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Genomic profiling of atypical meningiomas associates gain of 1q with poor clinical outcome

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Abstract

Atypical meningiomas exhibit heterogeneous clinical outcomes. It is unclear which atypical meningiomas require aggressive multimodality treatment with surgery and radiation therapy versus surgery alone to prevent recurrence. Detailed molecular-genetic characterization of these neoplasms is necessary to better understand their pathogenesis and to identify genetic markers. Oligonucleotide array comparative genomic hybridization was used to identify frequent genetic alterations in 47 primary atypical meningiomas resected at Massachusetts General Hospital between August 1987 and September 2006. Eighty five percent of samples exhibited loss of 22q, including the *NF2* gene. The second most frequent regions of loss were confined to the short arm of chromosome 1, particularly 1p33-p36.2 (70%) and 1p13.2 (64%). Other frequent regions of loss, detected in more than 50% of samples, included 14q, 10q, 8q, 7p, 21q, 19, 9q34, and 4p16. Frequent regions of gain were detected along 1q (59%), 17q (44%), 9q34 (30%) and 7q36 (26%). Univariate marker-by-marker analysis of all frequently identified copy number alterations showed potential correlation between gain of 1q and shorter progression free survival. Given the heterogeneous treatment outcomes of atypical meningioma, investigation of large-scale and focal genomic alterations in multi-institutional efforts may help clarify molecular-genetic signatures of clinical utility.

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Authors' contributions. DG participated in the study design, reviewed patient charts, collected clinical information, set up the clinical data base, collected samples and isolated DNA. DE and RAB participated in statistical analyses, SG carried out bioinformatic analyses, GAS participated in DNA isolation, probe preparations and microarray hybridizations, FGB and JSL participated in study design and provided clinical input, DNL conceived of the study, participated in study design, provided clinical and histopathological input. GM participated in study design, performed microarray hybridizations, carried out data analysis and drafted the manuscript. All authors contributed to the edits and approved the final manuscript.

Keywords

atypical meningioma; array comparative genomic hybridization

Introduction

Meningiomas comprise up to 30% of intracranial neoplasms (1–3). Approximately 10–40% of meningiomas correspond to atypical (WHO grade II) and anaplastic (grade III) subtypes, which are associated with less favorable clinical outcomes (4–6). These non-benign subtypes have been associated with two-fold increased relative risk of local failure and four-fold relative excess risk of death (7,8). Among them, the atypical subtype (grade II) comprises up to 35% of meningiomas (3,4,9,10). By the World Health Organization (WHO) criteria, grade II atypical meningiomas exhibit four or more mitotic figures per ten high-power fields or have at least three other histologic features associated with higher grade (architectural sheeting, necrosis, prominent nucleoli, hypercellularity and high nuclear:cytoplasmic ratio). WHO grade III malignant meningiomas exhibit frank histological malignancy or twenty mitotic figures per 10 high-power fields. According to the 2000 and 2007 WHO classifications, brain invasion does not necessarily imply WHO grade III meningioma; in the absence of frank anaplasia, meningiomas exhibiting brain invasion behave most like atypical meningiomas (11) and correspond to WHO grade II (3). These changes in the WHO grading systems have resulted in increased diagnosis of atypical meningioma (4,9,11,12). For example, upon re-assigning grade based on WHO 2000 criteria versus WHO 1993 criteria, 35% of previously diagnosed grade III meningiomas were re-assigned to grade II whereas few original grade II meningiomas were re-assigned (13). Hence, understanding the clinical significance of the diagnosis of atypical meningioma is assuming increasing importance.

Primary treatment for meningiomas consists of maximal safe surgical resection. Although outcomes for benign meningiomas after surgery are favorable and those for the rarer anaplastic (malignant; grade III) subtype are uniformly poor, outcomes for atypical meningiomas are variable (1,2,10). Progression-free survival (PFS) at 5 years after definitive treatment has been reported to be 20%–50% when atypical and anaplastic meningiomas are combined and 38%–62% when atypical meningioma is evaluated alone (1,2,14). In addition, atypical meningioma is associated with 57% cause-specific survival at 15 years, compared to 86% for benign meningioma (15). But, in many cases, the significant individual variability in clinical behavior of atypical meningiomas cannot be accounted for by known clinical or pathological variables. Given the broad range of reported outcomes after radical surgery (1,16) it remains unclear which atypical meningiomas will recur and which patients will benefit from additional treatment options such as adjuvant postoperative radiation therapy. We reasoned that detailed molecular-genetic characterization of these neoplasms could provide a better understanding of their pathogenesis and potentially reveal prognostic and therapeutic markers. In turn, such knowledge could further help guide clinical management and decrease inappropriate treatment, thereby minimizing treatment failure and reducing treatment-related toxicity. We therefore undertook a comprehensive genomic analysis of a series of atypical meningiomas.

Meningiomas are among the most studied human solid tumors by karyotype analysis. Among characterized genetic alterations, loss of an entire chromosome 22 is commonly reported in meningiomas and was among the first recurring cytogenetic alterations ever described in human solid tumors (17). Alteration of the neurofibromatosis type 2 gene (*NF2*) on 22q12 is associated with 30–60% of all sporadic meningiomas (18). Notably, however, relatively few studies have focused specifically on the genetic characteristics of atypical meningiomas. In addition to loss on 22q, loss of 1p is a second common chromosomal abnormality in atypical meningioma (19–22). Two main target regions on 1p36 and 1p32-p34 have been identified

based on high resolution microsatellite markers (23). The putative 8.2 megabase minimal region of deletion on 1p36 includes TP73, CDKN2C, RAD54L and ALPL as potential candidate genes (24–28). The region of deletion on 1p33-34 has been further narrowed to 2.8 megabases using *in silico* sequence analysis and deletion mapping with microsatellite markers (29,30). Other regions of chromosomal alteration reported in atypical meningioma include losses of 6q, 10, 14q, 18q and gains of 1q, 9q, 12q, 15q, 17q and 20q (19,22,31,32).

