Concurrent occurrence of an inherited 16p13.11 microduplication and a
de novo 19p13.3 microdeletion involving *MAP2K2* in a patient with
developmental delay, distinctive facial features, and lambdoid synostosis

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ABSTRACT

A female patient presented with developmental delay, distinctive facial features, and congenital anomalies, including a heart defect and premature lambdoid synostosis. The patient showed a paternally inherited 16p13.11 microduplication and a *de novo* 19p13.3 microdeletion involving the mitogen-activated protein kinase kinase 2 gene (*MAP2K2*), in which mutations cause the cardio-facio-cutaneous (CFC) syndrome. Reports of patients with overlapping 19p13.3 microdeletions of this region describe similar clinical manifestations including distinctive facial features: prominent forehead, horizontal/down-slanting palpebral fissures, long midface, pointed chin/angular jaw, sparse eyebrows, and underdeveloped cheekbones. Some of these findings overlapped to that of the patients with 16p13.11 microduplications and CFC syndrome. Although craniosynostosis was occasionally observed in patients with dominant-negative mutations in RAS/MAP kinase signaling genes (RASopathies) related to CFC syndrome, it was also reported in two patients with 16p13.11 microduplications. Genetic contributions of both chromosomal aberrations were discussed.

KEYWORDS: 16p13.11 microduplication; 19p13.3 microdeletion; *MAP2K2*; lambdoid synostosis

Introduction

Microdeletions in chromosome 19 are rare. There are some reports of the patients with overlapping deletion of 19p13.3, in which the mitogen-activated protein kinase kinase 2 gene (*MAP2K2*) is located (Al-Kateb et al., 2010; de Smith et al., 2011; Nowaczyk et al., 2014). *MAP2K2* is recognized as a gene related to cardio-facio-cutaneous (CFC) syndrome (MIM#115150) and involved in RAS/MAP kinase signaling. Most patients with gene mutations in RAS/MAP kinase signaling exhibit the presence of activating gain-of-function mutations in related genes (Nava et al., 2007), and the functional consequence of such gene mutations are recognized as RASopathies (Tidyman and Rauen, 2009; Aoki et al., 2016). Accordingly, loss-of-function mutations were rare in *MAP2K2* and it is still controversial how *MAP2K2* haploinsufficiency is related to clinical phenotypes of the patients with 19p13.3 microdeletion.

16p13.11 microduplication is recurrently identified not only in patients with neurological features but also in normal individuals (Nagamani et al., 2011; Tropeano et al., 2013). The existence of low-copy repeats in the both ends of the breakpoints is the reason why reciprocal deletions/duplications in this region were recurrently identified. Phenotypic penetrance of this duplication is clearly incomplete and carrier parents are often asymptomatic.

In this study, we identified an inherited 16p13.11 microduplication and a *de novo* 19p13.3 microdeletion in a patient with developmental delay and congenital malformations including lambdoid synostosis. We will discuss genetic contributions of both chromosomal aberrations in this patient.

Clinical report

The female patient, who presented at 1 year and 3 months of age, is the only child of healthy parents and her family history was unremarkable. At 38 weeks of gestation, she was born by caesarean section for dilatation of the cerebral ventricles and polyhydramnios associated with a single umbilical artery and torsion of the umbilical cord. At birth, her weight was 2586 g (10~25th centile) and occipitofrontal circumference (OFC) was 32.5 cm (mean), indicating standard proportions.

