High-resolution karyotype reveals a maternally inherited complex rearrangement involving chromosomes 4p, 5p, and 12q with an inverted insertion and 12q loss.

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Introduction

Background: Complex chromosome rearrangements (CCRs) are defined as rearrangements involving three or more chromosomes. CCRs can be further classified into subgroups based on complexity, often defined by the number of chromosomes and number of breaks involved. The most straightforward form consists of three breakpoints on three different chromosomes with more complex rearrangements involving inversions, insertions, or derivative chromosomes comprising three or more chromosomes. CCRs can be balanced or unbalanced. Unbalanced CCRs are more often associated with phenotypic findings than balanced CCRs.

Clinical Background

Case study: Here, we present a summary of lab findings for a 19-year-old patient referred for autoimmune hemolytic anemia with a history of thrombocytopenia and leukopenia. Neoplastic karyotype performed on a bone marrow specimen at an outside laboratory identified an apparently balanced novel translocation between the short arm of chromosome 4 (4p) and the long arm of chromosome 12 (12q) (Figure 1). As this translocation was present in all cells examined and not known to be associated with hematological malignancy in the literature, a germline origin was suspected.

Figure 1 _ Neoplastic partial karyotype (proband) showing an apparently balanced rearrangement between chromosomes 4p and 12q. Neoplastic karyotype from an outside laboratory shows an apparently balanced rearrangement between the short arm of chromosome 4 and the long arm of chromosome 12, suspected to be constitutional. No abnormality was reported for chromosome 5, likely due to the lower resolution of the karyotype (reported as 400 band level).

Methods

Testing: Routine chromosome analysis performed on cultured fibroblast cells confirmed a germline origin and revealed chromosomal aberrations at 4p14, 5p15.1, and 12q14 in all cells examined (figure 2). There was also high suspicion of a more complex rearrangement involving a portion of the long arm of chromosome 12q14q21 material inserted into the 5p15.1 locus. Fluorescence *in situ* hybridization (FISH) studies using subtelomeric-specific probes (Vysis ToTelVysion, Abbott, Inc.) for these chromosomes further supported this finding (figure 3). DNA was isolated (Qiagen®), and SNP microarray was performed using the CytoSNP-850K Beadchip (Illumina®). The sole microarray finding was a 234 kilobase (kb) interstitial loss of 12q21.31q21.32, which is intragenic to the *MGAT4C* gene and was classified as a variant of unclear significance (figures 4, 5). Parental testing was recommended to determine if the CCR was inherited or had arisen *de novo*. A high-resolution (650 band level) maternal karyotype and SNP microarray performed on peripheral blood confirmed the CCR in our patient was maternally inherited and further elucidated the nature of the rearrangement (figure 6).