Array-based comparative genomic hybridization (aCGH) detects DNA copy number changes and provides a global assessment of molecular events in the genome (33). Multiple studies using chromosomal CGH have been reported in the meningioma literature (19,22,32,34). However, these studies used fewer samples, lack the improved resolution of aCGH, have not elucidated specific genes or loci associated with chromosomal changes and have not specifically studied large, carefully annotated series of atypical meningioma. Array CGH data can be integrated with underlying genome annotations, allowing identification of associations between clinical parameters, such as progression and death, and candidate tumor suppressor or oncogene loci. The potential clinical utility of aCGH-based studies is maximized with inclusion of tumor samples from patients with substantial clinical follow-up. To improve our understanding of meningioma genetics and to identify potentially useful prognostic markers for use in the setting of atypical meningioma, we studied a large series of atypical meningiomas using a comprehensive aCGH approach.

Materials and Methods

Tumor Samples and Clinical Data

The inclusion criteria for the study were: 1) diagnosis of atypical meningioma on primary resection; 2) frozen tissue in the brain tumor repository, and 3) at least 6 months of clinical follow-up. Exclusion criteria included history of prior brain irradiation and age < 18 years. The Massachusetts General Hospital Brain Tumor Repository contained fresh frozen tumor specimens from 85 cases of atypical meningioma treated surgically between August 1987 and September 2006. Histopathologic diagnosis of atypical meningioma was made by neuropathologists on original paraffin-embedded surgical specimens using WHO criteria: four or more mitotic figures per ten high-power fields; or at least three of five other histologic features (architectural sheeting, necrosis, prominent nucleoli, hypercellularity and high nuclear:cytoplasmic ratio) (35). Presence of atypical meningioma within the banked tissue was confirmed by an independent pathologist using hematoxylin-and-eosin stains of the frozen material. Informed consent for use of tissue was obtained from each patient at the time of resection. Medical records of each patient were reviewed for demographic information, tumor characteristics, treatment details, tumor progression and death under a protocol approved by the Institutional Review Board. Thirty-eight cases were excluded for the following reasons: recurrent tumor (23 cases), history of prior brain irradiation (8 cases), lack of follow-up (6 cases), age < 18 years (1 case). The study included the remaining 47 cases of primary atypical meningioma from the tumor bank (with at least 6 months clinical follow-up). Of the 47 cases, 25 (53%) were men and 22 (47%) were women with a median age at diagnosis of 59 years (range 31–90). The diagnosis of atypical meningioma was confirmed by review of hematoxylin and eosin-stained sections from formalin-fixed, paraffin-embedded tissue sections from each case. Only samples with 80–90% tumor cells were used for DNA extraction. All samples were anonymized and a database with detailed clinical follow-up information was created using File Maker Pro. Radiographic PFS was measured from the date of primary surgery until the date of first documented radiographic recurrence of tumor after gross total resection or growth of residual disease after subtotal resection or death, whichever occurred first. The median radiographic follow-up was 29 months (95% CI 24–55 months). Radiographic progression was

seen in 13 patients. The estimated median PFS period for all patients was 56 months (95% CI 35 months-not estimable).

aCGH

Genomic DNA was isolated from 47 primary atypical meningioma samples and from normal whole blood from 10 anonymous donors using routine protocol. Array CGH was performed to determine DNA copy number changes using Agilent Human 105K oligonucleotide microarrays following the manufacturer's instructions (http://www.home.agilent.com/agilent/home.jspx). Genomic coordinates for this array are based on the NCBI build 36, March 2006 freeze of the assembled human genome (UCSC hg18), available through the UCSC Genome Browser. This array includes a comprehensive probe coverage spanning both coding and non-coding regions, with emphasis on well known genes, promoters, micro RNAs, and telomeric regions and provides an average spatial resolution of 21.7 kb. For array hybridizations, 5 μg each of tumor and normal DNA were digested with Dpn II for 3 hours at 37°C and purified with PureLink PCR purification columns (Invitrogen). Purified tumor and normal DNA, 1 μg each, were labeled with Cy3-dCTP and Cy5-dCTP, respectively, using bioprime labeling kit (Invitrogen) following the manufacturer's instructions. Unincorporated nucleotides were removed using Sephadex G-50 columns. Labeled tumor and reference samples were precipitated with 100 μg of human Cot-1 DNA and resuspended in 250 μl of hybridization buffer provided in the Agilent oligonucleotide array CGH kit. Prior to hybridization the probe mixtures were denatured for 5 minutes at 95°C and incubated at 37°C for 30 minutes. The samples were then hybridized onto the oligonucleotide array in the Agilent SureHyb microarray hybridization chamber and the hybridization was carried out for 42 hours at 65°C. Arrays were then disassembled and washed as recommended by the manufacturer. Array slides were scanned in Axon 4000B microarray scanner using GenePix Pro 4.0 software. Microarray images were analyzed and data points were generated using the Feature Extraction software (version 9.1, Agilent Technologies) with linear normalization (protocol-v4_91). Data were subsequently imported into CGH Analytics software (version 3.4.40, Agilent Technologies) for visualization.

aCGH data analysis methods

DNA copy number alteration (CNA) was identified through dynamic thresholding of segmented aCGH data. First, circular binary segmentation (CBS) (36) was used to segment each hybridization into regions of common mean. Second, for each hybridization, the scaled median absolute deviation (MAD) across all segments was obtained. A default scaling factor of 1.48 was utilized. Finally, probes assigned to segments with mean value greater than 0.75 MADs were identified as gain. Likewise, probes corresponding to segments with mean value less than 0.75 MADs were identified as loss. Segment classification and choice of thresholds was based on the approach of Korkola et al. (2008) (37).

Statistical analysis

To identify minimal regions of common alteration across all hybridizations, the Genomic Identification of Significant Targets in Cancer (GISTIC) approach was utilized (38). Under this approach, regions from among the CBS identified segments that are aberrant more often than would be expected by chance are identified, with greater weight given to high amplitude events. GISTIC assigns each region of the genome two G-scores (Figure 2), each representing the combined frequency and amplitude of either losses or gains. It then compares these scores with similar scores generated from random permutations of the data to determine false discovery rate q-values (39), representing the likelihood of obtaining the observed G-scores from chance events alone. This approach has been shown to be more likely to identify regions highly associated with cancer pathogenesis (38). Threshold selection for the GISTIC procedure

was based, conservatively, on the maximum threshold for alteration (across all hybridizations) identified under the MAD approach described above; 0.4 was selected as the gain and loss threshold and 0.25 was selected as the significance threshold. Each analyzed CBS segment consisted of at least four markers. Segments that contained fewer than four markers were combined with the adjacent segment closest in segment value. A q-value was then obtained for each region. Each peak (i.e., region associated with a low q-value) was tested to determine whether the signal was primarily due to broad events, focal events or overlapping events of both types.

