Cytogenetic Analysis of Posterior Uveal Melanoma

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ABSTRACT: Cytogenetic analysis was performed on short-term cultures of primary tumor samples from seven patients with posterior uveal melanoma. Informative data were obtained from four patients, all of whom had a near-diploid chromosomal number and clonal chromosomal alterations. Analysis of one patient's tumor revealed monosomy 3 as the only cytogenetically distinguishable aberration. Trisomies of chromosome 8 and i(8)(q10) were detected in two other patients in combination with monosomy of chromosome 3. The fourth patient's karyotype displayed two different translocations. One translocation, der(6)t(6;8)(q12;q13.1), resulted in the over-representation of 8q13.1→qter and a partial monosomy of 6q12→qter; the other translocation, der(9)t(6;9)(p12;p23), produced a partial trisomy of 6p12→pter and a partial monosomy of 9p23→pter. These results support the view that the recurring pattern of chromosomal rearrangements in ocular melanoma is unique from that associated with cutaneous malignant melanoma. Furthermore, these results help confirm that chromosomes 3, 6, and 8 are nonrandomly altered in ocular melanoma.

INTRODUCTION

Posterior uveal melanoma (ciliary body and choroid) is the most common intraocular tumor of adult Caucasians. These tumors usually occur between the fourth and sixth decade of life and have a high incidence of metastasis [1, 2]. Unlike cutaneous melanoma, the severity of uveal melanoma does not correlate with patients' age [1]. In the world literature, 14 families have been documented with at least two members having uveal melanoma. However, it has not yet been established that this is an inherited disorder [1, 3]. Although the etiology of uveal melanoma is unknown, ultraviolet radiation, chemical agents, viruses, and trauma have been implicated in its development [1, 2, 4].

Relatively few cases of cytogenetic analysis of uveal melanoma have been reported to date. These studies have implicated monosomy 3, i(8)(q10), trisomy 8, and alterations in 6 as primary chromosomal alterations in the development of this tumor [5-9]. Recently, restriction fragment length polymorphism analysis has confirmed the cytogenetic results indicating loss of heterozygosity on chromosome 3 and over-representation of 8q [10]. Loss of alleles on chromosome 2 has also been reported [11], but there have been no reported cytogenetic alterations involving this chromosome. In this report we provide complete cytogenetic information on four previously unpublished cases of ocular melanoma.

MATERIALS AND METHODS

Tumor samples were obtained from freshly enucleated eyes of patients with posterior uveal melanoma. Patients were not treated with radiation prior to enucleation. The clinical data of the patients and the histologic characterization of the tumors are summarized in Table 1.

Immediately after enucleation the tumors were excised, mechanically minced, and treated with collagenase (2 µg/ml) at 37°C for 15 minutes, then cultured in RPMI 1640 media supplemented with 10% fetal bovine serum, L-glutamine (2 mM), and gentamicin (10 ng/ml). Incubation periods for the primary cultures varied from 3 to 10 days. To obtain metaphases, the cells were treated with velban (2.5 ng/ml) for 1 hour, trypsinized, and subjected to 35 minutes of hypotonic (0.38% KCl) solution. Finally, the cells were gradually fixed with cold 3:1 methanol:acetic acid. Air-dried slides were banded by the GTG method [12]. Phytohemagglutinin-stimulated peripheral blood cultures were used to confirm constitutional karyotypes.

Fluorescence in situ hybridization (FISH), as described by Pinkel [13], was used to help identify marker chromosomes. Previously G-banded chromosomes were destained in a series of ethanol and formaldehyde/PBS washes [14]. They were then hybridized with whole chromosome composite painting probes furnished by Imagenetics (Naperville, IL).

An average of 37 cells and nine karyotypes were examined from each sample. Results presented conform to ISCN recommendations [15, 16].

Table 1 Histopathology of patient samples

Patient No.	Age (yr)	Sex	Tumor thickness (mm)	Maximum scleral dimension (mm)	Cell type	Mitosis (#/40 hpf)[a]	Melanin content	Ciliary body involvement	Infiltration of scleral lamellae
M90-002	71	F	9.5	16	Mixed	30	Moderate	Yes	Yes[b]
M91-001	73	F	9.5	13	Spindle	1	Moderate	No	No
M91-003	39	M	8.0	11	Mixed	7	Moderate	Yes	Yes
M91-019	58	M	12.0	19	Mixed	0	High	Yes	No
M91-029	83	F	7.0	12	Mixed	4	Moderate	Yes	Yes
M91-044	57	M	11.5	18	Epithelioid	0	High	Yes	No
M91-045	81	M	8.0	15	Epithelioid	11	Low	Yes	Yes

[a] High-power field.

