生物信息工具在长岛型掌跖角化症研究中的应用

Application of bioinformatic analysis tools

on the research of

Nagashima-type Palmoplantar Keratoderma

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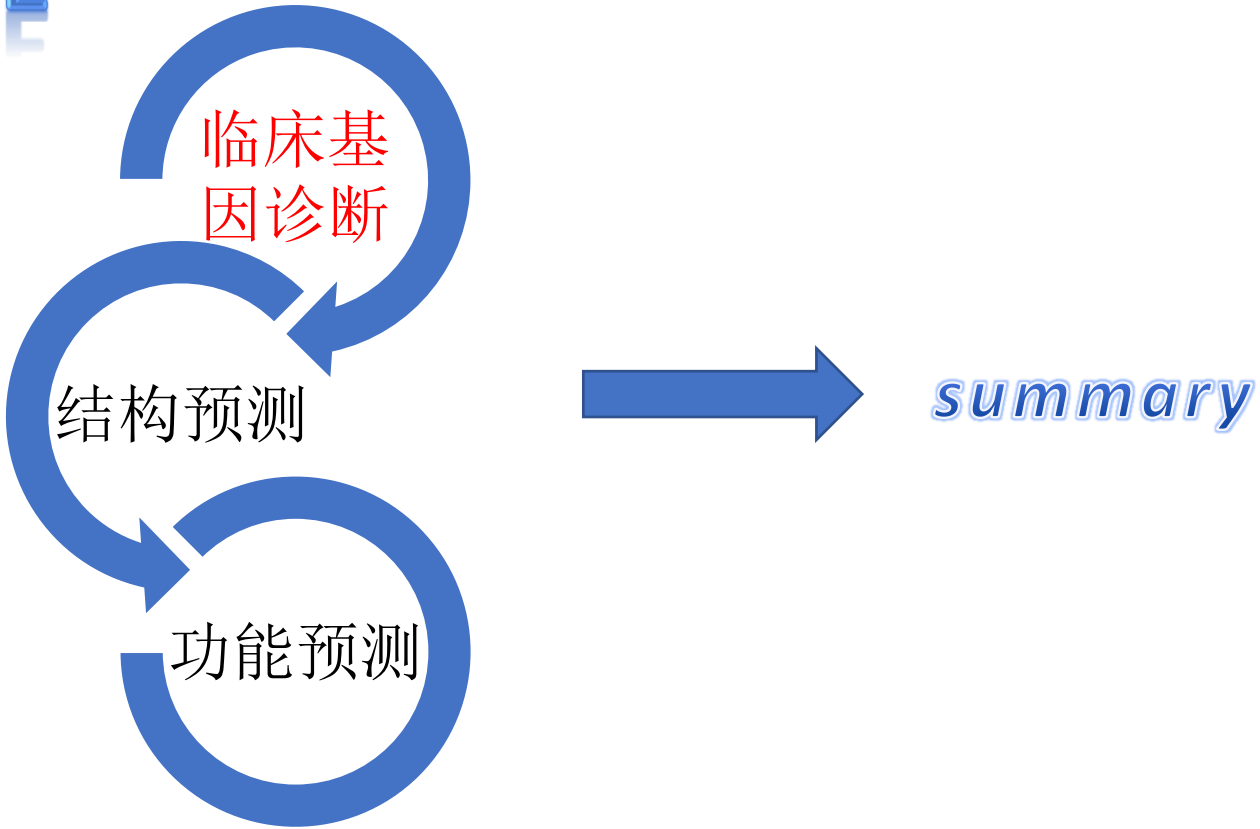
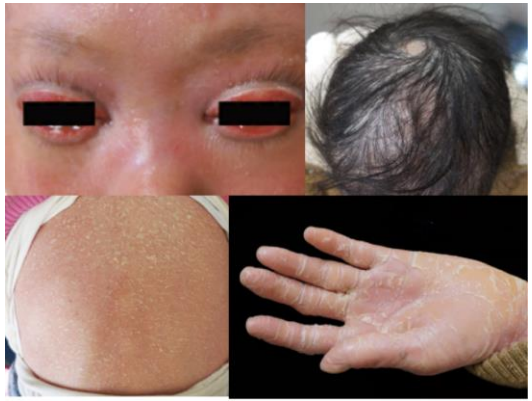
主 讲 人: 刘婷婷

