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17p13.3 microduplication including *CRK* leads to overgrowth and elevated growth factors: A case report

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Abstract

17p13.3 microduplications classified as class I duplications involving *YWHAE* but not *PAFAH1B1* (formerly *LISI*) and class II duplications which extend to involve *PAFAH1B1*, are associated with diverse phenotypes including intellectual disability and structural brain malformations. We report a girl with an approximately 1.58 Mb apparently terminal gain of 17p13.3, which contains more than 20 genes including the *YWHAE* and *CRK* genes (OMIM: 164762). She had increased growth factors accompanied by pathologic tall stature. In addition to these, she developed central precocious puberty at 7 years old. In individuals with class I 17p13.3 microduplications including *CRK*, we recommend biochemical evaluation of the growth hormone axis. Providers caring for these patients should be aware of their possible risk for the development of central precocious puberty.

Keywords

17p13.3 microduplications; *CRK* gene; growth factors; central precocious puberty

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Introduction

Microduplications of 17p13.3 have been previously reported to cause a diverse array of phenotypes most commonly involving intellectual disability, autism spectrum disorder, and/or structural brain malformations. They have previously been categorized as class I duplications involving *YWHAE* but not *PAFAH1B1* (formerly *LIS1*) and class II duplications which extend to involve *PAFAH1B1*.¹ Bi et al previously noted a macrosomia tendency in 3 out of 4 patients with a class I microduplication including *YWHAE*.² Although overgrowth was observed in other case reports, the largest case series failed to find a clear association with early overgrowth among 8 patients with class I duplications.³

We report a patient with a class I 17p13.3 microduplication and congenital macrosomia presenting to an Endocrinologist with overgrowth and increased growth velocity in childhood. This patient was also found to have elevated growth factors and pituitary hyperplasia. The duplication involves *YWHAE* and *CRK*. This case report suggests a possible mechanism for overgrowth in patients with 17p13.3 microduplications involving *CRK*.

Clinical Report

The probanda was originally referred to the Genetics clinic at 2 years old. She was delivered at 39 weeks gestational age by a planned, repeat cesarean section. Pregnancy was complicated by uterine bleeding one month prior to delivery requiring mom to be placed on bed rest. At birth, the patient was large for gestational age (LGA) with birth weight 4 kg and length 52.5 cm. There were no acute medical problems in the neonatal period.

Neonatal examination was positive for dysmorphic features including a thick neck, low set nipple and long torso. When compared to her length and weight, her head circumference was small. A karyotype done shortly thereafter showed 46,XX with an inverted duplication of the 17p13.3 region. FISH analysis using a probe specific to the subtelomeric sequence on the short arm of chromosome 17 (Tel 17p; Abbott Molecular) showed one signal on the normal chromosome 17 homologue and a possibly slightly larger signal on the abnormal chromosome 17, indicating a possible duplication of this region 46,XX,dup(17)(p13.3p13.2)(Tel 17p++,LIS1+,Tel 17q+)]. Physical features at 2 years old included a flattened nose with a broad base and prominent philtrum, head with normal shape and closed sutures, normal appearing ears with no pits, creases or skin tags. Musculoskeletal and neurological examinations were normal. Chromosomal microarray, which had been performed in the interim, confirmed the submicroscopic duplication at 17p13.3.

She was referred to the Endocrinology clinic at 4 years and 3 months old for a growth evaluation (Figure 1) as parents were concerned about her tall stature. On physical examination her height was 121.7 cm (>97th percentile for age) and weight 20.5 kg (92nd percentile for age). Her mid parental height (MPH) was 172.8 cm (95th percentile for age). Facial dysmorphism included a flat nasal bridge with a tubular nose. Investigation for her tall stature, included growth factors (IGF1 and IGFBP3), which were both elevated (Table 1). Thyroid function testing was normal. Magnetic resonance (MR) brain was normal.

