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CASE REPORT

A 47, XXY patient and Xq21.31 duplication with features of Prader–Willi syndrome: results of array-based comparative genomic hybridization

Pornpoj Pramyothin · Manop Pithukpakorn · Richard F. Arakaki

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Abstract A man diagnosed with 47, XXY during childhood presents an appearance similar to that of Prader–Willi syndrome with hypogonadism and gynecomastia, developmental delay, and short stature and obesity. Array-based comparative genomic hybridization revealed duplication at Xq21.31 in addition to his abnormal karyotype. This duplication was also found in his mother who appeared normal. We raise the possibility that the phenotype in this patient is a combination of both extra X chromosome and Xq21 duplication.

Keywords Klinefelter syndrome · Xq duplication · Prader–Willi-like syndrome · DNA microarrays

Introduction

Klinefelter syndrome (KS) occurs in approximately 1.2 to 1.53 per 1,000 live born male births [1, 2]. The prototypic phenotype of a 47, XXY patient is well recognized. Patients demonstrate seminiferous tubule dysgenesis, androgen deficiency, and unsuppressed follicle-stimulating and luteinizing hormones. Affected men often are taller than predicted because of their longer legs, and typically have infertility, small testes and penis, decreased facial and pubic hair, and gynecomastia.

P. Pramyothin · R. F. Arakaki (✉)
Department of Medicine, John A. Burns School of Medicine, University of Hawaii, 677 Ala Moana Boulevard, #1024, Honolulu, HI 96813, USA
e-mail: rfarakak@hawaii.edu

M. Pithukpakorn
Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

In this report, we describe a patient with KS genotype but with phenotype similar to that of Prader–Willi syndrome (PWS), with short stature, stage 3 obesity, polyphagia and developmental delay. A report on this patient at 7 years of age has been published [3]. This report details this patient's development into adulthood, and investigation with array-based comparative genomic hybridization (array CGH) and X inactivation studies, which identified an additional genetic abnormality apart from 47, XXY, duplication on the long arm of X chromosome at Xq21.31.

Results: clinical report

This 20-year-old patient was born by cesarean delivery after an uncomplicated 40-week gestation to a G_1 mother of mixed Caucasian, Japanese, and Mexican ancestry. There was a history of antiepileptic drug use in the first trimester but the medication was stopped when the pregnancy was discovered. The prenatal ultrasound and glucose tolerance test were within normal limits. The patient's birth weight was 5 lb and 10½ oz, his infancy was uncomplicated except for asthma requiring intermittent beta-agonist inhaler therapy.

At 3 years of age, the patient began to gain weight and developed signs and symptoms of Cushing syndrome. The diagnosis of hypercortisolemia was excluded with biochemical testing, and the patient was suspected of having PWS and evaluated at 7 years of age. His weight was over the 95th percentile with a height at the 10th percentile. The diagnosis of PWS was ruled out as DNA probe for the 15q11-12 PWS region and methylation studies failed to detect any abnormalities. Fragile X syndrome was excluded as no FMR-1 gene mutation was found. However, the

patient's karyotype was found to be 47, XXY and the diagnosis of KS was made [3].

His growth and development into adulthood has been atypical of KS. Although hypogonadism and small testes were observed, he continued to be mentally challenged and morbidly obese. At the age of 16, he developed diabetes mellitus type 2, which has remained poorly controlled over the years despite 380 units of daily insulin and maximal dosage of insulin-sensitizing medications. Persistent hyperglycemia was attributed to "sneaking" consumption of sweets and other carbohydrates along with non-compliance to insulin injections, which is seen frequently in PWS patients with diabetes. Due to his developmental delay, special education was required. At 20 years of age the patient lives with his family who provide assistance with activities of daily living. Three-generation pedigree revealed no consanguinity. His mother has hypertension, rheumatoid arthritis, hypothyroidism, and Sjogren's syndrome. His father has no medical problems. His 15-year-old younger brother was diagnosed with attention-deficit hyperactivity disorder. No other family member exhibits similar findings as those seen in this patient.

