

GAIN OF CHROMOSOME ARM 17q AND ADVERSE OUTCOME IN PATIENTS WITH NEUROBLASTOMA

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ABSTRACT

Background Gain of genetic material from chromosome arm 17q (gain of segment 17q21–qter) is the most frequent cytogenetic abnormality of neuroblastoma cells. This gain has been associated with advanced disease, patients who are ≥ 1 year old, deletion of chromosome arm 1p, and amplification of the *N-myc* oncogene, all of which predict an adverse outcome. We investigated these associations and evaluated the prognostic importance of the status of chromosome 17.

Methods We compiled molecular cytogenetic analyses of chromosome 17 in primary neuroblastomas in 313 patients at six European centers. Clinical and survival information were collected, along with data on 1p, *N-myc*, and ploidy.

Results Unbalanced gain of segment 17q21–qter was found in 53.7 percent of the tumors, whereas the chromosome was normal in 46.3 percent. The gain of 17q was characteristic of advanced tumors and of tumors in children ≥ 1 year of age and was strongly associated with the deletion of 1p and amplification of *N-myc*. No tumor showed amplification of *N-myc* in the absence of either deletion of 1p or gain of 17q. Gain of 17q was a significant predictive factor for adverse outcome in univariate analysis. Among the patients with this abnormality, overall survival at five years was 30.6 percent (95 percent confidence interval, 21 to 40 percent), as compared with 86.0 percent (95 percent confidence interval, 78 to 91 percent) among those with normal 17q status. In multivariate analysis, gain of 17q was the most powerful prognostic factor, followed by the presence of stage 4 disease and deletion of 1p (hazard ratios, 3.4, 2.3, and 1.9, respectively).

Conclusions Gain of chromosome segment 17q21–qter is an important prognostic factor in children with neuroblastoma. (N Engl J Med 1999;340:1954–61.)

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GENETIC studies have an important role in formulating the prognosis in children with neuroblastoma, because certain acquired genetic abnormalities in the tumor cells correlate closely with the clinical outcome.¹ Established indicators of the aggressiveness of the tumor and poor outcome include the deletion of the short arm of chromosome 1 (1p),² the amplification of the *N-myc* gene,³ and near diploidy or near tetraploidy.² Conversely, the presence of 1p, single copies of *N-myc*,

near triploidy, and expression of the *TRK* gene⁴ are significantly associated with a favorable prognosis.

Rearrangements of chromosome 17 in neuroblastoma cells, particularly those resulting in a gain of material from the long arm of the chromosome (17q), were first described in the early 1980s,^{5,6} but the extent of such abnormalities in neuroblastoma cells was not apparent until fluorescence in situ hybridization and comparative genomic hybridization were used to study neuroblastoma cells.^{7–15} These methods revealed that the gain of material from chromosome 17 is the most frequent genetic abnormality of neuroblastoma cells, with an incidence ranging from 63 to 83 percent. This gain may consist of an entire chromosome 17 (e.g., tetrasomy 17 in a triploid tumor) or of only the distal segment of the long arm, 17q21–qter. Such a partial gain is strongly associated with risk factors: age of more than one year, presence of advanced disease, deletion of 1p, amplification of the *N-myc* gene, and unfavorable ploidy.

The principal mechanism underlying partial gain of 17q is an unbalanced translocation, with a variety of partner chromosomes.^{7–9,15} The segment on the partner chromosome distal to the breakpoint is lost, and a segment of 17q translocates to that site. The translocated segment is in effect an extra copy, because the cell also contains two or more normal chromosomes 17. Hence, these translocations result in unbalanced gain of the distal segment of 17q. The most common site of the translocation is 1p, where the gain of the distal portion of 17q is linked with the loss of 1p (Fig. 1A and 1B).

