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RESEARCH ARTICLE

The First Report of Genetic Characteristics and Polymorphisms in the Shadow of Prion Protein Gene (*SPRN*) in Bats

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ABSTRACT

The shadow of prion protein (Sho), encoded by the shadow of prion protein gene (*SPRN*), is a prion-like protein that shares structural and cellular localization similarities with the prion protein (PrP). Emerging evidence suggests that Sho may act as a modulator of PrP misfolding. However, prion diseases have not been reported in bats, and the role of *SPRN* genetic variation in this potential resistance remains unexplored. Genomic DNA was extracted from 100 bat samples, and the *SPRN* gene was amplified and sequenced to identify variations. Genetic analysis, including genotype, allele, haplotype frequencies, and linkage disequilibrium (LD), was performed to characterize the bat *SPRN* gene. Comparative protein sequence alignment across several species was conducted to assess evolutionary conservation. The structural properties of bat Sho and the impact of non-synonymous substitutions on bat Sho were evaluated using structural modeling tools. The functional effects of identified variants were predicted using multiple *in-silico* approaches. A total of 20 polymorphisms were identified in the coding region of the bat *SPRN* gene, including 11 non-synonymous variants. Notably, the bat *SPRN* gene displayed a distinct polymorphism pattern compared to both prion-susceptible species and putatively prion-resistant species. Comparative analysis demonstrated that bat Sho shares high sequence similarity with other mammalian Sho. Computational analyses predicted that certain low-frequency variants, including W9R, G68E, and G76E, may influence protein stability and Sho–PrP interactions. This study represents the first report describing *SPRN* gene polymorphisms in bats, offering baseline insights for future studies on prion biology.

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INTRODUCTION

Prion diseases are fatal neurodegenerative disorders caused by the conformational conversion of the normal cellular prion protein (PrP^{C}) into an abnormal, *β*-sheet-rich isoform (PrP^{Sc}), leading to spongiform neuropathology and clinical neurodegeneration (Prusiner, 1998). These diseases affect a broad range of hosts, including Creutzfeldt-Jakob disease (CJD) in humans, scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease (CWD) in cervids (Jeong and Kim, 2014; Abdul *et al.*, 2026). Despite differences in host and clinical presentation, all prion diseases share the pathogenic mechanism of PrP^{C} misfolding and aggregation. However, the mechanisms of PrP conversion and its physiological roles remain poorly understood.

The shadow of prion protein (Sho), which is encoded by the shadow of prion protein gene (*SPRN*), was first identified through *in-silico* analysis (Premzl *et al.*, 2003). Sho is a member of the prion protein family and exhibits structural similarities to PrP^{C}, particularly within the N-terminal and the central regions of PrP^{C}, as well as in the conformational and assembly characteristics of the PrP^{C} hydrophobic domain (HD) (Daude *et al.*, 2010). In addition to structural similarities, both PrP^{C} and Sho are glycosylphosphatidylinositol (GPI)-anchored glycoproteins mainly expressed in the central nervous system (CNS), with neuroprotective properties (Watts *et al.*, 2007; Sakthivelu *et*

al., 2011). A previous study has shown that protein levels of Sho are reduced in the brains of clinically ill mice infected with the Rocky Mountain Laboratory (RML) strain of prions (Watts *et al.*, 2007). As glycosylphosphatidylinositol (GPI)-anchored proteins, PrP^{C} and Sho are likely localized to the outer surface of the cell membrane and physically interact with each other at micromolar affinity (Ciric *et al.*, 2015). These findings suggest that Sho contributes to the conversion of PrP^{C} to PrP^{Sc}, supporting its role as a modulator of the prion misfolding process. However, the influence of Sho on the folding pathway remains unclear. Analogously to the prion protein gene (*PRNP*), polymorphisms in *SPRN* have been investigated as potential genetic modifiers of prion disease susceptibility across species. Indeed, multiple studies have linked *SPRN* variants to prion disease outcomes in various hosts: for example, a single base-pair insertion was detected in patients with variant CJD (Beck *et al.*, 2008), a specific 5-base insertion in the 3' untranslated region of caprine *SPRN* shows a strong association with scrapie susceptibility in goats (Peletto *et al.*, 2012), and a 12-bp deletion in the hydrophobic region of bovine *SPRN* was identified in a cow affected by atypical BSE (Gurgul *et al.*, 2012). These findings support the idea that *SPRN* polymorphisms can influence prion disease vulnerability, complementing the primary effect of *PRNP* genotype.