At his most recent evaluation, he was 20-year-old. His height was 63 in. (<3rd percentile), weight was 290 lbs (>97th percentile), and BMI was 51.4 kg/m^2. His examination revealed a pleasant, attentive young man who had speech limited to a few short sentences. His face was full with erythema in the malar area, and he had increased posterior cervical fat accumulation. His breasts were Tanner stage 4 (Fig. 1). No axillary or pubic hair was noted. He had a small penis and, and his testes measured 4 cc (1.5 cm × 1.5 cm × 2 cm) bilaterally with diffusely firm consistency. The neurological examination was unremarkable. Evaluation of reproductive hormones revealed low total testosterone level of 81 ng/dl (NL range 262–1593 ng/dl), and elevated FSH level of 25.5 mIU/ml (NL range, 1–8 mIU/ml) and LH level of 17.6 mIU/ml (NL range 2–12 mIU/ml), consistent with primary hypogonadism. His prolactin level was 8.7 ng/ml (NL range 2.6–13.1 ng/ml). His hemoglobin A1c levels over the previous year have remained above 10.0% (normal glucose tolerance range 4–6%).

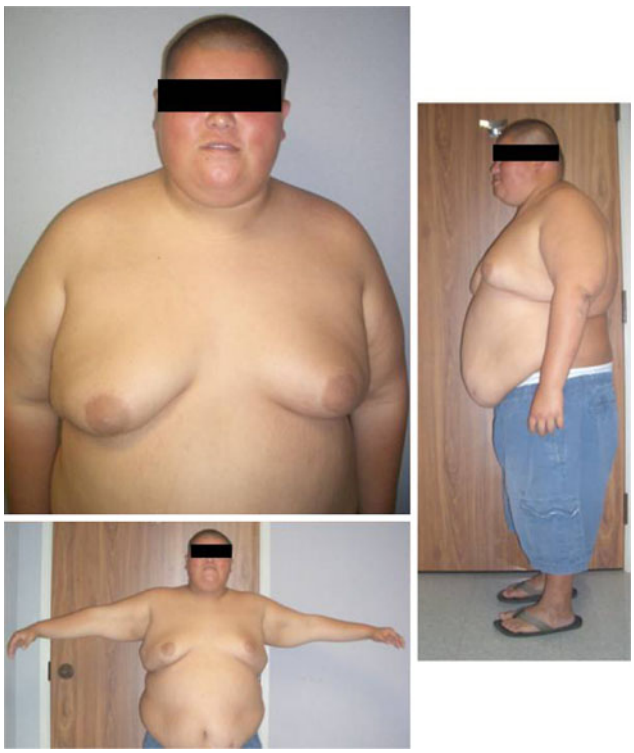

Fig. 1 A 20-year-old patient with Kleinfelter Syndrome who presented atypically with short stature and obesity stage 3. The figures also demonstrate lack of axillary hair and Tanner stage 4 breast development

Results and methods

Array CGH was performed at the Georgia Esoteric and Molecular Diagnostic Labs (Augusta, GA, USA) using Spectral Chip^{TM} 2600 BAC array (Perkin Elmer Life and Analytical Sciences). The test detects gain or loss of chromosome region ranging from 100 kb to 2 Mb. Duplication of the X chromosome consistent with KS was detected in this patient with array CGH. Interestingly, a single clone gain, *CTB188I17*, on the long arm of X chromosome at Xq21.31 was demonstrated. This was confirmed by fluorescence in situ hybridization (FISH; Fig. 2). Thus, the patient's genotype is described as 47, XXY, dup(X) (q21.31). This same duplication was demonstrated by array CGH and FISH in the mother, while no abnormality was detected in the father. Duplications in the long arm of the X chromosome have been found to account for PWS phenotype [4] and the possibility that X inactivation plays a significant role in gene expression in this patient and his mother was assessed using methylation-specific polymerase chain reaction (MS-PCR) [5]. However, as a result of homozygosity of *FMR1* and *AR* repeats in both subjects, X inactivation pattern could not be determined.