[b] Extrascleral extension.

RESULTS

Primary tumor samples were obtained from seven patients with posterior uveal melanoma. It was possible to obtain analyzable metaphases from four samples. The cytogenetic results are summarized in Table 2.

M90-002

Figure 1 illustrates the stemline karyotype of case M90–002: 46,XX, – 3, + i(4)(p10),inv(8)(p21.3q11.2),i(8)(q10), + i(8)(q10), der(16)t(16;21)(q11;q11), – 17, – 21, + mar. Seventy-nine per cent of the cells analyzed demonstrated the stemline karyotype, whereas 7% had nonclonal rearrangements, and 14% were karyotypically normal. Monosomy for chromosome 3 and i(8)(q10), resulting in a net gain of 8q and a monosomy of 8p (Fig. 6), were the most common chromosomal aberrations observed in this case. Cytogenetic analysis of peripheral blood cells from this patient was also performed and the inv(8) was found in all cells examined; demonstrating this to be a constitutional alteration.

M91-003

Analysis of the tumor sample of this patient displayed no cytogentically visible structural chromosomal rearrangement, but did reveal monosomy 3 in 88% of the cells examined.

M91-029

The stemline karyotype of case M91-029 demonstrated monosomy 3 and an additional chromosome 8, or alternatively, an i(8)(q10) as clonal chromosomal alterations (Table 2). A representative karyotype of the stemline: 46,XX, – 3, + 8 was identified in 58% of the cells analyzed (Fig. 2). In 8% of the cells there was an i(8)(q10) instead of + 8, resulting in a significant over-representation of 8q (inset to Fig. 2; Table 2). The 15p + chromosome identified in this case was further analyzed using C-banding and the additional material on the p arm was identified as C-band positive heterochromatin (data not shown). The 15p + was also observed in karyotypes from normal cells of the patient, confirming that this was a constitutional polymorphism.

Table 2 Summary of cytogenetic results

Patient No.	% of cells analyzed	Karyotype
M90-002	79	46,XX, – 3, + i(4)(p10),inv(8)(p21.3q11.2),i(8)(q10), + i(8)(q10), der(16)t(16;21)(q11;q11), – 17, – 21, + mar[22]
	14	46,XX,inv(8)(p21.3q11.2)[4]
	7	NC[2]
M91-001	ND	
M91-003	88	45,XY, – 3[29]
	3	46,XY[1]
	9	NC[3]
M91-019	ND	
M91-029	58	46,XX, – 3, + 8[31]
	8	46,XX, – 3, + i(8)(q10)[4]
	4	45,XX, – 3, + 8,der(8)t(8p;14p), – 10[2]
	30	NC[16]
M91-044	74	46,XY,idic(1)(pter→q23::q23→pter),der(6)t(6;8)(q12;q13.1),der(9)t(6;9)(p12;p23)[26]
	26	46,XY,der(6)t(6;8)(q12;q13.1),der(9)t(6;9)(p12;p23)[9]
M91-045	ND	

