Delineation of Commonly Deleted Chromosomal Regions in Meningiomas by High-Density Single Nucleotide Polymorphism Genotyping Arrays

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Despite recent advances in the identification of the cytogenetic profiles of meningiomas, a significant group of tumors still show normal karyotypes or few chromosomal changes. The authors analyzed the cytogenetic profile of 50 meningiomas using fluorescence in situ hybridization and high-density (500 K) single nucleotide polymorphism (SNP) arrays. Our results confirm that del(22q) (52%) and del(1p) (16%) (common deleted regions: 22q11.21-22q13.3. and 1p31.2-p36.33) are the most frequent alterations. Additionally, recurrent monosomy 14 (8%), del(6q) (10%), del(7p) (10%), and del(19q) (4%) were observed, while copy number patterns consistent with recurrent chromosomal gains, gene amplification, and copy number neutral loss of heterozygosity (cnLOH) were either absent or rare. Based on their overall SNP profiles, meningiomas could be classified into: (i) diploid cases, (ii) meningiomas with a single chromosomal change [e.g., monosomy 22/del(22q)] and (iii) tumors with $\geq$2 altered chromosomes. In summary, our results confirm and extend on previous observations showing that the most recurrent chromosomal abnormalities in meningiomas correspond to chromosome losses localized in chromosomes 1, 22 and less frequently in chromosomes 6, 7, 14, and 19, while chromosomal gains and cnLOH are restricted to a small proportion of cases. Finally, a set of cancer-associated candidate genes associated with the *TP53, MYC, CASP3, HDAC1*, and *TERT* signaling pathways was identified, in cases with coexisting monosomy 14 and del(1p). © 2012 Wiley Periodicals, Inc.

INTRODUCTION

In the last decades, increasing knowledge has accumulated about the cytogenetic abnormalities of meningiomas. Early reports showed that monosomy 22/22q− is by far the most common cytogenetic alteration of meningiomas and that complex karyotypes, associated or not with monosomy 22/del(22q), are rare. Among other abnormalities, deletion of chromosome arms 1p and 6q as well as monosomy 14 and/or losses of the sex chromosomes are much more frequent than both chromosomal gains and tetraploidy (Simon et al., 2007; Guan et al., 2008) as confirmed by more sensitive techniques such as fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH) arrays (Buckley et al., 2002). Altogether, these findings support the notion that monosomy 22 or del(22q) in association with mutation of the *NF2* gene is the most frequent abnormality involved in meningiomas while del(1p) and monosomy 14 are the most informative alterations to predict recurrence (Maillo et al., 2007; Nakane et al., 2007; Ketter et al., 2008). Despite this, relatively limited data exist about the relevant genes and combinations of genes involved in these chromosomal alterations.

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Genome-wide analysis through high-density single nucleotide polymorphism (SNP) arrays provides large scale approaches for detailed characterization of these alterations, through the identification of copy number (CN) changes and loss of heterozygosity (LOH) for millions of SNP, even in the presence of copy number neutral LOH (cnLOH) (Bacolod et al., 2009). While SNP arrays have been used in the characterization of many types of tumors, to the best of our knowledge, few studies have been reported so far in which they have been applied to analyze large series of meningiomas. In fact, until now only 100K SNP arrays have been used to characterize an atypical meningioma (Krupp et al., 2008) and 18 meningiomas have been studied with 500K arrays (Goutagny et al., 2010).

In this article, the authors report on the cytogenetic profile of 50 meningiomas obtained by high-density SNP arrays, interphase FISH (iFISH), and microsatellite markers. The goal was to define more precisely the specific regions of these chromosomes, which are altered in common in these tumors. Overall, our results confirm and extend previous observations by delineating those chromosomal regions, which are systematically deleted in meningiomas with losses of chromosomes 22, 1p, 6q, 7p, and 14, pointing out the potential relevance of cancer-associated genes coded in such chromosomal regions.

MATERIALS AND METHODS

Patients and DNA Samples

Fifty meningioma patients, who gave their informed consent to participate according to the Helsinki Declaration, were included in this study. Histopathological diagnosis and classification of meningioma were established according to the WHO criteria (Table 1). In all cases DNA was obtained from paired fresh tumor tissues and peripheral blood (PB) samples, using the QIAamp DNA mini kit (Qiagen, Hilden, Germany).

Copy Number (CN) Changes and Loss of Heterozygosity (LOH) by SNP Arrays

A total of 500,568 SNPs were analyzed for paired tumoral ($n = 50$) and normal PB ($n = 50$) DNA samples using the GeneChip Human Mapping 250K Nsp and 250K Sty arrays (Affymetrix, Santa Clara, CA) according to the manufacturer's instructions. The SNP call rate per array was always $\geq$92% (range 92–99.8%). Overall, 200 ".CEL" files were analyzed with the GCOS software (version 1.3, Affymetrix), the Copy Number Analysis Tool (CNAT v4.0, Affymetrix), the dChip 2007 software (http//www.dchip.org; Dana Farber Institute, Boston, MA) and the GeneChip Genotyping Analysis software (GTYPE 4.1; Affymetrix). The CNAG software (Tokyo University, Tokyo, Japan; Nannya et al., 2005) was also used to distinguish between homozygous and heterozygous deletions. Tumor DNA that showed mitotic recombination losses and reduplication or chromosomal non-disjunction with a CN value of two was considered as carrying copy neutral LOH (cnLOH). The relationship between genes involved in cancer, which are coded in the commonly lost chromosomal regions was evaluated through the Ingenuity Pathways Analysis software (Ingenuity Systems®, www.ingenuity.com).

Comparative Genomic Hybridization Arrays

A custom-made CGH array with 3,495 DNA-clones (median size of 200 kb; average spacing of $\approx 10^3$ kb) covering the 22 autosomes plus the X chromosome (kindly provided by the Sanger Institute; Cambridge, UK) was used in a subset of 20 paired PB/tumoral DNA samples. CGH arrays were hybridized using previously reported protocols (Espinosa et al., 2008).

