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SHORT COMMUNICATION

A novel missense mutation in the HECT domain of *NEDD4L* identified in a girl with periventricular nodular heterotopia, polymicrogyria and cleft palate

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We identified a novel *de novo* heterozygous missense mutation in the *NEDD4L* gene (NM_015277: c.2617G>A; p.Glu873Lys) through whole-exome sequencing in a 3-year-old girl showing severe global developmental delay, infantile spasms, cleft palate, periventricular nodular heterotopia and polymicrogyria. Mutations in the HECT domain of *NEDD4L* have been reported in patients with a neurodevelopmental disorder along with similar brain malformations. All patients reported with *NEDD4L* HECT domain mutations showed periventricular nodular heterotopia, and most had seizures, cortex anomalies, cleft palate and syndactyly. The unique constellation of clinical features in patients with *NEDD4L* mutations might help clinically distinguish them from patients with other genetic mutations including *FLNA,* which is a well-known causative gene of periventricular nodular heterotopia. Although mutations in the HECT domain of *NEDD4L* that lead to AKT-mTOR pathway deregulation in forced expression system were reported, our western blot analysis did not show an increased level of AKT-mTOR activity in lymphoblastoid cell lines (LCLs) derived from the patient. In contrast to the forced overexpression system, AKT-mTOR pathway deregulation in LCLs derived from our patient seems to be subtle.
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INTRODUCTION

Malformations of cortical development are etiologically heterogeneous and include several disorders induced by the disruption of each cortical development step.[1] For instance, periventricular nodular heterotopia (PNH) appears due to abnormal neuronal migration, while polymicrogyria is the result of abnormal postmigrational development. Genetic studies have identified several genetic mutations underlying malformations of cortical development, which is frequently observed among the symptoms of a genetic syndrome.[2] Mutations in genes within the phosphatidylinositol-3-kinase (PI3K)-AKT-mTOR pathway (mTOR pathway) cause a wide range of developmental disorders.[3,4] Recently, mutations in the HECT domain of the *NEDD4L* gene were reported that lead to mTOR pathway deregulation, resulting in PNH.[5] Here, we report a novel *de novo* heterozygous missense mutation in the HECT domain of *NEDD4L* (NM_015277:c.2617G>A; p.Glu873Lys) identified by whole-exome sequencing in a Japanese 3-year-old female patient with PNH, polymicrogyria, severe global developmental delay, infantile spasms and cleft palate.

CASE REPORT

Herein we report a female patient, born at 41-week gestation, to unrelated, healthy Japanese parents. She was born as a first child, and both pregnancy and delivery were uneventful. Birth weight was 2986 g (34th percentile), length was 48 cm (14th percentile) and head circumference was 33 cm (30th percentile). She had cleft palate and patent foramen ovale, but no syndactyly. She was referred to us at 4 months of age due to hypotonia, unstable neck and difficulty maintaining eye contact. At 8 months of age, she developed symptomatic infantile spasms. Brain magnetic resonance imaging showed bilateral perisylvian polymicrogyria and PNH (Figure 1a), and electroencephalogram identified hypsarrhythmia.

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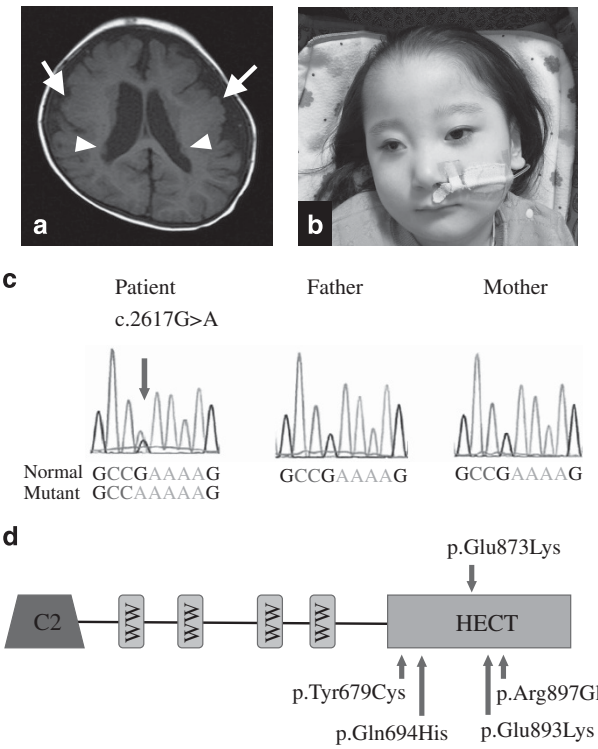