She returned to the Endocrinology clinic for follow up at 5 years and 5 months old Mom reported cognitive delays, behavioral aggression and the development of pain to her limbs during cold weather suggestive of Raynaud's phenomenon. At that time, her height was 131.3 cm (> 97th percentile for age) and weight was 23.7 kg (92nd percentile for age). Her annualized growth velocity was 9.2 cm (> 97th percentile for age). Growth charts in Figure 3, illustrate that her height was consistently > 97th percentile for age between ages 2 to 5 years old and weight was mainly near the 95th percentile for age. Father's height was 182.9 cm (81st percentile) and mother's height was 177.8 cm (> 97th percentile).

Repeat lab testing again showed elevated growth factors with IGF1 363 (50- 300 ng/ml) and IGFBP3 5.2 (1.1- 5.2 µg/ml) [Table 1]. A glucose suppression test done to rule out the possibility of a growth hormone secreting tumor was normal. A MR brain evaluation showed pituitary hyperplasia. A repeat bone age (BA) evaluation showed bone age advancement with a reading between 7 years to 7 years and 10 months old at a chronological age of 5 years and 4 months (1 SD of 11.18 months). Prior BA evaluation done at a chronologic age of 4 years was consistent with that of a 6 years and 10 months old female (1 SD of 8.9 months), hence, also advanced.

The patient was referred to the National Institutes of Health (N.I.H.), Bethesda, MD for further evaluation by a pediatric endocrinologist with expertise in overgrowth syndromes. At the NIH, testing in accordance with research protocol 97- CH- 0076- *A Clinical and Genetic Investigation of Pituitary Tumors and Related Hypothalamic Disorders* was performed, including SNP chromosomal microarray, and results were similar to those mentioned previously. Under the protocol 97- CH- 0076 at the N.I.H., transthoracic echocardiogram showed dilated coronary sinus suggestive of persistent left superior vena cava. Thyroid ultrasound showed a small diffusely abnormal thyroid and renal ultrasound showed enlarged kidneys with possibly mal-rotated kidneys.

At a follow up clinic visit at 7 years and 8 months old, months after she returned from the N.I.H., mom expressed concern regarding the onset of puberty. Continued behavioral aggression was controlled by non-pharmacologic means, such as positive reinforcement, with good effect noted. Physical examination now showed Tanner Stage II breast development on the right side, whereas the left side was Tanner Stage I.

Growth factor testing, IGF 1 529 (133- 849 ng/ml), IGFBP 3 5.9 (1.4- 6.2 µg/ml) was normal. Gonadotropin testing showed LH 0.62 (normal < 0.15 µIU/ml, Tanner Stage I), FSH 1.98 (1.4-2.5 µIU/ml, Tanner Stage II). A GnRH stimulation test was performed out of suspicion for true central precocious puberty and results are shown in Table 2. Again a glucose suppression test (results not shown) result was normal.

MRI evaluation now showed a pubertal appearing pituitary gland and no masses. Bone age evaluation continued to show advancement, 12 years old at a chronologic age of 7 years and 8 months (1 SD of 10 months). An abdominal ultrasound performed to exclude liver or adrenal pathology as being the cause for her elevated estradiol level was normal, whereas pelvic ultrasound showed a pubertal configuration to both the ovaries and uterus.

Her parents elected that she be treated with the monthly preparation of Lupron Depot Peds®, 11.25 mg mainly for psychosocial reasons, considering that with puberty, there could be an exacerbation of her baseline behavioral problems. Her annualized growth velocity, was decreased to a pre-pubertal level after therapy for six (6) months with Lupron Depot Peds®.

Chromosomal and molecular analyses

Chromosome analysis was performed on peripheral blood using standard high resolution G-band techniques. Each of the 20 cells analyzed had one abnormal chromosome 17 with an inverted duplication of a small region on the short arm of this chromosome. No other consistent structural or numerical abnormalities were detected.

Using probes specific to the subtelomeric regions on the short (TelVysion 17p; Abbott Molecular) and long (TelVysion 17q; Abbott Molecular) arms of chromosome 17, the abnormal chromosome 17 showed a more intense and slightly larger signal for the short arm subtelomeric probe, indicating a probable duplication of this area of the chromosome. Both the abnormal chromosome 17 and the normal chromosome 17 homologue showed one signal each with the 17q subtelomeric probe which is the normal signal pattern. Parental peripheral blood karyotypes were normal, so the finding was considered to be *de novo*. The final result was reported as 46, XX, dup(17)(p13.3p13.2)dn.ish dup (17)(p13.3p13.2)(Tel 17p+ +, LIS1+, Tel 17q+).

