Meta-Analysis Suggests Association of L-myc EcoRI Polymorphism with Cancer Prognosis

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ABSTRACT
The L-myc EcoRI polymorphism is a noncoding variation in the second intron of the L-myc gene, resulting in S and L alleles. Individuals carrying the S allele tend to have poor prognosis and increased risk of several tumor types, although controversial results have been reported. A meta-analysis of 36 studies on L-myc EcoRI genotyping, including 3563 patients with different types of cancer and 2953 controls, was performed. In lung cancer patients the S/S genotype was significantly associated with lymph node metastasis [odds ratio (OR), 2.8; 95% confidence interval (CI), 1.8–4.3], distant metastasis (OR, 4.7; 95% CI, 2.4–9.2), and stage (OR, 2.3; 95% CI, 1.2–4.4). No association was observed between the S/S genotype and cancer (OR, 1.1; 95% CI, 0.8–1.4). In patients with other cancers, the S/S genotype was significantly associated with tumor recurrence (OR, 2.8; 95% CI, 1.4–6.0), whereas no significant association was seen for the other prognostic parameters. When all types of cancer were examined together, the S/S genotype was associated with lymph node metastasis (OR, 2.3; 95% CI, 1.6–3.3), distant metastasis (OR, 2.9; 95% CI, 1.8–4.6), clinical stage (OR, 1.8; 95% CI, 1.2–2.9), and cancer risk (OR, 1.25; 95% CI, 1.07–1.45). The meta-analysis suggests that the L-myc EcoRI polymorphism is a marker of tumor prognosis in lung cancer and possibly in other types of cancer.

INTRODUCTION
In clinical practice, it is well established that patients with tumors of the same histotype and with similar clinical and pathological features differ greatly in prognosis. Variations in cancer aggressiveness and progression have been associated mainly with somatic alterations accumulated in the developing lesions (1, 2). However, recent results have raised the possibility that hereditary factors can also play a role in tumor progression. For example, survival rates of patients with hereditary nonpolyposis colorectal cancer differ significantly from those of patients with sporadic forms of colorectal cancer (3).

L-myc is a member of the myc gene family, which also includes c-myc and N-myc. These genes encode transcription factors that affect cell proliferation, apoptosis, differentiation, and tumor development (reviewed in Refs. 4 and 5). A T/G variation located in the second intron of the L-myc gene creates an EcoRI polymorphism that produces short (S) and long (L) fragments after enzymatic digestion. This noncoding polymorphism was the first genetic variation found to be associated with patients’ outcome in sporadic cancer (6). Since then, several similar studies have been carried out in different tumor types, producing contradictory data on the role of this polymorphism as a prognostic indicator in cancer patients. Some reports confirmed the significant association between L-myc polymorphism and cancer outcome (7, 8), whereas others observed no significant effects (9, 10). However, most of the studies were of small size, including less than 100 cases, and they may have been too underpowered to detect modest but real effects of L-myc polymorphism, as hypothesized for common variants that may contribute in determining disease susceptibility (11). Meta-analysis of genetic association studies is a common approach to overcome the problem of small sample size of individual studies. We chose this approach to examine whether L-myc EcoRI polymorphism is a potential marker of prognosis and/or risk in cancer patients.

METHODS
A Medline search was conducted for studies reported up to December 2003 on L-myc and prognosis or risk of different types of cancer. Key words used were “cancer risk,” “prognosis,” “stage,” “survival,” “case-control,” and “L-myc,” “MYCLI,” or “Lmyc.” Studies on prognosis were eligible if they had determined the distribution of L-myc EcoRI (T3109G in GenBank, accession no. M19720 sequence) genotypes according to clinical parameters. Studies on cancer risk had to include L-myc distribution in both cases and controls to be considered in the meta-analysis. For each study, the date of publication, type of cancer, ethnic group, number of subjects, genotype frequency of cases/control, covariates, main results, and data on genotype frequency of cases/controls were abstracted. Data on disease outcomes were tabulated as discrete (binary) end points. Data on disease outcome included in the analysis were as follows: clinical stage, lymph node metastasis, distant metastasis, and tumor recurrence. Clinical stage grouping depended on the criteria used in the original papers that allowed division only into stage I versus other stages for lung cancer and stage I/II versus higher stages for “other cancers”; lymph node metastases were separated into two groups (n = 0, no metastasis; and n > 1, presence of metastases). Data were checked for consistency with the
published article or with information provided by the investigators and converted into a standard format for incorporation into a central database.

On the basis of the extracted binary data, crude odds ratios (ORs) and their 95% confidence intervals (CIs) have been calculated for the association between the L-myc EcoRI polymorphism and cancer or prognostic parameters. Adjusted ORs for the pooled association between the L-myc EcoRI polymorphism and cancer or prognostic parameters were calculated using the Mantel-Haenszel procedure, with homozygosity of the L allele (L/L genotype) serving as the reference group, as already done in previous literature and because the L allele was the most frequent allele in the general population controls. Hardy-Weinberg equilibrium was tested by the $\chi^2$ method (12).