Results

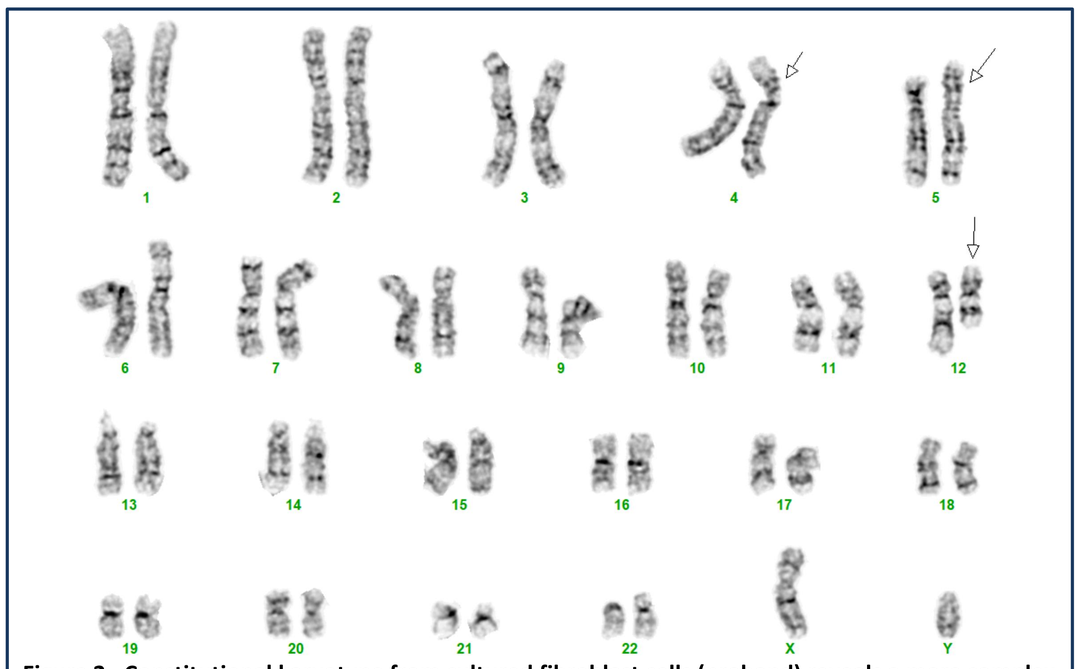

Figure 2 _Constitutional karyotype from cultured fibroblast cells (proband) reveals a more complex rearrangement between chromosomes 4p, 5p, and 12q.
G-banded karyotype (400 Band level) on fibroblast cells shows a CCR between chromosomes 4, 5, and 12 with chromosome 12q material inserted into chromosome 5p. This was described as an unbalanced translocation between chromosomes 4p14 and 12q21. The remaining material from chromosome 12 (12q14 to 12q21) appears interstitially inserted into chromosome 5 at 5p15.1.

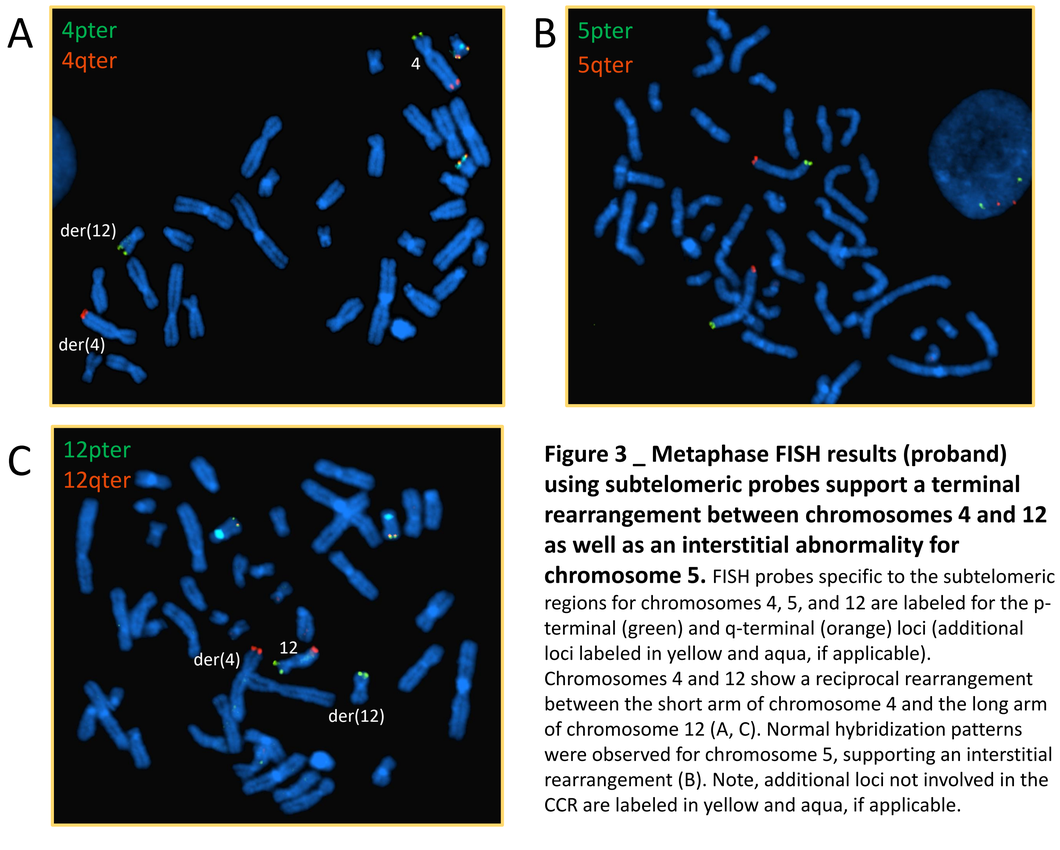

Figure 3 _ Metaphase FISH results (proband) using subtelomeric probes support a terminal rearrangement between chromosomes 4 and 12 as well as an interstitial abnormality for chromosome 5. FISH probes specific to the subtelomeric regions for chromosomes 4, 5, and 12 are labeled for the p-terminal (green) and q-terminal (orange) loci (additional loci labeled in yellow and aqua, if applicable). Chromosomes 4 and 12 show a reciprocal rearrangement between the short arm of chromosome 4 and the long arm of chromosome 12 (A, C). Normal hybridization patterns were observed for chromosome 5, supporting an interstitial rearrangement (B). Note, additional loci not involved in the CCR are labeled in yellow and aqua, if applicable.