Interphase Fluorescence in Situ Hybridization Analyses

To confirm the presence of specific chromosomal numerical changes, 26 BAC/PAC probes used previously in the CGH array were fluorescently labeled for iFISH analyses (Table 2). All clones were provided by the Sanger Institute, except for chromosome arm 14q clones ($n = 11$ clones) that were obtained from the BACPAC Resources Children's (Hospital Research Institute, Oakland, CA) and they were specifically assessed in a total of 20 of the 50 tumor samples.

PCR Analysis of Microsatellite Markers

To confirm LOH involving specific areas of 22q, 11 microsatellite markers were analyzed in a total of 14 cases, including three tumors with del(22q) and four with monosomy 22. Primer sequences were obtained from the UniSTS database (www.ncbi.nlm.nih.gov), except for one marker corresponding to the *NF2* gene (Legoix et al., 1999). For multiplex amplification

TABLE 1. Cytogenetic Profiles Defined by the Copy Number Changes Identified for Each Individual Chromosome/Chromosomal Region in Meningioma Tumor Samples and its Relationship with Patient Age, Gender, and Tumor WHO grade ($n = 50$)

Case *N*	Patient		WHO tumor grade	Chromosome copy number profile by SNP arrays
	Age	Gender		
01	46	F	I	DIPLOID
02	73	F	I	DIPLOID
03	40	F	I	DIPLOID
04	77	M	I	DIPLOID
05	42	F	I	DIPLOID
06	69	F	I	DIPLOID
07	42	F	I	DIPLOID
08	43	F	I	DIPLOID
09	42	F	I	DIPLOID
10	69	F	I	DIPLOID
11	62	F	I	DIPLOID
12	54	F	I	DIPLOID
13	63	F	I	DIPLOID
14	54	M	I	DIPLOID
15	47	F	I	DIPLOID
16	61	F	I	DIPLOID
17	66	M	**II**	DIPLOID
18	56	M	**III**	DIPLOID
19	65	M	I	+X
20	33	F	I	del(6)(q24.1→qter)
21	77	M	I	−22
22	34	F	I	−22
23	49	F	I	−22
24	69	F	I	−22
25	42	F	I	−22
26	58	F	I	del(22)(pter→q13.31)
27	54	F	I	−22
28	69	F	I	−22
29	66	F	I	−22
30	65	F	I	del(22)(q11.21→qter)
31	65	F	I	−22
32	75	F	I	−22
33	78	F	I	−22
34	53	F	I	−22
35	56	F	I	−22
36	57	F	I	−22
37	80	F	I	del(1)(pter→p31.3),del(7) (q11.21→q11.23::q21.12→q31.1)
38	66	F	I	−22,−X
39	26	F	I	del(1)(pter→34.3::q32.1→qter), del(2)(p23.3), del(19)(pter→p13.13),−22
40	68	M	I	del(1)(pter→p31.2),-6,-14, −22
41	82	F	I	−1(p36.33→p11.2),p,−7(p22.3→p11.1),p,−11, del(18) (q11.2→q12.1::18q12.2→qter),−22
42	83	F	I	−1p(p36.33→p11.2), del(3)(pter→p12.2), −4(p16.3→p11),p,del(4) (q32.3→qter), −6(q11.1→q27), −q,−7(p22.3→p11.1),p, del(19)(pter→p13.11)
43	84	F	I	−1(p36.33→p11.2),p, **cnLOH**1q(q12→q44), −−8,−10,−14,−18,−22
44	62	F	III	−6, −7(p22.3→p11.1),p, −14,del(19)(pter→p13.11), del(22)(pter→q11.21::q11.22→qter)
45	53	F	I	−1(p36.33→p11.2)p, +1(q12→q44)q,del(3) (p22.1→p12.3), −6(q11.1→q27),q,del(21) (q11.2→q21.1::q22.2-qter), del(22)(q11.22→qter)
46	61	M	I	+13, −14,−22
47	77	F	I	+3, +20, −22
48	58	M	II	+5,+20
49	78	M	I	−1(p36.33→p11.2),−p,−3 (p26.3→p11.1),p,del(4)(pter→p13), + 4p12, + 4q(q13.1→q22.1),del(5) (pter→p15.33),del(7)(pter→p14.1), del(11) (p11.2→p12::p12→p15.4), +17q21.31-qter, del(22)(q11.23→qter)
50	52	M	I	+8,+13,+17

WHO: World Health Organization (Acta Neuropathol 2007;114:97–109). SNP: single nucleotide polymorphism; cnLOH: copy neutral loss of heterozygosity.

TABLE 2. BAC/PAC Clones (*n* (=26) Uused to Vvalidate CN (Ccopy Nnumber) Rresults Oobtained with the SNP – Arrays by Interphase Fluorescence in Situ Hybridization

BAC/PAC clone	Chromosome	Chromosome band	Localization		Size (N. of bp)
			Starting bp	Ending bp	
RP4-575L21	1[a]	p36.22	10,075,630	10,169,954	94,324
RP11-114B7		p35.1	32,874,030	33,046,828	172,798
RP1-42M2	7[a]	p22.1	6,102,813	6,103,621	808
RP11-149I2	9[a]	p21	21,851,433	22,046,818	195,385
RP11765C10	10[a]	q23	89,705,200	89,888,619	183,419
CTD2120P12		q11.2	20,238,025	20,398,600	160,575
RP11433O19		q13.1	33,950,156	34,130,673	180,517
RP11-73E17		q13.2	34,343,505	34,485,026	141,521
RP11700L24		q23.3	64,190,857	64,415,593	224,736
RP11-746C4		q24.2	72,480,956	72,642,631	161,675
RP11-31E3		q24.3	73,034,707	73,200,904	166,197
RP11-536A5	14[b]	q24.3	74,082,799	74,277,120	194,321
RP11410E23		q31.2	83,142,999	83,279,501	136,502
RP11532E10		q32.33	103,230,563	103,442,515	211,952
RP11493E18		q32.33	103,877,829	104,082,121	204,292
RP11-84H9		q32.33	105,325,858	105,501,944	160,575
CTD-2547N9	19[a]	p13.2	8,490,674	8,703,884	213,210
CTC-444D3		p13.2	9,041,365	9,197,603	156,238
CTD-2623N2		p13.2	9,688,758	9,689,294	537
RP11-197O4		p13.2	10,248,852	10,410,584	161,733
CTC-539A10		p13.2	10,699,023	10,699,695	673
CTD-2639E6		q13.33	53,951,044	54,181,980	230,937
XX-p273a17	22[a]	q11.21	16,347,244	16,490,323	143,079
Z99716		q13.2	40,582,730	40,803,595	220,865
RP3-437M21		q13.2	41,529,087	41,529,734	647
Z82245		q13.32	49,425,787	49,521,447	95,660

