Brief Report

Interstitial deletions in the proximal regions of 6q: 12 original cases and a literature review

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SUMMARY   Interstitial microdeletions in the proximal region of the long arm of chromosome 6 are rare. Herein we have reported 12 patients with developmental delays associated with interstitial microdeletions in 6q ranging from q12 to q22. The microdeletions were detected by chromosomal microarray testing. To confirm the clinical significance of these deletions, genotype-phenotype correlation analysis was performed using genetic and predicted loss-of-function data. *SIM1* was recognized as the gene responsible for developmental delay, particularly in Prader-Willi syndrome-like phenotypes. Other genes possibly related to developmental delay were *ZNF292*, *PHIP*, *KCNQ5*, and *NUS1*. To further establish the correlation between the genotype and phenotype, more patient information is required.

Keywords   chromosomal microarray testing, 6q interstitial deletions, developmental delay

1. Introduction

Interstitial microdeletions in the proximal region of the long arm of chromosome 6 are rare. In 1997, Hopkins *et al.* reported three new cases and reviewed 57 previously reported cases of partial deletions on 6q and classified them into three phenotypic groups: proximal [del(6)(q11–q16)], intermediate [del(6)(q15–q25)], and distal [del(6)(q25–qter)] (*1*). Although there were some characteristic features unique to each phenotypic group, recognizable clinical entities were not established at that time. In 2007, Klein *et al.* reported three patients with 6q deletions (*2*). Two of them were obese and showed signs of hypotonia and developmental delay, which were described as Prader-Willi syndrome (PWS)-like phenotypes; *SIM1* (MIM* 603128) located in the deleted region, was suspected to be a candidate gene for the PWS-like phenotype. In 2012, Rosenfeld *et al.* reported 12 new cases of interstitial 6q deletions and four of the cases had PWS-like phenotypes (*3*). These findings suggest that there is a correlation between the occurrence of the PWS-like phenotype and *SIM1* deletion. This is further supported by other reports of patients with similar genotypic and phenotypic findings (*4,5*).

Similarly, deletions in certain regions were associated with characteristic findings. So far, in our ongoing research on genomic copy number analysis using chromosomal microarray testing, we have encountered 12 patients who showed interstitial microdeletions in 6q. Herein, the genotype-phenotype correlation was assessed.

2. Patients and Methods

This study was performed in accordance with the Declaration of Helsinki and approved by the ethics committee of this institution. Written informed consent was obtained from the families of the patients. Blood samples were collected from the patients; additionally, blood samples were also collected from the parents of the patients when it was necessary for further investigation. Genomic copy number variations were analyzed using the Agilent Microarray system (Agilent Technologies, Santa Clara, CA, USA), as previously described (*6*). Based on the results, patients with 6q interstitial microdeletions were included in this study.

The clinical information of the patients was obtained from their attending doctors. The correlation between the genotype and phenotype was then investigated using a genome map in which the deleted regions were depicted (Figure 1). Gene information was evaluated using Online Mendelian Inheritance in Man® (OMIM; *https://www.omim.org/*). Predicted loss-of-function was also used for the evaluation. The genomic coordinate referred to was the GRCh37/hg19 genome build.

3. Results and Discussion

Interstitial deletions involving the long arm of chromosome 6 were identified in 12 patients: six males and six females, ranging in age from 1 to 21 years (Table 1). Although all the patients showed developmental delays, the extent of developmental delay varied among the patients. The deleted regions are shown on the genome map in Figure 1. For further evaluation, we summarized the genes from the deleted regions and the probability of loss-of-function intolerance (pLI), shown in Supplemental Table S1 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=112*) (7). The candidate genes listed were those related to autosomal dominant traits and had a pLI score of 1. The genes listed are shown in Figure 1, in which genome map is captured from the UCSC genome browser (*https://genome.ucsc.edu/*).