In an additional attempt to assess common patterns across hybridizations, unsupervised hierarchical clustering of samples was also conducted. Following segmentation and classification, data were further reduced, with out compromising the continuity and breakpoints, to facilitate downstream analyses (40). The log2 ratios from this reduced dataset were then clustered using the Cluster and Treeview program (41). The distance matrix was computed using the Pearson distance and clustered using gplots module within Bioconductor (http://www.r-project.org/).

To assess association of DNA copy number alteration with radiographic progression free survival in primary tumors, univariate marker-by-marker analyses were conducted under the Cox proportional hazards model. The effects of gain and loss were assessed separately. That is, for the analysis evaluating the effect of gains (losses), each marker was classified as either gain or no-gain (loss or no-loss). Assignment of each marker was made according to the CBS-MAD classification (described above) of the CBS segment to which the given marker belonged. Only markers identified as potentially informative were utilized in each analysis. Potentially informative markers were defined as those with some variation in gain or loss across all hybridizations; it was assumed that markers with nearly uniform gain or loss (or absence thereof) across all hybridizations were not potentially informative with regard to progression. Hence, markers whose percentage of gain/loss across all hybridizations exceeded 90% or was less than 10% were omitted from the analysis. To adjust for multiple testing via the false discovery rate, the q-value (distinct from the q-value obtained under the GISTIC analysis described above) for each test was obtained (42).

Results

Patient and tumor characteristics

Between 1987 and 2006, atypical meningiomas comprised 20.9% (171/817) of all surgically resected meningiomas collected in the MGH brain tumor repository. The percentage of atypical meningiomas increased over time, corresponding with refinements in meningioma grading, including WHO classifications in 1993 and 2000: 1987–1993: 17.6% (56/318), 1994–2000: 22.1% (64/290), 2001–2006: 24.4% (51/209). Fifty percent of the atypical meningiomas collected between 1987–2006 had frozen tissue available for evaluation (n=85). The 47 atypical meningioma cases included in this study consisted of 25 males (53%) and 22 females (47%) (Table 1). Ninety percent of patients (42/47) were Caucasian. Median age at diagnosis was 59 years (range, 31–90 years). Seventy-four percent (34/46) of patients had no or only minor comorbidities (comorbidity score 0, 1). Twenty-six percent (12/46) of patients had multiple/major comorbidities (comorbidity scores >1). Information about tumor size was available in the neuroradiology or operative reports for 41/47 cases. Median tumor diameter was 5.5 cm (range, 2–11.6 cm) based on these reports. The majority of tumors were located in the convexity (28%) and parasagittal (28%) regions. Other regions of involvement included the falx (17%), sphenoid (15%), skull base (6%), olfactory groove (2%), orbit (2%) and cerebellopontine angle (CPA; 2%). Gross total resection (GTR) was achieved in 74% of cases (35/47), compared with 26% (12/47) subtotal resection (STR). Bone involvement was documented in 32% of cases (15/47). Radiographic evidence of bone involvement included hyperostosis, bone sclerosis

and/or osteolytic lesions. Intraoperative evidence of bone involvement included hyperostosis, extension of tumor mass into bone, and/or bony destruction. Bone samples were sent for histopathologic evaluation in 8/15 cases of bone involvement, with 100% concordance. Brain invasion was noted in only 4% (2/47).

Global DNA copy number alterations

Genomic copy number for each of over 99,000 probes was determined by calculating the log2 ratio of median signal intensities of the tumor and normal reference DNAs. A genome-wide view of the DNA copy-number alterations (CNAs) in 47 primary atypical meningiomas is shown in Figure 1. Most chromosomal arms undergo either loss or gain across a large proportion of the samples, suggesting high degree aneuploidy in the atypical meningioma genomes (Figure 1). Extensive erosion of telomeric regions was frequently observed for many chromosomes. There were no high level amplification or homozygous deletion events detected in this set of tumors. In order to identify consistent regions of copy-number alterations associated with gains and losses, and to identify minimal regions of loss and gain, we applied the statistical method GISTIC to the data set (figure 2). GISTIC identified 65 regions of losses along 35 chromosome arms (Figure 2a) and 5 regions of gains along 4 chromosome arms (Figure 2b) distributed throughout the genome. Several chromosome arms had more than one minimal region of loss or gain. For each alteration we selected the peak region (ie, the highest frequency and amplitude of events) as the region most likely to contain a cancer gene. Several tumor suppressor genes previously shown to have copy number changes in meningiomas, such as *TP73*, *NDRG2*, and *NF2*, were readily identified by GISTIC. Chromosomal locations, frequencies, genomic intervals, gene contents and candidate cancer genes of these changes are listed in Table 2. The size of deletions ranged from 400kb to 3 Mb and the number of genes mapping to these regions ranged from 1–137 respectively. There were only 5 regions of gains identified with the number of genes ranging from 2–42. Genes with known or possible function in cancer are listed in Table 2.

The most common genomic alteration was loss of 22q including the *NF2* gene (85% of samples). The second most frequent regions of loss were confined to the short arm of chromosome 1. Most cases displayed copy number alteration patterns consistent with complex structural rearrangements involving both arms. Two distinct regions of loss along 1p included 1p33-p36.2 (70%) and 1p13.2 (64%). Both regions spanned several megabases of DNA sequences and included 134 genes (Table 2). Other frequent regions of loss, detected in more than 50% of samples, included 14q (68%), 10q (55%), 8q (55%), 7p (54%), 21q (51%), 19 (51%), 9q34 (51%), and 4p16 (51%). Frequent gains were detected along 1q (59%), 17q (44%), 9q34 (30%) and 7q36 (26%).