Localization of each BAC/PAC clone is defined by the starting and ending base pair (bp) sequences for each chromosome region and its length (N. of bp).
[a]Clones provided by the Sanger Institute (Welcome Trust; Sanger Institute, Cambridge, UK).
[b]Clones for chromosome 14q provided by the BACPAC Resources Children's Hospital Research Institute (Oakland, CA, EEUU; http://bacpac.chori.org).

reactions, the Qiagen Multiplex PCR kit (Qiagen, Valencia, CA) was used according to the manufacturer's instructions. PCR products were analyzed using an ABI310 instrument (Applied Biosystems, Foster City, CA) and the GeneScan Analysis software (v2.1.1; Applied Biosystems). LOH was assessed by calculating the LOH index defined as the allele ratio of tumor DNA/normal DNA (height of the smaller/lighter peak divided by the height of the larger/heavier peak); cut-off values for the LOH index of <0.5 or >1.5 were used to define LOH.

RESULTS

Frequency of Chromosomal Abnormalities by SNP Arrays

Overall, SNP arrays showed a higher frequency of chromosomal losses ($n = 60$) than gains ($n = 10$) for the 50 meningiomas analyzed (Fig. 1). These involved 19/23 chromosomes and only chromosomes 9, 12, 15, and 16 were found to have no abnormalities.

Genetic losses were most frequent at 22q (52%), 1p (16%), 6 (10%), 7 (10%), 14 (8%), and 19 (6%). CN changes at chromosome 22 were consistent with monosomy 22 ($n = 21$) and del(22q) ($n = 5$). Regarding chromosome 1, losses were typically restricted to its short arm, with either complete ($n = 5$) or partial ($n = 3$) loss of 1p. In turn, losses of chromosome 6 were consistent with monosomy 6 ($n = 2$) and either complete ($n = 2$) or partial del(6q) (n = 1), respectively. Similarly, CN changes involving chromosome 7 consisted of del (7p) ($n = 4$) and del(7q) ($n = 1$). All four cases that showed chromosome 14 losses had monosomy 14 (Fig. 1). Other chromosomal losses recurrently detected in our series included del(3p) ($n = 3$), del(4p) ($n = 2$), −11/del(11q) ($n = 2$), del(18q) ($n = 2$), and del(19p) ($n = 3$).

Chromosomal gains involved chromosomes 1, 13, 17, and 20 ($n = 2$ each), and chromosomes 3,

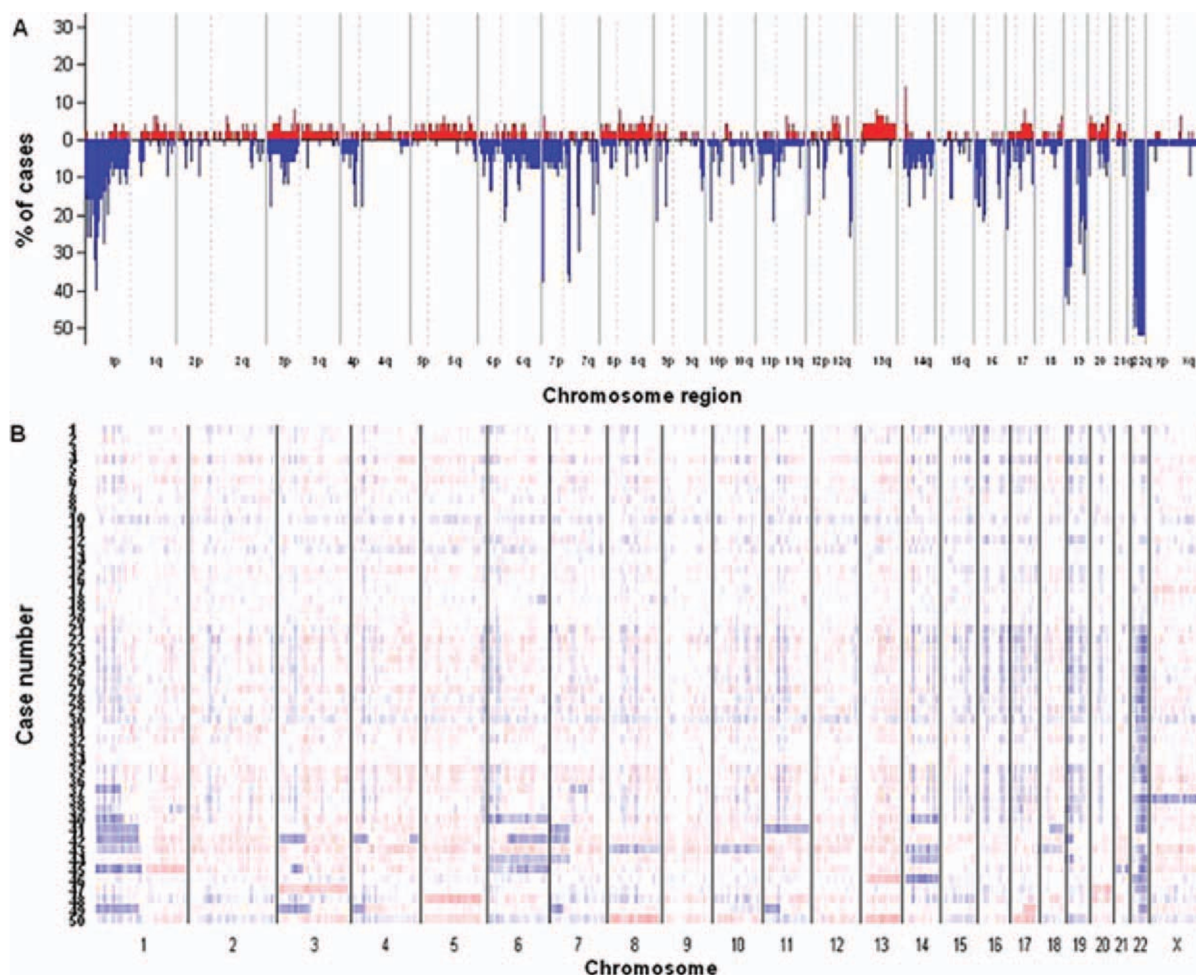

Figure 1. Frequency and extent of copy number changes for individual chromosomes and chromosomal region in meningioma tumors ($n = 50$) as detected by 500 K SNP arrays. Panel A shows the overall frequency of gains and losses (y-axis) identified for the 23 human chromosomes delineated by vertical lines (red and blue lines, respectively) for each individual loci analyzed within each chromosome (x-axis). In panel B, a heat map is displayed where the y-axis represents individual tumor samples and the x-axis represents each specific chromosomal region analyzed; this heat map is colored from white to dark blue or dark red depending on whether a normal, decreased or increased copy number was detected, respectively.

