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Detection of Mutually Exclusive Mosaicism in a Girl with Genotype-Phenotype Discrepancies

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Abstract

Discordance between clinical phenotype and genotype has multiple causes, including mosaicism. Phenotypes can be modified due to tissue distribution, or the presence of multiple abnormal cell lines with different genomic contributions. We have studied a 20-month-old female whose main phenotypes were failure to thrive, developmental delay, and patchy skin pigmentation. Initial chromosome and SNP microarray analysis of her blood revealed a non-mosaic ~24 Mb duplication of 15q25.1q26.3 resulting from the unbalanced translocation of terminal 15q to the short arm of chromosome 15. The most common feature associated with distal trisomy 15q is prenatal and postnatal overgrowth, which was not consistent with this patient's phenotype. The phenotypic discordance, in combination with the patchy skin pigmentation, suggested the presence of mosaicism. Further analysis of skin biopsies from both hyper- and hypopigmented regions confirmed the presence of an additional cell line with the short arm of chromosome X deleted and replaced by the entire long arm of chromosome 15. The Xp deletion, consistent with a variant Turner Syndrome diagnosis, better explained the patient's phenotype. Parental studies revealed that the alterations in both cell lines were de novo and the duplicated distal 15q and the deleted Xp were from different parental origins, suggesting a mitotic event. The possible mechanism for the occurrence of two mutually exclusive structural rearrangements with both involving the long arm of chromosome 15 is discussed.

Keywords

distal 15q duplication; Xp deletion; SNP microarray analysis; tissue specific mosaicism

INTRODUCTION

Since the first case reported by Fujimoto [Fujimoto et al., 1974], more than 70 cases with distal 15q duplication have been described in the literature [Gutierrez-Franco Mde et al.,

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2010]. Although the phenotypic findings may vary from case to case depending upon the length and location of the duplicated portion of chromosome 15q and the coincident monosomic component of another chromosome, the phenotypic findings are very consistent for the distal terminal duplications with proximal breakpoints ranging from 15q25.1 or 15q26.1 to the terminus. The clinical features seen in the majority of mixed cases and all pure cases with the duplication of this region include prenatal and postnatal overgrowth, intellectual disability, characteristic craniofacial dysmorphism, and renal anomalies [Tatton-Brown et al., 2009]. Such phenotype-genotype correlation resulted in the delineation of the 15q overgrowth syndrome.

Deletion of the short arm of chromosome X (Xp) is another well-characterized genetic disorder. Generally, almost all females with partial Xp deletion have short stature and some have Turner syndrome (TS) phenotypic features [Fryns et al., 1981; Ogata et al., 2001]. Females with the deletion of the entire short arm of the X chromosome are also associated with gonadal dysgenesis. The short stature seen in these patients is considered to be the result of haploinsufficiency for the *SHOX* gene, within the pseudoautosomal region on Xp [Rao et al., 1997].

Here we present a case of a 20 month old female who carried mutually exclusive mosaicism for both distal 15q duplication and Xp deletion.

MATERIALS AND METHODS

Clinical Report

A 20 month old female infant was the second child born to healthy, non-consanguineous African-American parents. She was delivered spontaneously at 37 5/7 weeks of gestation. All prenatal exams were normal. Maternal age was 28 years old and paternal age was 30 years old at delivery. Maternal height is 172.72 cm and paternal height is 175.26 cm.

After birth, she had postnatal failure-to-thrive, falling from the 12th centile for weight at birth to the 3rd centile during the first month of life. She was growing consistently along the 3rd centile for weight until 6–7 months of age, when her rate of weight gain began to plateau. Her length had consistently remained between the 10–50th centile, and her occipital frontal circumference (OFC) had increased from the 4th centile at birth to around the 53 centile at 11 months. At 20.5 months of age, she had a weight of 8.11 kg (0.01 centile), length of 80 cm (18.44 centile), and OFC of 46cm (22.78 centile). The detailed measurements during this period of time are present in the growth chart (Fig. 1).

Physical examination revealed that she was a very thin child with mild facial dysmorphism, including fine sparse hair throughout with patchy areas of hair loss, prominent anti-tragus of the ears bilaterally, bitemporal narrowing, and relative hypertelorism (IPD-75th centile). Her skin was remarkable for hypopigmented macules and patches scattered on her trunk and extremities in a linear distribution along Blaschko's lines. Other features included central and peripheral hypotonia, development delay and speech delay.

Cytogenetic Analysis

Standard chromosome analysis by Giemsa-trypsin banding was performed on metaphase spreads prepared from PHA stimulated peripheral blood, and cultured fibroblast from the skin biopsy obtained from the hyperpigmented region. Additional metaphase cells were studied by a series of fluorescence in situ (FISH) analyses using the probe mapping within the duplicated region of 15q25.1-qter (G248P8864C11), another probe mapping within the deleted region of Xp21.2 (RP11-89L23), and a control probe within the long arm of chromosome X (RP11-526E6).

