Journal of Human Genetics (2019) 64:499–504
https://doi.org/10.1038/s10038-019-0579-3

BRIEF COMMUNICATION

Leucine-485 deletion variant of *BRAF* may exhibit the severe end of the clinical spectrum of CFC syndrome

Sato Suzuki-Muromoto[1] · Takuya Miyabayashi[1] · Koki Nagai[2] · Saeko Yamamura-Suzuki[1] · Mai Anzai[1] · Yusuke Takezawa[1,3] · Ryo Sato[1] · Yukimune Okubo[1] · Wakaba Endo[1] · Takehiko Inui[1] · Noriko Togashi[1] · Atsuo Kikuchi[3] · Tetsuya Niihori[2] · Yoko Aoki[2] · Shigeo Kure[3] · Kazuhiro Haginoya[1]

Received: 10 October 2018 / Revised: 5 February 2019 / Accepted: 6 February 2019 / Published online: 6 March 2019

Abstract

The genotype–phenotype correlation in *BRAF* variant in cardio-facio-cutaneous (CFC) syndrome is not clearly defined. Here we report a case with a severe clinical phenotype and a novel *BRAF* variant, p.Leu485del. The present case showed severe intellectual disability, impaired awareness, hyperekplexia, involuntary movements, early onset refractory seizures, and delayed myelination on brain magnetic resonance imaging as well as a polycystic and dysplastic kidney, which are previously unreported anomalies in CFC or RAS/mitogen-activated protein kinase syndromes related to *BRAF* variant. CFC syndrome, especially caused by *BRAF* variant, should be included in the differential diagnosis of patients with developmental and epileptic encephalopathies and hyperekplexia. Furthermore, we need to keep in mind that missense variants or the deletion of Leucine-485 may be associated with severe symptoms.

Introduction

Cardio-facio-cutaneous (CFC) syndrome (OMIM 115150) is a multiple congenital anomaly first described by Reynolds et al. [1]. Typical features of CFC syndrome include facial abnormalities (sparse and friable hair, high forehead with bitemporal constriction, hypoplastic supraorbital ridges, downslanting palpebral fissures, a depressed nasal bridge, posteriorly angulated ears with prominent helices), heart defects (pulmonary stenosis, atrial septal defects, hypertrophic cardiomyopathy), and ectodermal abnormalities (hyperkeratotic skin, ichyosis-like condition). In addition, diverse phenotypic characteristics have been reported, which include perinatal problems (polyhydramnios, prematurity), growth failure, neurological problems (seizures, hypotonia, brain anatomical abnormalities), and gastrointestinal problems [2–3].

KRAS, BRAF, MAP2K1, and *MAP2K2*, which interact with the RAS/mitogen-activated protein kinase (MAPK) pathway, regulate cell differentiation, proliferation, and apoptosis and have been reported as causative genes of CFC syndrome [4–7]. BRAF is a cytoplasmic serine/threonine kinase that is activated by binding to RAS. Among the CFC syndromes, *BRAF* variant is the most frequently identified (approximately 43% [8] or 35% [9] of patients studied). However, the genotype–phenotype correlation in *BRAF* variant is still not clearly defined. Here we report a case with a severe clinical phenotype caused by a novel microdeletion of Leucine-485.

Case report

This 2-year-old Japanese boy was the second child of healthy and nonconsanguineous parents. As he was diagnosed with a polycystic kidney by intrauterine

These authors contributed equally: Sato Suzuki-Muromoto, Takuya Miyabayashi, Koki Nagai

Supplementary information The online version of this article (https://doi.org/10.1038/s10038-019-0579-3) contains supplementary material, which is available to authorized users.