The first patient was an 8-year-9-month-old girl (patient 1) who was born by cesarean section owing to an unequal infant pelvis. The patient showed distinctive features. Her development was delayed;

the patient started walking independently at 2 years of age and speaking at 4 years of age. At 6 years of age, she developed epilepsy, for which she had to take antiepileptic drugs. A radiological examination of the brain revealed no abnormalities.

The second patient, a 13-month-old boy (patient 2), could turn over, but not sit up; this indicated delayed motor function development. Furthermore, he had language delay. Physical examination revealed distinctive features, which included coarse scalp hair. Magnetic resonance imaging (MRI) of the brain showed no apparent abnormalities.

The third patient was a 17-month-old girl (patient 3) who could not walk unaided, which indicated a developmental delay. She also showed growth deficiency with -2 SD parameters and microcephaly with -3 SD parameters.

The prenatal history of a 21-year-old male patient (patient 4) revealed amniotic fluid overload in the 8th month of pregnancy. There were several congenital anomalies, such as congenital duodenal atresia, inguinal hernia, and cleft palate. In addition, there were also urogenital anomalies present that included bilateral hypoplastic kidneys, renal enuresis, and cryptorchidism. An external strabismus was observed later. His motor skills were limited to sitting, and he had not yet acquired substantial language skills; this indicated a severe psychomotor developmental delay. He developed epilepsy and neuropsychiatric features, including sleep disorder and self-injurious behavior, such as hitting his eyes with his hands. Episodes of hypothermia were also observed.
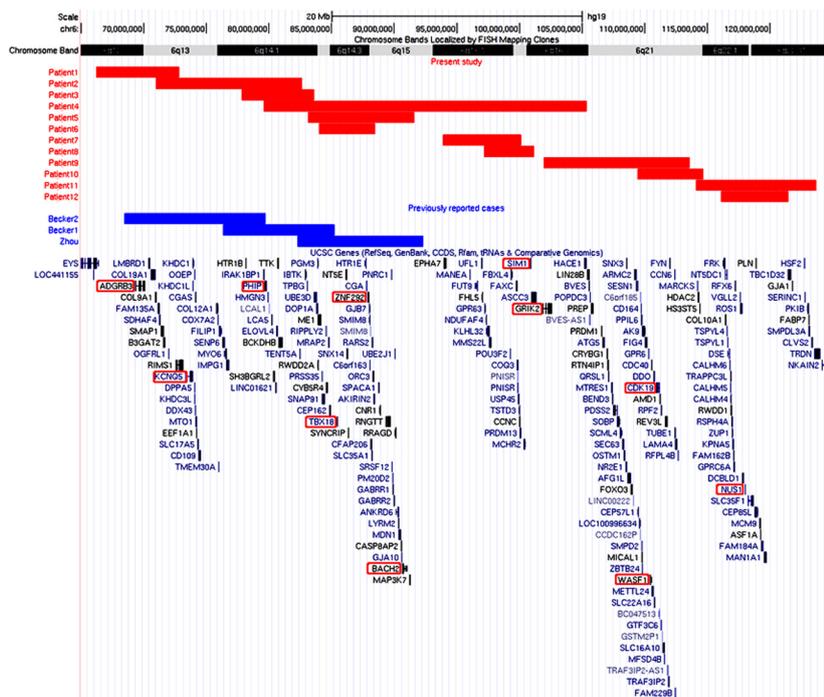

Figure 1. Genome map of 6q captured from the UCSC genome browser. Regions of the identified deletions are depicted by custom tracks with rectangles; the red and blue are for regions of the deletions identified in this study and previous studies, respectively. The genes discussed in the text are highlighted using red circles.