4, 5, 8 and also chromosome X in females (one tumor each). Patterns consistent with gene amplification or homozygous deletions were not detected for any chromosome among all 50 tumors analyzed, except for one case that showed cnLOH of chromosome arm 1q.

According to the overall pattern of CN changes identified, meningiomas could be classified into three groups: (i) tumors in which no extensive CN changes were identified (diploid profile; $n = 18$, 36%; with 3/18 cases showing microdeletions of chromosomes 5, 7, 9, 10 11, 12, 15, and/or 19); (ii) tumors with only one chromosome altered ($n = 18$; 36%), which typically consisted of -22/del(22q) and; (iii) cases displaying complex karyotypes with ≥ 2 altered chromosomes ($n = 14$; 28%); in this latter group, meningiomas showed either only chromosomal losses ($n = 9$; 18%) or less frequently, coexistence of chromosomal losses and gains ($n = 3$; 6%) or just chromosomal gains ($n = 2$; 4%; Fig. 2).

Localization and Extent of Recurrent Chromosomal Alterations

The extent of the most common deletions are illustrated in Figure 3. The common deleted region for del(1p) was pter→1p34.2 (from 742,429 to 40,866,917 bp positions) which contains multiple cancer-associated genes (e.g., *CASP9*, *HDAC1*, *PIK3CD*, *TNFRSF1B*; Table 3). Of note, two cases (cases 43 and 45) that had del(1p) also displayed gains of 1q, associated in one case (case 43) to cnLOH (Fig. 3).

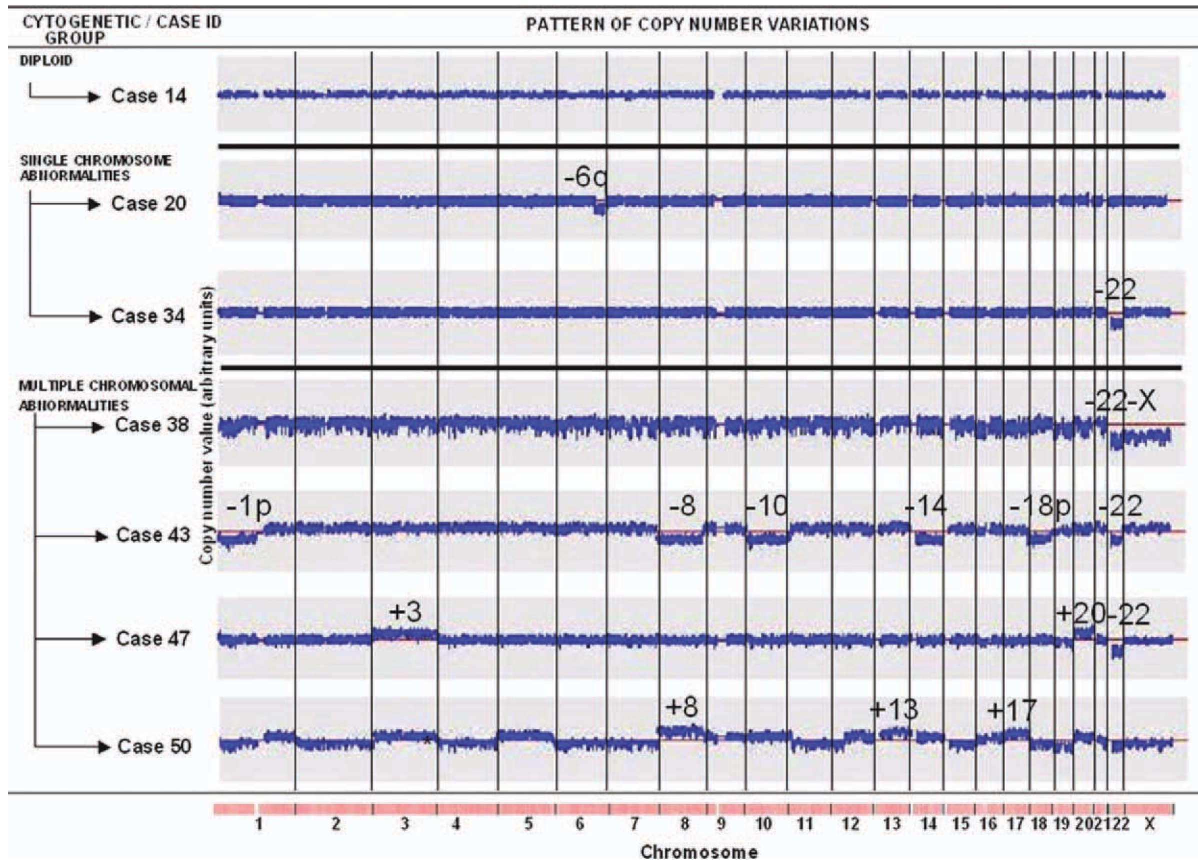

Figure 2. Copy number variation (CNV) profiles for the 23 human chromosomes representative of the different patterns observed in meningiomas. The seven cases displayed are representative of the three major cytogenetic groups of meningiomas detected: (1) diploid tumors ($n = 18$; case 14); (2) meningiomas with only one altered chromosome with either gains ($n = 1$), partial losses ($n = 1$; case 20) of a single chromosome or monosomy 22/del(22q) ($n = 16$; case 34), and; (3) tumors with multiple chromosomal changes corresponding to chromosomal losses ($n = 8$; cases 38 and 43), to both chromosomal gains and chromosomal losses ($n = 4$; case 47) and to chromosomal gains ($n = 2$; case 50). The red line indicates where the hybridization signal would fit a normal diploid CN pattern.