SNP Microarray Analysis

Genomic DNA was extracted from uncultured peripheral blood and cultured fibroblasts from the skin biopsies collected from the hypopigmented region and hyperpigmented region respectively in a standard manner. Maternal DNA was extracted from the fixed cytogenetic cell suspensions according to previously described methods [Amorim et al., 2007]. SNP array was performed using the Illumina Human 850K Bead Chip (Illumina Inc., San Diego, CA) on these DNA samples, according to the protocol described before [Conlin et al., 2012]. The copy number alterations were visually inspected and manually detected using the BeadStudio software according to the change of the logR ratio and the B allele frequency, in combination with a CNV detection tool [Gai et al., 2010]. The B allele frequencies for each sample were also examined for imbalance of two alleles as the indicators of mosaicism, which was calculated as described previously [Conlin et al., 2010]. The genotype calls were exported from the software and were compared between mother and child pair to determine the parental origins of the 15q duplication and Xp deletion. All coordinates are in hg19.

RESULTS

Non-Mosaic Distal 15q Duplication Detected in the Peripheral Blood

SNP array analysis performed on the peripheral blood revealed a ~24 Mb duplication of chromosome 15q25.1-qter in all cells examined (Fig. 2A). The duplication contains more than 70 genes including *IGF1R* (OMIM# 147370).

Follow-up chromosomal analysis identified a derivative chromosome 15 (Fig. 3A), which showed additional chromosomal material replacing the short arm of one chromosome 15 in all cells examined. FISH analysis demonstrated two copies of distal 15q25.1 on this derivative chromosome 15. This confirmed that the duplicated segment of 15q detected by SNP array was derived from the unbalanced translocation of the chromosome segment distal to q25.1 to the short arm of the same chromosome (Table I).

Mosaicism Detected in the Skin Biopsy Tissue

SNP array and cytogenetic analysis performed on the skin biopsy of both hyper- and hypopigmented regions demonstrated the presence of two cell lines, but at different percentages in the two skin biopsies. In the hyperpigmented region, around 50–55% of cells examined contained the same distal 15q duplication as seen in the blood cells (Fig. 2A). In addition, a second cell line containing a ~57 Mb deletion on chromosome Xp22.33p11.21 was detected in 35–40% cells (Fig. 2B). Chromosome analysis found 45 chromosomes, with

the majority of Xp replaced by the entire 15q. In this cell line, no distal duplication of 15q was observed (Fig. 3B). This finding was also confirmed by FISH analysis. A total of 120 metaphases were screened and a third cell line, including normal cells, was not detected. The same chromosome abnormalities were detected in the hypopigmented skin region but the percentage for each cell line was different. In this sample, 70–75% of cells examined showed the abnormal chromosome 15 with distal 15q duplication (Fig. 2A) while only 15–20% cells contained the Xp deletion (Fig. 2B).

In light of the skin findings, the blood was reanalyzed for low level mosaicism for the Xp deletion, which was not detected (Fig. 2B). No run of homozygosity (ROH) was observed on chromosome X in this tissue, indicating that two normal X chromosomes were biparental in origin, and not a result of rescue of the abnormal X chromosome. A comprehensive constitutional karyotype and abnormalities reflecting all cell types was summarized in the Table I.

Parental Study

In order to rule out the possibility of a familial rearrangement leading to these abnormal cell lines, chromosome analysis was also performed on the peripheral blood from this patient's parents. The karyotypes were normal for both parents, without the detection of any balanced chromosome rearrangement, including a translocation or inversion.

SNP array analysis was also performed on the maternal DNA. A total of 1,250 informative SNPs from the distal 15q duplication region, and 1,486 SNPs from the deleted Xp region were identified. The results suggest the duplicated 15q is maternal in origin, while the deleted Xp is paternal in origin. In addition, the genotyping results from the remaining parts of chromosome X and 15 suggest biparental origins in all tissue samples. These findings suggested that the two cell lines were formed independently.

DISCUSSION

In this report, we describe a unique patient with co-occurrence of mutually exclusive mosaicism for a distal 15q duplication and Xp deletion. This patient presented with postnatal failure to thrive, developmental delay, mild facial dysmorphism and patchy hypopigmented and hyperpigmented lesions of the skin. Initial analysis revealed a non-mosaic pure duplication of 15q25.1q26.3 in her blood sample. Although this patient showed developmental delay and mild facial dysmorphism, her phenotype including growth failure had minimal overlap with the clinical features of 15q overgrowth syndrome [Tatton-Brown et al., 2009].

Discordance between clinical phenotype and genotype can result from multiple reasons including mosaicism. Mosaicism can modify the phenotypes due to its tissue distribution, or in some cases, due to the presence of multiple cell lines with different genomic contributions [Biesecker and Spinner, 2013]. In this patient, the phenotypic discordance, in combination with the patchy skin pigmentation suggested the presence of mosaicism, prompting us to analyze DNA from skin biopsies to look for other genetic changes.

