COMMENTARY

Pas1 haplotype-dependent genetic predisposition to lung tumorigenesis in rodents: a meta-analysis

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Rodent species and strains show wide variations in susceptibility to lung tumorigenesis. In mice, hierarchical clustering of 29 inbred laboratory strains by pulmonary adenoma susceptibility 1 (Pas1) locus polymorphisms separated the strains into either an A/J- or a C57BL/6J-type Pas1 haplotype. A pooled analysis (including >8500 mice) of studies on spontaneous and chemically induced lung tumorigenesis in these strains revealed a significantly higher risk of spontaneous lung tumors [odds ratio (OR) 12.17; 95% confidence interval (CI) 9.00-16.45] as well as of chemically induced lung tumors (OR 15.14; 95% CI 12.51-18.31) in the A/J-type haplotype. Strain differences were observed with six different carcinogens, suggesting that Pas1 locus activity is carcinogen-independent. Thus, the present meta-analysis indicates a link between the genetic control of spontaneous and chemically induced lung tumor susceptibility in mice. The Pas1 susceptibility allele is frequent in the population of inbred mouse strains, whereas a counterpart appears to be absent or rare in rat and hamster strains. These findings might help in the interpretation of results of rodent carcinogenicity bioassays and assessing the risk of lung carcinogenesis from chemicals.

Introduction

Laboratory rodents represent mammalian models for the risk assessment of potential chemical carcinogenicity in humans (1). However, rodent species and strains often vary widely in susceptibility/resistance to either spontaneous or chemically induced tumorigenesis, complicating the extrapolation of results obtained in these species to humans. The identification and characterization of the genetic factors involved in susceptibility/resistance to tumorigenesis in rodents would enable a more rationally based risk assessment of potential carcinogenicity of chemicals. Moreover, dissection of the genetic characteristics of rodent strains might improve interpretations of any kind of carcinogenicity results obtained in these models.

Chromosomal mapping studies of lung cancer modifier loci in mice has implicated the pulmonary adenoma susceptibility 1 (Pas1) locus, mapping in the distal region of chromosome 6, as a major determinant of quantitative variations in chemically induced lung tumor multiplicity (2-4). Additional loci may affect inherited resistance (5) or susceptibility to chemically induced lung tumor multiplicity or progression (6), pointing to a polygenic control of susceptibility to lung tumor development in the mouse (7).

To date, no overall quantitative assessment has been carried out to define the role of the Pas1 locus in determining the lung tumor risk of mouse strains treated with different chemical carcinogens nor have genetic linkage studies been carried out to define the role of Pas1 in spontaneous lung tumor susceptibility. However, although several studies have reported the incidence of spontaneous or chemically induced lung tumors in mouse strains, either for comparative purposes or for carcinogenesis bioassays, most mouse carcinogenesis studies include a relatively small number of animals per group. A meta-analysis of available studies, an approach commonly used to overcome the problem of small sample size of individual studies, would allow a more precise assessment of the quantitative effects of the Pas1 locus on spontaneous as well as chemically induced lung tumor response.

Our recent study on positional cloning of the Pas1 locus indicates that in mouse laboratory strains the Pas1 locus consists of a conserved haplotype block of 468 kb containing six putative Pas1 candidate genes (8). Thus, the recent availability of the Pas1 haplotype distribution pattern for most strains tested for lung carcinogenesis (8), together with the published studies on spontaneous and chemically induced lung tumorigenesis, make it feasible to quantitatively estimate association of the Pas1 locus with spontaneous and chemically induced lung tumorigenesis.

We used the meta-analysis approach to examine whether and to what extent the Pas1 haplotype is a potential determinant of lung cancer risk in inbred mouse strains. In rats and hamsters, for which no overall assessment of strain susceptibility/resistance to lung tumorigenesis has been available, we conducted a literature search of spontaneous incidence of lung tumors in these rodents for a comparison of lung tumorigenesis susceptibility with that in mice.

Lung tumor phenotypes and mouse Pas1 locus haplotype

A Medline search was conducted for studies reported as of March 2004 on lung carcinogenesis in mouse strains. Key words used were: lung, pulmonary, cancer, tumor, adenoma, strain(s), inbred, mice, rats, hamsters. Potentially informative studies quoted in the extracted papers were also examined. In two studies on spontaneous lung tumor incidence in mice, including six strains (9,10), only the percentage tumor incidence, but not the number of mice, was reported: in those cases, we arbitrarily estimated the number of mice as 30 (Table I).