2019.01.12

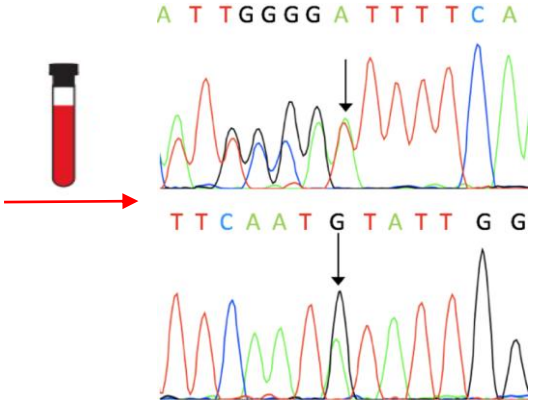
OUTLINE



单基因遗传病临床诊断



临床症状

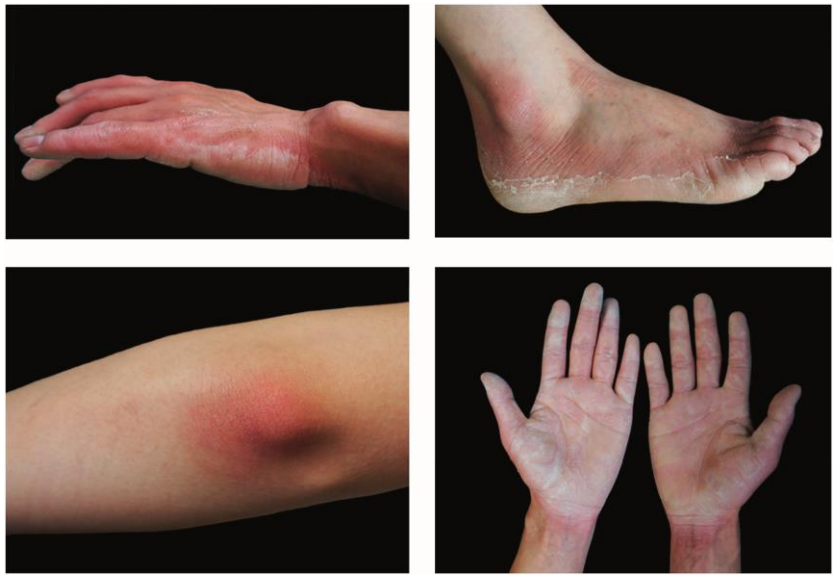


基因检测

基因型与表型共分离病例报道

长岛型掌跖角化症（NPPKs）

- 长岛型掌跖角化症，又称Nagashima掌跖角化症（NPPKs），是一种日本人首先命名的遗传性掌跖角化症。
- 主要表现为双手、双足的皮肤红斑、增厚、伴有水后掌跖角质发白、肿胀，及手足多汗。足部多汗通常会激发真菌感染，部分患者还伴有肘及膝盖部位的皮肤红斑、角化。
- 研究现状：中国人和日本人中携带*SERPINB7*致病基因比例较高（多达3%）且无治疗药物。



遗传类型和基因：

OMIM®

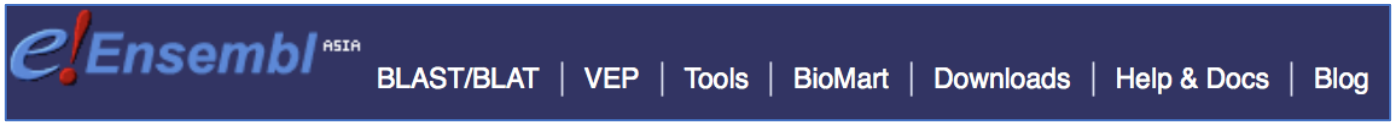
Online Mendelian Inheritance in Man®

Phenotype-Gene Relationships

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key	Gene/Locus	Gene/Locus MIM number
18q21.33	Palmoplantar keratoderma, Nagashima type	615598	AR	3	SERPINB7	603357

本病为*SERPINB7*基因突变所致的**常染色体隐性遗传病**，*SERPINB7*基因是**丝氨酸蛋白酶抑制剂**家族成员之一，主要通过抑制蛋白酶活性来发挥生物功能。

转录本下载：



下载human的各个外显子序列

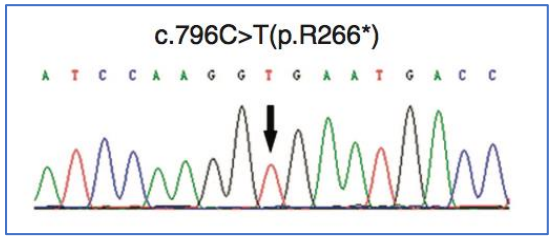
引物设计：

BLAST®

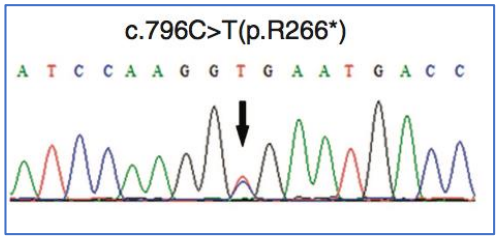
Basic Local Alignment Search Tool

设计出特异性好，Gc含量合适的引物

测序结果：



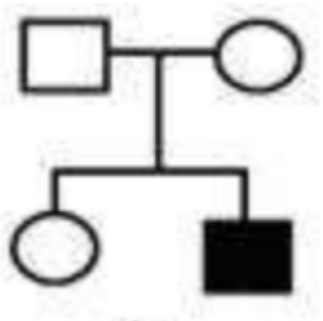
患者：纯合突变



父母：杂合突变

共分离：

C/T

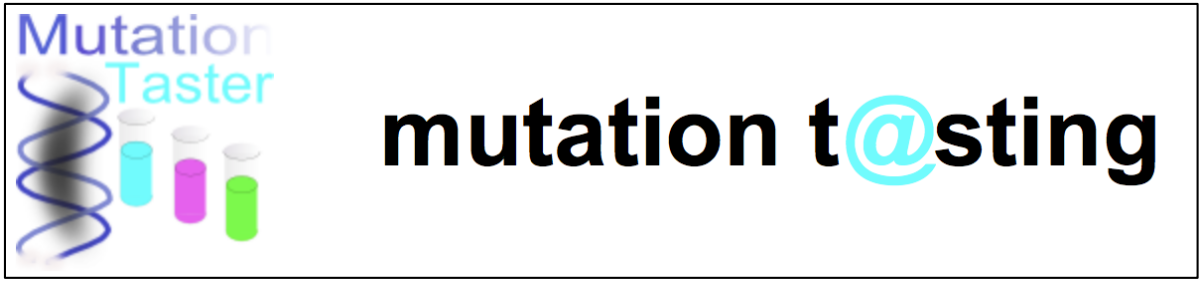


C/T

T/T

患者父母均正常，携带有一个突变
患者出现症状，且带有两个突变

突变预测：



相关疾病数据库中注释信息

序列与蛋白结构

氨基酸保守性

家系	碱基突变	氨基酸改变	SIFT	Polyphen-2	Mutation Taster	功能预测
1	c.2759A>G	p.N920S	0.000	0.884	0.999	致病

Mutation Taster:

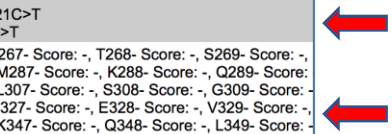
ATCCAAGG[C/T]GAATGACC

Prediction disease causing

Summary

- amino acid sequence changed
- heterozygous in TGP or ExAC
- known disease mutation: rs142859678 (pathogenic)
- protein features (might be) affected
- splice site changes
- truncated protein (might cause NMD)

analysed issue	analysis result
name of alteration	no title
alteration (phys. location)	chr18:61471522C>T show variant in all transcripts IGV
HGNC symbol	SERPINB7
Ensembl transcript ID	ENST00000398019
Genbank transcript ID	NM_001261830
UniProt peptide	O75635
alteration type	single base exchange
alteration region	CDS
DNA changes	c.796C>T cDNA.1121C>T g.51354C>T
AA changes	R266*, M267- Score: -, T268- Score: -, S269- Score: -, Score: -, M287- Score: -, K288- Score: -, Q289- Score: Score: -, L307- Score: -, S308- Score: -, G309- Score: - Score: -, I327- Score: -, E328- Score: -, V329- Score: -, Score: -, K347- Score: -, Q348- Score: -, L349- Score: - Score: -, K367- Score: -, D368- Score: -, D369- Score: -
position(s) of altered AA if AA alteration in CDS	266 (frameshift or PTC - further changes downstream)
frameshift	no



