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Interpreting aCGH-defined karyotypic changes in gliomas using copy number status, loss of heterozygosity and allelic ratios

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Abstract

We have used SNP mapping arrays to simultaneously record copy number changes, loss of heterozygosity and allele ratios (ploidy) in a series of 13 gliomas. This combined analysis has defined novel amplification events in this tumor type involving chr1:241544532-243005121 and chr18:54716681-54917277 which contain the AKT3 and ZNF532 genes respectively. The high resolution of this analysis has also identified homozygous deletions involving chr17:25600031-26490848 and Chr19:53883612-55061878. Throughout the karyotypes of these tumors, the combined analysis revealed counter intuitive relationships between copy number and LOH that requires reinterpretation of the significance of copy number gains and losses. It was not uncommon to observe copy number gains that were associated with loss of heterozygosity as well as copy number losses that were not. These events appeared to be related to ploidy status in the tumors as determined using allelic ratio calculations. Overall, this analysis of gliomas provides evidence for the need to perform more comprehensive interpretation of the CGH data beyond copy number analysis alone to evaluate the significance of individual events in the karyotypes.

Keywords

CGH; glioma; amplification; deletion; copy number

Introduction

The application of array comparative genomic hybridization (aCGH) to the analysis of genetic changes in cancer cells has become widespread, and is the platform of choice in most cases because of the comprehensive, high resolution provided. While CGH has been available in various forms for over a decade (Kallioniemi, et. al., 1992, Pinkel, et. al., 1998), the ability to define loss of heterozygosity (LOH) in parallel, using the same arrays, has only recently become possible with the development of arrays featuring polymorphic probes in their design (Affymetrix, 2007). In this application, the presence of LOH is assessed through statistical analysis of the frequency of contiguous homozygous alleles along the chromosomes compared to reference standards (Lo, et. al., 2008). Where the hybridization intensity can be measured at each of the two alleles on the array, it is also possible to generate allelic ratios (AR), which can be used to determine ploidy status in the tumors (Gardina, et. al., 2008). This combined analysis provides a significant improvement over the majority of array CGH platforms where copy number abnormalities (CNAs) are defined relative to a diploid reference standard. The primary restriction for accurate ploidy analysis in this case is probably the experimental protocol, which calls for a standard amount of DNA to be assayed. Such procedures will approximately equalize the total signal regardless of whether the original cells were diploid

or, for example, tetraploid. In addition, analytical procedures tend to normalize the overall signal approximate to those found in the reference population, which is almost exclusively diploid. As a result, computational methods relying on the total DNA signal will tend to grossly underestimate the actual copy number of tetraploid samples, since the presumptive baseline signal is set at two rather than four which makes it difficult to distinguish absolute ploidy status from relative copy number changes. Determining ploidy status in the analysis of tumor cells can be important in interpreting the consequences of copy number losses in particular, where a loss which generates LOH suggests the exposure of a recessive mutation in a tumor suppressor gene (Cavenee. et. al., 1983). This is not the case for single copy loss from a tetraploid chromosome complement where heterozygosity will be maintained and the consequence of the loss is harder to interpret.

Endoreduplication of the chromosome complement often occurs during tumor development making the tumor cells essentially tetraploid. Losses and gains of chromosome regions can occur before or after this endoreduplication, and these events can be detected through the combined analysis of CN, LOH and AR status in the tumor, thus providing a more complete interpretation of the karyotype. The combined analysis using these integrated approaches, however, are not commonplace and correct interpretation of the data often requires some supervised analysis of the data in the context of the biology. We have developed a streamlined approach to this analysis (Cowell and Lo, 2009) and in this study, we have used the Affymetrix SNP mapping arrays to define complex events seen in glioma karyotypes and, as a result of this combined analysis, suggest ways to interpret the parallel profiles. We have previously demonstrated that all but the smallest CNAs can be readily detected using any of the 50K, 100K, 250K and 500K platforms (Lo, et. al., 2008), although the current trend is to use the 6.0 SNP mapping array for most applications. However, regardless of the specific arrays used, the overall strategy for interpretation of the results follows the same guidelines. Using these approaches we now demonstrate the utility of the combined analysis to perform comprehensive characterization of the genetic events that have occurred in gliomas.

