

ORIGINAL ARTICLE

## Chromosome 1p and 11q Deletions and Outcome in Neuroblastoma

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### ABSTRACT

#### BACKGROUND

Neuroblastoma is a childhood cancer with considerable morbidity and mortality. Tumor-derived biomarkers may improve risk stratification.

#### METHODS

We screened 915 samples of neuroblastoma for loss of heterozygosity (LOH) at chromosome bands 1p36 and 11q23. Additional analyses identified a subgroup of cases of 11q23 LOH with unbalanced 11q LOH (unb11q LOH; defined as loss of 11q with retention of 11p). The associations of LOH with relapse and survival were determined.

#### RESULTS

LOH at 1p36 was identified in 209 of 898 tumors (23 percent) and LOH at 11q23 in 307 of 913 (34 percent). Unb11q LOH was found in 151 of 307 tumors with 11q23 LOH (17 percent of the total cohort). There was a strong association of 1p36 LOH, 11q23 LOH, and unb11q LOH with most high-risk disease features ( $P < 0.001$ ). LOH at 1p36 was associated with amplification of the *MYCN* oncogene ( $P < 0.001$ ), but 11q23 LOH and unb11q LOH were not ( $P < 0.001$  and  $P = 0.002$ , respectively). Cases with unb11q LOH were associated with three-year event-free and overall survival rates ( $\pm$ SE) of  $50 \pm 5$  percent and  $66 \pm 5$  percent, respectively, as compared with  $74 \pm 2$  percent and  $83 \pm 2$  percent among cases without unb11q LOH ( $P < 0.001$  for both comparisons). In a multivariate model, unb11q LOH was independently associated with decreased event-free survival ( $P = 0.009$ ) in the entire cohort, and both 1p36 LOH and unb11q LOH were independently associated with decreased progression-free survival in the subgroup of patients with features of low-risk and intermediate-risk disease ( $P = 0.002$  and  $P = 0.02$ , respectively).

#### CONCLUSIONS

Unb11q LOH and 1p36 LOH are independently associated with a worse outcome in patients with neuroblastoma.

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**N**EUROBLASTOMA IS A CANCER OF EARLY childhood in which genomic changes in the tumor correlate with its behavior and outcome in patients.<sup>1,2</sup> The algorithm devised by the Children's Oncology Group for risk assessment in cases of neuroblastoma has been successful in distinguishing patients with aggressive disease from those with a high likelihood of cure after surgery or even observation alone. The algorithm stratifies patients into three subgroups with expected low, intermediate, and high risks of death from neuroblastoma. This system involves the use of the clinical factors of age at diagnosis, tumor stage, and the results of the Shimada method of histopathological classification, as well as the biologic factors of amplification status of the *MYCN* oncogene and DNA index.<sup>1</sup> Amplification of *MYCN*, which plays a critical part in neurodevelopment and occurs in about 20 percent of cases of neuroblastoma, was one of the first tumor-derived genetic markers that was shown to be of clinical value, and it continues to provide important prognostic information.

Whereas patients in the low-risk subgroup have an overall survival rate of more than 95 percent,<sup>3</sup> patients in the high-risk subgroup have a rate of long-term survival of less than 40 percent despite dose-intensive, multimodal therapy.<sup>4-6</sup> These differences reflect the heterogeneity of neuroblastoma. For example, many high-risk tumors have *MYCN* amplification, but more than 60 percent do not,<sup>7</sup> suggesting that there are other genetic pathways in the development of high-risk neuroblastoma.

Loss of heterozygosity (LOH; loss of one allele at a polymorphic locus) at chromosome arms 1p and 11q occurs frequently in neuroblastoma.<sup>8-12</sup> Previous studies have suggested that there is an association between LOH at 1p36 or 11q23 and features of high-risk neuroblastoma.<sup>8-11,13,14</sup> Whereas 1p36 LOH was found to be associated with *MYCN* amplification, 11q23 LOH was rarely observed in tumors with this abnormality.<sup>8,14</sup> An independent association of 1p36 LOH with decreased event-free survival has also been reported, but these studies did not include all of the prognostic factors currently in use.<sup>9,13</sup> Given that 11q23 LOH occurs primarily in tumors without *MYCN* amplification, we hypothesized that 11q23 LOH could be a useful prognostic marker, especially in cases defined as associated with low or intermediate risk. Therefore, we determined the allelic status at chromosome

arms 1p and 11q in a large series of neuroblastomas accrued from recent cooperative-group clinical trials.

