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Case Report

A rare intrauterine onset growth retardation syndrome caused by mosaic 19p13.3 microduplication: evaluation of GH/IGF- 1 axis and GH therapy response

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ABSTRACT

Background. 19p13.3 microduplication syndrome is a newly defined intrauterine onset growth retardation syndrome characterized by microcephaly, moderate intellectual disability, speech delay, and mild dysmorphic features. The *PIAS4* gene located in this region plays a crucial role as a transcriptional co-regulator in various cellular pathways including STAT, p53/TP53 and growth hormone (GH) signaling and mutations in this gene are thought to be responsible for clinical features.

Case. We present a 10 year-old girl with intrauterine onset growth retardation, microcephaly, and mild facial dysmorphic features. Treatment with GH was started at 4 years and 9 months of age targeting the severe short stature (-3.65 standard deviation score, SDS) since she had significant IGF-1 response to exogenous GH. Microarray study demonstrated a 19p13.3 microduplication of 4.4 Mb. FISH analyses revealed mosaic extra signals (27.5% on blood lymphocytes, and 47% on buccal epithelium) of 19p13.3 region. At the age of 10, her height was at -2.37 SDS, and she had mild intellectual disability which has been described in 19p13.3 microduplication syndrome.

Conclusion. We present here a patient with typical findings of 19p13.3 microduplication syndrome and also with a prominent response to GH treatment, which has not been reported previously in this syndrome.

Key words: 19p13.3 microduplication, intrauterine growth retardation, microcephaly, *PIAS4*, growth hormone.

The p13.3 locus on chromosome 19 is 6.9 Mb in length and contains 307 genes. Duplications and deletions of this region were reported in about 50 patients, the majority of which were deletions.[1-6] Siggberg et al.[3] reported microcephaly, intrauterine onset growth retardation and 0.8 Mb-long duplication on 19p13.3 (chr:19: 1952590_6908729) in three patients from a family with similar clinical features (microcephaly, moderate to severe intellectual disability, speech delay and dysmorphic findings) and defined the 19p13.3 microduplication syndrome. Andries et al.[7] also earlier reported a case that has similar characteristics of the syndrome with pure terminal duplication of the short arm of chromosome 19. Novikova et al.[5] tried to identify the genetic mechanisms of the syndrome and suggested that *PIAS4* (Protein Inhibitor of Activated STAT Protein 4), *ZBTB7A* and *MAP2K2* genes of this region are responsible for the characteristic findings of this syndrome. It was hypothesized that *PIAS4* gene duplication has the essential role for microcephaly and intrauterine growth retardation.

Herein, we report on a patient with both 19p13.3 microduplication and a positive growth response to growth hormone (GH) treatment, which were not associated previously.

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Case Report

A 12 month-old girl was admitted due to her atypical facial appearance and microcephaly (Fig.1). Her parents were first cousins once removed. Due to oligohydramnios and fetal intrauterine growth restriction, fetal karyotype analysis was performed and revealed normal at 25 weeks of gestation. The pregnancy was complicated by gestational diabetes mellitus. The patient was born at 36 weeks and 4 days of gestation. Her birth height, weight, and head circumference (HC) were 38 cm (-3 standard deviation score, SDS), 1370 gr (-2.6 SDS), and 27.8 cm (-3 SDS), respectively.

Physical examination at age 1 showed dysmorphic features including distinctive facial appearance with round facies, brachycephaly, sparse hair, bitemporal narrowing, highly arched sparse eyebrows, upslanted short palpebral fissures, low set large ears, and micrognathia with chin dimple (Fig. 1a,b). Her height, weight, and HC were 67cm (-2.29 SDS), 6400 gr (-3.4 SDS), and 38.5 cm (-5.7 SDS), respectively. Biochemical tests of blood and urine, abdominal ultrasonography (USG), echocardiography and cranial MRI were normal. She gained head control at 3 months of age, sat without support when 11 months-old and walked when 2.5 years-old. At 13 months of age, the Denver-II test result was 73%, compatible developmental delay.