Materials and Methods

GeneChip® 250K Mapping Arrays

For each tumor sample, 250ng of DNA was processed according to the Affymetrix GeneChip® Mapping 500K Assay Manual (www.affymetrix.com). Briefly, 250ng of high-quality genomic DNA was digested with NspI and ligated to custom adaptors. Adaptor-ligated restriction fragments were then amplified by PCR, fragmented and labeled as described by Kennedy et al., (2003). Samples were hybridized to GeneChip Human Mapping 250K Nsp arrays using an Affymetrix Hybridization Oven 640. Washes and staining of the arrays were performed with an Affymetrix Fluidics Station 450, and images were obtained using an Affymetrix GeneChip Scanner 3000. A reference sample was included for quality control.

Copy Number and LOH Analysis

Genotype calls were determined using GeneChip® Genotyping Analysis Software (GTYPE 4.0) Bayesian Robust Linear Model with Mahalanobis distance (BRLMM) algorithm with the default settings as described previously (Lo, et. al., 2008). Copy number estimations and LOH states were determined using the Chromosome Copy Number Analysis Tool (CNAT 4.0) without Gaussian smoothing. The unsmoothed log₂ ratio for each SNP was then used to segment the array using Circular Binary Segmentation (CBS) using DNACopy software.

The 'LOH score', which is used to define loss of heterozygosity (Lo, et. al., 2008), is calculated by multiplying the reference homozygous rate for a contiguous stretch of SNPs, using an in-house script, that has been classified as having likely LOH using the Hidden Markov Model

that is incorporated into CNAT 4.0.1. The reference homozygosity rate was obtained from the allelic frequencies derived from 50 phenotypically normal individuals from the HapMap project (data available at <http://www.affymetrix.com>). These samples were used as normal controls for the calculation of both LOH and Copy Number. The calculated p-value represents the likelihood that a contiguous stretch of SNPs would be homozygous by chance alone, based on the allelic frequencies defined by the control samples. The LOH Score is then calculated using the $-\text{Log}_{10}(\text{pLOH}_{\text{sample}})$.

Allelic Ratios were generated following guidelines described by Gardina, et. al., (2008). Allele signal summaries and genotypes were generated from CEL files using the command-line program apt-probeset-genotype.exe (v. 1.8.5). The files were processed using the BRLMM algorithm implemented in Affymetrix Power Tools (APT) with a quantile sketch normalization of 50,000 points and no background correction. 250K Nsp arrays from 50 female samples from the International Hap Map project were used in the normalization to provide baseline reference signals. Contrasts (d) were generated by the following relationships, bound by $[-1,1]$:

$$d = \frac{S_A - S_B}{S_A + S_B}$$

Where S_A and S_B represent signal summaries of alleles A and B, respectively. Allelic ratios were calculated:

$$AR = \begin{cases} \frac{d_s}{\text{Max}\{d_r\}} & \text{for } d_s > 0 \\ \frac{d_s}{\text{Min}\{d_r\}} & \text{for } d_s < 0 \\ 0 & \text{for } d_s = 0 \end{cases}$$

Where d_s and d_r represent contrasts of sample and reference, respectively. To assign confidence of each allelic ratio, 2-D Gaussian mixture modeling using the expectation maximization (EM) algorithm was performed using the R library 'mclust' on the reference signals. SNPs were excluded if the Gaussian mixture model yields component mixtures not equal to 3 (AA, AB and BB clusters). The density estimate of each sample is estimated using the 'Dens' function within the mclust library using the sample S_A and S_B . The confidence measure is the $\log_{10}(\text{density}(\text{SNP}_{is}))$ where i is the index of SNP and s is the sample. Allelic ratios were then filtered using a confidence measure threshold of -4 .

Tumor samples

Tumor samples used in this study were obtained from the Roswell Park tumor bank and were collected under IRB approved protocols. Freshly acquired samples were snap frozen in liquid nitrogen and then processed for DNA preparation using standard phenol-chloroform procedures.

