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The importance of phase analysis in multiexon copy number variation detected by aCGH in autosomal recessive disorder loci

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Abstract

Cohen Syndrome (CS) is a rare autosomal recessive disorder caused by homozygous or compound heterozygous pathogenic variants in *VPS13B*, also known as *COH1*. Over 100 pathogenic variants in *VSP13B*, primarily truncations, and copy number variants, have been found in patients with CS. Here, we present an 11-month-old girl with CS caused by two multi-exonic small deletions in *VSP13B* in *trans*. Array comparative genomic hybridization has revolutionized the field of genome copy number analysis down to the exonic level, however it has its limitations. It cannot detect balanced structural variation nor determine the phase of copy number variants. Heterozygous multi-exonic copy number variation in autosomal recessive genes should be interpreted in the context of a clinical phenotype, and, if warranted, phase analysis should be performed before sequence analysis for that gene is pursued. This patient emphasizes the need of obtaining clinical information and determining the phase in multi-exonic copy number variants for accurate diagnosis and risk counseling.

Keywords

Array CGH; Cohen Syndrome

1 | INTRODUCTION

In recent years, the importance of copy number variants (CNVs) in disease has been appreciated. While array comparative genome hybridization (aCGH) technology has advanced rapidly and allowed for the higher resolution and better detection of CNVs, it is still important to critically evaluate aCGH results. The increase in resolution and coverage of aCGH is of particular importance for genes implicated in autosomal recessive disorders that are subject to copy number variation.

The gene *VPS13B*, located on chromosome 8q22.2 and consisting of 62 exons encoding a putative transmembrane protein of 4,022 amino acids, is an example of a gene often affected by genomic variation. In 2003, homozygous or compound heterozygous pathogenic variants in *VPS13B*, also known as *COH1*, were found to be causative of Cohen Syndrome (CS),

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characterized by several clinical phenotypes including facial dysmorphism (including high-arched eyelids and short philtrum), microcephaly, obesity, intellectual disability, retinopathy, and neutropenia (Kolehmainen et al., 2003). Since then, over 100 pathogenic variants in *VSP13B*, primarily truncations, and copy number variants, have been found in patients with CS. In addition to single nucleotide variants, both genomic deletions and duplications have been found in patients with CS, suggesting the region is susceptible to genomic rearrangements (Wang, Falk, Wensel, & Traboulsi, 2006).

Using clinical aCGH, we identified a non-contiguous multi-exonic loss in *VPS13B* in an 11-month-old female with clinical features of CS. After discussing the clinical phenotype of the patient with the clinician, phase analysis, using parental DNA, by aCGH and FISH analysis using fosmid probes mapping to the deleted regions in the patient's cells showed that there were in fact two intragenic *VPS13B* deletions of 130 and 66 kb in *trans* (on opposite chromosomes), one inherited from each parent. This patient highlights the importance of small CNVs in autosomal recessive disorders and their interpretation in aCGH.

2 | CLINICAL REPORT

The patient was a product of an in vitro fertilization pregnancy to a 36-year-old mother and 41-year-old father (Supplemental Figure S1). She was born at 35 weeks via Cesarean delivery due to intrauterine growth restriction (IUGR). Her healthy male twin had no IUGR and is phenotypically normal. The patient's birth weight was 3 lbs 8oz and she was hospitalized for 2 weeks for small size. At 3 months of age, she was diagnosed with left hip dysplasia for which she was treated with a brace. Developmental screening at 3 months of age was considered normal, however, at 10 months of age she was found to have global developmental delay. At the time of her first visit at 11-months-of age, her weight was on the 2nd centile (7.6 kg, Z-score −2.05), her height was on the 30th centile (72 cm, Z-score −0.53), and her OFC <1st centile (41.7 cm, Z-score −2.66). All centiles and Z-scores are based on 2,000 Centers for Disease Control and Prevention (CDC) growth charts. The patient had microcephaly, brachycephaly,and plagiocephaly. Her face was notable for a round shape, small maxilla with slightly down slanting palpebral fissures, high arched eyelids, a short philtrum, and large ears. Both the high-arched eyelids and short philtrum are consistent with CS. Additionally, she had laryngomalacia. She was also found to have persistent neutropenia. This symptomatology was considered suggestive of CS.

3 | MATERIALS AND METHODS

Written informed consent was obtained from the patient's parents. The data presented in this manuscript are not considered research at Baylor College of Medicine, as a single individual is investigated, and no generalizable conclusions can be drawn from this study. The investigators did, however, obtain signed consent for the publication of identifiable information, including the patient's photograph.