The Pas1 locus haplotype was assigned to each strain based on our previous analysis (8) of 54 genetic markers located in a 468 kb region on chromosome 6.
Mouse strain clustering by *Pasl* haplotype

Hierarchical cluster analysis of 29 inbred mouse laboratory strains using 54 genetic markers mapping within the 468 kb *Pasl* haplotype region (8) produced a dendogram revealing two separate branches, A/J-type and C57BL/6J-type, based on *Pasl* haplotype (Figure 1A). The *Pasl*-derived strain dendogram separated laboratory strains into two major branches carrying either the A/J-type or the C57BL/6J-type haplotype, respectively. The branch of mice with the *Pasl* resistance allele included 12 strains, whereas the branch representing the *Pasl* susceptibility allele included 17 strains (open and filled lines, respectively, in Figure 1A). These results, consistent with previous studies (11,12), indicated that the *Pasl* susceptibility allele is frequent (allelic frequency ~ 0.6) in inbred laboratory mouse strains. The *Pasl*-related dendogram reflects strain relatedness at the *Pasl* locus, which is different from strain genealogy based on strain history or on whole genome polymorphisms (13). Indeed, some pairs of strains with close genealogy and genome-wide similarity carry a different *Pasl* haplotype (Figure 1B).

The dendogram shows that intra-group similarity was much higher for mice with the C57BL/6J-type *Pasl* haplotype (open lines) than for A/J-type *Pasl* haplotype mice (filled lines), which can be divided into several subgroups. This observation reflects the higher intra-group variability of the genetic polymorphisms in A/J-type than C57BL/6J-type *Pasl* haplotype mice (8), possibly due to the presence of slightly different *Pasl* alleles among the A/J-type strains. In light of the apparent complex and multigenic nature of the *Pasl* locus, constituted by a cluster of genes and polymorphisms, it is possible that the locus contains internal cancer modifiers whose polymorphisms arose and evolved independently of the haplotype in several A/J-type strains, whereas the *Pasl* haplotype remained stable in C57BL/6J-type mice. Further studies in appropriate genetic crosses, including two strains belonging to different sub-branches of the A/J-type *Pasl* haplotype, may clarify this hypothesis.

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**Table I.** Spontaneous incidence of lung tumors in mouse inbred strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Haplotype type</th>
<th>Mice with lung tumors (%)</th>
<th>Total no. of mice</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>129</td>
<td>A/J</td>
<td>1</td>
<td>149 (15)</td>
<td></td>
</tr>
<tr>
<td>A/J</td>
<td>A/J</td>
<td>82</td>
<td>182 (16,17,30)</td>
<td></td>
</tr>
<tr>
<td>BALB/c</td>
<td>A/J</td>
<td>33</td>
<td>901 (31,32)</td>
<td></td>
</tr>
<tr>
<td>CBA</td>
<td>A/J</td>
<td>17</td>
<td>681 (15,33)</td>
<td></td>
</tr>
<tr>
<td>FVB/N</td>
<td>A/J</td>
<td>36</td>
<td>121 (34,35)</td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td>A/J</td>
<td>5</td>
<td>128 (15)</td>
<td></td>
</tr>
<tr>
<td>O20/A</td>
<td>A/J</td>
<td>41</td>
<td>136 (36)</td>
<td></td>
</tr>
<tr>
<td>STS/A</td>
<td>A/J</td>
<td>24</td>
<td>88 (36)</td>
<td></td>
</tr>
<tr>
<td>MA/MyJ</td>
<td>A/J</td>
<td>42</td>
<td>310 (10)</td>
<td></td>
</tr>
<tr>
<td>P/J</td>
<td>A/J</td>
<td>3</td>
<td>30 (30b)</td>
<td></td>
</tr>
<tr>
<td>RF/J</td>
<td>A/J</td>
<td>26</td>
<td>115 (9,37)</td>
<td></td>
</tr>
<tr>
<td>RIIRS/J</td>
<td>A/J</td>
<td>34</td>
<td>112 (36)</td>
<td></td>
</tr>
<tr>
<td>SWR/J</td>
<td>A/J</td>
<td>47</td>
<td>437 (19,38)</td>
<td></td>
</tr>
<tr>
<td>AKR/J</td>
<td>C57BL/6</td>
<td>0</td>
<td>71 (20,39)</td>
<td></td>
</tr>
<tr>
<td>C3H</td>
<td>C57BL/6</td>
<td>9</td>
<td>408 (15,16,19,40)</td>
<td></td>
</tr>
<tr>
<td>C57BL/10</td>
<td>C57BL/6</td>
<td>3</td>
<td>93 (15,16)</td>
<td></td>
</tr>
<tr>
<td>C57BL/6</td>
<td>C57BL/6</td>
<td>3</td>
<td>729 (19,31)</td>
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<tr>
<td>C57BR/cdJ</td>
<td>C57BL/6</td>
<td>3</td>
<td>30 (9b)</td>
<td></td>
</tr>
<tr>
<td>C57L</td>
<td>C57BL/6</td>
<td>0</td>
<td>101 (17)</td>
<td></td>
</tr>
<tr>
<td>CEJ</td>
<td>C57BL/6</td>
<td>0</td>
<td>17 (41)</td>
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<tr>
<td>DBA/1 J</td>
<td>C57BL/6</td>
<td>3</td>
<td>30 (10)</td>
<td></td>
</tr>
<tr>
<td>DBA/2</td>
<td>C57BL/6</td>
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<td>118 (15)</td>
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<tr>
<td>NZB/B1gd</td>
<td>C57BL/6</td>
<td>0</td>
<td>535 (42)</td>
<td></td>
</tr>
<tr>
<td>SJL/J</td>
<td>C57BL/6</td>
<td>5</td>
<td>30 (9)</td>
<td></td>
</tr>
</tbody>
</table>

*Age at the end of the observation: 18–24 months, except for AKR mice (10–15 months).