Soon after birth, she was noted as having distinctive facial features including broad forehead, arched eyebrows, coarse hair, flat nasal bridge, telecanthus, epicanthus, up-swept nose, long philtrum, flattened upper lip, and a pointed chin (Fig. 1A). In the early neonatal period, her sucking ability was poor and tube feeding was required. At 3 months, an echocardiography was performed due to a heart murmur resulting in identification of an atrial septal defect and quadricuspid aortic valve. At 10 months, frequent episodes of sleep apnea were noted. Brain magnetic resonance imaging revealed abnormalities including thin corpus callosum, dilatation of the lateral ventricles, and Chiari malformation type I. Cranial computed tomography revealed lambdoid synostosis (Fig. 1B), which was suspected of causing narrowing of the posterior cranial fossa and subsequent amygdala herniation, and later resulted in obstructive hydrocephalus (Fig. 1C and D). At the age of 10 months, her height was 67.9 cm (3~10th centile), weight was 7.96 kg (25~50th centile), and OFC was 47.5 cm (>97th centile). She showed mild developmental delay with a developmental quotient of 69 as evaluated by the Enjoji developmental test. At 15 months, surgical operation for craniosynostosis was performed.

Molecular karyotype

This study was performed in accordance with the Helsinki declaration. We obtained permission from the ethical committee of Tokyo Women's Medical University. After obtaining written informed consent from the patient's family, peripheral blood samples were collected. DNA was then extracted using QIAamp DNA extraction kit (QIAGEN, Hilden, Germany). Chromosomal microarray testing was performed using the Agilent SurePrint 60K array (Agilent Technologies, Santa Clara, CA) as described (Shimajima et al., 2015). The results showed a genomic copy number gain and loss described as $arr\ 16p13.11(15131723_16249607) \times 3, 19p13.3(3104902_4392250) \times 1$ (GRCh37/hg19) (Supplemental Fig. S1). Examination of the parents revealed the 16p13.11 duplication in her father. Since the 19p13.3 deletion was not detected in either parent, it was confirmed to *be de novo or germline mosaicism in a parent*.

Discussion

In this study, we identified *a chromosome 16p13.11 microduplication as well as a chromosome 19p13.3 microdeletion* in a patient with developmental delay and congenital malformations including distinctive facial features and lambdoid synostosis.

The genome map *shown* in Figure 2 depicts the 19p13.3 deletion region identified in the present patient compared with reported deletions. In 2010, Al-Kateb et al. reported a patient with a 1.93 Mb microdeletion in 19p13.3 *and severe developmental delay and distinctive features*. A smaller 0.174 Mb deletion was identified in *another* patient by de Smith (2011). Subsequently, Nowaczyk et al. (2014) reported seven patients with 19p13.3 microdeletions. Among these 7 patients, the smallest deletion was

0.06 Mb in size and only *MAP2K2* was included in the shortest region overlap (Fig. 2). All of these patients showed overlapping deletions and common facial features, i.e. prominent forehead, horizontal/down-slanting palpebral fissures, long midface, pointed chin/angular jaw, and underdeveloped cheekbones (Table 1). The present patient also shows similar features. Thus, these similar clinical features can be attributed to *MAP2K2* haploinsufficiency.

Mutations in the *MAP2K2* gene are responsible for CFC syndrome, which manifests intellectual disability, heart defects, and ectodermal abnormalities, such as sparse, friable hair, hyperkeratotic skin lesions, and a generalized ichthyosis-like condition (Narumi et al., 2007; Aoki et al., 2008). Facial features of patients with CFC syndrome include a high forehead with bitemporal constriction, hypoplastic supraorbital ridges, downslanting palpebral fissures, a depressed nasal bridge, and posteriorly angulated ears with prominent helices (Aoki et al., 2008). In accordance with the report (Lee et al., 2011), frequencies of the accompanying features were summarized in Table 1, which show some overlapping features between CFC syndrome and *MAP2K2* haploinsufficiency, such as sparse eyebrows and long philtrum. On the other hand, long midface and pointed chin were rather specific for patients with *MAP2K2* haploinsufficiency, while they do not present other features considered characteristic of the CFC syndrome, such as webbed neck and pulmonary valve abnormalities.

Mutations in different genes cause RASopathies by a gain of function mechanism rather than haploinsufficiency that may be linked to different clinical outcome. Dominant-negative mutations in the protein tyrosine phosphatase non-receptor type 11 gene (*PTPN11*) are associated with Noonan syndrome while inactivating mutations in the same gene cause metachondromatosis, a clinical entity very different from Noonan

syndrome (Bowen et al., 2011). Similar to *PTPN11* mutations, the different dysregulations of the Ras/MAPK pathway, either caused by gain-of-function or haploinsufficiency mechanisms of *MAP2K2* mutations, may lead to different clinical pictures.

