Deciphering genomic inversion events using optical mapping

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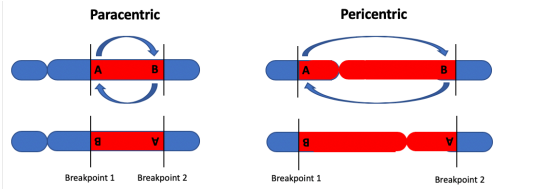
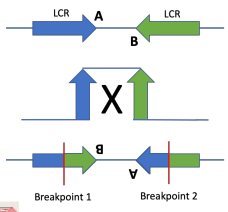
Background

Genomic inversions are a class of **DNA structural variation (SV)** that usually presents with **two breakpoints that occur in *cis*** and a subsequent 180-degree longitudinal turn of DNA.



Copy-number neutral inversions are challenging to resolve using classical methods for SV detection as there may be **no immediately obvious genomic alteration** to infer orientation of the copy number neutral event.

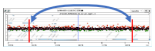
Inversions are often **flanked by inverted repeats** and have **breakpoints embedded within repeat regions** further complicating analysis.



Adapted from Carvalho *et al.*, 2016

Problems Characterizing Inversions

Traditional Chromosomal Analysis
5-10 MB Resolution



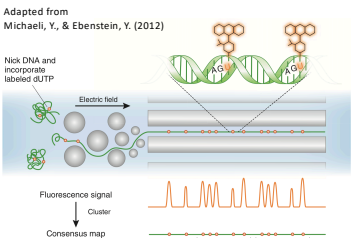
Array Comparative Genomic Hybridization (aCGH)
Does not detect copy-number neutral events

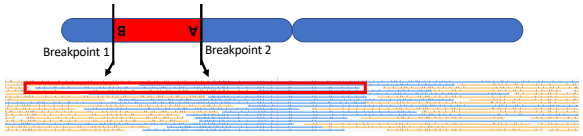
Paired-End Short-Read Sequencing

Genomic Inversions can also be accompanied with other **complex genomic rearrangements** further complicating analysis.

Optical Mapping

Optical mapping (OM) is a new technology that involves **tagging sequence motifs** along **unbroken DNA strands** >100 kb in length **preserving the architecture of SVs** harboring more than one breakpoint junction in *cis*.



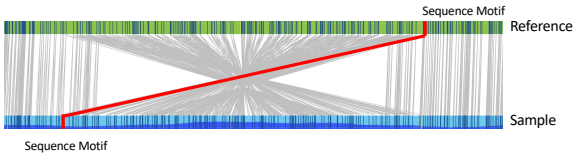


Rationale

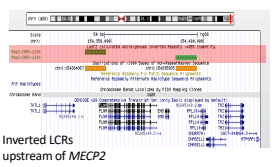
Given the mounting evidence for inversion rearrangements contributing to **disease pathogenesis,** we sought to employ optical mapping **in combination with other genomic technologies** to gain a more comprehensive picture of the **genomic architecture** at a given locus.

Methods

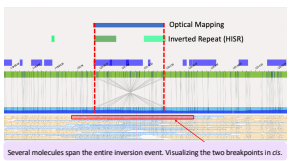
DLS Optical Mapping was performed on the following cohorts: • DUP-TRP/INV-DUP at the *MECP2* Locus (N=2) • Pericentric and multiple paracentric inversions on Chr6 (N=1) • Control Sample (N=1)



Given that inversions can be **mediated by repeats**, we incorporated a custom database, **Highly Similar Intrachromosomal Repeats (HSIRs)**, allowing for the **visualization of inverted repeat regions genome-wide**.



Inverted LCRs upstream of *MECP2*



Several molecules span the entire inversion event. Visualizing the two breakpoints in *cis*.

Custom high-resolution **array comparative genomic hybridization** as well as **short-read whole genome sequencing** was performed to compliment data obtained through OM.