Figure 1 Patient's brain magnetic resonance imaging (MRI), photograph and the identified *NEDD4L* mutation. Brain MRI was acquired at 8 months of age. Her parents gave written consent for publication of the photograph. (**a**) Axial slice of T1-weighted image showing bilateral periventricular nodular heterotopia (arrow head) and polymicrogyria (arrow). (**b**) Photograph of the patient taken at 3 years of age showing distinctive facies. (**c**) Sanger sequencing of the *NEDD4L* mutation. The patient has a heterozygous c.2617G>A mutation (arrow) not present in the parents. (**d**) Previously published mutations in the HECT domain of *NEDD4L* associated with periventricular nodular heterotopia (red arrows, below) and our patient's mutation (blue arrow, above). A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Administration of adrenocorticotropic hormone and sodium valproate resolved her clinical spasms and hypsarrhythmia within a month. However, focal seizures gradually increased and infantile spasms relapsed at 16 months of age. Re-administration of adrenocorticotropic hormone improved her spasms. By the time this paper was written, she was 3 years old and seizure-free under antiepileptic medication (sodium valproate and zonisamide). Her body weight, height and head circumference were within 3rd–10th percentile. She had facial dysmorphic features of frontal upsweep hair, sparse eyebrow, upslanting palpebral fissure, low insertion of the columella, and thin upper and lower lips (Figure 1b). Her head and neck became stable at 17 months of age, and she started to show rolling over at the same time, but she was not able to sit or speak. She required tube feeding as she refused to take food, even though she was able to swallow. She also showed disturbed sleep rhythm.

MATERIALS AND METHODS

Genomic DNA was extracted from the patient and her parents from peripheral blood leukocytes by a standard procedure.[6] Proteins were obtained from Epstein-Barr (EB) virus-transformed lymphoblastoid cell lines (LCLs) established from the patient and healthy controls leukocytes. Trio-based whole-exome sequencing was performed as previously described.[7] The mutation was confirmed by Sanger sequencing of PCR-amplified products. Western blot was performed in triplicates using the conventional method,[8] with primary antibodies against Akt (pan) (#4691), phosphorylated Akt (p-Akt; Ser473; #4060), S6 ribosomal protein (#2217), phosphorylated S6 (p-S6; Ser240/244; #5018) and GAPDH (#5174) (Cell Signaling Technology, Danvers, MA, USA). Densitometric quantification was performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA, https://imagej.nih.gov/ij/). Mean ± s.d. were calculated, and two-sided Student's *t*-test was performed to determine the statistical significance with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan).[9] $P<0.05$ was considered significant. This study was approved by the institutional review board of Nagoya City University Graduate School of Medical Sciences, and written informed consent was obtained from the patient's parents.

RESULTS

The results of total reads by exome sequencing ranged between 73.6 and 82.8M reads, and the mean depth of target region was 90.5–100.6. We identified a *de novo* heterozygous missense mutation (c.2617G>A; p.Glu873Lys) in the HECT domain of the *NEDD4L* gene (NM_015277), which was confirmed by Sanger sequencing (Figure 1c). This mutation was not listed in public databases (for example, ExAC) or in our in-house whole-exome database (639 Japanese individuals). The mutation was predicted to be pathogenic by *in silico* analysis as probably damaging (Polyphen-2: score = 0.999) and deleterious (SIFT: score = 0). The raw CADD score was 7.63 and scaled C-score was 35, indicating the pathogenicity. We analyzed the expression level of p-AKT and p-S6, downstream effectors of the mTOR pathway,[10] in LCLs by western blot analysis. Neither p-AKT nor p-S6 expression was significantly different in LCLs derived from the patient compared to that from the controls (Figure 2).

DISCUSSION

In this study, we identified a novel missense mutation in the HECT domain of *NEDD4L*. Briox *et al.*[5] first reported seven patients with a mutation in this domain. All the patients in that study showed PNH, and most displayed hypotonia, intellectual disability, seizures, syndactyly and cleft palate (Table 1). The clinical features of our patient showed similarities to those previously reported, confirming that a mutation in *NEDD4L*, at least in the HECT domain, causes a recognizable neurological disorder with abnormal neuronal migration. Additionally, dysmorphic facies, as shown in our patient, could also be characteristic. However, as such features have not been reported so far, a larger number of patients are needed before a conclusion is drawn.

Regarding PNH, a representative gene causing PNH is *FLNA*.[11] *FLNA* is responsible for PNH and otopalatodigital syndrome, which are allelic disorders. Whereas otopalatodigital syndrome is characterized by cleft palate and digital complications, PNH-associated *FLNA* mutations are not commonly associated with cleft palate, syndactyly or polymicrogyria. Thus, *NEDD4L*-associated PNH could be clinically discriminated from that of *FLNA*.

