Cytogenetics into Cytogenomics: SNP Arrays Expand the Screening Capabilities of Genetics Laboratories

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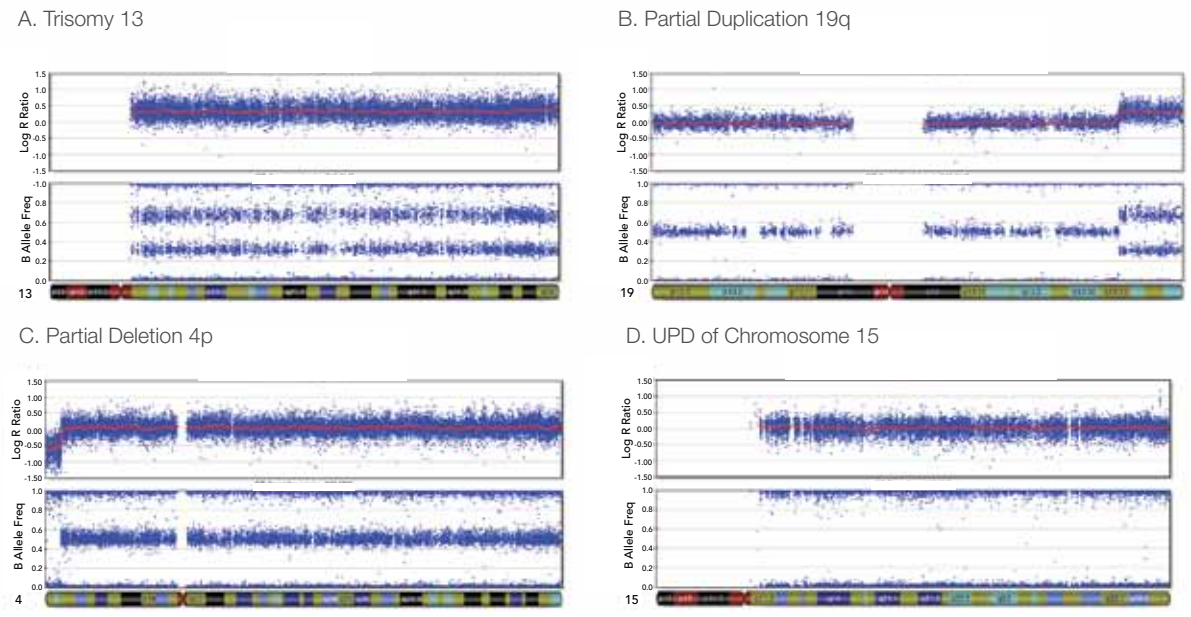
Introduction

Array technology is rapidly taking over the cytogenetics laboratory, resulting in greatly improved screening and validation capabilities*1,2.* While CGH (comparative genomic hybridization) array platforms have been the most widely used to date, SNP technology is now coming into its own for genetic testing. Our laboratory has been validating the Illumina® HumanHap550 BeadChip for use in cytogenetic evaluations. In a study of more than 200 patients, we were able to validate all of the known abnormalities with deletions or duplications previously identified using standard cytogenetics, as well as discover numerous abnormalities that were below the resolution of standard cytogenetic tests. In addition, we have identified hidden mosaics, patients with uniparental disomy, and complex patterns of meiotic crossing over that led to chromosomal aberrations, all of which could not be detected by standard cytogenetics or by comparative CGH methods.

Validation of Known Abnormalities

As a first step to validating the Illumina platform for screening and validation testing, we analyzed a panel of patients with known cytogenetic abnormalities previously identified by standard cytogenetics, fluorescence in situ hybridization (FISH), or multiplex-ligation–dependent probe amplification. We were able to confirm the abnormalities in all patients with known deletions or duplications, including 60 subtelomeric alterations, 43 interstitial deletions, 16 whole-chromosome abnormalities, and 4 supernumerary markers. Nine cytogenetic alterations could not be identified using the SNP array, including balanced

Figure 1: Cytogenetic Analysis Results From the Humanhap550 Beadchip



Each grouping shows the Log R ratio in the top and the B-allele frequency ratio below. (A) Trisomy of chromosome 13. (B) A partial duplication of chromosome 19 (7.6 Mb) derived from a translocation. (C) A deletion of chromosome 4p (5.8 Mb) consistent with a diagnosis of Wolf-Hirschhorn syndrome. (D) Uniparental disomy (UPD) for chromosome 15. The power of the combined use of B-allele frequency and Log R ratio is evident in the UPD15 patient, where Log R ratio is normal, but the lack of heterozygosity indicates UPD with two copies of the same maternal chromosome (maternal isodisomy).

translocations, ring chromosomes, and inversions. None of these abnormalities involved copy number variations.