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## METHODS

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### STUDY DESIGN AND PATIENTS

Eligible patients were those in whom a diagnosis of neuroblastoma had been made between July 1985 and July 2003 and who were registered for a biology study with the Children's Cancer Group (CCG B973), the Pediatric Oncology Group (POG 9047), or the Children's Oncology Group (COG ANBL00B1). The only inclusion criteria were the availability of outcome data and both tumor and nontumor genomic DNA. Tumors were classified according to the International Neuroblastoma Staging System (INSS).<sup>15</sup> Treatment was assigned according to risk group on the basis of evaluation of the patient's age at diagnosis and the INSS stage and *MYCN*-amplification status of the tumor. Other covariables included the Shimada histopathological category and the DNA index (described below). In general, patients with low-risk disease (INSS stages 1, 2, and 4S) were treated with surgery or observation only.<sup>3,16-18</sup> Patients with intermediate-risk disease (those with biologically favorable stage 3 tumors and infants with stage 4 tumors and nonamplified *MYCN*) were treated with surgery and adjuvant chemotherapy of moderate intensity.<sup>19</sup> High-risk patients (those with biologically unfavorable stage 3 tumors, infants with stage 4 tumors and amplified *MYCN*, and all patients one year of age or older with stage 4 tumors) were treated with neoadjuvant regimens of dose-intensive induction chemotherapy with alkylating agents and platinum, delayed resection of the primary tumor, radiation therapy at the primary tumor site, and in most patients, a regimen of myeloablative consolidation chemotherapy followed by autologous stem-cell rescue.<sup>4</sup>

The institutional review board of the Children's Hospital of Philadelphia approved this study, and investigators at all participating institutions obtained informed consent for a biologic study before specimens were obtained.

### Samples and Biologic Studies

Immediately after surgical removal, tumor samples were snap-frozen or placed in tissue-culture media and shipped to a central reference laboratory for

studies of tumor biology. The amplification status of *MYCN* was determined with the use of immunohistochemical analysis,<sup>20</sup> fluorescence in situ hybridization,<sup>21</sup> or Southern blotting.<sup>22</sup> Histopathological analysis was performed according to central review with the use of the method of Shimada and colleagues.<sup>23</sup> The DNA index was defined with the use of flow cytometry, as previously described.<sup>24</sup> DNA from the tumor and blood or uninvolved bone marrow was prepared with the use of anion-exchange chromatography (Qiagen).

#### *Allelic Status of Chromosome Arms 1p and 11q*

We first screened tumor samples for LOH at 1p36 and 11q23 using a panel of fluorescently labeled microsatellite markers, as previously described (chromosome 1: *D1S243*, *D1S468*, *D1S2145*, *D1S1646*, *D1S3720*, and *GGAA30B06*; chromosome 11: *D11S1760*, *D11S1338*, *D11S4090*, *D11S908*, *D11S4127*, *D11S925*, *D11S4094*, and *D11S4191*).<sup>8,9,12</sup> When possible, markers were combined in multiplex fluorescence screening panels. Samples for which there were equivocal results underwent repeated screening in a conventional uniplex polymerase chain reaction (PCR). Individual samples were analyzed with up to 35 additional markers for chromosome arm 1p and 59 additional markers for chromosome 11 (Table 1 of the Supplementary Appendix, available with the full text of this article at [www.nejm.org](http://www.nejm.org)) to confirm LOH status and map the region of deletion. Electrophoresis was performed with the use of a DNA-sequencing instrument (PerkinElmer–Applied Biosystems; model 377 or 3730) and analyzed with ABI software packages (GeneScan with Genotyper or GeneMapper). LOH at an individual marker was considered to be present when a comparison of the allelic intensity of fluorescence electropherograms yielded a score of less than 0.5 or more than 2.0 (indicating a 50 percent reduction in intensity of one tumor allele), as previously described.<sup>8,9,12</sup> A sample was considered to have LOH at 1p36 or 11q23 if there were at least two informative markers at that locus showing LOH. During the assessment of allelic status, investigators were blinded to the characteristics of the patients and to outcome data.

We distinguished among samples with LOH at every marker along chromosome 11 (referred to as whole-chromosome 11 LOH) and samples with LOH at markers on 11q with retention of 11p material (referred to as unbalanced LOH, or unb11q LOH). The assignment of the status of whole-chro-

mosome 11 LOH and unb11q LOH required the presence of at least two informative markers on 11p or proximal 11q in addition to the two or more informative markers at 11q23. This distinction was not relevant for chromosome 1, because the deletion of this entire chromosome was essentially never observed in our earlier work or in the extensive literature on comparative genomic hybridization.<sup>25,26</sup>

#### *Statistical Analysis*

Tests of association were performed with the use of Fisher's exact test. Survival curves were constructed according to the methods of Kaplan and Meier,<sup>27</sup> with standard errors according to the method of Peto,<sup>28</sup> and comparisons of the survival curves were performed with a two-sided log-rank test. Failures, or events, for the event-free survival analysis were defined as relapse, disease progression, a secondary cancer, or death. Events for the progression-free survival analysis were defined as relapse or disease progression. The time to an event was calculated as the time from study enrollment to the occurrence of the first event or the time to the last contact with the patient if no event occurred. The time to an event for the overall survival analysis was calculated as the time from study enrollment until the time of death or the time of last contact if the patient was alive. Event-free survival, progression-free survival, and overall survival rates were calculated as the rates  $\pm$ SE.

