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Marta Piqueras, Samuel Navarro, Adela Cañete, Victoria Castel, Rosa Noguera. Prognostic value of partial genetic instability in Neuroblastoma with  $\leq 50\%$  neuroblastic cell content.. *Histopathology*, 2011, 59 (1), pp.22. 10.1111/j.1365-2559.2011.03899.x . hal-00651158

**HAL Id: hal-00651158**

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Submitted on 13 Dec 2011

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**Prognostic value of partial genetic instability in  
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Journal:	<i>Histopathology</i>
Manuscript ID:	HISTOP-04-10-0207.R1
Wiley - Manuscript type:	Original Article
Date Submitted by the Author:	12-Jul-2010
Complete List of Authors:	Piqueras, Marta; University of Valencia, Pathology Navarro, Samuel; University of Valencia, Pathology Cañete, Adela; Hospital Infantil Universitario La Fe, Paediatric Oncology Unit Castel, Victoria; Hospital Infantil Universitario La Fe, Paediatric Oncology Unit Noguera, Rosa; University of Valencia, Pathology
Keywords:	neuroblastoma, FISH, TMA, genome instability, prognostic factors



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**Title:** Prognostic value of partial genetic instability in Neuroblastoma with  $\leq 50\%$  neuroblastic cell content.

**Concise title:** Partial genomic instability in Neuroblastoma

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**Grants:** This study was supported by grants from Instituto Carlos III Madrid, Spain (contract RD06/0020/0102 and contract FIS PI06/1576) and Fundación AECC.

## ABSTRACT

**Aims.** Better understanding of neuroblastoma genetics will improve with genome-wide techniques. However it is not adequate to perform these analyses in samples with less than 60% neuroblastic cell content. We evaluated the utility of FISH on tissue microarrays (TMA) in detecting partial genetic instability (PGI), focussing on samples with  $\leq 50\%$  neuroblastic cells.

**Methods and results.** Alterations of 11q and 17q were detected by FISH on 369 neuroblastic samples included in TMA. Status of *MYCN* gene and 1p36 region was previously established by FISH diagnosis. Partial genetic instability (PGI) was defined as the ratio between segmental genetic alterations detected and number of genetic markers diagnosed in each tumor. 14.6% of primary tumors harbored 11q deletions, whereas 42.6% showed 17q gain. PGI was established in 260 primary tumors, 67 of them contained  $\leq 50\%$  neuroblasts. Outcomes were statistically worse for patients whose tumors presented high PGI ( $p < 0.0001$ ). Multivariate analysis revealed moderate and high PGI as prognostic factors.

**Conclusions.** In our cohort, univariate and multivariate analysis confirmed the effect of PGI in patient outcome. PGI established by FISH on TMA is a useful method to identify high risk patients whose tumors have  $\leq 50\%$  neuroblastic cell content.

**Keywords:** neuroblastoma, FISH, TMA, genome instability, prognostic factors.

## Introduction

Neuroblastoma is the most common tumor among children under 1 year of age worldwide<sup>1</sup>. This malignant neoplasm arises from neural crest cells<sup>2</sup>. Neuroblastoma patients show a wide spectrum of clinical behavior, from rapid progression to maturation into benign ganglioneuroma with spontaneous regression<sup>1</sup>. This clinically heterogeneous nature of neuroblastoma is also reflected in part by its genetic heterogeneity<sup>3</sup>.

Over the last two decades, several genetic aberrations have been identified in neuroblastic tumors that are strongly associated with outcome<sup>4</sup>. Therefore, genetic and molecular findings are routinely incorporated into therapeutic decision making<sup>3</sup>. Amplification of *MYCN* gene occurs in 20 to 25% of primary tumors and is a genetic marker for poor outcome<sup>3</sup>. Nevertheless, the majority of metastatic neuroblastomas do not show amplification of this oncogene. 11q loss is present in 15-22% of primary neuroblastoma, but rarely seen in tumors with *MYCN* amplification. It is associated with reduced progression-free survival<sup>5, 6</sup>. The **International Neuroblastoma Risk Group (INRG)** Classification System, published in 2009, includes *MYCN* amplification and 11q aberrations as prognostic genetic markers<sup>4</sup>. 1p36 loss can be identified in 25-35% of neuroblastic tumors. This deletion correlates with *MYCN* amplification and predicts an increased risk of relapse in patients with localized tumors<sup>6</sup>. Unbalanced 17q gain is the most common genetic aberration, present in more than half of neuroblastomas, and has been found to constitute an independent parameter for poor outcome<sup>3</sup>.