Cluster analysis

Figure 3a illustrates the two groups that resulted from the unsupervised hierarchical clustering of unfiltered aCGH data described above. Results of the hierarchical clustering are presented in Figures 3a and 3b. In Figure 3a, rows correspond to probes and columns correspond to subjects. Gain of 1q was much more common in cluster 2, shown on the right in Figure 3a (25 of 30 cases), than in cluster 1 (3 of 17 cases). Loss of 18q12.3-q21.31 in 7 of 17 in cluster 1, compared with 3 of 30 cases in cluster 2. The known tumor suppressor gene *DCC* (deleted in colorectal carcinoma) maps to the minimal region of 18q deletion. This distinction between cluster 1 and cluster 2 is further displayed in Figure 3b which consists of a heatmap based on the distance matrix underlying the hierarchical clustering. Along the left vertical axis, red indicates 18q loss. Along the upper horizontal axis, red indicates 1q gain. The heatmap shows two large clusters associated with gain of 1q and loss of 18q (Fig 3b). This finding confirms the mutual exclusivity of these two alterations in atypical meningiomas.

Correlation with clinical outcome

To evaluate relationships between DNA copy number alterations and clinical end points, such as PFS, we chose to perform univariate marker-by-marker analysis for all probes showing gain or loss throughout the genome. On univariate marker-by-marker analysis, multiple markers with DNA copy number gains on 1q were found to be significantly associated with shorter PFS ($p < 0.05$). Figure 3c shows marker-by-marker p-value (red) and q-value (blue) results for copy number gains across all chromosome arms for potentially informative markers. Correlation between loss on 18q and longer PFS was also investigated. However, due to the small number of observed PFS events, no statistically significant association was identified for 18q loss.

Minimal region of 1q gain

GISTIC approach identified two regions of frequent CNAs along 1q. The first one maps to 1q25.1 and the peak region (chr1:172161698–172889501) spans 727 kilobases of DNA sequence, includes 30 probes and 2 genes (Table 2). The second peak region (chr1:182,779,475–197,636,068) of gain along 1q25.3-q32.1 spans 1.48 megabases of DNA sequence, includes 75 markers and 42 genes (Table 2).

Discussion

In this study, we profiled a cohort of 47 clinically annotated primary atypical meningiomas using high resolution oligonucleotide aCGH. The goals of this study were to generate a high resolution genomic map that would allow identification of genes targeted by frequent loss and gain of DNA sequences, and to develop prognostic markers for atypical meningioma risk stratification. We analyzed data obtained by aCGH to explore the relationship between CNAs and PFS. As a result of this analysis, we found a significant correlation between the gain of 1q and PFS.

Eighty-five percent of atypical meningiomas exhibited loss of 22q, including the *NF2* gene. The second most frequent regions of loss were confined to the short arm of chromosome 1, particularly 1p33-p36.2 (70%) and 1p13.2 (64%). Other common regions of loss, detected in more than 50% of samples, included 14q, 10q, 8q, 7p, 21q, 19, 9q34, and 4p16. Frequent regions of gain were detected along 1q, 17q, 9q34 and 7q36. Unsupervised clustering of aCGH data revealed two groups of atypical meningioma: one group was composed of the majority of cases with 1q gain and the other group was composed of the majority of the cases with 18q loss (Fig 3a, b). Loss of genetic information from 1p may represent early progression-associated changes in the transition from benign to atypical meningioma (39). Analysis of the data in the current study revealed focal regions of frequent loss on 1p36.32 (70%) and 1p13.2 (64%). The *TP73* gene, encoding a p53-related protein, is located at 1p36.2 and may represent a candidate gene warranting further investigation for its potential role in atypical meningioma tumorigenesis. Other studies of atypical meningioma using loss of heterozygosity and/or comparative genomic hybridization analysis have reported loss on 1p in 40%–86% (21, 22, 32, 43) of cases.

Evaluation of chromosome 1 copy number imbalances using a genomic tiling path method of aCGH on 82 meningiomas revealed frequent, aberrations of chromosome 1 of varying type (deletions and amplifications), size (monosomy and homozygous deletion) and distribution (44). Deletion on 1p was the most frequent alteration, ranging from whole p-arm deletion (120 MB) to homozygous loss (3.5 Mb). Three distinct candidate loci on 1p and one locus on 1q exhibited tumor-specific aberrations of potential importance for tumorigenesis. Aberrations of 1q were less common and were always accompanied by full or partial loss of 1p. Elsewhere in the literature, gains on 1q have been shown to occur in 30%–50% of meningioma cases (19,22). Our data revealed chromosomal gains on 1q in 59% of atypical meningioma cases.

The 1q gain centered on two regions: 1q25.1 (chr1:172,161,698-172,889,501) and 1q25.3-q32.1 (chr1:182,779,475-197,636,068). The 1q25.1 interval includes two genes: *RC3H1* (roquin) encodes a highly conserved member of the RING type ubiquitin ligase protein family and *RABGAP1L* (RAB GTPase activating protein 1-like isoform A) encodes a 298-amino acid GTPase-activating protein, containing a putative phosphotyrosine binding domain (45). The 1q25.3-q32.1 interval includes 42 genes, some of which may have relevance to tumorigenesis: *EDEM3* (ER degradation enhancer, mannosidase alpha like), which ensures that only properly folded proteins are retained in the cell; *RNF2* (ring finger protein 2), a polycomb protein, part of the multiprotein complexes involved in transcriptional control; *PRG4* (proteoglycan 4), a large proteoglycan synthesized by chondrocytes; *TPR* (nuclear pore complex associated protein), which encodes a large coiled-coil protein that is required for the nuclear export of mRNAs and some proteins, with oncogenic fusions of the 5′ end of this gene with several different kinase genes occuring in some tumors; *PTGS2* (prostaglandin endoperoxidase synthase 2 precursor), a key enzyme in prostaglandin biosynthesis, the expression of which is deregulated in epithelial tumors; *CDC73* (parafibromin), a member of the PAF protein complex, which associates with the RNA polymerase II subunit POLR2A and with a histone methyltransferase complex (46); *ASPM* (abnormal spindle)-like, microcephaly), a human ortholog of the *Drosophila* ‘abnormal spindle’ gene, which is essential for normal mitotic spindle function in embryonic neuroblasts (47); *LHX9* (Homo sapiens LIM homeobox 9), which encodes a member of the LIM homeobox gene family of developmentally expressed transcription factors; *NEK7* (Homo sapiens NIMA (never in mitosis gene a)-related kinase 7), which shares high amino acid sequence identity with the gene product of the Aspergillus nidulans ‘never in mitosis A’ gene and which controls initiation of mitosis. The potential functional relevance of these genes in the progression of atypical meningioma is not known and will require further investigation.