Mutations in the HECT domain of *NEDD4L* cause deregulation of mTOR pathway and affect neurogenesis, migration and terminal translocation resulting in malformations of cortical development.[5] We previously showed that the upregulation of the mTOR pathway could be demonstrated in LCLs derived from patients with an mTOR pathway mutation.[8] LCLs derived from our patient did not show an upregulation of the mTOR pathway activity based on the expression level of p-AKT and p-S6. A possible reason for these contradictory findings is differences in the experimental design. In contrast to the forced expression system performed by Briox *et al.*,[5] the dysregulation of mTOR pathway in LCLs might be insufficient to be detected by

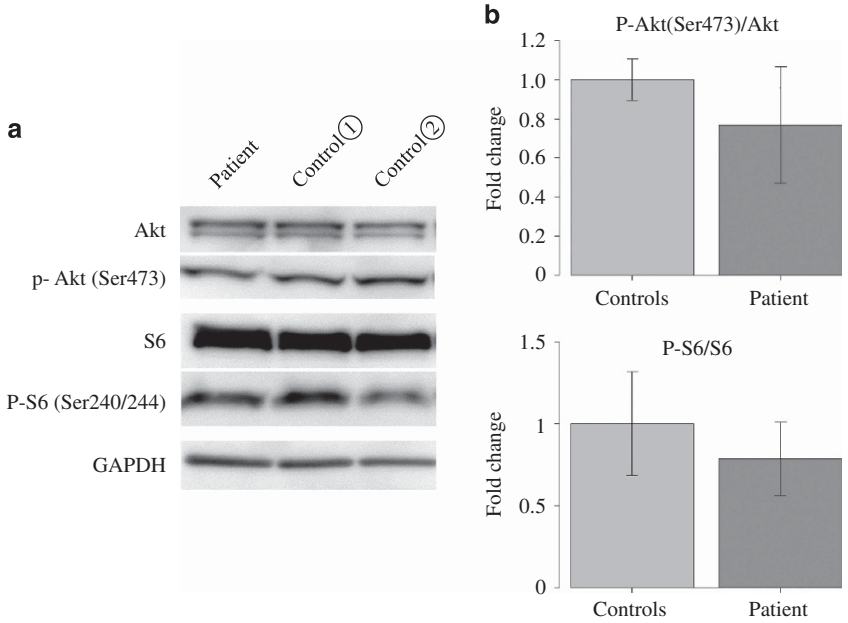

Figure 2 (**a**) Western blot results of protein extracts from lymphoblastoid cell lines from the patient and healthy controls. (**b**) Densitometry of the western blot experiments. The expression levels of phosphorylated AKT (p-AKT; Ser473) were not significantly different between the patient and controls ($P=0.271$). The expression level of phosphorylated S6 (p-S6; Ser240/244), a marker of mTOR pathway activation, was also similar ($P=0.391$). Error bars represent s.d. of the mean. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Table 1 Clinical features of patients with mutations in the HECT domain of *NEDD4L*

	Patient	*Briox et al.*[5]
Sex	Female	Female 4: male 3
Hypotonia	+	5/5
Syndactyly	–	6/7
Cleft palate	+	6/7 (including 1 patient with bifid uvula)
Last examination	3 years	4 months–12 years
HC	–1.1 s.d.	–0.8 ± 1.3 s.d. (microcephaly (<–2 s.d.): 2)
Height	–1.6 s.d.	–0.4 ± 1.7 s.d. (short stature (<–2SD): 2, high stature (> + 2SD): 1)
Developmental delay	+ (severe)	7/7 (severe: 3)
Seizures	+ (infantile spasm)	4/7 (infantile spasm: 1)
Brain MRI		
PNH	+ (bilateral)	7/7 (all cases are bilateral)
MCD	+ (PMG)	3/7 (PMG:1, cerebral atrophy: 1, frontal cortical dysplasia: 1)

Abbreviations: HC, head circumference; MCD, malformations of cortical development; MRI, magnetic resonance imaging; PMG, polymicrogyria; PNH, periventricular nodular heterotopia.

western blot analysis. The alternative possibility is the difference of the tissues or timing. Regulation of the mTOR pathway by *NEDD4L* might be crucial only in nervous system but not in blood cells at a certain developmental period. The expression level of NEDD4L in mouse cortex was reported to show a peak at embryonic day 16.5, a developmental stage of brain proliferation and migration.

In conclusion, a mutation in the HECT domain of *NEDD4L* might cause a clinically recognizable syndrome. Further experiments are required to determine how *NEDD4L* regulates the mTOR pathway and coordinates the process of neural development.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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