RESULTS

Table 1 lists the 36 articles examined in the present analysis, including 14 studies on lung cancer and 27 studies on other cancers (some articles included separate studies on different cancer types or different ethnicity, totaling 41 comparisons). Five papers were excluded because the L-myc EcoRI genotypes were either not reported by clinical data or incompletely reported (i.e., two genotypes together) or because alleles but not genotypes were reported (Table 2).

The studies were heterogeneous in their design, with a case-only, a case–control, or a mixed design. The mean size of the studies was 88 cases/study (median, 70 cases; range, 13–381 cases), with 10 (24%) of 41 studies exceeding 100 cases and only 2 studies exceeding 200 cases. Overall, 18 (44%) of 41 studies reported a statistically significant genetic association of the L-myc genotype with one or more clinical parameters or with cancer risk; 8 of these 18 studies were carried out in Asian populations and the remaining 10 in Caucasians (Table 1). Both of the larger studies reported a positive association (Table 1).

In controls, the allelic frequency of the S allele was 0.458 in Caucasians (1661 subjects), 0.486 in Asians (1224 subjects), and 0.69 in a small number (13 individuals) of African-Amer-
Lung Cancer. In lung cancer studies, seven of eight studies showed a positive association between the S/S genotype and lymph node metastasis (Fig. 1). Overall, patients with the S/S genotype showed a significantly increased risk of lymph node metastasis compared to patients with the L/L genotype (OR, 4.3; 95% CI, 2.4–9.2; Fig. 1). Similar results were observed in patients with the L/S genotype (OR, 2.3; 95% CI, 1.2–4.4; Fig. 3), and it was the same for patients with the L/L genotype (OR, 1.8; 95% CI, 1.1–3.1; not shown). Of four studies examining clinical stage, three found a positive association with the S allele.

Case–control studies indicated no significant association between lung cancer and the S/S genotype (OR, 1.1; 95% CI, 0.8–1.4), or the L/S genotype (OR, 1.1; 95% CI, 0.9–1.4; not shown).

Association between L-myc EcoRI polymorphism and lung cancer patients’ survival was observed in four of four studies carried out in the Asian population (7, 8, 13, 14), but it was not confirmed in three of three studies carried out in the Caucasian population (9, 10, 15). No individual data were available in the reports; therefore, no combined analysis on survival was possible.

Other Cancers. When we considered all non-lung cancer types combined together, no significant association was observed between L-myc polymorphism and lymph node metastasis (six studies in bladder, esophagus, gastric, and oral cancer), and tumor stage (seven studies in esophagus, gastric, etc.)
hepatocellular, oral, and renal cancer). Association between the S/S genotype and distant metastasis (seven studies in bladder, breast, gastric, hepatocellular, and renal cancer) showed an OR of 1.8 (95% CI, 1.0–3.4).

Four studies were available on tumor recurrence, including 251 subjects with bladder, oral, or renal cancer or glioma. All of the studies showed a positive association between the S allele and tumor recurrence. Combined analysis showed a significant association between tumor recurrence and L-myc EcoRI polymorphism, (OR, 2.8; 95% CI, 1.4–6.0 for the S/S genotype, Fig. 4; OR, 2.2; 95% CI, 1.1–4.4 for patients with the L/S genotype). A significant trend was observed ($\chi^2 = 9.2, P < 0.05$).

It was possible to conduct a combined analysis for cancers of certain sites, when more than one study was present; no association was found between the L-myc polymorphism and lymph node metastasis in gastric cancer; between the L-myc polymorphism and distant metastasis or stage in breast and renal cancer. The association with cancer was present in three studies (OR, 1.9; 95% CI, 1.1–3.1 for the S/S genotype), and esophageal cancer in two studies (OR, 2.3; 95% CI, 1.3–4.3 for the S/S genotype).

If all types of cancer were examined together, the Mantel-Haenszel OR for lymph node metastases with the S/S genotype was 2.3 (95% CI, 1.6–3.3) in 14 studies including 1085 subjects; the OR for the S/S genotype with distant metastasis was 2.9 (95% CI, 1.8–4.6) in 12 studies on 921 subjects, with clinical stage was 1.8 (95% CI, 1.2–2.9) in 11 studies on 753 subjects (Fig. 5). Considering all 32 case-control studies together (2358 cases and 2626 controls), a statistically significant association between cancer and the S/S genotype was observed (OR, 1.25; 95% CI, 1.07–1.45; Fig. 5).