Discussion

Array CGH has only recently become available as a specific and sensitive technique to detect DNA copy number change in patients with genetic disorders [6, 7]. In order to investigate additional genetic abnormalities apart from the underlying 47, XXY karyotype in this young man with PW-like phenotype, array CGH was performed. Results

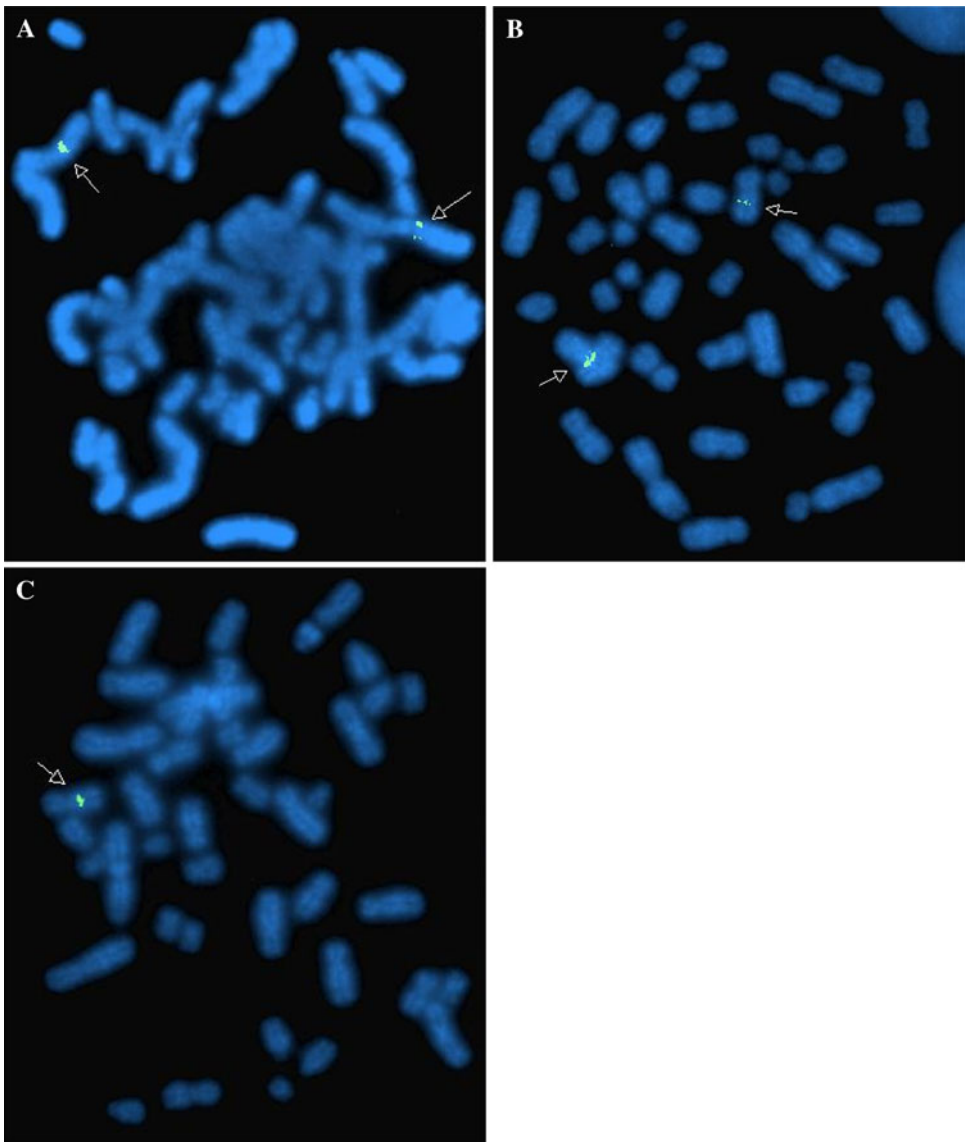

Fig. 2 FISH using a probe localizing to Xq21 region. Both proband (**a**) and mother (**b**) show identical localization in contrast to the father (**c**)

revealed duplication at Xq21.31 which was inherited from his heterozygous mother who has a normal phenotype.