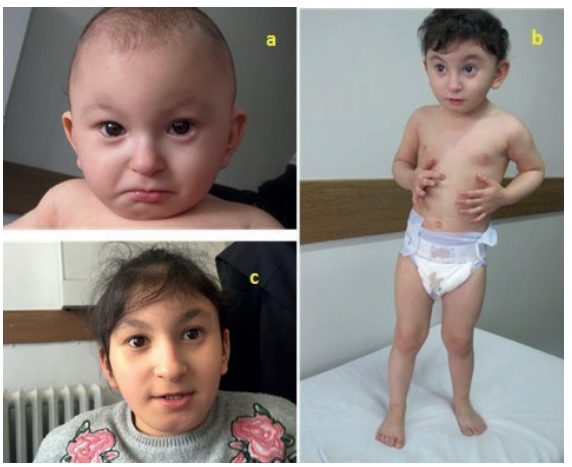

Fig. 1. The patient at 12 months (a), 42 months (b) and 8 years (c) of age. Note microcephaly, round facies, bitemporal narrowing, sparse hair, sparse and highly arched eyebrows, upslanted and short palpebral fissures, low set and large ears, thin upper lip and micrognathia with a chin dimple (a,b). She developed a long face with aging (c).

At the age of 4 years and 9 months, her height and weight were 91 cm (-3.25 SDS) and 12.5 kg (-2.57 SDS). Her bone age was compatible with the standards of 2.5 years. The GH response to GH stimulation test with clonidine was higher than 10 ng/ml, showing sufficient response. Due to her severe growth retardation, GH axis was reevaluated by IGF generation test to demonstrate if there was an IGF-1 response to exogenous GH. GH with a dose of 0.1 mg/kg/day was injected subcutaneously for 4 days. After the GH injections, baseline IGF-1 level (130.4 ng/ml, -0.6 SDS) reached the peak level (216.1 ng/ml, +0.9 SDS) with an increase ratio of 66% at the 5th day, showing a significant IGF-1 response to exogenous GH. Thus, GH treatment (0.2 mg/kg/week) was started. After the 1st year of treatment her height SDS was -3.16 and height velocity was 8.4 cm/year which was -3.65 SDS and 5.5 cm/year before treatment. After the 2nd year of the treatment her height SDS was -2.98 and height velocity was 7.1 cm/year. Growth velocity SDS in the first and second years of treament were +1.42 SDS and +0.79 SDS indicating a good response to the treatment. A positive affect on head circumference was not noted during GH treatment.

At 10 years of age, on the last examination, she was still receving GH treatment. Her height was 123.2 cm (-2.37 SDS). Her response to GH treatment was 7 cm/year (+0.61 SDS) with an IGF-1 response of 398.7 ng/ml (+2.35 SDS). Dysmorphic features including long facies, thin long fingers with wide fingernails, prominent bilateral sacral dimple, and skin eczema were noted (Fig 1c). She was receiving special education and was unable to read and write, yet.

Cytogenetic and molecular cytogenetic studies

Written informent consent was obtained from the parents. The patient had a normal

female karyotype in a peripheral blood sample (46,XX). Both SNP-array and array-CGH analyses of the genomic material extracted from peripheral blood cells revealed a 4.4 Mb-long microduplication of the 19p13.3 locus (Chr19: 259395_4615348) (Fig.2a,b). To define the location of the duplicated region, FISH analysis on metaphase spreads prepared from cultured lymphocytes was performed by using 19p subtelomeric and Smith Magenis / Miller Dieker (SM/MD) probes (dJ564C11, RAI1/ PAFAH1B1, Aquarius®, Cytocell Cambridge, UK). The analyses revealed an extra signal of 19p13.3 region on the short arm of chromosome 17 as a mosaic pattern (11/40 metaphases; 27.5%), and normal signal of SM/MD specific probe on the 17p region (Fig.3a, b, c). The FISH analysis of the buccal smear cells revealed the mosaicism ratio as 47%. The chromosomal analysis and FISH study of the parents excluded balanced translocations.