There is a paucity of data evaluating the clinical significance of genomic abnormalities in atypical meningioma. To our knowledge, this is the first comprehensive genomic analysis of a large clinically-annotated cohort of atypical meningiomas. Previous studies have focused on genetic progression of meningiomas and relatively small numbers of atypical meningiomas have been included (22,32). The overall pattern of losses and gains detected in our study is consistent with what has been reported in the general meningioma literature. However, the increased resolution and the relatively large sample of atypical meningiomas allowed us to identify novel large-scale and focal chromosomal alterations. Most importantly, each case evaluated in the current study was fully annotated in a clinical database with information regarding treatment course and clinical endpoints. This provided the opportunity to evaluate for potential clinically relevant genomic markers.

Analysis of all frequently identified CNAs showed a significant correlation between gain of 1q and shorter PFS ($p<0.05$). Gain of 1q has previously been associated with increased recurrence in neuroblastoma, Wilms’ tumor and ependymoma (48–50) and reduced overall survival in multiple myeloma, medulloblastoma and Ewing sarcoma (51–53). To our knowledge this is the first study to correlate chromosome 1q gain with clinical endpoints in atypical meningioma.

Although an association of 1q gain with shorter PFS was evident in our series of tumors and although the sample size was large relative to other atypical meningioma investigations, the study remained under-powered to reveal other clinical associations of identified CNAs. Clarification of molecular-genetic signatures useful in atypical meningioma diagnosis and treatment will therefore require investigational efforts on even larger clinically annotated series. Given the increasing diagnosis and heterogeneous treatment outcomes of atypical meningioma, such efforts are warranted and should become a priority to guide clinical

management, decrease inappropriate treatment, minimize treatment failure and reduce undue treatment-related toxicity in this disease.

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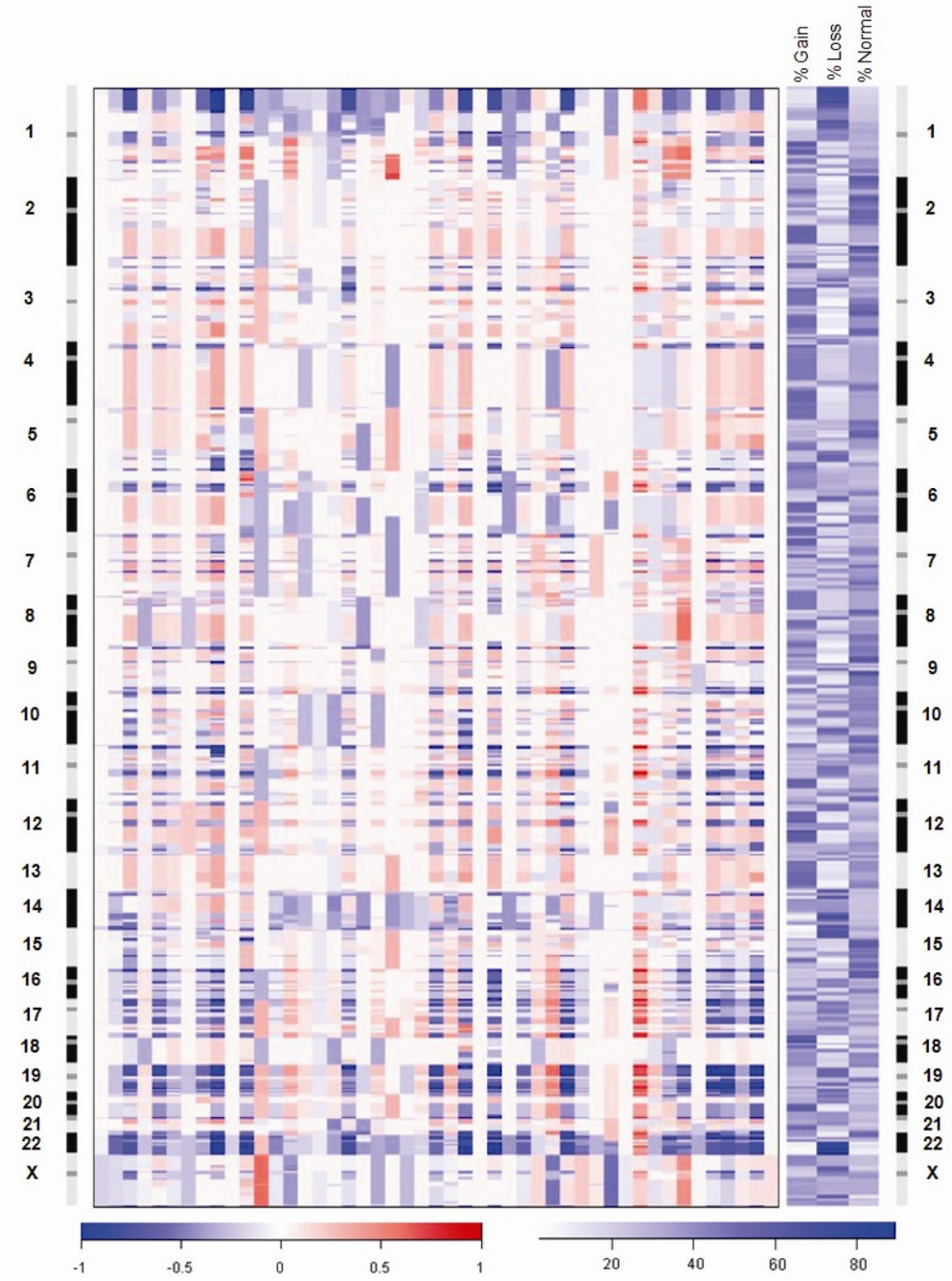

Figure 1.