### Table 2: Studies excluded from the present meta-analysis

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Ethnicity</th>
<th>Cases</th>
<th>Controls</th>
<th>Covariates</th>
<th>Reasons for exclusion</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mironov</td>
<td>1994</td>
<td>Caucasian</td>
<td>21</td>
<td>gastric</td>
<td>Metastasis</td>
<td>No genotype by clinical data</td>
<td>(60)</td>
</tr>
<tr>
<td>Kato</td>
<td>1996</td>
<td>Asian</td>
<td>82</td>
<td>gastric</td>
<td></td>
<td>L/S+S/S genotype</td>
<td>(61)</td>
</tr>
<tr>
<td>Ko</td>
<td>1999</td>
<td>Asian</td>
<td>99</td>
<td>colorectal</td>
<td>Stage, metastasis, survival, differentiation</td>
<td>Only S/S genotype</td>
<td>(62)</td>
</tr>
<tr>
<td>Mendoza</td>
<td>2000</td>
<td>Asian</td>
<td>97</td>
<td>lung</td>
<td>Histology, stage</td>
<td>Alleles, no genotype</td>
<td>(63)</td>
</tr>
<tr>
<td>Spinola</td>
<td>2001</td>
<td>Caucasian/Asian</td>
<td>199/108</td>
<td>lung</td>
<td>Survival in Caucasian samples</td>
<td>No genotype by clinical data</td>
<td>(10)</td>
</tr>
</tbody>
</table>

### Table 3: Distribution of L-myc EcoRI alleles among controls of the included studies

<table>
<thead>
<tr>
<th>First author (Ref.)</th>
<th>Year</th>
<th>Population</th>
<th>Genotype</th>
<th>Allele frequency</th>
<th>HWE, $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chenevix-Trench</td>
<td>1989</td>
<td>Caucasian</td>
<td>LL 46</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Ishizaki (33)</td>
<td>1990</td>
<td>Asian</td>
<td>LS 72</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Ko</td>
<td>1990</td>
<td>Asian</td>
<td>SS 43</td>
<td>0.49</td>
<td>0.009</td>
</tr>
<tr>
<td>Saranath (32)</td>
<td>1990</td>
<td>Caucasian</td>
<td>LL 17</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Tefre (9)</td>
<td>1990</td>
<td>Caucasian</td>
<td>LS 63</td>
<td>0.49</td>
<td>0.015</td>
</tr>
<tr>
<td>Dolcetti (37)</td>
<td>1991</td>
<td>Caucasian</td>
<td>SS 20</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Murakami (39)</td>
<td>1992</td>
<td>Asian</td>
<td>LS 30</td>
<td>0.49</td>
<td>0.031</td>
</tr>
<tr>
<td>Weston (36)</td>
<td>1992</td>
<td>Caucasian, African-Americans</td>
<td>LS 24</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Taylor (40)</td>
<td>1993</td>
<td>Caucasian</td>
<td>LS 22</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Crossen (42)</td>
<td>1994</td>
<td>Caucasian</td>
<td>LS 23</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Weston (43)</td>
<td>1994</td>
<td>Caucasian</td>
<td>LS 22</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Weston (43)</td>
<td>1994</td>
<td>African-Americans</td>
<td>LS 4</td>
<td>0.49</td>
<td>0.035</td>
</tr>
<tr>
<td>Young (41)</td>
<td>1994</td>
<td>Caucasian</td>
<td>LS 12</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Ge (8)</td>
<td>1996</td>
<td>Asian</td>
<td>LS 12</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Hsieh (46)</td>
<td>1996</td>
<td>Asian</td>
<td>LS 22</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Chernisha (49)</td>
<td>1998</td>
<td>Caucasian</td>
<td>LS 18</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Fernandez (48)</td>
<td>1998</td>
<td>Caucasian</td>
<td>LS 49</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Shibuta (47)</td>
<td>1998</td>
<td>Asian</td>
<td>LS 55</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Ejarque (50)</td>
<td>1999</td>
<td>Caucasian</td>
<td>LS 45</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Kondratieva (51)</td>
<td>2000</td>
<td>Caucasian</td>
<td>LS 52</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Shibuta (53)</td>
<td>2000</td>
<td>Asian</td>
<td>LS 55</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Togo (52)</td>
<td>2000</td>
<td>Caucasian</td>
<td>LS 13</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Kuminoto (56)</td>
<td>2002</td>
<td>Asian</td>
<td>LS 134</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Shih (7)</td>
<td>2002</td>
<td>Asian</td>
<td>LS 54</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Yaylim (55)</td>
<td>2002</td>
<td>Caucasian</td>
<td>LS 16</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Isbir (58)</td>
<td>2002</td>
<td>Caucasian</td>
<td>LS 29</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Dlugosz (57)</td>
<td>2002</td>
<td>Caucasian</td>
<td>LS 29</td>
<td>0.49</td>
<td></td>
</tr>
</tbody>
</table>

a HWE, Hardy-Weinberg equilibrium. Only $P$ values < 0.05 are reported.
DISCUSSION