*Group size estimated as 30 mice when only percentages were reported in the quoted papers.

*Number of mice in Ryan et al. (9) estimated as 30.
Modulation of spontaneous and chemically-induced lung tumorigenesis in mice by *Pas1* haplotype

The specific *Pas1* haplotype was assigned to mouse strains for which lung tumor incidence and multiplicity data were available. Substrains, which in most cases represent the same strains maintained by different institutes/laboratories, were considered genetically equivalent to the parental strain. Since some lung tumor susceptibility data but no genomic DNA were available for the progenitor Dba and C57Bl strains, the C57BL/6J-type *Pas1* haplotype was assigned to these strains because all the substrains originating from these strains (DBA/1 and DBA/2 from Dba, and C57BL/10 and C57BL/6 from C57Bl) carry that *Pas1* haplotype.

Tables I–III summarize the data on spontaneous and chemically induced lung tumor incidence and multiplicity from published studies. Since, with a few exceptions (3), in most of the strains and crosses gender only marginally affects lung tumor susceptibility (14), data from mice of both sexes were pooled to obtain an overall assessment of strain susceptibility.

Spontaneous lung tumor incidence data were collected from 20 studies that included 13 strains with the A/J-type and 11 strains with the C57BL/6J-type *Pas1* haplotype (Table I). The mean size of the experimental groups was 123 mice/group (median 78 mice/group, range 17–632 mice/group). In mouse strains carrying the A/J-type *Pas1* haplotype, 965 of 3110 (31%) mice developed lung tumors during their lifetime, with the incidence in individual studies ranging from 0 to 86%. In decreasing order, strains A/J, SWR/J, MA/MjJ, and O20 showed the highest (>40%) spontaneous lung tumor incidences. In mice carrying the C57BL/6J-type *Pas1* haplotype, overall spontaneous lung tumor incidence was 66 of 2162 (3.1%), with incidences in individual studies ranging from 0 to 16%. The C3H strain showed the highest (9%, range 4–16%) spontaneous lung tumor incidence. The difference between mice carrying the A/J-type and C57BL/6J-type *Pas1* haplotype in overall incidence was highly statistically significant (negative logarithm of P value (–log P) = 168, Fisher’s exact test).

Chemically induced lung tumor incidences collected from 14 studies included 7 strains with the A/J-type and 10 strains

