Clonazepam as an Effective Treatment for Epilepsy in a Female Patient with *NEXMIF* Mutation: Case Report

Masashi Ogasawara^{a, b, c} Eiji Nakagawa^a Eri Takeshita^a Kohei Hamanaka^d
Satoko Miyatake^d Naomichi Matsumoto^d Masayuki Sasaki^a

^aDepartment of Child Neurology, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan; ^bDepartment of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan; ^cDepartment of Genome Medicine Development, Medical Genome Center, National Center of Neurology and Psychiatry, Kodaira, Japan; ^dDepartment of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan

Established Facts

- The *NEXMIF* gene is located in the X chromosome, and mutations in *NEXMIF* cause X-linked intellectual disability.
- Thus far, only 24 female patients with *NEXMIF* mutations have been reported. Of these 24 patients, 20 have epilepsy.
- Only 2 of the female patients with *NEXMIF* mutations and epilepsy are well controlled by anti-epileptic drugs.

Novel Insights

- We were able to control the epilepsy of a female patient with the *NEXMIF* mutation with clonazepam.
- Sodium valproate was also effective but caused secondary amenorrhea.
- On the basis of a review of previously published cases, 4 female and 4 male patients share the same *NEXMIF* mutations thus far. Even with the same mutations, female patients have more severe epilepsy than male patients.

Keywords

Epilepsy · Facial dysmorphism · Intellectual disability · Obesity · X-linked inheritance

Abstract

The *NEXMIF* (*KIAA2022*) gene is located in the X chromosome, and hemizygous mutations in *NEXMIF* cause X-linked intel-

lectual disability in male patients. Female patients with heterozygous mutations in *NEXMIF* also show similar, but milder, intellectual disability. Most female patients demonstrate intractable epilepsy compared with male patients, and the treatment strategy for epilepsy is still uncertain. Thus far, 24 female patients with *NEXMIF* mutations have been reported. Of these 24 patients, 20 also have epilepsy. Until now, epilepsy has been controlled in only 2 of these female patients.

We report a female patient with a heterozygous de novo mutation, NM_001008537.2:c.1123del (p.Glu375Argfs*21), in *NEXMIF*. The patient showed mild intellectual disability, facial dysmorphism, obesity, generalized tonic-clonic seizures, and nonconvulsive status epilepticus. Sodium valproate was effective but caused secondary amenorrhea. We successfully treated her epilepsy with clonazepam without side effects, indicating that clonazepam might be a good choice to treat epilepsy in patients with *NEXMIF* mutations.

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Introduction

NEXMIF (KIAA2022) is located in the X chromosome, and hemizygous mutations in *NEXMIF* cause X-linked intellectual disability [Cantagrel et al., 2004]. Male patients with *NEXMIF* mutations have moderate to severe intellectual disability, autistic spectrum disorders, mental developmental delay, epilepsy, microcephaly, and facial dysmorphisms [Van Maldergem et al., 2013]. Recently, female patients with heterozygous mutations in *NEXMIF* also show similar symptoms [de Lange et al., 2016; Farach and Northrup, 2016]. Female patients with *NEXMIF* mutations usually show milder intellectual disability, but they often have intractable epilepsy compared with male patients [de Lange et al., 2016]. Thus far, 24 female patients with *NEXMIF* mutations have been reported. Of these 24 patients, 20 also had epilepsy, including generalized tonic, myoclonic, and absence seizures as well as Jeavons syndrome (eyelid myoclonia with absence seizures) [de Lange et al., 2016; Farach and Northrup, 2016; Borlot et al., 2017; Webster et al., 2017; Lambert et al., 2018; Samanta and Willis, 2020]. Because there are only a few reports regarding patients with *NEXMIF* mutations, therapeutic management for epilepsy in patients with *NEXMIF* mutations is not well established. Here, we report a patient diagnosed with intellectual disability, mild dysmorphic facial features, obesity, and epilepsy associated with a heterozygous *NEXMIF* mutation, NM_001008537.2:c.1123del (p.Glu375Argfs*21).