Table 1. Clinical features of the patients in this study

Items	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12
Age	8y9m	1y2m	1y5m	21y	1y6m	3y2m	2y3m	16y4m	5y7m	3y10m	4y10m	13y9m
Gender	Female	Male	Female	Male	Male	Male	Female	Female	Male	Female	Male	Female
Chromosome band	6q12q13	6q13q14.1	6q14.1	6q14.1q16.3	6q14.1q15	6q14.2q15	6q16.1	6q16.1q16.3	6q16.3q21	6q21q22.1	6q21q22.31	6q22.1q22.31
Start*	65,485,026	67,176,848	76,392,293	72,891,116	81,135,362	84,025,024	92,902,137	96,533,420	98,273,562	108,670,734	113,020,897	114,656,502
End*	73,838,151	85,971,380	84,606,179	110,736,632	93,665,050	88,452,718	101,255,262	101,724,035	117,068,093	115,143,797	125,341,725	123,074,922
Deletion Size (Mb)	8.4	18.8	8.2	37.8	12.5	4.4	8.4	5.2	18.8	6.5	12.3	8.4
Gestational age	NA	39w1d	39w3d	38w0d	NA	40w6d	39w5d	38w4d	37w6d	39w6d	39w0d	40w5d
Birth weight	NA	2976	3135	1970	NA	3194	2860	3048	2156	3554	3165	2595
Birth length	NA	47.6	49.5	NA	NA	50.5	48	NA	46	52	49.1	46.2
Birth occipitofrontal circumference	NA	32.9	33	NA	NA	35	30.4	NA	32	34	32.5	31
Developmental delay	+	+	+	+	+	+	+	±	+	+	+	+
DQ/IQ			DQ 79	NA	+	DQ 38	DQ below 35	IQ 76	DQ 35	DQ60	DQ below 35	DQ54
Growth deficiency	-	-	+	NA	-	-	-	-	+	-	+	-
Microcephaly	-	-	+	+	-	-	-2.6SD	-	-	-	+	-
Epilepsy	+	-	-	+	-	-	-	-	-	-	+	+
MRI brain abnormalities	-	-	NA	NA	+	-	-	-	NA	+	NA	+
Gastrointestinal anomalies	-	-	-	+	+	-	-	-	+	-	-	-
Genital anomalies	-	-	-	+	+	+	-	-	-	-	-	-
Distinctive facia features												
Low-ser-ears	-	+	+	-	-	+	+	-	-	-	+	-
Epicanthus	-	-	+	-	-	+	-	-	-	+	+	-
Blepharophimosis	-	-	-	-	-	+	+	-	-	-	-	-
Micrognathia	-	+	+	-	+	+	+	-	+	-	+	+
Flat nasal bridge	-	-	-	-	-	+	-	-	-	-	+	-
Wide ala nasi	-	-	-	-	-	-	-	-	-	+	+	-
Hypertelorism	-	-	-	-	-	+	+	-	-	+	+	-
Other findings				Hypoplastic kidney, renal enuresis		Cryptorchidism, ptosis		Hypothyroidism, precocious puberty, obesity				

y, years; m, months; w, weeks; s, days; NA, not available, DQ, developmental quotient; IQ, inteligent quotient, *Genomic cordinate corresponds to GRCh37/hg19.

The fifth patient, an 18-month-old boy (patient 5), had no remarkable perinatal history. The patient showed psychomotor developmental delays from early infancy. There were several abnormal findings, such as blephaophimosis, small jaw, hypertrichosis on the back, tapered phalanges, long first toe, accessory ears, abnormal dentition, hypodontia, soft larynx, and left optic nerve hypoplasia. Gastroesophageal reflux disease was also observed. Brain MRI showed corpus callosum hypoplasia and delayed myelination. However, there were no abnormal results from the laboratory tests for inborn errors of metabolism, mitochondrial disease, Pompe's disease, Fabry disease, and mucopolysaccharidosis. Auditory testing revealed no abnormalities.

Motor development in a 3-year-old boy (patient 6) was delayed; he achieved head control at 8 months, independent sitting at 12 months, crawling at 12 months, and independent walking at 32 months. His language development was severely delayed, and he could not speak comprehensibly. He showed distinctive features that included flat occiput, hypertelorism, epicanthus, bilateral ptosis, strabismus, flat basal bridge, low-set ears, small mouth, short neck, and bilateral cryptorchidism.

