Three-generation familial chromosomal insertion identified in a proband with the unbalanced form presenting with multiple congenital anomalies

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Introduction

Chromosomal insertions are uncommon structural rearrangements involving two or more chromosomes and are considered complex chromosomal rearrangements (CCRs). Insertions can occur either as an interchromosomal or intrachromosomal translocations. Most balanced forms of insertions do not have any phenotypic consequence; however, a carrier can pass an unbalanced form of the rearrangement, resulting in trisomy or monosomy for the areas involved. Unbalanced rearrangements can cause variable phenotypes dependent on the chromosomal region's gene content. High-resolution karyotype analysis can identify insertions, but its ability depends on the size and capability to discern differences to the expected banding patterns of the chromosome areas involved. SNP array technology (Illumina Inc ®., USA) combined with Fluorescence *in situ* hybridization (FISH) may help in characterizing unbalanced insertions.

Clinical Background

Here, we describe a ten-year-old female presenting with short stature, multiple congenital anomalies, significant developmental delay, cognitive delay, and growth abnormalities. She was a product of a near-term birth and experienced significant intrauterine growth retardation. She displayed significant hypotonia; a neurological assessment and MRI were both unremarkable. She had a small patent ductus arteriosus as a neonate, which spontaneously resolved. In addition to her hypotonia, she exhibited significant symptoms of gastroesophageal reflux. An endoscopy revealed significant reflux and erosion of the distal esophagus and underwent treatment. A review of family history did not identify other family members affected with short stature or developmental concerns. Prior workup from two outside laboratories included conventional G-banded karyotype (550 band level) performed on peripheral blood, which was unremarkable. Fluorescence *in situ* hybridization (FISH) studies using standard DiGeorge probes (TUPLE1/HIRA, Vysis) and subtelomeric-specific probes (ToTelVysion, Vysis) proved unremarkable on metaphase cells. Despite normal cytogenetic results, there was still a high clinical suspicion of a chromosomal abnormality contributing to the proband's various congenital anomalies. Therefore, a Single Nucleotide Polymorphism (SNP) array using the Illumina® 850k BeadChip was performed on peripheral blood received in our laboratory.

Results

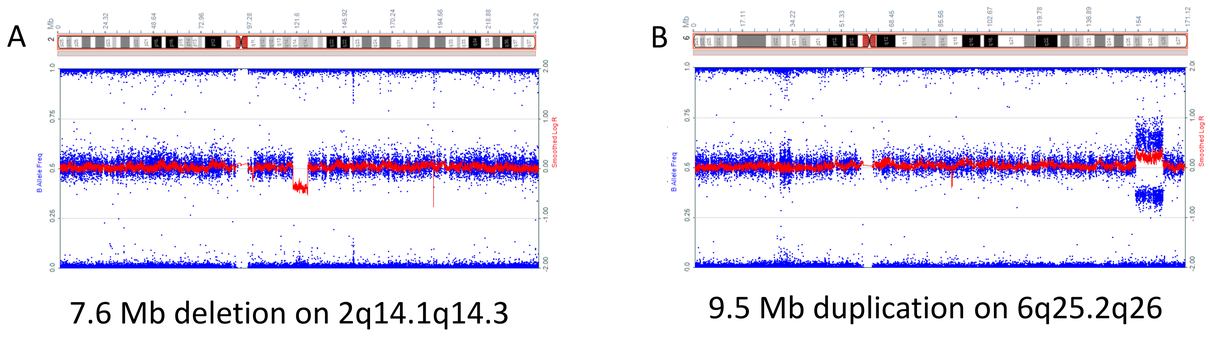
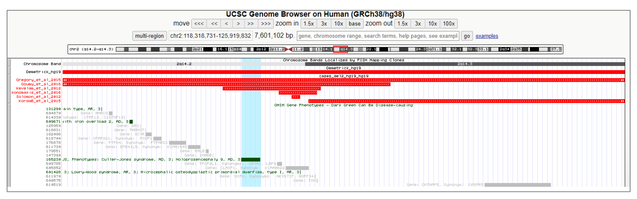


Figure 1. SNP array analysis performed on the proband reveals two interstitial pathogenic copy number events.

