Genetic and functional characterization of complex chromosomal rearrangements in a family with multisystem anomalies

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Abstract

We present here a family whose inherited complex chromosomal rearrangements (CCRs) were evaluated using both traditional molecular cytogenetic approaches including karyotyping, fluorescence in situ hybridization (FISH), chromosome microarray analysis (CMA), as well as two novel methods including optical genome mapping (OGM) and proximity ligation sequencing (Hi-C). Both OGM and Hi-C defined the breakpoints of CCR in the proband and her mother at higher resolution than the conventional test methods. We also performed transcriptome and methylome analyses of this family, and identified the biological pathways associated with the proband's phenotypes.

Introduction

Conventional cytogenetic tools have specific limitations with each method, such as karyotyping is a single cell whole genome assay but having limited resolutions, FISH is a targeted assay with limited coverage, and cytogenomic microarray analysis can detect CNAs and copy neutral LOH (cnLOH) with high resolution but not able to detect balanced rearrangements. Some NGS methods also have limitations, such as low pass whole genome has low resolution due to limited read length and depth [1] and not able to detect copy neutral LOH nor balanced rearrangements while long-read sequencing technology is limited by the requirement for high molecular weight DNA, relatively high error rate, lack of mature computational analysis tools and high sequencing cost [2].

OGM and Hi-C are two high-throughput technologies that capture ultra-long-range contiguity information and enable precise detection of all types of SVs in a single assay. OGM, is an imaging-based method that produces DNA fingerprints spanning very large genomic regions [3]. Hi-C is a chromatin conformation analysis to capture chromatin contacts within the nucleus by proximity ligation followed by NGS [4]. Although based on different principles, both technologies potentially allow for improving test accuracy with high resolution.

We present here a family whose inherited complex chromosomal rearrangements (CCRs) were evaluated using both traditional molecular cytogenetic approaches and two novel methods, OGM and Hi-C. The indication for testing in the proband was significant intellectual disability and an immune deficiency. Her mother was unaffected. We also performed transcriptome and methylome analyses of this family, and identified the biological pathways associated with the proband's phenotype.

Methods and Materials

Here, we report on genome, transcriptome and methylome data analyses from a female patient with idiopathic thrombocytopenic purpura (ITP) and developmental delay who has an inherited complex chromosomal rearrangement. Genotype were done for the proband and her mother with conventional cytogenetic methods and two novel methods, OGM (Figure 1A) and Hi-C (Figure 2B). Expression analyses and methylation analysis were done for this patient and her mother and compared to normal controls to identify dysregulated pathways due to chromosomal rearrangements.

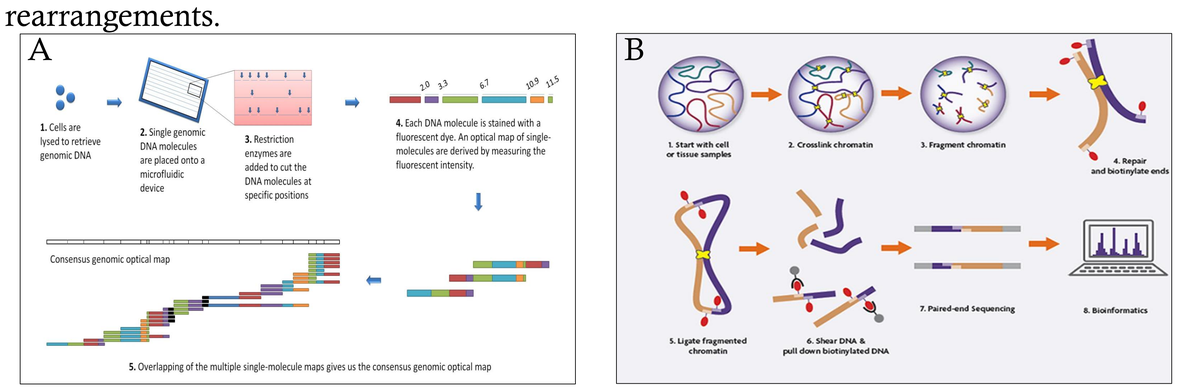


Figure 1. Workflow for OGM and Hi-C. A) Workflow for OGM. Blood samples are collected and lysed to retrieve large size genomic DNA molecules. DNA is subsequently labelled with a label pattern that spans the whole genome and is unique to each individual sample. Multiple molecules are used to create consensus genome maps representing different alleles from the sample. The sample's unique optical genome map is aligned to the reference genome and differences are automatically called, allowing for detection of structural variations in a genome wide fashion. (Image modified from: https://wiki.com). B) Workflow for Hi-C. Blood cells are cross-linked with formaldehyde, digested with a restriction enzyme, the 5' overhang is filled with a biotinylated residue, blunt-end fragments are ligated under dilute conditions, DNA fragments are sheared and selected with streptavidin beads. The library containing proximity ligated fragments is analyzed by paired-end high throughput sequencing. (Image from Boxer, Lisa. "Hi-C: Genome-wide Chromosome Conformation Capture." (2010).)