In 1995, Caron¹⁶ reported that 20 of 53 neuro-

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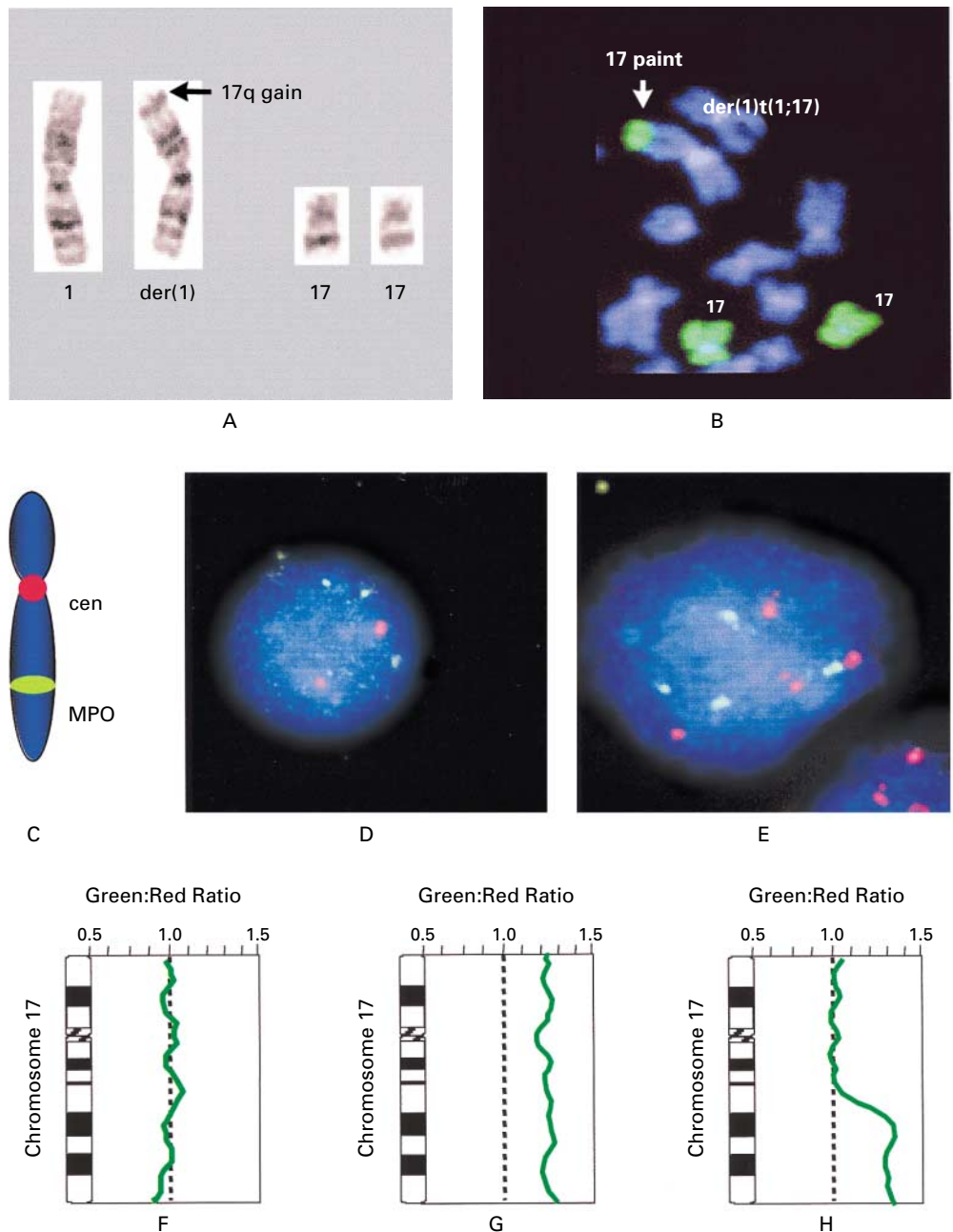


Figure 1. Techniques Used to Determine Chromosome 17 Status.

Panel A shows the results obtained with cytogenetic analysis. G-banding identifies a gain of 17q12–qter as an unbalanced translocation with 1p. Panel B shows the results obtained with fluorescence in situ hybridization of cells in metaphase. A paint probe for chromosome 17 identifies a gain of genetic material from chromosome 17 on the short arm of chromosome 1 in addition to two normal copies of chromosome 17. Panel C shows the results obtained with fluorescence in situ hybridization of cells in interphase in which rhodamine-labeled centromeric probe and fluorescein-labeled myeloperoxidase (MPO) probe have been used. Panel D shows the nucleus of a tumor with 17q gain by fluorescence in situ hybridization of cells in interphase (2 centromeric signals [cen], 3 MPO signals). Panel E shows a whole chromosome gain in a triploid tumor, also by fluorescence in situ hybridization of cells in interphase (4 centromeric signals, 4 MPO signals). Panel F shows the results of comparative genomic hybridization for chromosome 17 with a green:red ratio of 1.0, indicating no gain with respect to ploidy. In Panel G the color ratio shifted to 1.25, indicating gain of a whole chromosome. Panel H shows results of comparative genomic hybridization indicating gain of distal q arm (q21–qter).