CNV analysis by CytoSNP-850k arrays. DNA was processed on the Infinium CytoSNP-850K v1.3 BeadChip, and analyzed with Karyostudio v1.4 using the standard cluster files on a research basis to clarify the original clinical report generated from an earlier chip version (610k array; hg18). A) SNP array plot showing an interstitial 7.6 Megabase (Mb) deletion between 2q14.1 and 2q14.3 (chr2:118,318,731-125,919,832;hg19). B) SNP array plot showing an interstitial 9.5 Mb duplication between bands 6q25.2 and 6q26 (chr6: 153,822,736-163,320,125;hg19). The microarray has an overall probe spacing of ~1.8 kilobases (Kb). A high-resolution karyotype was recommended for the proband and the parents to evaluate for the possibility of a complex rearrangement. Genetic counseling was also recommended to discuss the possibility of recurrence risk for her parents.

A



B

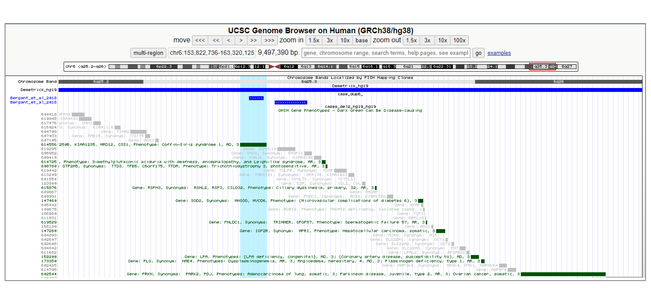
Phenotypes	Delayed growth	Brain anomalies	Heart anomalies	DD	Dysmorphic features	Other features
Our patient	+	-	+ (resolved as neonate)	+		IUGR, severe GI reflux
KordaB, et al (2015)	+	+(microcephaly)			+	left non functional kidney
Goumy, et al (2016)		+	-	+	+	
Kevelam, et al (2012)	+	-		-	+	large liver, right stomach
Oikonomakis, et al (2016)					+	ASD
Gregory, et al (2015)	+					
Solomon, et al (2012)	+			-		left polydactyly

DD; developmental delay, IUGR; intrauterine growth restriction, GI; gastrointestinal, ASD; autism spectrum disorder

Figure 2. Comparison of the 2q loss and candidate genes of interest.

(A) UCSC Genome Browser (GRCh37/hg19) showing the loss (Demetrick_1) found in our patient compared to previously reported variants. Pure 2q14 deletions are rare. A total of 55 genes are included in the 2q deletion, five of which are predicted to exhibit haploinsufficiency: *CLASP1, GLI2, INHBB, EN1*, and *TFCP2L1* (pHaplo scores of >0.82; Decipher). Only one is a green OMIM-disease gene and fully contained in the loss: *GLI2* (highlighted in blue) with a haploinsufficiency score of 3 out of 3 (ClinGen); loss-of-function variants are associated with GLI2-related diseases, which may display high phenotypic variability. (B) The table displays selected cases from the literature with overlapping losses inclusive of the *GLI2* gene. Our patient shares delayed growth with other cases, but lacks other noted features, raising the possibility of her being on the milder end of the genotype-phenotype spectrum.

A



B

Phenotypes	DD	Other features
Our patient	+	IUGR, GI
Bergant et al 2018	+	Suspected disorder of glycosylation

DD; developmental delay, IUGR; intrauterine growth restriction, GI; gastrointestinal

Figure 3. Comparison of the 6q gain and candidate genes of interest.