The initial microarray was performed using the SignatureSelect OS 105K v1.1 oligonucleotide array which was based on the Agilent 105K array (Signature Genomic Laboratories, Spokane, WA). The results were consistent with an approximately 1.58 Mb apparently terminal gain of 17p13.3, [arr cgh 17p13.3(53,448-1,629,788)×3] (Figure 2). This region contained more than 20 genes, including the *CRK* gene (OMIM: 164762), and the genomic gain was categorized as likely pathogenic. Nucleotide locations were based on UCSC Genome Browser Build hg18 March 2006.

The follow up array at the N.I.H. was performed using the Affymetrix CytoScan HD assay, containing over 2.67 million probes including 1.9 million copy number probes and 750 thousand single nucleotide polymorphism (SNP) probes (Affymetrix, Santa Clara, CA). The array results were consistent with the previous array results, and showed an approximately 1.6 Mb terminal gain of 17p13.3, including more than 20 genes [arr cgh 17p13.3(525-1,629,882)×3], again categorized as pathogenic. In addition, a 54 kb loss at 3p12.1 of unknown significance was observed [arr cgh 3p12.1(85,119,040-85,173,415)×1]. This deletion involved an intron of *CADM2*. No parental testing was done as the variant was considered to be unlikely to be clinically significant. Nucleotide locations are based on UCSC Genome Browser Build hg 19 February 2009.

Discussion

Here we describe the endocrine phenotype of a probanda with 17p13.3 microduplication who has tall stature. She was found to have pituitary hyperplasia and persistently elevated growth factors consistent with excessive growth hormone secretion by somatotrophs. Previous authors have noted a tendency for childhood overgrowth in class I

microduplications including *CRK*.⁴⁻⁵ This patient raises the possibility for a central hormonal mechanism for the overgrowth previously observed in these patients. Previous case reports did not provide detailed information regarding endocrinologic evaluation of their patients. Structural brain anomalies, such as hypoplasia of the corpus callosum and the cerebellar vermis³, have been described, but this is the first reported case of a patient with a 17p13.3 microduplication with known involvement of the anterior pituitary gland. Marfanoid habitus has been described as a rare “unusual variant phenotype” in patients with 17p13.3 microduplication syndrome. Although our patient was tall, she was not felt to have a marfanoid habitus by the evaluating dysmorphologist at 5 years of age.

CRK may be a good candidate gene for tendency to overgrowth. The Crk family of adapter proteins are homologs of the v-Crk oncogene that induces sarcomas in chickens.⁶ Although Crk family members have historically been implicated in a number of signal transduction events, more recently studies have begun to show that Crk plays a role in aggressive and malignant behavior of human cancers.⁷ CrkII, an alternatively spliced isoform, has been reported to interact and be phosphorylated by insulin-like growth factor receptor.⁸ Locking Crk in the phosphorylated state has been shown to prevent Crk from initiating adipocyte differentiation which is hypothesized to be due to interruption of IGF-1 mediated pathways.⁹

Postnatal overgrowth was interestingly observed in 3 out of 4 patients with class I 17p13.3 microduplications by Bi et al.² The one patient in whom overgrowth was not seen had a normal copy number of *CRK* and *MYOIC*.² A single case of a small deletion involving *CRK* but not *YWHAE* showed significant postnatal growth retardation with significant catch-up response following growth hormone treatment.¹⁰ This patient had a normal MRI of the pituitary gland, delayed bone age (bone age greater than 2 standard deviations below the mean bone age) and growth retardation. No constitutional *CRK* point mutations in humans have been described in the medical literature.

Curry et al described the largest cohort of patients with class I microduplications involving *YWHAE* and *CRK*. Of the ones for whom height measurements are available, only 2 out of 8 had overgrowth (height 90th percentile).³ Therefore, the growth phenotype is variable. This may be explained by incomplete penetrance. Patients with larger class II duplications, which also include *CRK*, do not demonstrate overgrowth, therefore other genes in the region have an effect on the growth phenotype.