Results and Discussion

In this study we used DNA from 13 previously uncharacterized GBM samples which were hybridized to a single Nsp1 array from the Affymetrix 500K platform. The arrays were processed for CNA, LOH and AR as described previously (Gardina, et. al., 2008, Lo, et. al., 2008). A comprehensive summary describing the CNAs detected in this study are given in table 1. During the course of this analysis, however, it became clear that the interpretation of the consequences of the karyotypic changes using copy number alone can potentially be misleading in defining the genetic events that occur in the tumors. Specific circumstances that

required supervised interpretation of the results by combining the total capabilities of the SNP mapping arrays are discussed below.

Amplification and Homozygous Deletions

Amplification events are considered to reflect consequences of focal increases in DNA content which are typically associated with a specific increase in expression levels for genes within the amplicons. As such, amplifications are distinct from increased copy number of whole chromosomes or chromosome regions within the karyotype. Identification of amplified regions using aCGH is straightforward, although the estimated level of the amplifications can be more difficult to establish using SNP arrays compared with BAC arrays, for example (Lo, et. al., 2008). We suggest that this is due to dye saturation considerations using the oligonucleotide arrays which consistently report amplification levels that are far lower than BAC arrays. Even though the exact levels of amplification cannot be accurately defined using SNP mapping arrays, they can be readily distinguished from simple gains of chromosome regions because their $\log_2$ ratios are typically > 1.0 and the extent of the amplicon is highly restricted to small regions along the chromosome (Figure 1). Even if the amplification events are associated with double minute chromosomes (Cowell, 1982), they still appear as focal amplifications in the aCGH profiles. In this study, we identified those common amplification events seen in gliomas involving the EGFR (7p) and PDGFRA (4q) loci (table 1) with similar frequencies described previously for GBM (10, 13). The less frequent 1q amplicon involving the MDM4 locus was also seen in one tumor in this series (Table 1; Figure 1). Conveniently, the gene content of these regions can be obtained by entering the extent of the amplicon described in table 1 into the genome browser at <http://genome.ucsc.edu/cgi-bin/hgGateway>. In addition to these common events, we also detected an amplicon on 1q which involves the chr1:241544532-243005121 interval (figure 1). Interestingly, this amplicon was present in the same tumor (13682) showing amplification events involving the DMD4 locus (figure 1) as well as amplicons on 7p11 and 12q13 (figure 1). This observation, given the overall rare nature of some of these amplicons, raises the issue whether some tumors show an amplifier genotype that makes these events more likely within a given tumor. The novel amplicon in 1q in this tumor involves an ~1.5 Mbp region containing only 6 genes which includes the AKT3 gene (figure 2), which is known to be associated with tumor progression (Bellacosa, et. al., 2005). The amplification event seen on 12q in this tumor revealed several tandemly arranged, independent amplicons (figure 1), which could be resolved into discrete units because of the high resolution of the array (Table 1). This fine mapping, as we have shown previously (10, 13), when combined with gene expression studies, can give a more accurate assessment of the individual genes within the interval that are related to the amplification events. Interestingly, one of these amplicons included the MDM1 and MDM2 genes (figure 2), suggesting potential cooperativity between different members of this family of genes and the more usually amplified MDM4 gene on 1q in this tumor. Finally, a small amplicon was seen in tumor 13747 involving the chr18:54716681-54917277 region (figure 2), which is another novel amplicon revealed through the high resolution of the array, although the significance of this observation is less clear since the only gene in this region is the largely uncharacterized ZNF532 gene.

The specific amplification events seen throughout the glioma genomes should theoretically be associated with LOH, if the amplification events involved a single allele of the target gene(s), regardless of the ploidy level in the cell. In the majority of cases, however, LOH scores for the amplified regions rarely exceed the value of 20 (figure 1), which, based on previous comparisons between tumor and normal tissue from the same individuals, we have suggested is a lower cut off for LOH (10). Since the LOH score reflects the statistical likelihood that a contiguous series of alleles could be homozygous by chance, the smaller the region, the fewer the SNPs in that region, and so potentially the lower the LOH score. As such, LOH analysis

may be of limited value in defining either the region of amplification or demonstrating mono-allelic amplification events using this platform if the amplicons are small.

