**A viable mouse model for Netherton syndrome based on mosaic inactivation of *Spink5* gene**

Petr Kasparek, Zuzana Ileninova, Radka Haneckova, Ivan Kanchev, Irena Jenickova, and Radislav Sedlacek

**Supplementary Material**

**Supplementary Table 1** Sequencing of Spink5 mutant alleles in Spink5-TALPSP mice.

|  |  |  |
| --- | --- | --- |
| Nucleotide sequence | | Alleles per mouse |
| wt | CATACCGCAGTAGATGTGAACTGTGTGCTGAGAATGCGTGAGTACCC |  |
| Lesional skin  Spink5-TALPSP 1 | CATACCGCAGTAGA-------TGTGTGCTGAGAATGCGTGAGTACCC -7 nt | 1 |
| CATACCGCAGTAGATGTGAACTGT-------------GTGAGTACCC -13 nt | 2 |
| CATACCGCAGTAGATGTGAACTGTGTG-----CATGCGTGAGTACCC -5 nt | 3 |
| CATACCGCAGTAGATGTGAACTGTGTG-----CATGCGTGAGTACCC -5 nt | 3 |
| CATACCGCAGTAGATGTGAACTGT-------------GTGAGTACCC -13 nt | 2 |
| CATACCGCAGTAGATGTGAACTGTGTG-----CATGCGTGAGTACCC -5 nt | 3 |
| nLesional skin  Spink5-TALPSP 1 | CATACCGCAGTAGATGTGAACTGTGTGCTGAGAATGCGTGAGTACCC wt | 3 |
| CATACCGCAGTAGATGTGAACTGTGTGCTGAGAATGCGTGAGTACCC wt | 3 |
| CATACCGCAGTAGATGTGAACTGTGTG---AGA---CGTGAGTACCC -6 nt | 1 |
| CATACCGCAGTAGATGTGAACTGTGTG---AGAATGCGTGAGTACCC -3 nt | 2 |
| CATACCGCAGTAGATGTGAACTGTGTG---AGAATGCGTGAGTACCC -3 nt | 2 |
| CATACCGCAGTAGATGTGAACTGTGTGCTGAGAATGCGTGAGTACCC wt | 3 |
| Lesional skin  Spink5-TALPSP 2 | CATACCGCAGTAGATGTGAACTGTGT-----GAATGCGTGAGTACCC -5 nt | 3 |
| CATACCGCAGTAGATGTGAACTGTGT-----GAATGCGTGAGTACCC -5 nt | 3 |
| CATACCGCAGTAGATGTGAACTGTGT-----GAATGCGTGAGTACCC -5 nt | 3 |
| CATACCGCAGTAGATGTGAACTGTGTG-----CATGCGTGAGTACCC -5 nt | 3 |
| CATACCGCAGTAGATGTGAACTGTGTG-----CATGCGTGAGTACCC -5 nt | 3 |
| CATACCGCAGTAGATGTGAACTGTGTG-----CATGCGTGAGTACCC -5 nt | 3 |
| nLesional skin  Spink5-TALPSP 2 | CATACCGCAGTAGATGTGAACTGTGTGCTGAGAATGCGTGAGTACCC wt | 4 |
| CATACCGCAGTAGATGTGAACTGTGTGCTGAGAATGCGTGAGTACCC wt | 4 |
| CATACCGCAGTAGATGTGAACTGTGT-----GAATGCGTGAGTACCC -5 nt | 2 |
| CATACCGCAGTAGATGTGAACTGTGTGCTGAGAATGCGTGAGTACCC wt | 4 |
| CATACCGCAGTAGATGTGAACTGTGTGCTGAGAATGCGTGAGTACCC wt | 4 |
| CATACCGCAGTAGATGTGAACTGTGT-----GAATGCGTGAGTACCC -5 nt | 2 |

DNA was obtained from lesional and non-lesional areas of two Spink5-TALPSP mice (Spink5-TALPSP A and B). PCR products were amplified as described in Figure legend 2E and cloned into pGEM-T easy vector (Promega, Fitchburg, WI, USA) according to manufacturer´s instructions. Six independent clones obtained from each DNA template were analysed by Sanger sequencing.

**Legends**

**Supplementary Figure 1** Schematic representation of the Spink5 gene.

TALEN-target sequence (TS) is located in the exon5 of *Spink5*. Binding sites for left (TALEN-Sp5-L) and right left (TALEN-Sp5-R) TALENs is marked with red color.

**Supplementary Figure 2** Histological analysis of Spink5-TALPSP lesional and non-lesional skin.

Hematoxylin and eosin stained section obtained from Spink5-TALPSP skin. Lesional epidermis (flanked by red arrowheads) is characterized by acanthosis, parakeratosis and severe intrafollicular hyperkeratosis (white arrows). Surrounding non-lesional epidermis (black arrowhed) does not show any prominent skin abnormalities; scale bar = 500 μm. Hematoxylin and eosin staining was performed as described in Figure Legend 3B.

**Supplementary Figure 3**  Keratin 6 expression in Spink5-TALPSP lesional and non-lesional skin.

Strong upregulation of keratin 6 expression was found only in the lesional areas (red arrowheads), but not in non-lesional epidermis (black arrowhead); scale bar = 500 μm. Keratin 6 staining was performed as described in Figure Legend 3C.

**Supplementary Figure 4** qRT-PCR analysis of Klk expression.

Expression levels of Klk5, Klk7 and Klk14 genes in wildtype (wt) and Spink5-TALPSP lesional (Sp5-Les) skin were quantified using qRT-PCR analysis. mRNA levels were normalized to TBP expression.

**Supplementary Figure 5** Analysis of proteolytic activity by casein gel zymography.

Epidermal extracts obtained from P1 Spink5-TALPSP pups show increased caseinolytic activity (red arrowhead) in comparison to wt samples. Epidermal samples were frozen in liquid nitrogen, homogenized and diluted in 1M acetic acid (Lach:Ner, Neratovice, Czech Republic). 4 μg of protein were loaded on 12,5% polyacrylamide gel with 0,05% casein (Sigma Aldrich, St Louis, MO, USA) and separated by electrophoresis. The gels were first incubated for 2 h at 37 °C in 2,5% Triton X-100 (Sigma Aldrich, St Louis, MO, USA) and then for 24 h at 37 °C in 0,1 M Tris-HCl (Sigma Aldrich, St Louis, MO, USA). The gels were stained with 1% Coomasie Blue for 2 h.