blastomas (38 percent) had gain of 17q and that patients with such tumors had a significantly worse event-free survival at two years than those without this feature. However, when the series was expanded sufficiently to allow multivariate analysis,¹⁷ the predictive power of the 17q gain was marginal and was not apparent when 1p or N-*myc* status was added to the model. Using cytogenetic techniques and fluorescence in situ hybridization, Łastowska et al.¹⁸ identified 17q gain in 28 of 45 neuroblastomas (62 percent) and found that overall survival for patients with 17q gain was 13.5 percent at three years, as compared with 100 percent for those without this abnormality. Multivariate analysis revealed a powerful independent effect of 17q gain after other clinical and genetic variables were taken into account.

In this study, we sought to confirm the association of unbalanced 17q gain with other indicators of high risk in patients with neuroblastoma and to test the hypothesis that this abnormality is an independent predictor of tumor aggressiveness and poor clinical outcome. To this end, we assembled a large series of analyses in which the status of chromosome 17 was known and related this information to well-established clinical and genetic risk factors and to rates of relapse and mortality.

METHODS

Tumors

Inclusion in the study was based solely on the ability to define the status of chromosome 17. The data on 313 patients with neuroblastoma for which the status of chromosome 17 in tumor cells was established were collected from six European centers. The tumor stage was classified according to the International Neuroblastoma Staging System.¹⁹ Similar treatment regimens were used in the various centers for patients of the same age and with the same stage of disease.

Genetic Analysis

Four centers provided results of comparative genomic hybridization. A complete description of this procedure is reported elsewhere.^{10-14,20,21} In brief, tumor DNA and normal DNA from reference tissue were extracted and labeled by nick translation for differential fluorescence detection (fluorescein vs. rhodamine). Equal amounts of tumor and reference DNA were mixed and hybridized to lymphocytes in metaphase. Images were captured by a photomultiplying camera, and image-analysis software was used to calculate the ratio of red to green fluorescence along chromosome lengths. Control experiments (competitive hybridization of differentially labeled samples of normal DNA) were carried out to establish the fluorescence-ratio thresholds for gain or loss of chromosome regions.

Three centers contributed results from cytogenetic analysis supplemented by fluorescence in situ hybridization.^{9,15} Chromosomes were prepared and banded according to standard protocols. In some cases, the status of chromosome 17 was confirmed by fluorescence in situ hybridization of cells in metaphase by using chromosome 17 paints in conjunction with either Oncor myeloperoxidase probe, which maps to 17q21.3-q23, or yeast artificial-chromosome probes mapping to 17q.

Oncor probes for either TP53 (locus 17p13) or chromosome 17 centromere in combination with differentially labeled myeloperoxidase probe were used for interphase fluorescence in situ hybridization at one center. Determining relative numbers of red

and green signals in tumor nuclei allows a balanced status of 17q to be distinguished from unbalanced gain (Fig. 1C, 1D, and 1E).

Examples of the results obtained with these techniques are shown in Figure 1. Genetic factors other than the status of 17q were assessed at individual centers by combinations of techniques. The status of 1p was determined by comparative genomic hybridization or by fluorescence in situ hybridization with the use of combinations of a pericentromeric probe for chromosome 1 with probes derived from the 1p36 region,²² analysis of DNA microsatellite markers by the polymerase chain reaction,²³ or cytogenetic analysis. The status of N-*myc* was determined by comparative genomic hybridization, fluorescence in situ hybridization, or Southern blotting, and ploidy was determined by flow cytometry or cytogenetic analysis.