✉ Sato Suzuki-Muromoto
sato.suzuki@kmf.biglobe.ne.jp

[1] Department of Pediatric Neurology, Miyagi Children's Hospital, Sendai 989-3126, Japan

[2] Department of Medical Genetics, Tohoku University School of Medicine, Sendai 980-8574, Japan

[3] Department of Pediatrics, Tohoku University School of Medicine, Sendai 980-8574, Japan

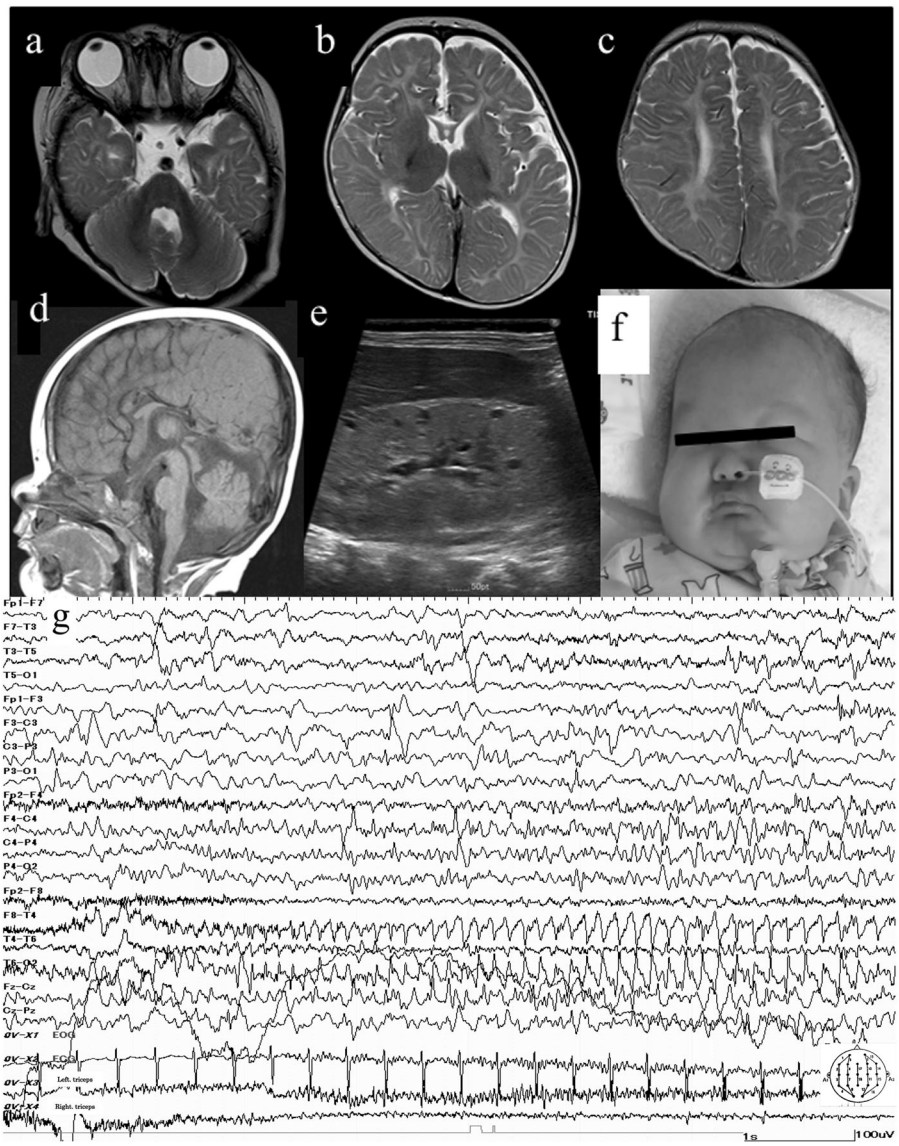

Fig. 1 Brain magnetic resonance imaging, axial T2WI (**a–c**), sagittal T1WI (**d**) at 1 year, echography of the right kidney (**e**), facial features (**f**), and ictal EEG at 1 year (**g**). Delayed myelination and reduced volume of the white matter as well as severe hypoplasia of corpus callosum body to the splenium are remarkable (**a–d**). Unclear cortico-medullary junction and multiple cysts are evident (**e**). Sparse hair and eyebrows, broad forehead, coarse face, depressed nasal bridge, short chin, and short neck are characteristic findings (**f**). The ictal EEG showed focal spikes bursts in the left occipital area spreading to the left temporal area, when he showed facial myoclonus followed by bilateral tonic seizures. Multifocal spikes are also noted (**g**)

ultrasonography, his mother received amniocentesis to treat polyhydramnios at 28 weeks gestational age. He was born after early rupture of the membrane at a gestational age of 30 weeks and 4 days. His birth weight, body length, and occipito-frontal head circumference (OFC) were 1815 g (+2.0 SD), 37 cm (−1.3 SD), and 29.5 cm (+2.0 SD), respectively.