Multivariate analyses were performed with the use of a Cox proportional-hazards regression model<sup>29</sup> to identify variables that were independently predictive of outcome. A stepwise, backward model-building procedure was used to identify the variables retained in the Cox model, with a P value of less than 0.05 considered to indicate statistical significance. The patient cohort analyzed in each model was made up of all patients for whom complete data were available for the variables in the model.

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## RESULTS

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### **CHARACTERISTICS OF THE PATIENTS**

Clinicopathological characteristics of the patients and the tumors are detailed in Table 1. The cohort we studied was representative of the population with neuroblastoma as a whole.<sup>7</sup> With a median follow-up of 3.01 years, the three-year event-free and overall survival rates ( $\pm$ SE) for the entire cohort were 70 $\pm$ 2 percent and 89 $\pm$ 2 percent, respectively.

**Table 1. Number and Proportion of Patients with 1p36 LOH, 11q23 LOH, and unbl1q LOH, According to Characteristics of Patients and Tumors.\***

Variable	All Patients†		1p36 LOH‡		11q23 LOH (All Types)‡		Unbalanced 11q LOH‡	
	No. (%)	No. (%)	P Value	No. (%)	P Value	No. (%)	P Value	
All patients	915	209 (23)		307 (34)		151 (17)		
Age								
<365 days	337 (37)	52 (15)		100 (30)		29 (9)		
≥365 days	578 (63)	157 (27)	<0.001	207 (36)	0.07	122 (21)	<0.001	
INSS tumor stage								
1	198 (22)	15 (8)		43 (22)		7 (4)		
2	158 (18)	18 (11)		42 (27)		13 (8)		
3	160 (18)	31 (19)		56 (35)		23 (14)		
4	335 (37)	129 (39)	<0.001	152 (45)	<0.001	102 (30)	<0.001	
4S	50 (6)	11 (22)		9 (18)		4 (8)		
Unknown	14	—		—		—		
MYCN status								
Nonamplified	760 (84)	100 (13)		282 (37)	<0.001	137 (18)	0.002	
Amplified	145 (16)	108 (74)	<0.001	22 (15)		12 (8)		
Unknown	10	—		—		—		
Shimada histopathologic category								
Favorable	472 (57)	46 (10)		138 (29)		44 (9)		
Unfavorable	361 (43)	132 (37)	<0.001	143 (40)	0.002	93 (26)	<0.001	
Unknown	82	—		—		—		
DNA ploidy								
Hyperdiploid	445 (67)	67 (15)		149 (33)	0.007	64 (14)		
Diploid	221 (33)	57 (26)	0.001	51 (23)		38 (17)	0.36	
Unknown	249	—		—		—		
1p36 status								
No loss	689 (77)	NA		232 (34)		107 (16)		
LOH	209 (23)			70 (33)	1.00	40 (19)	0.24	
Unknown	17							
11q23 status								
No loss	606 (66)	138 (23)			NA		NA	
LOH	307 (34)	70 (23)	1.00					
Unknown	2	—						
Unbl1q LOH status								
Not unbalanced	758 (83)	167 (22)			NA		NA	
Unbalanced	151 (17)	40 (26)	0.24					
Unknown	6	—						
COG risk group								
Low	379 (43)	34 (9)		88 (23)		22 (6)		
Intermediate	145 (16)	12 (8)		53 (37)		19 (13)		
High	362 (41)	155 (43)	<0.001	155 (43)	<0.001	106 (29)	<0.001	
Unknown	29	—		—		—		

\* LOH denotes loss of heterozygosity, INSS International Neuroblastoma Staging System, NA not applicable, and COG Children's Oncology Group. Two-sided P values were calculated with the use of Fisher's exact test. For tumor stage, the P value is for the comparison between stage 4 and all other stages combined, and for risk group, for the comparison between high-risk disease and low-risk and intermediate-risk disease combined. Percentages in every row are for cases with LOH among those for which data were available within a given subgroup of patients.

† Percentages are of patients with known values.

‡ Percentages in every row are for cases with LOH among those for which data were available within a given subgroup of patients.

A risk group could not be assigned in 29 cases owing to missing data.

*Frequency and Distribution of 1p36 and 11q23 LOH*  
LOH at chromosome arm 1p was detected in 209 samples (23 percent) (Table 1), with a common region of deletion at 1p36.3.<sup>12</sup> There were significant associations between 1p36 LOH and the presence of the adverse prognostic factors age of 365 days or more ( $P<0.001$ ), INSS stage 4 disease ( $P<0.001$ ), MYCN amplification ( $P<0.001$ ), unfavorable Shimada histologic category ( $P<0.001$ ), diploidy ( $P=0.001$ ), and high-risk Children's Oncology Group status ( $P<0.001$ ).