Bats are unique mammals known for their exceptional longevity and robust immune systems, and they play important ecological and epidemiological roles as natural reservoirs for numerous zoonotic pathogens (Cooper *et al.*, 2024; Morales *et al.*, 2025). They harbor a wide range of emerging viruses, including coronaviruses (SARS-CoV-2), filoviruses (Ebola), henipaviruses (Nipah), and lyssaviruses (rabies) (Brüssow, 2025). However, no evidence of transmissible spongiform encephalopathy (TSE) has been reported in bats, either under natural or experimental conditions. Recent ecological perspectives suggest that hematophagous bats could act as potential carriers or transmission bridges of prions through blood-feeding behavior, highlighting a potential yet unexplored interface between bat biology and prion disease transmission (Frank *et al.*, 2026). Given the role of Sho in prion disease, investigating the bat *SPRN* gene may reveal potential adaptations related to putative resistance to prion disease. To date, the *SPRN* gene remains uncharacterized in bats, and the impact of its genetic variation on prion susceptibility is unclear, particularly in species without reported prion disease.

In this study, we analyzed *SPRN* gene polymorphisms in bats and performed comparative analyses of Sho protein sequences across species susceptible to prion diseases and species without reported natural prion disease. We further investigated the distribution of *SPRN* variants and assessed their potential structural and functional impacts, including their influence on Sho–PrP interactions.

MATERIALS AND METHODS

Sample preparation: The intestine and liver tissue samples of bats were acquired for this research from the National Institute of Environmental Research in the Republic of Korea and the College of Veterinary Medicine at Jeonbuk National University. The samples consisted of multiple bat species and species identification was performed based on the information provided by the source institution (Table 1). The samples were collected across geographic regions in South Korea.

Genetic analysis of the bat *SPRN* gene: Genomic DNA was extracted from 100 bat tissue samples using a Bead Genomic DNA Prep Kit (BioFACT™, Korea). The bat *SPRN* gene was amplified by PCR using primers designed based on the bat *SPRN* sequence in GenBank (Gene ID: 117035939), with the following sequences: 5'-CCGGTCCTCAGAAGCTGATG-3' (forward primer) and 5'-CACGTCCCCACTGTGGAG-3' (reverse primer). PCR amplification generated a 559-bp fragment covering the *SPRN* coding region from c.-59 to c.-95, including the entire ORF. The optimal annealing temperature was determined to be 63°C, following the provided protocol for BioFACT™ Taq DNA Polymerase (BioFACT Co., Korea). The PCR amplicons were purified using the FavorPrep™ GEL/PCR Purification Kit (Favorgen Biotech Corp., Taiwan) and subsequently submitted to sequencing on an ABI 3730XL analyzer (Applied Biosystems, USA). All single nucleotide polymorphisms (SNPs) were confirmed by manual inspection of sequencing chromatograms using FinchTV software (version 1.4, Geospiza Inc., USA) to ensure genotyping accuracy.

Multiple sequence alignment of Sho across mammalian species: Clustal Omega was used for protein alignment of Sho across species (Sievers *et al.*, 2011). All protein sequences were sourced from GenBank, including humans (NP_001378903.1), cattle (AAY83885.1), goats (AGU17009.1), sheep (NP_001156033.1), horses (XP_023492126.1), dogs (XP_038296952.1), rabbits (XP_008268877.2), chickens (A2BDG0.1), and bats (this study).