Model: *complex_aae*, prob: 0.999999999969084 (classification due to ClinVar, real probability is shown anyway)

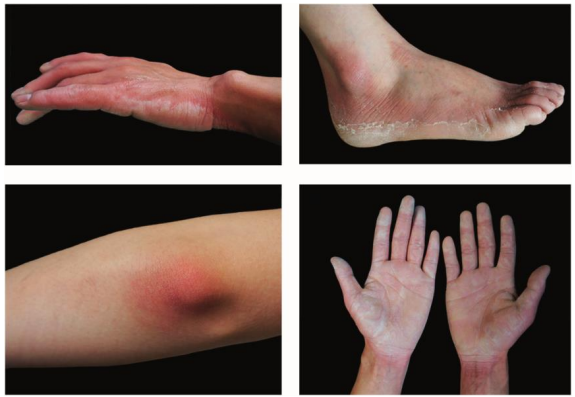
相关病例报道：

				Allele 1		Allele 2		
Patient	Sex	Age (years)	Onset age	Nucleotide change	Amino-acid change	Nucleotide change	Amino-acid change	Clinical features in addition to PPK
1	Female	25	8 months	c.796C>T	p.R266*	c.796C>T	p.R266*	Mild erythrokeratoderma on the elbows and knees
2	Male	13	At birth	c.796C>T	p.R266*	c.796C>T	p.R266*	Tinea pedis, hyperhidrosis
3	Male	17	6 months	c.796C>T	p.R266*	c.796C>T	p.R266*	Tinea pedis, hyperhidrosis
4	Female	24	6 months	c.796C>T	p.R266*	c.796C>T	p.R266*	Tinea pedis, hyperhidrosis
5	Male	11	1 year	c.796C>T	p.R266*	c.650-653delCTGT	p.S217Lfs*7	Tinea pedis, hyperhidrosis
6	Female	8	1 year	c.796C>T	p.R266*	c.455G>T	p.G152V and predicted splicing alternation	Mild erythrokeratoderma affecting the elbows and knees, mild pruritus
7	Male	30	4 years	c.522-523insT	p.V175Cfs*46	c.522-523insT	p.V175Cfs*46	Onychomycosis and tinea pedis, hyperhidrosis

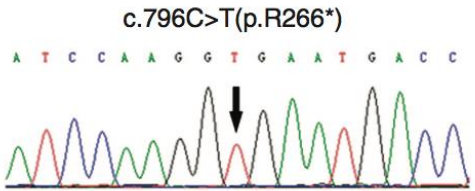
Abbreviation: PPK, palmoplantar keratoderma.

