Gain of Distal Chromosome Arm 17q Is Not Associated with Poor Prognosis in Neuroblastoma

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ABSTRACT

Purpose: In several studies, gain of the distal long arm of chromosome 17 was shown to be a frequent and prognostically relevant factor in neuroblastoma, in addition to MYCN amplification (MNA) or 1p deletion. We asked whether this observation could be confirmed in a German cohort.

Experimental Design: To evaluate the frequency and prognostic impact of 17q gain, we investigated tissue samples from 193 neuroblastoma patients by the use of fluorescence in situ hybridization. The DNA probe (MPO) was located in distal 17q in the region of interest as used by several groups. To analyze the association of patients’ outcome with the breakpoint position within 17q, we used the more proximal DNA probe ERBB2 in 17q21 on a selected number of cases. Gain was defined as an excess of 17q material compared with the chromosome 17 centromere in at least 50% of the analyzed tumor cells. In addition, alterations in chromosomes 1p, 3p, and 11q, as well as MYCN status, were determined to describe the interrelationship between the different parameters and to evaluate an independent prognostic influence.

Results: Gain of 17q was found in 61% of the investigated tumors. An additional 23% displayed an excess of 17q in less than half of all cells. Gain correlated with stage 4 disease (P = 0.003) and with other chromosomal alterations, such as 1p (P < 0.001), 3p (P = 0.01), 11q (P = 0.001), and MNA (P = 0.016), as well as with increased patient age (P = 0.01). Outcome was not different between patients with 17q gain compared with those without, however. A prognostic influence could not be delineated in all stages or in localized or in stage 4 subgroups or in the MYCN nonamplified patient cohort. Outcome did not differ between patients with additional 17q material in <10% of the cells or in >70%.

INTRODUCTION

An excess of chromosomal material of distal 17q in neuroblastoma tissue was first described approximately 20 years ago (1, 2), but for more than a decade, little attention was paid to this aberration. With the development of new molecular cytogenetic techniques, this aberration was shown to be present in approximately half of all primary neuroblastoma and thus was much more frequent than the intensively investigated loss in 1p or MNA2 (3–6). Chromosome analysis of cell lines and primary tumors revealed that the additional 17q material is most often the consequence of unbalanced translocations between chromosome 17 and the translocation partners 1 or 11. Thus, gain of 17q is frequently associated with loss in 1p or 11q (7). The breakpoints in 17q were often located between the centromere and 17q21 and span to the telomere; therefore, the size of the translocated segment varied strongly but consistently included 17q23-qter (8–11). In a recent study it was shown that gain with a breakpoint proximal to the gene ERBB2 in 17q21 was correlated with a better survival than gain with a more distal breakpoint (12). 17q gain was shown to be associated with several parameters of poor prognosis, such as advanced stage disease, MNA, age >1 year, and di-/tetraploid chromosome content and was proved as a significant independent prognostic marker in neuroblastoma in a large multicenter study combining results that were obtained by different methods (5). To date, there have been no published investigations for 17q gain in neuroblastoma with a single technique in a large cohort. Therefore, we here present the first analysis of a considerable number of neuroblastoma tumors by use of FISH to evaluate the frequency and the prognostic impact of 17q gain.

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2 The abbreviations used are: MNA, MYCN amplification; FISH, fluorescence in situ hybridization; EFS, event-free survival; CGH, comparative genome hybridization.
MATERIALS AND METHODS

Tumor tissue of 193 unselected patients was investigated for unbalanced 17q gain. Samples were collected from more than 50 children’s hospitals participating in the German cooperative trials NB 85, NB 90, and NB 97. Tissue was obtained as touch preparations (n = 150), cut sections (n = 4), or bone marrow aspirates (n = 39). Eighty-seven percent (168 of 193) of the tumors were investigated at diagnosis, 5% (10 of 193) at relapse, and 8% (15 of 193) during therapy. Stage was classified according to the INS: stage 1, 20% (n = 39); stage 2, 15% (n = 28); stage 3, 10% (n = 20); stage 4, 47% (n = 90); and stage 4s, 8% (n = 16).

Median follow-up of patients without events was 16 months.