Discussion

19p13.3 microduplication syndrome is a newly defined intrauterine onset growth retardation syndrome characterized by microcephaly, moderate intellectual disability, speech delay, and mild dysmorphic features.[1-6] The 19p13.3 region includes *PIAS4, ZBTB7A*, and *MAP2K2* genes, which were thought to be responsible for the characteristic findings of the syndrome. It was hypothesized that PIAS4 gene duplication has the essential role for microcephaly and intrauterine growth retardation.[5,6] PIAS4 protein is a member of the E3 SUMO-protein ligase family. It regulates histone modifications and plays a crucial role in transcriptional coregulation, genetic stability, and various cellular pathways like STAT, p53/TP53, Wnt, and steroid hormone signaling pathways.[8-13] Burn et al.[12] injected PIAS1, PIAS2, PIAS3, and PIAS4 RNAs into early frog embryos and observed failure in blastopore closure, shortened body

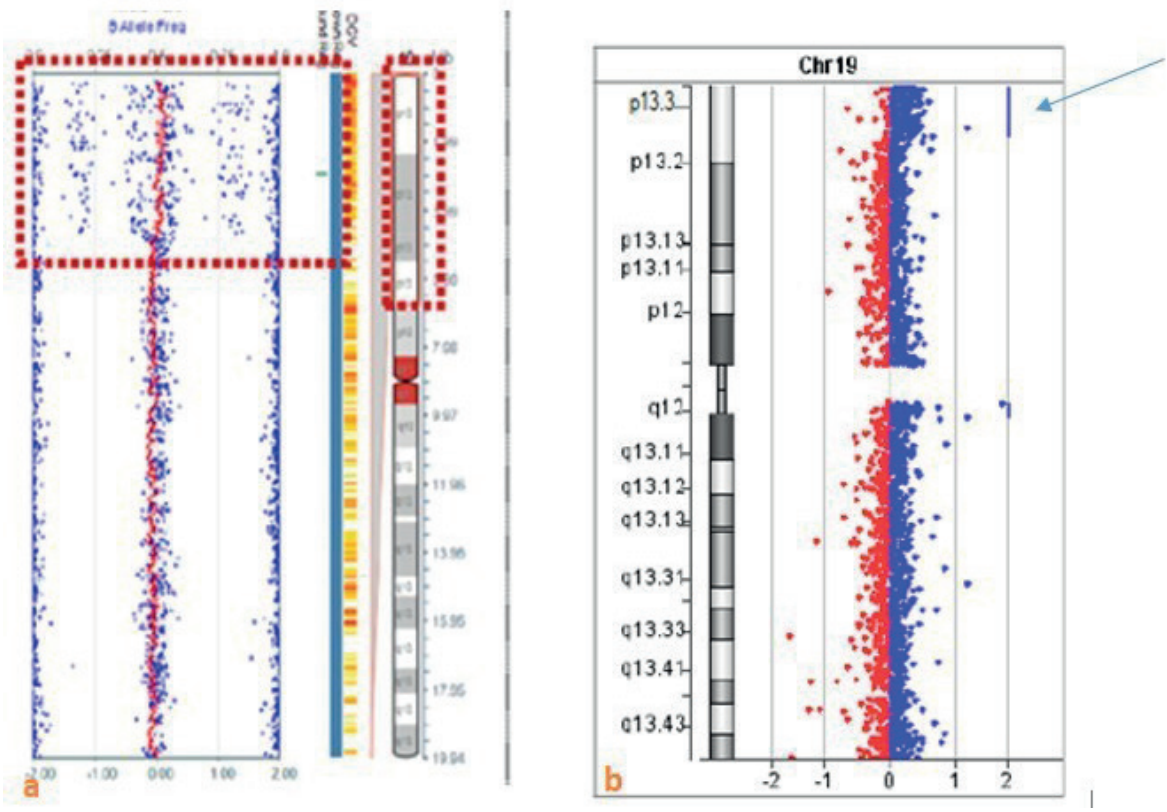

Fig. 2. SNP Array (a) and Array-CGH (b) analysis revealed a mosaic 4.4 Mb microduplication of 19p13.3 (Chr19: 259.395 – 4.615.348).