Definition of the Status of Chromosome 17

The status of chromosome 17 was defined as either gain of 17q or normal. Gain of 17q denoted the gain of segment 17q21-qter. The defining characteristics of 17q gain were a breakpoint in 17q11-q21 and an extra copy of the 17q segment distal to this breakpoint in the chromosomal complement of the cell, resulting in an increase in the number of copies of distal 17q as compared with 17p. These gains almost always result from unbalanced translocations. Normal status denoted no unbalanced gain of 17q relative to 17pter-q12. It included tumors showing no gain or loss of any part of chromosome 17 (e.g., two intact copies of 17 in a diploid nucleus or three in a triploid nucleus) and tumors showing gain of an entire chromosome 17 in relation to the ploidy level of the cell (e.g., four copies in a triploid nucleus). In these cases there is no chromosome breakpoint, and there is a normal ratio of 17p to 17q.

Statistical Analysis

Logistic-regression analysis was used to test the consistency of the findings from the various centers. Descriptive statistical analysis used χ^2 tests to identify the biologic characteristics of the combined data set. In particular, the distribution of 17q gain was determined in relation to established clinical and genetic factors.

The Kaplan-Meier method²⁴ and the log-rank test were used to estimate survival and progression-free survival. The predictive significance of age, tumor stage, ploidy, and 1p, N-*myc*, and 17q status were tested in univariate analyses. After performing a stratified subgroup analysis, we applied a stepwise Cox proportional-hazards model (successively rejecting nonsignificant variables) to test the hypothesis that 17q status has an independent influence on outcome and to quantify the influence of 17q status with respect to other clinical and genetic factors. In a separate analysis, a multivariate model incorporating tumor stage, age, and N-*myc* status was extended to include 17q status in order to test the additional predictive power that the variable of 17q status provides.

RESULTS

Most of the data were compiled from the results of comparative genomic hybridization (68.7 percent); in the remaining 31.3 percent of cases, the status of chromosome 17 was determined by standard cytogenetic analysis supplemented with metaphase fluorescence in situ hybridization or by two-color interphase fluorescence in situ hybridization. Data on 154 of the 313 tumors (49.2 percent) have been previously reported.^{9-12,14,15}

The median age of the patients at the time of diagnosis was 2.2 years. The median duration of follow-up for survivors was 2.5 years (interquartile range, 1.0 to 4.4). Of the 313 tumors, 119 were from infants less than one year of age and 194 were from children

TABLE 1. CLINICAL AND GENETIC CHARACTERISTICS OF 313 PATIENTS WITH NEUROBLASTOMA ACCORDING TO 17q STATUS.*

CHARACTERISTIC	NORMAL 17q (N=145)	GAIN OF 17q (N=168)	P VALUE†
	no. of patients (%)		
Tumor stage‡			
1, 2, or 4S	100 (69)	24 (14)	
3	24 (17)	25 (15)	
4	21 (14)	119 (71)	<0.001
Age			
<1 yr	88 (61)	31 (18)	
≥1 yr	57 (39)	137 (82)	<0.001
Chromosome 1p			
Normal	97 (79)	44 (31)	
Deleted	26 (21)	98 (69)	<0.001
Not evaluated	22	26	
N-myc gene			
Single copy	126 (91)	84 (52)	
Amplified	13 (9)	78 (48)	<0.001
Not evaluated	6	6	
Ploidy‡			
Diploid	21 (42)	47 (62)	<0.001
Tetraploid	3 (6)	19 (25)	<0.001
Triploid	26 (52)	10 (13)	
Not evaluated	95	92	

*Percentages include all patients who could be evaluated for a particular characteristic.

†P values were calculated with the χ^2 test.

‡The test for trend with 2 df was used for tumor stage and ploidy.

one or more years of age. According to the criteria of the International Neuroblastoma Staging System, 44 of the tumors were classified as stage 1, 35 as stage 2, 49 as stage 3, 140 as stage 4, and 45 as stage 4S.

Supplementary analyses established the status of 1p in 265 of the tumors (84.7 percent); of these, 124 (46.8 percent) showed 1p deletion. Assessment of the number of N-myc copies was performed for 301 (96.2 percent), and gene amplification was detected in 91 of these tumors (30.2 percent). Ploidy could be classified in 126 tumors (40.3 percent); of these, 68 (54.0 percent) were within the near-diploid range, 36 (28.6 percent) were near-triploid, and 22 (17.5 percent) were near-tetraploid.