As mentioned above, although most losses of chromosome 22 were consistent with monosomy 22, five tumors showed overlapping del(22q): del(22)(q11.21-q13.31), del(22)(q11.21-qter), del(22)(q11.23-qter), del(22)(q11.22-qter), and del(22)(q11.23-qter), respectively (Fig. 3). A region close to the centromere was retained in four of these five cases, while in the remaining patient, del(22q) expanded from the centromere to the 22q13.31 region. Thus, the common deleted region on chromosome 22 consisted of del(22)(q11.23→q13.31) with a length of 23,145,208 bp (from 22,410,163 to 45,555,371 bp positions), which systematically included 12 cancer-associated genes (Table 3).

For chromosome 6, the common deleted segment in those three cases that had del(6q) was localized between 6q24.1 and 6qter (from 139,275,856 to 170,747,902 bp position) where the *ESR1* and *IGF2R* cancer-associated genes are located (Fig. 3; Table 3). For chromosome 7, 4/5 cases showed del(7p) – common deleted region: del(7)(pter→7p13, from 141,322 to 43,338,377 bp – where 153 genes (e.g., the *RAC1* and *RALA* cancer-associated genes) are located and one case displayed del(7)(q11.21→11.23) (Fig. 3). Monosomy 14 included loss of one copy of 19 cancer-associated genes (Fig. 4; Table 3).

CGH arrays, iFISH, and Microsatellite Analyses

Overall, CGH array profiles were concordant with SNP array results, with complete match in 14/20 cases evaluated (70%). In the remaining six tumors, discordant results were found for a limited number of chromosomes. Further iFISH and microsatellite marker ($n = 11$) analyses directed to the study of chromosome 22q were then performed. The microsatellite studies confirmed the presence of del(22q) by SNP arrays but not by CGH arrays in two cases (cases 31 and 49; Fig.

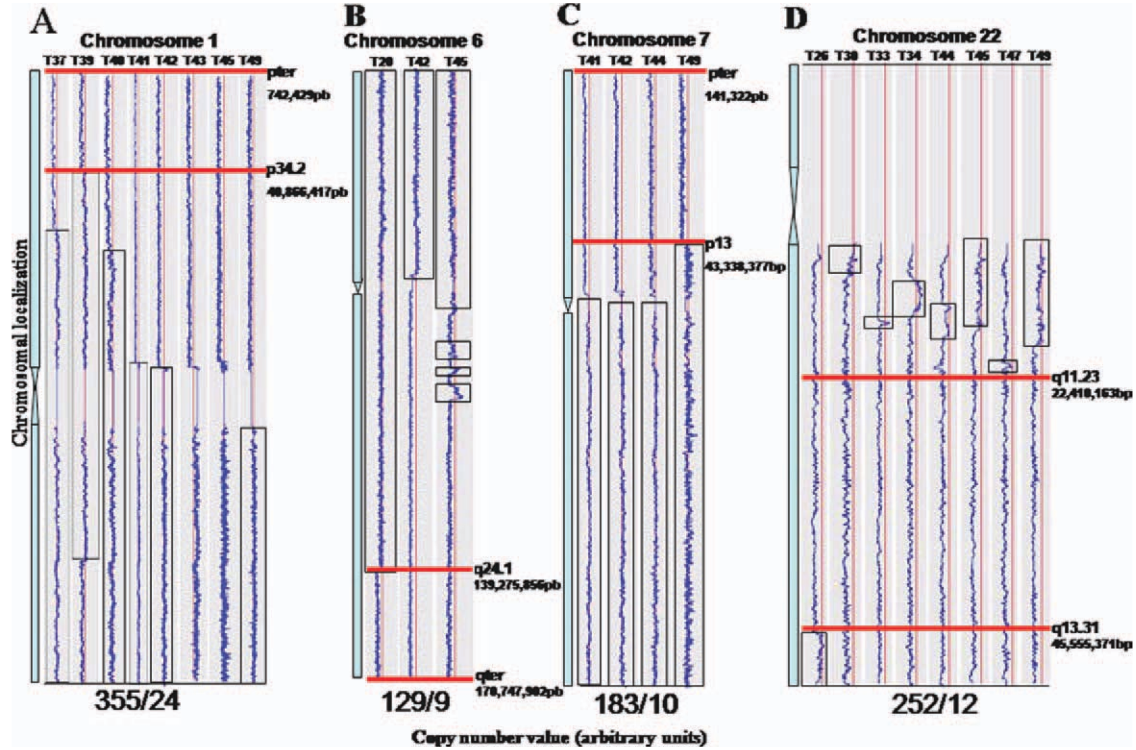

Figure 3. SNP array profiles and extent of the chromosomal regions deleted in common in two or more tumors. The common deleted regions for recurrent losses detected for chromosomes 1, 6, 7, and 22 are shown in panels A, B, C, and D, respectively. In all panels, copy number values for each individual tumor (*x*-axis) are plotted against the physical position of each individual SNP analyzed along the corresponding chromosome (*y*-axis). As shown, deletions of different sizes were found in the short arms of chromosomes 1 (Panel A) and 7 (Panel C) as well as for the long arms of chromosomes 6 (Panel B) and 22 (Panel D) expressed in base pairs. Boxes are drawn over the retained areas, out of which the common deleted regions are displayed between horizontal red lines. At the bottom of each graph, the number of all genes as well as cancer-associated genes located in the deleted segments are shown, respectively.

