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Mate Pair Testing for Targeted Chromosome Rearrangements

HOT TOPIC / 2017

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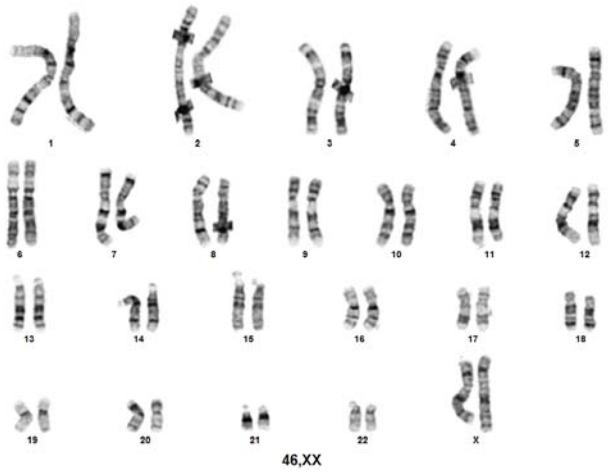
Disclosure

- None

Utilization Management

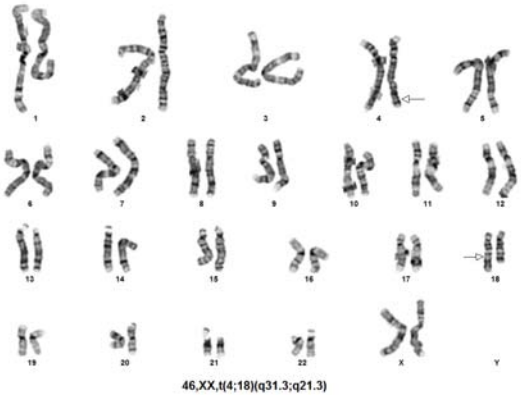
- As you view this presentation, consider the following important points regarding testing:
  - How is the testing going to be used in your practice?
  - When should the tests be used?
  - How will results impact patient management?

Introduction: Chromosomes and Cytogenetics



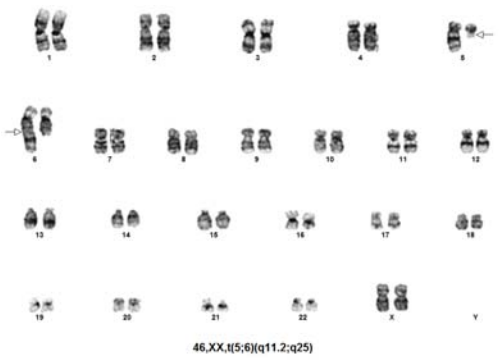
Chromosome Rearrangements

- In a congenital (germline) setting:
  - Phenotypically normal individual, miscarriages/infertility/phenotypically abnormal offspring
  - Phenotypically abnormal individual, the rearrangement MIGHT be causing/contributing to the phenotype (significance uncertain)



Chromosome Rearrangements

- In a neoplastic (cancer) setting, can result in:
  - Chimeric (fusion) genes
  - Disruption of a tumor suppressor gene
  - Activation of an oncogene via position effects
- Can impact diagnosis, prognosis, and/or therapy



- However, chromosome studies CANNOT identify what genes are at/near the breakpoints of a rearrangement
- Some rearrangements associated with neoplasms are “classic” and the genes can be inferred and/or confirmed using other techniques (FISH, RT-PCR, etc)
- For most, there was not, until recently, a clinical test available for further characterization
- Mate Pair Sequencing has made this possible

Mate Pair Sequencing (MPseq)

- A specialized library preparation technique followed by whole genome, paired-end next-generation sequencing (NGS)
- Input DNA is large (3-5 kilobases as opposed to 200-500 kilobases, which is standard for most NGS applications)
- Allows for efficient detection of structural variation in the genome at lower sequencing depth than other NGS methods