Results

1. Karyotype and FISH analysis identified a CCR with at least 4 breakpoints in the proband and 7 breakpoints in her mother involving chromosomes 1, 7 and 11.

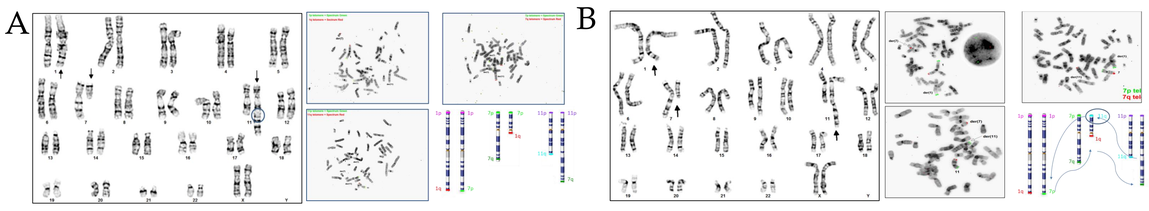


Figure 2. Chromosomal and FISH analysis of proband (A) and her mother (B). G-banding method at 750 bands per haploid was performed. Two color FISH using the telomeric probes set on metaphase cells show localizations of 1p, 1q, 7p, 7q, 11p and 11q on chromosome 1, the der(1), chromosome 7, the der(7), chromosome 11 and the der(11). The schematic representation in (C) shows localization of FISH signals on the ideograms of the respective chromosomes involved in the rearrangement.

Results

2. CMA showed a terminal duplication of 7pter-p22 and 3 interstitial deletions of 1q44, 7q11 and 11q25 in the proband but a balanced genome in her mother.

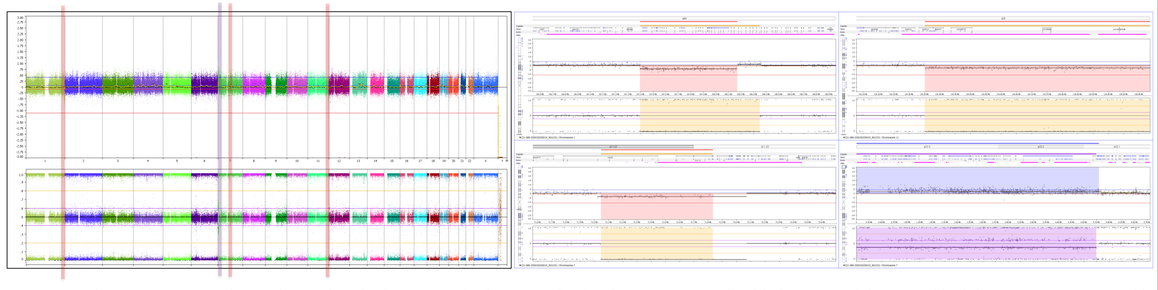


Figure 3. The SNP array results in the proband, showing a duplication of 4.8 Mb in 7pter-p22 (highlight in purple), a 775 kb deletion in 1q44, a 788kb deletion in 7q11 and a 735kb deletion in 11q25. No copy number change was detected in her mother.

3. OGM and Hi-C defined the breakpoints and identified chromosomal configurations of CCR in the proband and her mother at high resolutions.