Abbreviations: NC = non-clonal aberrations; ND = no analyzable metaphases obtained.
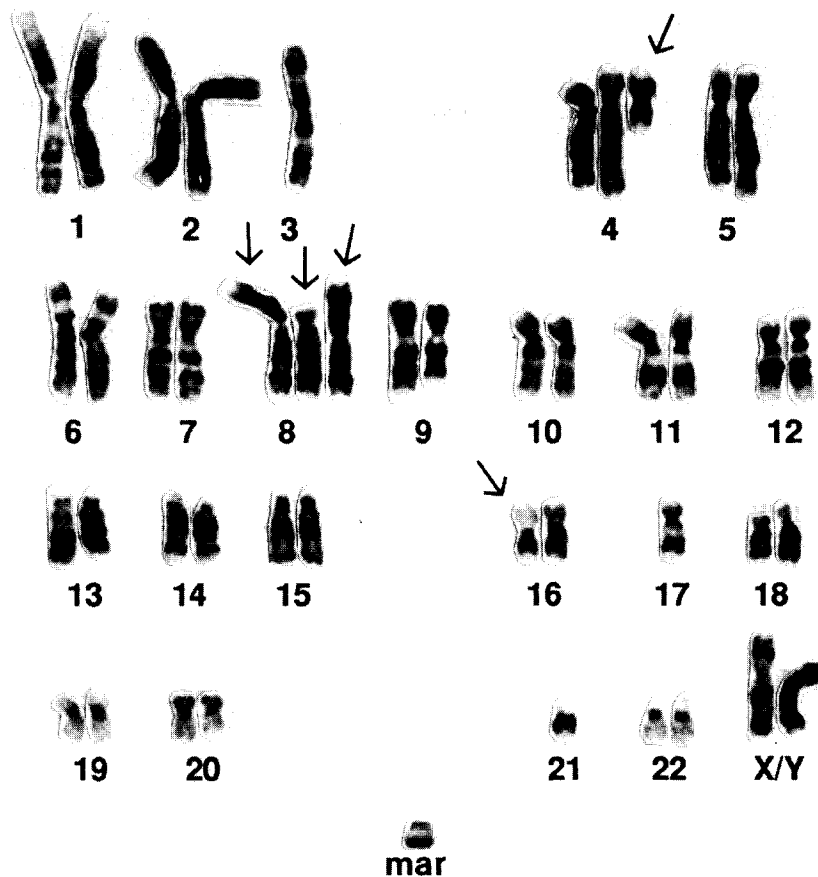
Figure 1 A representative karyotype from case M90-002. 46,XX, − 3, + i(4)(p10),inv(8)(p21.3q11.2),i(8)(q10), + i(8) (q10),der(16)t(16;21)(q11;q11), −17, − 21, + mar. The arrows indicate the marker chromosomes.

M91-044

The stemline karyotype of case M91-044 included two different translocations, resulting in partial trisomies for 6p and 8q and partial monosomies for 9p and 6q, and a novel isodicentric chromosome 1 (Table 2; Fig. 3). Seventy-four per cent of the cells had the isodicentric chromosome, whereas the remaining 26% only had the two translocated chromosomes. The der(6)t(6;8)(q12;q13.1) chromosome resulted in a partial trisomy of 8q13.1→qter and a partial monosomy of 6q12→qter; the der(9)t(6;9)(p12;p23) chromosome yielded a partial trisomy of 6p12→pter and a partial monosomy of 9p23→pter. The breakpoints of the nonreciprocal translocations were identified following comparison of the translocated chromosomes to their respective normal chromosomes and confirmed by FISH (Fig. 4).

The novel marker chromosome was shown to have markedly different contraction rates on either side of the chromosome (Fig. 5). Low-resolution banding made it difficult to characterize the marker chromosome (Fig. 5A); however, it was possible to discern that the marker was composed entirely of chromosome 1 using FISH (Fig. 5C). Comparison of high-resolution banding of the marker chromosome 1 to a normal chromosome 1 of equivalent length suggested that

it was an isodicentric chromosome with the breakpoint located in band q23 (Fig. 5B). This unusual pattern of differential contraction has been previously identified in other human cancers [17].

In summary, monosomy 3 was the sole cytogenetic abnormality present in one of the tumors analyzed in this study. Two cases had a trisomy for chromosome 8 or, alternatively, an i(8)(q10), along with monosomy 3 as the primary aberrations. Another case showed two novel translocations and a unique isodicentric chromosome. The der(6)t(6;8)(q12;q13.1) chromosome resulted in a partial trisomy and monosomy of 8q13.1→qter and 6q12→qter, respectively; the der(9)t(6;9) (p12;p23) chromosome gave rise to a partial trisomy of 6p12→pter and a partial monosomy of 9p23→pter. The isodicentric marker chromosome, idic(1)(q23), exhibited a differential rate of contraction on either side of the breakpoint. Additionally, a subclone of this sample contained only the two translocations.