Actually, genetic markers can be identified separately by karyotyping, fluorescence in situ hybridization (FISH), loss of heterozygosity and polymerase

chain reaction, among others. However, these analyses are time consuming, their interpretation may be difficult and not all markers can be studied simultaneously<sup>7</sup>. Within the last decade, whole-genome analysis techniques, such as comparative genomic hybridization (CGH), have been developed. These methods enable the study of DNA copy number gains or losses over the entire genome in one single experiment<sup>3</sup>. In fact, the INRG Biology Subcommittee suggests using pan/multigenomic techniques, such as **multiplex ligation-dependent probe amplification (MLPA)** or **array-comparative genomic hybridization (aCGH)**, enabling a routinely analysis of all relevant genomic loci of neuroblastoma<sup>8</sup>. A genomic profile with whole chromosome gains or losses is related to good prognosis. Unbalanced chromosome translocations leading to segmental chromosome gains or losses characterized another profile, with intermediate prognosis. Finally, tumors with *MYCN* amplification and segmental chromosome alterations showed aggressive clinical behavior<sup>9</sup>. Thus, future therapies will involve specific treatment protocols based on the molecular signature for individual patients<sup>2</sup>.

However, molecular biology-based methods, including whole-genome analysis techniques, require tumor cell content greater than 60% and not all tumor samples meet this characteristic<sup>8</sup>. Nevertheless, in our laboratory experience, the robustness of MLPA results is maintained even in neuroblastic tumors with tumor cell content  $\leq 50\%$ <sup>10, 11</sup>. Even so, it has not so far been possible to perform this type of analysis in neuroblastic samples containing  $\leq 50\%$  of tumor cells, so therapeutic stratification of these patients based on risk estimation could be inadequate.

Alternative systems are needed to establish the most complete genetic information of these patients with minimum time and product costs.

In a previous paper, we demonstrated the utility and reliability of Tissue Microarrays (TMAs) in detecting genetic alterations by FISH in neuroblastic tumors<sup>12</sup>. In this study we evaluate whether the status of 4 individual genetic markers (*MYCN* gene, 11q, 1p36 region and 17q) could provide information about the stability of the tumor genome and if this could then be used as an alternative method to whole-genome analysis techniques in tumors with  $\leq 50\%$  of neuroblastic cell content. To evaluate the prognostic relevance, we performed FISH analyses in neuroblastoma samples included on 12 TMAs and studied the relationship with patient outcome.

## Materials and methods

A total of 369 neuroblastoma samples from 341 consecutive patients sent to the Spanish Reference Center for Biological Studies of Neuroblastoma (Department of Pathology, University of Valencia) between January 1, 1996 and December 31, 2004, were selected; of which 291 were primary tumors, 15 relapses and 63 post-treatment samples. Informed consent was provided by a parent or guardian for each patient enrolled in this study.

### TMA's AND FISH

Twelve TMA's were constructed as previously reported<sup>12</sup>. Two cores, 1mm diameter each, were included from each tumor. Aberrations in 11q arm and 17q arm status were detected by FISH on TMA's. The following commercial DNA probes were used: *ATM* (11q22) red/ SE 11 green and *MPO* (17q23) iso 17q red/ *p53* (17p13) green (Kreatech Biotechnology, Amsterdam, The Netherlands). *MYCN* gene and 1p36 region status were already known as they had been routinely diagnosed by FISH on imprints for risk assessment using the following commercial DNA probes: *MYCN* (2p24) red/  $\alpha$ -satellite 2 green and 1p36 midisatellite green/chromosome 1 satellite red (Q-BIOgene, Amsterdam, The Netherlands). Genetic alteration was diagnosed when more than 15% of neuroblasts of the sample showed aberration, applying our own system to detect genetic alterations in formalin-fixed paraffin-embedded tissues (FFPE), as previously described, and following the ENQUA and INRG definitions for chromosome aberrations in neuroblastic tumors<sup>12, 13</sup>.

A new variable was created: *partial genetic instability* (PGI), defined as the ratio between the number of segmental genetic alterations detected and the number

of genetic markers studied successfully in each tumor. The instability grade was established in the subgroup of primary tumors with 3 or 4 genetic markers diagnosed: minimum PGI (0 or 1 genetic aberration diagnosed); moderate PGI (2 genetic alterations detected); high PGI (3 or 4 genetic aberrations).