Our patient was discovered to have pituitary hyperplasia from as early as 5 years old which progressed to the gland having a pubertal configuration at 7 years old. Since her MRI pituitary was normal at 4 years old, this suggests that the pituitary findings which developed at 5 years old were not congenital. Since *CRK* signaling has been implicated also in tumorigenesis, specifically in the regulation of tumor size and local invasion, it is also plausible that pituitary hyperplasia, specifically somatotroph hyperplasia as evidenced by an increase in IGF-1, could lie along a pathway of the hyperplasia- dysplasia sequence.¹¹ It has been shown previously that pituitary adenomas develop from hyperplastic cells within the pituitary gland as supported by the fact that there is often coexistence of both hyperplastic and adenomatous tissue with a zone of transition from hyperplasia to neoplasia.¹²

Somatotroph hyperplasia usually occurs in the setting of excess Growth Hormone Releasing Hormone (GHRH) produced by the hypothalamus however, our patient's GHRH level was 14 (normal 5- 18 pg/mL, Interscience Institute, Inglewood, CA).^{13,14} Since it is believed that the pathogenesis of pituitary tumors is from the initial hyperplasia of pituitary cells in response to hyperstimulation, it is plausible that CRK signaling could somehow be associated with hyperstimulation of pituitary cells resulting in somatotroph hyperplasia, a physiologic process of increased cell number.¹¹ This hyperstimulation would be independent of GHRH in our patient. The fact that our patient's glucose suppression test result was normal suggests an absence of either a pituitary adenoma or carcinoma as the cause for our patient's increased IGF 1 levels and is in keeping with pituitary cell hyperplasia.¹⁵

Our patient also developed central precocious puberty (CPP) at 7 years old. The etiology of this could be viewed as either CRK signaling causing an activation of the hypothalamic pituitary ovarian axis independently or that it could be related to our patient's increased growth velocity and bone age advancement. We, however, speculate that CRK signaling may be associated with a perturbation of the growth hormone – IGF 1 axis and this in turn led to a cascade of events which included bone age advancement as well as an increased growth velocity and tall stature. CPP then was the ultimate sequel of an advanced bone age.

In conclusion, the phenotype of 17p13.3 microduplications is variable. Our case adds to a growing body of evidence that CRK could be a candidate gene for early overgrowth but more similar-sized duplication cases are needed to further elucidate this hypothesis. We propose that physicians who are caring for patients with 17p13.3 microduplications including *CRK* should consider biochemical evaluation of the patient's growth hormone axis and also be aware that they may be at risk for the development of CPP.

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Figure 1. Patient near initial Endocrine evaluation with an older sibling

Figure 2.

Microarray result confirming the patient's 17p13.3 microduplication [arr cgh 17p13.3(53,448->1,629,788)×3]. The region and oligonucleotide probes highlighted in pink reach denote significance for a gain of this region. The microarray analysis was performed using a SignatureSelect OS 105K v1.1 oligonucleotide array which was based on the Agilent 105K array (genome browser build hg18 Mar 2006). The genes listed in blue were current at the time of analysis. The genes, *YWHAE* and *CRK*, are indicated by the red circle.

Figure 3. Patient's growth charts showing height for age in cm and weight for age in kg

Table 1
Growth factors from first two clinic visits

Test	Result (initial presentation)	Reference range	Result (1 st follow up visit)	Reference range
IGF1	328	50-300 ng/ml	363	50-300 ng/ml
IGFBP3	5.1	1.0-4.7 µg/ml	5.2	1.1-5.2 µg/ml

Table 2
Results of the GnRH stimulation testing

Test	Baseline	Reference range	60 mins post Leuprolide acetate	Reference range
LH	1.15	<0.15 mIU/mL	16.49	Pubertal > 8 mIU/mL
FSH	5.22	1.4-2.5 mIU/mL	10.97	-
Estradiol	75	< 15 pg/ml	234	-