DISCUSSION

Informative cytogenetic analysis was performed on four of seven posterior uveal melanomas. As recognized in earlier
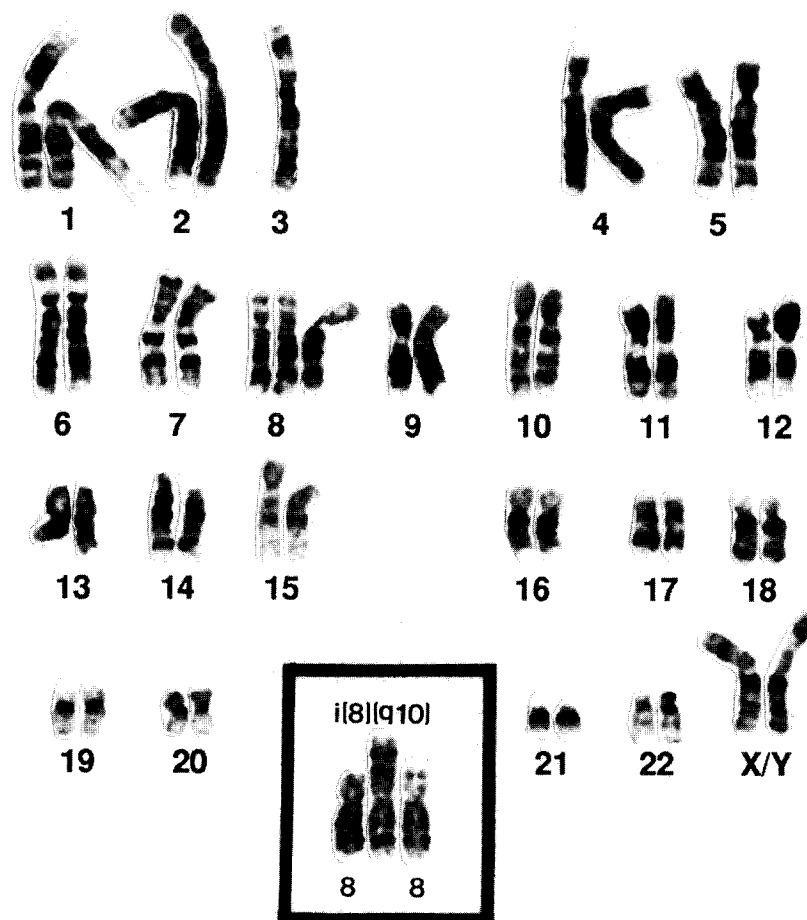
Figure 2 An example of a karyotype from case M91-029. 46,XX, - 3, + 8. The inset shows an example of two copies of normal chromosome 8 in addition to an i(8)(q10) present in a subpopulation of cells.

studies, chromosomes 3, 6, and 8 are frequently altered in this malignancy [5-10]. Loss of an entire chromosome 3 was the sole visible cytogenetic aberration observed in case M91-003 (Table 2). This is the first reported incidence of mono-somy 3 as the only chromosomal abnormality in uveal mela-noma, further suggesting the importance of chromosome 3 in the etiology of this disorder. Case M90-002 was shown to display monosomy of chromosome 3 and an i(8)(q10) as clonal aberrations. In addition, a combination of monosomy 3 and trisomy 8, or alternatively, an i(8)(q10), were the major abnormalities observed in case M91-029. The final case, M91-044, illustrated two different translocations with chromo-somes 6 and 8 [der(9)t(6;9)(p12;p23) and der(6)t(6;8)(q12; q13.1)], which supports the importance of loci on 6 and 8q in the development or progression of this disorder.

Including the four cases reported in this article, there are now a total of 26 cases of cytogenetically characterized posterior uveal melanomas published in the world literature [5-9]. Figure 6 presents a summary of the reported clonal chromosomal alterations, including a net loss of chromo-somes 3 and 6q and a net gain of 6p and 8q, observed in all published cases. To date, 50% (13/26) of cases are monosomic for chromosome 3; 31% (8/26) have aberrations producing a partial monosomy of 6q as well as an over-representation of 6p; 62% (16/26) have multiple copies of 8q, and 23% (6/26) of the cases revealed a loss of 8p. Over- and under-representa-tion of loci on chromosomes 6 and 8 might be important in the mechanisms governing this malignancy. Many of the cases had varying combinations of the aberrations observed with these three chromosomes. The only ocular melanoma with a sole clonal alteration to date is from patient M91-003 in this report, who exhibited a monosomy 3 as its only clonal chromosomal aberration.

