiplex PCR analysis using two sets of primers for exon 1B and exon 2 of *CDKL5* showed no band for exon 1B (Fig. 2A), thereby confirming the nullisomy of this region. Both parents of Patient 1 declined trio analysis.

aCGH analysis identified chromosomal aberrations in the *CDKL5* region in three female patients (Fig. 1, Table S3). Because male reference DNA was used in this study, genomic copy numbers of the normal female X chromosome regions showed $\log_2$ ratio of +1. Therefore, a $\log_2$ ratio of "0" indicates the same genomic copy numbers with the male reference sample, indicating a partial monosomy of this region in these patients. For Patients 2 and 3, identified aberrations were confirmed by FISH by detecting only one signal with RP11-106N3 and CTD-2335C24, respectively, indicating deletions in this region (Fig. 2B,C). For Patient 4, one of the targeted signals of CTD-2335C24 was weaker than the other, indicating a partial deletion of the targeted region (Fig. 2D). For Patients 2 and 3, the deletion region involved four genes: *CDKL5*; X-linked juvenile retinoschisis protein gene (*RS1*), which is responsible for X-linked juvenile retinoschisis (MIM #312700); protein phosphatase with EF hand calcium-binding gene (*PPEF1*); and phosphorylase kinase alpha 2 gene (*PHKA2*), which is responsible for X-linked hepatic glycogen storage disease (MIM #300798). For Patient 3, the deleted region involved the

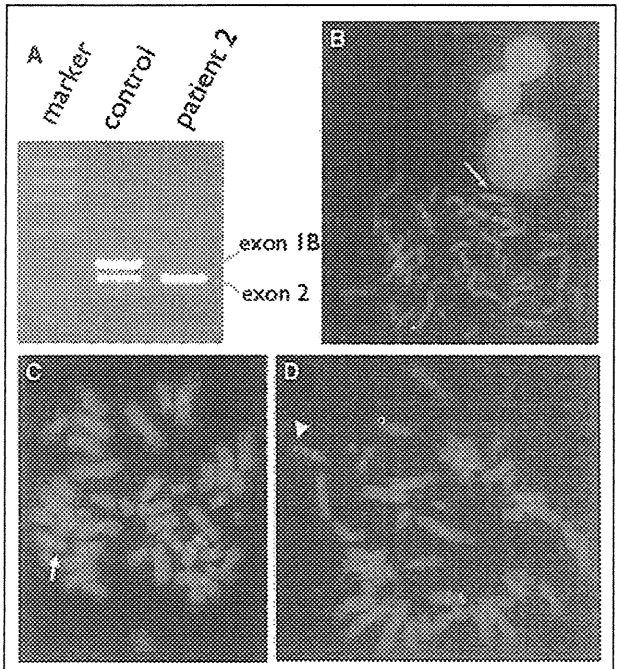


**Figure 2.**
Validations of genomic copy number aberrations. (**A**) Multiplex PCR amplification indicates deletion of exon 1B in Patient 1. The marker lane shows HaeIII digested $\varphi$X174 DNA. (**B**, **C**) FISH analysis indicates loss of the green signal on one of the X chromosomes (arrows). For Patient 2 (**B**) and Patient 3 (**C**), RP11-106N3 and CTD-2335C24 are used for the targets, respectively. Patient 4 (**D**) shows a week green signal labeled on CTD-2335C24 (arrowhead), indicating a partial deletion within CTD-2335C24 region.