Genomic DNA was extracted from whole blood (Chemagen kit, PerkinElmer chemagen Technologie GmbH, Germany) from the patient and both parents. Array CGH was performed in the patient and each parent to determine inheritance and phase using a custom

designed array manufactured by Agilent Technologies (Santa Clara, CA), as described previously (Boone et al., 2010). Fluorescence in situ hybridization (FISH) analyses with the fosmid clones, (obtained from the Human Genome Center at Baylor College of Medicine) were performed to confirm the array analysis showing that the deletions are in *trans* as a quality control measure. Briefly, fosmid DNA was isolated using the Perfectprep mini kit (Eppendorf, Hamburg, Germany) and labeled using Spectrum Orange or Spectrum Green Vysis dUTPs (Vysis, Inc., Des Plaines, IL) according to the manufacturer's protocol. Hybridization was performed using standard FISH protocol. In parallel with aCGH, exome sequencing was performed as described previously (Yang et al., 2013, 2014). A heterozygous benign variant c.10049C>T p.(Thr3350Ile) (rs138127778) in *VPS13B* was identified, which is found in 1% of European Americans. No pathogenic variants in other disease-related genes were identified.

4 | RESULTS

Chromosomal microarray analysis identified two deletions spanning 130 and 66 kb (hg19 positions 100168626–100298366 and 100479509–100545102), respectively, on chromosome 8q22.2 (Figure 1a,b). This region contains only 1 gene, *VPS13B*. To determine if these were de novo and if they were in *cis*, on the same chromosome, or in *trans*, on opposite chromosomes, microarray analysis was performed on the parental samples. This identified a 130 kb deletion, involving exons 15 through 19, inherited from the father, and a 66 kb deletion, involving exons 24 through 30, inherited from the mother (Figure 1c, Supplemental Table S1). The parental DNA was run on one array, using the mother as a control sample and father as a test sample, with expected log ratio changes as in Table S1. Analysis by FISH confirmed the presence of the deleted regions in *trans* in the patient (Supplemental Figure S2).

5 | DISCUSSION

With increasing resolution and coverage of aCGH, and now that CNVs can be assessed with exome sequencing also, it is important to critically evaluate CNV results with the patient's phenotype. This is especially important for genes implicated in autosomal recessive disorders that are subject to copy number variation, such as *VPS13B*.

Accurate identification of intragenic CNVs is highly dependent on using the appropriate technology. Here, a high-resolution exon targeted aCGH was first used, as it is a first tier test for intellectual disability and chromosomal losses are known to be causative in CS. With a lower resolution array, these intragenic CNVs can easily be missed. As this is a recessive disorder, exome sequencing was performed in parallel with aCGH to ensure no pathogenic variants were also contributing to the patient's phenotype. Depending on the process that is used, it may be possible to identify these large, non-contiguous, multi-exonic deletions by exome sequencing as well, although that was not performed at the time the patient was evaluated. When two or more non-contiguous intragenic CNVs are detected there are three interpretive possibilities: 1) the CNVs are in *cis*; 2) The CNVs are in *trans*; 3) Complex rearrangement such as an inversion, translocation, or an insertion that can be in *cis* or *trans*. At this point it is important to correlate the clinical data with the laboratory results in order

to interpret the findings. In the present patient, the clinical presentation of CS with two non-contiguous deletions in the VPS13B gene and a negative exome sequencing report suggested that the two deletions may be in *trans*. Typically, our laboratory uses aCGH on parental samples to determine the inheritance of CNVs and in this family, it was found that both the parents are carriers for a deletion in *VPS13B*, and their child inherited both these deletions. FISH was also performed to confirm the finding though at this point it was not necessary but this was done to rule out additional rearrangements. The use of FISH could be valuable if the CNV is more than 20 kb to determine the phase in the patient cells, which is an important consideration for intragenic, non-contiguous, or contiguous deletions that may be in *trans*.

While this is the first report of two deletions in *trans* resulting in CS, the phenomenon of two deletions in *trans* resulting in an autosomal recessive disorder is not unique to *VPS13B*. Similar findings have been seen in patients with exon rearrangements in *PARK2* (Kim et al., 2012). Exon rearrangements account for up to 60% of *PARK2* mutations, which are causative of early onset Parkinson disease (EOPD) with autosomal recessive inheritance. In a study of 114 Korean patients with EOPD, more than 80% diagnosed with contiguous multi-exon deletions were found to be actually in *trans*, once the phase of CNVs was determined (Kim et al., 2012). This suggests that previous studies indicating that the patients' EOPD was caused by contiguous CNVs may actually have been multiple CNVs in *trans*. These patients and the current patient with CS emphasize that while molecular diagnoses can lead to answers, clinical information must be considered when interpreting these results. As these large multi-exonic CNVs can appear contiguous, it is important to consider if the clinical phenotype suggests that phase of the CNVs may play a role. This is particularly important for large genes known to be responsible for recessive disorders that are subject to multi-exonic CNVs.