Genomic profiles of 47 primary atypical meningiomas generated by oligonucleotide array CGH. Each column in the left panel represents a tumor sample and rows represent losses and gains of DNA sequences along the length of chromosomes 1 through X as determined by the segmentation analysis of normalized log2 ratios. The color scale ranges from blue (loss) through white (two copies) to red (gain). The right panel indicates the frequencies of gain and loss of oligonucleotide probes on a probe-by-probe basis for all autosomes and the X chromosome. The color scale ranges from white (no changes) to blue (frequent changes). Loss of 22q12.2 including the *NF2* gene was the most frequent alteration observed in 85% of the atypical meningiomas.

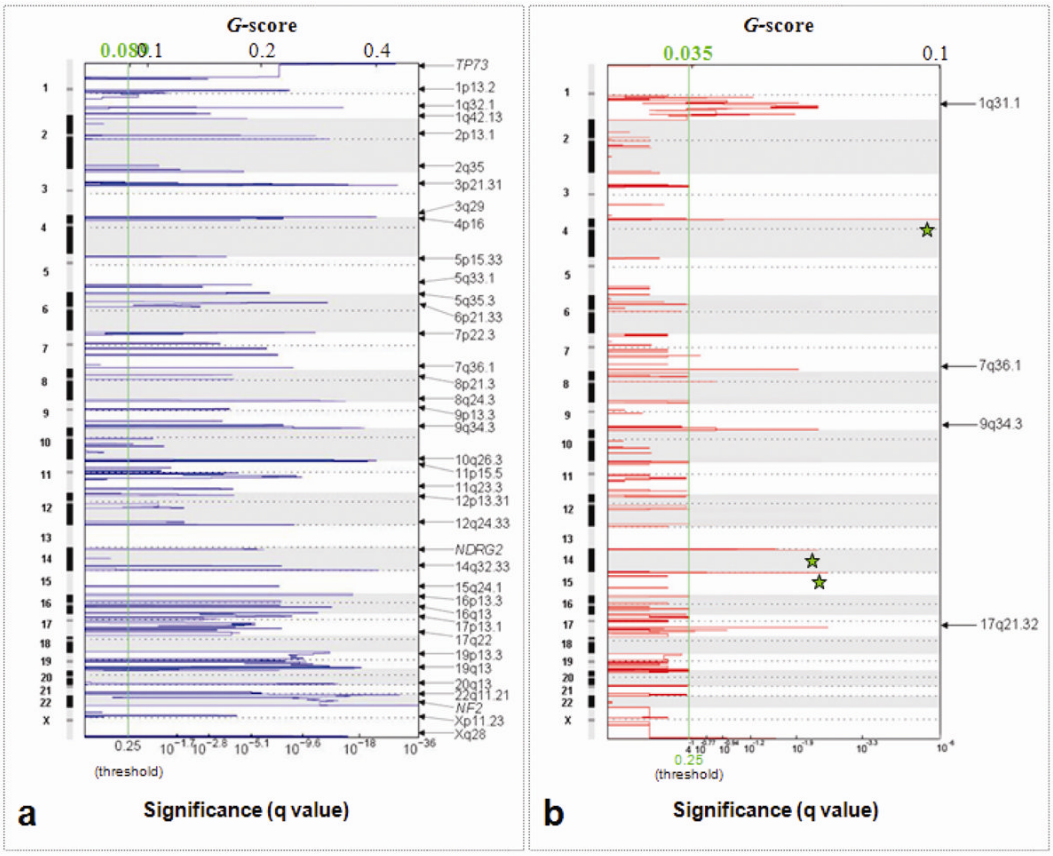

Figure 2.

GISTIC analysis of copy number alterations in atypical meningiomas. The statistical significance of the aberrations identified by GISTIC are displayed as false discovery rate q values to account for multiple hypothesis testing (q values; green line is 0.25 cut-off for significance). Scores for each alteration are plotted along x axis and the genomic positions are plotted along y axis; dotted lines indicate the centromeres. **a)** Loss of both broad and focal regions are identified by GISTIC (copy number threshold = log2 ratio ≤ 0.4 for broad and ≤ 0.1 for focal events). Sixty five broad regions of losses were identified. Three focal events indicated by the gene names were identified in the background of broad regions. **b)** GISTIC reveals five broad regions of gain (copy number threshold = log2 ratio ≥ 0.4). Green stars indicate known or presumed copy number polymorphisms.