(A) UCSC Genome Browser (GRCh37/hg19) showing the gain (Demetrick_1) found in our patient to previously reported variants. The interstitial duplication on 6q contained 89 genes, four of which are predicted to exhibit triplosensitivity: *ARID1B, SCAFB, WTAP,* and *ZDHHC14* (pTriplo scores of >0.80; Decipher). Only one is a green OMIM-disease gene fully contained in the gain: *ARID1B* has a triplosensitivity score of 0 out of 3 (ClinGen). De novo truncating variants are associated with ARID1B-related disorders. (B) The table displays selected cases from the literature with overlapping gains inclusive of the *ARID1B* gene. Our patient shares developmental delay but lacks a suspected disorder of glycosylation.

Figure 4. Partial G-banded karyotype reveals a cryptic CCR in the proband.

A retrospective high-resolution karyotype (650 band level) was performed, revealing a subtle change within chromosome 2 at the same bands identified by the SNP array. The partial karyotype shows the derivative 2 with the normal homolog for comparison. As the event did not result in a significant difference in the expected banding, visualization of the insertion was difficult to discern by karyotype alone. A CCR was suspected. Ideograms were generated using Cydas (Hiller et al., 2004).

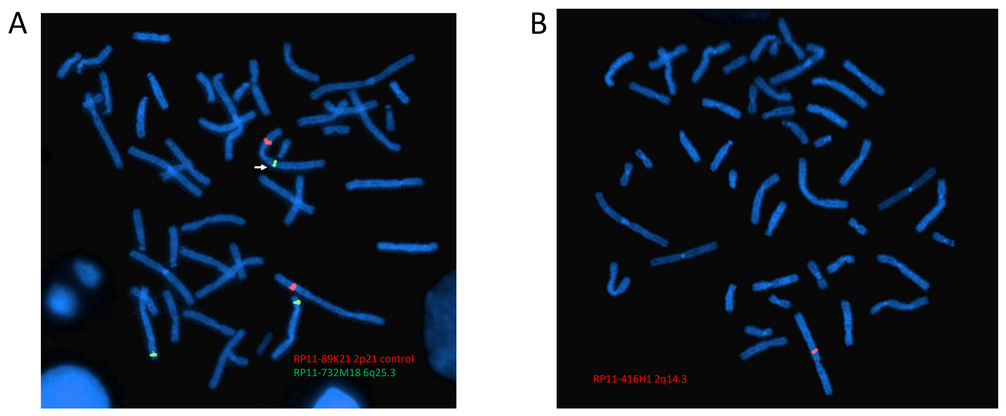


Figure 5. Metaphase FISH confirms the insertion event in the proband.

A) To clarify the orientation for the affected loci, metaphase FISH was performed using custom probes RP11-89K21 (2q14.3; orange) and RP11-732M18 (6q25.3; green) that map within both copy number changes. FISH confirmed an unbalanced insertion involving chromosomes 2 and 6, whereby the gain of chromosome 6 material was inserted into the deleted loci on chromosome 2 (see white arrow). B) FISH with probe RP11-416H1 (orange) confirmed the 2q deletion found by the SNP array evident with a single signal present.

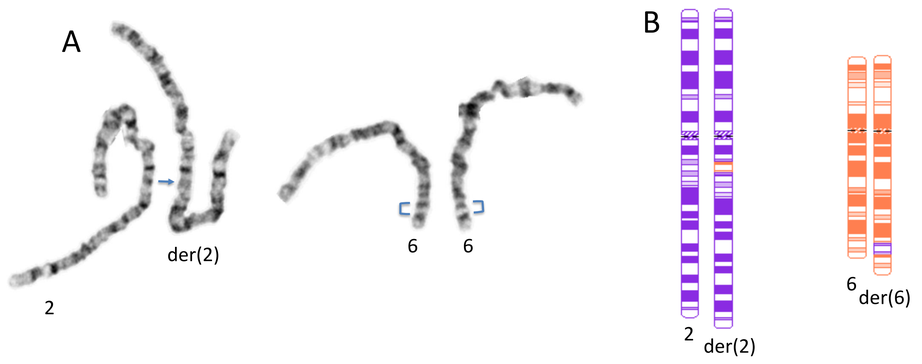


Figure 6. Maternal karyotype supports a reciprocal insertion.