Children and adults, male and female, with various duplications in the long arm of X chromosome have been reported with Prader–Willi-like phenotype that includes obesity, short stature, and intellectual impairment similar to our patient. Of particular interest is a recent report of a 4-year-old 46, XY male with Xq21.1–q21.31 duplication [4]. The genotype and phenotype of the 4-year-old in their report appears particularly similar to our patient in early childhood. Observed clinical features include polyphagia, early onset obesity, and developmental delay, with the exception of short stature, as his height was reported at 75th percentile. This patient also received a provisional diagnosis of PWS before molecular studies confirmed otherwise, a situation which is very similar to that of our patient's. With this first (and only) observation of duplication at the Xq21 locus in a patient with Prader–Willi-like phenotype, our patient is another example of this association, albeit the presence of an additional X chromosome cannot be ignored or disregarded.

The phenotypic presentation of KS has been found to be associated with degrees of X chromosome inactivation as well as CAG_n repeat lengths of the AR sequence [8]. Both random and non-random X inactivation occurs not only in 46, XX females, but also in 47, XXY and 48, XXYY Klinefelter patients [9]. Skewed or non-random inactivation of the duplicated X chromosome occurs in females and this phenomenon appears to protect them from genetic imbalance caused by the duplication, which could explain our patient's and his mother's phenotype. Unfortunately, the X inactivation studies with MS-PCR with amplified

fragments of polymorphic repeat-containing region on *FMR 1* and *AR* genes were not informative due to homozygosity of the alleles at both loci.

At least seven loci of mental retardation syndromes encompassing the duplication in the Xq21.31 region were identified from in silico analysis. Clinical features reminiscent of PWS were described in mental retardation X-linked syndromic 7 (MRXS7; OMIM 300218)[1] [10] and Wilson–Turner syndrome (WTS; OMIM 309585)[2] [11], localized at Xp11.3-q22 and Xp21.1-q22, respectively. The two syndromes share an overlap region on the X chromosome, and also share similar clinical features including mental retardation, obesity, tapering fingers, small feet, and hypogonadism. However, gynecomastia was described only in WTS but not in MRXS7. Additionally, the Xq21.31 duplication may well represent a copy-number variant (CNV) that does not contribute to the phenotype of this subject and as discussed, an incidental finding in his mother [12]. With the development of array CGH, an increasing number of CNVs have been detected with sizes ranging from 1 kb to 3 Mb (GenBank). In silico analysis revealed at least three CNVs within the Xq21.31 band, ranging in size from 0.27 to 0.41 Mb (UCSC Geneome Browser).[3] Thus, there remains the possibility that the Xq21.31 duplication in this patient represents a CNV and his phenotype exclusively attributed to KS. Moreover, review of potential candidate genes in this region does not appear to explain our patient's phenotype. The known *KLHL4* and *DACH2* genes have been implicated in mullerian aplasia [13] and the *TGIF2LX* gene has testis-specific expression and probably involved in spermatogenesis (Ensembl Genome Database).[4]

Obesity, metabolic syndrome, type 2 diabetes, and intellectual disability have been described within the clinical spectrum of KS and may be related to androgen deficiency in this condition [14], although this would not entirely explain our patient's short stature. In summary, we report on a 47, XXY patient with PW-like presentation to have Xq21.31 duplication as revealed by array CGH.

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