After birth, endotracheal intubation was performed for respiratory management

He undertook nasal-directional positive airway pressure after extubation, but could not withdraw due to frequent apneic spells. Increased apneic spells and muscle stiffness became evident at day 33. At the same time, irritability, an exaggerated startle response, and involuntary movements appeared. His startle response was accompanied with facial flash and apnea, which was exaggerated by tactile or acoustic stimuli. His involuntary movements consisted of intermittent fragmentary myoclonic movements of the extremities, eyelid myokymia, and jaw closing dystonia. Electroencephalography (EEG) at 2 months of age showed equivocal sharp waves in the right temporal area. Startle response and myoclonic movement were not correlated with epileptic discharges. There was no giant sensory evoked potential. The auditory brainstem response was normal. At the age of 4 months, he developed epileptic seizures, which consisted of facial myoclonic movements and tachycardia, tonic–clonic seizures of the upper limbs, and generalized tonic seizures. The interictal EEG showed multifocal spikes in the frontal, central, and temporal areas independently, and ictal EEG showed secondary generalization from their focal discharges (Fig. 1g). We diagnosed

him with peculiar encephalopathy with a mixture of focal epilepsy, hyperekplexia, and involuntary movements of unknown cause. Although several drugs were used (vitamin B6, folic acid, lidocaine, clonazepam, valproic acid, carbamazepine, perampanel, and piracetam), his startle response, involuntary movements, and seizures were poorly controlled. Because of his frequent startle response, involuntary movements, and seizure-disturbed effective ventilation, he had a tracheostomy at 5 months. Since then, he has been cared for under continuous mechanical ventilation. Brain magnetic resonance imaging (MRI) at 1 year showed delayed myelination, reduced volume of the white matter, and hypoplasia of the genu to the tail of the corpus callosum (Fig. 1a–d).

At present, he is 2 years and 6 months old, he has severe psychomotor retardation with no head control or social smiles, visual pursuit or voluntary movements of the extremities. His awareness is persistently impaired. He has peripheral hypotonia with joint contractures of the elbow, wrist, and knee, as well as pes equinus. He shows severe mixed quadriplegia, startle response, tremor-like myoclonus of the extremities, lip and eye lid myokymia, exaggerated tendon reflexes, and neck rigidity, as well as jaw closing dystonia. Growth failure is also remarkable; his body weight, height, and OFC are 6 kg (−3.4 SD), 60 cm (−5.6 SD), and 41 cm (−3.3 SD), respectively. He has some dysmorphic features (sparse hair and eyebrows, broad forehead, coarse face, depressed nasal bridge, short chin, and short neck; Fig. 1f) and multiple anomalies (hypoplasia of the thorax, atrial septal defect, supravalvular pulmonary stenosis, polycystic and dysplastic kidney (Fig. 1e), cryptorchidism, and ejaculatory duct obstruction). There are no overt skin abnormalities. His startle response is somewhat better than neonatal period. However, involuntary myoclonic movements and seizures are difficult to control despite anticonvulsants (phenobarbital, lacosamide, gabapentin, clobazam) or a ketogenic diet.

We excluded metabolic abnormalities, e.g., mitochondrial disease, sulfite oxidase deficiency, or peroxisomes diseases, from several laboratory tests or ophthalmologic examination. The analyses of causative genes for hyperekplexia, including *GLRA1*, *SLC6A5*, *GLRB*, *SLC32A1*, and *SLC6A*, were unremarkable. To explore the molecular diagnosis to clarify his etiology, we performed trio-based whole-exome sequencing and identified a de novo deletion in the *BRAF* gene (c.1453_1455del p.Leu485del) (NM_004333), which was confirmed by Sanger sequencing. No other pathogenic mutation was observed.