Yin J et all . J Invest Dermatol . 2014

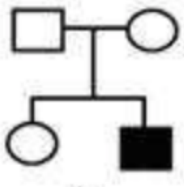
长岛型掌跖角化症的临床基因诊断：



临床症状：双手、双足的皮肤红斑、增厚、伴有水后掌跖角质发白、肿胀，及手足多汗

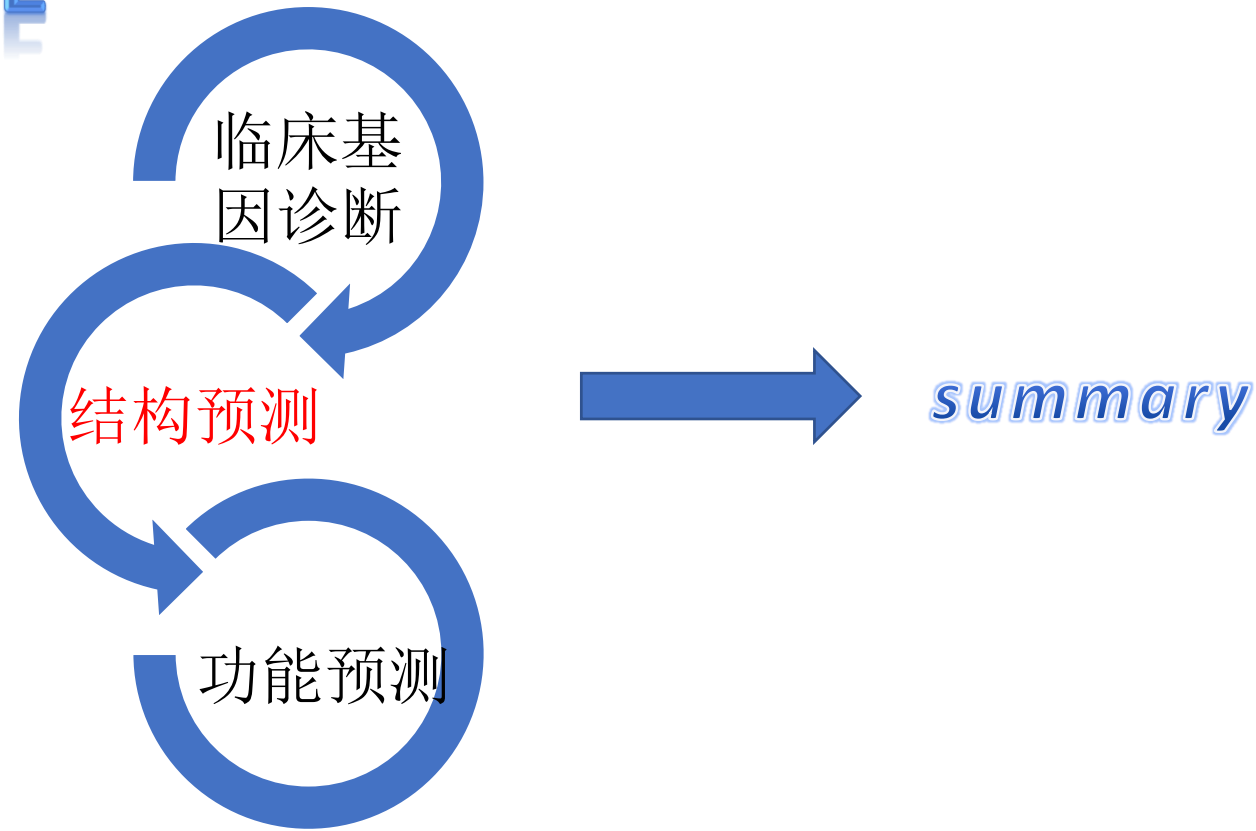


基因诊断：
患者：纯合突变
父母：携带杂合突变



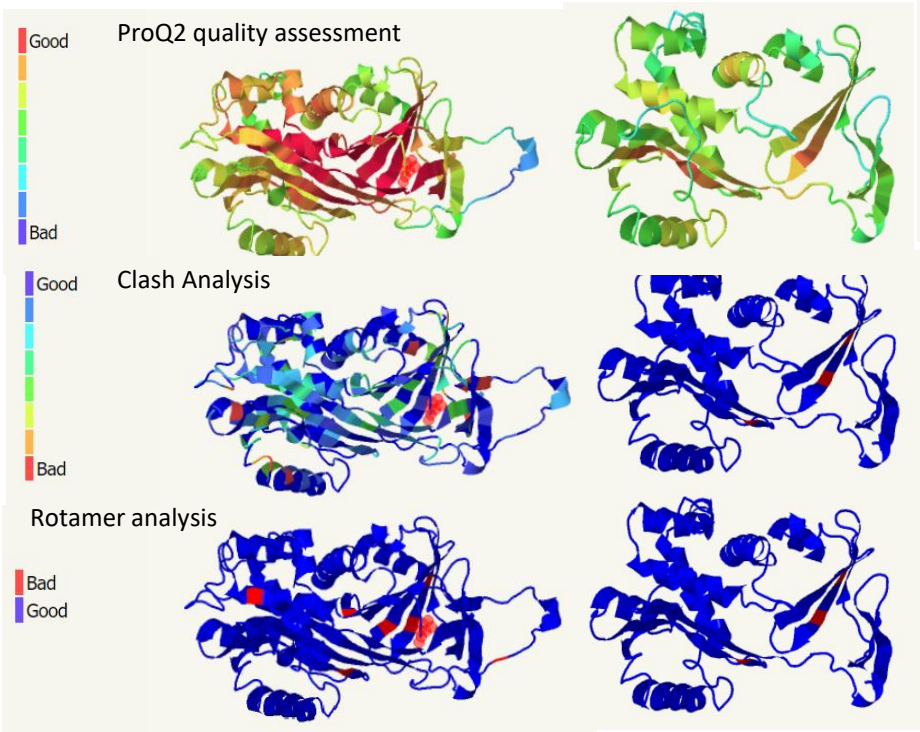
基因型与表型符合共分离
相关病例报道

OUTLINE