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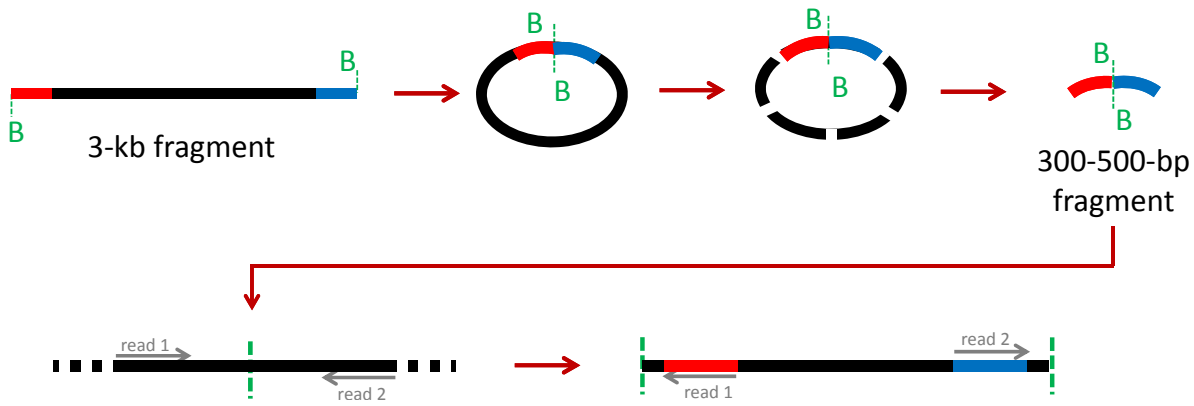


Reads should map to same chromosome,
~3-kb apart, in specific orientation

"Discordant Fragments"

- Paired reads map either to different chromosomes or the same chromosome but unexpected locations and/or orientations
  - Translocations
  - Inversions

- Possible to tease out, by looking at the mapping patterns and genomic location (breakpoint usually distinguishable within 2-3 kb), what the rearrangement is and what genes are affected

Three Clinical Assays Live to Date:

- MTRBL / MatePair, Targeted Rearrangements, Congenital
  - Congenital: peripheral blood
- MTRBM / MatePair, Targeted Rearrangements, Hematologic
  - Hematologic neoplasms: bone marrow (preferred) or blood
- MTRTI / MatePair, Targeted Rearrangements, Oncology
  - Oncology: solid tumors, fresh/frozen tissue or cell cultures (NO FFPE)

Mate Pair Clinical Assays

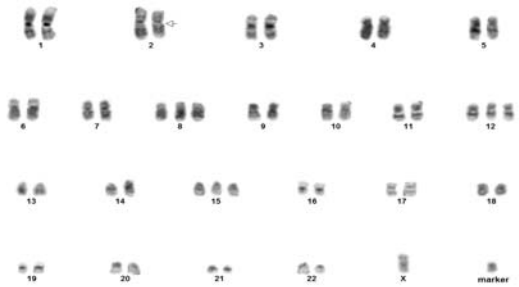
- MTRBL (constitutional blood), MTRBM (hematologic neoplasm), MTRTI (solid tumor):
  - Follow-up/add on test when a chromosome rearrangement by FISH or chromosomes is identified and there is possible utility in characterizing the rearrangement further
  - FOCUSED analysis (NOT a whole genome test)
  - Copy of previous test results REQUIRED in order to determine the target rearrangement for analysis
- These assays are NOT:
  - Whole genome tests
  - A replacement for any other test we offer
  - Stand-alone tests
  - Useful for monitoring minimal residual disease (MRD)
  - Useful for detecting point mutations/single nucleotide variants (SNVs)