To analyze the effect of p.Leu485del on the ERK pathway, we conducted reporter gene assays for measuring transactivation of ELK, which is a downstream transcription factor of ERK (see Supplementary Methods). We transfected *BRAF* expression constructs with pFR-luc trans-

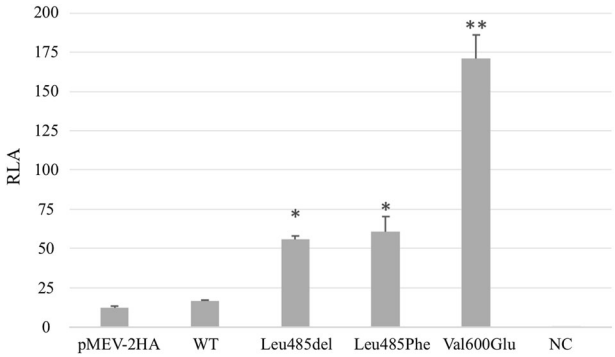

Fig. 2 Stimulation of ELK transcription in *BRAF* mutations. Relative luciferase activity (RLA) in cells expressing *BRAF* p.Leu485del, p.Leu485Phe, and p.Val600Glu was significantly higher than that in cells expressing wild-type *BRAF*. Representative data from three experiments performed in triplicate are shown. Date are shown as mean ± SE of three wells of cells. RLA relative luciferase activity, WT wild-type, NC negative control. *$p < 0.05$, **$p < 0.0001$ versus BRAF wild-type by Tukey–Kramer tests.

reporter vector, pFA2-Elk1, and phRL-null in NIH3T3 cells. Relative luciferase activity (RLA) in cells transfected with *BRAF* p.Leu485del was significantly higher than that in cells expressing wild-type *BRAF* (Fig. 2). Compared with the p.V600E mutant, the p.Leu485del and p.Leu485Phe mutants showed intermediate activity as described by Wan et al. [10]. These results suggest that the p.Leu485del, as well as p.Leu485Phe, causes increased ELK transactivation, suggesting activation of the ERK pathway.

Discussion

The present case provides some outstanding findings: (1) a novel *BRAF* gene variant, p.Leu485del; (2) a severe clinical phenotype, including severe intellectual disability, impaired awareness, hyperekplexia, involuntary movements, early onset refractory seizures, and delayed myelination on brain MRI; and (3) previously unreported malformation in CFC syndrome or RAS/MAPK syndromes related to *BRAF* variant, including a polycystic and dysplastic kidney.

BRAF is found predominantly in neural tissues, testis, and melanocytic and hematopoietic cells and is a key regulator of the RAS/MAPK pathway, which is important for cell proliferation, growth, and death [5, 11]. The brain MRI in CFC syndrome caused by *BRAF* variant was described only in 16 patients among 117 CFC syndrome patients reported [2, 6, 7, 12–15]. Most details were not described, but among them, delayed myelination was reported in two patients, both had Leucine-485 variant. Including present case, these MRI findings indicate that Leucine-485 plays a critical role in the neurodevelopmental process. Missense variants of Leucine-485 have been reported in five patients [6, 12–14] (Table 1). Adachi et al. [13] described a girl with

Table 1 Detailed clinical summary and findings of the patient and previously reported patients

	Present case	Aizaki et al. [12]	Adachi et al. [13]	Yoon et al. [14]		Niihori et al. [6]
Mutation of *BRAF*	c. 1453-1455del pL485del	c. 1454T>C p.L485S	c. 1454T>C p.L485S	L485S	L485F	c. 1455G >C p. L485F
Sex	Male	Female	Female	Male	Male	Female
Brain imaging	Cortical atrophy, HCC, delayed myelination	Brain atrophy, HCC, delayed myelination	Cortical atrophy, HCC, delayed myelination	NC	Type I Chiari malformation	NC
Seizure onset	1 month	2 months	Day 0	4 months	2 weeks	None
Seizure description	Facial myoclonus, tonic-clonic, generalized tonic seizure	Brief tonic seizures	Brief tonic spasms, tonic-clonic, myoclonic, complex partial seizures	Generalized tonic-clonic, complex partial, infantile spasms	Complex partial, secondary generalized, absence	
Electroencephalogram	Multifocal spikes, continuous spikes	High voltage slow waves with multifocal sharp waves, hypsarrhythmia	Hypsarrhythmia, continuous high-voltage spike or polyspikes	NC	NC	ND
Seizure prognosis	Uncontrolled, multiple drug medication	Partial improvement by ketogenic diet, multiple drug medication	Uncontrolled, multiple drug medication	Multiple drug medication	Multiple drug medication	
Other movement	Hyperekplexia, myoclonus	NC	Dystonia, athetosis, myoclonus	NC	NC	NC
Associated anomaly	Hypoplasia of thorax, atrial septal defect, supravalvular pulmonary stenosis, polycystic and dysplasia of kidney, cryptorchidism, and ejaculatory duct obstruction	Pulmonaly valve stenosis, hypertrophic cardiomyopathy, atrial septal defect	Narrow chest, hypertrophic cardiomyopathy	NC	NC	Mitral abnormality
Developmental milestones (age examined)	No head control, no visual pursuit (2 years)	No purposeful movement (4 months)	Severe motor and intellectual disabilities (4 years)	Died at 26 months	Severe intelligence disability (7 years)	Moderate retardation