Results (continued)

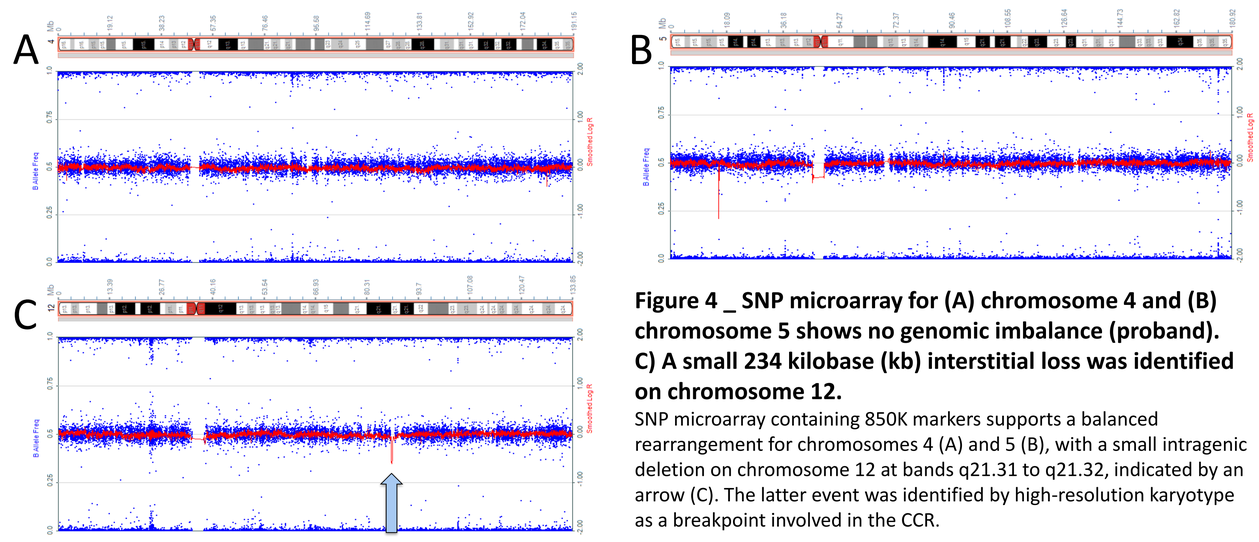

Figure 4 _ SNP microarray for (A) chromosome 4 and (B) chromosome 5 shows no genomic imbalance (proband). C) A small 234 kilobase (kb) interstitial loss was identified on chromosome 12.
SNP microarray containing 850K markers supports a balanced rearrangement for chromosomes 4 (A) and 5 (B), with a small intragenic deletion on chromosome 12 at bands q21.31 to q21.32, indicated by an arrow (C). The latter event was identified by high-resolution karyotype as a breakpoint involved in the CCR.

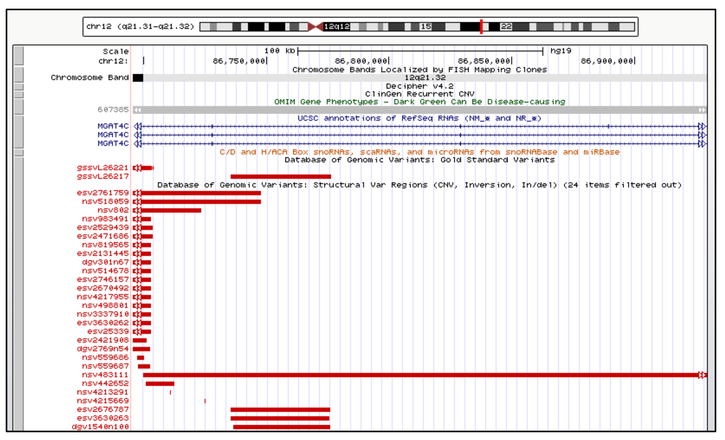

Figure 5 _ The 234 kb loss at 12q21.31q21.32 identified by SNP microarray shows an intragenic deletion of the *MGAT4C* gene, classified as a variant of unclear significance.
The deletion (chr12:86,695,712-86,929,340; GRCh37) includes part of the *MGAT4C* gene (exons 3 to 5 out of 9 total exons; NM_001351285.2). This gene is not currently associated with disease (OMIM). A larger overlapping loss is noted in DGV (nsv483111; 1/39 controls). Similarly sized and overlapping losses are not enriched in databases of genomic imbalance and disease in humans. In silico tools do not predict dosage sensitivity (Decipher). ClinGen CNV-Loss Calculator predicts VUS (score 0 out of 0). This loss may be related to the CCR identified by karyotype; however, it may not result in a clinically significant dosage imbalance.