Structural modeling and comparative analysis of Sho: The tertiary structures of Sho from bat and other species were predicted using AlphaFold2 (ColabFold version 1.5.5) (Mirdita *et al.*, 2022). To visualize the predicted changes in amino acid substitutions of the bat Sho structure, we used the Swiss-PdbViewer program (version 4.1) (Guex and Peitsch, 1997). Structural similarity between proteins was evaluated using US-align by calculating TM-score and root mean square deviation (RMSD) (Zhang *et al.*, 2022). Protein–protein interactions were predicted using HawkDock, with binding free energy evaluated by MM/GBSA (Weng *et al.*, 2019). The binding free energy and interaction stability were further evaluated using the MM/GBSA method. The bat PrP structure was used as the receptor, while bat Sho (wild-type and mutant variants) was used as the ligand.

The effect of amino acid substitutions in the bat *SPRN* gene using *in-silico* evaluation: Several computational tools, including MutPred2, MUpro, SIFT and SNPs&GO, were used to evaluate the effects of non-synonymous substitutions in the bat *SPRN* gene. MutPred2 predicts the molecular and phenotypic impact of amino acid variants (Pejaver *et al.*, 2020). MUpro assesses mutation-induced changes in protein stability (Cheng *et al.*, 2006). SIFT evaluates whether substitutions are tolerated or deleterious based on evolutionary conservation (Ng and Henikoff,

2003). SNPs&GO classifies variants as neutral or deleterious by integrating protein features and functional annotations from Gene Ontology (GO) (Capriotti *et al.*, 2013).

Statistical analysis: Hardy–Weinberg equilibrium (HWE) was assessed using the Chi-square test (Hosking *et al.*, 2004). Haplotype and linkage disequilibrium (LD) analyses were performed using Haploview version 4.2 (Broad Institute, USA), with LD evaluated by the r^2 value (Barrett *et al.*, 2005).

RESULTS

Identification of novel polymorphisms in the *SPRN* gene of bats: Novel polymorphisms in the bat *SPRN* gene were identified through genotyping analysis of PCR sequence results from 100 bat samples. A total of 20 SNPs were identified in the open reading frame (ORF) region within exon 2, including 11 non-synonymous variants and nine synonymous mutations (Fig. 1; Table 2). Table 2 presents the genotype and allele frequencies of the 20 polymorphisms and their HWE status. All SNPs were in accordance with HWE, except for c.390A>G (P<0.0001). We also investigated the LD among the *SPRN* polymorphisms displayed in Table 3, and 15 strong LDs were identified. Additionally, three major haplotypes (>1%) were identified (Table 4), with TCGCTCGGGTGGACTTCCAG being the most frequent (56.4%).

Table 1: Detailed information on bat samples investigated in the present study

Bat species	Number of samples, n	Tissue type
Eptesicus serotinus	3	Intestine
Miniopterus schreibersii	11	Intestine, liver
Murina leucogaster	2	Intestine
Myotis aurascens	4	Intestine
Myotis macrodactylus	2	Liver
Myotis petax	7	Intestine
Pipistrellus abramus	9	Intestine
Pipistrellus alaschanicus	3	Intestine
Rhinolophus ferrumequinum	58	Intestine, liver
Vespertilio sinensis	1	Intestine
Total	100	

Table 2: Genotype and allele frequencies of the shadow of prion protein gene (*SPRN*) polymorphisms in 100 bats