LOH at chromosome band 11q23 was detected in 307 samples (34 percent) (Table 1 and Fig. 1). Unb11q LOH was present in 151 samples (50 percent of those with 11q23 LOH; 4 cases could not be classified). The pattern of LOH for each tumor sample was consistent with the presence of a single region of deletion. All but three deletions included chromosome band 11q23; all but five overlapped with the previously implicated region within 11q23.3.<sup>8</sup>

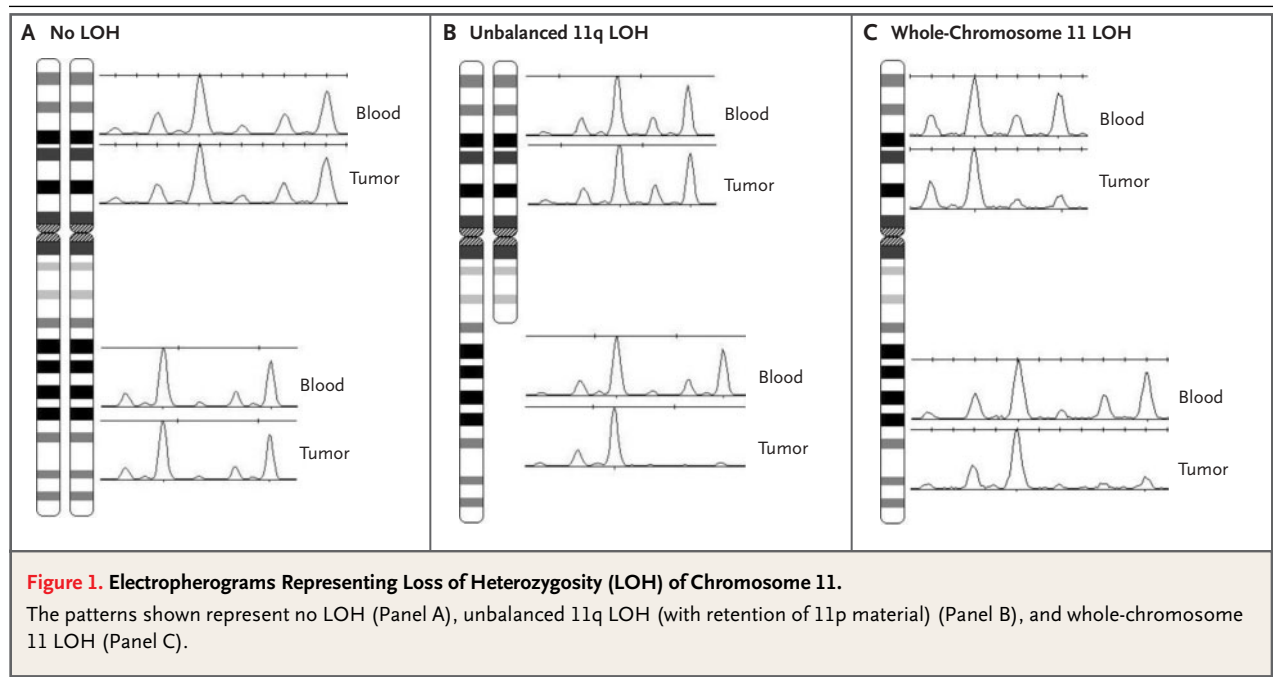
LOH at chromosome band 11q23 (without regard to 11p material [307 cases]) was significantly associated with the presence of the adverse prognostic factors of INSS stage 4 disease ( $P<0.001$ ) and unfavorable Shimada histologic category ( $P=0.002$ ) but also with the favorable prognostic factor of hy-

perdiploidy ( $P=0.007$ ) (Table 1). The subgroup of these cases defined as unb11q LOH (151 cases) had significant associations with the presence of the adverse prognostic factors age of 365 days or more ( $P<0.001$ ), INSS stage 4 disease ( $P<0.001$ ), and unfavorable Shimada histologic category ( $P<0.001$ ). Both the 11q23 LOH group and the unb11q LOH subgroup were significantly associated with tumors that did not have MYCN amplification ( $P<0.001$  and  $P=0.002$ , respectively).

*Effect of 1p36 and 11q23 LOH on Patients' Outcomes*

A univariate analysis of patients' outcomes showed that LOH at chromosome band 1p36 was significantly associated with a decreased probability of survival. Patients in whom tumors showed 1p36 LOH had three-year event-free and overall survival rates of  $47\pm4$  percent and  $64\pm4$  percent, respectively, as compared with  $77\pm2$  percent ( $P<0.001$ ) and  $85\pm2$  percent ( $P<0.001$ ), respectively, in patients in whom tumors did not have 1p36 LOH (Table 2 and Fig. 2A and 2B).

As compared with cases in which 11q23 LOH was not found, cases with 11q23 LOH (without regard to 11p material) were associated with a decreased probability of event-free survival ( $63\pm3$  percent vs.  $74\pm3$  percent,  $P=0.003$ ); the difference in overall survival ( $77\pm3$  percent vs.  $82\pm2$  percent) however, was not statistically significant ( $P=0.07$ ). Unb11q LOH (151 cases) was strongly associated



**Table 2. Results of Univariate Analysis of Event-free and Overall Survival Rates.\***

Cohort and Marker	No. of Patients	3-Yr Event-free Survival %	P Value	3-Yr Overall Survival %	P Value
<b>All patients</b>					
1p36			<0.001		<0.001
No loss	689	77±2		85±2	
LOH	209	47±4		64±4	
Unb11q LOH status					
Not unbalanced	758	74±2	<0.001	83±2	<0.001
Unbalanced	151	50±5		66±5	
<b>MYCN not amplified</b>					
1p36			<0.001		0.05
No loss	644	79±2		87±2	
LOH	100	62±6		83±5	
Unb11q LOH status					
Not unbalanced	617	82±2	<0.001	91±2	<0.001
Unbalanced	137	52±5		68±5	

\* Plus-minus values are rates ±SE. Two-sided P values were calculated with the use of the log-rank test. LOH denotes loss of heterozygosity.

