Integration of chromosomal microarray analysis for precise clinical diagnosis and risk stratification of pediatric neoplastic disorders

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Introduction

The 5-year survival rate for pediatric neoplastic disorders has increased dramatically in the last 40 years, with 84.8% overall, specifically 90.6% for acute lymphoblastic leukemia (ALL) and 76.8% for neuroblastoma. These improvements are mainly due to the correlation of morphologic, immunophenotypic, cytogenetics, and outcome data to risk-stratified consolidation chemotherapy regimens, an effort led by the Children's Oncology Group (COG). Structural genomic abnormalities, including balanced chromosomal rearrangements, copy number gains and losses and copy-neutral loss-of-heterozygosity (CN-LOH) represent an important category of diagnostic, prognostic and therapeutic markers for pediatric cancer patients' treatment and management planning. Chromosomal microarray analysis (CMA), due to its higher resolution, no requirement for dividing cells and unique CN-LOH detection has been increasingly incorporated into clinical testing algorithms for neoplastic disease diagnostic workup. It has been reported that CMA reveals additional genomic abnormalities in around 14-54% of hematological malignancy cases that are otherwise normal/ non-informative by karyotype and fluorescence in situ hybridization (FISH).

Here we present three clinical scenarios to demonstrate how CMA facilitates the detection of submicroscopic copy number abnormalities (CNAs), adds precision with regards to breakpoint locations and the gene content, and further refines the risk stratification for pediatric neoplastic disorders.

Case Report 1

Case 1: Chromosome analysis and anaplastic lymphoma kinase (ALK) rearrangement FISH were ordered on a 1-year-old male with neuroblastoma. Chromosome analysis identified additional material of unknown origin on both 1p32 and 9p13, a deletion of 6q13q23 and 9q22q34, and double minute chromosomes. ALK rearrangement FISH was negative. Prompted by the physician, further CMA analysis clarified that the additional material on chromosome 1 is derived from der(1)t(1;17)(p33;q21) and the double minutes are derived from MYCN amplification. The der(1)t(1;17)(p33;q21) results in loss of distal 1p and gain of distal 17q which is a recurrent finding in neuroblastoma and indicates unfavorable prognosis7. MYCN gene amplification, the most prognostic relevant genetic alteration in neuroblastoma, is associated with high-risk neuroblastic tumors and poor patient prognosis7. CMA confirmed loss of material at chromosome 6q, 9p, and 9q, and further refined the breakpoints. CMA additionally showed a gain of 20p13 and a region of homozygosity on 9p.

Reference

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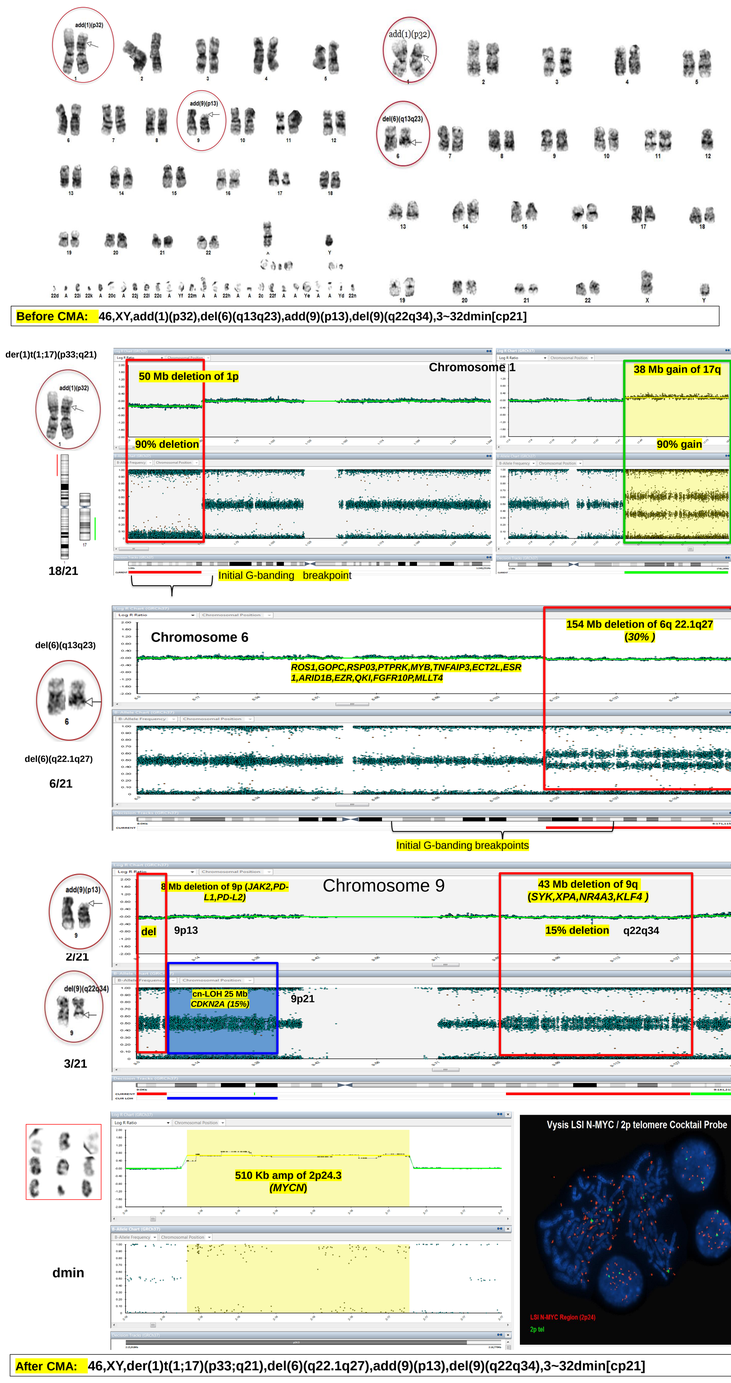
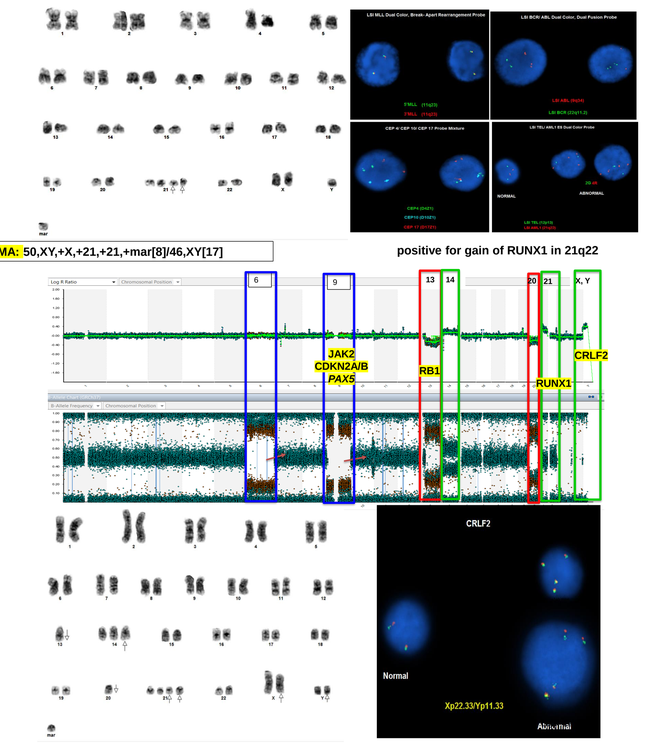
Case Report 1-continued