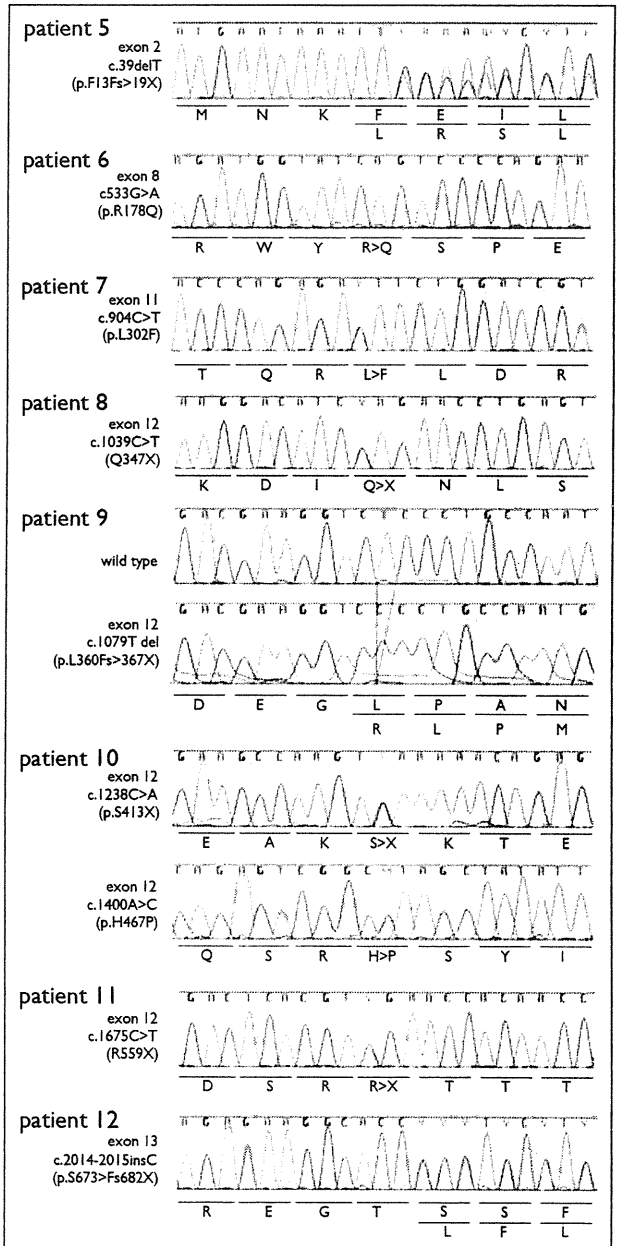


**Figure 3.**
Electrophoresis of the direct sequencing. Alphabetic symbols indicate amino acids. For Patients 5, 9, and 12, lines above the sequences indicate reference amino acid sequences, and lines below the sequences indicate amino acid changes caused by the mutations.

**Table 1. Summary of the clinical features and the identified *CDKL5* mutations in the patients reported in this study**

| No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gender | M | F | F | F | F | M | F | F | M | F | F | F |
| Initial concerns | EE | EE | EE | EE | EE | EE | EE | EE | EE | EE | EE | EE |
| Age at examination | 6 m | 2 y 7 m | 4 y 2 m | 2 y 7 m | 8 m | 1 y 9 m | 4 y 7 m | 2 y 6 m | 2 y | 2 y 1 m | 1 y 4 m | 1 y 4 m |
| Physical examination | | | | | | | | | | | | |
| Birth weight (g) | 3,458 | 3,016 | 2,400 | 2,716 | 2,612 | 3,800 | 2,560 | 3,352 | 3,228 | 2,955 | 3,250 | 2,976 |
| OFC at birth (cm) | 36.0 | 36.0 | 32.0 | 32.0 | 30.3 | 34.0 | NT | NT | 36.0 | NT | 33.0 | 33.5 |
| Microcephaly | – | – | + | + | + | – | – | – | – | – | – | + |
| Deceleration of head growth | – | – | – | – | + | – | – | – | – | – | – | + |
| Neurologic features | | | | | | | | | | | | |
| Hypotonia | – | – | + | + | + | + | +/– | +/– | + | +/– | + | + |
| Autistic features | NT | NT | + | NT | NT | – | +/– | + | NT | + | + | NT |
| Stereotype movement | NT | NT | + | + | + | – | – | + | NT | + | – | NT |
| Development | | | | | | | | | | | | |
| Sitting | – | – | – | – | – | – | – | + | – | – | – | + |
| Walking | – | – | – | – | – | – | – | – | – | – | – | – |
| Best motor development | Bedridden | Bedridden | Turn over | Turn over | Bedridden | Bedridden | Bedridden | Sit | Bedridden | Turn over | Turn over | Sit |
| Speech | – | – | – | – | – | – | – | – | – | – | – | – |
| Seizure | | | | | | | | | | | | |
| Age at onset of seizure | 1 m | 1 m | 2 m | 1.5 m | 2 w | 2 w | 4 d | 2 m | 3 m | 3 w | 6 m | 6 w |
| Persistent epilepsy | + | + | + | + | + | + | – | + | + | + | + | + |
| Seizure type | Infantile spasms | Infantile spasms | Spasms, focal Sz, myoclonia | Spasms, focal Sz | Spasms, focal Sz | Epileptic spasms | Infantile spasms | Tonic–clonic convulsion | Infantile spasms | Tonic–clonic convulsion | Tonic–clonic convulsion | Epileptic spasms |
| Radiologic examination | | | | | | | | | | | | |
| Brain MRI | Cerebral atrophy | Cerebral atrophy | Cerebral atrophy | Mild cerebral atrophy | Cerebral atrophy | Bifrontal-diffuse atrophy | Very mild cerebral atrophy | Mild frontal lobe atrophy | Cerebral atrophy | Cerebral atrophy | Frontal lobe atrophy and delayed myelination | Mild cerebral atrophy |
| Hypoperfusion revealed by SPECT | NT | NT | Left frontal | NT | No abnormality | Right frontal | Right temporal | Left frontal | No abnormality | NT | Frontal and left parietal | NT |