The present patient also showed a 16p13.11 **microduplication**, which has been associated with a range of neurodevelopmental disorders including autism, attention deficit/ hyperkinetic disorder, intellectual disability and schizophrenia (Tropeano et al., 2013). Some of the patients also showed predominant skeletal features (craniosynostosis, polydactyly, and joint hypermobility) and cardiac/aortic malformations (Nagamani et al., 2011). Frequencies of the phenotypic complications were included in Table 1.

Chiari malformation type I identified on the present patient (Fig. 1D) was considered as a secondary consequence to premature closure of the lambdoid suture, a rare type of craniosynostosis. In the literature, there are several reports of premature craniosynostosis in association with RASopathies (Kratz et al., 2009; Brasil et al., 2012; Takenouchi et al., 2014; Addissie et al., 2015). However, this finding was observed in two patients with 16p13.11 microduplications. Thus, this may be derived from 16p13.11 microduplications rather than *MAP2K2* haploinsufficiency.

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Disclosure

We certify that we have read the journal's position regarding issues pertaining to ethical publication, and affirm that this report is consistent with the guidelines. The authors have no conflicts of interest to declare.

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Figure legends

Fig. 1. Clinical features of the patient. (A) Facial findings at 14 months of age, indicating prominent forehead, down-slanting palpebral fissures, telecanthus, long midface, pointed chin/angular jaw, sparse eyebrows, and underdeveloped cheekbones. (Written informed consent to publish this image was obtained.) (B) Three-dimensional computed tomography of the head examined at 1 month of age indicates bilateral lambdoid synostosis (arrows). (C and D) Brain magnetic resonance imaging examined at 14 months. Axial (C) and sagittal (D) images show dilatation of the bilateral ventricles and Chiari malformation type I, respectively.

Fig. 2. Genome map around 19p13.3 comparing the deletion regions in different patients. *MAP2K2* is located in the depicted common deletion region. Blue and red bars indicate the deletion regions in reported cases and in the present patient, respectively. Black rectangles indicate the locations of the genes discussed in the text. A green line indicates shortest region overlap of 19p13.3 microdeletions.

Figure 1

Figure 2

19p13.3

Al-Kateb *et al.* 2010

de Smith *et al.* 2011

Nowaczyk *et al.* 2014

Pt 1

Pt 2

Pt 3

Pt 4

Pt 5

Pt 6

Pt 7

Present Patient

GNA11 ■

GIPC3 ■

MAP2K2 ■

TBXA2R ■

CACTIN ■

RAX2 ■

EEF2 ■

3.0

4.0

5.0 (Mb)

Table 1

Table 1 Clinical features of the patients with 19p13.3 deletions in comparison with frequency reported in *MEK1 / MEK2* mutation positive CFC patients