Of the 313 tumors, 145 (46.3 percent) had a normal 17q status and 168 (53.7 percent) showed 17q gain. Twenty-six percent of the neuroblastomas from the patients less than one year old showed 17q gain, as compared with 71 percent of the tumors from the older children. The proportions with 17q gain according to tumor stage were as follows: 20 percent in stage 1, 17 percent in stage 2, 51 percent in stage 3, 85 percent in stage 4, and 20 percent in stage 4S.

To assess the consistency of the data among centers, the results of genetic analyses (the status of 1p, N-myc and 17q) were studied by logistic-regression

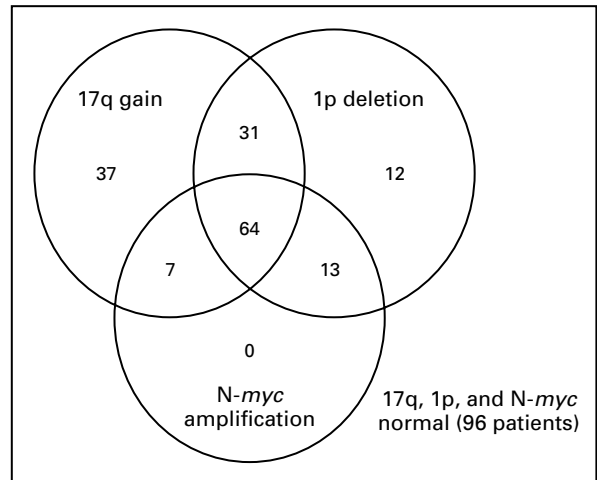


Figure 2. Interrelation of 17q Gain, 1p Deletion, and N-myc Amplification in 260 Patients with Neuroblastoma.

analysis. No significant differences between laboratories were detected ($P>0.1$ for all analyses).

Correlations with Other Clinical and Genetic Prognostic Factors

Table 1 shows the relation between clinical and genetic factors and the status of 17q. Gain of 17q was strongly associated with stage 4 disease ($\chi^2=114$, $P<0.001$ for the comparison with all other stages combined). It was also significantly associated with an age of one year or more at presentation ($\chi^2=59$, $P<0.001$). Gain of 17q was strongly associated with 1p deletion ($\chi^2=61$, $P<0.001$), N-myc amplification ($\chi^2=53$, $P<0.001$), and diploidy or tetraploidy ($\chi^2=24$, $P<0.001$), but not triploidy. Figure 2 shows the interrelation of 17q gain, N-myc amplification, and 1p deletion for the 260 tumors in which the presence or absence of all three abnormalities was known. The status of 17q, 1p, and N-myc was normal in 96 tumors, but these tumors had other acquired genetic changes.

Univariate Analysis of Survival

At five years, the overall survival for the 313 children was 55.9 percent (95 percent confidence interval, 48 to 63 percent), and progression-free survival was 46.4 percent (95 percent confidence interval, 39 to 54 percent). With a median follow-up of 24 months, the projected overall 5-year survival of the 168 patients with 17q gain was 30.6 percent (95 percent confidence interval, 21 to 40 percent), as compared with 86.0 percent (95 percent confidence interval, 78 to 91 percent) for the 145 patients whose tumors had normal 17q (Fig. 3A). This difference was significant ($P<0.001$).

In a univariate analysis of survival, age, tumor stage, 1p status, N-myc status, and ploidy were all signifi-