Fifty-one patients were screened for catecholamine metabolites in the German neuroblastoma screening trial (1995–2000; Ref. 13). Twenty-eight tumors were detected after a positive screening result (true-positive screening patients); 23 patients showed a negative screening result but developed a neuroblastoma later (false-negative screening patients). Before analysis, tumor cell content was determined in each preparation by a pathologist to ensure that only tissue with a minimum of 80% tumor cells was enclosed. We used FISH on interphase nuclei of all analyzed tumor cells. Nuclei with a questionable hybridization pattern (centromere associations or centromere signals of strongly varying size) were not counted. We evaluated between 100 and 250 nuclei per case.

Additionally, abnormalities in chromosome arms 1p, 3p, and 11q were investigated as well as the MYCN oncogene with use of the following DNA probes: D1Z1+D1Z2, 3p tel+D3Z1, D11Z1+MLL, and D2Z+n-myc. All FISH probes were obtained from Oncor (Gaithersburg, MD) except for D1Z1 and D1Z2 (American Type Culture Collection, Manassas, VA).

MNA was defined as at least a 5-fold higher number of MYCN copies in relation to the copy number of chromosome 2. Alterations in chromosomes 1p, 3p, and 11q were defined as described elsewhere (14, 15). The somy status of chromosome 1 was determined because it is strongly associated with tumor ploidy.

Statistical Analyses. To compare variables of interest, the $\chi^2$ test, Fisher’s exact test, or Mann–Whitney U-test was used where appropriate. Kaplan–Meier estimates for EFS were calculated and compared by the log-rank test. For multivariate analysis, Cox’s proportional hazards regression model built on EFS was used.

Recurrence, progression of disease, and death from disease were counted as events. Death resulting from therapy-related complications was not counted as an event, but was censored for EFS analysis.

RESULTS

Additional material in 17q was found in 84% (163 of 193) of all cases; however, only 61% (118 of 193) displayed the excess in >50% of all analyzed tumor cells and were thus counted as gain according to our definition. Tumors with 17q gain were found less often in localized stages [stage 1, 51% (20 of 39); stage 2, 64% (18 of 28); stage 3, 50% (10 of 20)] and 4s (31%; 5 of 16) compared with stage 4 (72%; 65 of 90; $P = 0.003$). The associations between 17q gain and other frequent chromosomal aberrations are shown in Table 1, and the interrelationships among the different aberrations are summarized in Fig. 1. Concerning the chromosome content, 17q gain was associated with di-/tetrasomy 1 (78 of 113; 63%) and was seen less frequently in trisomic tumors (27 of 65, 42%; $P < 0.001$). The age of patients with 17q gain was significantly increased (median age, 20 versus 13 months; $P = 0.01$).

Of 51 patients who participated in the German screening trial, 17q gain was found in 24 of 28 tumors (86%) with a positive screening result and thus more frequently compared with those with a negative result (14 of 23; 61%; $P = 0.057$). Of the 17 cases with 17q gain who where additionally investigated for a proximal/distal breakpoint, 9 of 17 showed a gain in the distal and proximal part (reaching 17q21), whereas 8 of 17 cases displayed the gain only in the more distal region.

Table 1 Association between 17q gain and other frequent chromosomal aberrations

<table>
<thead>
<tr>
<th>MYCN (n = 192)</th>
<th>1p (n = 188)</th>
<th>3p (n = 145)</th>
<th>11q (n = 168)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17q gain</td>
<td>78%</td>
<td>57%</td>
<td>80%</td>
</tr>
<tr>
<td>No gain</td>
<td>22%</td>
<td>43%</td>
<td>20%</td>
</tr>
<tr>
<td>$P = 0.016$</td>
<td>$P &lt; 0.001$</td>
<td>$P = 0.01$</td>
<td>$P = 0.001$</td>
</tr>
</tbody>
</table>

a Amplified; Nonampl., not amplified; Del., deletion; Nondel., no deletion.
Fig. 1 A, 17q gain and 1p loss and/or MNA. Of 185 investigated patients, 128 (69%) showed at least one alteration. Of 113 children with 17q gain, 48 (42%) also demonstrated 1p loss and/or MNA. B, 17q gain and 11q loss and/or MNA. Of 174 investigated patients, 126 (72%) showed at least one alteration. Of 110 children with 17q gain, 72 (65%) also demonstrated 11q loss and/or MNA.