Polymorphisms	Genotype Frequencies, n (%)			Allele Frequencies, n (%)		HWE
	M/M	M/m	m/m	M	m	
c.25T>C (W9R)	99(99.0)	1(1.0)	0(0.0)	199(99.5)	1(0.5)	0.9599
c.54C>T (L18L)	98(98.0)	2(2.0)	0(0.0)	198(99.0)	2(1.0)	0.9195
c.80G>A (G27D)	99(99.0)	1(1.0)	0(0.0)	199(99.5)	1(0.5)	0.9599
c.82C>T (R28C)	97(97.0)	3(3.0)	0(0.0)	197(98.5)	3(1.5)	0.9191
c.84T>C (R28R)	99(99.0)	1(1.0)	0(0.0)	199(99.5)	1(0.5)	0.9599
c.130C>T (R44C)	99(99.0)	1(1.0)	0(0.0)	199(99.5)	1(0.5)	0.9599
c.135G>A (G45G)	99(99.0)	1(1.0)	0(0.0)	199(99.5)	1(0.5)	0.9599
c.149G>T (R50L)	98(98.0)	2(2.0)	0(0.0)	198(99.0)	2(1.0)	0.9195
c.203G>A (G68E)	99(99.0)	1(1.0)	0(0.0)	199(99.5)	1(0.5)	0.9599
c.221T>C (V74A)	99(99.0)	1(1.0)	0(0.0)	199(99.5)	1(0.5)	0.9599
c.227G>A (G76E)	99(99.0)	1(1.0)	0(0.0)	199(99.5)	1(0.5)	0.9599
c.276G>A (G92G)	98(98.0)	2(2.0)	0(0.0)	198(99.0)	2(1.0)	0.9195
c.312A>G (A104A)	99(99.0)	1(1.0)	0(0.0)	199(99.5)	1(0.5)	0.9599
c.318C>T (G106G)	99(99.0)	1(1.0)	0(0.0)	199(99.5)	1(0.5)	0.9599
c.341T>C (V114A)	99(99.0)	1(1.0)	0(0.0)	199(99.5)	1(0.5)	0.9599
c.343T>C (Y115H)	99(99.0)	1(1.0)	0(0.0)	199(99.5)	1(0.5)	0.9599
c.369C>T (D123D)	99(99.0)	1(1.0)	0(0.0)	199(99.5)	1(0.5)	0.9599
c.388C>T (P130S)	96(96.0)	3(3.0)	1(1.0)	195(97.5)	5(2.5)	0.8383
c.390A>G (P130P)	68(68.0)	8(8.0)	24(24.0)	144(72.0)	56(28.0)	<0.0001
c.402G>A (L134L)	99(99.0)	1(1.0)	0(0.0)	199(99.5)	1(0.5)	0.9599

M/M: major homozygote; M/m: heterozygote; m/m: minor homozygote; M: major allele; m: minor allele

Table 3: Linkage disequilibrium (LD) analysis of the 20 *SPRN* polymorphisms of bats

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	-																			
2	0	-																		
3	1	0	-																	
4	0	0.157	0	-																
5	0	0	0	0	-															
6	0	0.497	0	0	0	-														
7	0	0	0	0.33	0	0	-													
8	0	0	0	0	0	0	0	-												
9	0	0	0	0.33	0	0	1	0	-											
10	0	0	0	0.33	0	0	0	0	0	-										
11	0	0	0	0	0	0	0	0	0	0	-									
12	0.497	0	0.497	0	0	0	0	0	0	0	0	-								
13	0	0	0	0	0	0	0	0.497	0	0	0	0	-							
14	0	0	0	0.33	0	0	0	0	0	1	0	0	0	-						
15	0	0	0	0.33	0	0	1	0	1	0	0	0	0	0	-					
16	0	0	0	0	0	0	0	0.497	0	0	0	0	1	0	0	-				
17	0	0	0	0.33	0	0	1	0	1	0	0	0	0	0	1	0	-			
18	0	0.089	0	0.054	0	0	0	0	0	0	0	0	0	0	0	0	0	-		
19	0.004	0.014	0.004	0.021	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.004	0.007	0.007	0.004	0.007	0.009	-	
20	0	0	0	0	0	0	0	0	0	0	0	0.497	0	0	0	0	0	0	0.007	-