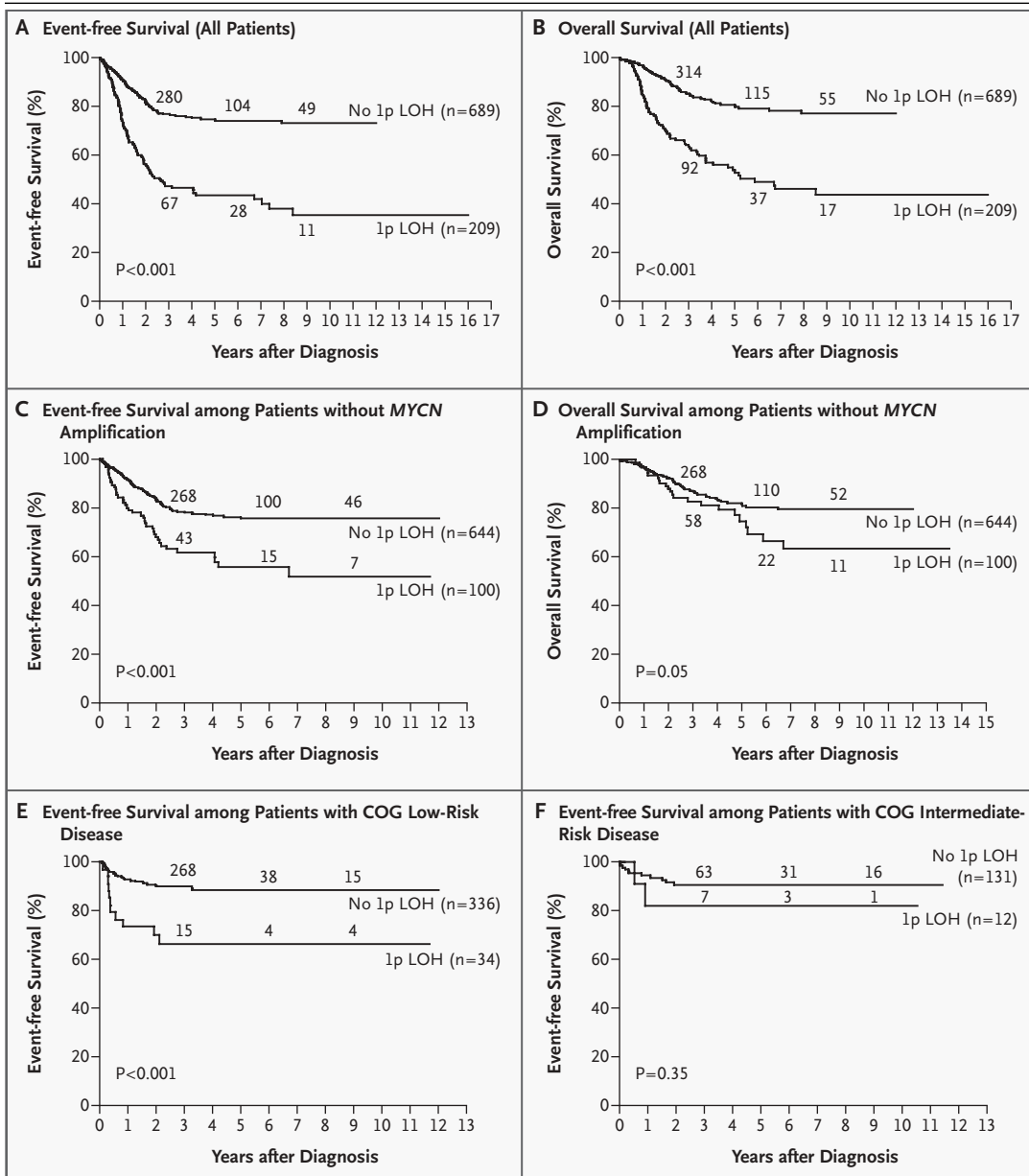
with both decreased event-free and decreased overall survival (Fig. 3A and 3B). Patients whose tumors showed unb11q LOH had three-year event-free and overall survival rates of 50±5 percent and 66±5 percent, respectively, as compared with 74±2 percent (P<0.001) and 83±2 percent (P<0.001) in the group that did not have unb11q LOH (Table 2).

In 40 cases, both 1p36 and unb11q LOH were detected. The patients with these tumors had a three-year event-free survival rate of 36±10 percent; the 274 cases with only one or the other aberration had a three-year event-free survival rate of 52±4 percent (P=0.10).