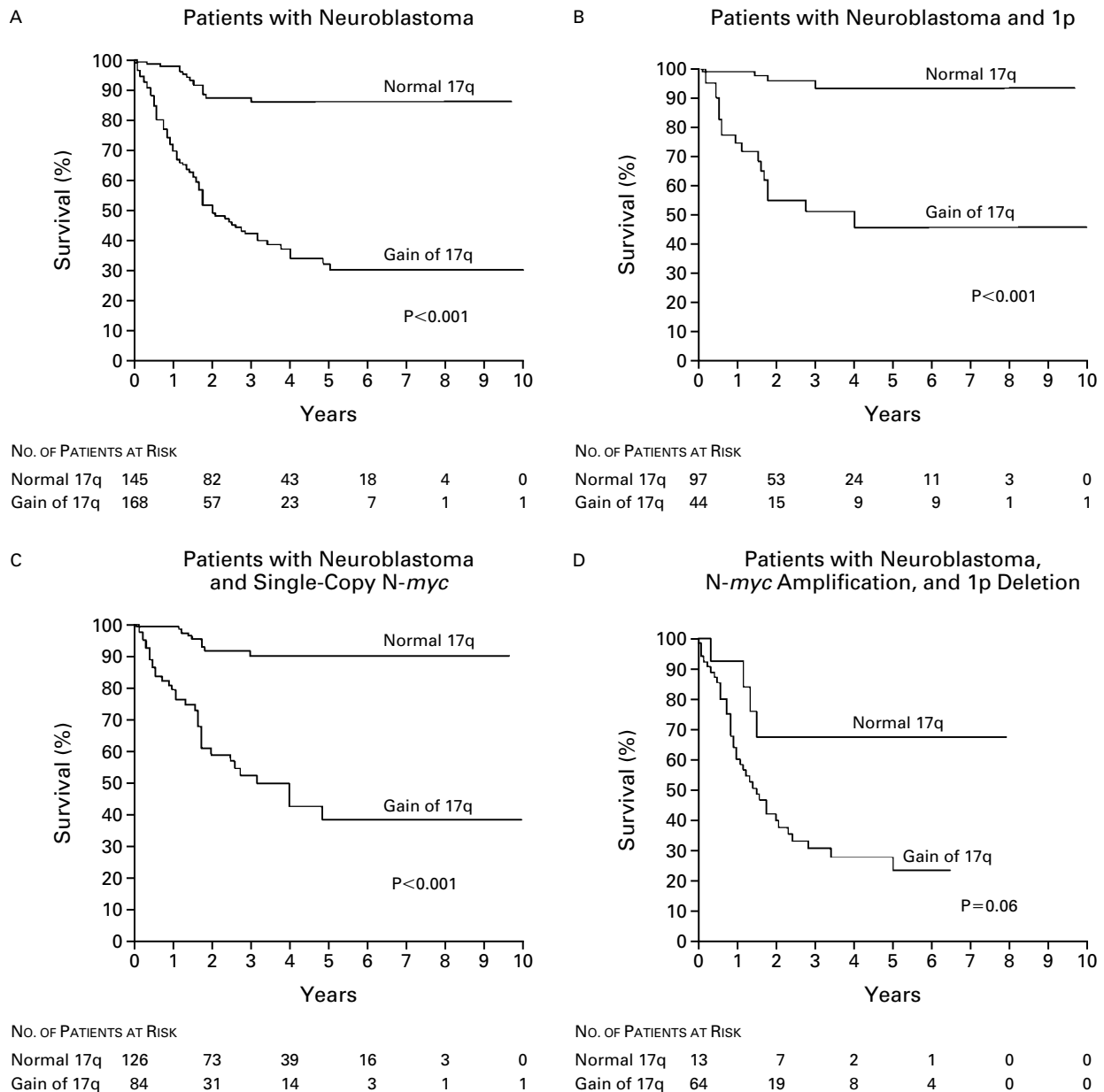


Figure 3. Survival Rates According to the Status of Chromosome 17q.

cantly associated with outcome ($P < 0.01$ according to the likelihood-ratio test) (Table 2). The discriminative power of the status of 17q was significant in subgroups in which 1p was not deleted (Fig. 3B) or *N-myc* was not amplified (Fig. 3C) ($P < 0.001$ for both). Gain of 17q in tumor cells was associated with significantly poorer outcomes in patients of any age and with tumors of stages 1, 2, 3, or 4S (Table 3). In the subgroup of tumors showing simultaneous 1p

deletion and *N-myc* amplification, there was a non-significant trend toward additional adverse effects with 17q gain (Fig. 3D).

Multivariate Analysis of Survival

A stepwise Cox proportional-hazard procedure was applied, incorporating age, tumor stage, and status of 1p, 17q, and *N-myc*, to the 260 cases in which all three genetic factors were known. Ploidy was not in-

TABLE 2. SURVIVAL RATES OF 313 PATIENTS WITH NEUROBLASTOMA ACCORDING TO CLINICAL AND GENETIC FACTORS IN UNIVARIATE ANALYSIS.*

