

SNP Allele Differentiation Provides Independent Confirmation of Genomic Imbalance

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Abstract

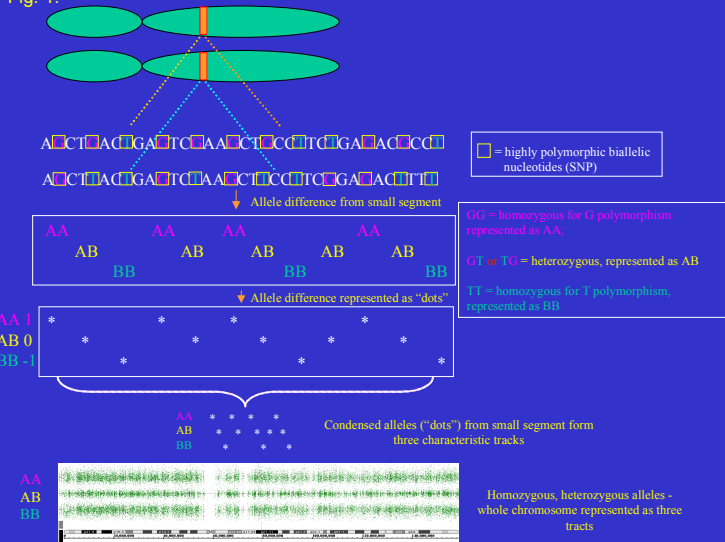
The whole genome 1.8 million feature SNP/copy number microarray (Affymetrix, Inc.) provides high-resolution detection of DNA copy number changes in individuals with idiopathic mental retardation and developmental delay. One of the unique features of the SNP based microarray is the specific SNP allele difference confirmation of DNA copy number changes and designation of copy neutral homozygosity correlated with UPD and consanguinity. The SNP allele call is not dependent on copy number changes and therefore provides a secondary confirmation of genomic aberrations. The specific allele designation was found to be essential for the precise identification of true gain and loss. This polymorphic nucleotide allele difference provides confirmation by identifying regions of loss of heterozygosity associated with deletions, allele specific dosage gain associated with duplications, and long contiguous stretches of homozygosity (LCSH) associated with UPD and consanguinity. For example, only one SNP allele possibility exists for all targets within a deletion interval (i.e., no heterozygosity, only an A or B allele). The absence of heterozygosity becomes confirmation of the deletion segment. Four possible SNP genotype tracts are generated across duplicated intervals (AAA, AAB, ABB, BBB). The four dosage ratio tracts of these genotypes provide confirmation of the duplicated chromosomal segment. Three SNP tracts (AA, AB, BB) would indicate the absence of a true duplication. The SNP allele dosage differentiation is thus a powerful independent confirmation of chromosome copy number aberrations and reduces the need for secondary follow-up in the proband. We present characteristic patterns of allele specific copy number analyses of microdeletions (including nullisomy X), duplications and copy neutral LOH associated with LCSH. Further applications would be to use the SNP allele dosage percentage to evaluate constitutional low-level mosaicism and DNA amplification in oncology.

Introduction

Whole genome wide analysis of millions of biallelic single nucleotide polymorphisms (SNPs) have been used to assess DNA copy number changes resulting from chromosome deletions and duplications and copy number neutral loss of heterozygosity (LOH). The latter is important in cancer clonal evolution and in determinations of inbreeding or UPD. Allelic imbalance results in LOH, secondary to the complete loss of an allele while duplications will increase the copy number of one allele relative to the other, if the SNP is polymorphic at that site.

Relative allele difference is calculated from polymorphic allele calls and dosage and is represented as a simple algebraic formula $A - B = 0$ (Fig.1, see below). The DNA dosage component is required to differentiate deletion-based LOH from copy neutral LOH found in long contiguous stretches (LCSH) in inbreeding and UPD. The relative allele difference can also be used to assess mosaicism and amplification in cancer (data not shown). Since the allele difference is proportional, it can be used as an independent confirmation of copy number changes across all SNPs in a copy number change interval.

Fig. 1.



AA - 0 = Homozygote AA and assigned by the computer software at position 1 on the output graph.
A - B = Heterozygote AB and assigned by the computer software at position 0 on the output graph.
0 - BB = Homozygote BB and assigned by the computer software at position -1 on the output graph.

Methods

The Affymetrix SNP microarray version 6.0 genechip (1.8 million SNP and CN targets) with GTC 2.1 software was used to determine DNA copy number changes and allele difference.

Results

Examples of allele difference consistent with autosomal and X chromosome deletions and duplications, and mosaicism are presented. In each case, the allele difference, the log2 ratio and copy number state (CN) are shown (Figs 2-9).

Fig. 2. Normal Chr 12. Regions of AA and BB Homozygosity, and AB Heterozygosity

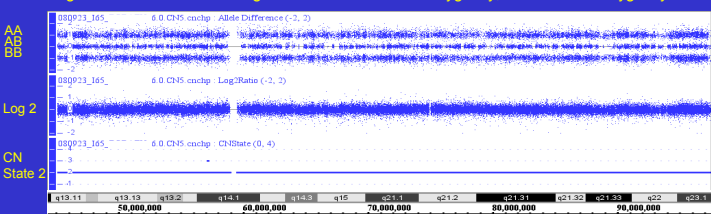


Fig. 3. Terminal and Interstitial Deletion of Chromosome 5p. LOH. Only A or B allele within deleted segments (no heterozygosity).