1, c.25T>C; 2, c.54C>T; 3, c.80G>A; 4, c.82C>T; 5, c.84T>C; 6, c.130C>T; 7, c.135G>A; 8, c.149G>T; 9, c.203G>A; 10, c.221T>C; 11, c.227G>A; 12, c.276G>A; 13, c.312A>G; 14, c.318C>T; 15, c.341T>C; 16, c.343T>C; 17, c.369C>T; 18, c.388C>T; 19, c.390A>G; 20, c.402G>A. Bold font indicates strong LD (r^2>0.333). The values below the diagonal indicate the r^2 values.

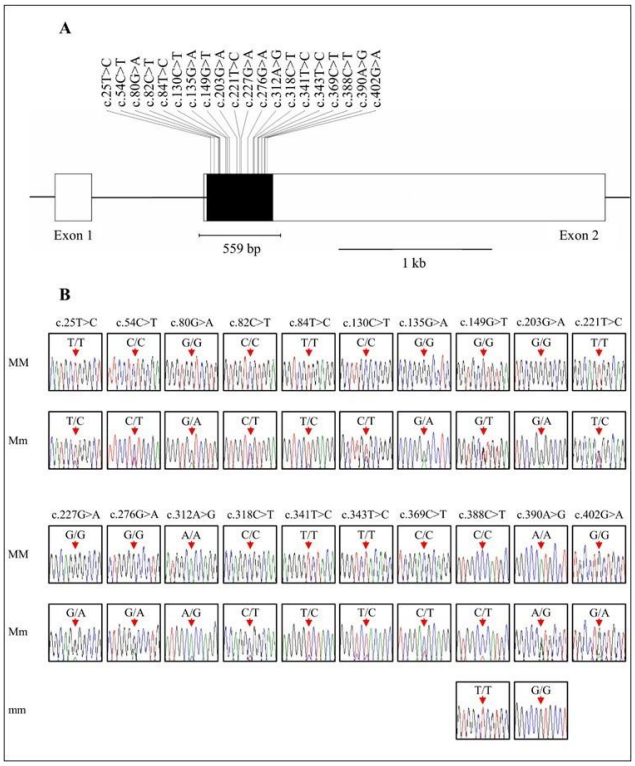

Fig. 1: Identification of single-nucleotide polymorphisms (SNPs) in the *SPRN* gene of the bats. (**A**) The gene map illustrates the genomic structure and the distribution of the bat *SPRN* gene. The open reading frame (ORF) located at the beginning of exon 2 is represented by a black box, while the white boxes represent the 5′ and 3′ untranslated regions (UTRs). The PCR product region analyzed in this study is shown as a horizontal line with caps at both ends, and 20 novel polymorphisms are indicated by vertical lines above this gene. (**B**) The electropherograms of 20 SNPs in the bat *SPRN* gene are shown. The position of each polymorphism found in this study is marked by the top arrow. The sequence peaks are represented in different colors for four nucleotide bases: green – adenine (A), red – thymine (T), blue – cytosine (C) and black – guanine (G)

Table 4: Haplotype frequencies of the 20 *SPRN* polymorphisms in bats

Haplotypes	Frequency, %
TCGCTCGGGTGGACTTCCAG	56.4
TCGCTCGGGTGGACTTCCGG	36.6
TCGCTCGGGTGGACTTCTGG	1.4
Others*	5.6

Others* contain rare haplotypes with <1.0%.

Cross-species sequence comparison of the shadow of prion protein: Multiple sequence alignment of Sho across species revealed notable variation in protein length. Among them, bat Sho (148 aa) exhibits a sequence length comparable to that of horse and dog (147 aa) (Fig. 2). Sequence identity analysis indicated that bat Sho shares the highest similarity with human Sho (82.24%), followed by horse (77.48%) and dog (76.32%). In addition, sequence analysis identified 15 species-specific amino acids in bat Sho (Fig. 2). Key structural features of Sho were conserved across species, including the N-terminal signal peptide, arginine-glycine-rich (RG-rich) region, and N-X-T glycosylation motif. In contrast, the alanine-rich hydrophobic region, a homologous segment between Sho and PrP, exhibited substantial variation among species (Fig. 2).