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Strain</th>
<th>Haplotype type</th>
<th>No. of mice with lung tumors (%)</th>
<th>Total no. of mice</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,5,6-Dibenzanthracene</td>
<td>A</td>
<td>A/J</td>
<td>108 (100)</td>
<td>108</td>
<td>(18)</td>
</tr>
<tr>
<td></td>
<td>C57L</td>
<td>C57BL/6</td>
<td>11 (24)</td>
<td>46</td>
<td>(18)</td>
</tr>
<tr>
<td></td>
<td>7,12-Dimethylbenz[a]anthracene</td>
<td>129/SvJ</td>
<td>A/J</td>
<td>2 (12)</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>A/J</td>
<td>A/J</td>
<td>62 (94)</td>
<td>66</td>
<td>(43,44)</td>
</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>A/J</td>
<td>49 (78)</td>
<td>63</td>
<td>(43,44)</td>
</tr>
<tr>
<td></td>
<td>CBA</td>
<td>A/J</td>
<td>30 (65)</td>
<td>46</td>
<td>(44)</td>
</tr>
<tr>
<td></td>
<td>FVB/J</td>
<td>A/J</td>
<td>12 (71)</td>
<td>17</td>
<td>(43)</td>
</tr>
<tr>
<td></td>
<td>C3H</td>
<td>C57BL/6</td>
<td>27 (42)</td>
<td>64</td>
<td>(43,44)</td>
</tr>
<tr>
<td></td>
<td>C57BL</td>
<td>C57BL/6</td>
<td>1 (2)</td>
<td>48</td>
<td>(44)</td>
</tr>
<tr>
<td></td>
<td>C57BL/6J</td>
<td>C57BL/6</td>
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<td>16</td>
<td>(43)</td>
</tr>
<tr>
<td></td>
<td>DBA/2J</td>
<td>C57BL/6</td>
<td>2 (13)</td>
<td>16</td>
<td>(43)</td>
</tr>
<tr>
<td></td>
<td>Diethylnitrosamine</td>
<td>SWR/J</td>
<td>A/J</td>
<td>18 (72)</td>
<td>25</td>
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<td>AKR/J</td>
<td>C57BL/6</td>
<td>6 (24)</td>
<td>25</td>
<td>(39)</td>
</tr>
<tr>
<td></td>
<td>C57BL/6J</td>
<td>C57BL/6</td>
<td>3 (14)</td>
<td>21</td>
<td>(39)</td>
</tr>
<tr>
<td></td>
<td>Dimethylnitrosamine</td>
<td>A/Sn</td>
<td>A/J</td>
<td>40 (78)</td>
<td>51</td>
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<td>C3H</td>
<td>C57BL/6</td>
<td>19 (18)</td>
<td>107</td>
<td>(16)</td>
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<tr>
<td></td>
<td>C57BL/10ScSnA</td>
<td>C57BL/6</td>
<td>4 (8)</td>
<td>53</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>N-Ethyl-N-nitrosourea</td>
<td>A/Bcr</td>
<td>A/J</td>
<td>38 (48)</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>SWR/J</td>
<td>A/J</td>
<td>44 (40)</td>
<td>109</td>
<td>(22)</td>
</tr>
<tr>
<td></td>
<td>AKR/J</td>
<td>C57BL/6</td>
<td>12 (14)</td>
<td>86</td>
<td>(22)</td>
</tr>
<tr>
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<td>C57BL/6J</td>
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<td>6 (7)</td>
<td>85</td>
<td>(22)</td>
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<td>C57BL/Bcr</td>
<td>C57BL/6</td>
<td>10 (23)</td>
<td>44</td>
<td>(23)</td>
</tr>
<tr>
<td></td>
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<td>C57BL/6</td>
<td>5 (7)</td>
<td>75</td>
<td>(22)</td>
</tr>
<tr>
<td></td>
<td>DBA/2J</td>
<td>C57BL/6</td>
<td>2 (2)</td>
<td>87</td>
<td>(22)</td>
</tr>
<tr>
<td></td>
<td>DBA/Bcr</td>
<td>C57BL/6</td>
<td>17 (23)</td>
<td>75</td>
<td>(23)</td>
</tr>
<tr>
<td></td>
<td>Urethane</td>
<td>129/J</td>
<td>A/J</td>
<td>8 (89)</td>
<td>9</td>
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<tr>
<td></td>
<td>A/J</td>
<td>A/J</td>
<td>145 (100)</td>
<td>145</td>
<td>(14,20,21)</td>
</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>A/J</td>
<td>103 (87)</td>
<td>118</td>
<td>(11,21,45)</td>
</tr>
<tr>
<td></td>
<td>CBA/H</td>
<td>A/J</td>
<td>30 (79)</td>
<td>38</td>
<td>(20)</td>
</tr>
<tr>
<td></td>
<td>RIIS/J</td>
<td>A/J</td>
<td>7 (70)</td>
<td>10</td>
<td>(21)</td>
</tr>
<tr>
<td></td>
<td>SWR</td>
<td>A/J</td>
<td>220 (96)</td>
<td>230</td>
<td>(11,19,21)</td>
</tr>
<tr>
<td></td>
<td>AKR/J</td>
<td>C57BL/6</td>
<td>5 (9)</td>
<td>57</td>
<td>(20,21)</td>
</tr>
<tr>
<td></td>
<td>C3H</td>
<td>C57BL/6</td>
<td>170 (32)</td>
<td>525</td>
<td>(19,21,45)</td>
</tr>
<tr>
<td></td>
<td>C57BL/10</td>
<td>C57BL/6</td>
<td>5 (26)</td>
<td>19</td>
<td>(21)</td>
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<tr>
<td></td>
<td>C57BL/6</td>
<td>C57BL/6</td>
<td>31 (13)</td>
<td>248</td>
<td>(19–21)</td>
</tr>
<tr>
<td></td>
<td>C57BR/cdJ</td>
<td>C57BL/6</td>
<td>0 (0)</td>
<td>10</td>
<td>(21)</td>
</tr>
<tr>
<td></td>
<td>C57L/J</td>
<td>C57BL/6</td>
<td>1 (10)</td>
<td>10</td>
<td>(21)</td>
</tr>
<tr>
<td></td>
<td>DBA/2J</td>
<td>C57BL/6</td>
<td>5 (25)</td>
<td>20</td>
<td>(21)</td>
</tr>
<tr>
<td></td>
<td>DBAf</td>
<td>C57BL/6</td>
<td>10 (32)</td>
<td>31</td>
<td>(45)</td>
</tr>
<tr>
<td></td>
<td>NZB/BINJ</td>
<td>C57BL/6</td>
<td>3 (25)</td>
<td>12</td>
<td>(21)</td>
</tr>
</tbody>
</table>
with the C57BL/6J-type \( \text{Pas}1 \) haplotype; mice were treated with any of six different chemical carcinogens (Table II). In mice carrying the A/J-type \( \text{Pas}1 \) haplotype, chemical carcinogen treatment induced lung tumors in 916 of 1132 (80.9%) mice, with a range over individual studies of 11.8–100%. On the other hand, lung tumors developed in 355 of 1780 (20.0%) carcinogen-treated mice carrying the C57BL/6J-type \( \text{Pas}1 \) haplotype, with a range of 0–57%. The difference between mice carrying the A/J-type and C57BL/6J-type \( \text{Pas}1 \) haplotype in overall chemically induced incidence was highly significant (\( \log P = 24.1 \), Fisher’s exact test). Heterogeneity of the studies precluded assessment of lung carcinogenic potency among the six different chemicals. However, \( N \)-ethyl-\( N \)-nitrosourea appears to be relatively less efficient as a lung carcinogen, in both A/J-type and C57BL/6J-type \( \text{Pas}1 \) haplotype mice.