## STATISTICAL METHODS

Neuroblastic cell content was divided in 3 categories: 10-50%, 51-60%, > 60%.

The association between percentage of neuroblastic cells and presence of genetic alterations was examined using Pearson correlation models with a 3 x 2 contingency table. Two variables were considered correlated at a p-value smaller than 0.05. Clinical variables included stage and age at diagnosis. Stage of disease was dichotomized into two categories: localized (1, 2 and 4S) and advanced (3 and 4). Age was treated as a dichotomous variable: less than 18 months and equal to or greater than 18 months. Morphological and biological variables included grade of differentiation, *MYCN* amplification, 1p36 deletion, 11q alteration and gain of 17q. Events for event-free survival (EFS) analysis were considered as relapse or death from disease. Time to event for EFS was calculated as the time from diagnosis until the time of first event, or until the time of last patient contact if no event occurred. Time to event for overall survival (OS) analysis was the time from diagnosis until death, or until the time of last contact if the patient was alive. Univariate analyses were performed using Kaplan-Meier to generate survival curves, which were compared using a log-rank test to identify statistically significant predictive factors of EFS and OS. For multivariate analysis, Cox's proportional hazards regression models were used to identify the most highly statistically significant variable. P values lower

than 0.05 were considered statistically significant. All EFS and OS values are reported at the 9-year time point with the standard error.

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## Results

Neuroblastic cell content from 369 analyzed samples ranged between 10% and more than 80% (figure 1, A-F). Of the 291 primary tumors, 260 had 11q diagnosis; 38/260 (14.6%) harbored 11q deletions (figure 1, G). Of the 260 diagnosed cases, 68 presented up to 50% of neuroblastic cell content: 6 tumors with 11q deletion and 62 tumors with normal 11q status (table 1). Neuroblastic cells content was not associated with status of 11q arm ( $p=0.173$ ). 17q status was known in 258 of the 291 primary tumors, detecting 17q gain in 110/258 (42.6%) (figure 1, H). Among the 68 diagnosed tumors with  $\leq 50\%$  neuroblastic cell content, 22 showed 17q gain and 46 cases 17q normal status (table 1). Percentage of neuroblasts was not correlated with status of 17q arm ( $p=0.135$ ).

### PARTIAL GENETIC INSTABILITY (PGI)

The status of 4 genetic markers was considered: *MYCN* gene, 1p36 region, 11q arm and 17q arm. At least 3 of these genetic markers were diagnosed in 260 of the 291 primary tumors. Fifty-eight tumors harbored only 1 genetic aberration, most of the cases (42/58) with 17q gain and none with *MYCN* gene amplification. 12/32 tumors with 2 genetic alterations presented 17q gain-11q deletion, whereas 8/32 cases harbored *MYCN* gene amplification-1p36 deletion. Among the tumors with 3 genetic alterations, *MYCN* gene amplification/1p36 deletion/17q gain was the most frequent combination. 8/260 cases showed both *MYCN* gene amplification and 11q deletion, with or without another genetic alteration.

The PGI grade was established in these 260 primary tumors. More than 25% (67/260) had  $\leq 50\%$  neuroblasts content. Forty-six cases (46/260) had high

partial genetic instability, and almost 22% (10/46) had  $\leq 50\%$  neuroblastic cells content (table 1). Moreover, there was no difference between PGI of tumors on neuroblastic cells content ( $p=0.333$ ).

## STATISTICAL RESULTS

The mean age at diagnosis in this study was 23.06 months (range, 1 day to 227 months). EFS and OS median follow-up were 51.07 and 54.15 months respectively. Outcome related to clinical and genetic factors is summarized in tables 2 and 3. Age 18 months at diagnosis, advanced stages of disease, *MYCN* aberrations, 1p36 deletion, 17q gain and 11q deletion were unfavorable prognostic factors in the whole cohort by univariate statistical analysis (table 2 and 3).

Patients with localized tumors had better EFS and OS than patients with advanced disease in the subgroup of tumors with  $\leq 50\%$  neuroblastic cells content ( $p<0.0001$ ). EFS and OS were significantly higher for patients without *MYCN* alterations than those with *MYCN* gain or amplification in  $\leq 50\%$  neuroblastic cell subgroup ( $p<0.0001$ ). Similarly, statistically significant differences in EFS and OS were observed based on 1p36 status in the subgroup of tumors with lower percentages of neuroblastic cells ( $p<0.0001$ ).