Homozygous deletions are frequently indicative of the location of tumor suppressor genes (TSGs) and discovering these events in tumor cells is an important observation facilitated by the high resolution of oligonucleotide arrays. In this series of tumors, however, the only frequent homozygous deletion (table 1) involved the CDKN2A (p16), as we have reported before (Lo, et. al., 2008, Rossi, et. al., 2005). Evidence for two novel homozygous deletions was also seen. The first, which was identified in tumor 13682, involved a hemizygous deletion of the Chr17:24393508-26550868 region (Figure 3), with an average $\log_2$ ratio of ~ -0.3 and which contains >25 genes. Within this homozygous region there was a smaller deletion of chr17:25600031-26490848 with an average $\log_2$ ratio of ~ -0.62 indicating a homozygous loss. This region contains 14 genes (figure 3) and also spans the first 4 exons of the NF1 gene, which was recently shown to be mutated in gliomas (CGARN, 2008). The structure of this homozygous deletion, being located within a more extensive hemizygous deletion involving the flanking region, is similar to events frequently seen associated with p16 loss in 9p12 (10). The second deletion involved Chr19:53883612-55061878 in tumor 15343 (figure 3). In this case the homozygous deletion was also located within a region of hemizygous deletion spanning Chr19:53082236-56024482, suggesting each event had occurred on a different homolog of chromosome 19. Even though this deletion spans only 1.2 Mbp, it is still a gene dense region (figure 3) which includes the BAX and BCL2L12 genes associated with apoptosis.

Discordancy between LOH and copy number values

Chromosome losses are considered particularly relevant to the identification of TSGs when they are accompanied by LOH (see Introduction). The analysis identified several tumors where virtually all CNA_{loss} regions also showed LOH, suggesting they had a largely diploid chromosome number (table 1). These are straightforward interpretations that are also borne out by the AR profiles as we described previously (Gardina, et. al., 2008). The ARs evaluate the relative hybridization levels at each of the different alleles at each SNP and the $\log_2$ ratios can be used to define the alleles in the LOH region. Thus, calls of only A or B alleles would be expected where the loss was on a diploid background. Alternatively, if the allelic ratios suggest AAB or BBA allelotypes, then this would suggest a tetraploid background (Gardina, et. al., 2008). In other tumors, none of the CNAs were associated with LOH, suggesting a tetraploid or higher ploidy status (table 1). This ploidy level is also evident from the $-\log_2$ ratios, which were greater when the losses were associated with LOH (Figure 1) and this was confirmed using the AR analyses. Occasionally, mixtures of these observations were seen within the same karyotype, suggesting that the CNA_{loss} that correlates with LOH (figure 4) occurred before endoreduplication and those that did not occurred after the event. However, within these karyotypes these associations did not always hold true, especially where the CNA involved an interstitial region of the chromosome (figure 4).

Gains of chromosome regions are often assumed to represent a mechanism of increasing gene dosage events for critical genes within the region. However, in some circumstances, complex rearrangements generating subregional gain of chromosome regions may have alternative interpretations which may be overlooked using copy number analysis alone. This inconsistency between LOH and CN values is highlighted by the analysis of the distal part of chromosome 13 in tumor 13180 (figure 4), where LOH is, in fact, associated with a CNA_{gain} . In this case the $\log_2$ ratio demonstrates three copies for the region but homozygosity scores indicate that they are all derived from the same chromosome. The remainder of the chromosome shows a CNA_{loss} but heterozygosity is retained (figure 4). Since the gain extends to the 13qter region, this event, which would not have been identified using CN analysis alone, is assumed to result from chromosome non disjunction of a translocation event which results in three copies of the

distal 13q region being retained and one copy of the proximal region being lost. LOH involving the distal region of 17p was the most common event in this series associated with a copy number neutral (CNN) profile (figure 4). This region contains the TP53 gene which is frequently mutated in gliomas (Ohgaki, et. al., 2004), although the extent of the LOH region cannot be accurately determined using homozygosity analysis alone, because of the possibility of constitutional homozygosity in the vicinity of the breakpoint (Lo, et al., 2008).