The seventh patient, a 27-month-old girl (patient 7), had a small jaw as a distinctive feature. She could crawl, but could not grasp or speak significantly, indicating psychomotor developmental delay. Ophthalmological examination revealed interocular dissection. A neurological examination revealed generalized hypotonia. Brain MRI showed no abnormalities. Moreover, there were no abnormalities found upon routine laboratory examination. She is now 15 years of age. The patient had severe intellectual disability with microcephaly and was not obese.

The eighth patient was a 16-year-4-month-old girl (Patient 8) who was diagnosed with psychomotor developmental delay and hypothyroidism at 14 months of age. She developed precocious puberty and was treated with leuplin, but later she became amenorrheic. This patient was 151 cm tall (-1.3 SD) and weighed 79 kg (+2.6 SD), which indicated obesity.

The ninth patient was a 5-year-7-month-old boy (patient 9) who showed developmental delay. At 2 years of age, he started to walk independently and had no significant speech, indicating delayed development. The patient had a small jaw and was short in stature. Gastroesophageal reflux disease, sleep apnea syndrome, and scoliosis were also observed.

The tenth patient, a 3-year-10-month-old girl (patient 10), had occipital flatness, bilateral eye openings, lamina propria, auricular deformity, low nasal apex, wide nasal bridge, bilateral middle fingers, and curly hair. Brain MRI showed corpus callosum hypoplasia. At the time of examination, her height, weight, and occipitofrontal circumference were 107 cm (+2.3 SD), 18.1 kg (+1.7 SD), and 47.3 cm (-1.2 SD), respectively; this indicated a relatively large stature.

A 4-year-10-month-old boy (patient 11) had sufficient head and neck control; however, he was not ambulatory and has no significant speech, indicating a developmental delay. Physical examination revealed microcephaly, occipital flatness, bilateral eye openings, lamina propria, low auricular position, flat nasal dorsum, cleft palate, and a small mandible. He experienced epileptic seizures, with loss of consciousness for a few seconds, and was taking antiepileptic drugs.

The twelfth patient, a 13-year-9-month-old girl (patient 12), had developmental delay hand tremors. Brain MRI revealed an enlarged cerebellar fissure. EEG abnormalities were also observed. The patient did not experience any epileptic seizures.

According to the genome map (Figure 1), the deleted regions in two of the patients (patients 4 and 7) included *SIM1*, which was confirmed to contribute to obesity and PWS-like features when loss-of-function variants were present in the coding region (*8*). Indeed, patient 7 showed PWS-like phenotypes, including obesity; however, patient 4 showed a much more severe developmental delay than that of patient 7. The PWS-like phenotypes could not be distinguished owing to the large region of deletion in 6q, that was observed in this study.

Additionally, patient 4 exhibited urinary tract abnormalities. The overlapping regions of the deletions in patients 4, 5, and 6 included *TBX18* (MIM* 604613), which is related to "congenital anomalies of kidney and urinary tract" (MIM# 143400). However, *TBX18* variants identified in humans with congenital anomalies of kidney and urinary tract exert a dominant-negative effect rather than haploinsufficiency (*9*). Thus, we considered that the urinary tract abnormalities observed in patient 4 were not related to the deletion of *TBX18*, which explained why patient 5 did not show any urinary tract abnormalities.

The overlapping regions of deletion in patients 4, 5, and 6 included *ZNF292* (MIM* 616213), which is related to "intellectual developmental disorder, autosomal dominant 64" (MIM# 619188). Therefore, the haploinsufficiency of *ZNF292* may have contributed to the developmental delay observed in patients 4 and 5 (*10,11*). Furthermore, Zhou *et al.* (2017) reported a patient with developmental delay in association with an overlapping region of a deletion from 6q14.1 to 6q15; this suggested that the haploinsufficiency of *ZNF292* is responsible for the delayed development (*12*).