Outcome was statistically worse for patients whose tumors had high PGI, for both EFS and OS, in the whole cohort as well as in the subgroup of tumors with  $\leq 50\%$  neuroblastic cells content ( $p<0.0001$ ) (figure 2, A and B). In the whole cohort, nine-year EFS was  $31\pm 8.7\%$  for patients suffering tumors with high PGI compared with  $84.8\pm 4.5\%$  for those with tumors with minimum PGI (table 2).

Similarly, 9-year OS was  $36.8 \pm 12.4\%$  for patients with tumors with high PGI compared with  $92.1 \pm 2.7\%$  for those cases with minimum PGI (table 3).

Multivariate analysis **performed in whole cohort** based on EFS including clinical variables and individual genetic alterations revealed stages 3 and 4, *MYCN* amplification and 11q deletion as significant prognostic factors. Similarly, stages 3 and 4 and *MYCN* amplification were considered as factors with significant prognostic value in the multivariate analysis based on OS (table 4).

Furthermore, multivariate analysis **performed in whole cohort** based on EFS including clinical variables and PGI revealed stages 3 and 4, moderate PGI and high PGI as significant prognostic factors. Multivariate analysis based on OS again revealed moderate PGI and high PGI as significant prognostic factors together with 17q gain (table 5).

## Discussion

Several techniques can identify recurrent segmental alterations simultaneously, but their efficacy is limited by the tumor cell content of the sample<sup>3, 10, 11</sup>. In fact, an important limitation of pan/multigenomic techniques is that they require a tumor cell content of more than 60% in the sample<sup>8</sup>. This study focused on the utility of FISH on TMA in establishing a possible prognostic value of a combination of representative genetic markers and whether this is an adequate tool for stratifying patients with neuroblastoma, including tumors with  $\leq 50\%$  neuroblastic cells.

Our suggested partial genetic instability model is different to patient genomic profile obtained with pan/multigenomic techniques, because we only considered 4 representative genetic markers: *MYCN* gene, 1p36 region, 11q and 17q arms. FISH can diagnose segmental aberrations with low neuroblastic cell content and the use of TMA allows us to study a higher number of neuroblastic tumors with particular characteristics, as tumors with low neuroblastic cell content<sup>14</sup>.

In our study, alterations of 11q and 17q arms were independent of neuroblastic cells content. 26% of cases with 11q and 17q known status had  $\leq 50\%$  of neuroblasts. Furthermore, 25,7% tumors with different grades of PGI had  $\leq 50\%$  of neuroblastic cell content, thus we would have been unable to obtain a partial genomic profile of these tumors using other techniques.

As a first step, the prognostic value of clinical and biological variables included in the INRG Risk Classification System was evaluated to know if our whole cohort was statistically representative.

Age 18 months at diagnosis, advanced stages of disease, *MYCN* aberrations, 1p36 deletion, 17q gain and 11q deletion were unfavorable prognostic factors in our cohort by univariate statistical analysis, in agreement with previous reports<sup>4</sup>.

<sup>6</sup>. Multivariate analysis was performed including all individual genetic alterations as well as clinical variables. Stages 3 and 4, *MYCN* amplification and 11q deletion was the combination of factors with more impact in EFS and OS. These results were similar to previously published data <sup>4, 15</sup>.

Partial genetic instability in TMA was defined to infer the genomic profile by FISH. 17q gain was the most frequent genetic aberration detected in primary tumor with minimum PGI. In fact, this is the most common genetic alteration in neuroblastomas<sup>3</sup>. In our cohort, 17q gain was present in 42.6% of all primary tumors analyzed, compared to the 48% of the INRG report<sup>4</sup>. This small difference may be explained by the number of patients: the INRG report included 8,800 patients in contrast with our 260 primary tumors. Three of our cases harbored only 11q loss. In general, deletions of 11q have been identified in 15-22% neuroblastic primary tumors<sup>5</sup>. The frequency of 11q-alterations in our cohort was 14.6%, slightly less than the 21% published in the INRG report <sup>4</sup>.