Analysis of the subgroup of cases without amplification of MYCN showed that both 1p36 LOH and unb11q LOH were highly associated with decreases in both event-free survival (P<0.001 for both) and overall survival (P=0.05 and P<0.001, respectively) (Table 2 and Fig. 2C, 2D, 3C, and 3D). Within the risk groups defined by the Children's Oncology Group, 1p36 LOH was associated with shortened event-free survival among low-risk patients, whereas unb11q LOH was associated with shortened event-free survival within both the low-risk and intermediate-risk groups (Table 3 and Fig. 2E, 2F, 3E, and 3F). We also analyzed progression-free survival in these cohorts. The three-year progression-free survival rate for low-risk and intermediate-risk patients combined was 73±8 percent for those with 1p36 LOH, as compared with 91±2

percent for those without it (P=0.002); the progression-free survival rate was 75±10 percent for patients with unb11q LOH, as compared with 90±2 percent for patients without it (P=0.006) (Table 3, and Fig. 1 of the Supplementary Appendix). The differences in overall survival rates among patients with and without unb11q LOH within both of these groups were not statistically significant (P=0.09 and P=0.15) (Table 3).

In a multivariate analysis, unb11q LOH was found to be independently associated with decreased event-free survival (Table 4). INSS stage 4 disease, MYCN amplification, and an unfavorable Shimada histologic category were also independently significant in this model. The age of the patient, the DNA index, 1p36 LOH, and 11q23 LOH without regard to 11p material (307 patients) were not independently associated with event-free survival. Unb11q LOH was not significantly associated with overall survival after adjustment for INSS stage 4 disease, MYCN amplification, unfavorable Shimada histologic category, and DNA index; however, there was a trend toward independent significance with unb11q LOH that was not seen with 1p36 LOH. In low-risk and intermediate-risk patients, MYCN amplification, an unfavorable Shimada histologic category, unb11q LOH, and 1p36 LOH were all independently associated with decreased progression-free survival (Table 5).



**Figure 2. Event-free and Overall Survival According to 1p36 Loss of Heterozygosity (LOH).**

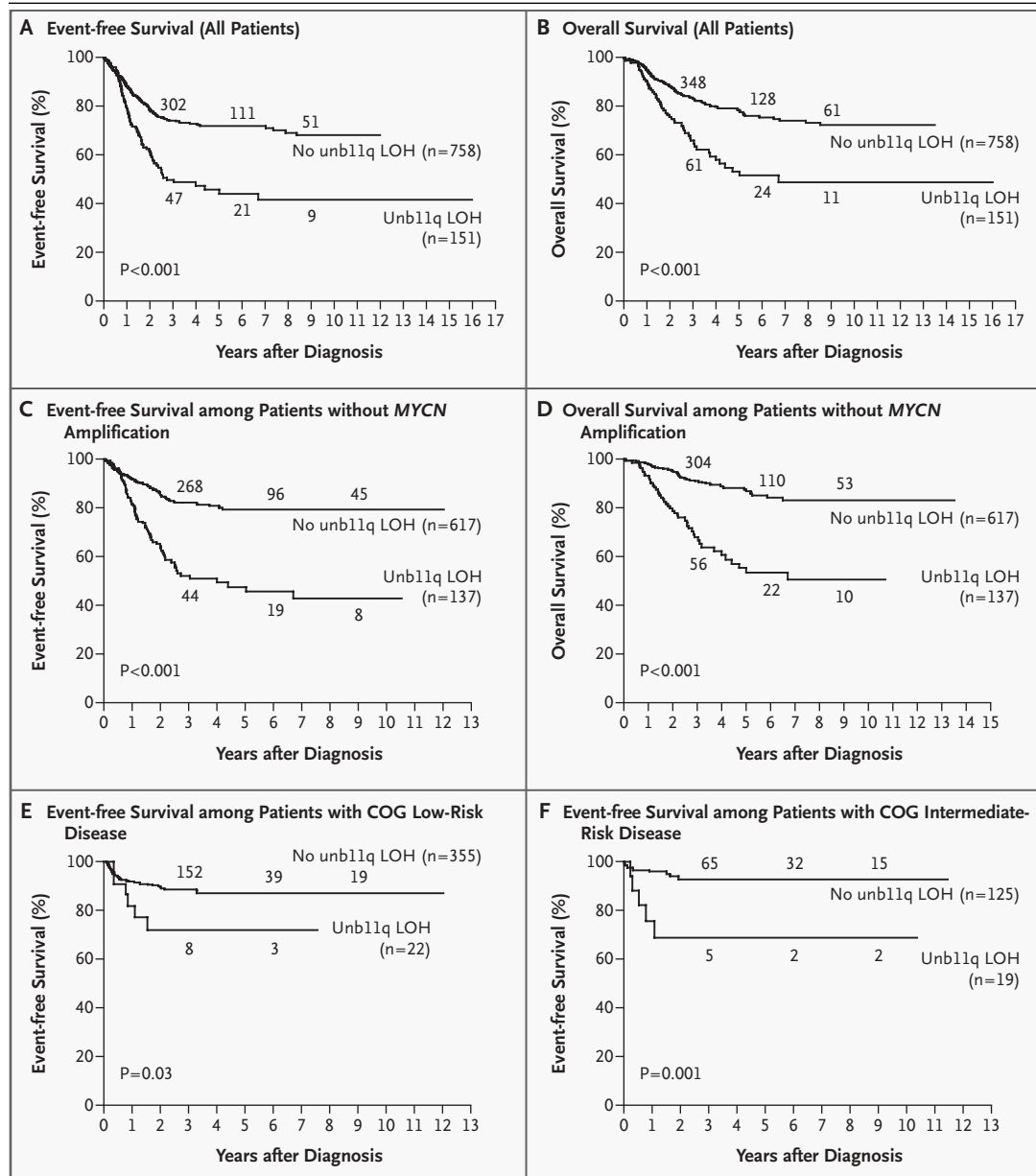
The rates of event-free and overall survival are shown for all patients (Panels A and B), event-free and overall survival for those whose tumors did not have MYCN amplification (Panels C and D), and event-free survival for those with low-risk disease, as defined by the Children's Oncology Group (COG) (Panel E), and intermediate-risk disease (Panel F). The numbers of patients at risk for an event are shown along the curves. Two-sided P values were calculated with the use of the log-rank test.