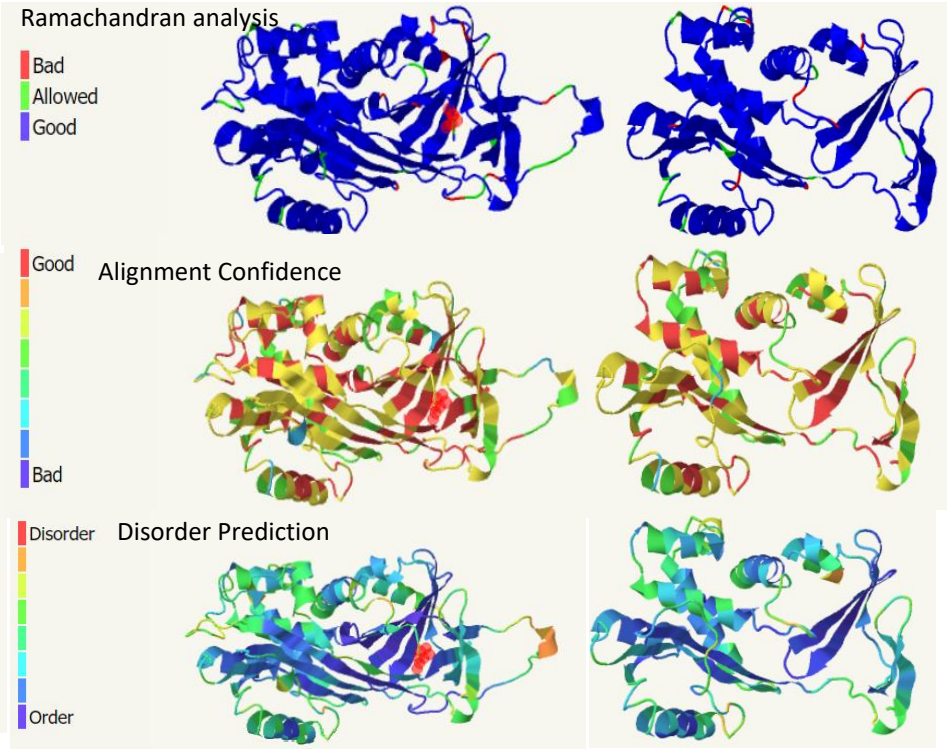
基于同源模建的结构分析——Quality

SerpinB7 SERPINB7_p.R266*



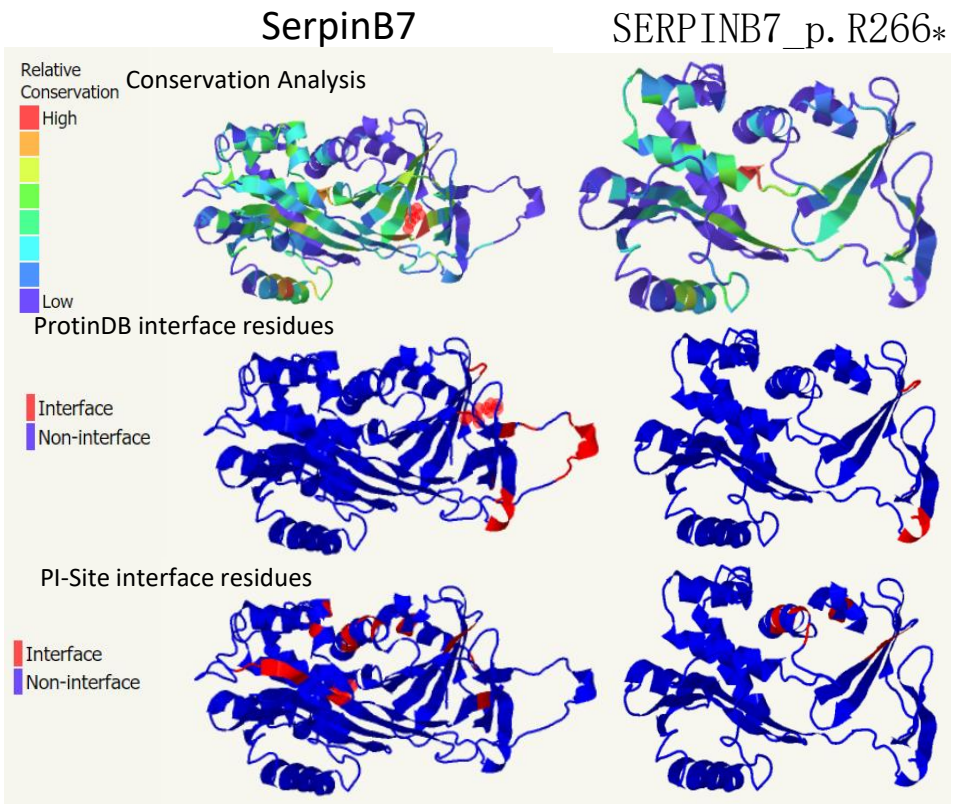
Confidence: 100.0% 100.0%

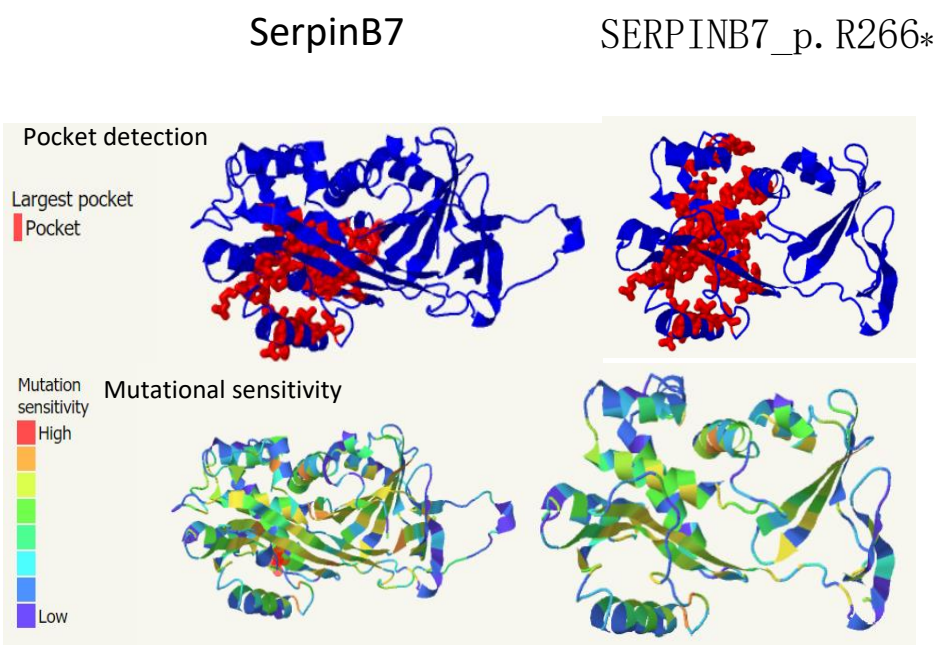
SerpinB7 SERPINB7_p.R266*

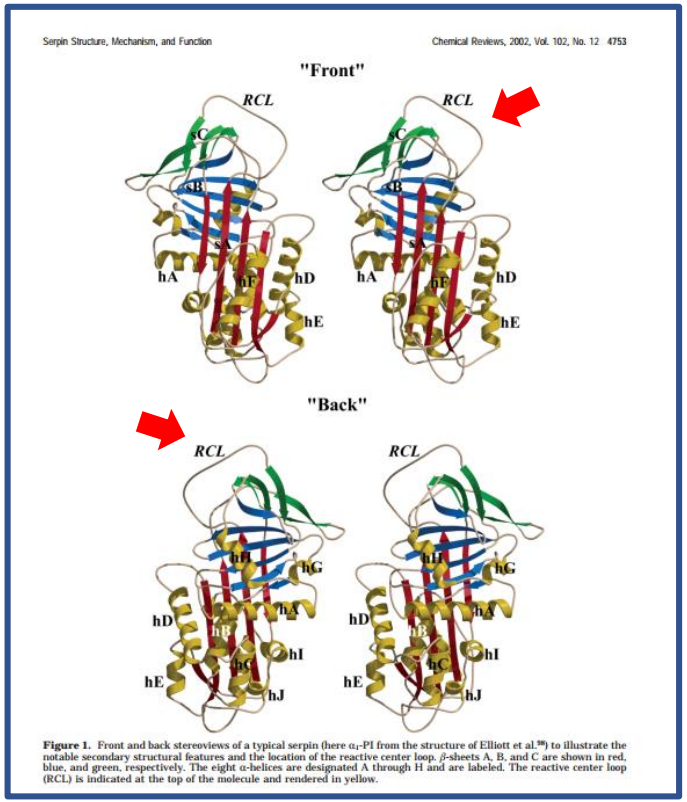