No primary tumor showed only *MYCN* amplification. Of cases displaying moderate genetic instability, the presence of both *MYCN* amplification and 1p36 deletion in the same sample was one of the most frequent combinations, confirming the association of these genetic aberrations<sup>5</sup>. Interestingly, 11q deletion-17q gain was the most common genetic combination, suggesting unbalanced translocations between chromosomes 11 and 17, in agreement with previous studies<sup>16</sup>. On the other hand, the whole cohort contained only 8 cases showing both *MYCN* amplification and 11q deletion, with or without another genetic aberrations, in agreement with the idea that these aberrations are rarely seen together in neuroblastic tumors <sup>6</sup>.

Recently, a multivariate analysis attributed the presence of segmental aberrations as having the most power in predicting relapse<sup>7</sup>. In our study, univariate statistical analysis showed that PGI was an unfavorable prognostic factor in the whole cohort as well as in subgroup of tumors with  $\leq 50\%$  neuroblastic cells content: EFS and OS for patients whose tumors had high PGI were statistically worse than EFS and OS for those with minimum PGI. Moreover, when the multivariate analysis was repeated in our whole cohort to also include PGI, the individual genetic alterations *MYCN* amplification and 11q deletion, included in the first multivariate analysis, were substituted by moderate and high PGI as genetic markers with more impact on patient survival. Our results confirmed the effect of partial genomic instability in patient outcome, in agreement with the report of Janoueix-Lerosey et al.<sup>7</sup>.

International efforts are needed to identify the most significant genetic markers and techniques for their evaluation in all patients. The combination of FISH and TMA could be a useful method to identify the main genetic aberrations in patients whose tumors have  $\leq 50\%$  neuroblasts content. This method can distinguish between clinically relevant minimum PGI and moderate/high PGI, allowing the patient to be included in new therapeutic protocols based on partial genetic alterations<sup>17, 18</sup>. A further challenge is integrated analysis for determining the prognostic value of combining genomic features of the tumor including gene copy number profiling and epigenetic markers along with the currently used clinicobiological factors for risk stratification<sup>19</sup>.

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**Table 1.** Status of 11q, 17q arm and PGI grade of primary tumors diagnosed by FISH on TMAs.

Marker	Status	Patients (%)	Neuroblasts content			P value
			10-50% <i>n</i> (%)	51-60% <i>n</i> (%)	> 60% <i>n</i> (%)	
11q	Normal	222 (85.4)	62 (91.2)	24 (77.4)	136 (84.5)	0.173
	Deletion	38 (14.6)	6 (8.8)	7 (22.6)	25 (15.5)	
	<i>TOTAL</i>	<i>260 (100)</i>	<i>68 (100)</i>	<i>31 (100)</i>	<i>161 (100)</i>	
17q	Normal	148 (57.4)	46 (67.6)	17 (54.8)	85 (53.5)	0.135
	Gain	110 (42.6)	22 (32.4)	14 (45.2)	74 (46.5)	
	<i>TOTAL</i>	<i>258 (100)</i>	<i>68 (100)</i>	<i>31 (100)</i>	<i>159 (100)</i>	
PGI grade	Minimum	181 (69.6)	49 (73.1)	17 (54.8)	115 (71)	0.333
	Moderate	33 (12.7)	8 (11.9)	7 (22.6)	18 (11.1)	
	High	46 (17.7)	10 (14.9)	7 (22.6)	29 (17.9)	
	<i>TOTAL</i>	<i>260 (100)</i>	<i>67 (100)</i>	<i>31 (100)</i>	<i>162 (100)</i>	

Abbreviations: **n, number of patients**; PGI, partial genomic instability.

**Table 2.** Clinical characteristics related to EFS in the whole cohort versus tumors with  $\leq 50\%$  neuroblastic cells content.