**DISCUSSION**

The ability to detect risk factors at diagnosis and tailor therapy accordingly could make the treatment of cancer more effective and less toxic than it has been. Among these risk factors are tumor-cell markers, of which MYCN amplification, HER2/neu

overexpression, and certain translocations (e.g., BCR-ABL, PAX-FKHR) have proved useful. Our findings regarding the association of 1p36 and unb11q LOH with the survival of children with neuroblastoma suggest that these variables should be incorporated into clinical trials.

Although MYCN amplification is a hallmark of



**Figure 3. Event-free and Overall Survival According to Unbalanced 11q Loss of Heterozygosity (unb11q LOH).**

The rates of event-free and overall survival are shown for all patients (Panels A and B), event-free and overall survival for those whose tumors did not have MYCN amplification (Panels C and D), and event-free survival for those with low-risk disease, as defined by the Children's Oncology Group (COG) (Panel E) and intermediate-risk disease (Panel F). The numbers of patients at risk for an event are shown along the curves. Two-sided P values were calculated with the use of the log-rank test.

aggressive disease in patients with neuroblastoma, 60 percent of high-risk tumors do not have this aberration. Furthermore, aggressive disease will ultimately develop in a subgroup of patients within the low-risk and intermediate-risk groups despite the lack of MYCN amplification. Since 11q23 LOH oc-

curs almost exclusively in tumors without MYCN amplification, we postulated that it may be a useful marker for tumors that are aggressive but lack MYCN amplification. The decreased probability of survival associated with unb11q LOH should be considered in the light of the tendency of 11q23

**Table 3. Univariate Analysis of Event-free, Overall, and Progression-free Survival Rates According to the Clinical Risk Groups.\***

Cohort and Marker	No. of Patients	3-Yr Event-free Survival %	P Value	3-Yr Overall Survival %	P Value	3-Yr Progression-free Survival† %	P Value
<b>Low-risk patients</b>							
1p36			<0.001		0.54		<0.001
No loss	336	90±2		96±2		90±2	
LOH	34	66±10		100		66±10	
Unb11q LOH status			0.03		0.09		0.03
Not unbalanced	355	89±2		98±1		89±2	
Unbalanced	22	72±14		87±10		72±14	
<b>Intermediate-risk patients</b>							
1p36			0.35		0.41		0.69
No loss	131	91±4		94±3		94±3	
LOH	12	82±13		100		91±10	
Unb11q LOH status			0.001		0.15		0.01
Not unbalanced	125	93±3		96±3		96±3	
Unbalanced	19	69±17		86±13		80±15	
<b>High-risk patients</b>							
1p36			0.01		0.007		
No loss	201	50±4		64±4			
LOH	155	41±5		54±5			
Unb11q LOH status			0.67		0.87		
Not unbalanced	253	47±4		60±4			
Unbalanced	106	44±6		60±6			

\* Plus-minus values are rates ±SE. Two-sided P values were calculated with the use of the log-rank test. LOH denotes loss of heterozygosity, and NA not applicable.

† Progression-free survival was not analyzed for high-risk patients.

LOH to occur in tumors without MYCN amplification. In fact, the proportion of patients whose tumors had MYCN amplification was higher in the subgroup of 750 patients (133 patients; 18 percent) who did not have unb11q LOH (and who had a better overall outcome) than in the subgroup of 149 patients (12 patients; 8 percent) who had unb11q LOH (and had a worse outcome) (P=0.002).