Continued

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**Table 1. Continued**

| No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mutation | | | | | | | | | | | | | |
| Location | Exon 1 | Whole exons | Large deletion after exon 4 | Large deletion after exon 16 | Exon 2 | Exon 8 | Exon 11 | Exon 12 | Exon 12 | Exon 12 | Exon 12 | Exon 12 | Exon 13 |
| Nucleotide change | | | | | c.39delT | c.533G>A | c.904C>T | c.1039C>T | c.1079delT | c.1238C>G | c.1400A>C | c.1675C>T | c.2014-2015insC |
| Amino acid change | | | | | p.F13Fs>19X | p.R178Q | p.L302F | p.Q347X | p.L360Fs>367X | p.S413X | p.H467P | p.R559X | p.S673>Fs682X |
| Domain | | | | | | Catalytic | | | | | | | |
| Inheritance | NT | De novo | De novo | De novo | NT | De novo | NT | NT | NT | De novo | De novo | NT | De novo |
| Novel/recurrent | Novel | Novel | Novel | Novel | Novel | Novel | Novel | Recurrent | Novel | Novel | Novel | Recurrent | Novel |
| Population study | NT | NT | NT | NT | None | None | None | None | None | None | None | None | None |
| Previous reports | | | | | | | | Artuso et al. (2010) | | | | Sartori et al. (2009) | |

M, male; F, female; EE, epileptic encephalopathy; y, years; m, months; w, weeks; d, days; OFC, occipitofrontal circumference; NT, not tested; Sz, seizures; SPECT, single-photon emission computed tomography.

latter half of *CDKL5* after exon 4. Patient 4 also showed a partial *CDKL5* deletion after exon 16, and *RS1*, which was encoded in the antisense direction. For Patients 2, 3, and 4, both parents were negative for these deletions, indicating de novo origin.

There were no other known pathogenic aberrations in these four patients. In the other two patients, genomic copy number aberrations in the region of the platelet-activating factor acetylhydrolase gene (*PAFAH1B1*), which is responsible for lissencephaly, were identified (Shimojima et al., 2010). The remaining 119 patients showed no genomic copy number aberrations and were included in the cohort study for *CDKL5* mutations.

### *CDKL5* nucleotide alterations

In the 119 patients, eight pathogenic mutations were identified (including six novel and two recurrent mutations), which consisted of three nonsense mutations, three frameshift mutations, and two missense mutations (Fig. 3, Table 1). Aristaless-related homeobox gene (*ARX*; MIM #300382) was not found in any of the male patients. Five patients with RTT who did not show *MECP2* mutations also did not show mutations in *CDKL5*. No control samples showed any of the nucleotide alterations identified in this study (Table 1).

Although Patient 10 showed a nonsense mutation (p.S413X), an additional missense mutation (p.H467P) was also identified in exon 12. Neither alteration was found in parents, indicating de novo occurrence of both mutations. Because a similar missense mutation (p.H467R) was reported to be a nonpathogenic mutation, p.H467P is also expected to be a nonpathogenic mutation (Evans et al., 2005).

### Clinical description

Brain magnetic resonance imaging (MRI) of the patients with *CDKL5* alterations is shown in Fig. 4. Many patients showed frontal dominant cerebral atrophy. All clinical data including the findings of neuroimaging are summarized in Table 1. The ability to sit autonomously was the maximum gross motor development achieved by these patients, and none of the patients acquired speech ability, indicating severe developmental delay. Only the oldest patient (Patient 7; 4 years and 7 months old), who had a missense mutation, showed seizure control after 3 years of age; all the other patients had persistent seizures.

## Discussion

Using aCGH analyses, Erez et al. (2009) identified partial *CDKL5* deletions in female patients with early onset intractable epilepsy. Mei et al. (2010) identified four patients who had total or partial deletions in *CDKL5*. However, those studies included only female patients. In comparison, the aim of our study was to identify candidate