Further analysis of the skin biopsies confirmed the presence of mosaicism for two cell lines, one cell line with distal 15q duplication, as well as additional cell line with a deletion of most of Xp, resulting from an unbalanced translocation between chromosome X and 15. The difference in the percentage of cell line with 15q duplication detected by metaphase cell analysis and array analysis may be due to the growth advantage of cells carrying 15q duplication. Since this derivative chromosome X carries the whole 15q, in order to achieve a relatively functional balance, the normal chromosome X should be inactive in this cell line. Such asymmetric inactivation can cause monosomy Xp but avoid monosomy 15, which is generally lethal. In this patient, the monosomy Xp in the second cell line can better explain her growth failure. Distal 15q duplication in the first cell line may account for her other phenotypes such as developmental delay, and dysmorphic features. To date, no cases with co-occurrence of distal 15q duplication and Xp deletion have been reported. The two disorders are mutually exclusive in associated phenotypes regarding growth. As we know, overgrowth was described only in 75% of mixed cases with distal 15q duplication arisen from a parental balanced translocation. For some of these cases, no overgrowth or even growth retardation was reported. It has been hypothesized that IGF1R function in these individuals was modified because of the concurrence of monosomy of another chromosome segment [Van Allen et al., 1992]. Such modification could also happen in the current patient with Xp deletion resulting in the decreased dosage of *SHOX* gene.

Parental studies demonstrated that the 15q and Xp alterations in both cell lines were de novo and not the result of parental balanced rearrangement or recombinant chromosome. Since the total ratio of two cell lines in both skin biopsy tissues was slightly less than 100% by array analysis, we hypothesized that there might be an intermediate cell line to bridge two abnormal ones; however, further screening of 120 metaphases failed to identify the third cell line, which also ruled out low level mosaicism (<3%) at 95% confidence [Hook, 1977]. SNP array genotyping results indicated that the duplicated distal 15q was maternal in origin and the deleted Xp was paternal in origin. Two abnormalities involving chromosomes deriving from the different parents strongly suggest that they were two separate events, and at least one of them occurred during mitosis. Since there are two normal X chromosomes in the 15q duplication cell line, we assume that the unbalanced translocation involving two homologous chromosomes 15 was the first event. Similar chromosomal findings have been reported as a recombinant-like chromosome, and arise in meiosis or are postzygotically mediated by non-allelic homologous recombination (NAHR), nonhomologous end joining, or telomere transposition [Rivera et al., 2013]. The formation of the derivative chromosome X resulting in Xp deletion is either a secondary event from the further rearrangement of the derivative chromosome 15 for rescue purpose during mitosis, or may have occurred simultaneously as the derivative 15q in a different cell population; however, the lack of a normal cell line supports the 15q rescue mechanism.

In conclusion, the discordance between the genotype and phenotype enabled us to detect the co-occurrence of two mutually exclusive de novo mosaic structural abnormalities, distal 15q duplication and Xp deletion, in the same patient. This case illustrates that phenotypic discordance with a detected genomic alteration could be explained by hidden mosaicism. It also presents an interesting example of two distinct alterations involving translocation of

different genomic segments from the same chromosome resulting in opposite phenotypic effects which further confounded the clinical phenotype prediction in this patient.

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FIG. 1.
Growth chart for the weight showing post-natal failure-to-thrive in this patient.

FIG. 2.

SNP genomic profile of both logR ratio (top) and B allele frequency (bottom) showed the identification of a ~24 Mb duplication of chromosome 15q25.1-qter or arr[hg19] 15q25.1q26.3 (78,833,758-102,461,162)x3 in all cells from the peripheral blood sample; about 70–75% cells from hypopigmented skin region; and around 50–55% cells from hyperpigmented skin region. Only the end of the p arm and the distal portion of the q arm is displayed in this figure (A). A ~57 Mb deletion of chromosome Xp22.3p11.21 or arr[hg19] Xp22.33p11.21 (60,814-56,958,934)x1 was not detected in the peripheral blood sample, but was identified in around 15–20% cells from hypopigmented skin region, and 35–40% cells from hyperpigmented skin region by the genome wide SNP array analysis (B). Only the p arm and part of the q arm of chromosome X are displayed in this figure.

FIG. 3.

Cytogenetic analysis demonstrated two cell lines. One cell line contained the derivative chromosome 15 with a duplicated distal 15q replacing the short acrocentric arm of the same chromosome. This abnormality was also confirmed by FISH with distal 15q labeled in red (A). The second cell line contained one normal chromosome 15 and one derivative chromosome X with Xp replaced by almost the entire 15q. In FISH study, the green signal mapping within the Xp was replaced by the red signal mapping within the distal 15q (B).

TABLE I

Comprehensive Changes and Their Parental Origin Detected in All Cell Types

Samples	Number of metaphases with given karyotype		Array findings		
	46,XX,der(15)(15;15)(p11.2;q25.1),ish der(15)(G248P8864C11++)	46,X,der(X)(X;15)(p11.21;q10),ish der(X)(RP11-89L23-,DXZ1+,RP11-526+,G248P8864C11+)	15q duplication (%)	Xp deletion (%)	
Blood	10	0	100	0	
Darker skin	113	5	50-55	35-40	
Lighter skin	NA	NA	70-75	15-20	