Distribution of *SPRN* polymorphisms in prion-susceptible and putatively prion-resistant species: Previously reported genetic variants within the coding sequence of the *SPRN* gene were compared between prion-susceptible species and species without reported natural prion disease, together with newly generated bat data (Fig. 3). Overall, prion-susceptible species tend to harbor a higher number of SNPs within the *SPRN* coding region, particularly including non-synonymous variants. In contrast, species in which natural prion disease has not been reported generally exhibit fewer SNPs and lack non-synonymous substitutions. However, avian species (Pekin duck, pheasant, and quail) and bats did not follow this pattern, exhibiting relatively high SNP numbers, including multiple amino acid variants, despite no clear evidence of natural prion disease.

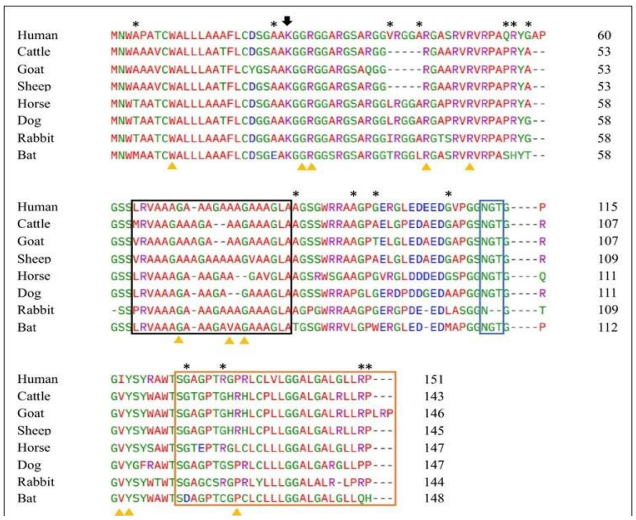

Fig. 2: Multiple sequence alignment of the shadow of prion protein in bats and other species. Colors represent the chemical characteristics of amino acids: blue, acidic; magenta, basic; red, small and hydrophobic; green, hydroxyl, sulfhydryl, amine, and glycine. The black arrow indicates the beginning of the basic repeats. Asterisks indicate specific amino acids of bats. Yellow arrows indicate non-synonymous polymorphisms in the bat *SPRN* gene. The black box indicates the hydrophobic alanine-rich region. The blue box indicates the N-X-T motif. The orange box indicates the signal sequence of the GPI anchor of Sho

Structural prediction and comparison of the bat Sho with those of other species: To date, no complete 3D structure of Sho has been determined by nuclear magnetic resonance (NMR) or X-ray crystallography. Therefore, AlphaFold2 was used to predict Sho structures across nine species shown in Fig. 4. The predicted models showed that Sho structures are largely conserved despite sequence variation, with all species containing two α-helices (α1 and α2) connected by a flexible coil region (Fig. 4B). To quantitatively assess structural similarity, we used US-align to compare the wild-type bat Sho structure with those of other species. The results showed that all pairwise comparisons yielded TM-scores over 0.2, with RMSD values ranging from 4 to 6 Å, indicating a generally similar spatial arrangement of secondary structure elements (Table 5). Comparison between wild-type bat Sho and the HT3 haplotype showed no substantial changes in overall structure, despite a slight elongation of the second α-helix in the HT3 variant (Fig. 4A). Consistently, the similar TM-score (0.312) and RMSD (5.37 Å) values further support the minimal structural impact of the HT3 variant (Table 5).