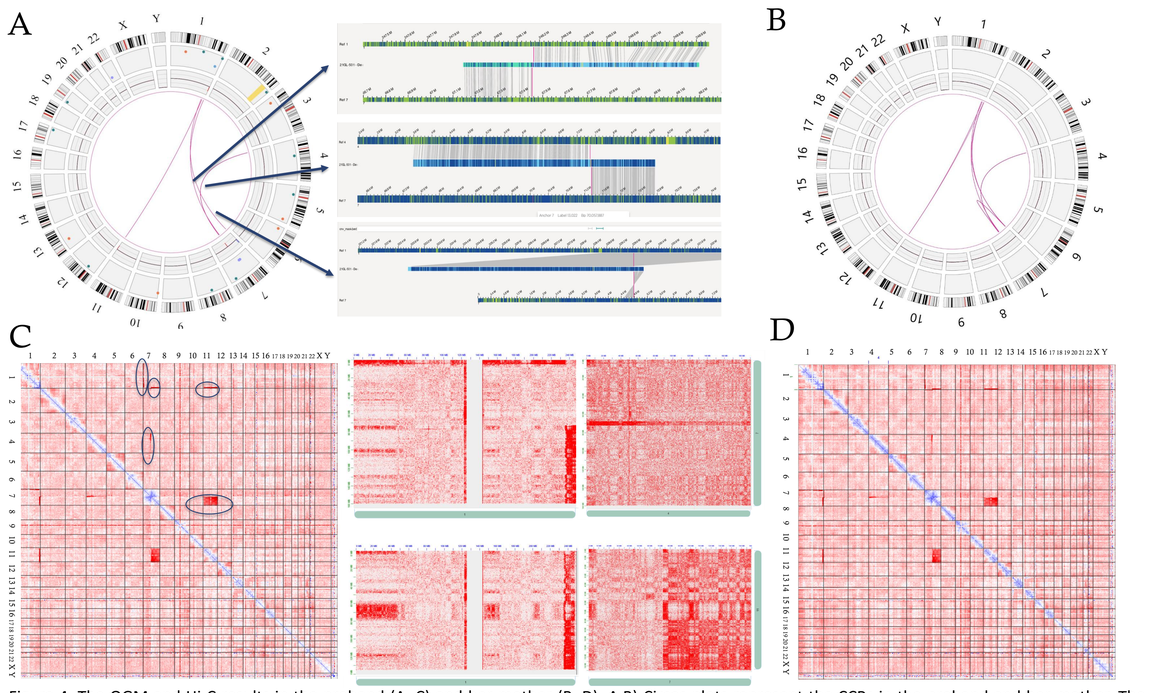


Figure 4. The OGM and Hi-C results in the proband (A, C) and her mother (B, D). A,B) Circos plots represent the CCRs in the proband and her mother. The outer ring of the plot shows chromosomes 1-22, X and Y. and the inner ring shows the CRs detected by OGM. The red spikes show the intra-chromosomal translocations. Left panel of A shows the rearrangements around the translocation break points. C,D) Hi-C heatmaps represent the CCRs in the proband and her mother. Left panel of A shows the translocations between chromosome 1, chromosome 4, chromosome 7 and chromosome 11.

5. Transcriptome and methylome analysis identified the functional pathways affected by the CCRs.

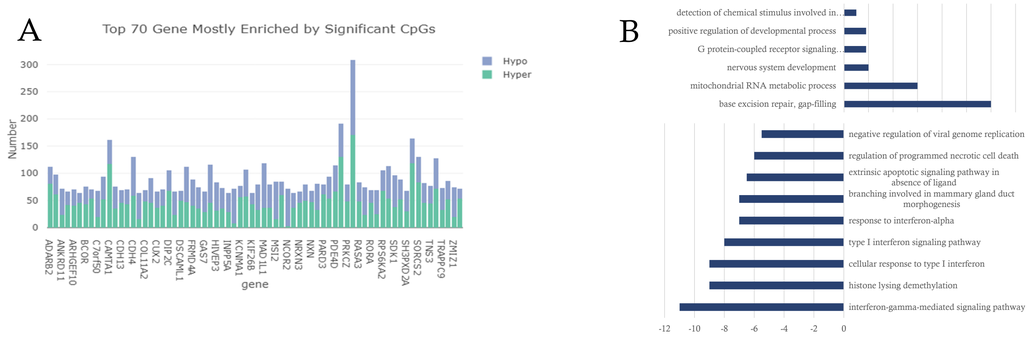
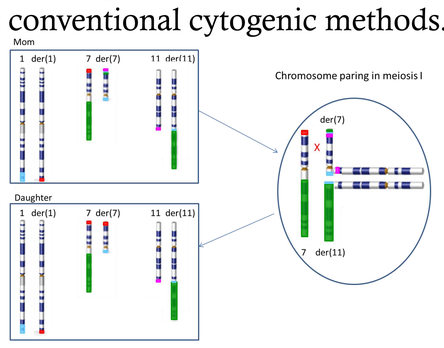


Figure 5. A) Differentially methylated genes identified by Illumina Infinium MethylationEPIC array in the proband. Top 70 genes enriched with significant CpGs are listed. Hypomethylated CpGs are colored by blue and hypermethylated CpGs are colored by green. B) Functional pathways affected identified by RNA-seq in the proband. Up-regulated genes are enriched in immune response associated pathways while down regulated genes are enriched in neuronal development associated pathways.

Conclusion and Discussion

- We demonstrated the use of OGM and Hi-C as tools for detection of chromosomal rearrangements and copy number alterations in constitutional disorders.
- OGM and Hi-C detected novel chromosomal rearrangements and implicated genome organizations in proband and her mother at a high resolution in addition to CCRs detected by conventional cytogenic methods.



- This integrative study of proband's genomics uncovered key biological pathways associated with proband's phenotype.

References

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