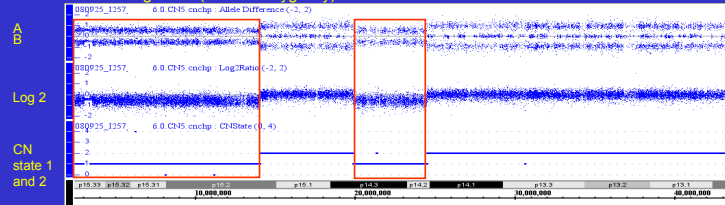


Fig. 4. Interstitial Duplication of Chromosome 17p. AAA, AAB, ABB and BBB alleles. All allele combinations show equal dosage separation (1.5, 0.5, -0.5, -1).

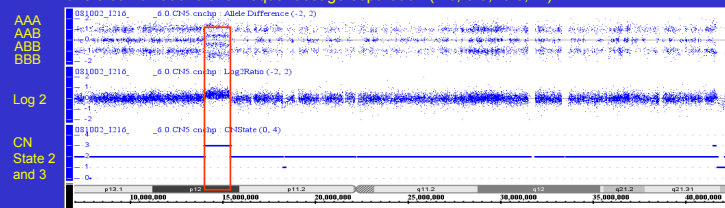


Fig. 5. Interstitial Deletion of the X chromosome in a Male. No A or B present (nullisomy)

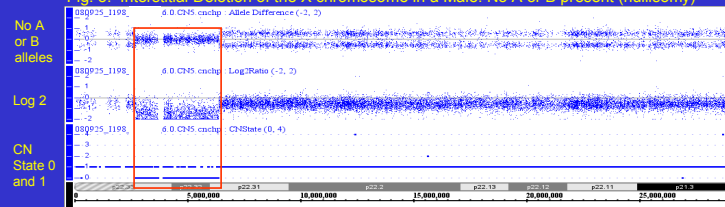


Fig. 6. Interstitial Duplication of the X chromosome in a Male. AA and BB only

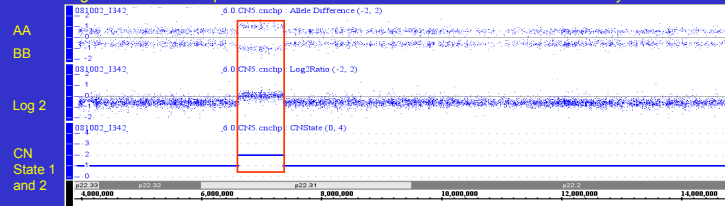


Fig. 7. UPD 6. *Copy number state 2. No heterozygosity Only AA or BB

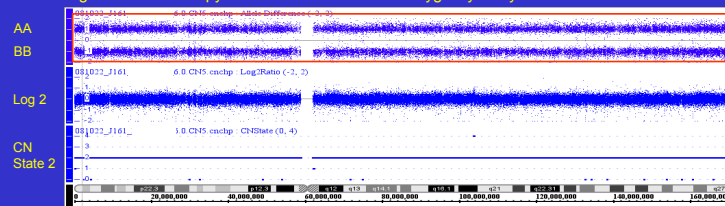


Fig. 8. Chromosome 13 somatic deletion pattern. Log 2 and all allele combinations show proportionally reduced dosage based on level of mosaicism with characteristic five allele tract combinations (AA,AB,BB/A,B). Interphase FISH showed 60% deletion while chromosomes were normal, suggesting that lymphocytes were either not involved or not stimulated.

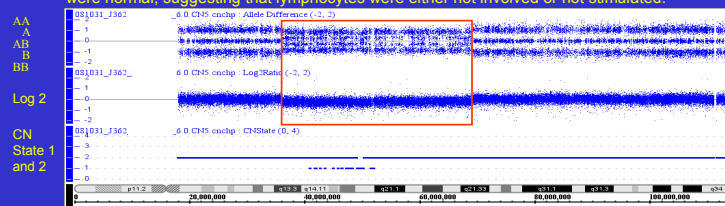
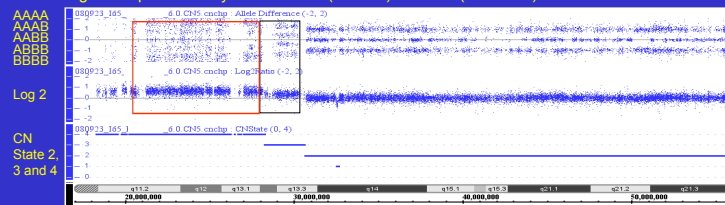


Fig. 9. Supernumerary idic 15. Five (red box) and four (black box) allele combinations



Discussion

We have demonstrated that the allele difference supports the diagnosis of autosomal and sex chromosome deletions/duplications at our current 200kb threshold, and UPD/consanguinity associated with large contiguous stretches of homozygosity. The allele difference also has the potential to detect mosaicism by measuring the dosage and ratio of alleles within an interval. This feature allowed the detection of a deletion mosaic that would have been missed by a dosage only microarray. The SNP allele dosage differentiation is a powerful secondary internal analysis for the confirmation of DNA dosage copy number changes that are not available on standard BAC and Oligo microarrays. The independent confirmation of chromosome copy number changes in many cases reduces the need for secondary follow-up in the proband.