VARIABLE AND CATEGORY	NO. OF PATIENTS	5-YEAR SURVIVAL		P VALUE†
		% (95% CI)‡		
Age				
<1 yr	119	82	(72–89)	
≥1 yr	194	42	(33–51)	<0.001
Tumor stage§				
1, 2, or 4S	124	91	(84–95)	
3	49	62	(44–76)	
4	140	25	(16–36)	<0.001
Chromosome 17q				
Normal	145	86	(78–91)	
Unbalanced gain	168	31	(21–40)	<0.001
Chromosome 1p				
Normal	141	77	(67–85)	
Deleted	124	31	(21–42)	<0.001
N- <i>myc</i> gene				
Single copy	210	69	(60–77)	
Amplified	91	28	(16–41)	<0.001
Ploidy§				
Diploid	68	35	(21–50)	0.008
Tetraploid	22	44	(13–72)	
Triploid	36	88	(71–95)	

*Survival estimates and comparisons exclude patients who were not evaluated for the particular category.

†P values were calculated with the log-rank test.

‡CI denotes confidence interval.

§Comparisons of tumor stage and ploidy were calculated by the log-rank test for trend.

cluded in the multivariate analysis because of the relative paucity of data (ploidy was established for only 40 percent of the tumors). Age and the status of N-*myc* were excluded from the final model (score test, P=0.4 and P=0.2, respectively). Remaining predictors of adverse outcomes were 1p deletion (hazard ratio, 1.9; 95 percent confidence interval, 1.1 to 3.2; P=0.02), stage 4 disease (hazard ratio 2.3; 95 percent confidence interval, 1.3 to 4.0; P=0.004), and 17q gain (hazard ratio, 3.4; 95 percent confidence interval, 1.7 to 6.6; P<0.001). When a multivariate model incorporating age, tumor stage, and N-*myc* status was extended to include the status of 17q, a significant increase in predictive power was apparent (likelihood-ratio statistic, +15.0; P<0.001).

DISCUSSION

The selection of the 313 neuroblastomas that were included in this series was based on the ability to determine the status of chromosome 17. With regard to clinical characteristics, this series of patients is broadly typical of other series of patients with this tumor.²⁵ We found that 54 percent of the tumors showed gain of 17q12–qter (i.e., a gain of chromosomal material from the distal end of the long arm of chromosome 17), whereas 46 percent had either

TABLE 3. SURVIVAL RATES OF 313 PATIENTS WITH NEUROBLASTOMA STRATIFIED ACCORDING TO 17q STATUS AND CLINICAL AND GENETIC FACTORS.*

VARIABLE AND CATEGORY	NO. OF PATIENTS	5-YEAR SURVIVAL		P VALUE†
		NORMAL 17q	17q GAIN	
		% (95% CI)‡		
Age				
<1 yr	119	94 (84–98)	49 (26–69)	<0.001
≥1 yr	194	77 (61–87)	27 (17–38)	<0.001
Tumor stage				
1, 2, or 4S	124	95 (87–98)	75 (50–89)	0.01
3	49	81 (50–94)	45 (22–66)	0.01
4	140	52 (26–74)	22 (13–33)	0.09
Chromosome 1p				
Normal	141	93 (82–98)	45 (26–62)	<0.001
Deleted	124	63 (40–79)	22 (12–35)	0.01
N- <i>myc</i> gene				
Normal	210	90 (81–95)	38 (21–54)	<0.001
Amplified	91	67 (34–86)	22 (12–36)	0.05
Ploidy				
Diploid	68	83 (57–94)	19 (7–34)	<0.001
Tetraploid	22	100	35 (7–66)	0.4
Triploid	36	100	49 (13–78)	0.001

*Survival estimates and comparisons exclude patients who were not evaluated for the particular category.

†P values were calculated with the log-rank test.

‡CI denotes confidence interval.

no gain of chromosome 17 or an additional copy of the whole chromosome.

Our results confirm that 17q gain in neuroblastoma is associated with advanced-stage disease and with tumors in older children rather than infants. Gain of 17q was very strongly linked to both 1p deletion and N-*myc* amplification; indeed, N-*myc* amplification was not found in any tumor without 1p deletion, 17q gain, or both.

In the univariate analysis of survival, the status of 17q was a significant predictor of clinical outcome, as were other clinical and genetic factors. In subgroup analyses, 17q gain was a significant predictive factor within tumor stage, in both infants and children one year old or more, and in cases in which N-*myc* was not amplified or 1p was not deleted. In the stepwise multivariate analysis, the status of 17q was the most significant predictive factor for clinical outcome, followed by stage 4 disease and the status of 1p. The status of N-*myc* and age were not statistically significant in this analysis.