HCC hypoplasia of corpus callosum, *NC* not commented, *ND* not done

frequent involuntary movements, early onset refractory seizures, and delayed myelination on brain MRI similar to our case. However, based on the frequent involuntary movements, hyperekplexia, associated anomalies, and high needs of nursing care, our case can be described as the severe end of the clinical spectrum of Leucine-485 variant.

Several reports have shown that conditional ablation of BRAF in mice results in deficits in extracellular signal-regulated kinase (ERK) activation, a MAPK signaling pathway, and severe neurological impairments, such as dysmyelination, defective oligodendrocyte differentiation, progressive loss of coordination, tremors, and ataxia [16–18]. These loss-of-function phenotypes are partially consistent with the present case. However, contrary to the total ablation of BRAF in mice, one study described *BRAF* p.Q241R knock in mouse showing a gain-of-function mutation [19], which had a CFC-like phenotype, including craniofacial abnormalities (mandibular hypoplasia), kyphosis and ossification in the interparietal bone, multiple heart defects, liver necrosis, and edema. Another report stated that ERK activation via BRAF signaling plays a critical role in renal cystogenesis in *PKD2* transgenic mice (*PKD2* is the causative gene of human autosomal dominant polycystic kidney disease) [20].

In this study, we showed that p.Leu485del variant indicated activation of the ERK pathway. The result was identical to our previous report of two *BRAF* variants (p.L485F and p.K499E) of CFC syndrome [6]. From these findings, we can anticipate that the *BRAF* gain-of-function variant plays an important role in the phenotype of CFC syndrome, including renal cyst formation. But, as we showed in past research, the severity of phenotypes cannot explain only by the activity of ERK pathway [6]. We need more case accumulation to know the pathomechanism affecting the severity of CFC syndrome.

In conclusion, CFC syndrome, especially caused by *BRAF* variant, should be included in the differential diagnosis of patients with developmental and epileptic encephalopathies and hyperekplexia. We need to keep in mind that among the *BRAF* variants, point mutation or the deletion of Leucine-485 may be associated with severe symptoms of CFC syndrome.