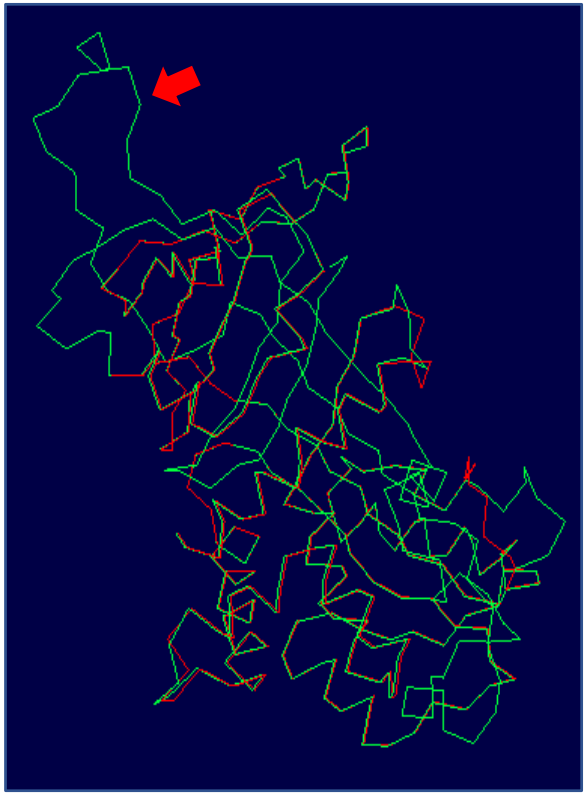
Coverage: 97% 97%

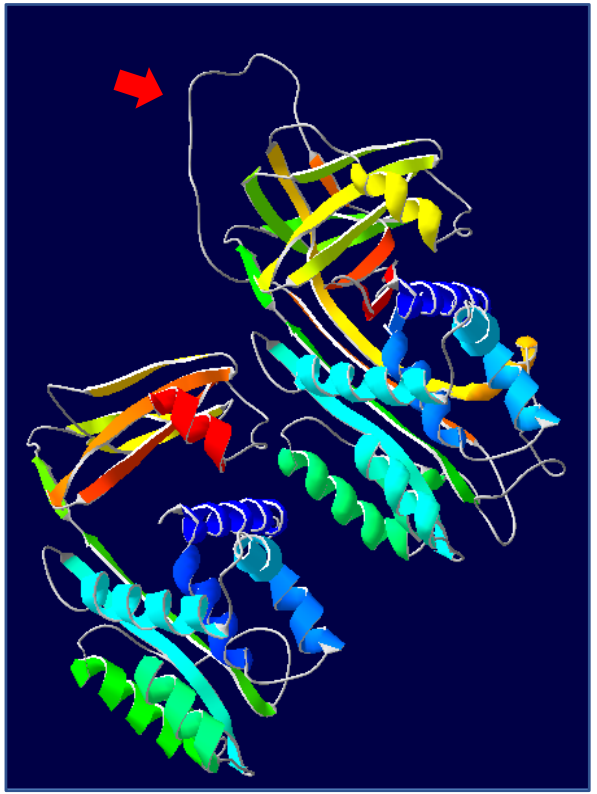
基于同源模建的结构分析——Function











The reactive center loop (RCL) is indicated at the top of the molecule and rendered in yellow

Gettins PG. Chem Rev. 2002

nb atoms involved:262 RMS:1.53?