Although neuroblastoma cells contain a variety of chromosomal aberrations, abnormalities of chromosome 17 are the most common. Moreover, gain of 17q21–qter is strongly associated with tumor progression. We found significant correlations between

17q gain and established clinical and genetic risk factors. As noted previously,^{6-9,15} a direct relation between 1p deletion and 17q gain is the well-recognized unbalanced translocation t(1p;17q); it seems likely that this rearrangement accounts for a high proportion of 1p deletions in patients with neuroblastoma. Further cytogenetic data will be necessary to clarify the contribution of translocations of 17q to other sites of reported loss of heterozygosity in neuroblastoma cells.

In this study, N-*myc* amplification was not found in any tumor without concurrent 1p deletion, 17q gain, or both. Caron also showed N-*myc* amplification to be a subcategory of 1p loss and 17q gain, albeit in a much smaller number of tumors.¹⁶ This finding suggests that N-*myc* amplification is a late event in the sequence of genetic lesions contributing to neuroblastoma.

The relation between 17q gain and N-*myc* amplification is obscure. Juxtaposition of amplified N-*myc* and 17q material has repeatedly been observed in neuroblastoma cell lines,^{7,8} but this finding is very rare in primary tumors, in which N-*myc* amplification usually takes the form of double minute chromosomes; involvement of 17q in these structures has not been demonstrated. The functional interrelations of 17q and N-*myc* (and 1p) remain to be clarified.

A number of potentially important genes, such as *nm23-H1* and the survivin gene (which are involved in inhibiting apoptosis) and the gene for nerve growth factor receptor (which is underexpressed in neuroblastoma cells),²⁶ are located in the commonly gained segment of 17q. Investigations of such genes may reveal the mechanism of the effect of 17q gain on the behavior of neuroblastoma cells. Preliminary mapping has identified at least seven breakpoints within the proximal half of 17q in neuroblastomas and neuroblastoma cell lines^{9,26}; this finding argues against a specific gene event (e.g., gene fusion), but coupled with the gain of 17q material, may implicate a gene dose effect. Loss of heterozygosity at the translocation partner sites may also be important.

The implications of our findings for clinical management follow from the increasing tendency to tailor therapy on an individual basis according to risk factors detected at the time of diagnosis. The objective is to direct the most intensive treatments to children with the most aggressive tumors, while sparing other children from the adverse effects of unnecessarily intensive therapy. For neuroblastoma, N-*myc* gene amplification has an important role in determining risk and is used internationally to stratify therapy for infants and for children with localized tumors. Specifically, infants with stage 4S disease without N-*myc* amplification who are clinically well are simply observed, whereas those with N-*myc* amplification with identical clinical features receive intensive chemotherapy followed by surgery, radiotherapy, and consolidation with myeloablative therapy.^{27,28} However, the pres-

ence of N-*myc* amplification does not ensure completely accurate prognostic grouping, since 20 percent of infants with metastatic disease and N-*myc* amplification in the tumor are long-term survivors and 20 percent of patients without N-*myc* amplification have recurrence of tumors (Gerrard M: personal communication).

The ongoing Localized Neuroblastoma European Study is investigating the hypothesis that 1p allelic loss is predictive of tumor recurrence in patients with localized resectable (stage 2) neuroblastomas without N-*myc* amplification. Preliminary results suggest that not all recurring tumors have 1p deletion. Therefore, additional molecular genetic markers are needed to identify which tumors with normal 1p and no amplification of N-*myc* are likely to recur.

In our series, gain of 17q emerged as a more important indicator of adverse outcome than any other clinical or genetic factor, including 1p deletion and N-*myc* amplification. The presence of this abnormality identified a larger proportion of aggressive tumors and was more closely linked to outcome than other markers. Furthermore, the status of 17q was informative in all tumor stages and was predictive of the outcome in infants and older children with neuroblastoma and in patients with tumors in which N-*myc* and 1p were both normal. We propose that the detection of 17q gain in individual tumors at the time of diagnosis should be accorded a priority equal to that of the determination of the status of 1p and N-*myc*. In addition, we suggest that investigation of the status of chromosome 17 should be incorporated into future clinical trials in patients with neuroblastoma.

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