Additionally, the overlapping region of the deletions in patients 4 and 5 included *BACH2* (MIM* 605394), which is related to "immunodeficiency 60 and autoimmunity" (IMD60) (MIM# 618394). Previous reports determined that missense variations of *BACH2* contributed to the dominant negative effects; therefore, *BACH2* was excluded from this analysis (*13*).

The shortest region of overlapping deletions was observed in patients 2, 3, and 4. This region included *PHIP* (MIM* 612870), which is known to cause Chung-Jansen syndrome (CHUJANS; MIM# 617991), a clinical

entity characterized by global developmental delay in infants, impaired intellectual development or learning difficulties, behavioral abnormalities, dysmorphic features, and obesity (*14*). Thus, it is plausible that *PHIP* haploinsufficiency contributed to the neurodevelopmental delay observed in patients 2, 3, and 4.

Becker *et al.* reported two patients with microdeletions in 6q (*15*). One of the patients (patient 2) showed a more proximal deletion than that observed in the patient reported by Zhou *et al.* (*12*). The deleted region included *ADGRB3* (MIM* 602684). *ADGRB3* is a member of the adhesion-G protein-coupled receptor family and is mostly expressed in the brain (*16*). Although the pLI score of *ADGRB3* was "1", which indicated intolerance for haploinsufficiency, previously reported *ADGRB3* variants were biallelic. Thus, it is unknown whether *ADGRB3* is related to the developmental delay observed in patient 1.

The haploinsufficiency of the other genes located in the proximal regions of 6q may be related to the clinical features observed in this study. *KCNQ5* (MIM * 607357), located on 6q13, is related to "intellectual developmental disorder, autosomal dominant 46" (MIM# 617601) (*17*). Loss-of-function variants in *KCNQ5* were predicted to lower the seizure threshold by decreasing the repolarization reserve; therefore, it is possible that *KCNQ5* haploinsufficiency contributed to the clinical features that were observed in patient 2.

Additionally, *GRIK2* (MIM* 138244) was located in the deleted regions in patients 4 and 9. It is related to "neurodevelopmental disorder with impaired language and ataxia and with or without seizures" (MIN# 619580). However, the identified variants of *GRIK2* were related to gain-of-function (*18*); thus, the haploinsufficiency of *GRIK2* would not have contributed to the clinical features of these patients.

WASF1 (MIM* 605035) and *CDK19* (MIM* 614720) were in the deleted regions that were observed in patients 9 and 10. *WASF1* is responsible for "neurodevelopmental disorder with absent language and variable seizures" (MIM# 618707). Previously reported *WASF1* variants were nonsense variants, and they were considered to have dominant negative effects, which were a consequence of evading nonsense mediated decay (*19*). *CDK19* is associated with "developmental and epileptic encephalopathy 87" (MIM# 618916). All previously reported variants of *CDK19* were dominant negative missense variants that resulted (*20*). Therefore, it is difficult to attribute the developmental delay observed in patients 8 and 9 to *WASF1* or *CDK19* haploinsufficiency.

The overlapping region of the deletions observed in patients 9 and 10 was proposed as the etiology for acro-cardio-facial syndrome (MIM 600460) (*21,22*); however, none of the patients showed any symptoms related to acro-cardio-facial syndrome.

Furthermore, *NUS1* (MIM* 610463) was located in the deleted regions observed in patients 10 and 11. *NUS1*

is responsible for "intellectual developmental disorder, autosomal dominant 55, with seizures" (MIM# 617831). Because previously reported *NUS1* variants were related to loss of function, haploinsufficiency was considered the pathogenic mechanism (*23*).

4. Conclusion

Among the genes in the long arm of chromosome 6 (chr6:65,485,026-125,341,725), only five genes (*SIM1*, *ZNF292*, *PHIP*, *KCNQ5*, and *NUS1*) were considered to be related to the developmental delay observed in the patients reported in this study.

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