As described above, increases in the copy numbers of 6p and 8q and a partial reduction of loci on 6q are observed in posterior uveal melanomas (Fig. 6). Griffin et al. reported a case in which a translocation, t(6;21)(p10;q10), resulted in a trisomy of 6p. Sisley et al. reported a case in which a trisomy of 6p21→pter resulted from a nonreciprocal translocation, t(6;11)(p21;p15), without any other abnormalities involving chromosomes 3 or 8. This is the smallest reported region of chromosome 6 observed to be over-represented in uveal mela-
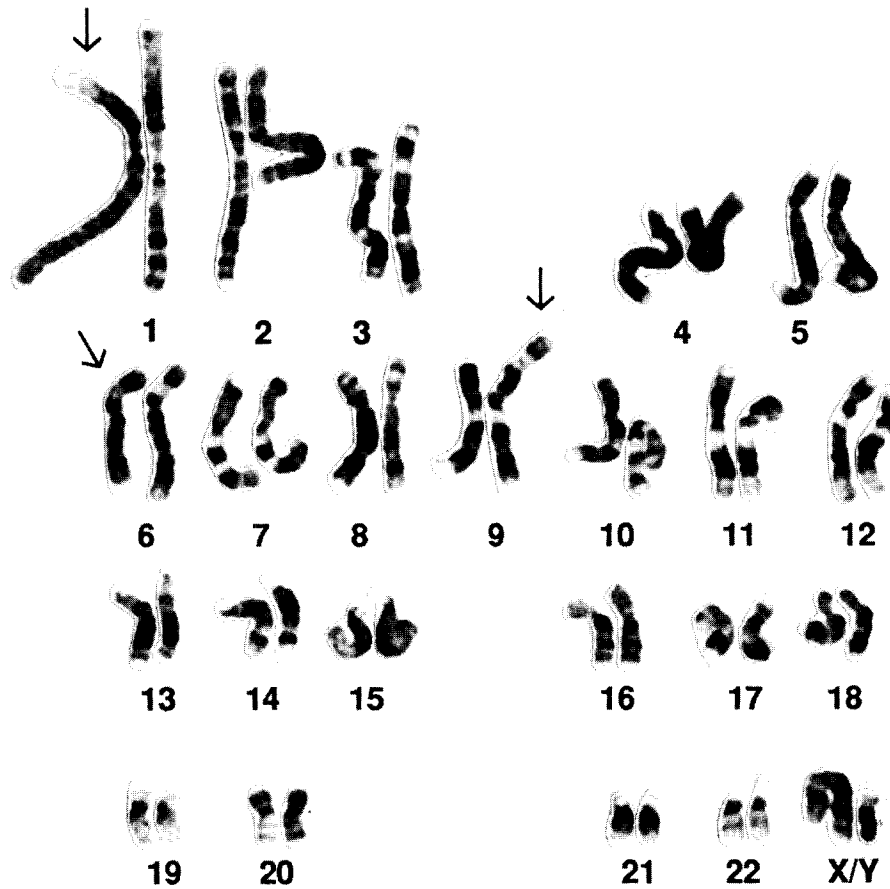
Figure 3 An example of a karyotype from case M91–044. 46,XY,idic(1)(q23),der(6)t(6;8)(q12;q13.1),der(9)t(6;9)(p12;p23). The arrows indicate the marker chromosomes.

noma (Fig. 6). The smallest over-represented region of 8q, (8q21→qter), and the smallest under-represented region of 6q, 6q22→qter, were reported by Prescher et al. (1990) (Fig. 6). These aforementioned studies, in addition to the data obtained from case M91-044, provide further evidence that