		Frequency in patients * with CFC syndrome (%)	Positive ratio in patients with 16p13.11 microduplications #	Patients with 19p13.3 deletions										Positive ratio in patients with 19p13.3 deletion syndrome	Present patient
				Al-Kateb et al. 2010	de Smith et al. 2011	Nowaczyk et al. 2014									
						P1	P2	P3	P4	P5	P6	P7			
Polyhydramnios		62	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	
Fetal macrosomia		52	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	—	
Short stature		79	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	±	
Macrocephaly		76	NA	+	+	+	—	—	+	+	—	+	6/9	+	
Failure to thrive		83	NA	—	—	+	+	+	+	+	+	—	6/9	±	
Facial anomalies		100	8/14	+	+	+	+	+	+	+	+	+	9/9	+	
	Prominent supraorbital ridge	45	NA	+	+	+	+	—	+	+	—	+	7/9	—	
	Sparse eyebrows	92	NA	+	+	—	—	+	+	—	+	+	6/9	+	
	Downslanting palpebral fissures	72	NA	+	—	+	+	+	—	+	—	+	6/9	+	
	Hypertelorism	77	NA	—	—	—	—	+	—	—	+	—	2/9	—	
	Epicanthal folds	54	5/9	—	—	—	—	+	—	—	—	—	1/9	+	
	Palpebral ptosis	67	NA	—	—	—	+	—	—	—	—	—	1/9	—	
	Low-set ears/thickened helix	90	4/9	—	—	—	+	—	—	—	—	—	1/9	+	
	Large, thick ear lobe	87	NA	—	—	—	NA	—	—	NA	—	—	0/7	—	
	Flat nasal bridge	83	NA	+	—	—	—	+	—	—	+	—	3/9	+	
	Thick lips/macrostomia	58	NA	±	±	±	±	±	±	±	±	±		±	
	Long/prominent philtrum	62	NA	—	—	—	—	+	—	—	+	—	2/9	+	
	Micrognathia	48	NA	—	—	—	—	—	—	—	—	—	0/9	—	
	Long midface	NA	NA	+	+	+	+	+	+	+	+	+	9/9	+	
	Pointed chin	NA	NA	+	+	+	+	+	+	+	+	+	10/10	+	
Congenital	heart defect	64	4/16	+	—	+	—	—	+	+	—	—	5/10	+	
	Pulmonary valve stenosis	40	0/16	—	—	—	—	—	—	—	—	—	0/9	—	
	Pulmonary valve dysplasia	3	0/16	—	—	—	—	—	—	—	—	—	0/9	—	
	Supravalvular PVS	3	0/16	—	—	—	—	—	—	—	—	—	0/9	—	
	Atrial/ventricular septal defect	18	1/16	—	—	+	—	—	+	+	—	—	0.4	+	
	Hypertrophic cardiomyopathy	33	NA	—	—	—	—	—	—	—	—	—	0/9	—	
	Others	NA	NA	Fallot							Fallot			Quadracuspid valve	
Skin anomalies		98													
	Keratosis pilaris facies	44	NA	NA	+	+	—	+	—	—	—		3/7	—	
	Cavernous angiomata	33	NA	—	—	—	+	+	—	—	+	+	4/9	—	
	Nevi	32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		—	
	Café-au-lait spots	17	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		—	
	Sparse hair	67	NA	+	—	+	—	+	—	—	+	—	4/9	+	
	Curly hair	84	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		—	
Musculoskeletal abnormalities		94	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		+	
	Short webbed neck	96	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		+	
	Pectus excavatum	74	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		—	
	Joint limitations/heperextension	54	6/12	NA	NA	NA	NA	NA	NA	NA	NA	NA		—	
	Craniosynostosis	NA	2/16	NA	NA	NA	NA	NA	NA	NA	NA	NA		+	
Cubitus valgus		37	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		—	
Thoracic anomalies		82	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		—	
Undescended testes		100	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		—	
Neurological		96												+	
	Intellectual disability	93	NA	+	—	+	+	+	+	+	NA	+	7/8	+	
	CNS abnormality	47	2/12	—	NA	+	+	—	—	—	—	—	2/8	+	
	Seizures	36	1/15	NA	NA	NA	NA	NA	NA	NA	NA	NA		—	
	Development delay	96	10/16	+	+	+	+	+	+	+	NA	+	8/8	+	
	Hypotonia	89	4/16	+	NA	—	+	+	+	—	—	—	4/8	+	
Ocular abnormalities		53	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
	Photophobia	27	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	—	
	Nystagmus	35	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	—	
	Strabismus	46	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	—	
	Myopia	22	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	—	
	Optic nerve hypoplasia	16	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	—	
	Cataract (bilateral)	15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	—	

*; Frequencies are referred to that reported by Dentici et al. (2009)
#; Positive ratio in patients with 16p13.11 **microduplications** were **calculated** according to the report by Nagamani et al. (2011)
NA; not available