The lack of MYCN amplification in tumors with 11q23 LOH contrasts with the findings regarding 1p36 LOH. Although 1p36 LOH was highly associated with a decreased probability of survival, there was a statistically significant overlap between tumors with 1p36 LOH and tumors with MYCN amplification.<sup>9,13</sup> The association between 1p36 LOH and MYCN amplification may partially explain why 1p36 LOH was not independently associated with survival after the adjustment for MYCN amplification in multivariate analyses. After the multivariate model was restricted to the low-risk and intermediate-

risk groups, which are made up almost entirely of patients with tumors that do not have MYCN amplification, 1p36 LOH was independently associated with progression-free survival, confirming our previous report.<sup>9</sup>

We distinguished tumors showing unb11q LOH from those in which every marker on chromosome 11 showed LOH, because hyperdiploid DNA content is common in neuroblastoma and presumably results from a defect in the mitotic machinery that causes random gains and losses of whole chromosomes.<sup>1,2</sup> This defect can result in monosomy for chromosome 11 or in multiple copies of one parentally derived homologue that can masquerade, in a PCR-based assay, as LOH. Thus, we excluded these cases because we concluded that they probably differed from those in the subgroup that had a targeted deletion. Data from conventional and array-based comparative genomic hybridization had previously shown that loss of the entire chromo-

**Table 4. Results of Multivariate Cox Model of Event-free Survival in 622 Patients for Whom Complete Data Were Available.\***

Variable	Hazard Ratio	P Value
INSS stage 4	2.41	<0.001
MYCN amplification	2.02	0.005
Unfavorable Shimada histologic category	2.36	<0.001
Unb11q LOH	1.84	0.009

\* Hazard ratios are for relapse, disease progression, second cancer, or death. INSS denotes International Neuroblastoma Staging System, and LOH loss of heterozygosity. The following variables were added to the model and found not to have statistical significance: a patient age of 365 days or more, a DNA index of 1, 1p36 LOH, and 11q23 LOH (without regard to 11p status).

**Table 5. Results of Multivariate Cox Model of Progression-free Survival in 492 Low-Risk and Intermediate-Risk Patients for Whom Complete Data Were Available.\***

Variable	Hazard Ratio	P Value
MYCN amplification	5.55	0.02
Unfavorable Shimada histologic category	2.69	0.001
Unb11q LOH	2.48	0.02
1p36 LOH	2.92	0.002

\* Hazard ratios are for relapse or disease progression. LOH denotes loss of heterozygosity. The following variables were added to the model and found not to have statistical significance: a patient age of 365 days or more, an International Neuroblastoma Staging System stage of 4, a DNA index of 1, and 11q23 LOH (without regard to 11p status).

some 1 does not occur,<sup>25,26</sup> and therefore, in this study, the distinction was relevant only to chromosome 11. Our findings strongly suggest that future applications of the results of this research to the treatment of neuroblastoma will require a global assessment of the status of LOH for chromosome 11 in order to maximize prognostic power. An assessment of whole-genome LOH and copy number, with the use of array-based probes, would result in a higher-throughput assessment of the complex genomic patterns present in neuroblastomas and their associations with clinical phenotype.

We have shown that 1p36 LOH and unb11q LOH are strongly associated with outcome in patients with neuroblastoma. The addition of these markers to the currently used prognostic variables may allow for more precise treatment recommendations. For example, because both 1p and unb11q LOH are independently predictive of worse progression-free survival in patients with low-risk and intermediate-risk disease, the Children's Oncology Group plans to use these markers to assign the

number of cycles of adjuvant chemotherapy in the hope of averting a relapse of disease. The effect of unb11q LOH with regard to overall survival in these subgroups was of borderline significance; however, the numbers within each subgroup are small, and future analyses with longer follow-up times are required. Further studies may ultimately show that certain low-risk patients with 1p36 LOH, unb11q LOH, or both, who are currently treated with surgery alone would benefit from adjuvant chemotherapy.

We do not know whether 1p36 LOH and unb11q LOH can facilitate the assignment of treatment for patients with high-risk disease. Recent data suggest that the prognostic effect of age is continuous in nature, and the Children's Oncology Group now recommends chemotherapy of decreased intensity for patients between 12 and 18 months of age who have metastatic disease with "biologically favorable" tumors.<sup>30</sup> Future clinical trials involving patients with high-risk neuroblastomas may be designed to stratify treatment intensity on the basis of aberrations in MYCN, 1p, 11q, other genomic loci such as 3p or 17q, or all of these, which are currently under study by the Children's Oncology Group. It is expected that the pattern of genomic aberrations present in the cancer cell, rather than any individual marker, will provide the most sensitive and specific prognostic information.

In summary, we have shown that 1p36 LOH is present in about 23 percent of primary neuroblastomas, is highly associated in univariate analyses with a poor outcome, and is independently predictive of worse progression-free survival in low-risk and intermediate-risk patients. We have also shown that unb11q LOH is present in about 17 percent of primary neuroblastomas, predominantly in those without MYCN amplification, and that this LOH is an independently significant marker of decreased event-free and progression-free survival. The clinical usefulness identification of 1p36 LOH and unb11q LOH is currently applicable to patients with localized disease whose tumors do not show MYCN amplification, since patients with metastatic disease, MYCN amplification, or both, for the most part already receive the most aggressive therapy.

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