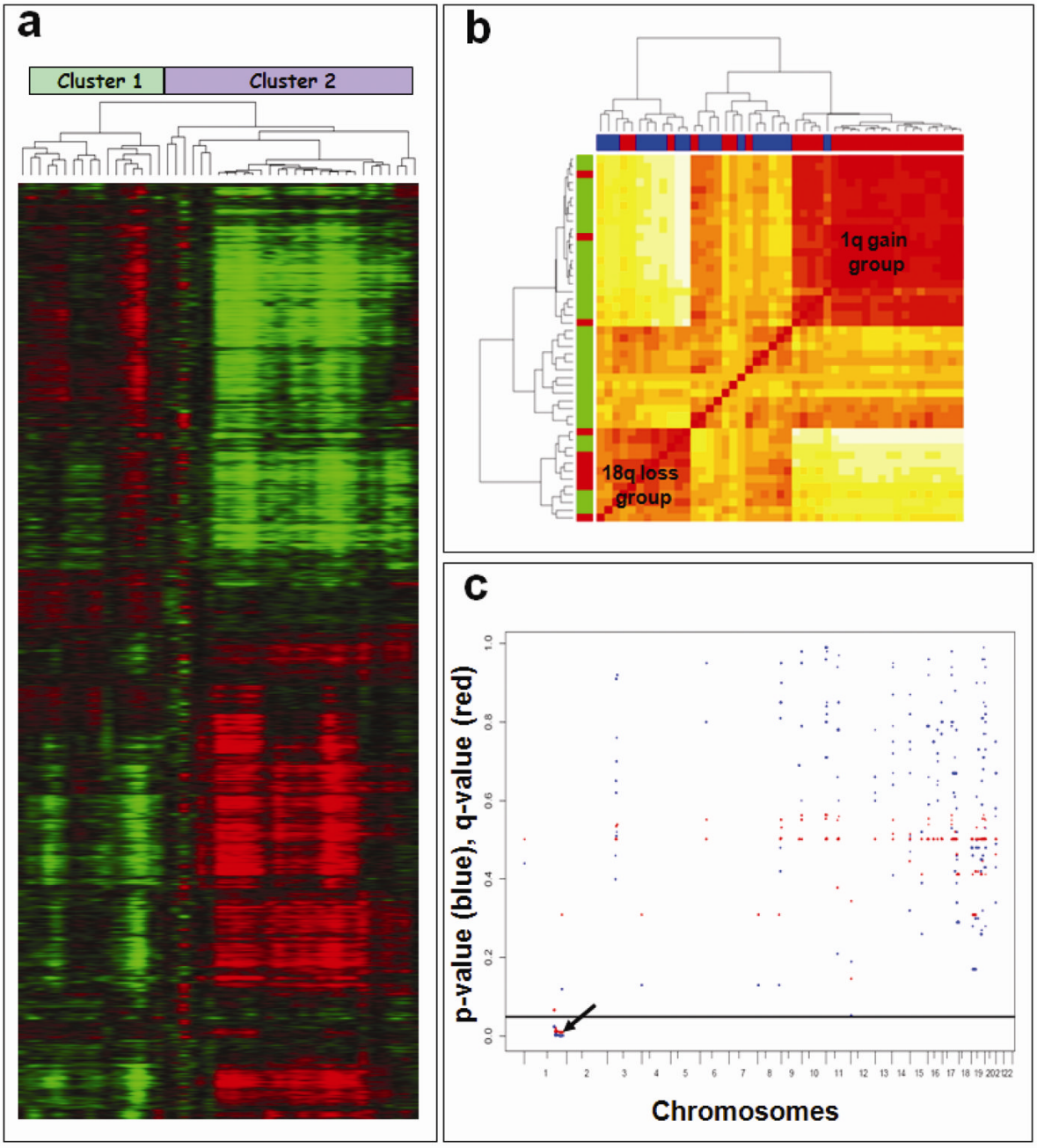

Figure 3.

Cluster analysis of copy number alterations in atypical meningiomas. **a)** Unsupervised hierarchical clustering of CGHsmooth transformed dataset derived from 47 primary atypical meningiomas. Copy number values are color coded as follows: green (loss), black (normal) and red (gain). The pattern of dendrogram suggests two major genomic subgroups of atypical meningiomas. **b)** The distance matrix of all copy number alterations included in the heatmap (Pearson distance). Majority of the samples with 1q gain (indicated in Red on the horizontal side bar) and 18q loss (indicated in Red on the vertical side bar) cluster together. **c)** Marker-by-marker assessment of DNA copy number gains reveals association between 1q gain and shorter progression-free survival. P-values (Cox model) are denoted in red. Q-values are

denoted in blue. The horizontal black line at 0.05 denotes the chosen significance level. Long tick marks along the bottom axis denote chromosome ends whereas short tick marks denote the centromere.

Table 1

Patient and tumor characteristics.

Characteristic	Number of patients
All	47
Gender	
Male	25 (53%)
Female	22 (47%)
Race	
Caucasion	42 (90%)
African American	1 (2%)
Asian	2 (4%)
Hispanic	2 (4%)
Median age	59 yrs (range, 31–90)
Comorbidity Score	
0, 1	34 (74%)
> 1	12 (26%)
Median tumor size	5.5 cm (range, 2 cm – 11.6 cm)
Tumor location	
Convexity	13 (28%)
Falx	8 (17%)
Sphenoid	7 (15%)
Skull base	3 (6%)
Parasagittal	13 (28%)
Olfactory groove	1 (2%)
Orbit	1 (2%)
CPA	1 (2%)
Resection	
GTR	35 (74%)
STR	12 (26%)
Brain invasion	
Yes	2 (4%)
No	45 (96%)
Bone involvement	
Yes	15 (32%)
No	32 (68%)

GTR = Simpson grade I-III. STR = Simpson grade IV.

Table 2

Broad and focal regions of losses and gains in atypical meningioma identified by GISTIC analysis.

Cytoband	Broad or focal	Peak region (Mb)	q value	Frequency (%)	Number of genes in peak	Known cancer genes
Copy Number Losses						
1p36.32	both	chr1:1810386-2847377(probes 67:111)	1.8236E-32	70	18	TP73
1p13.2	focal	chr1:111326775-111897939(probes 3987:4016)	1.55E-10	64	11	
1q32.1	focal	chr1:200252797-200463129(probes 6396:6404)	7.53E-16	40	5	
1q42.13	both	chr1:225643390-226726883(probes 7266:7322)	0.000485	40	19	
1q44	both	chr1:246911252-247249719(probes 8035:8044)	6.70E-07	49	6	
2q35	broad	chr2:216778698-220212213(probes 14809:14987)	0.00819	47	65	WNT6, CDK5R2
2q37.3	broad	chr2:238972240-242951149(probes 15638:15805)	1.63E-06	47	40	
2p13.3	both	chr2:71595156-72012567(probes 10400:10409)	0.0004362	21	1	
2p13.1	both	chr2:74536424-74606525(probes 10532:10542)	2.33E-10	32	5	
2q11.2	broad	chr2:96035700-96341316(probes 11018:11032)	2.32E-13	45	9	
3q29	broad	chr3:196924341-197049555(probes 22343:22348)	4.53E-22	40	2	
3p21.31	broad	chr3:50156214-50688743(probes 17620:17660)	4.13E-17	49	23	TUSC2&4, RASSF1
3p21.1	broad	chr3:51948378-52059380(probes 17723:17733)	1.04E-26	49	9	PARP3, PCBP4, DUSP7
4p16.3	broad	chr4:146101-2164782(probes 22466:22566)	4.16E-08	51	35	
4p16.1	broad	chr4:5508457-9468999(probes 22705:22864)	1.60E-05	51	35	
5p15.33	both	chr5:1034536-1570159(probes 28100:28126)	0.0011558	40	7	TERT
5q31.3	both	chr5:140995024-141299875(probes 32197:32210)	9.42E-05	33	5	PCDH1
5q33.1	both	chr5:148181565-150843781(probes 32452:32597)	0.0043584	34	35	PDGFRB, IL17B
5q35.3	broad	chr5:175886313-177743535(probes 33368:33458)	3.32E-06	40	39	FGFR4
6p21.33	broad	chr6:31654033-32398882(probes 34782:34856)	1.25E-13	55	55	
7q36.1	broad	chr7:150277900-150468342(probes 44309:44322)	2.27E-09	47	10	
7p13	broad	chr7:43911979-45251561(probes 40723:40789)	2.39E-05	45	26	
7q11.23	broad	chr7:71945710-76475359(probes 41411:41566)	4.47E-09	50	58	
7p22.3	broad	chr7:962086-1559228(probes 39157:39190)	6.43E-12	54	12	
7q22.1	broad	chr7:99327158-102122525(probes 42354:42510)	3.84E-09	52	65	
8q24.3	broad	chr8:145534920-145756763(probes 49163:49181)	1.01E-16	55	17	VPS28, FOXH1