Figure 4 Examples of non reciprocal translocation chromosomes from case M91-044. A) The t(6;8)(q12;q13.1) chromosome is compared to normal chromosomes 6 and 8. B) Comparison of t(6;9)(p12;p23) with normal chromosomes 6 and 9.
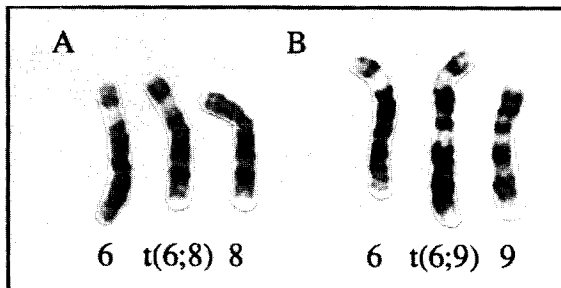
regions 6p21→pter, 6q22→qter, and 8q21→qter are potentially important in this malignancy. A partial monosomy of 9p was observed only in case M91-044, suggesting that it was potentially important in the development of uveal melanoma in this patient. Further cytogenetic and molecular analyses need to be performed to investigate the possible involvement of loci on 9p in posterior uveal melanoma.

Although abnormalities of chromosome 1 are the most frequent alterations in cutaneous malignant melanoma [18], there are surprisingly few reported for uveal melanoma. No consistent net gain or loss of any portion of chromosome 1 has been observed. The most unusual alteration of chromosome 1 reported to date was seen in our case M91-044, in which there was an isodicentric chromosome 1. We observed a differential banding pattern on either side of the breakpoint (Fig. 5), and it is possible that this translocation might have been responsible for the abnormal pattern of chromatid condensation. Similar differential condensation has been reported previously [17], although the mechanism responsible for this change has not been determined.

In addition to chromosome 1, nonrandom rearrangements of chromosomes 6, 7, and 11 are frequently associated with
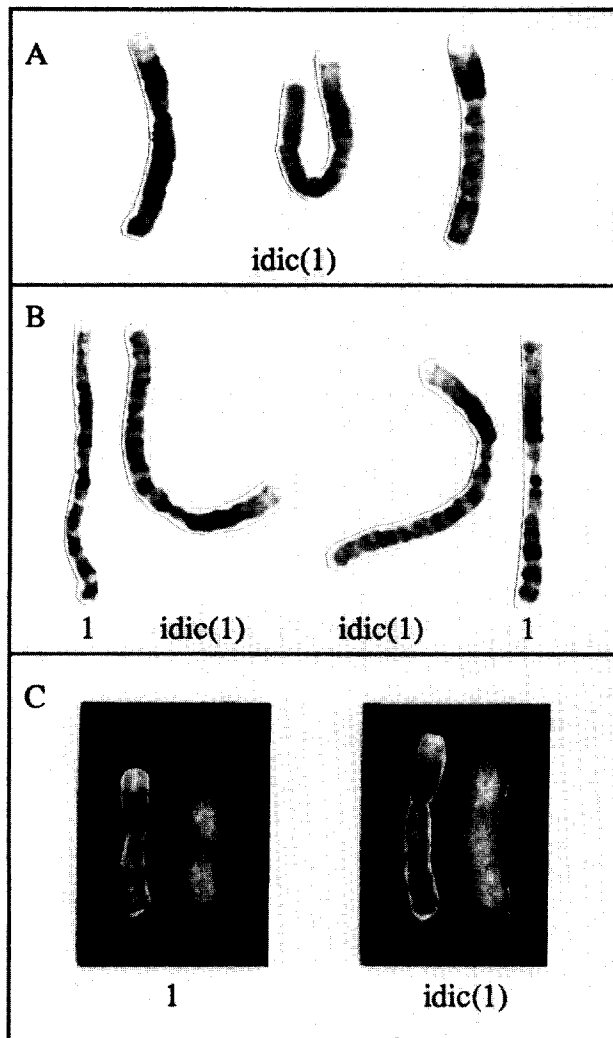
Figure 5   Idic(1)(q23) from case M91–044. A) Examples of low-resolution G-banding isodicentric chromosome 1 from three different cells. B) Comparison of high-resolution G-banded isodicentric chromosome 1 with a normal chromosome 1. C) The normal and isodicentric chromosome 1 were analyzed sequentially by G-banding followed by FISH. The chromosomes were identified with biotinylated chromosome painting probes.

cutaneous melanoma. In contrast, chromosome 3, 6, and 8 are the most consistently altered in posterior uveal melanoma. Chromosome 6 is the only common chromosome that is rearranged in these two types of melanoma. Although 6q is most frequently involved with cutaneous melanoma [18], 6p and 6q are altered in uveal melanoma. The distinct cytogenetic characteristics of cutaneous and uveal melanomas indicate that different loci and, therefore, defects in different genes are likely to be involved in the genesis and progression of these cancers.