Lung tumor multiplicity data from 11 studies included 14 strains with the A/J-type and 12 strains with the C57BL/6J-type \( \text{Pas}1 \) haplotype (Table III). Overall, >1079 carcinogen-treated mice were analyzed. Three different chemical carcinogens were used, although most of the studies were carried out with urethane. The mean size of single experiments was 27 mice per strain, treatment and study (median 23, range 6–108), however, the number of treated mice was not reported in 11 experiments (Table III). In mice carrying the A/J-type \( \text{Pas}1 \) haplotype, chemical carcinogen treatment induced 13.2 ± 3.1 (mean ± SE) lung tumors/mouse, whereas 0.6 ± 0.2 lung tumors/mouse were induced in similarly treated mice carrying the C57BL/6J-type \( \text{Pas}1 \) haplotype (\( P < 0.001 \), ANOVA).

Of note, the 129 (Tables I–III), LP and P/J (Tables I and III) strains show a consistently relatively low lung tumor susceptibility in spite of their allocation to the susceptible A/J-type haplotype group (Figure 1A). The reason for this apparent discrepancy may be the strength and number of resistant alleles carried by these strains at pulmonary tumor modifier loci. We have observed that 129/SvJ and LP/J mice carry lung tumor resistance alleles, inhibiting in a semi-dominant way the genetic susceptibility of the A/J strain (14).

We then conducted a pooled analysis using only the studies that included both A/J-type and C57BL/6J-type \( \text{Pas}1 \) haplotype strains, which were similarly kept as untreated groups or treated with chemical carcinogen. Based on the incidence data, we estimated the \( \text{Pas}1 \) haplotype-associated odds ratios (ORs) in the individual studies and then in all studies combined. All seven studies on spontaneous lung tumor incidence showed an OR > 1 for mice carrying the A/J-type \( \text{Pas}1 \) haplotype compared with C57BL/6J-type mice (Figure 2A). ORs ranged from 1.59 (15) to 17.67 (16), except for the

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**Table III.** Chemically induced lung tumor multiplicity in mouse inbred strains