A) N=185

B) N=174

Three parameters normal in 57 tumors (31%)

Three parameters normal in 48 tumors (28%)

able prognosis could be delineated in our patient cohort. To avoid artificial mistakes attributable to a distorted threshold (which defines a gain), we also calculated with a lower (gain, >30% cells with 17q excess) and a higher (>70%) threshold. The three-year EFS was 0.50 ± 0.06 for patients of all stages with additional material in >30% of the cells (n = 145 of 193; 75%) and 0.62 ± 0.08 for those without gain (n = 48 of 193; 25%; P = 0.77). When we used a threshold of 70%, 3-year EFS was 0.44 ± 0.07 (n = 81 of 193; 42%) for patients with tumor specimens of all stages with gain versus 0.62 ± 0.06 (n = 112 of 193; 58%; P = 0.61). Finally, we categorized four groups according to the percentage of cells with 17q gain: group 1, <10%; group 2, 10–39%; group 3, 40–69%; group 4, 70–100%. There was no difference in outcome among the four groups (3-year EFS, all stages: group 1, 0.51 ± 0.11 (n = 31); group 2, 0.7 ± 0.09 (n = 35); group 3, 0.66 ± 0.09 (n = 46); group 4, 0.44 ± 0.07 (n = 81); P = 0.45; Fig. 2D). When untreated primary tumors were analyzed separately or patients found by mass screening were excluded from the calculations, we could not find a prognostic impact of this aberration within the subgroups. There was no difference between patients (all stages) with and without gain, not only according to the EFS but also according to the overall survival (P = 0.48; Fig. 2E). In the MYCN-amplified or single-copy group (all stages), 17q gain was not a reliable factor for discriminating between good and poor prognosis (data not shown). The same was true for the subgroups with and without 11q loss. Fig. 3 describes the outcome for patients with regard to the status of 17q, MYCN, and 11q. Patients with alterations in 11q or MNA had a poor prognosis either with or without 17q gain. In the MYCN- and 11q-normal group, 17q status did not discriminate between good and poor outcome.

In the patient group with 17q gain extending to 17q21, five of nine showed an event to date (follow up, 9–48 months) compared with four of eight in the group with a more distal gain (follow up, 11–50 months).

Finally, we performed a multivariate analysis including the chromosomal and clinical parameters 17q gain and MYCN and 11q status as well as stage. This analysis showed that the well-known prognostic factor MYCN status and 11q status, but not 17q gain or stage, are significant markers for predicting outcome (Table 2).

DISCUSSION

This study represents the largest analysis for alterations in 17q to date using FISH as the only technique, which is used as a routine diagnostic procedure. Most other data about 17q gain were obtained from CGH studies, which usually define the thresholds for chromosomal gains or losses in ~50% and more of all investigated cells (16). Therefore, tumors with specific gains or losses in far fewer than half of all cells would hardly be recognized in CGH profiles because of the dilution effect of “normal” cells. We therefore decided to use a threshold for 17q gain of ≥50% of the analyzed tumor nuclei. This is supported by the assumption that a prognostically relevant aberration results in a growth advantage for the cell (in any way) and may therefore be expected in the majority of cells. Thus, a much lower threshold might not be reasonable.

The portion of tumors with 17q gain in our cohort was in accordance with the percentage found in other studies including CGH analyses (5, 17, 18). Furthermore, we could confirm the previously described association between 17q gain and other chromosomal aberrations, such as MNA and 1p and 11q loss, as well as patient age (3, 5, 18, 19). It was a remarkable finding that a substantial number of tumors (23%) showed additional 17q material in fewer than half of all cells, which was not counted as gain according to our definition and would partially be missed by CGH analyses. Such a strong heterogeneity in the cell population has never been observed for alterations in 1p, 3p, or 11q. Possibly 17q gain provides a growth advantage for the tumor cells in a less intense manner than the other aberrations do. In contrast to the closely corresponding overall frequency between our investigation and the multicenter study of Bown et al. (5), the stage-specific frequency was totally different. In our analyses, 71% of stage 4 tumors displayed gains in contrast to 85% in the multicenter study (5). Using microsatellite marker analysis, Mora et al. (20) also recently described a lower portion of stage 4 tumors with 17q gain (48%). In another CGH analysis of 22 stage 3 and 4 patients, unbalanced 17q gains were seen in...
only 57% (21). Conversely, 51% of our stage 1 samples contained additional 17q material, compared with only 20% in the multicenter cohort (5). Consistent with our results, a Swedish FISH study on 48 tumors (4) detected 17q gain in 52% of the localized and 71% of stage 4 tumors; however, the threshold defining a gain was much lower. Discrepancies between results obtained by FISH and by CGH might be explained in part by the use of different thresholds to define a gain. Thus it is difficult to compare results from FISH and CGH. Nevertheless, in our cohort, we found 31 patients with no gain but the same outcome as those with gain in a high percentage of cells, as shown in Fig. 2D. The position of thresholds is therefore one, but probably not the most, critical point.