In summary, we present a report of two small deletions in *trans*, in the *VPS13B* gene, in which autosomal recessive mutations or deletions are responsible for CS. Our findings highlight the importance of high-resolution aCGH and the critical analysis of aCGH data surrounding genes that are often impacted by genomic variation. Furthermore, our findings emphasize the need to understand the clinical phenotypes when interpreting diagnostic data and determining the appropriate assays to identify the molecular changes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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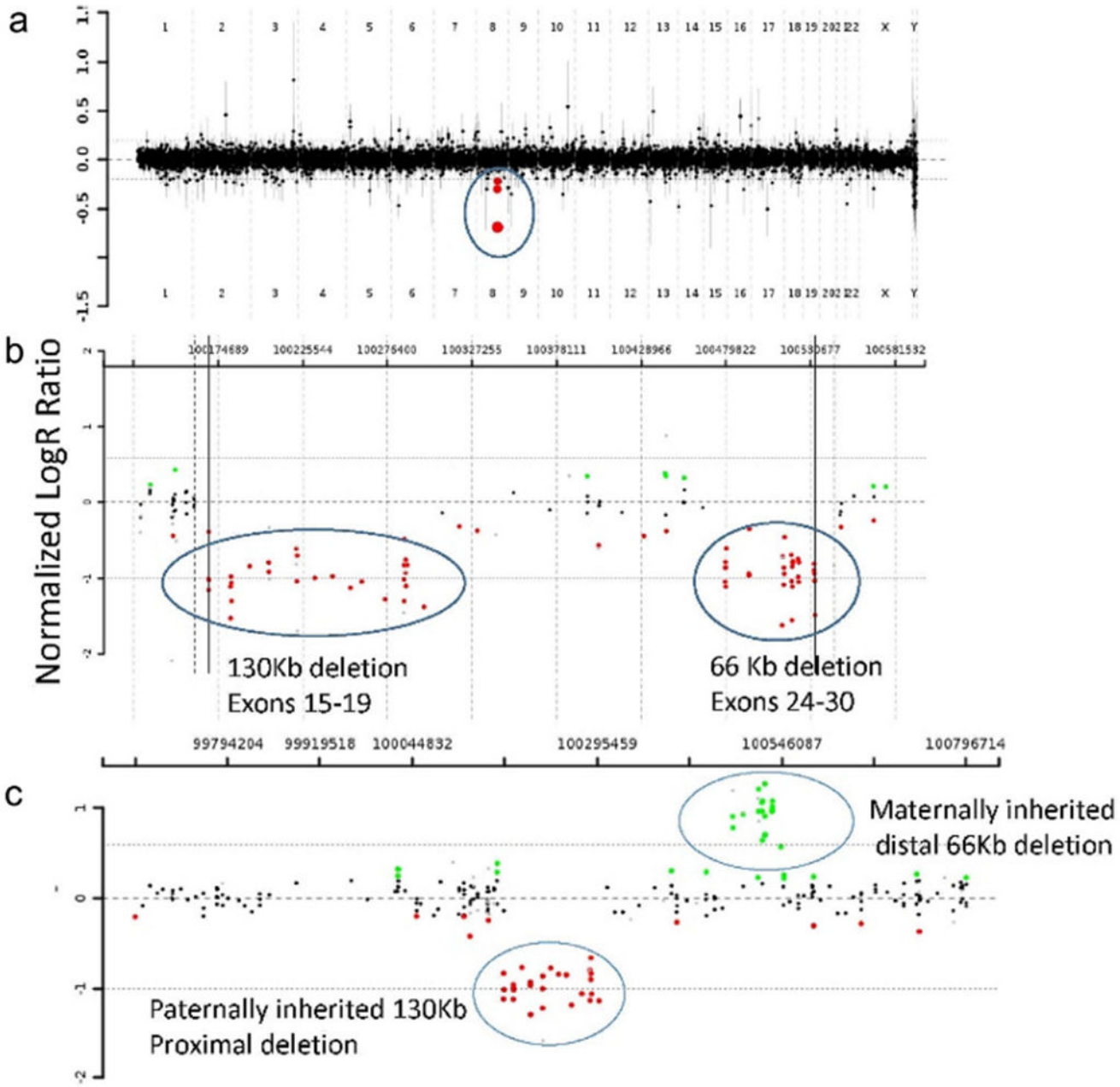

FIGURE 1.

a: Clinical array Comparative Genomic Hybridization plot showing deletion region on chromosome 8q22.2 in the patient. b: Zoomed-in deleted region in clinical aCGH, showing what appears to be a non-consecutive deletion on chromosome 8p22.2 in the patient. c: Parental aCGH plot showing both parental deletions. The maternal sample was used as the control sample while the paternal sample was used as the test sample, with expected log ratios seen in Supplemental Table S1. The paternal deletion of 130 kb is indicated by the log ratio of −1.0 while the maternal deletion of 66 kb is indicated by the log ratio of 1.0. [Color figure can be viewed at wileyonlinelibrary.com]