绿：SerpinB7

红：SerpinB7_mutation

黄：两者重叠部分

SerpinB7_mutation

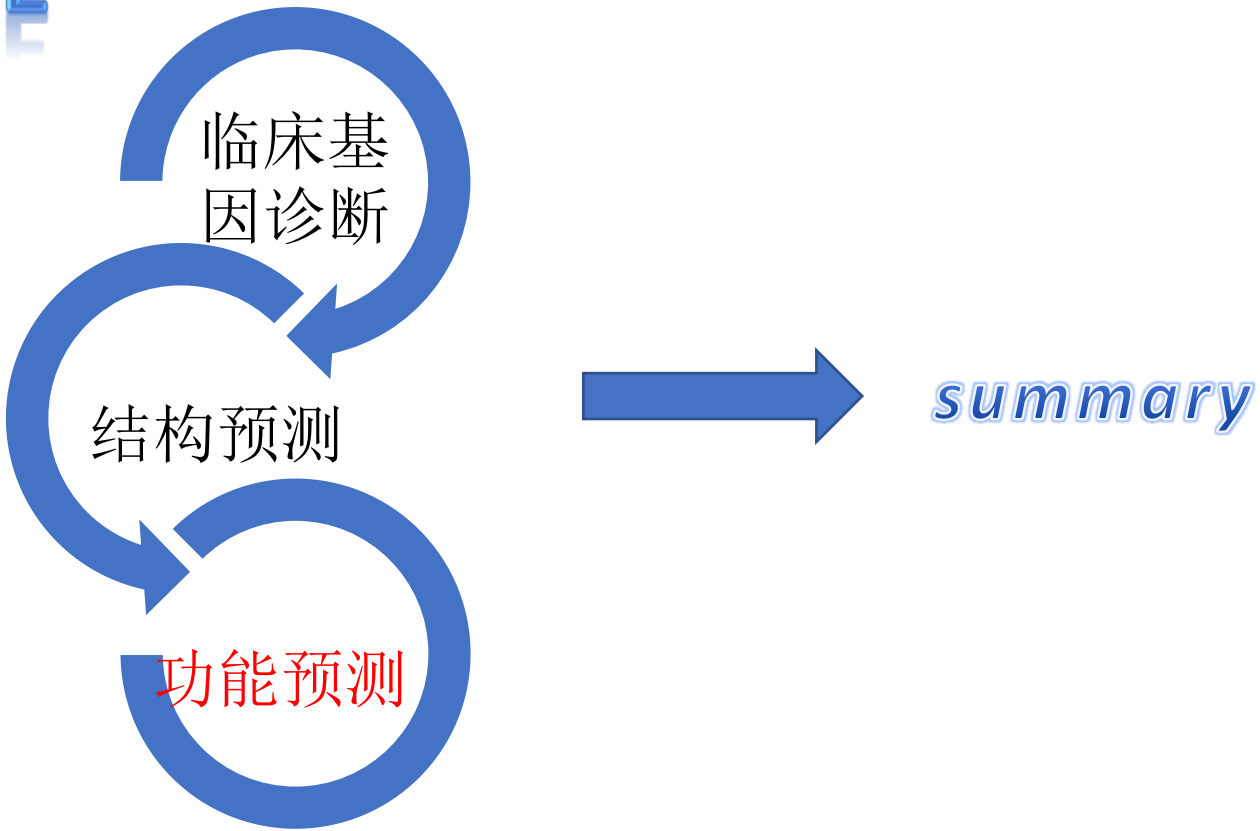
丢失RCL

预测结果分析

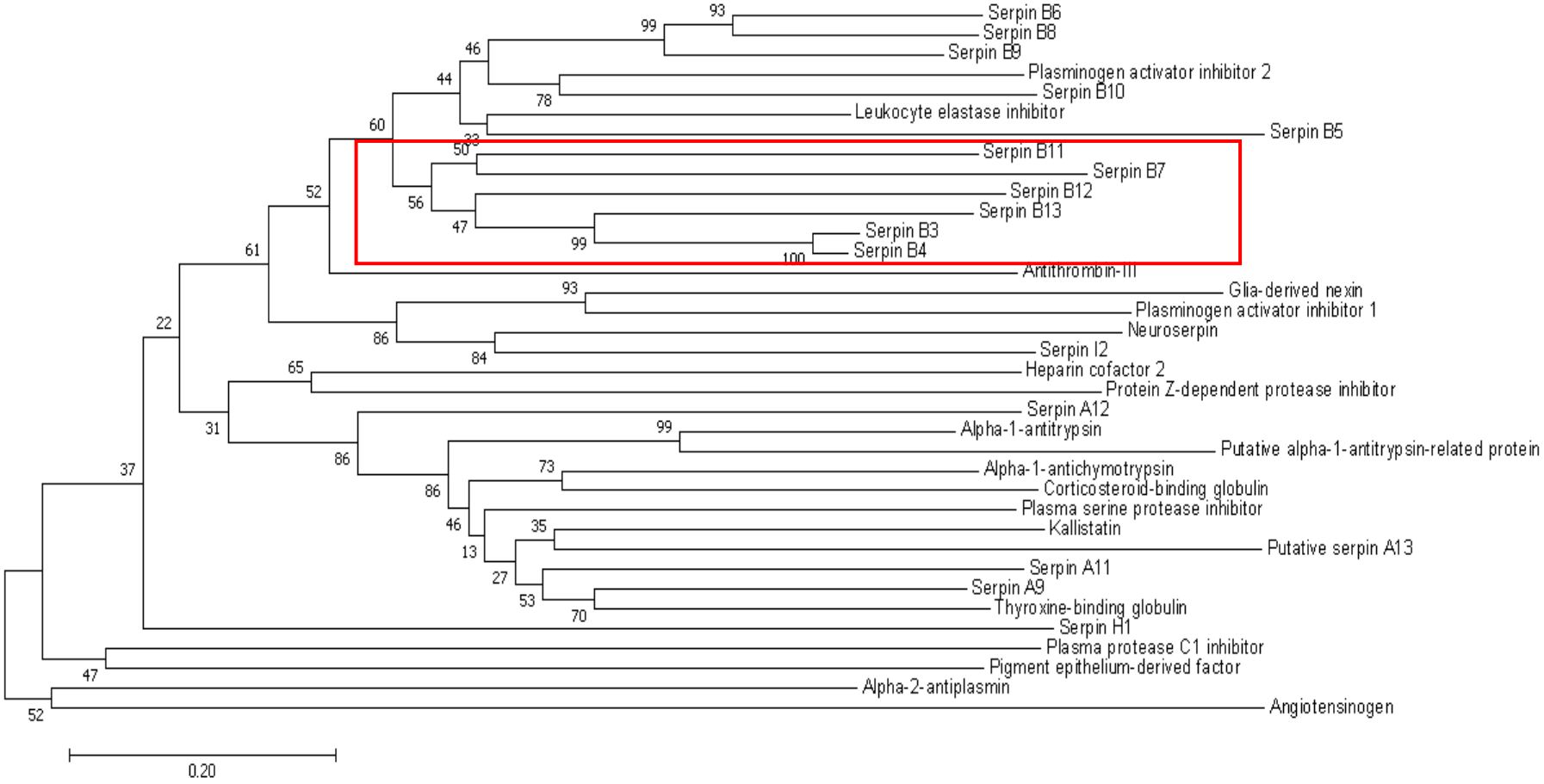
- The protease-inhibitory activity of serpins is dependent on the reactive site loop to form a covalent bond with target proteases.
- The center of the reactive site loop (P1–P1’) is located at amino acids **347–348** of SERPINB7, and the entire region of the reactive site loop (P17–P50, corresponding to the amino acid region 331–352 of SERPINB7) is predicted to be absent in all of the mutant proteins.
- Thus, all of the mutations identified in this study presumably result in a complete loss of the protease inhibitory activity of SERPINB7.

Kubo A et all. Am J Hum Genet. 2013

OUTLINE



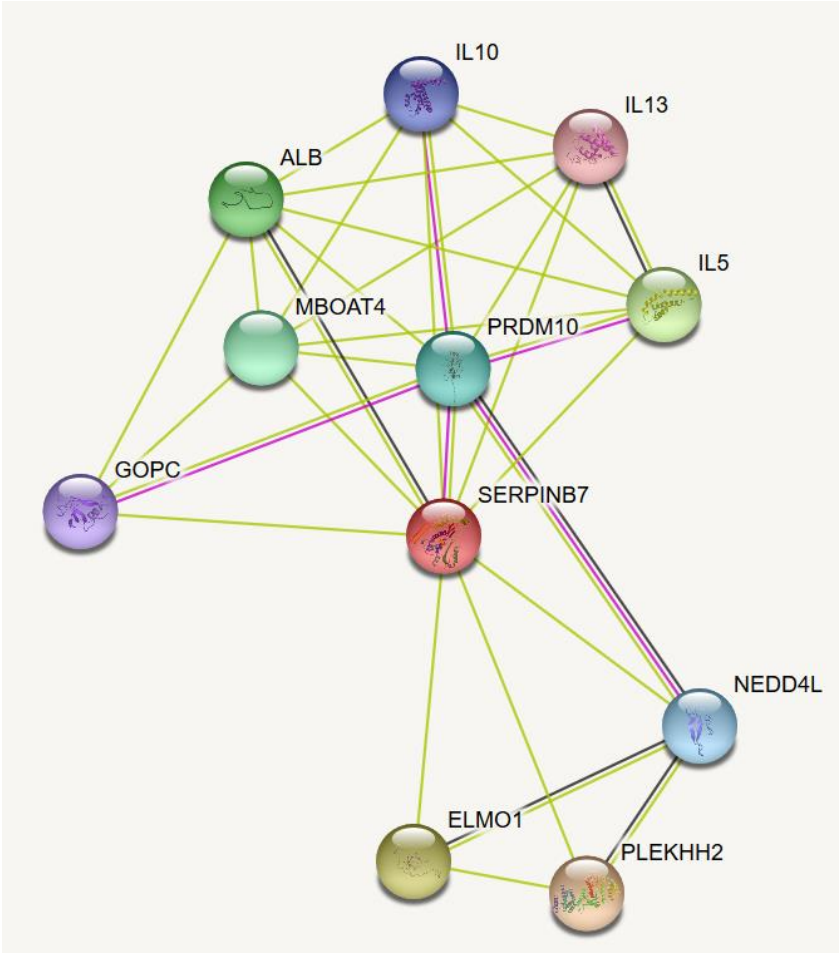
Evolutionary relationships of serpin family in human