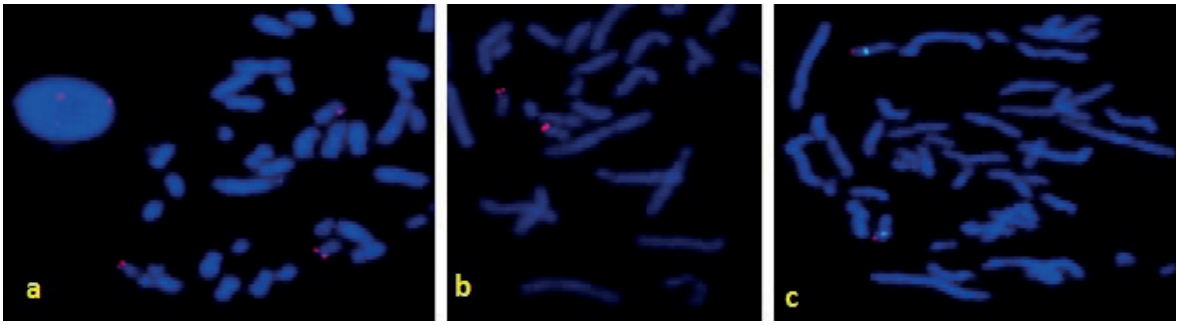

Fig. 3. FISH analyses revealed a third signal of 19p13.3 on the short arm of chromosome 17 in mosaic (in 11/40 metaphases) (a), among normal cells with two normal signals of 19p13.3 (b). FISH with the Miller Dieker syndrome-specific probe; green centromeric signals indicate chromosomes 17 (c).

axis, and defective head formation. *MAP2K2* is a regional gene potentially functioning in mitogenic activity, and mutations may cause growth retardation. Monoallelic mutations of this gene is associated more with cardiofasciocutaneous syndrome. *PIAS4* which encodes a member of the E3 SUMO-protein ligase family is thought to be the strongest candidate gene associated with growth retardation in 19p13.3 microduplication syndrome. Recently a case with single nucleotide variant in *PIAS4* that has common characteristics of 19p13.3 microdeletion syndrome was reported, supporting the hypothesis of *PIAS4* effect on head circumference and growth.[6] Nevado et al.[1] also evaluated the clinical findings of eleven patients with 19p13.3 microdeletion and two patients with 19p13.3 microduplication in a study in 2015. While one of the patients with microduplication without *PIAS4* duplication was normocephalic and short statured, the other patient with *PIAS4* duplication had microcephaly (-4 SDS) and short stature. They associated 19p13.3 region duplications with microcephaly as well as 19p13.3 region deletions with macrocephaly; and named both conditions as the 19p13.3 microdeletion/ microduplication syndrome.

We detected a *de novo* 4.4 Mb microduplication of 19p13.3 region by array-CGH analysis in a girl with microcephaly and IUGR. This duplication may originate by *de novo* unequal crossing over, or by abnormal segregation from parental balanced translocation or inversion. Since the parental karyotypes were normal, 19p13.3 duplication occurred as *de novo*. FISH analysis which was performed for defining the location of this duplicated region, revealed a mosaic 19p13.3 insertion on the chromosome 17p. This unbalanced insertion had caused a 19p13.3 microduplication, without 17p deletion. Mosaicism refers to a different genetic content in a group of cells from other cells in the body that arises during development. The percentage of cells with the mutation is related to the developmental stage at which the mutation arises.[14] The ratio between affected and unaffected cells or the corresponding cell types correlate with the severity of clinic outcome and probably with the response to growth hormone treatment.

The patient presented here also had mild intellectual disability with aggressive behaviour patterns, speech delay, elongated face, sparse hair and eyebrows, bitemporal narrowing, short and upslanted palpebral fissures, thin upper lip, micrognathia, chin dimple, and long fingers. 19p13.3 microduplication syndrome has been reported in 9 patients to date. Table I summarizes the clinical findings in our patient and in previously reported patients with pure 19p13.3 duplications.[1-6] The majority of subjects including our patient had microcephaly (7/10). One of the normocephalic patients had a duplication without *PIAS4* gene, and the other patient with borderline microcephaly had a partial *PIAS4* gene duplication.[1-6] All patients had developmental delay and/or intellectual

Table I. The molecular and clinical findings of present and reported patients with 19p13.3 microduplication.