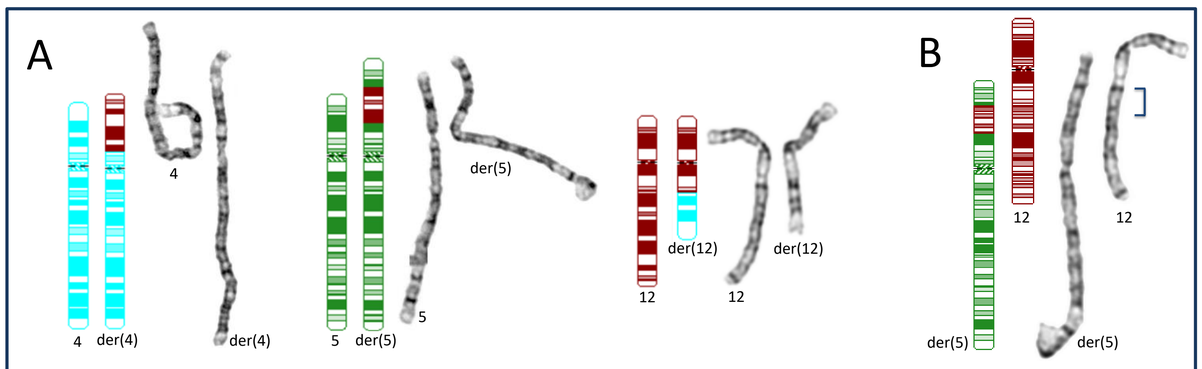

Figure 6 _ Partial maternal high-resolution karyotype from peripheral blood shows (A) derivative chromosomes 4, 5, and 12 with normal homologs for comparison and corresponding ideograms and (B) the derivative 5 chromosome with the normal 12 chromosome highlighting the inverted insertion of 12q21.31q13.3.
The high-resolution chromosomes in this figure range in resolution from 650 to 750 band level. The inverted segment between 12q21.31q13.3 is indicated by the bracket and estimated to be approximately 25 megabases (Mb) in size. Ideograms were generated using Cydas (Hiller et al., 2004) at 550 Bands (A) and 800 Bands (B).

ISCN Nomenclature

Proband 400 Band Level	46,XY,der(4)t(4;12)(p14:q21),ins(5;12)(p15.1;q?14q21),der(12)t(4;12)(p14;q?14)
Mother 650 Band Level	46,XX,der(4)t(4;12)(p14;q21.31),ins(5;12)(p14.3;q21.31q13.3),der(12)t(4;12)(p14;q13.3)

Figure 7 _ ISCN nomenclature for proband and mother describing each derivative chromosome independently and reflecting the cytoband differences between low and high-resolution karyotypes.
The ISCN acknowledges the difficulty in describing CCRs using standard ISCN guidelines and states that the nature of these rearrangements may need to be described in words to achieve optimal clarity. For this study, each derivative chromosome was described separately as a derivative chromosome for improved clarity using ISCN guidelines at time of reporting.

Discussion

- This study highlights the continued utility of high-resolution karyotypes (≥550 band level) in assessing CCRs. The high-resolution maternal karyotype was essential in elucidating the nature of the rearrangement in this family by clarifying cytobands and identifying that the orientation of the inserted 12q13.3q21.31 material within chromosome 5p14.3 is inverted.
- Although these findings do not currently explain the patient's phenotype, other small deletions or gene disruptions from the rearrangement cannot be excluded. Emerging laboratory methods such as long-read sequencing or optical genome mapping may help assess this possibility. It is possible that the small deletion at 12q21.31q21.32 occurred secondary to the CCR formation.
- This case study confirms prior literature reports that inherited CCRs are often maternal in origin. As carriers of CCRs are at an increased risk of abnormal pregnancy outcomes and infertility, genetic counseling is recommended to discuss recurrence risk. Although female carriers of CCR are at an increased risk for abnormal pregnancy outcomes, the mother reportedly had four phenotypically normal children with no documented history of recurrent miscarriages. Male carriers of CCR are at increased risk of infertility, and other family relatives, specifically the siblings of this patient, may be carriers of this CCR and should be evaluated using high-resolution karyotype and FISH testing.

References

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