Cytoband	Broad or focal	Peak region (Mb)	q value	Frequency (%)	Number of genes in peak	Known cancer genes
8p21.3	broad	chr8:22009601-22164198(probes 45357:45369)	0.0001077	43	9	
9q32	both	chr9:115818205-116113463(probes 52044:52060)	0.0003482	21	4	KIF12
9q34.11	broad	chr9:129185484-131549479(probes 52562:52702)	2.34E-08	47	60	CDK9, ENG
9q34.3	broad	chr9:138978944-139324935(probes 53067:53093)	1.29E-20	51	28	
9p13.3	broad	chr9:34231161-35777161(probes 50268:50363)	0.0001502	42	40	FANCG
10q26.3	broad	chr10:133846906-135145826(probes 57675:57746)	1.08E-14	55	23	INPP5A
11p15.5	broad	chr11:1-1515184(probes 57758:57844)	2.21E-23	49	49	HRAS
11q23.3	broad	chr11:118043425-119502992(probes 61928:62001)	9.65E-05	44	29	
11q12.1	broad	chr11:56850038-57193055(probes 59612:59634)	5.27E-05	36	12	
11q13.1	broad	chr11:66780417-67594725(probes 60196:60243)	2.66E-10	49	29	
12q24.33	broad	chr12:130850076-131736884(probes 67143:67185)	1.67E-09	47	9	
12p13.31	broad	chr12:6616267-6903061(probes 62836:62865)	8.56E-05	49	18	
14q32.33	broad	chr14:104589610-105050674(probes 73764:73792)	4.85E-24	65	10	JAG2, CRIP1 &2
14q11.2	both	chr14:23956846-24348799(probes 70746:70760)	9.88E-07	68	8	NDRG2
14q32.12	both	chr14:90665049-90858909(probes 73154:73162)	3.26E-08	68	3	
15q24.1	broad	chr15:72051988-73168030(probes 76042:76114)	5.47E-08	40	26	PML
16p13.3	broad	chr16:1-3105058(probes 77207:77422)	4.80E-18	51	137	TSC2, SOX8
16p11.2	broad	chr16:27341930-31408441(probes 78244:78442)	3.71E-08	47	117	MAPK3
16q13	broad	chr16:56068807-56658226(probes 78870:78898)	4.33E-08	49	13	DOK4
16q22.1	broad	chr16:65749906-66612926(probes 79108:79177)	2.29E-14	49	41	
16q24.3	broad	chr16:86172468-88827254(probes 79912:80047)	2.70E-12	49	45	IL17C, FANCA, CDK10
17p11.2	broad	chr17:20618475-20845678(probes 80907:80912)	0.0006169	45	2	
17q21.2	broad	chr17:34989301-39828444(probes 81445:81756)	4.14E-06	45	47	STAT3
17q22	broad	chr17:53603427-53812876(probes 82242:82252)	3.26E-08	34	7	SUPT4H1
17q23.3	broad	chr17:58901630-60399623(probes 82454:82525)	0.0006169	32	30	
17q25.1	broad	chr17:70825746-70934827(probes 82864:82868)	4.05E-05	32	1	
17p13.1	broad	chr17:7217430-8017270(probes 80410:80478)	3.13E-09	45	42	TP53
19p13.3	broad	chr19:1-3747215(probes 85650:85832)	4.74E-14	52	109	
19q13.12	broad	chr19:40168317-41398102(probes 86948:87029)	1.50E-08	51	56	
19q13.33	broad	chr19:54489423-56393197(probes 87670:87779)	5.53E-14	51	60	

Cytoband	Broad or focal	Peak region (Mb)	q value	Frequency (%)	Number of genes in peak	Known cancer genes
19q13.42	broad	chr19:59337767-60853685(probes 87917:87997)	7.33E-20	51	42	
19q13.43	both	chr19:63654029-63811651(probes 88142:88157)	2.00E-18	51	9	
20p13	both	chr20:3576278-3741605(probes 88329:88344)	5.47E-08	45	9	
20q13.33	broad	chr20:61334277-61867592(probes 90310:90342)	2.15E-15	45	21	
21q22.3	broad	chr21:44375425-46717428(probes 91623:91770)	1.52E-06	51	29	
22q11.21	broad	chr22:18070267-18691703(probes 91909:91947)	2.71E-30	78	15	SEPT5
22q12.2	both	chr22:27,901,842-28,852,291(probes 92365:92430)	3.16E-37	85	17	NF2
Xq28	focal	chrX:152278550-152830969(probes 97921:97955)	2.97E-17	57	19	TREX2
Xp11.23	focal	chrX:47780775-49008093(probes 94924:95005)	5.97E-05	55	43	GATA1
		Copy Number Gains				
1q25.1	broad	chr1:172161698-172889501(probes 5543:5570)	0.0098287	55	2	
1q31.1	broad	chr1:182779475-197636068(probes 5944:6019)	0.004383	59	42	
7q36.1	broad	chr7:150277900-150468342(probes 44309:44322)	0.0098287	26	10	CDK5
9q34.3	broad	chr9:138940849-139254452(probes 53064:53086)	0.004383	30	34	COBRA1
17q21.32	broad	chr17:41604766-42212584(probes 81839:81848)	0.0027607	44	7	WNT3