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Strain</th>
<th>Haplotype type</th>
<th>Lung tumor multiplicitya</th>
<th>Total no. of mice</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>1,2,5,6-Dibenzanthracene</td>
<td>A</td>
<td>A/J</td>
<td>75.4</td>
<td>108 (18)</td>
<td>(18)</td>
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<tr>
<td></td>
<td>C57L</td>
<td>C57BL/6</td>
<td>0.3</td>
<td>46 (18)</td>
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</tr>
<tr>
<td>( N )-Ethyl-( N )-nitrosourea</td>
<td>A</td>
<td>A/J</td>
<td>32.5</td>
<td>56 (4)</td>
<td>(4)</td>
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<td>CBA/A</td>
<td>A/J</td>
<td>5.6</td>
<td>39 (46)</td>
<td>(46)</td>
</tr>
<tr>
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<td>P/J</td>
<td>A/J</td>
<td>1.8</td>
<td>30 (46)</td>
<td>(46)</td>
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<td>SM/J</td>
<td>A/J</td>
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<td>35 (46)</td>
<td>(46)</td>
</tr>
<tr>
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<td>C57BL/6 J</td>
<td>C57BL/6</td>
<td>2.6 ± 1.5</td>
<td>86 (4,46)</td>
<td>(4,46)</td>
</tr>
<tr>
<td></td>
<td>C57BR/cdj</td>
<td>C57BL/6</td>
<td>1.7</td>
<td>43 (46)</td>
<td>(46)</td>
</tr>
<tr>
<td>Urethane</td>
<td>129/J</td>
<td>A/J</td>
<td>2.1</td>
<td>9 (21)</td>
<td>(21)</td>
</tr>
<tr>
<td></td>
<td>A/J</td>
<td>A/J</td>
<td>27.5 ± 2.7</td>
<td>126 (14,21,47)</td>
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</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>A/J</td>
<td>3.3 ± 0.5</td>
<td>95 (11,21,45)</td>
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<tr>
<td></td>
<td>CBA</td>
<td>A/J</td>
<td>1.7 ± 0.6</td>
<td>40 (47,48)</td>
<td>(47,48)</td>
</tr>
<tr>
<td></td>
<td>LP/J</td>
<td>A/J</td>
<td>1.1</td>
<td>NR (48)</td>
<td>(48)</td>
</tr>
<tr>
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<td>MA/MyJ</td>
<td>A/J</td>
<td>8.9</td>
<td>NR (48)</td>
<td>(48)</td>
</tr>
<tr>
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<td>NPG/N</td>
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<td>28.3</td>
<td>NR (48)</td>
<td>(48)</td>
</tr>
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<td>O20/A</td>
<td>A/J</td>
<td>12.1</td>
<td>20 (49)</td>
<td>(49)</td>
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<td>PL/J</td>
<td>A/J</td>
<td>2.0</td>
<td>NR (48)</td>
<td>(48)</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>A/J</td>
<td>6.7 ± 5.0</td>
<td>48 (21,47)</td>
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<tr>
<td></td>
<td>SM/J</td>
<td>A/J</td>
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<td>NR (48)</td>
<td>(48)</td>
</tr>
<tr>
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<td>ST/J</td>
<td>A/J</td>
<td>3.2</td>
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<td>(48)</td>
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<tr>
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<td>SWR/J</td>
<td>A/J</td>
<td>20.1 ± 5.1</td>
<td>41 (11,21)</td>
<td>(11,21)</td>
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<td>AKR</td>
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<td>0.1 ± 0.1</td>
<td>11 (21,48)</td>
<td>(21,48)</td>
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<td>C3H</td>
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<td>19 (21)</td>
<td>(21)</td>
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<td>C57BL/6</td>
<td>C57BL/6</td>
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<td>27 (21,48)</td>
<td>(21,48)</td>
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<td>0.9</td>
<td>35 (47)</td>
<td>(47)</td>
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<td>10 (21)</td>
<td>(21)</td>
</tr>
<tr>
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<td>(21)</td>
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<td>C58/J</td>
<td>C57BL/6</td>
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<td>NR (50)</td>
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<td>DBA/2J</td>
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<td>20 (21)</td>
<td>(21)</td>
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<td>C57BL/6</td>
<td>0.5</td>
<td>31 (45)</td>
<td>(45)</td>
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<td>NZB/BINJ</td>
<td>C57BL/6</td>
<td>0.3</td>
<td>12 (21)</td>
<td>(21)</td>
</tr>
<tr>
<td></td>
<td>SIM/J</td>
<td>C57BL/6</td>
<td>0.3</td>
<td>NR (48)</td>
<td>(48)</td>
</tr>
</tbody>
</table>

aMean ± SE.

bNR, not reported.
extremely high OR = 981, observed in the pioneer study of Heston (17). Overall, mouse strains with the A/J-type \( \text{Pas}1 \) haplotype showed a significantly higher risk of spontaneous lung tumors than did C57BL/6J-type \( \text{Pas}1 \) mice [OR 12.17; 95% confidence interval (CI) 9.00–16.45] (Figure 2A). All 11 studies on chemically induced lung tumor incidence showed an OR for mice with the A/J-type \( \text{Pas}1 \) haplotype compared with C57BL/6J-type mice (Figure 2B). ORs ranged from 3.08 to 52.55, except for a single study comparing A/J with C57L mice treated with 1,2,5,6-dibenzanthracene, which showed a very high OR due to the 100% tumor incidence in the highly susceptible A/J mice and the low lung tumor response of C57L mice (18; Table II). Excluding this study, the chemical treatment associated with the highest ORs was urethane (range 42.95–52.55) (19–21; Table II); N-ethyl-N-nitrosourea was associated with the lowest ORs (range 3.08–8.34) (22,23). Overall, mouse strains with the A/J-type \( \text{Pas}1 \) haplotype showed a significantly higher risk of chemically induced lung tumors compared with C57BL/6J-type \( \text{Pas}1 \) mice (OR 15.14; 95% CI 12.51–18.31) (Figure 2B).

Meta-analysis of lung tumor incidence and multiplicity according to \( \text{Pas}1 \) haplotype in inbred mouse strains indicated that the \( \text{Pas}1 \) locus is significantly linked with strain susceptibility to both spontaneous and chemically induced lung tumorogenesis (Figure 2). These results provide a genetic link between susceptibility to both spontaneous and chemically induced lung tumor susceptibility, which appear to be modulated by a common genetic mechanism rather than by a specific chemical carcinogen. In the chemical carcinogen studies urethane was most frequently used, but five other carcinogens of different chemical classes also produced the same pattern of \( \text{Pas}1 \) haplotype-linked lung tumor incidence (Tables II and III). Indeed, the similar OR values observed for spontaneous (OR 12.17) (Figure 2A) and chemically induced (OR 15.14) (Figure 2B) lung tumorigenesis support the hypothesis that phenotypic effects of the \( \text{Pas}1 \) locus are carcinogen-independent.