Results

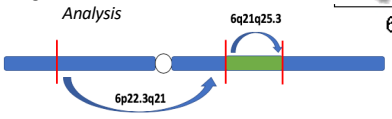
Multiple *de novo* inversions involving chromosome 6

Clinical Review:

- Delayed neuro-psychomotor development
- Craniofacial dysmorphisms
- Congenital heart disease

1

Original Chromosomal Analysis



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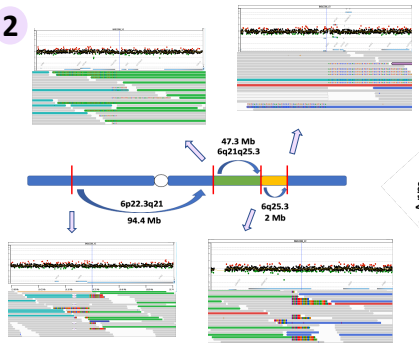

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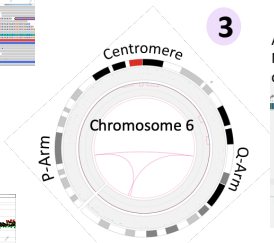
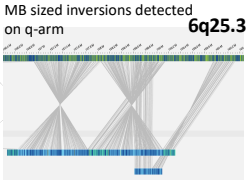


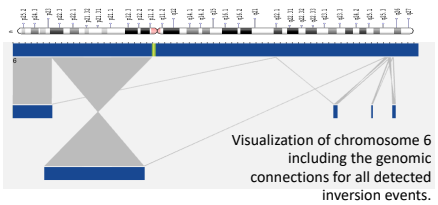
An additional cluster of ~1 MB sized inversions detected on q-arm



Large inversion events (>5MB) confirmed through optical mapping

Whole genome sequencing detected the presence of an additional inversion **below the resolution of karyotyping**

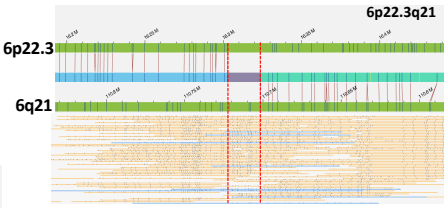
Custom **high-resolution array comparative genomic hybridization (aCGH)** confirmed **no CNVs present** elsewhere at the junction.



Visualization of chromosome 6 including the genomic connections for all detected inversion events.

Proposed Structure

Visualization of inversion between 6p22.3q21



Several molecules **>300 kb in length span both sides of the inversion event**

Methodology	Data Obtained
Karyotyping (1)	6q22.3q21, 6q21q25.3
High-Resolution Array (2)	No CNVs Present
Genome Sequencing (2)	6p22.3q21, 6q21q25.3, 6q25.3 + 6q25.3
Optical Mapping (3)	6p22.3q21, 6q21q25.3, 6q25.3, 6q25.3 + Resolved Structure

Inverted Triplications at the *MEP2* Locus

Custom high-resolution array CGH gives precise location **genomic copy number** changes in the ***MECP2* locus**

Optical mapping provides a **complimentary tool** to infer genomic architecture for a region with **complex genomic rearrangements**

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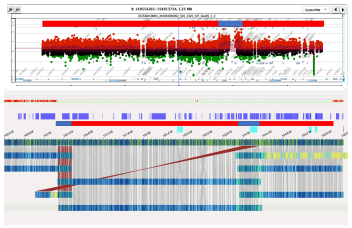

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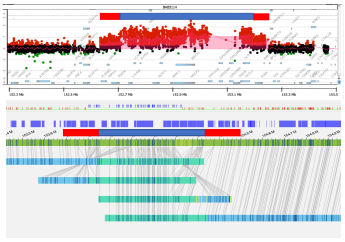

Triplication
Duplication

Conclusions

The visualization of long lengths of **unbroken DNA** through optical mapping is critical in properly studying an SV with **two breakpoints in *cis*.**

Optical mapping provides a **complementary tool** to characterize previously challenging structural variants

The use of technologies that can can **visualize single molecules** that **span inverted repeats** may be necessary to visualize inversions that are **invisible to other methods.**

As the role of structural variants in human disease becomes more clear new methods like optical mapping may be required to study a structural aberration in its totality.

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