Features		Ishikawa (2)	Novikova (5)	Orellana (4)			Siggberg (3)	Nevado (1)		Tenorio (6)	Present Patient
				Patient 1	Patient 2	Patient 3		Patient 1	Patient 2		
Age (yrs)		3	11	10	2.5	39	9	6	3	5	1
Gender		F	M	M	M	F	M	F	F	F	F
Duplication size (Mb)		6.1	0.83	4.95	4.95	4.95	0.81	2.39	1.479	3.04	4.3
Including PIAS4 gene		+	Partial	+	+	+	+	+	-	+	+
Microcephaly		+	+	+	+	+	+	+	-	-	+
Birth	Length (SDS)	-3.3	NA	-0.9	-1.0	+1.1	-2.6	NA	NA	-1.35	-3.2
	Weight (SDS)	-2.9	NA	-2.1	-2.1	-1.0	-1.9	NA	NA	-1.39	-2.8
	HC (SDS)	-2.2	NA	NA	NA	NA	-2.2	NA	NA	-0,85	-3.2
Last examination	Height (SDS/age)	-5.0 / 3yrs	-1,6 / 11yrs	NA	-0.6 / 6yrs	-0.2 / 22 mo	–1.5 / 2yrs	Short	Short	NA	-2.8 / 8yrs
	Weight (SDS/age)	-3.4 / 3yrs	-2,0 / 11yrs	NA	+0.8 / 6yrs	-0.2 / 22 mo	NA	NA	NA	NA	-1.4 / 8yrs
	HC (SDS/age)	-4.0 /3	-1.7 /11	NA	-2.6 /6	-2.1 / 22 mo	NA	-4.4 / 6	N/3yrs	NA	-4.7 /8
Intellectualdisability		+	+	+	+	+	+	+	+	+	+
Speech delay		+	+	+	+	+	+	+	+	+	+
Hypotonia		NA	+	NA	NA	NA	NA	+	Mild	+	-
Behaviour phenotype		NA	ADHD	VSA	VSA	VSA	Adequate	ADHD	NA	NA	Aggre-sive
Elongated face		-	+	+	NA	+	NA	NA	NA	NA	+
Prominent forehead		NA	+	+	+	+	NA	+	+	NA	-
Temporal narrowing		-	-	-	NA	-	NA	-	-	NA	+
Sparse hair and eyebrow		+	+	-	NA	-	NA	NA	NA	NA	+
Short palpebral fissure		+	+	+	NA	+	NA	NA	NA	NA	+
Upslanted palpebral fissure		-	+	+	NA	+	NA	NA	+	NA	+
Hypertelorism		+	+	-	NA	+	NA	-	NA	NA	-
Thin upper lip		-	+	+	+	+	NA	+	-	NA	+
Micrognathia		+	+	+	NA	+	NA	NA	NA	NA	+
Chin dimple		-	-	-	NA	-	NA	NA	NA	NA	+
Small mouth		+	+	+	NA	+	NA	NA	NA	NA	-
Long finger		NA	-	+	+	+	NA	NA	NA	NA	+
GH treatment		-	-	-	-	-	-	-	-	-	+

ADHD: attention-deficit/hyperactivity disorder, GH: growth hormone, NA: not-available, VSA: very sociable and affectionate; mo: months, yrs: years, N: normal, HC: head circumference, SDS: standard deviation score.

disability. Common craniofacial findings were prominent forehead (6/7), sparse hair and eyebrows (3/5), upslanted palpebral fissures (5/6), short palpebral fissures (5/5), thin upper lip (6/8), small mouth (4/5), micrognathia (5/5), and long fingers (4/5). Other uncommon features were early-onset puberty, joint abnormalities (hip dislocations, hypermobility), and cardiac defects.[2-4]

Growth hormone stimulation test of the patient presented here revealed adequate GH responses. Despite normal baseline IGF-1 levels we performed IGF-1 stimulation test to see the increment of IGF-1 in response to GH, which showed a significant increase and led to treatment with GH. GH treatment improved growth in our patient in the short term and she achieved a good response. To our knowledge, the response to GH treatment in patients with 19p13.3 microduplication syndrome has not been examined to date. However long term effects and final height might show a real benefit of GH treatment. It is difficult to explain the exact mechanism of growth failure in this disorder in which several genes are located adjacently. It is also difficult to explain why our patient responded to GH because there was no GH nor IGF-1 deficiency. Especially *PIAS4*, a transcriptional coregulator in various cellular pathways including STAT, p53/TP53 and growth hormone (GH) signalling, may be involved in growth failure by affecting post receptor signalling of GH. Although IGF-1 levels increased after GH stimulation showing a functional receptor, the patient might have had a partial insufficiency in post receptor signalling, thus achieving higher GH levels following administration of GH might have improved her growth.

In conclusion, we have described typical findings of 19p13.3 microduplication syndrome in a patient with mosaic 19p13.3 microduplication, and demonstrated that there may be a prominent response to GH treatment in this syndrome.

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