Factor	9-year EFS whole cohort			9-year EFS tumors $\leq 50\%$ nbs		
	N	% $\pm$ SE	P value	N	% $\pm$ SE	P value
Age (months)						
< 18	166	80.7 $\pm$ 5.4		32	79.1 $\pm$ 7.8	
$\geq 18$	89	48.5 $\pm$ 6.1	<0.0001	37	54 $\pm$ 9.8	0.095
INSS Stage						
1, 2, 4S	130	87.3 $\pm$ 5.1		33	90.2 $\pm$ 6.6	
3, 4	122	51.6 $\pm$ 5	<0.0001	34	41.4 $\pm$ 10.4	<0.0001
Differentiation						
Gang, int GNB	19	83.1 $\pm$ 11		16	78.8 $\pm$ 13.4	
d NB	13	72.7 $\pm$ 13.4		6	60 $\pm$ 21.9	
pd NB, u NB	206	68.4 $\pm$ 4.5	0.306	36	58 $\pm$ 9	0.201
MYCN gene						
Not amplified	194	79.8 $\pm$ 4.2		51	78.7 $\pm$ 6.4	
Gain	12	40.5 $\pm$ 16.5		3	66.7 $\pm$ 27.2	
Amplified	49	38 $\pm$ 8.1	<0.0001	15	19.4 $\pm$ 15.2	<0.0001
1p36 region						
Not deleted	177	81.8 $\pm$ 4.3		44	83.8 $\pm$ 6.2	
Deleted	74	41.2 $\pm$ 6.7	<0.0001	22	26.3 $\pm$ 13.2	<0.0001
17q arm						
No gain	126	77.2 $\pm$ 6.6		39	76 $\pm$ 7.6	
Gain	100	58.2 $\pm$ 5.5	0.001	21	49.1 $\pm$ 13	0.065
11q arm						
Not deleted	194	76.2 $\pm$ 4.3		54	70.8 $\pm$ 7.1	
Deleted	35	36.8 $\pm$ 9.1	<0.0001	6	50 $\pm$ 20.4	0.138
PGI						
Minimum	157	84.8 $\pm$ 4.5		42	80 $\pm$ 6.9	
Moderate	30	52 $\pm$ 9.9		8	46.9 $\pm$ 18.7	
High	42	31 $\pm$ 8.7	<0.0001	10	-	0.004

Abbreviations: nbs, neuroblastic cells; EFS, event-free survival; N, number of patients; SE, standard error; INSS, International Neuroblastoma Staging System; gang, ganglioneuroma; int GNB, intermixed ganglioneuroblastoma; d NB, differentiating neuroblastoma; pd NB, poorly differentiated neuroblastoma; u NB, undifferentiated neuroblastoma; PGI, partial genetic instability.

**Table 3.** Clinical characteristics related to OS in the whole cohort versus tumors with  $\leq 50\%$  neuroblastic cells content.

Factor	9-year OS whole cohort			9-year OS tumors $\leq 50\%$ nbs		
	N	% $\pm$ SE	P value	N	% $\pm$ SE	P value
Age (months)						
< 18	161	89.7 $\pm$ 3		31	79.7 $\pm$ 10.7	
$\geq 18$	86	58.1 $\pm$ 6.5	<0.0001	35	60.9 $\pm$ 10.7	0.135
INSS Stage						
1, 2, 4S	128	95.1 $\pm$ 2.7		33	92.9 $\pm$ 6.9	
3, 4	116	60.8 $\pm$ 5.6	<0.0001	31	47.5 $\pm$ 12.9	<0.0001
Differentiation						
Gang, int GNB	19	90.9 $\pm$ 8.7		16	88.9 $\pm$ 10.5	
d NB	13	77.9 $\pm$ 14.1		6	53.3 $\pm$ 24.8	
pd NB, u NB	198	77.3 $\pm$ 3.5	0.338	33	63.8 $\pm$ 10.3	0.159
MYCN gene						
Not amplified	193	88 $\pm$ 2.8		51	83 $\pm$ 6.9	
Gain	12	69.3 $\pm$ 15		3	66.7 $\pm$ 27.2	
Amplified	42	32.4 $\pm$ 14.2	<0.0001	12	-	<0.0001
1p36 region						
Not deleted	174	90.9 $\pm$ 2.7		44	86.2 $\pm$ 6.9	
Deleted	68	44.1 $\pm$ 8.8	<0.0001	19	-	<0.0001
17q arm						
No gain	124	87.1 $\pm$ 3.9		38	77.6 $\pm$ 9	
Gain	94	69 $\pm$ 5.5	0.003	19	64.2 $\pm$ 14.4	0.381
11q arm						
Not deleted	187	83.2 $\pm$ 3.5		52	74.4 $\pm$ 8.1	
Deleted	34	56.8 $\pm$ 9.2	<0.0001	5	60 $\pm$ 21.9	0.126
PGI						
Minimum	154	92.1 $\pm$ 2.7		42	83 $\pm$ 7.3	
Moderate	31	66.2 $\pm$ 9.3		7	53.6 $\pm$ 20.1	
High	36	36.8 $\pm$ 12.4	<0.0001	8	-	0.005

Abbreviations: nbs, neuroblastic cells; OS, overall survival; N, number of patients; SE, standard error; INSS, International Neuroblastoma Staging System; gang, ganglioneuroma; int GNB, intermixed ganglioneuroblastoma; d NB, differentiating neuroblastoma; pd NB, poorly differentiated neuroblastoma; u NB, undifferentiated neuroblastoma; PGI, partial genetic instability.