TABLE 3. DNA Sequences Recurrently Deleted in Tumors with Losses of Chromosomes 1, 6, 7, 14, and 22. Associated Cancer Genes Included in the Deleted Chromosomal Regions

	Chromosomal region deleted in common				
Chromosome	Band	Start (bp)	End (bp)	Size (Mb)	Genes
Chr1	pter-1p34.2	742,429	40,866,417	40	*CASP9, CDC42, CSF3R, E2F2; ENO1, FABP3, FGR, HDAC1; KDM1A, LCK, MASP2, MTOR; PAFAH2, PIK3CD, PRKCZ; RPS6KA1,TARDBP, TNFRSF1B; TNFRSF8, TNFRSF9, TP73*
Chr6	6q24.1-qter	139,275,856	170,747,902	31	*ESR1, EZR, IGF2R, LPA; PARK2, PLG, SOD2,TBP*
Chr7	pter-7p13	141,322	43,338,377	43	*ADCYAP1R1, HOXA9, IL6; MEOX2, RAC1, RALA*
Chr14	14q11.2-q32.33	19,272,965	106,356,482	87	*ACIN1, AKT1, APEX1; ARHGAP5, BDKRB2, BMP4; ESR2,HIF1A,HNRNPC, HSP90AA1,MAX,NFKBIA, PSEN1,PSMA6,SEL1L, SERPINA1, SERPINA3, TSHR, YY1*
Chr22	22q11.23-q13.41	22,410,163	45,555,371	23	*CHEK2, CSF2RB, EP300; FBLN1, MIF, MYH9, NF2; PARVG, PPARA, RAC2; TIMP3, XRCC6*

5). Analysis of microsatellite markers on 22q showed a high degree of agreement with the results obtained by SNP arrays. Accordingly, except for case 8, full concordance was observed in all nonaltered cases showing allele retention as well as in all tumors presenting LOH, due to monosomy 22, either as the only chromosomal abnormality or in the context of a complex karyotype (Table 4).

DISCUSSION

In human cancer, deviation of individual cells from normal gene dosage typically involves oncogenes in regions of gains and tumor suppressor genes in deleted areas. Therefore, detailed characterization of the altered chromosomal regions is crucial for the identification of informative cancer-associated chromosomal regions and the relevant genes they contain. Detailed analysis of the

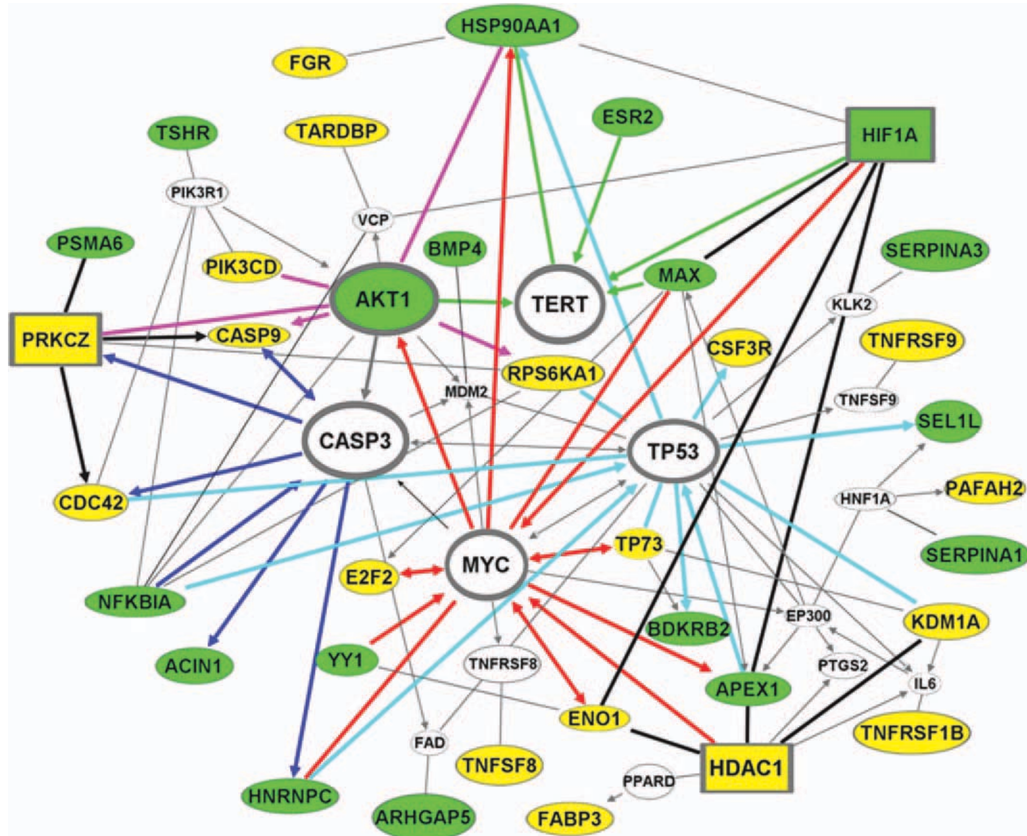

Figure 4. Schematic representation of the network of interactions observed between cancer-associated genes located in chromosome 14 and chromosome arm 1p. Interaction network between those cancer-associated genes mapping to chromosome 14 (genes highlighted in green) and 1p (genes highlighted in yellow) deleted in common in meningiomas with monosomy 14/del(1p). Different colors and subtypes of lines were used to indicate the relationship between genes: an ended line indicates "binding," ended line with an arrow indicates "acts on," doubled ended line with a vertical line plus an arrow indicates "inhibits and acts on." The MYC pathway relationships are drawn as red lines, *TP53* as cyan, *TERT* as green, *CASP3* as blue lines and *HDAC1* as magenta lines. The multiple direct relationships among the *PRKCZ, HIF1A*, and *HDAC1* genes located in chromosomes 1p and 14 are highlighted as black lines.

altered chromosomal regions through high-density genetic screening has not been reported so far in large series of meningiomas. In this article, the authors report a series of 50 meningiomas analyzed by high-density SNP arrays. Overall our results showed the presence of CN alterations in most meningiomas, with two clearly different SNP array profiles characterized by single – e.g., del(22q) or monosomy 22 – versus multiple chromosomal changes, and they confirm that del(22q) monosomy22 and del(1p) are the most frequent alterations.

In the first group of tumors characterized by single chromosomal changes, these typically consisted of del(22q) or monosomy 22. These results further support the notion that NF2 located at 22q12.2 may be involved in the pathogenesis of this subgroup of meningiomas, since it was constantly included in the common deleted region, even when interstitial deletions were observed. Other candidate cancer-associated genes mapping in the deleted region of chromosome 22 included the *CHEK2*, *EP300*, and *PPARA* genes related to the *TP53* pathway (Morimura, 2006; Chrisanthar et al., 2008; Teufel DP, 2009), and the *PARVG* gene associated with cell adhesion (Olski et al., 2001; Yoshimi et al., 2006). Previous studies suggest the existence of LOH involving small regions of chromosome 22, which are missed by microsatellite mapping, presumably because of their relatively low density (Ueki et al., 1999; Wozniak, 2008), but this could not be confirmed in our study by high-density SNP arrays.