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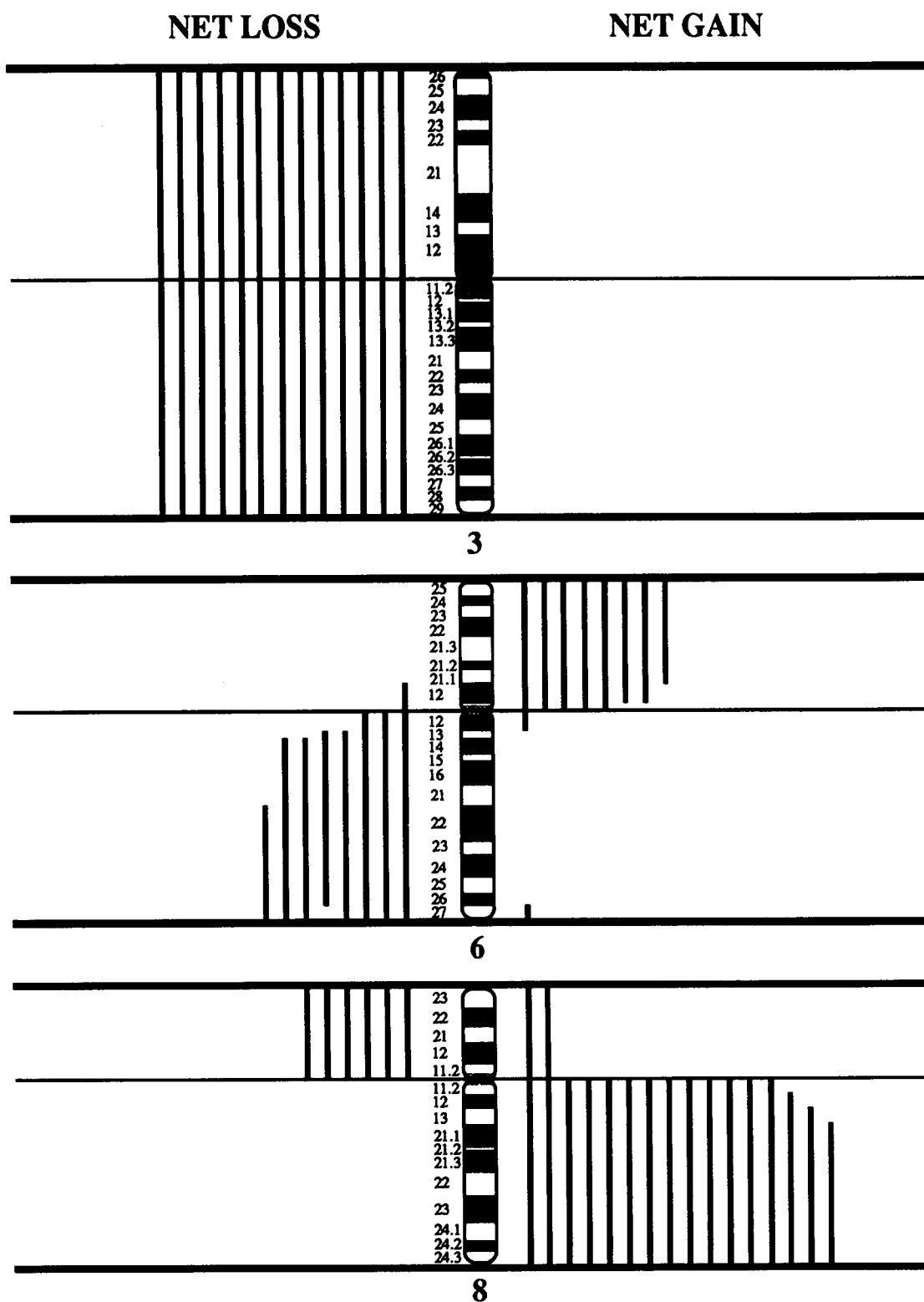
Figure 6 Summation of the recurring aberrations involving chromosomes 3, 6, and 8 from 26 reported cases of uveal melanoma [5–9]. This representation is based on the combined clonal numerical and structural abnormalities reported.