

# Loss of Heterozygosity at 19q13.3 Is Associated with Locally Aggressive Neuroblastoma<sup>1</sup>

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## ABSTRACT

A genome-wide allelic analysis of neuroblastoma (NB) revealed a previously undescribed increased incidence of loss of heterozygosity (LOH) on chromosome arm 19q13 primarily affecting stages 3 and 4N disease. Further allelic analysis of chromosome 19q13 in a cohort of 116 NB patients using 17 polymorphic microsatellite markers identified the shortest common region of loss between *D19S606* and *D19S112* at 19q13.3. In some cases, clonal LOH at 19q13 was acquired during the course of disease, and deleted clones remained after cytotoxic therapy. In multivariate analysis, 19q13 LOH was associated with overall survival in local-regional International Neuroblastoma Staging System stages 1, 2, and 3 patients and was specifically present in tumors at the site of recurrence.

## INTRODUCTION

NB<sup>3</sup> is a pediatric cancer that arises from precursor cells of the peripheral sympathetic nervous system. It is clinically heterogeneous with at least three well-recognized patterns of disease: (a) infants with widespread disease that can spontaneously regress without medical intervention; (b) systemic disease with widespread metastasis that responds to cytotoxic therapy but frequently becomes resistant to medical treatment with poor outcome, mainly because of distant metastasis; and (c) LR NB characterized by lack of distant metastases. Most patients with LR NB have a good prognosis without cytotoxic therapy; however, some LR tumors will recur locally and often become resistant to cytotoxic therapy. These patients finally succumb to uncontrollable local disease.

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<sup>3</sup> The abbreviations used are: NB, neuroblastoma; LR, local-regional; LOH, loss of heterozygosity; INSS, International Neuroblastoma Staging System; SRO, shortest region of overlap.

We performed a genome-wide allelic analysis to identify clinically useful markers of disease progression for each group of NB. This analysis revealed a previously undescribed region of LOH for NB on chromosome arm 19q. An expanded study of 19q LOH in a large sample of NB cases showed that 19q13 LOH occurred primarily in a defined subgroup of high-risk NB patients with propensity for local recurrence.

## MATERIALS AND METHODS

**Patient Materials and Clinical Characteristics.** One hundred fifty-seven NB tumors from 116 patients managed at Memorial Sloan-Kettering Cancer Center, including 10 patients with INSS (1) stage 4s, 45 patients with LR disease (INSS stages 1, 2, 3), and 61 patients with stage 4 were analyzed. Multiple tumor samples obtained over time or from multiple sites at the same surgical procedure were available from 18 patients. All samples were evaluated histologically, and only specimens with >50% tumor cell content were studied, most with >80% of tumor cells. Standard treatment consisted primarily of surgical resection for LR disease and stage 4s disease (2) and the use of an intensive multimodality regimen for stage 4 (3).

**LOH Analysis.** Genomic DNA from frozen tumors, bone marrow, and peripheral blood was extracted using standard procedures. Polymorphic microsatellite loci were identified in the Genome Database, and fluorescently labeled primers were obtained from Research Genetics (Birmingham, AL). Map locations were taken from the Lawrence Livermore National Laboratories for chromosome 19 as a primary source.<sup>4</sup> Additional information was taken from NIH Genemap99 for chromosome 19q,<sup>5</sup> Marshfield Clinic genetic map,<sup>6</sup> The University of Southampton genetic maps,<sup>7</sup> and maps published previously from Smith *et al.* (4). Allelic analysis was performed as described previously (5).

**Statistics.** The association between event-free survival, defined as relapse, and survival, defined as the time to death or last follow-up and clinicobiological variables, was assessed using the log-rank test (6). Those factors that were potentially predictive of event-free survival and overall survival were entered into a multivariate analysis using the Cox proportional hazards model (7). Survival curves were generated using the method of Kaplan and Meier (8). All statistical calculations were performed using S-Plus 2000 (Mathsoft, Inc., Seattle, WA).