This meta-analysis indicated a significant association between L-myc EcoRI polymorphism and lymph node metastasis, distant metastasis, and clinical stage in lung cancer subjects. For all of these variables, the S/S genotype was associated with the poor prognostic value, as compared with the L/L genotype. The heterozygous L/S genotype showed an intermediate risk. We could not perform a combined analysis of survival data because the frequency of censored individuals (not extractable from the articles) would affect the outcome and, therefore, re-analysis of the pooled raw data would be required to obtain an unbiased overall result. However, all four available studies in Asian lung cancer patients showed a significant association of the S/S L-myc genotype with poor survival (7, 8, 13, 14), whereas all three lung studies in Caucasian resulted negative (9, 10, 15). These findings may indicate an ethnic-specific effect of the L-myc EcoRI polymorphism on lung cancer patients’ survival.

For other cancer types, a significant association with L-myc was found only for tumor recurrence. However, the other prognostic parameters (lymph node and distant metastasis, tumor stage) showed a consistent, although not statistically significant, increased risk for worse prognosis in subjects with the S/S genotype. Therefore, there was an agreement between lung cancer and non-lung cancer studies, i.e., the S/S genotype being associated with poor prognosis. Other risk factors, such as smoking or hormonal levels, may have confounded the association between L-myc and outcomes, and these factors may differ according to cancer site, or their inclusion in different studies may have varied. However, we were not able to collect or control for such factors in the present analysis.

In case–control studies, a slight but significant association with cancer was observed when all studies were combined.

Meta-analyses suffer from several limitations, such as potential heterogeneity of the studies in the diagnostic criteria, patient selection, laboratory methods (16). These combined analyses, however, present the advantage of an overall assessment, in heterogeneous samples from the human population, of the potential role of a given polymorphism in a specific disease, largely increasing the power of single studies. Indeed, most of the confounding variables present in individual studies, such as population stratification and population-specific linkage disequilibrium (LD), are expected to balance and reduce their effects in a combined, overall assessment of association (17–19).

The results of this meta-analysis support the hypothesis that a genetic variation in either L-myc or in a flanking gene determines tumor progression, especially in lung cancer, and might slightly affect cancer risk.

Normal expression of the L-myc gene is restricted to specific tissues and developmental stages (20). The L-myc gene is frequently amplified and overexpressed in small cell lung cancer, but not in a broad range of tumor types (21). Therefore, a possible explanation of the results of this meta-analysis relies on...
LD of the L-myc EcoRI polymorphism with a nearby gene that affects tumor prognosis. The distance over which significant linkage disequilibrium is maintained between single nucleotide polymorphisms in the human population remains controversial, with estimates ranging from 5 kb (22) to 500 kb (23). The distribution of LD is, however, highly irregular and population-specific LD patterns may be observed (24) and may provide an explanation for the difficulty in replicating association studies.

Scanning of the L-myc chromosomal region for polymorphisms in LD with the L-myc EcoRI polymorphism might enable the identification of the functional polymorphism(s) and of the gene affecting tumor prognosis in different types of cancer.

The closest genes flanking L-myc are TRIT1 (tRNA isopentenyltransferase) and an unnamed gene coding for the hypothetical protein FLJ14490. The latter protein has no known function or protein domain that might be associated with a putative biochemical activity. The plant homologue of the human TRIT1 gene is involved in the synthesis of phytohormones that regulate plant development and physiology (25, 26). Slightly more distant L-myc flanking genes have been reported to be coamplified and/or rearranged (RLFP rearranged L-myc fusion; PPIE, peptidyl-prolyl cis-trans isomerase E; Refs. 27 and 28) with L-myc in some tumors or tumor cell lines. Their biochemical function or their role in cancer, if any, is not known.

In conclusion, the present results of the meta-analysis showed a convincing significant association between the L-myc EcoRI polymorphism and tumor prognosis and would, therefore, encourage carrying out large-scale case-only association studies for L-myc and flanking polymorphisms and tumor prognosis. Such studies may allow accessing the specific gene functions of the L-myc region and the genetic mechanisms linked to tumor prognosis, thus providing a first step in the design of specific therapeutic strategies to control cancer progression.

REFERENCES


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Fig. 5 Meta-analysis of effects of L-myc EcoRI polymorphism on tumor progression parameters and cancer risk in all types of cancers, in patients with S/S L-myc genotype minus those in patients with L/L L-myc genotype. Diamond, overall Mantel-Haenszel-adjusted odds ratio (center) for results of all studies combined. Extremes of diamond, 95% confidence interval (CI).


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