Function of serpin family members phylogenetic closely related to Serpin B7

Protein Name	UniProt Accession	Function
Serpin B7	O75635	Might function as an inhibitor of Lys-specific proteases. Might influence the maturation of megakaryocyteS via its action as a serpin.
Serpin B3	P29508	May act as a papain-like cysteine protease inhibitor to modulate the host immune response against tumor cells. Also functions as an inhibitor of UV-induced apoptosis via suppression of the activity of c-Jun NH2-terminal kinase (JNK1).
Serpin B4	P48594	May act as a protease inhibitor to modulate the host immune response against tumor cells.
Serpin B11	Q96P15	Has no serine protease inhibitory activity, probably due to mutations in the scaffold impairing conformational change.
Serpin B12	Q96P63	Inhibits trypsin and plasmin, but not thrombin, coagulation factor Xa, or urokinase-type plasminogen activator.
Serpin B13	Q9UIV8	May play a role in the proliferation or differentiation of keratinocytes.

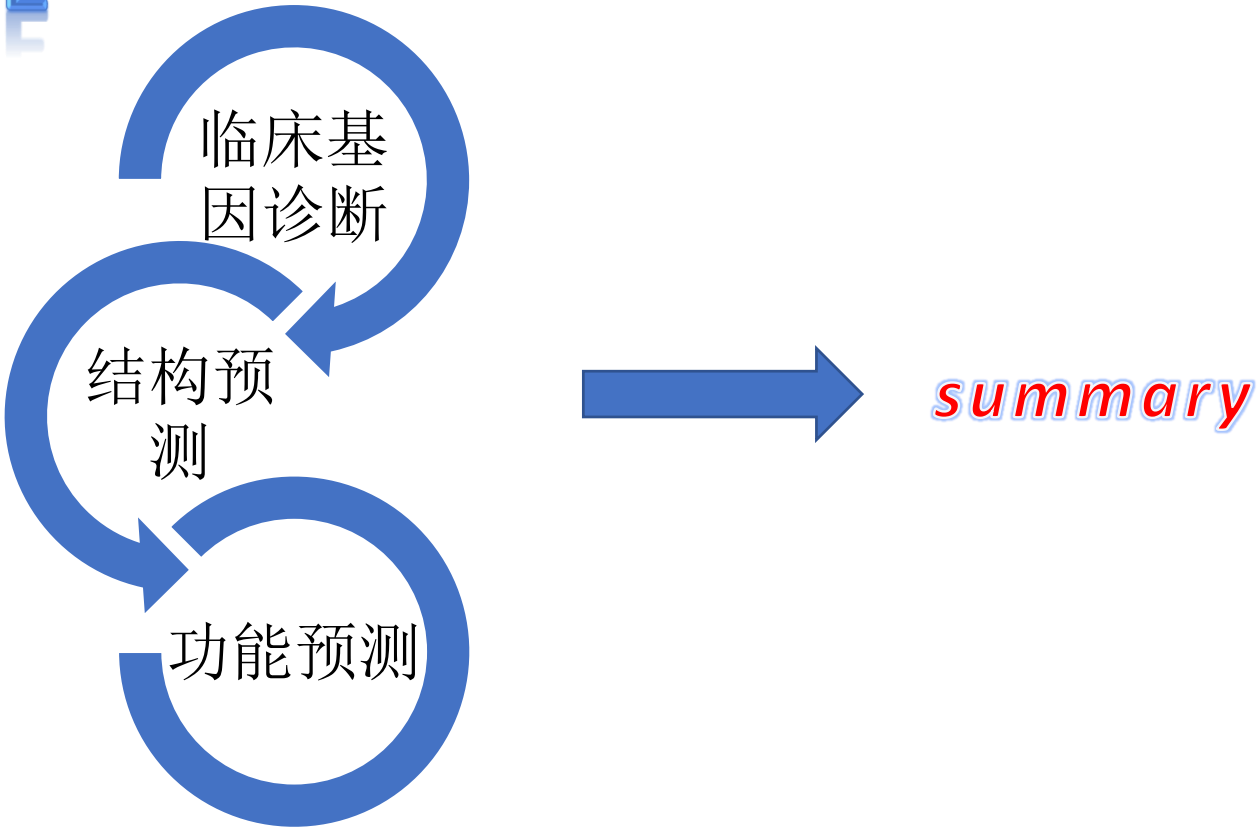
Protein-Protein Interactions prediction through STRING



- ✓ The putative homologs of SERPINB7 had interaction with PRDM10 in other species.

- ✓ The putative homologs of SERPINB7 and ALB have co-expression relationship.

OUTLINE



Summary

- 通过Phyre2对SERPINB7 p.R266*突变前后蛋白结构的预测，推测NPPKs疾病的发生有可能与reactive site loop的缺失有关。
- 生物信息学工具分析为SERPINB7的**互作蛋白**及**功能**给出启示；
- 可以在细胞或动物水平将SERPINB7 RCL的氨基酸位点进行定点突变，来研究NPPKs疾病的发生机理。