

## SUPPLEMENTARY FILES

### Material

Comparison of the amino-acid sequences of the PROE and IGLL1 proteins of pigeons with their homologous proteins in geese and ducks.

Local alignments of the amino-acid sequences of pigeon PROE (XP\_005514568) and IGLL1 (XP\_005503921) proteins were performed against non-redundant (NR) protein sequences from a database of goose and duck taxids using the BLASTp tool. Identity (ID), similarity (SIM), and percentage coverage were used to compare the amino-acid sequences of the pigeon PROE and IGLL1 proteins with their homologous proteins in geese and ducks.

### Results

#### Comparison blast

The PROE amino-acid sequences showed a minimum coverage of 73%, with the percentage of identity ranging from 61 to 81% and the percentage of similarity from 68 to 88% (Table A1, Appendices). The duck PROE protein has an amino acid sequence closer to that of pigeons than geese. The alignments of the IGLL1 amino-acid sequences showed a minimum coverage of 99%, a percentage of identity ranging from 65 to 89%, and a percentage of similarity from 77 to 89% (Table A1, Appendices). The IGLL1 proteins of goose and duck showed highly conserved amino-acid sequences (Coverage: 100%, ID, 81%, SIM: 89%).

Table A1.

Antigenic Pigeon proteins (Accession Number)	Alignment Pigeon / Goose	Alignment Pigeon / Duck	Alignment Goose/ Duck
Pigeon PROE (XP_005514568)	Proproteinase E-like (XP_013056116) ID: 61% SIM: 68% Coverage: 86%	Proproteinase E-like (XP_027303168) ID: 81% SIM: 88% Coverage: 88%	BLASTp (XP_013056116/XP_027303168) ID: 73% SIM: 74% Coverage: 73%
Pigeon IGLL1 (XP_005503921)	Immunoglobulin light chain V-J-C region (AMS75113); 231 aa ID: 66% SIM: 80% Coverage: 99%	Immunoglobulin lambda light chain (CAA57568); 230 aa ID: 65% SIM: 77% Coverage: 99%	BLASTp (AMS75113/CAA57568) ID: 81% SIM: 89% Coverage: 100%

Table A1. Alignment of amino-acid (aa) sequences of pigeon, goose, and duck proteins.

Abbreviations: ID: identity, SIM: similarity. Alignments were conducted using BLASTp tools

freely available at <https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins>

Table A2:

	Recombinant proteins	AUC [95% CI]	Threshold index value	Sensitivity (%) [95%CI]	Specificity (%) [95%CI]	P-value (K-test)	PPV (%)	NPV (%)
Chronic FDL patients vs controls (excluding BFL)	r-PROE	0.90 [0.82-0.97]	0.505	75.0 [55.0-90.0]	86.7 [73.3-96.7]	3.33e-05	78.95	83.87
	r-IGLL1	0.80 [0.66-0.92]	0.325	55.0 [35.0-75.0]	93.3 [83.3-100]	2.731e-06	84.62	75.68
Acute FDL patients vs controls (excluding BFL)	r-PROE	0.85 [0.67-1.0]	0.615	72.7 [45.5-100]	100 [100-100]	0.002723	100	90.91
	r-IGLL1	0.68 [0.43-0.89]	0.440	36.4 [9.1-63.64]	100 [100-100]	0.007672	100	81.08

Table A2: Diagnostic accuracy of serum IgG antibody against r-PROE (Proproteinase E) and r-IGLL1 (Immunoglobulin lambda-like polypeptide-1) for chronic and acute FDL patients. The threshold index value was derived from the ROC analysis. PPV, positive predictive value ; NPV, negative predictive value.

Figure A1:

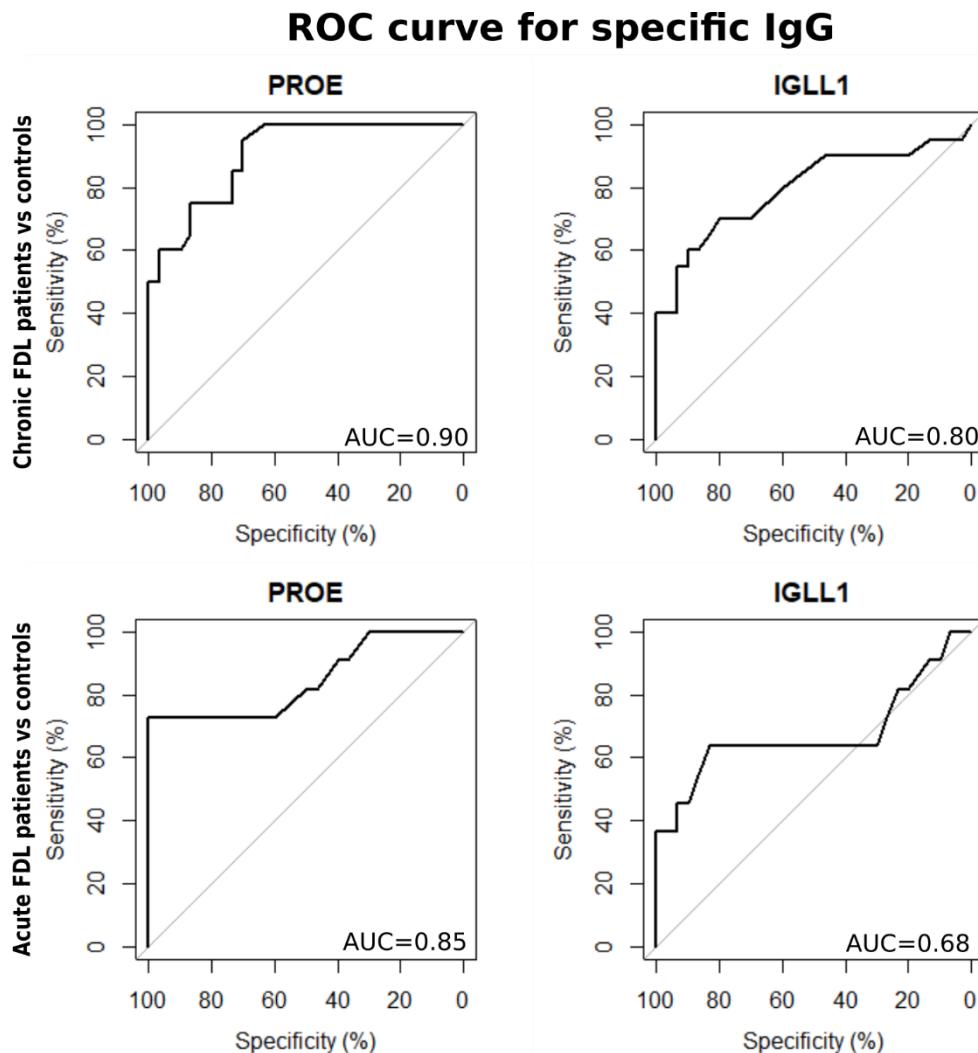


Fig A1 : ROC curves for the titres of serum IgG against r-PROE and r-IGLL1 to compare the index values between patients with chronic and acute FDL and controls.