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CASE REPORT

Nine de novo duplications affecting both maternal and paternal chromosomes and an inherited 15q11.2 deletion, in a patient with developmental delay

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Key Clinical Message

A patient with developmental delay and nine, de novo, tandem duplications affecting eight different chromosomes that arose on both maternal and paternal chromosomes indicating a vulnerable zygotic or early postzygotic period of development for these errors, potentially affected by genetic and nongenetic factors.

Keywords

Chromosomal microduplication, de novo copy number variations, developmental delay, multiple congenital anomalies

Introduction

Constitutional copy number variation (CNV) is a major source of germline genomic variation that has been associated with various clinical outcomes, such as intellectual disability, developmental delay, and multiple congenital anomalies [1–4]. Most pathogenic CNVs are either recurrent, arising by nonallelic homologous recombination (NAHR) between different regions throughout the genome, or nonrecurrent, arising by DNA replication errors and usually displaying microhomologies at the breakpoint junctions [5, 6].

Recurrent constitutional deletion and duplication CNVs affect various regions in the genome and have been reported in several recognized genomic syndromes, such as 16p11.2 deletion syndrome, 1q21.1 deletion and duplication syndromes, to name a few [7, 8]. Nonrecurrent CNVs can arise presumably anywhere in the genome and are also associated with diverse phenotypic abnormalities. Most are simple deletions or tandem duplications, but some of these CNVs are complex chromosomal rearrangements affecting single chromosome regions or multiple chromosomes [2, 3, 6]. Such complex rearrangements may account for a large proportion (perhaps up to 65%) of nonrecurrent CNVs that are associated with genomic disorders [9]. However, multiple de novo large (>100 Kb) CNVs affecting different chromosomes have rarely been observed in a single patient. Herein, we report one patient with multiple de novo duplications affecting different chromosomes and a paternally inherited deletion.

Methods

HumanCytoSNP-12 BeadChip

The HumanCytoSNP-12 BeadChip (300K probes) assay was performed according to the Infinium HD Ultra protocol (Illumina, San Diego, CA). Briefly, 200 ng of genomic DNA was used for whole-genome amplification at 37°C for 20–24 h. The amplified DNA was enzymatically fragmented, purified, loaded on the HumanCytoSNP-12 BeadChip, and allowed to hybridize at 48°C for 16–24 h. Subsequently, the hybridizing DNA on the BeadChip was labeled by enzymatic single base extension and

incorporated nucleotides were detected using fluorescently labeled antibodies. Stained BeadChips were scanned using a HiScan (Illumina). Data were generated with GenomeStudio (Illumina) and analyzed with Nexus Copy Number software version 6 (BioDiscovery, Hawthorne, CA). All CNVs >100 Kb were interrogated.

Fluorescence in situ hybridization

Metaphase and interphase fluorescence in situ hybridization (FISH) were performed for all eight large duplications and the deletion using BAC probes (BlueGnome, Cambridge, United Kingdom). Slides were prepared and hybridized according to standard laboratory procedures. Slides were dehydrated through graded ethanol. Probes were mixed per manufacture protocols and applied to each slide, which was then sealed with a coverslip and hybridized in a HYBrite (Abbott Molecular, Des Plaines, IL) apparatus using a 2 min denaturation step at 75°C followed by overnight hybridization at 37°C. Slides were washed in 0.4 × SSC buffer at 73°C for 2 min and 2xSSC/0.1% NP-40 at room temperature for 1 min. Slides were counterstained with DAPI II (Abbott Molecular). Metaphase and interphase cells were identified on a Leica DM5500D scope (Leica Microsystems, Buffalo Grove, IL) and images were captured by Cytovision software. Written informed consent was obtained from the patient's family.