Another inconsistency between LOH and CN involves small regions of loss as shown in figure 5. Single copy deletions may or may not show LOH depending on the extent of the deletion, since the LOH score depends on the statistical evaluation of the possibility of a homozygous region occurring by chance. If the region is small and/or the frequencies of the rare alleles within the region are low, then the LOH score will be reduced as shown for the Chr2:137312890-142832335 deletion in Figure 5. In this case, two LOH peaks are seen within the deleted region with scores between 25–35, but heterozygous calls within the region deflate the score. In contrast, the deletion on chromosome 4 (figure 5) shows a perfect correlation between CN and LOH in the same tumor (15343), with an LOH score of >70. These observations suggest that this tumor has a baseline diploid karyotype, as evidenced by the CNN LOH for the chr17:pter-71348737 region, but loss of heterozygosity in the region showing a single copy gain (figure 5).

The events associated with chromosome 19 in tumor 15343 (figure 5) illustrate another example where the relationship between LOH and CN can be complex. In this case, a subregional gain of the proximal and distal parts of this chromosome are seen with normal CN levels for the intervening region. LOH analysis, however, demonstrates that this intervening CNN region is homozygous. This observation is consistent with the interpretation that LOH has probably occurred through the generation of trisomy for chromosome 19, possibly duplicating a chromosome that carries a mutant gene within this region, followed by a deletion of the region from one of these chromosomes carrying the normal gene(s). On the long arm, not only is the deleted region Chr19:53156790-56044749 associated with LOH but the adjacent more distal CNN region also shows LOH. In this case, the break seen within the LOH score profile is associated with a homozygous deletion spanning the Chr19:53883612-55061878 region. This complex profile suggests that chromosome 19 has been involved in multiple rearrangement events and their accurate interpretation is important because of the frequent involvement of this chromosome in gliomagenesis (von Deimling, et. al., 1994).

In summary, from our aCGH analysis using SNP mapping arrays, it is clear that, even though the relationship between loss of genetic material often coincides with LOH, there are sufficient examples where this is not the case, making it important to be cautious when interpreting the consequences of these events. This is also true for observations of copy number gains, since we have shown that these events can also be associated with LOH making the interpretation of the consequences very different. As the application of SNP mapping arrays to the study of tumors evolves, it is clearly becoming necessary, therefore, to carry out all of the analyses that these platforms offer.

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Figure 1. Examples of amplification events seen in this glioma series comparing copy number (shown above in each panel) with LOH scores (shown below)

In (A) two amplification events are seen on 1q in tumor 13682, although the LOH scores in these regions do not exceed the significance values of 20 (see text) as indicated by the arrows. In (B) the amplification event seen on chromosome 7p in the same tumor does not show LOH either, although LOH scores >20 are consistently seen corresponding to the regions showing copy number gain on proximal and distal 7q, but not the intervening region. The multiple small amplicons seen in this tumor on 12q (C), although showing some coincident LOH peaks, the LOH scores are not >20. Since the LOH scale varies from profile to profile, the suggested significance of LOH=20 is indicated by the arrows on the left.

Figure 2. Summary overview of the gene content associated with novel amplicons

In (A), the gene content of the novel amplicon seen on distal 1q (*) in tumor 13682, reveals only six genes, including AKT3, within the region of amplification. The more proximal amplicon contains the MDM4 gene. In (B) the total gene content of the extended region containing the multiple amplicons is shown. The gene content in each of the precisely defined amplicons on 12q in tumor 13682 can be obtained by processing the data in table 1 through the UCSD genome browser (see materials and methods). In (C), analysis of the novel amplicon on 18q seen in tumor 13747 reveals that the only currently annotated gene in the region is ZNF532.

Figure 3. Homozygous deletions in gliomas

Analysis of the CNA_{loss} on 17q, in tumor 13682, demonstrates a hemizygous deletion spanning the Chr17:24393508-26550868 region with a homozygous loss, Chr17:26937933-26490848, within this region. The gene content of the homozygous deletion is shown relative to the human genome map where the homozygous region encompasses numerous gene including the 5' end of the NF1 gene. In (B) a complex series of events on chromosome 19 in tumor 15343 result in alternating regions of copy number gain and copy number neutral regions. On 19q, a hemizygous deletion, Chr19:53082236-56024482, is seen with a homozygous deletion, Chr19:53883612-55061878, located within it. The gene content of the homozygous deletion is shown below.