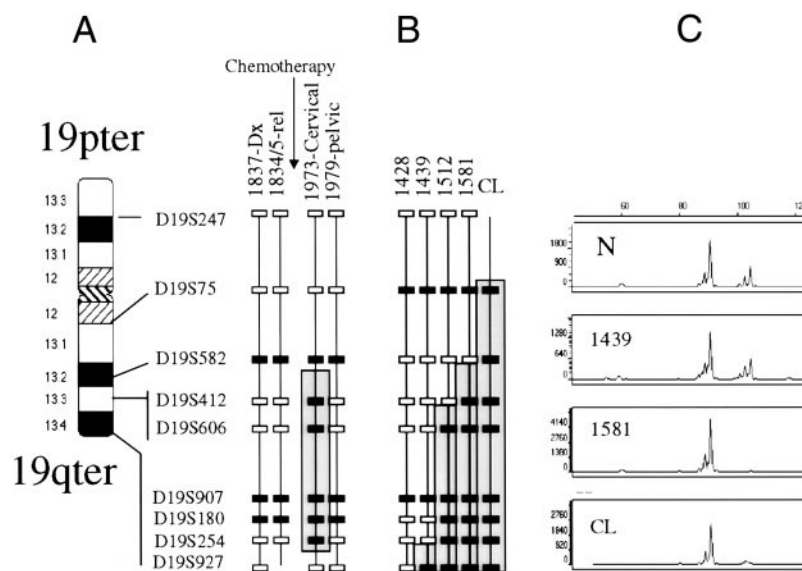
<sup>4</sup> Internet address: <http://greengenes.llnl.gov/genome-bin/>.

<sup>5</sup> Internet address: <http://www.ncbi.nlm.nih.gov/genemap/mapcgi?bin> or <http://www.ncbi.nlm.nih.gov/80/cgi-bin/Entrez/maps.cgi?org=hum&chr=19&MAPS=thon>.

<sup>6</sup> Internet address: <http://research.marshfieldclinic.org/genetics/MapMarkers/maps/IndexMapFrames.html>.

<sup>7</sup> Internet address: [http://cedar.genetics.soton.ac.uk/public\\_html/index.html](http://cedar.genetics.soton.ac.uk/public_html/index.html).





**Fig. 4** A, allelic analysis of multiple samples from the same patient. Tumors 1837 (at diagnosis) and 1834 and 1835 (first relapse) from the paraspinal region retained heterozygosity at 19q13. Postchemotherapy tumors from the cervical region (tumor 1973) had LOH at 19q13, but tumor from the pelvic area (tumor 1979) retained heterozygosity at 19q13. The patient subsequently relapsed only in the cervical region. Chromosome 19 ideogram on the left with microsatellite markers used in this analysis is indicated. Each column of boxes represents one case. □, retention of heterozygosity; ▤, noninformative markers; ■, LOH. Gray shaded region, shortest region of common loss. B, allelic analysis of tumors from multiple relapses and one cell line derived from the last tumor from the same patient. Although the first tumor did not show 19q13 LOH, subsequent relapse samples and derived cell line show deletions increasing in size. Chromosome 19 ideogram on the left with microsatellite markers used in this analysis is indicated. Each column of boxes represents one case. □, retention of heterozygosity; ▤, noninformative markers; ■, LOH. Gray shaded region, shortest region of common loss. C, example of electropherograms from the same case of B analyzed over multiple recurrences with the same marker *D19S412* showing acquisition/selection of 19q13 LOH over time.

Among stage 4 patients, only eight had LOH at 19q13, and four of the eight had large bulky 4N disease. It is of interest that stage 4N NB does not metastasize to bone or bone marrow like classic stage 4 disease and in this way is more similar to bulky LR NB. Six of the eight (including the four stage 4N) stage 4 patients with 19q13 LOH had tumor recurrence at or near the primary site. This rate of local primary recurrence (56%) was high compared with the reported rate of recurrence for other patients (16%) treated with N6 and N7 therapy during the same time period (9). Four patients treated with N7 therapy are alive and well, progression free. The other four treated with less intense regimens died.

**Clonal 19q13 LOH in NB Can Be Acquired and Is Closely Associated with Local Recurrence.** Nineteen (16%) of the 116 patients relapsed in the primary site after complete or very good partial responses to initial therapy, including 13 (28%) of the 45 LR and 6 (10%) of the 61 stage 4 cases. 19q13 LOH was found in 12 (63%) of the 19 relapsed cases.