A) Partial G-banded karyotype (650 band level) showed a subtle reciprocal insertion between chromosomes 2 and 6, with normal homologs for comparison. The banding pattern on chromosome 6 was indistinguishable by karyotype resolution (indicated by brackets). B) The ideograms on the right illustrates the rearrangement. Ideograms were generated using Cydas (Hiller et al., 2004).

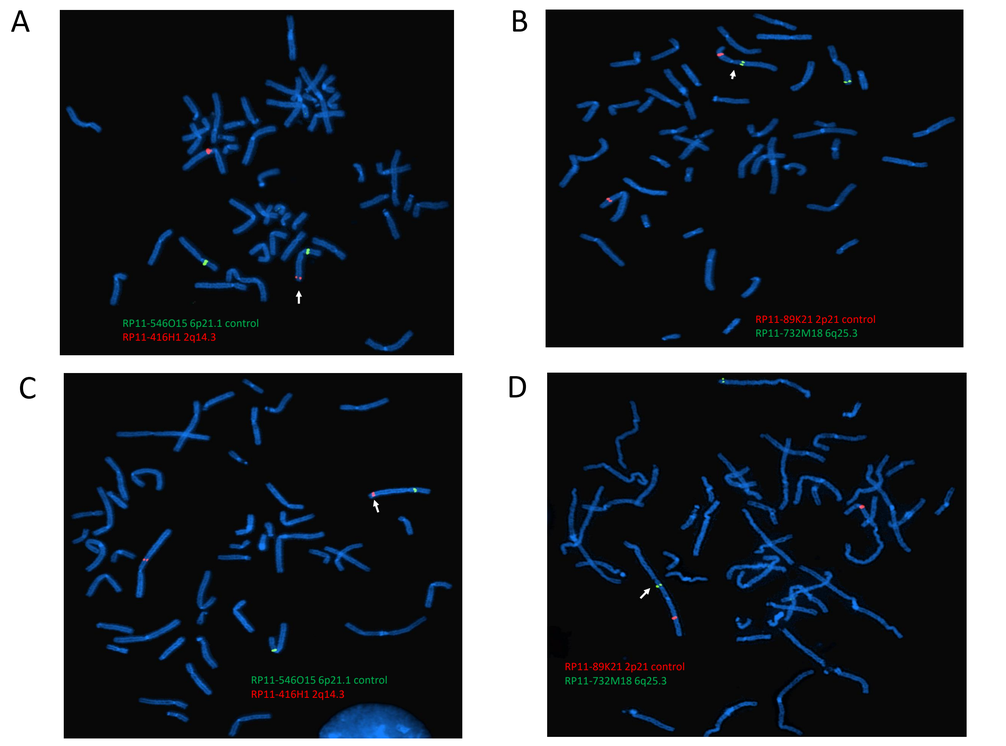


Figure 7. Metaphase FISH confirms the insertion segregated in the maternal lineage (mother, maternal grandfather).

A, B) Metaphase FISH showing an insertion of probe RP11-416H1 (orange) into chromosome 6 (green control probe). Metaphase FISH showing the insertion of probe RP11-732M18 (green) into chromosome 2 (orange control probe). Combined, these FISH studies (A, B) confirm the reciprocal insertion in the proband's mother. FISH and karyotype performed on the maternal grandfather (C, D), utilizing the same informative probes also showed a reciprocal insertion, demonstrating that the event occurred in three generations, with the proband's form being unbalanced.

Discussion

- Utilizing complementary methods, we confirmed the suspected chromosome abnormality in the proband.
- Using karyotype and FISH we were able to show that the mother and grandfather of the proband carried an apparently balanced reciprocal insertion.
- Additional family members should be tested to see if they are carriers of this balanced insertion.
- Other technologies, such as optical genome mapping (OGM), could be employed to further clarify the rearrangement.