Results

Clinical findings

The patient is a 16-year-old female who was the 6 pound, 7 oz product of a 42 week gestation born by normal, spontaneous vaginal delivery to a 17-year-old primigravid woman after an uncomplicated pregnancy. The mother smoked cigarettes for the first 2–3 months of pregnancy, and prenatal ultrasound at 3 months was normal. Concern regarding development of major motor milestones arose at 12 months and head circumference was at the 3rd centile. Head MRI was unremarkable. She began crawling at 14 months, pulled to stand at 26 months, and independent steps were not taken until 28 months of age. At 32 month of age, she was not assisting in dressing or undressing herself, did not perform any imitative behavior and would not follow directions. Self-induced head banging and hand waving behaviors occurred when she appeared to be frustrated. Neuropsychological screening placed her at approximately the 9-month-old level. Family history is significant for paternal dyslexia and need for special education classes throughout high school; a maternal cousin has cerebral palsy and delay in acquisition of motor milestones; a paternal uncle required special education classes. Physical exam findings at that time revealed a head circumference at the 3rd centile, height at the 10th centile, and weight at the 5th centile. Parental head circumferences were both normal (56 cm). Notable dysmorphic features included small cranium with sloping forehead, deep-set eyes, and flattened zygomas. The right side of the face was slightly longer in the vertical dimension by comparison and she had bilateral epicanthal folds and a prominent nasal bridge. The mouth appeared large and the palate and teeth were normal. Ears were normally formed and placed, with no pretragal tags or pits. Neurologic exam for tone, reflexes, and gait were normal. A chromosome analysis performed when the patient was 30 months old, revealed 46, XX, add(7)(q33). Parental chromosome analyses were normal. The identity of the additional chromosomal material was not determined. Follow-up evaluation at 8 years of age revealed very poor expressive language development with only two words. Receptive language was better, but very limited, including understanding "no" and recognizing her name. She could feed herself with her fingers, was unable to dress herself and potty training had not been accomplished. Hearing tests were normal, and her health was generally good. At that visit a prominent nasal bridge was appreciated, and she had numerous misaligned teeth. She had dextro-scoliosis from T8 to T12 of 13 degrees and levo-curvature from T12 to L3 of 12 degrees. At 16 years of age she had no improvement in expressive language. She has had frequent episodes of unexplained crying and inconsolability. She dresses and feeds herself, and there has been no loss of milestones. She has constipation and has had no seizures. Menstruation has not begun. During the exam she was tearful and avoided eye contact. Hyperreflexia was noted. MRI revealed no syrinx or cord compression, no vertebral segmentation anomaly or fusion, scoliosis and an aberrant right subclavian artery.

Cytogenetic and molecular analyses

Chromosomal microarray analysis (CMA) detected a female chromosome profile with 12 aberrations affecting 10 different chromosomes (Table 1). CMA on both parents revealed that the 15q11.2 deletion (546 kb) was inherited from the father, whereas the 10p14 duplication (131 Kb) and 16p12.2 deletion (149 Kb) were inherited from the mother. Nine duplications in the patient occurred de novo. The inherited 10p14 duplication and 16p12.2 deletion were considered benign CNVs and were excluded from further analysis. [10–13] Thus, 10 of these aberrations were considered potentially clinically significant including nine regions of duplication affecting chromosomes X, 3, 4, 7, 8,

Table 1. Twelve CNVs found in one patient. SNP analysis revealed the parental origin of each aberration.

Chromosome band	GRCh37/hg19 coordinates x copy number	Size (Mb)	Parental origin	Gene number (OMIM gene)	Reported similar CNV	Reported phenotype
Xp11.4p11.3dn	(40618904–45179762) × 3	4.6	Paternal	22 (12)	a 4.6 Mb duplication [22]	SD, ID, M, MY, S
3p24.1p23dn	(26797958–31529089) × 3	4.7	Maternal	13 (7)	No CNV	NA
4p11dn	(48735747–49053522) × 3	0.318	Maternal	4 (0)	Yes	Unknown[1]
7q33q34dn	(136624320–141093612) × 3	4.5	Paternal	53 (19)	No CNV	NA
8p21.2p12dn	(25159885-29932284) × 3	4.8	Maternal	51 (27)	No CNV	NA
9p24.1p23dn	(6027931–10603845) × 3	4.6	Paternal	13 (4)	No CNV	NA
9p21.3dn	(20014872–24527357) × 3	4.5	Maternal	42 (23)	No CNV	NA
10p14	(6658517–6789463) × 3	0.131	Maternal[2]	1 (0)	Yes	Benign[3]
10q24.33q25.1dn	(105666745–110387632) × 3	4.7	Paternal	17 (6)	No CNV	NA
15q11.2	(22754322–23300172) × 1	0.546	Paternal[4]	35 (4)	Numerous deletions/duplications	Various[5]
16p12.2	(21591157–21740231) × 1	0.149	Maternal[2]	4 (3)	Yes	Benign[3]
19q13.2q13.31dn	(39379906–43465171) × 3	4.1	Paternal	145 (86)	No CNV	NA

Dn, de novo; SD, speech delay; ID, mild intellectual disability; M, macrocephaly; MY, myopia; S, mild scoliosis.
[1]Two similar deletions and one duplication have been reported at this genomic location [13].
[2]Inherited from the mother.
[3]Both deletions and duplications have been reported at this genomic location [13].
[4]Inherited from the father.
[5]Associated with various neurodevelopmental abnormalities [17–21].

9, 10, and 19 and one deletion affecting chromosome 15q11.2 (Table 1; Figs 1A, B; Fig. S1).

Metaphase FISH analysis for probes mapping to 9p21.3 (BAC G100264R; Red) and 19q13.2-q13.3 (BAC G100203G; Green) showed no evidence for translocation or insertion of the 9p21.3 and 19q13.2-q13.3 regions to alternative chromosomal locations (Fig. 1C top). Interphase FISH analysis utilizing these two BAC probes showed three hybridization signals within 9p21.3 and 19q13.2-q13.3 indicating duplications of these regions (Fig. 1C bottom).