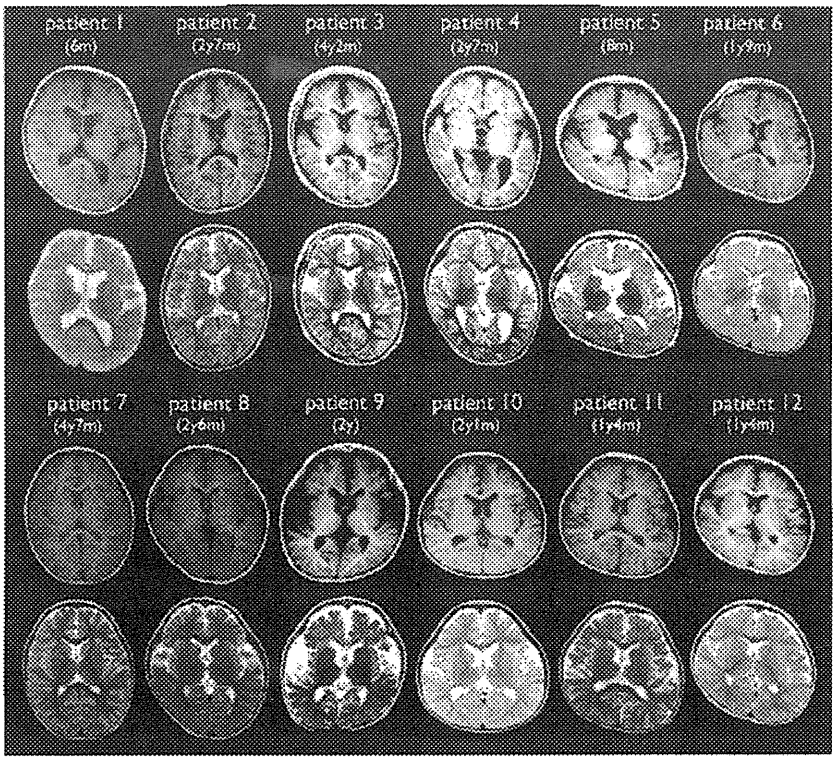


**Figure 4.**
Brain MRI findings of the patients. $T_1$- (up) and $T_2$-weighted (bottom) MRI indicates frontal atrophies in many patients, except for Patient 7. In Patient 6, spoiled gradient echo (SPGR) is shown instead of $T_1$.
*Epilepsia* © ILAE

genetic causes of early epileptic encephalopathy, and thus we recruited patients of both genders. Genomic copy numbers of whole chromosomes were comprehensively analyzed and submicroscopic chromosomal abnormalities of the *CDKL5* region were identified in both genders. The male patient (Patient 1) showed a partial deletion of *CDKL5*. Patients 2 and 3 showed large deletions in which the four neighboring genes, *CDKL5*, *RS1*, *PPEF1*, and *PHKA2*, were included. *RS1* and *PHKA2* are responsible for X-linked diseases, and the function of *PPEF1* is unknown. The remaining Patient 4 showed partial deletions of *CDKL5* and *RS1*. Therefore, phenotypic features of Patients 2, 3, and 4 suggest a causal role for *CDKL5* deletions in early epileptic encephalopathy. Despite the gender difference and the deleted size differences, the clinical severities of the patients with *CDKL5* deletions were similar between genders and similar to those of patients previously reported to have partial or total deletion of *CDKL5* (Van Esch et al., 2007; Erez et al., 2009; Bahi-Buisson et al., 2010; Mei et al., 2010).

Previously, *CDKL5* mutations were shown to affect mainly female patients, and their frequency has been estimated as approximately 9–28% in female patients with early onset seizures (Bahi-Buisson et al., 2008b; Nemos et al., 2009). However, those studies mainly included female patients. Elia et al. (2008) identified *CDKL5* mutations in three male patients with early onset epileptic encephalopathy. Male patients with *CDKL5* mutations or deletions have also been reported by others (Fichou et al., 2009; Sartori et al., 2009). In our study, initial identification of *CDKL5* deletions in both male and female patients with early epileptic encephalopathy prompted us to analyze *CDKL5* nucleotide sequences of both genders, and the results revealed nucleotide changes in two male patients and six female patients. We observed that the clinical severity of the disease did not differ between males and females. Therefore, male as well as female patients with early onset epileptic encephalopathy should be tested for *CDKL5* mutations.

Because *CDKL5* is located on Xp22.13, genetic traits of *CDKL5* alterations have been considered to be X-linked dominant, just as *MECP2* mutations are responsible for the majority of RTT cases, a neurologic disorder occurring almost exclusively in females. The rare male patients with *MECP2* mutations showed severe mental retardation but no RTT phenotype (Gomot et al., 2003). In comparison, there are no phenotypic differences between male and female patients with *CDKL5* mutations or deletions. Bahi-Buisson et al. (2008b) suggested that phenotypic heterogeneity does not correlate with the nature or the position of the mutations or with the pattern of X-chromosome inactivation. Indeed, no clear genotype–phenotype correlation between these factors has been established. Therefore, an important question is why clinical severity is the same between the genders. Based on previous reports, we know that the absence of CDKL5 protein is not lethal in males, and *CDKL5*