**Table 4.** Multivariate analysis including clinical variables and individual genetic alterations.

Factor	EFS		OS	
	Hazard ratio	P value	Hazard ratio	P value
3 and 4 stage (INSS)	3.58	0.002	4.74	0.008
<i>MYCN</i> amplification	3.045	0.0001	4.067	0.0001
11q deletion	2.015	0.029	2.115	0.052

Abbreviations: EFS, event-free survival; OS, overall survival; SE, standard error; INSS, International Neuroblastoma Staging System.

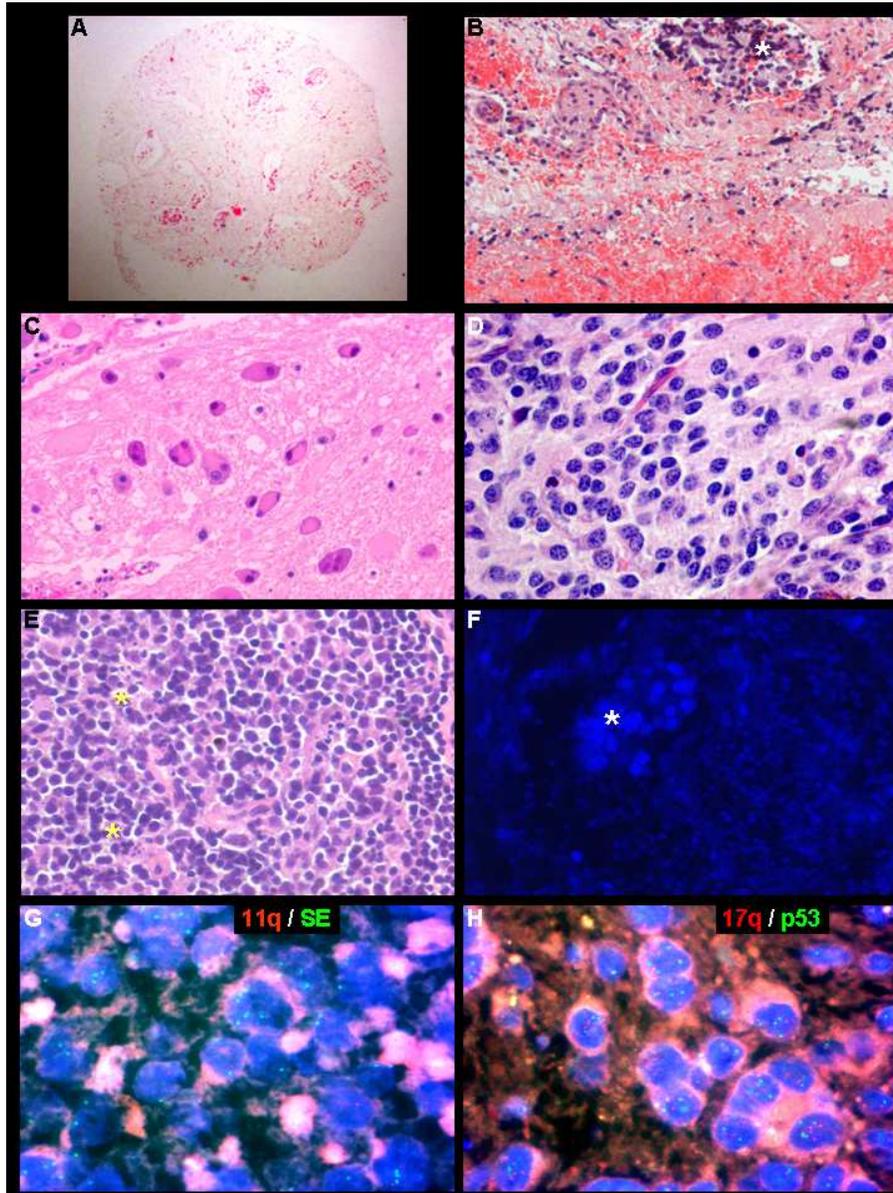
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**Table 5.** Multivariate analysis including clinical variables and partial genetic instability.