The second group of cytogenetically altered meningiomas showed complex karyotypes with between 2 and 9 different abnormal chromosomes, with or without monosomy 22/del(22q). In line with previous observations, 1p, 6q, 7p, and 14 were more frequently altered in these cases (Henn et al., 2003; Ketter et al., 2007; Guan et al., 2008). Remarkably, the regions deleted in common at 6q and 7p are also shared by other

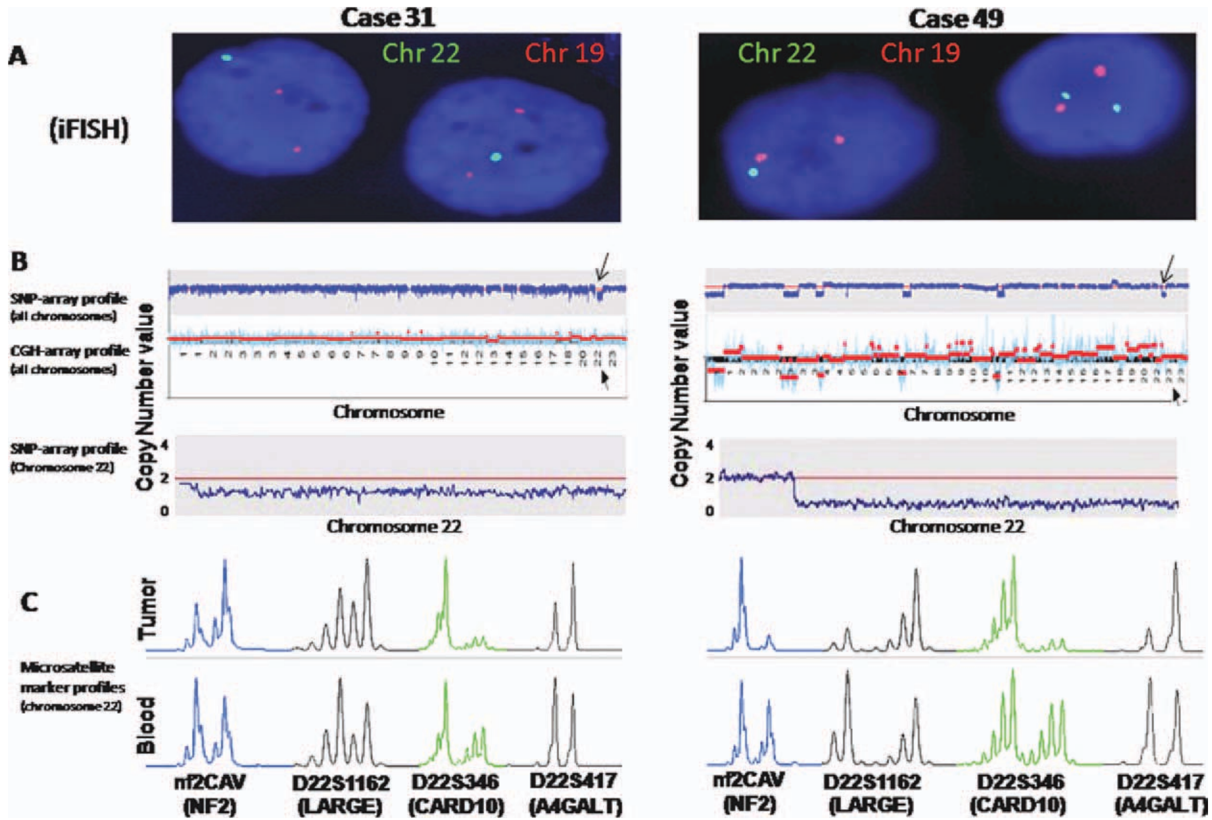

Figure 5. Comparative analysis of the cytogenetic results obtained with different methods in two cases showing loss of chromosome 22. Illustrating iFISH (Panel A), SNP and CGH array (Panel B), and microsatellite marker profiles (Panel C) obtained for the two discrepant tumors (cases 31 and 49), which were diploid by CGH arrays but recurrently showed loss of a chromosome 22 and del(22) by the other approaches (iFISH, SNP arrays, and microsatellite analysis of LOH for 4 microsatellite markers analyzed – *nf2CAV* (*NF2*), *D22S1162* (*LARGE*), *D22S346* (*CARD10*), and *D22S417* (*A4GALT*).

malignant tumors (Trost et al., 2007; Klatte et al., 2009). Except for chromosome 14, whose abnormalities systematically consisted of monosomy 14, commonly deleted regions were identified for the other chromosomes and involved cancer-related genes like *ESR1* and *IGF2R* in chromosome 6 and *RAC1* and *RALA* in chromosome 7. Noteworthy, when the authors searched for direct interactions among cancer-associated genes, seven genes deleted in cases with monosomy 14, and another nine genes which are lost in common in meningiomas with coexisting del(1p), emerged as directly related to four common signaling pathways involved in cell growth and survival and in DNA repair. These include: (i) genes of the TERT/estrogen pathway that regulate both cell senescence and chromosomal repair; (ii) genes that act in the execution phase of cell apoptosis regulated by caspase 3 (e.g., *CASP9*, *PRKCZ*, *AKT1*, and *PSMA6*); (iii) genes involved in down-regulation of cell proliferation, survival, and/or DNA repair through induction of *TP53* (Bon et al., 2009; Petroulakis et al., 2009; e.g., *HDAC1* and *APEX1*); and (iv) genes involved in down-regulation of cell proliferation through *MYC* (e.g., *HDAC1* and *HIF1A*). Altogether, these findings suggest a potential role for the combined deletion of these genes in conferring poor-prognosis to meningiomas with coexisting monosomy 14 and del(1p) in their ancestral tumor cell clone (Cerni, 2000; Bodvarsdóttir, 2007), potentially associated with increased cell proliferation and survival and diminished DNA and telomere repair.