Figure 4. Examples of discordant LOH relative to copy number

The typical association between copy number loss and LOH are shown in (A) for region Chr11:24065510-42207074 in tumor 13682. In tumor 13180, the distal region of 13q (B) shows a copy number gain, but the same region carries a highly significant LOH score, demonstrating homozygosity for this region. Significantly, the proximal part of 13q shows a copy number loss but this is not associated with LOH (see text). On chromosome 17 in the same tumor (C) a copy number neutral region at the distal tip of 17p is associated with a highly significant LOH score and in this case the copy number gain on 17q is not associated with LOH.

Figure 5. Small regions of loss may sometimes show significant LOH scores

In examples from tumor 15343, the deletion on chromosome 2q (A) shows two peaks on the LOH score which both exceed a score of 20 (arrow) although there is a break in the profile. For the deletion on 4q (B), however, the deleted region shows a consistent region of LOH with a score of ~60. In (C) the copy number neutral region of chromosome 17 shows a consistent LOH score across the entire region up to the transition point where a copy number gain is seen. In (D) two regions of chromosome 19 show significant LOH scores. On 19p the LOH is associated with a CNN region while on 19q the entire distal end of the chromosome arm shows LOH. This region spans a CNN region and encompasses the deletions described in figure 3.

Table 1
Summary of the losses gains and amplifications seen the GBM described in this study.

ID	segmental	gains	whole chromosomes		Amp
	losses		losses	gains	
13180 (4n)	chr2:221907848-qter			chr7:105274891-qter (LOH)	
	chr3:47292669-90252295			chr8:104695968-qter	
	chr3:175961730-qter			chr10:pter-32061117	
	chr4:57291670-qter			chr13:87159135-qter (LOH)	
	chr6:pter-27551936			chr17:49250880-qter	
	chr6:109313107-125407172				
	chr10:117692635-qter				
	chr11:pter-50174761				
	chr11:127896001-qter				
	chr13:pter-87159099				
	Chr17:pter-18229935 (CNN)				
	chr21:pter-33612283				
13279 (4n)	chr1:29957675-39276465		chr6		
	chr1:66322339-119861171		chr10		
	chr3:pter-174466476		chr16		
	chr5:49596616-qter		chr21		
	chr7:76576527-113794127				
	chr8:pter-31166747				
	chr11:116202554-qter				
	chr12:42260682-qter				
	chr13:37651676-qter				
	chr15:pter-47706362				
	chr15:67194579-78905813				
	Chr17:47115727-72336304				
		chr1:pter-29929562			
		chr1:142215052-190845386			
		chr1:238257848-qter			
		chr2:pter-136747699			
		chr4:48758236-86096585			
		chr4:142286086-165630506			
		chr5:pter-46419092			
		chr7:113797887-qter			
		chr12:pter-37352685			
		chr15:47912054-67175475			
		chr16:55043296-58443684			
		chr16:81816025-qter			
		Chr17:12922367-19109505			

ID	segmental losses	gains	whole chromosomes		Amp
			losses	gains	
13449		chr18:6616549-14061522 chr22:25219564-qter			
	chr5:27707626-29453179 chr9:20729123-27028948*		chr10	chr7	Chr4:54076876-55185567 chr7:53763279-56085787 chr7:53763279-56085787
13455		chr12:24458950-28474060			chr12:56095723-56963223
13682 (4n) mixed	chr1:57935204-qter	chr2:pter-26889482	chr10	chr15	chr1:202560013-203488282
	chr3:150537582-188605730	chr2:46090545-94914685	chr13		chr1:241544532-243005121
	chr4:59259221-qter	chr7:57824224-106345700	chr22		chr7:544464822-55936759
	chr7:pter-54575122	chr7:123252578-qter			chr12:58237233-58315221
	chr9:pter-43470441	chr9:43493496-qter			chr12:61309925-61326865
	chr9:21750254-24123646*	chr11:111647465-qter			chr12:61709259-61814976
	chr11:24065510-42207074	chr16:5466493-33847701			chr12:65102768-65367426
	chr12:49054606-qter	chr17:43389246-qter			chr12:66195382-66205238
	chr16:34060174-qter	chr19:32651846-qter			chr12:67388336-67561465
	chr17:5477022-6017765				chr12:67957425-68050635
	chr17:24393508-26550868*				chr12:68893334-69027002
	chr18:53387340-qter				
	chr20:43044754-qter				
13698 (2n)	chr1:pter-31690408		Chr:10	Chr:7	Chr7:54286784-55449355
	chr3:17342361-18938756				
	chr3:192324064-194687195				
	chr6:1001155-7196450				
	chr6:27222001-28315342 chr6:32710247-33153536				