**Spontaneous incidence of lung tumors in rats and hamsters**

Analysis of published studies on lifetime spontaneous lung tumor incidence in rats indicated available data for 11 rat strains and a total of 21 022 animals, with 1911 ± 639 (mean ± SE) rats/strain (Table IV). Rats have a low spontaneous lung tumor incidence, ranging from 0 to 1.9%. The F344 and Lewis strains revealed a slightly higher susceptibility than the other strains, with lung tumor incidences of 1.9 and 1.8%, respectively, as compared with 0.0–0.7% in the other nine strains. Few data on spontaneous lung tumor incidence of Syrian golden hamsters are available in the literature. Overall, lifetime spontaneous lung tumor incidences ranged from 0 to 0.8% in 1056 animals (Table IV).
The high overall spontaneous lung tumor incidence in mice (~19%, Table I, all strains combined) may reflect the high prevalence of the Ad-like $Pas1$ haplotype in the population of inbred mouse strains, since 17 of 29 (60%) strains carried that haplotype. The effects of the $Pas1$ locus in mouse spontaneous lung tumorigenesis, producing strong strain differences in susceptibility, contrast sharply with the lack of strain difference in other rodent species, particularly in rats, a species with a high number of strains and in common use for carcinogenicity bioassays. Indeed, spontaneous lung tumor incidence is homogeneously low in rat strains, ranging from 0 to 1.9% in 11 rat strains (Table IV). Comparison of mouse and rat incidences of spontaneous lung tumors suggested a much lower frequency of the susceptibility alleles at putative lung cancer modifier genes in the population of rat laboratory strains than in that of mouse strains. Thus, at present we have no evidence to support the existence of any ‘strong’ locus modulating genetic predisposition to lung tumorigenesis in rat laboratory strains. However, the low susceptibility of rats may also result from a high preponderance in this species of balancing alleles that confer resistance. Spontaneous lung tumor incidence is also low in hamsters (0–0.8%, Table IV), although few data from only a small number of strains of these animals are available.

A further justification of the cross-species comparison comes from the observation of the same prevalent histotype of spontaneous lung tumors in rats, hamsters and mice, i.e. adenoma or adenocarcinoma (see references in Tables I and IV). Thus, the existence of histotype-specific genetic elements cannot be invoked to explain the differences in lung tumor incidence among these species.

### Rodent species comparison of carcinogen-induced lung tumorigenesis

Comparison of rodent species for susceptibility to chemically induced lung tumorigenesis is hampered by the small number of studies available. However, a reasonable comparison may be carried out between the F344 rat strain and the B6C3F1 mouse hybrid, both species being routinely used at the National Toxicology Program (NTP). Thirty-eight NTP studies carried out in F344 rats and B6C3F1 mice showed clear evidence of lung carcinogenicity in either rats or mice (http://ntp-server.niehs.nih.gov/htdocs/Sites/LUNG.html). Among these 38 studies, 23 were positive in both F344 rats and B6C3F1 mice, six were positive only in B6C3F1 mice and nine were positive only in F344 rats. Thus, F344 rats and B6C3F1 mice showed a similar susceptibility to a variety of lung carcinogens. Since B6C3F1 mice originate from two parental strains both carrying the C57BL/6J-type $Pas1$ haplotype (Figure 1A), we could therefore infer that the F344 rat strain carries a $Pas1$ haplotype equivalent to that of C57BL/6J mice (i.e. a $Pas1$ haplotype conferring resistance to lung carcinogenesis). However, the low susceptibility of F344 rats to chemical induction of lung tumors may also derive from a preponderance of lung cancer resistance alleles.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>No. of animals with lung tumors (%)</th>
<th>Total no. of animals</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>ACI/N</td>
<td>0 (0.0)</td>
<td>459</td>
<td>(51,52)</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>1 (0.5)</td>
<td>200</td>
<td>(52)</td>
</tr>
<tr>
<td></td>
<td>Brown-Norway</td>
<td>4 (0.6)</td>
<td>680</td>
<td>(53–56)</td>
</tr>
<tr>
<td></td>
<td>CD</td>
<td>11 (0.4)</td>
<td>2523</td>
<td>(57)</td>
</tr>
<tr>
<td></td>
<td>Donryu</td>
<td>1 (0.5)</td>
<td>191</td>
<td>(51)</td>
</tr>
<tr>
<td></td>
<td>F344</td>
<td>137 (1.9)</td>
<td>7036</td>
<td>(58,59)</td>
</tr>
<tr>
<td></td>
<td>Lewis</td>
<td>14 (1.8)</td>
<td>769</td>
<td>(56,60)</td>
</tr>
<tr>
<td></td>
<td>Marshall</td>
<td>1 (0.5)</td>
<td>195</td>
<td>(52)</td>
</tr>
<tr>
<td></td>
<td>Osborne–Mendel</td>
<td>16 (0.7)</td>
<td>2145</td>
<td>(52,61)</td>
</tr>
<tr>
<td></td>
<td>Sprague-Dawley</td>
<td>17 (0.5)</td>
<td>3314</td>
<td>(62–64)</td>
</tr>
<tr>
<td></td>
<td>Wistar</td>
<td>19 (0.5)</td>
<td>3510</td>
<td>(65–67)</td>
</tr>
<tr>
<td>Hamster</td>
<td>CH</td>
<td>0 (0.0)</td>
<td>160</td>
<td>(68)</td>
</tr>
<tr>
<td></td>
<td>WH</td>
<td>1 (0.8)</td>
<td>131</td>
<td>(68)</td>
</tr>
<tr>
<td></td>
<td>AH</td>
<td>0 (0.0)</td>
<td>149</td>
<td>(68)</td>
</tr>
<tr>
<td></td>
<td>Syrian golden</td>
<td>5 (0.8)</td>
<td>616</td>
<td>(69,70)</td>
</tr>
</tbody>
</table>