In addition, metaphase FISH analysis for 7q33 (BAC G100228; Red) and 8p21.1 (BAC G100461G; Green) showed no evidence for translocation or insertion of the 7q33 and 8p12 regions to alternative chromosomal locations (Fig. 1D top) and three interphase hybridization signals for these two BAC probes indicating duplications of these regions (Fig. 1D bottom). Collectively, metaphase and interphase FISH analyses utilizing region-specific BAC probes confirmed the single deletion and eight large duplications, and showed that the large duplications were all located at the site of homology, presumably *in tandem*, and not inserted at a divergent position on the same or a different chromosome (Figs 1C, D; Fig. S2).

Evaluation of the parental origin of each aberrant CNV genomic material was performed using GenomeStudio B-allele SNP calls. SNP data analyses from the patient and both parents revealed that four of the nine de novo duplications originated on maternally derived chromosomes and five on paternally derived chromosomes (Table 1).

Discussion

Intellectual disability, developmental delay, and multiple congenital anomalies, are frequently associated with de novo constitutional CNVs, some of which are complex chromosomal rearrangements affecting single or multiple chromosomes [1–4, 6, 14, 15]. However, multiple de novo large CNVs (>100 Kb) involving different chromosomes have rarely been observed in a single patient. Liu et al. presented two patients with 8 and 11 de novo duplications (ranging from 104 Kb to 6.4 Mb and 211 Kb to 4.7 Mb, respectively). They suggested a postzygotic origin of these duplications based on biparental inheritance, similar to our patient, and suggestive of cellular DNA replication errors [16].

The collective effect of these chromosomal aberrations almost certainly explains the observed clinical phenotypes in this patient, however; the clinical significance of each of these aberrations individually, based on the genomic locations and gene content (Table S1), is currently unclear. Deletions and duplications similar to the 15q11.2 deletion in this patient have been reported more often in patients with congenital anomalies, behavioral and neurological problems, speech delay, and autism than in healthy parents and controls leading to a challenging clinical interpretation [17–21]. A duplication similar to the Xp11.4p11.3 duplication detected in this patient has been described in a female with speech delay, mild intellectual disability, macrocephaly, myopia, and mild scoliosis [22]. While preferential inactivation of the aberrant X chromosome in females