Most interestingly, around one-third of the cases did not show CN alterations by SNP arrays (Prowald et al., 2005; van Tilborg et al., 2006; Hansson et al., 2007). Potential misinterpretation of the data due to contamination of these tumor samples by nonaffected cells could be ruled out, since histopathological analyses of tissue sections mirror-cut to those used to prepare tumor DNA samples, systematically showed infiltration by ≥65% tumor cells. Despite our findings, more limited nucleotide changes (e.g., recurrent single point mutations) outside the SNP regions investigated cannot be ruled out, since they could go

TABLE 4. LOH Patterns of Chromosome Arm 22q in Meningiomas ($n = 14$) as Detected by Both SNP-Arrays ($n = 214$ SNPs) and Gene-Associated Microsatellite Markers ($n = 11$)

Genetic profile by SNP-arrays	Case ID	Heterozygosity status by SNP-arrays	Informative microsatellite markers ($n = 11$)	LOH status for chromosome 22q — gene-associated microsatellites (position in the genome) —										
				MN1 (q12.1)	NF2 (q12.2)		LARGE (q12.3)		CARD10 (q13.1)	POLR2F/SOX10 (q13.1)	FAM152B (q13.2)	A4GALT (q13.2)	ARHGAP8 (q13.31)	TTLL8 (q13.33)
				D22S535 **26498810** **26498919**	D22S929 **28354071** **28354208**	nf2CAV **28596431** **28596663**	D22S1172 **32004407** **32004670**	D22S1162 **32640944** **32641098**	D22346 36216346 36245495	D22S1156 **36711717** **36711872**	D22276 40326825 40346966	D22S417 **41434402** **41434575**	D22S1168 43476777 43637329	D22S1056 **48828657** **48824754**
Diploid Karyotype	3	NI/RET	**7**	NI	RET	RET	NI	RET	RET	NI	RET	RET	NI	RET
	5	NI/RET	**5**	NI	RET	RET	NI	NI	RET	RET	RET	NI	NI	NI
	7	NI/RET	**6**	NI	RET	NI	RET	NI	RET	NI	NI	RET	RET	RET
	8	NI/RET/**LOH**	**8**	NI	RET	RET	NI	RET	RET	RET	RET	RET	RET	NI
	9	NI/RET	**7**	NI	RET	NI	NI	RET	RET	RET	NI	RET	RET	RET
	11	NI/RET	**7**	RET	RET	RET	NI	RET	RET	RET	NI	NI	RET	NI
	14	NI/RET	**6**	RET	NI	RET	RET	RET	RET	NI	NI	NI	NI	RET
−22/del(22q)	25[b]	**LOH**/NI	**10**	**LOH**	NI	**LOH**	**LOH**	**LOH**	**LOH**	**LOH**	**LOH**	**LOH**	**LOH**	**LOH**
	26[a]	**LOH**/NI/RET	**7**	NI	**LOH**	**LOH**	NI	**LOH**	–	NI	**LOH**	**LOH**	**LOH**	RET[c]
	31[b]	**LOH**/NI/RET	**5**	NI	RET[d]	**LOH**	NI	**LOH**	LOH	NI	**NI**	**LOH**	**-**	NI
Complex Karyotype	38[b]	**LOH**/NI/RET	**7**	NI	NI	NI	**LOH**	**LOH**	**LOH**	NI	**LOH**	**LOH**	**LOH**	**LOH**
	41[b]	**LOH**/NI/RET	**8**	NI	**LOH**	**LOH**	**LOH**	NI	**LOH**	**LOH**	**NI**	**LOH**	**LOH**	**LOH**
	45[a]	**LOH**/NI/RET	**6**	**LOH**	**LOH**	**LOH**	NI	**LOH**	**LOH**	NI	**NI**	**LOH**	NI	NI
	49[a]	**LOH**/NI/RET	**6**	NI	NI	**LOH**	NI	**LOH**	**LOH**	NI	**LOH**	**LOH**	**NI**	**LOH**

LOH pattern found for microsatellite markers and the SNP-arrays are expressed for each gene area as: NI, non-informative homozygous pattern; LOH, LOH detected; and RET, retention of heterozygosity.
[a]del (22q).
[b]Monosomy 22.
[c]RET also found by SNP-arrays for the informative SNP analyzed for the same chromosomal region; in this tumor the 22q13.33 region was not deleted by SNP-arrays). Case 8 showed results compatible with a microdeletion.
[d]Discrepant result.

undetected with our approach; alternatively, other mechanisms, such as cell senescence and epigenetic changes occurring at early phases of the disease, could also play a role in long-term expansion of clonal cells in this subgroup of meningiomas (Hakin-Smith et al., 2001). Altogether, our findings further support the existence of different oncogenetic pathways in the ontogeny of meningiomas (Tabernero et al., 2009). In line with this hypothesis, only a fraction of all cases with complex karyotypes showed monosomy 22 or del(22q), and cases with multiple chromosomal gains in the absence of other alterations were also identified among this latter subgroup of meningiomas.

Apart from the alterations described, it had to be emphasized that in contrast to what occurs in malignant tumors including gliomas (Bergamaschi et al., 2008; Kuga et al., 2008; Konecny et al., 2009; Marx et al., 2009), our results demonstrate that cnLOH as well as gene amplification are either rare or absent in histologically benign meningiomas (Ueki et al., 1999; Wozniak, 2008). These results confirm and extend on previous observations, which indicate that despite tetraploidization may frequently occur in meningiomas, it is usually a secondary event which involves a minor fraction of all cells within a tumor.

In summary, in this study we confirm the cytogenetic heterogeneity of meningiomas and rule out the potential existence of genetic changes identifiable by high-density SNP arrays in around one third of the cases. In addition, we precisely define the commonly deleted regions for those chromosomes more frequently altered in the other meningiomas and identified a set of cancer-associated candidate genes involved in the caspase 3, *MYC/TP53*, and TERT/estrogen signaling pathways; these genes are simultaneously deleted in cases with both monosomy 14 and del(1p), a cytogenetic profile recurrently associated with poor prognosis in meningiomas. Further studies in which the functional role of these genes is investigated in detail are necessary to clarify their pathophysiological relevance and clinical impact.

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