Possible Scenario: MTRBM

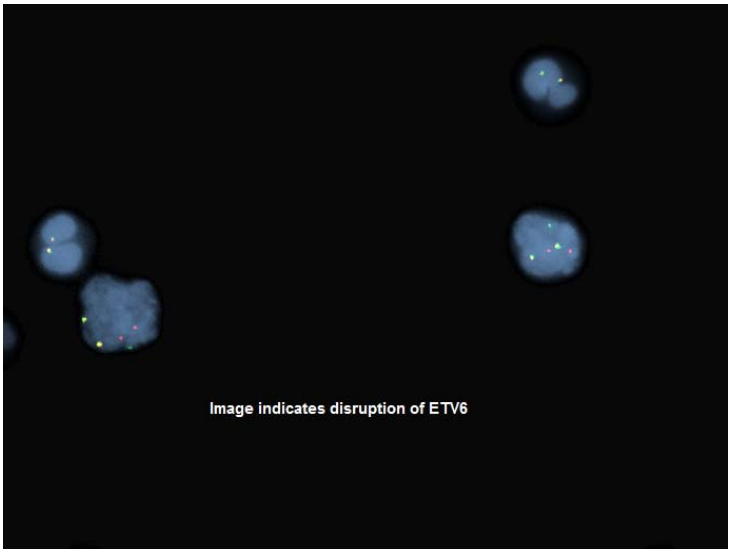
- 10-year-old female
- History of B-cell ALL, relapsed
- FISH studies with probe for *ETV6* had indicated possible 12;15 translocation
  - Unknown partner gene
  - Uncertain clinical significance

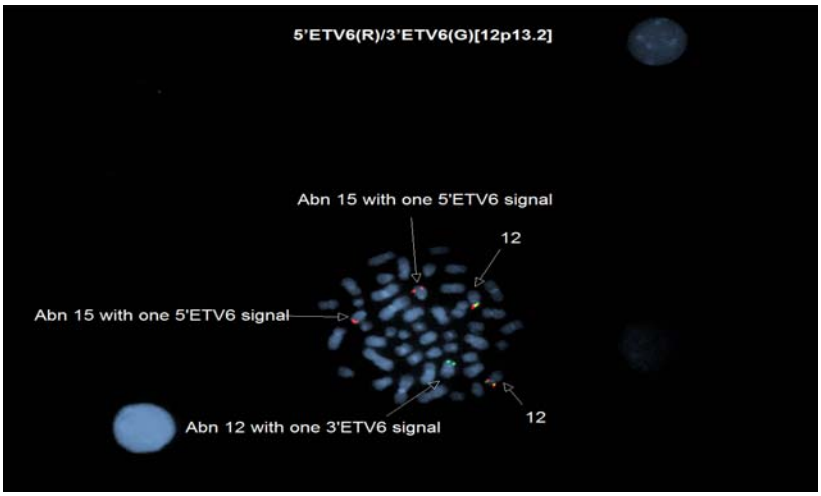
Bone Marrow: Chromosome studies

- 46-51,X,-X,del(2)(q13q21),+8,+12,+15,-17,+18,+0-2mar[cp20]