Case Report 2

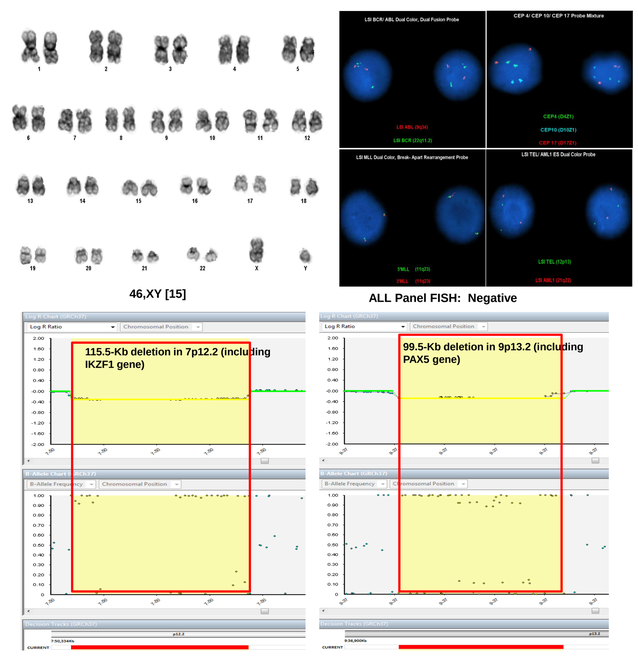
Case 2: Chromosome analysis and pediatric ALL FISH panel were ordered on a 3-year-old male for diagnostic ALL work-up. Both chromosome analysis showed an abnormal hyperdiploid clone (eight cells) with two extra copies of chromosome 21, gain of an extra copy of the X chromosome, a marker chromosome of uncertain origin (mar). FISH was positive for gain of RUNX1 in 21q22. Mistaking masked near-haploidy for hyperdiploidy could lead to erroneous risk classification and hence risk of treatment failure8, therefore, CMA was performed. CMA not only confirmed the hyperdiploid karyotype, but also revealed monosomy 20 and deletion of most of the long arm of chromosome 13 which are recurrent in ALL and have been associated with increased risk of relapse. In addition, SNP array also showed copy neutral loss of heterozygosity (CN-LOH) of chromosomes 6 and 9 which increase the risk of homozygous inactivation of tumor suppressor genes.

Case Report 2-continued



Case Report 3

Case 3: Chromosome analysis and pediatric ALL FISH panel were ordered on a 5-year-old male for diagnostic ALL work-up. Both chromosome and FISH panel showed normal results. Further CMA revealed a deletion in 7p12.2 including the IKZF1 gene and a deletion in 9p13.2 including the PAX5 gene. Deletions of the IKZF1 and PAX5 genes are associated with the BCR-ABL1-like high risk subtype of B-cell precursor acute lymphoblastic leukemia within the WHO classification system. IKZF1 abnormalities have been reported to predict a poor outcome in B-ALL.



Summary

In summary, Microarray technology enables accurate, cost-effective whole-genome analysis at a resolution significantly higher than that of conventional karyotyping and FISH. It provides additional disease specific and potentially clinically actionable genomic alteration information. The integration of chromosome microarray analysis into the cytogenetic diagnosis of pediatric malignancies and incorporation of such results into COG risk-stratification algorithms will further improve patient care.