The present evidence indicates that the susceptibility allele of the $Pas1$ locus is frequent in inbred mouse strains in which it determines a high genetic risk of lung tumors. On the other hand, a strong lung cancer susceptibility allele appears to be rare or absent in inbred rat strains and in the general human population.

### Spontaneous lung cancer in humans

In humans >90% of lung cancers occur in smokers, with the incidence of lung cancer in non-smokers being about 5 in 100 000 people/year (<0.5% lifetime incidence in humans). Thus, the frequency of a lung cancer predisposing allele(s) in the human population appears to be low. Preliminary case-control studies on the role of the homologous human $PAS1$ region (chromosome 12p12) in lung cancer have indicated a statistically borderline (24) or non-significant (25) association of a $Kras$ intronic polymorphism with lung cancer risk but a statistically significant association of the same polymorphism with cancer prognosis (24,25). These findings, although preliminary, contrast with the ORs of 12–15 observed in inbred mouse strains and suggest that in the general population genetic polymorphisms in the putative human $PAS1$ locus either do not exist or play a much smaller role in the genetic risk of lung cancer, as compared with the effects of the $Pas1$ locus in mouse inbred strains.

However, the low frequency of a lung cancer predisposing allele(s) in the general human population does not exclude the existence or a putative strong role of such alleles in particular individuals. Indeed, segregation analysis of families with lung cancer probands supports the involvement of a rare ‘major’ Mendelian factor that transmits a high risk of early onset lung cancer in a co-dominant manner (26–29). Nonetheless, establishing any role for the $PAS1$ locus in humans awaits identification of candidate functional polymorphisms.

The present evidence indicates that the susceptibility allele of the $Pas1$ locus is frequent in inbred mouse strains in which it determines a high genetic risk of lung tumors. On the other hand, a strong lung cancer susceptibility allele appears to be rare or absent in inbred rat strains and in the general human population.

### Conclusions

Although meta-analyses of carcinogenesis studies may suffer from several limitations related to potential heterogeneity of the studies with respect to diagnostic criteria, animal maintenance and diet and the laboratory methods used, combined analysis enables an overall assessment of the potential role of a given polymorphism, i.e. the $Pas1$ haplotype, in a specific disease, i.e. lung cancer, in heterogeneous samples from multiple laboratories and historical periods, greatly increasing the power over single studies. Indeed, most of the confounding variables present in individual studies are expected to balance...
out and produce less effect in a combined, overall assessment of association.

Contrasting results in different mouse strains or in different rodent species underscore the need to understand the strain- and species-specific mechanisms of lung carcinogenesis in experimental animals and their possible relevance for humans. Species-specific genetic variations might represent a major determinant of the spontaneous tumor spectrum and of the specific tumor response to chemicals.

A detailed strain and species comparison of the nucleotide sequence of the \textit{Pasl} region could highlight differences and similarities and perhaps provide a molecular genetic explanation of the differences in susceptibility to lung tumorigenesis.

Overall, the mechanism by which \textit{Pasl} exerts its effects does not appear to be chemical carcinogen-specific or linked with metabolic activation, but rather with control of the cell cycle, apoptosis or the inflammatory response. If the same type of genetic control of susceptibility/resistance to lung tumorigenesis operates in humans, population-based studies focused more on the role of polymorphisms of the latter class of genes than of genes involved in metabolic activation of chemical carcinogens might be valuable.

Understanding the genetic mechanisms underlying strain and species differences in spontaneous and chemically induced carcinogenesis is a necessary step in pursuing a rationally based risk assessment of chemical carcinogenicity in humans.

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References


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