For analysis of aberrations with the HumanHap550 BeadChip, we used the Log R ratio parameter (based upon intensity information) in combination with the B-allele frequency metric (based upon the genotype information). We found that this provided very powerful data for copy number analysis. In some cases, the combination of these two parameters allowed identification of an abnormality that would have been missed by either parameter alone (e.g., uniparental disomy). Examples of standard cytogenetic analyses made using the HumanHap550 BeadChip are shown in Figure 1. These include trisomy 13 (Figure 1A), a partial duplication of chromosome 19 in a patient with a derivative chromosome from a parental translocation (Figure 1B), deletion of chromosome 4p consistent with Wolf-Hirschhorn syndrome (Figure 1C), and uniparental disomy for chromosome 15 (Figure 1D). This last patient had a clinical diagnosis of Prader-Willi syndrome, which is caused by loss of a paternally expressed gene within 15q11. This disorder can be associated with either deletion of 15q11, maternal uniparental disomy or, in some cases, defects in the imprinting process. Standard cytogenetics can only identify deletions of 15q11, but use of SNP arrays enabled us to identify uniparental disomy in those cases where two copies of the same chromosome result in loss of heterozygosity.

Identification of Novel Deletions in Patients with Normal Chromosomes

We have studied a group of 100 patients who had previously undergone cytogenetic analysis. Findings for these individuals were reported as normal. In some cases, comparative genomic hybridization had also been carried out and reported as normal, although abnormalities were identified by CGH in a number of cases. In our laboratory, we set a cutoff of 20 consecutive SNPs on the HumanHap550 BeadChip, covering a region larger than 200 kb in order to call an abnormality as potentially pathogenic. We chose this cutoff because this size would be relatively straightforward to validate by FISH, which is in standard use in our laboratory. We recognize that only a small percentage of genes will actually cause a clinical phenotype when haploinsufficient, and at this time we are not able to predict the clinical consequences of smaller abnormalities involving one or very few genes. Since cytogenetic analysis is only able to identify deletions and duplications that are larger than 5–10 Mb, it is not surprising that our ability to identify deletions/duplications as small as 200 kb has greatly increased our rate of abnormal karyotypes. To date, either FISH or quantitative PCR has validated all abnormalities identified, and in all cases that parental samples were available, the abnormality was found to be *de novo*.

Identification of Hidden Mosaics and Meitoic Complexity

Many cytogenetic abnormalities may not occur in every cell, but rather as mosaics with some cells having a normal karyotype and others being abnormal. Identification of mosaics can be labor intensive, as enough cells have to be analyzed to permit discovery of the abnormal cell line. For example, it has been estimated that 30 cells need to be analyzed to detect 10% mosaicism, 50 cells to detect 5% mosaicism, etc. Furthermore, there may be bias against cells with chromosome abnormalities going through mitosis in cell culture, resulting in the abnormality being missed. The Illumina BeadStudio Genotyping Module allowed us to detect low-level mosaicism in a number of cases that were missed by cytogenetic analysis. The genotyping information in the form of B-allele frequency is especially useful in detection of mosaicism. Mosaicism can arise either by mitotic or meiotic mechanisms, resulting in different haplotypes of chromosomes in each cell line present in the patient. Using both the Log R ratio and the B-allele frequency, the level of mosaicism, as well as a clue into the mechanism behind the mosaicism, can be quickly determined.