Eight of the 17 cases with 19q13 LOH were found in the original diagnostic tumor biopsy. For these, the deletion was detected in all available subsequent specimens, regardless of cytotoxic therapy. In the other 9 cases, 19q13 LOH was detected either in relapse or in postchemotherapy specimens, and the diagnostic biopsy was not available for review. In 2 of these cases, the deletion was shown to be acquired (or selected) during progression because a prior tumor sample retained heterozygosity (Fig. 4).

In 2 cases where the deletion was found in only one of multiple samples from different sites, the relapse was at the site

of the tumor in which 19q13 LOH was detected (Fig. 4A). In one case, the deleted region increased in size with subsequent samples and in a cell line derived from the tumor (Fig. 4B). These results suggest that NB clones with 19q13 LOH have a propensity to regrow after surgical debulking and may be a marker of local aggressiveness.

## DISCUSSION

Loss of chromosome 19q13 is an uncommon finding in human tumors and has been most frequently reported in ovarian cancer (10) and gliomas (11). Interestingly, 1p36 and 19q13 LOH have demonstrated exquisite specificity for the oligodendroglial lineage among human gliomas and constitute early oncogenic events for these tumors (12, 13). Both NB and oligodendroglial tumors are believed to arise from neuroectodermal precursor cells (14), and both tumor types are characterized by a high incidence of 1p and a low incidence of p53 mutations (11, 15, 16). However, the clinical and biological significance of 19q13 LOH is quite different for these two tumors. Although the deletion represents an early event in oligodendroglial tumorigenesis and confers good prognosis related to chemosensitivity, 19q13 can be acquired or selected during NB progression and is associated with a high risk for local treatment failure and poor prognosis.

The frequent LOH at 19q in these tumors suggests the location of a tumor suppressor gene; however, none have yet been confirmed. The shortest common region of loss in oligo-

dendroglial tumors has recently been narrowed to a 1.4-Mb region between the markers *D19S412* and *D19S596* (4), a site overlapping the SRO identified for NB in this study (Fig. 3). Known genes in the region include *LIG1* (*ligase I, DNA, ATP-dependent*), *GRLF1* (*glucocorticoid receptor DNA binding factor 1*), *RPL18* (*ribosomal protein L18*), *HNF3G* (*hepatocyte nuclear factor 3, gamma*), and *ATP5G1* (*ATP synthase, H<sup>+</sup>-transporting, mitochondrial F0 complex, subunit C, isoform 1*). *LIG1* is one of four DNA repair/DNA metabolism genes that reside at 19q13.3, the other three (*XRCC1*, *ERCC1*, and *ERCC2*) are outside of the glioma and NB SRO. Other interesting candidate genes, such as the *glia maturation factor*  $\gamma$  (*GMF $\gamma$* ) and the *astrocytic NOVA1-like* gene or *ANOVA*, were previously unsuccessfully screened for mutations in glioma tumors (14, 17, 18). More recently, a narrower region has been suggested, extending the 150-Kb SRO between BAC 284K17 and PAC 310F22 contained within the 1.4-Mb region (4, 19). Nine new transcripts were identified in the 150-Kb region including: *C5R1*, *GLTSCR1* (*glioma tumor suppressor critical region 1*), *EHD2*, *GLTSCR2* (*glioma tumor suppressor critical region 2*), *SEPWI*, *CRX*, *STD*, *cytohesin-2*, and *synaptogyrin-4*. None of these transcribed genes showed mutations or aberrant expression in gliomas with 19q LOH (19). The clinical observation that NB tumors with 19q13 LOH recurred and were resistant to therapy suggested *BAX* at 19q13 as a candidate gene. Mutation analysis of the *BAX* gene (11) for 20 NB and 1 cell line revealed three different polymorphisms but no mutations. Furthermore, Western blot analysis for cases with 19q LOH failed to show differences in the amount of BAX protein compared with cases without 19q LOH (data not shown).

In this study, we found an association between 19q13.3 and bulky LR tumor without bone or bone marrow metastasis (most commonly clinical stages 3 and 4N), progression despite surgical debulking, and lack of response to standard cytotoxic therapy. LOH can be acquired during the course of the disease and is topographically directly associated with tumor recurrence. This subgroup may be responsible for part of the current controversies regarding conservative management of LR NB (2). There was statistical correlation between 19q13 LOH and outcome for patients with LR NB, suggesting that this marker may prove useful for risk-group classification of NB and assignment of appropriate therapy.

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