Dosage effect of zero to three functional LBR-genes in vivo and in vitro

Gravemann, S; Schnipper, N; Meyer, H; Vaya, A; Nowaczyk, M J; Rajab, A; Hofmann, W K; Salewsky, B; Tönnies, H; Neitzel, H; Stassen, H H; Sperling, K; Hoffmann, K

Abstract: The Lamin B receptor (LBR) is a pivotal architectural protein in the nuclear envelope. Mutations in the Lamin B receptor lead to nuclear hyposegmentation (Pelger-Huët anomaly). We have exactly quantified the nuclear lobulation in neutrophils from individuals with 0, 1, 2 and 3 functional copies of the lamin B receptor gene and analyzed the effect of different mutation types. Our data demonstrate that there is a highly significant gene-dosage effect between the gene copy number and the nuclear segmentation index of neutrophils. This finding is paralleled by a dose-dependent increase in LBR protein and staining intensity of the nuclear membrane in corresponding lymphoblastoid cell lines, which demonstrates a significant correlation on the protein level as well. We further show that LBR expression continually increases during granulopoiesis in vitro from human precursor cells with ovoid nuclei to multi-segmented neutrophil nuclei 11 days later, indicating relevance for regular human granulopoiesis. Altogether, LBR is a unique model that will allow the systematic study of gene-dosage effects and of modifying endogeneous and exogeneous factors on granulopoiesis.

DOI: [https://doi.org/10.4161/nucl.1.2.11113](https://doi.org/10.4161/nucl.1.2.11113)

Posted at the Zurich Open Repository and Archive, University of Zurich
ZORA URL: [https://doi.org/10.5167/uzh-46880](https://doi.org/10.5167/uzh-46880)

Originally published at:
DOI: [https://doi.org/10.4161/nucl.1.2.11113](https://doi.org/10.4161/nucl.1.2.11113)
<table>
<thead>
<tr>
<th>No. of functional LBR copies</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean value and standard deviation</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Supplemental Table 1. Correlation between the number of functional (wild type) LBR copies and the number of neutrophil nuclear segments according to the “Drittelregel”. Two individuals with zero, nine individuals with one, 12 individuals with two and three individuals with three functional (wild type) LBR copies were included. Fifty cells were analyzed from each individual. For each individual the number of cells with n=1 to n=6 nuclear segments, the total number of nuclear segments and the mean value of nuclear segments is shown.

(N) = null mutation; (S) = splice-site mutation; (R) = unknown mutation (probably regulatory)
<table>
<thead>
<tr>
<th>Experiment</th>
<th>ID</th>
<th>Genotype</th>
<th>signal intensity</th>
<th>LBR</th>
<th>β-Actin</th>
<th>LBR/β-Actin</th>
<th>Adjusted to control 96P125 = 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-01</td>
<td>8387</td>
<td>0 (S)</td>
<td>17599</td>
<td>134907</td>
<td>0.130</td>
<td>0.092</td>
<td></td>
</tr>
<tr>
<td></td>
<td>02P316</td>
<td>0 (R)</td>
<td>48554</td>
<td>128574</td>
<td>0.378</td>
<td>0.265</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8388</td>
<td>1 (S)</td>
<td>97214</td>
<td>105730</td>
<td>0.919</td>
<td>0.645</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8386</td>
<td>1 (S)</td>
<td>129161</td>
<td>130725</td>
<td>0.988</td>
<td>0.694</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96P125</td>
<td>2</td>
<td>194257</td>
<td>136359</td>
<td>1.425</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>01P233</td>
<td>3</td>
<td>187194</td>
<td>95042</td>
<td>1.970</td>
<td>1.383</td>
<td></td>
</tr>
<tr>
<td></td>
<td>01P146</td>
<td>3</td>
<td>231627</td>
<td>139955</td>
<td>1.655</td>
<td>1.162</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8388</td>
<td>0 (S)</td>
<td>6785</td>
<td>193489</td>
<td>0.035</td>
<td>0.042</td>
<td></td>
</tr>
<tr>
<td></td>
<td>02P316</td>
<td>0 (R)</td>
<td>63808</td>
<td>224584</td>
<td>0.284</td>
<td>0.341</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8388</td>
<td>1 (S)</td>
<td>108287</td>
<td>215001</td>
<td>0.504</td>
<td>0.604</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8386</td>
<td>1 (S)</td>
<td>140612</td>
<td>227958</td>
<td>0.617</td>
<td>0.739</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96P125</td>
<td>2</td>
<td>191137</td>
<td>229097</td>
<td>0.834</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>01P233</td>
<td>3</td>
<td>287908</td>
<td>217609</td>
<td>1.323</td>
<td>1.586</td>
<td></td>
</tr>
<tr>
<td></td>
<td>01P146</td>
<td>3</td>
<td>305156</td>
<td>280523</td>
<td>1.088</td>
<td>1.304</td>
<td></td>
</tr>
<tr>
<td>20-01</td>
<td>8387</td>
<td>0 (S)</td>
<td>7045</td>
<td>66320</td>
<td>0.106</td>
<td>0.067</td>
<td></td>
</tr>
<tr>
<td></td>
<td>02P316</td>
<td>0 (R)</td>
<td>32065</td>
<td>78119</td>
<td>0.410</td>
<td>0.257</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8388</td>
<td>1 (S)</td>
<td>73119</td>
<td>73710</td>
<td>0.992</td>
<td>0.621</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8386</td>
<td>1 (S)</td>
<td>65803</td>
<td>79658</td>
<td>0.826</td>
<td>0.517</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96P125</td>
<td>2</td>
<td>137360</td>
<td>86002</td>
<td>1.597</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>01P233</td>
<td>3</td>
<td>148029</td>
<td>58786</td>
<td>2.518</td>
<td>1.577</td>
<td></td>
</tr>
<tr>
<td></td>
<td>01P146</td>
<td>3</td>
<td>157492</td>
<td>81618</td>
<td>1.930</td>
<td>1.208</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Joint analysis</th>
<th>ID</th>
<th>Genotype</th>
<th>LBR/ β-Actin</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-01</td>
<td>8387</td>
<td>0 (S)</td>
<td>0.067</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td></td>
<td>02P316</td>
<td>0 (R)</td>
<td>0.288</td>
<td>0.038</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8388</td>
<td>1 (S)</td>
<td>0.623</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8386</td>
<td>1 (S)</td>
<td>0.650</td>
<td>0.096</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96P125</td>
<td>2</td>
<td>1.000</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>01P233</td>
<td>3</td>
<td>1.515</td>
<td>0.094</td>
<td></td>
</tr>
<tr>
<td></td>
<td>01P146</td>
<td>3</td>
<td>1.930</td>
<td>0.059</td>
<td></td>
</tr>
</tbody>
</table>

Supplemental Table 2: Protein quantification (Densitometry)