The cohort of patients in the multicenter study (5) was somewhat different from other study samples concerning the number of patients with MNA. Although the stage distribution

Fig. 2 EFS according to the status of 17q in all stages (A), stages 1–3 and 4s (B), and stage 4 (C). D. EFS of four patient groups with 17q gain in a specific percentage of tumor cells. E. Overall survival according to the 17q status in all stages. Patients censored for EFS or overall survival are indicated by △ (no gain) or ▲ (gain).
appeared to be representative, the portion of amplified tumors was higher (30%) compared with the 22% amplification in 3000 neuroblastomas reported by Brodeur et al. (22, 23). Thus, the patient sample in the multicenter study seemed to have a risk profile comparable to that of the German stage 4 subgroup in terms of MYCN status.

The most surprising finding in our investigation was the complete lack of correlation between 17q overrepresentation and a patient’s outcome, which was proposed by other authors (5, 6, 17, 18). Abel et al. (4) described an unfavorable prognosis for patients with 17q gain in localized stages and 4s but not in stage 4. This discrepancy might in part be the consequence of a much lower number of investigated tumors (48 and 53, respectively) in two studies (4, 6) compared with the present study (n/H11021193). The large portion of stage 4s patients in the cohort of Brinkschmidt et al. (6) suggests that the stage distribution was different. The analyses of Bown et al. (5) comprised a very large number of patients; however, they used a combination of two or three different techniques (CGH, FISH, and cytogenetic analyses) in seven centers to determine a possible 17q gain. As mentioned before, the CGH thresholds for chromosomal gains were not exactly the same in the different investigations, and conventional chromosome analyses might misinterpret additional material in some cases with unequivocal quality of tumor chromosomes. Nevertheless, we could not confirm a correlation with the prognosis even when lower (gain in >30%) or higher (>70%) thresholds were used.

Patients did not display a different outcome when stratified by 17q gain in all stages and in stage-specific subgroups, neither in the MYCN nonamplified group nor in the subset without 11q loss. Independent of the subgroup of neuroblastoma that was evaluated, there was no statistical association between a gain of distal 17q material and patients’ outcomes after FISH analyses. This was especially clear in the 51 patients detected by neuroblastoma screening. Children found by screening are known to have a favorable outcome. This group showed a high prevalence of 17q gain compared with the prognostically inferior false-negative patients.

Lastowska et al. (12) recently showed that patients with a more proximal breakpoint for 17q gain had better survival than those with a more distal one. Like in cohort studied by Lastowskas et al., the majority of our sample consisted of stage 4 tumors. Although we did not perform a statistical analysis because of the small amount of available tissue, we found progression of disease in five of nine patients with a more proximal breakpoint; this frequency was not lower than that for the group with a more distal breakpoint. Thus, in our few cases an association between a proximal breakpoint and a good prognosis could not be delineated. However, because of the small sample size, we cannot exclude that the difference in prognostic impact between our cohort and those of Bown et al. (5) is caused by unequal breakpoint distribution in 17q.

Finally, the multivariate analysis clearly revealed that 17q gain, although the most frequent chromosomal alterations in neuroblastoma, did not have a significant impact on prognosis. Outcome was reliably predicted by the status of MYCN and distal 11q, but not by 17q. Thus, in the German neuroblastoma cohort, gain of 17q determined by FISH analysis is not suitable as a prognostic marker.

### Table 2: Multivariate analysis of 163 patients

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>Exp (β)</th>
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<tbody>
<tr>
<td>MYCN</td>
<td>&lt;0.001</td>
<td>4.86</td>
</tr>
<tr>
<td>11q</td>
<td>&lt;0.001</td>
<td>3.35</td>
</tr>
<tr>
<td>17q</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Stage 1–3, 4s vs. 4</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

*NS, not significant.

Fig. 3 EFS according to the status of 17q, MYCN, and 11q. Patients with alterations in 11q and/or MNA either with or without 17q gain (b) displayed a significantly poorer outcome compared with the nonamplified and 11q-normal group either with or without 17q gain (a). alter., alteration.

![Graph showing EFS according to the status of 17q, MYCN, and 11q.](image_url)

**Note:** Our own data.
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