49,X,-X,del(2)(q13q21)+8,+12,+15,+mar

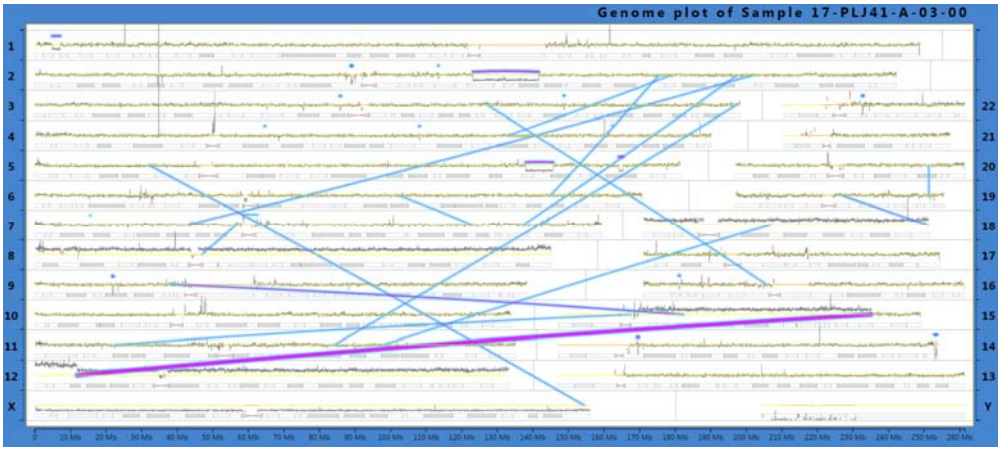


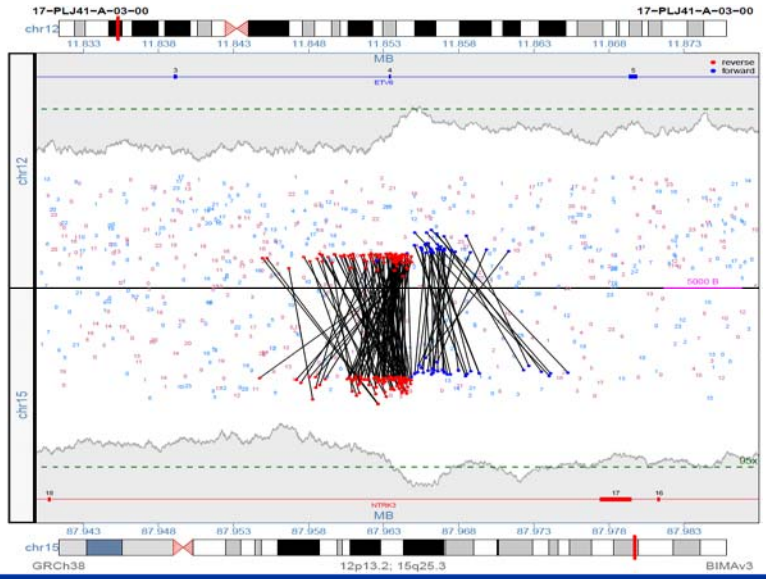


ETV6

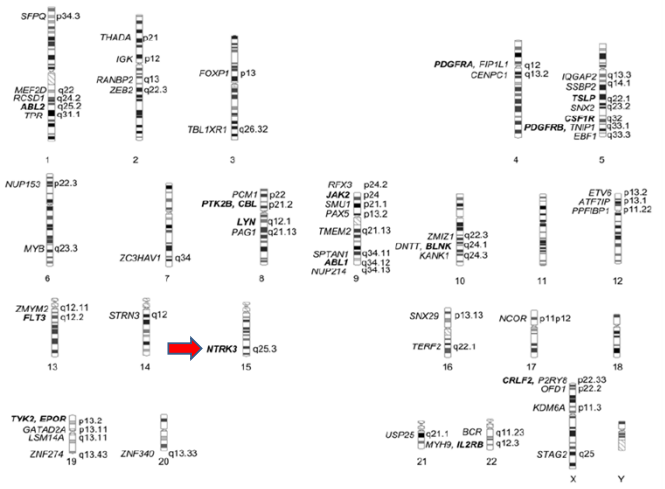
- Multiple partner genes
- Multiple tumor types (both solid and hematologic)
- Multiple mechanisms (constitutive activation, loss of function, activation of nearby oncogene, etc)
- By itself, uncertain significance for this patient

Mate Pair Result





Targetable Kinase Gene Fusions in B-ALL



Reshmi et al, Blood, 2017.

ETV6/NTRK3 Fusion

- Chimeric gene; exons 1-4 of *ETV6*, Exons 15-20 (tyrosine kinase domain) of *NTRK3*
- Rare; reported in patients with B-ALL and other neoplasms (high-grade glioma, secretory breast carcinoma, AML…)
- Targetable
  - Crizotinib, lestaurtinib, LOXO-101 (in clinical trials)

Possible Scenario: MTRTI

- Tumor cells from an 18-year-old woman are found to have an acquired inversion of chromosome X as a sole abnormality. Mate-pair whole-genome sequencing is requested to determine the genes at/near the breakpoints in order to subtype the neoplasm, provide prognostic predictions, and/or possibly identify therapeutic targets.

Possible Scenario: MTRBL

- A 6-month-old boy with a congenital malformation, developmental delay, and intellectual disability is found to have a 1;19 translocation by chromosome analysis. Neither of his parents has this same translocation. Mate-pair sequencing can be used to determine what genes are disrupted by the translocation in order to provide possible diagnostic information.

In Summary

- Mate Pair sequencing is a novel clinical assay used to characterize chromosome rearrangements
- Fills a gap in clinical testing
- Clinical applications: congenital (germ-line), hematologic malignancies, and solid tumors
- Can aid in diagnosis, prognosis (oncology), and choice of therapeutic regimen in some cases

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