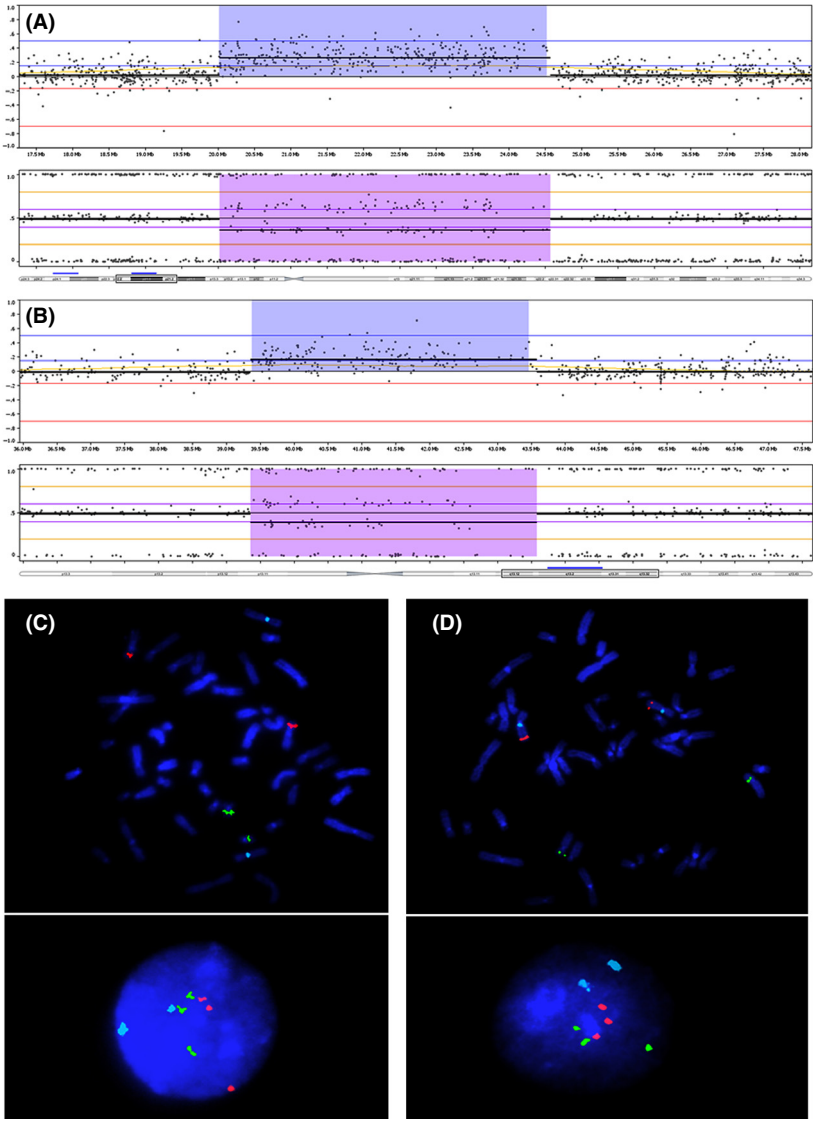

Figure 1. SNP chromosomal microarray and FISH analyses. Two representative examples of 1 copy gains detected by CMA. (A) Log R graph representing a duplication of 9p21.3 with a probe median of +0.26 highlighted in blue (top) and the corresponding allelic imbalance (highlighted in purple, bottom) detected by the B allele calls of SNPs, where heterozygous calls are outside the normal range of 0.4 to 0.6. (B) Log R graph representing a duplication of 19q13.2-q13.3 with a probe median of +0.17 highlighted in blue (top) and the corresponding allelic imbalance (highlighted in purple, bottom) detected by the B allele calls of SNPs. (C) FISH for 9p21.3 and 19q13.2-q13.3, and (D) FISH for 7q33 and 8p21.1. Top: metaphase FISH images showing no evidence for translocation or insertion of the 9p21.3 and 19q13.2-q13.3 (C) or 7q33 and 8p12 (D) regions to alternative chromosomal locations. Bottom: interphase FISH images showing three hybridization signals for probes mapping to 9p21.3 and 19q13.2-q13.3 (C), and for 7q34 and 8p12 (D) indicating a duplication of these regions. BAC probes used were: Red: BAC G100264R that hybridizes to 9p21.3 and BAC G100228 that hybridizes to 7q34. Green: BAC G100203G - 19q13.2 and BAC G100461G - 8p21.1. Aqua: CEP7 control probe to chromosome 7 pericentromeric alpha satellite DNA.

with Xp duplications often results in asymptomatic females, that patient exhibited a normal, nonskewed X inactivation pattern, offering a potential explanation for the phenotype. However, CNVs similar to the remaining eight de novo duplications detected in this patient have not been previously reported. [10–13]

Although we did not determine the precise DNA sequences at the duplication breakpoints in our patient, there are no large segmental duplications in the breakpoint regions in the reference genome, suggesting that they are nonrecurrent CNVs that arose from nonhomologous DNA repair. While the underlying mechanisms for

nonrecurrent CNVs have not yet been fully elucidated, several DNA-replication-based mechanisms, including fork stalling and template switching (FoSTes) and microhomology-mediated break-induced replication (MMBIR), have been proposed [6, 9]. Similar DNA-replication-based mechanisms and simple nonhomologus end joining have also been proposed for complex CNVs wherein numerous somatic genomic rearrangements, involving a single or multiple chromosomes, are acquired due to a single catastrophic event. These complex CNVs can resemble complex chromothripsis events first reported in certain types of cancer, especially bone cancers [23].

Using informative SNPs in the duplicated regions, we determined that four of the de novo duplications were maternal in origin and five had a paternal origin. While the duplications could have arisen in the germline of each parent, this pattern of inheritance is more suggestive of a zygotic or postzygotic origin during a vulnerable time early in development. Although an early postzygotic origin is a reasonable hypothesis, there was no indication of mosaicism, at least in DNA derived from blood cells that would be predicted from this model. A "CNV mutator' phenotype" resulting in multiple de novo rearrangements affecting different chromosomes has been proposed for patients similar to ours [16]. In our patient, this mechanism would have to be tissue and developmental stage restricted as we could not detect ongoing structural variation in two separate blood samples from our patient.

It is interesting that all the de novo abnormalities are gains and none were deletions, and that all (except for one at 318 Kb) were large (3–5 Mb). This could result from duplications having a milder phenotypic effect than deletions or might suggest a different mechanism for deletions or large duplications involving either the initiating DNA lesion or its repair processes in the cell(s) of origin. Considering that such events occurred on many different chromosomes suggests a transient mutagenic insult or dysregulation of chromosomal replication processes, and it is attractive to speculate the existence of genetic variants or a single environmental insult increasing the likelihood of such rare duplications.

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Conflict of Interest

No conflict of interest to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. A list of OMIM genes within 12 CNVs found in a single patient.
Figure S1. SNP chromosomal microarray data representing 10 genomic copy number changes of potential clinical significance detected in a single patient.
Figure S2. FISH images for four copy number gains showing three interphase FISH signals indicating a duplication of each locus and no evidence for translocation or insertion.