Factor	EFS		OS	
	Hazard ratio	P value	Hazard ratio	P value
3 and 4 stage (INSS)	3.050	0.009	-	-
PGI moderate	3.485	0.003	16.177	0.0001
PGI high	4.753	0.0001	42.479	0.0001
17q gain	-	-	0.283	0.027

Abbreviations: EFS, event-free survival; OS, overall survival; SE, standard error; INSS, International Neuroblastoma Staging System; PGI, partial genomic instability.

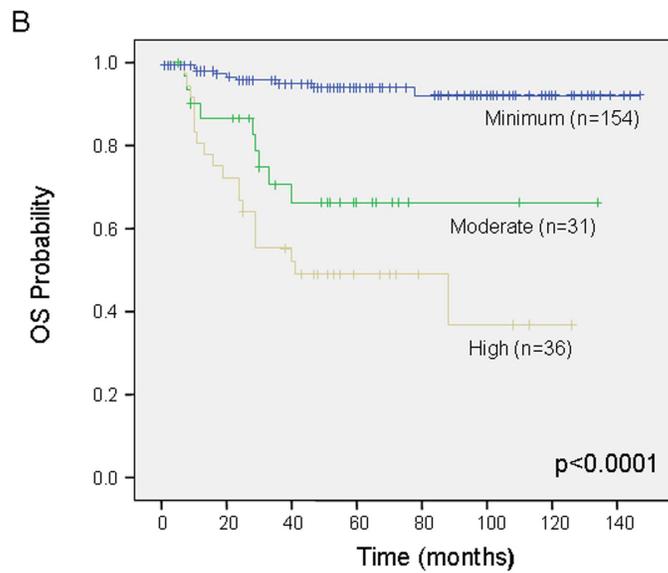
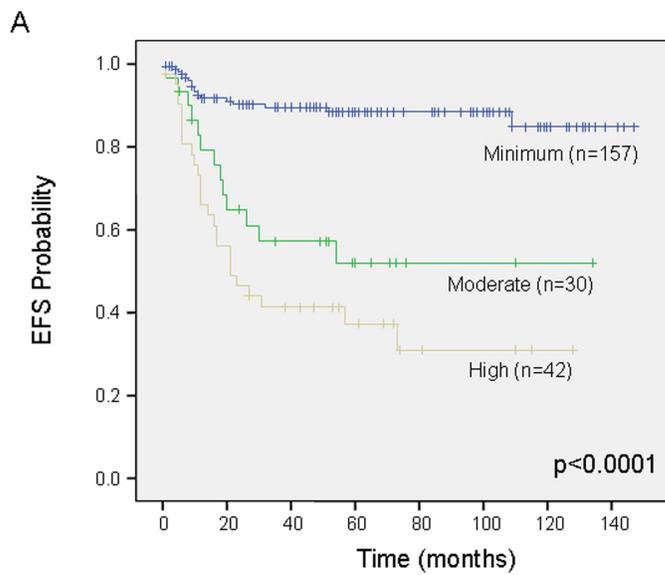
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190x254mm (96 x 96 DPI)

**Figure 1.** Images showing the variation of stroma and cell content in neuroblastomas. A-E, Hematoxylin-eosin staining; F-H, Fluorescence in situ hybridization. A, TMA core from sample with less than 50% of neuroblastic cells (40x). B, Hemorrhagic and necrotic areas with scant tumor cells (\*) (200x). C, Ganglioneuroblastoma associated with abundant stroma (neuropil) and neural cell differentiation (200x). D, Neuroblastic tumor with more than 50% of tumor cells (200x). E, Neuroblastoma with scant stroma and proliferation of neuroblastic cells associated with karyorrhexis (\*) (400x). F, abundant stroma with scant tumor cells (\*) (4'-6-Diamidino-2-phenylindole (DAPI) counterstaining) (400x). G, 11q (red) deletion (1000x). H, 17q (green) gain (1000x).

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**Figure 2.** Correlation between partial genetic instability (PGI) and patient outcome. Kaplan-Meier survival curves of event-free (A) and overall (B) survival. High PGI correlated with poor event-free ( $p < 0.0001$ ) and overall ( $p < 0.0001$ ) survival.

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### **Acknowledgements**

We thank Elisa Alonso (Pathology Department, Medical School, University of Valencia) for excellent technical assistance and Désirée Ramal (Paediatric Oncology Unit, Hospital Infantil Universitario La Fe, Valencia) for patient data management. This study was supported by grants from Instituto Carlos III Madrid, Spain (contract RD06/0020/0102 and contract FIS PI06/1576) and Fundación AECC.

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