We studied one patient who demonstrated a normal female karyotype in 20 cells, on cytogenetic analysis. Analysis using the HumanHap550 BeadChip revealed a hidden atypical mosaicism for trisomy of chromosome 14, a rare anomaly (Figure 2). The Log R ratio was elevated for this chromosome, indicating that approximately 50% of the cells in this patient's blood were trisomic for chromosome 14, while the other 50% contained the normal two copies of this chromosome. After seeing this result, we went back to the cytogenetic slides and studied additional cells. We found only 2 of 39 cells (5%) with a trisomy 14 karyotype, which we hypothesize reflects the lower mitotic rate for the abnormal cells. The B-allele frequency revealed a very complex genotype of these chromosomes and suggested a complex inheritance pattern. Modeling of this B-allele frequency suggests that two of the three chromosomes were inherited from one parent, instead of being derived post-zygotically. In addition, the unusual pattern in the B-allele frequency suggested that this extra chromosome had also undergone crossing-over in the parent before transmission to the patient, resulting in a chromosome with a mixed haplotype of the parental chromosomes. Using the parental genotypes obtained from a SNP array, we were able to verify that two of the patient's chromosomes were derived from both of the maternal chromosomes. We also identified chromosomal aberrations that were not observed using traditional cytogenetic techniques, either because of sampling error or cell-culture bias. In addition, we were able to highlight additional novel information that SNP genotypes can provide in understanding the mechanism behind the molecular karyotype.

Additional Information

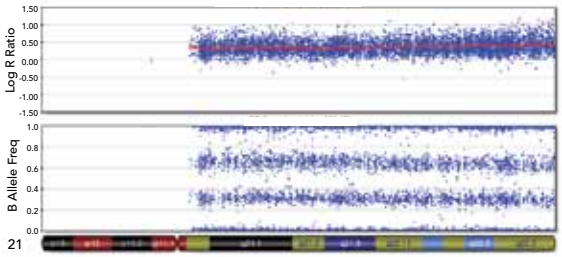
To learn more about Illumina's DNA analysis solutions, visit our website at www.illumina.com.

Reference

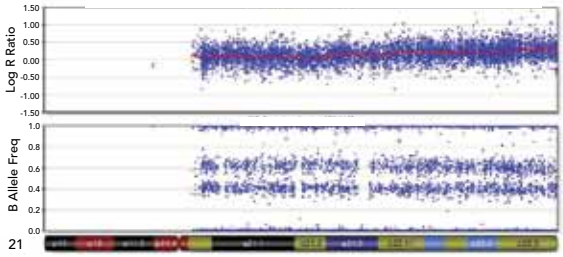
1. Cheung SW, Shaw CA, Yu W, Li J, Ou Z, et al. (2005) Development and validation of a CGH microarray for clinical cytogenetic diagnosis. Genet Med. 7: 422–432.
2. Shaffer LG and Bejjani BA (2006) Medical applications of array CGH and the transformation of clinical cytogenetics. Cytogenet Genome Res 115: 303–309.

Figure 2: Use of the Humanhap550 Beadchip Reveals a Rare Mosicism

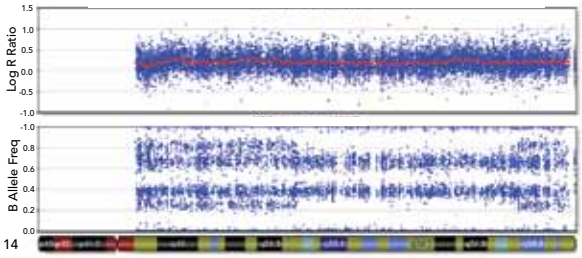
A. Trisomy 21



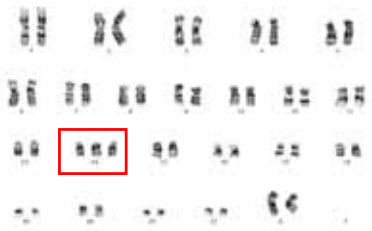
B. Mosaic Trisomy 21



C. Mosaic Trisomy 14 (complex inheritance)



D. Trisomy 14 Karyotype



Each grouping shows the Log R ratio in the top and the B-allele frequency ratio below. (A) Trisomy of chromosome 21 (Down Syndrome). (B) Mosaic trisomy 21 (~50%), both patients with only two haplotypes. (C) Mosaic trisomy 14 (~50%) with a complex inheritance pattern. The genotypes near the centromere and telomere indicate three unique haplotypes, while the central portion of the chromosome shows only two haplotypes. This B-allele frequency pattern is most likely due to recombination events during meiosis followed by non-disjunction. (D) The karyotype for (C) showing trisomy 14 in 2 out of 39 cells.

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