Author contributions SS-M and TM: study conceptualization, design, and manuscript preparation. KN, TN, and YA: functional analysis and interpretation of the data. SY-S, MA, YT, RS, TM, YO, WE, TI, NT: clinical evaluation. YT, AK, TN, and YA: gene analysis and interpretation of genetic analysis data. KH, YA, and SK: study concept, critical revision of the manuscript, and study supervision.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Reynolds JF, Neri G, Herrmann JP, lumberg B, Coldwell JG, Miles PV, et al. New multiple congenital anomalies/mental retardation syndrome with cardio-facio-cutaneous involvement–the CFC syndrome. Am J Med Genet. 1986;25:413–27.
2. Armour CM, Allanson JE. Further delineation of cardio-facio-cutaneous syndrome: clinical features of 38 individuals with proven mutations. J Med Genet. 2008;45:249–54.
3. Abe Y, Aoki Y, Kuriyama S, Kawame H, Okamoto N, Kurosawa K, et al. Costello and CFC syndrome study group in Japan. Prevalence and clinical features of Costello syndrome and cardio-facio-cutaneous syndrome in Japan: findings from a nationwide epidemiological survey. Am J Med Genet A. 2012;158A:1083–94.
4. Aoki Y, Niihori T, Narumi Y, Kure S, Matsubara Y. The RAS/MAPK syndromes: novel roles of the RAS pathway in human genetic disorders. Hum Mutat. 2008;29:992–1006.
5. Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. Nat Rev Cancer. 2003;3:459–65.
6. Niihori T, Aoki Y, Narumi Y, Neri G, Cave H, Verloes A, et al. Germline KRAS and BRAF mutations in cardio-facio-cutaneous syndrome. Nat Genet. 2006;38:294–6.
7. Rodriguez-Viciana P, Tetsu O, Tidyman WE, Estep AL, Conger BA, Cruz MS, et al. Germline mutations in genes within the MAPK pathway cause cardio-facio-cutaneous syndrome. Science. 2006;311:1287–90.
8. Narumi Y, Aoki Y, Niihori T, Neri G, Cave H, Verloes A, et al. Molecular and clinical characterization of cardio-facio-cutaneous (CFC) syndrome: overlapping clinical manifestations with Costello syndrome. Am J Med Genet A. 2007;143A:799–807.
9. Nava C, Hanna N, Michot C, Pereira S, Pouvreau N, Niihori T, et al. Cardio-facio-cutaneous and Noonan syndromes due to mutations in the RAS/MAPK signalling pathway: genotype-phenotype relationships and overlap with Costello syndrome. J Med Genet. 2007;44:763–71.
10. Wan PT, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, et al. Cancer Genome Project. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell. 2004;116:855–67.
11. Eychène A, Dusanter-Fourt I, Barnier JV, Papin C, Charon M, Gisselbrecht S, et al. Expression and activation of B-Raf kinase isoforms in human and murine leukemia cell lines. Oncogene. 1995;10:1159–65.
12. Aizaki K, Sugai K, Saito Y, Nakagawa E, Sasaki M, Aoki Y, et al. Cardio-facio-cutaneous syndrome with infantile spasms and delayed myelination. Brain Dev. 2011;33:166–9.
13. Adachi M, Abe Y, Aoki Y, Matsubara Y. Epilepsy in RAS/MAPK syndrome: two cases of cardio-facio-cutaneous syndrome with epilepticencephalopathy and a literature review. Seizure. 2012;21:55–60.
14. Yoon G, Rosenberg J, Blaser S, Rauen KA. Neurological complications of cardio-facio-cutaneous syndrome. Dev Med Child Neurol. 2007;49:894–849.
15. Sarkozy A, Carta C, Moretti S, Zampino G, Digilio MC, Pantaleoni F, et al. Germline BRAF mutations in Noonan, LEOPARD, and cardiofaciocutaneous syndromes: molecular diversity and associated phenotypic spectrum. Hum Mutat. 2009;30:695–702.
16. Galabova-Kovacs G, Catalanotti F, Matzen D, Reyes GX, Zezula J, Herbst R, et al. Essential role of B-Raf in oligodendrocyte maturation and myelination during postnatal central nervous system development. J Cell Biol. 2008;180:947–55.

17. Chen AP, Ohno M, Giese KP, Kühn R, Chen RL, Silva AJ. Forebrain-specific knockout of B-raf kinase leads to deficits in hippocampal long-termpotentiation, learning, and memory. J Neurosci Res. 2006;83:28–38.
18. Zhong J, Li X, McNamee C, Chen AP, Baccarini M, Snider WD. Raf kinase signaling functions in sensory neuron differentiation and axon growth in vivo. Nat Neurosci. 2007;10: 598–607.
19. Inoue S, Moriya M, Watanabe Y, Miyagawa-Tomita S, Niihori T, Oba D, et al. New BRAF knockin mice provide a pathogenetic mechanism of developmental defects and a therapeutic approach in cardio-facio-cutaneous syndrome. Hum Mol Genet. 2014;23:6553–66.
20. Park EY, Sung YH, Yang MH, Noh JY, Park SY, Lee TY, et al. Cyst formation in kidney via B-Raf signaling in the PKD2 transgenic mice. Biol Chem. 2009;284:7214–22.