Case Presentation

The 46-year-old woman is the first child born of nonconsanguineous parents with no family history of neurological disorders after an uncomplicated pregnancy and delivery. She was overdue at 40 weeks and 6 days. Her birth length was 51.5 cm (+0.93 SD), weight was 3,500 g (+1.18 SD), and head circumference was 35.5 cm (+1.48 SD). She became ambulant at 1 year and 7 months. At

3 years of age, she was found to have speech delay. At 8 years of age, she was diagnosed as having a mild intellectual disability. At 10 years, she developed generalized tonic-clonic seizures (GTCS), and an electroencephalogram (EEG) showed spikes in her right frontal lobe. She was diagnosed with epilepsy and was treated with sodium valproate (VPA), which resulted in her epilepsy being well controlled. At the age of 14, she had menarche, and at 20, her menstruation became irregular; thereafter, she had secondary amenorrhea for >10 years. She visited a gynecologist at 35 years of age and underwent norgestrel and ethinyl estradiol combination therapy without having had a hormone test or abdominal ultrasound. After the treatment, she had menstruation and also experienced left facial nerve paralysis. Her parents were concerned about using norgestrel and ethinyl estradiol due to facial nerve paralysis and stopped visiting the gynecologist. She sometimes had GTCS resulting from poor compliance of drugs. At 37 years of age, her treatment was changed from VPA to clonazepam (CZP). Since then, her menstruation restarted, and her seizures have been well controlled. After switching to CZP, she underwent abdominal ultrasound, which showed only fatty liver. At the age of 38, she had nonconvulsive status epilepticus. An ictal EEG showed generalized spikes, polyspikes, and sharp waves (Fig. 1e) because she had stopped taking an anti-epileptic drug (AED) for several days. Since then, she has remained on CZP and has not experienced seizures for >7 years.

On physical examination, she shows mild dysmorphic features such as prognathism, thick lower lip, depressed nasal bridge, and an open mouth (Fig. 1a, b). Her body height, body weight, and head circumference were 155.3 cm (−0.5 SD), 66.7 kg (body mass index, 27.7), and 54.7 cm (−0.2 SD), respectively. Neurological examination shows no abnormalities except for mild intellectual disability and left facial paralysis. An ophthalmologist confirmed that she does not have any ophthalmological abnormalities. She can speak in full sentences, write Chinese characters that are usually learned by 10-year-old elementary school students, and can complete easy tasks at work. An interictal EEG shows no apparent paroxysmal waves (Fig. 1f). T2- and T1-weighted brain magnetic resonance imaging does not show any abnormalities on her cortex, white matter, or the hypothalamic-pituitary-gonadal axis (Fig. 1c, d). We performed trio whole-exome sequencing as previously described elsewhere [Aoi et al., 2019] and identified a heterozygous de novo mutation, c.1123del (p.Glu375Argfs*21), in *NEXMIF*, which was previously reported in a male patient [Alarcon-Martinez et al., 2019]. We confirmed the mutation by Sanger sequencing. We also analyzed the X-chromosome inactivation pattern in our patient, which was 60:40.

Discussion/Conclusion

Our patient demonstrates mild intellectual disability, mild facial dysmorphism, obesity, and treatable epilepsy. Female patients with 100% skewed X-inactivation patterns show severe intellectual disability resembling the male phenotype [de Lange et al., 2016]. A male patient with the hemizygous mutation c.1123del (p.Glu375Argfs*21) in *NEXMIF* not only has milder motor developmental delay, facial dysmorphic features, and no

Fig. 1. **a, b** Frontal and profile views of the patient showing prognathism, thick lower lip, depressed nasal bridge, and asymmetric and open mouth. **c, d** Brain magnetic resonance imaging showing no abnormal signs. **e** Generalized spikes, polyspikes, and sharp waves are seen in ictal electroencephalogram. **f** No epileptic discharge is seen in interictal electroencephalogram.

episodes of epilepsy compared with other male patients, but also has torpedo maculopathy and increased extra-axial space, which were not observed in our patient [Alarcon-Martinez et al., 2019]. The X-inactivation pattern in our patient (60:40) and the milder phenotype in male patients with the same mutation in *NEXMIF* were compatible with our patient's milder intellectual disability and epilepsy.