ID	segmental losses	gains	whole chromosomes			Amp
			losses	gains		
13747 (2n)	<i>chr6:53615325-151341489</i>					
	<i>chr6:160019250-qter</i>					
	<i>chr9:21669152-22370211*</i>					
	<i>chr9:pter-38283902</i>					
	<i>chr11:pter-2397565</i>					
	<i>chr11:6481132-10053592</i>					
	<i>chr13:pter-54967126</i>					
	<i>chr13:57991463-62699886</i>					
	<i>chr13:68428665-70519125</i>					
	<i>chr13:89767419-92975450</i>					
	<i>chr13:112964990-qter</i>					
	<i>chr18:675574-25344247</i>					
	<i>chr18:35895685-38068767</i>					
13751 (4n)	<i>chr18:41756188-qter</i>					<i>chr7:54153661-54189336</i>
	<i>Chr19:352222775-qter</i>					<i>chr7:54696012-55673060</i>
	<i>chr22:17672446-qter</i>					<i>chr18:54716681-54917277</i>
14803 (4n)	<i>chr1:35212692-53653842</i>		<i>chr9</i> <i>chr10</i>	<i>chr7</i>		
			<i>chr10</i>	<i>chr2</i>		
	<i>chr3:17477922-18427543</i>		<i>chr10</i> <i>chr22</i>	<i>chr7</i> <i>chr20 4n</i>		
	<i>chr5:18064429-34895166</i>					
	<i>chr8:1855820-6986630</i>					
	<i>chr9:pter-31702257</i>					
	<i>chr12:5167939-8501490</i>					
	<i>chr12:10228644-17887500</i>					

ID	segmental losses	gains	whole chromosomes			Amp
			losses	gains	losses	
14948 (4n)	chr12:36768810-45729804					
	chr15:34477531-qter					
	chr19:50625333-57647938					
14948 (4n)	chr9:20265354-29234767	chr7:56161137-qter				chr7:54899473-56085787
		chr15:30231488-32447708				
15009 (2n)	chr1:pter-97814306	chr4:3165827-6933913			chr22	
	chr4:pter-3140220	chr4:15857774-32975430				
	chr4:6934988-15856952	chr4:37763190-66437855				
	chr4:32980408-37755689	chr8:63450713-69892366				
	chr4:66439872-qter	chr8:104988652-140810733				
	chr8:69910570-104964436	chr9:70312803-qter				
	chr8:140846433-qter	chr12:pter-235550				
	chr9:pter-23178152	chr12:36671779-40784667				
	chr11:pter-12518710					
	chr12:13632338-17028250					
	chr12:41055979-88695569					
	chr13:35585732-69100801					
15343 (2n)	chr2:137312890-142832335	chr17:71158961-qter			chr7	
	chr4:79292674-83923477	chr19:pter-10698677			chr20	
	chr5:70867397-72174208	chr19:18174515-46899465				
	chr6:66875325-qter					
	chr9:pter-27505967					
	chr9:20641453-23083611*					
	chr10:26074076-qter					
	chr14:29541738-qter					
	Chr17:pter-71158961					
	chr18:16935291-23662171					

ID	segmental losses	gains	whole chromosomes			Amp
			losses	gains		
	Chr19:10698677-18174515 chr19:53082236-56024482 chr19:53883612-55061878*					
15702 (2n)	chr1:pter-30861554 chr1:43811424-143261286 chr9:22319058-27743785 chr9:21087510-22318194*	chr1:14582452-qter chr20:50460887-qter	chr13 chr14 chr15			

Regions highlighted in red show LOH associated with copy number gains and those in blue are associated with CNN.
Regions highlighted in bold (*) represent homozygously deleted regions.