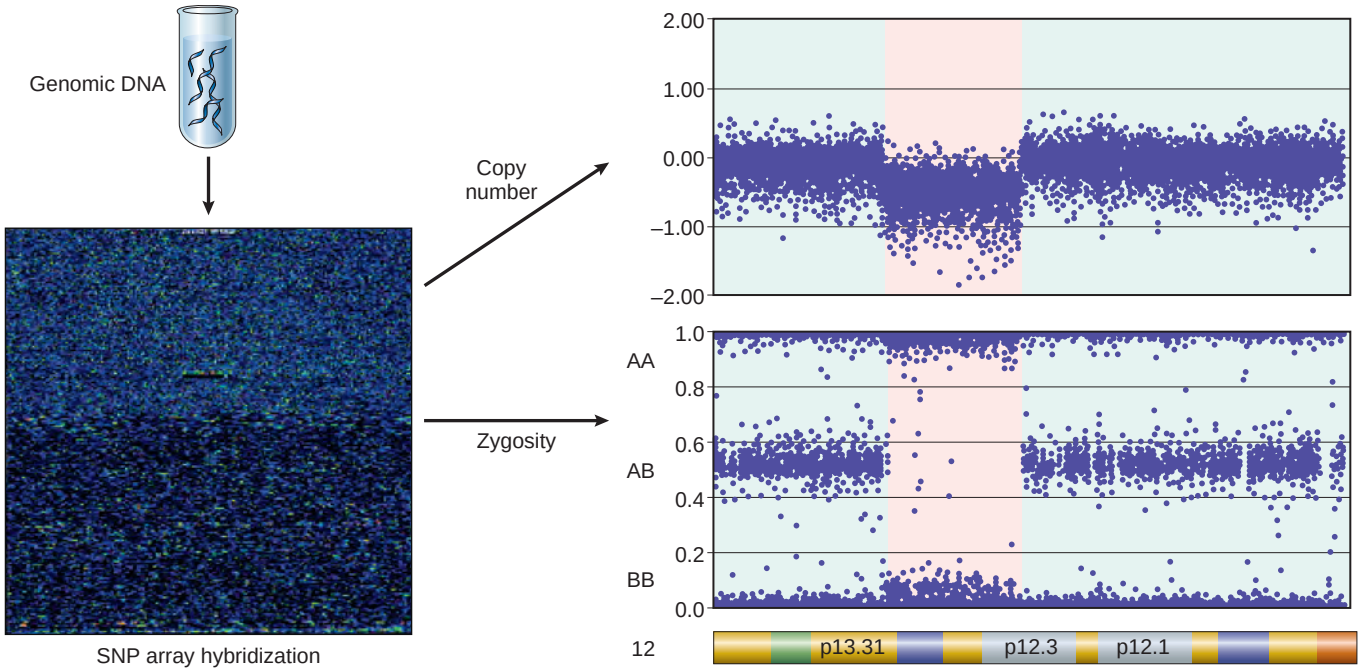

Figure 5-31 Analysis of copy number variation via SNP cytogenomic array. Genomic DNA is labeled and hybridized to an array containing potentially millions of probe spots. Copy number is determined by overall intensity and genotype is determined by allelic ratio. The example shown is the p arm of chromosome 12 in a pediatric leukemia. Here, the normal areas (green) show neutral (diploid) DNA content and the zygosity plot shows the expected ratio of AA, AB, and BB SNP genotypes. The anomalous area (red) shows decreased overall intensity, and the zygosity plot shows absence of the mixed AB genotype, indicating a full heterozygous deletion. (Modified from Paulsson K, et al: Genetic landscape of high hyperdiploid childhood acute lymphoblastic leukemia. PNAS 107(50):21719-24, 2010.)

(normal) DNA are labeled with two different fluorescent dyes. The differentially labeled samples are then co-hybridized to an array spotted with DNA probes that span the human genome at regularly spaced intervals, and usually cover all 22 autosomes and the sex chromosomes. At each chromosomal probe location, the binding of the labeled DNA from the two samples is compared. If the two samples are equal (i.e., the test sample is diploid), then all spots on the array will fluoresce yellow (the result of an equal admixture of green and red dyes). In contrast, if the test sample shows even a focal deletion or duplication, the probe spots corresponding to it will show skewing toward red or green (depending on gain or loss of material), allowing highly accurate determinations of copy number variants across the genome.

SNP Genotyping Arrays. Newer types of genomic arrays are based on a similar concept, but some or all of the probes are designed to identify single nucleotide polymorphism (SNP) sites genome-wide, which provides a number of advantages. As discussed earlier in Chapter 1, SNPs are the most common type of DNA polymorphism, occurring approximately every 1000 nucleotides throughout the genome (e.g., in exons, introns, and regulatory sequences). SNPs serve as both a physical landmark within the genome and as a genetic marker whose transmission can be followed from parent to child.

There are several testing platforms using different methodologies that allow SNPs to be analyzed genome-wide on arrays; details of these methods are beyond the scope of this discussion. Like CGH probes, these methods involving SNPs can be used to make copy number variations (CNV) calls, but by discriminating between SNP alleles at each particular location they also provide zygosity data (Fig. 5-31). The current generation of SNP arrays is quite comprehensive, with the largest containing greater than 4 million SNP probes. As a result, this technology is the mainstay of genome wide association studies (GWAS, described later).

In the clinical laboratory, SNP arrays are routinely used to uncover copy number abnormalities in pediatric patients when the karyotype is normal but a structural chromosomal abnormality is still suspected. Common indications include congenital abnormalities, dysmorphic features, developmental delay and autism. Here, the SNP data also proves useful. Typically, in areas of normal diploid copy number, the SNP results are roughly evenly split between homozygous and heterozygous calls. However, in anomalies such as uniparental disomy (e.g., in certain cases of Prader-Willi/Angelman syndromes), despite diploid copy number, the SNP calls in the affected region are all homozygous. SNP data can also help uncover other anomalies, such as mosaicism, which produces complex but distinctive skewing of zygosity plots.

Polymorphic Markers and Molecular Diagnosis

Clinical detection of disease-specific mutations is possible only if the gene responsible for the disorder is known and its sequence has been identified. If the exact nature of the genetic aberration is not known, or if testing for the primary defect is technically challenging or unfeasible, diagnostic labs can take advantage of the phenomenon of *linkage*. In humans, two DNA loci even 100,000 base pairs apart on