To get an overview of patients with *NEXMIF* mutations, we collected information regarding all male and female patients with *NEXMIF* mutations from the literature (online suppl. Table 1; for online suppl. material, see www.karger.com/doi/10.1159/510172) [Cantagrel et al., 2004; Van Maldergem et al., 2013; Athanasakis et al., 2014; Charzewska et al., 2015; Kuroda et al., 2015; Moyses-Oliveira et al., 2015; de Lange et al., 2016; Farach and Northrup, 2016; Borlot et al., 2017; Webster et al., 2017;

Lambert et al., 2018; Lorenzo et al., 2018; Alarcon-Martinez et al., 2019; Samanta and Willis, 2020]. To date, 24 female and 16 male patients with *NEXMIF* mutations have been reported. All of the female patients in this study, but only 4 of 16 male patients, were found to have de novo *NEXMIF* mutations. Overall, 96% of the female patients have truncating mutations and 2 of 13 female patients (15%) have skewed X-inactivation (Table 1). Further, 71% of the female patients with *NEXMIF* mutations have mild intellectual disability, and 19% of the male patients have mild intellectual disability (Table 1). In addition, 84% of the female patients have epilepsy and 18 of 25 female patients (72%) have intractable epilepsy; however, only 5 of 16 male patients (31%) have intractable epilepsy (Table 1). Generalized, myoclonic, absence, and GTCS are often noted in female patients (Table 1). In male patients, generalized, GTCS, and spasms

Table 1. An overview of patients with *NEXMIF* mutations

	Male	Female
Number of patients	16	25
Mutation		
De novo	4/16 (25%)	25/25 (100%)
Truncating	11/16 (69%)	24/25 (96%)
Complex rearrangements	2/16 (13%)	1/25 (4%)
Gross insertions	3/16 (19%)	0/25 (0%)
X-chromosomal inactivation		
Random	–	11/13 (85%)
Skewing	–	2/13 (15%)
Intellectual disability	16/16 (100%)	24/25 (96%)
Mild	3/16 (19%)	17/24 (71%)
Moderate	1/16 (6%)	2/24 (8%)
Severe	12/16 (75%)	5/24 (21%)
Autism	13/16 (81%)	11/23 (48%)
Dysmorphism	10/15 (67%)	9/23 (39%)
Microcephaly	5/14 (36%)	3/21 (14%)
Postnatal growth retardation	8/13 (62%)	4/22 (18%)
Obesity	3/13 (23%)	7/21 (33%)
Hypotonia	9/13 (69%)	9/22 (41%)
Spasticity	4/14 (29%)	0/21 (0%)
Epilepsy	9/16 (56%)	21/25 (84%)
Intractable	5/8 (62%)	18/21 (86%)
Controlled	3/8 (38%)	3/21 (14%)
Seizure type		
Generalized	5/9 (56%)	17/21 (81%)
Myoclonic	1/9 (11%)	16/21 (76%)
Absence	1/9 (11%)	14/21 (67%)
Tonic	0/9 (0%)	5/21 (24%)
Atonic	1/9 (11%)	8/21 (38%)
Clonic	0/9 (0%)	5/21 (24%)
GTCS	3/9 (33%)	9/21 (43%)
Focal	1/9 (11%)	4/21 (19%)
Spasm	3/9 (33%)	0/21 (0%)
Status epilepticus	0/9 (0%)	7/16 (44%)

are often observed (Table 1). Only 2 female cases with a milder epilepsy were controlled by VPA and topiramate, respectively, and 3 male cases were well controlled without using AED or by succeeding withdrawal using AED [Borlot et al., 2017; Lambert et al., 2018; Lorenzo et al., 2018]. Thus far, there are no concrete treatment strategies for epilepsy in patients with *NEXMIF* mutations. Although VPA has shown a good effect on 2 patients, including our case, our patient experienced amenorrhea as a side effect of VPA [Isojärvi et al., 1993]. Moyses-Oliveria et al. [2015] reported that one female patient with *NEXMIF* mutation had primary amenorrhea, but the details of this are not available. Eight of 13 male (62%) and 4 of 22 female patients (18%) also show postnatal growth retardation (Table 1), and Kuroda et al.

[2015] reported that a patient experienced central hypothyroidism. Ishikawa et al. [2012] and Kuroda et al. [2015] also suspected that *NEXMIF* mutation might be associated with hypothalamic dysfunction because *NEXMIF* expression was seen ubiquitously in mouse brain at any stage and was maintained in the ventral thalamic nucleus and ventromedial hypothalamic nucleus even in the adult stage [Ishikawa et al., 2012; Kuroda et al., 2015]. Because our patient experienced secondary amenorrhea for >10 years, we replaced VPA with CZP. Her menstruation restarted, and she has not experienced epilepsy for >7 years. CZP might be a good option to treat epilepsy in female patients with *NEXMIF* mutations because these patients might have hormone abnormalities.

Recently, *NEXMIF* knock-out (KO) mice have been reported [Gilbert et al., 2020]. *NEXMIF* KO mice recapitulate patients with *NEXMIF* mutations and showed reduced sociability and communication, deficits in learning and memory [Gilbert et al., 2020]. Furthermore, *NEXMIF* KO mice also have seizures including generalized, tonic-clonic, absence, and myoclonic seizures, which are similar to those observed in patients with *NEXMIF* mutations (Table 1). *NEXMIF* KO mice also showed a significant dysregulation in synaptic protein expression with a reduction in both glutamatergic and GABAergic synaptic proteins [Gilbert et al., 2020]. Reduced GABA signaling is thought to be one of the causative factors of epilepsy [Oakley et al., 2013]. As CZP acts as a GABA-enhancing drug [Oakley et al., 2013], CZP might have a good effect on epilepsy due to decreased GABAergic synaptic proteins caused by the loss of function of *NEXMIF*; however, further studies in animal model are necessary.

To the best of our knowledge, the 12 male patients with inherited *NEXMIF* mutations have at least 15 female relatives who have been genetically confirmed to be carriers of the same *NEXMIF* mutations or were suspected to carry the same *NEXMIF* mutations, based on their inheritance patterns [Cantagrel et al., 2004; Van Maldergem et al., 2013; Charzewska et al., 2015; Lambert et al., 2018; Lorenzo et al., 2018]. Among these 15 female relatives, 3 carriers, including P23 (online suppl. Table 1), were symptomatic, whereas the remaining 12 female carriers were asymptomatic. Because the disease is associated with loss of *NEXMIF* function, the majority of asymptomatic female carriers might be explained by a preferable X-inactivation pattern that primarily inactivates the mutant allele [Van Maldergem et al., 2013; Viggiano et al., 2013; de Lange et al., 2016]. In contrast, an

unfavorable X-inactivation pattern that primarily inactivates the wild-type allele could cause symptoms in female carriers [Viggiano et al., 2013; de Lange et al., 2016]. Previously, the frequency of epilepsy in patients with *NEXMIF* mutation was higher in female patients than in male patients (Table 1). However, de Lange et al. [2016] mentioned that this could be a result of selection bias. Thus far, 4 female (P12, P17, P23, and P25) and 4 male (P35, P38, P40, and P41) patients who share the same *NEXMIF* mutations have been reported (online suppl. Table 1). Lambert et al. [2018] reported familial cases, but the other cases are sporadic cases. All 4 male patients have severe intellectual disability, but 3 of the 4 female patients have mild to moderate intellectual disability, which is compatible with X-linked diseases [Lubs et al., 2012]. All 4 female patients had epilepsy; 2 of the 4 have refractory epilepsy, and the remaining 2 female patients were well controlled. On the other hand, only 2 out of the 4 male patients have epilepsy, which was well controlled in both. Furthermore, P38 reported only 1 episode of grand mal seizure and, therefore, does not take any AED. Although there are few reports of male and female patients who share the same mutation, female patients with mutations in *NEXMIF* might have more severe epilepsy. Understanding clinical discrepancy between male and female patients with mutations in *NEXMIF* might be important to treat these patients with *NEXMIF* mutations appropriately.

In conclusion, we report a female patient with a heterozygous de novo mutation, NM_001008537.2:c.1123del (p.Glu375Argfs*21), in *NEXMIF*. The patient shows mild intellectual disability, mild facial dysmorphism, obesity, GTCS, and nonconvulsive status epilepticus. We successfully treated her epilepsy with CZP without any side effects, indicating that CZP might be a good choice to treat epilepsy in patients with *NEXMIF* mutations.

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Statement of Ethics

Informed written consent for publication was obtained from the patient's parents. We also received permission to publish the patient's photographs. This study was approved by the ethical committees of the National Center of Neurology and Psychiatry (A2019-131).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.O., E.N., E.T., and M.S. managed the patient, drafted the article, and revised the final version. K.H., S.M., and N.M. contributed to sequence analysis. All authors read and approved the final manuscript.

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