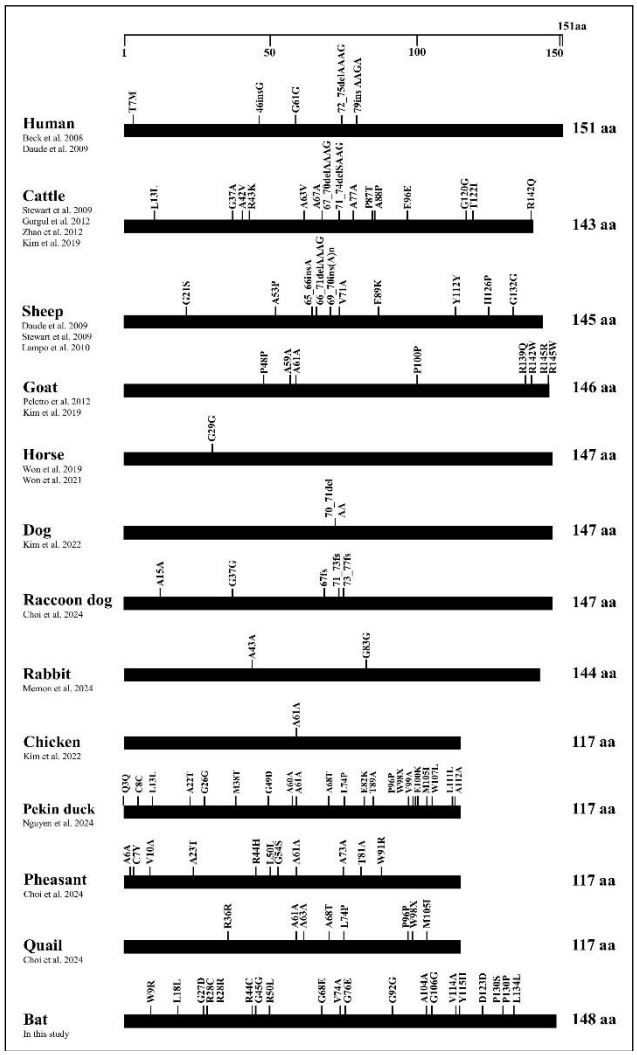

Fig. 3: Genetic polymorphism distribution within the open reading frame of the *SPRN* gene across multiple species. The figure illustrates reported genetic polymorphisms of the *SPRN* gene in humans (Beck *et al*., 2008; Daude *et al*., 2009), cattle (Stewart *et al*., 2009; Gurgul *et al*., 2012; Zhao *et al*., 2012; Kim *et al*., 2020), sheep (Daude *et al*., 2009; Stewart *et al*., 2009; Lampo *et al*., 2010), goats (Peletto *et al*., 2012; Kim *et al*., 2019), horses (Won *et al*., 2019; Won *et al*., 2021), dogs (Kim *et al*., 2022), raccoon dogs (Choi *et al*., 2024a), rabbits (Memon *et al*., 2024), chickens (Kim *et al*., 2022), Pekin ducks (Nguyen *et al*., 2024), pheasants (Choi *et al*., 2024b), quail (Choi *et al*., 2024c), and bats (this study). The horizontal bars represent the length of the Sho protein in each species

Table 5: Structural similarity of the wild-type Sho of bats with Sho of several species using US-align

Species	Protein accession	TM-score	RMSD
Human	NP_001378903.1	0.28917	5.88 Å
Cattle	AAY83885.1	0.27444	5.88 Å
Goat	AGU17009.1	0.26945	6.11 Å
Sheep	NP_001156033.1	0.3143	5.18 Å
Horse	XP_023492126.1	0.24582	4.18 Å
Dog	XP_038296952.1	0.26473	5.58 Å
Rabbit	XP_008268877.2	0.33086	5.10 Å
Bat (Haplotype 3)	This study	0.31204	5.37 Å

Effects of non-synonymous SNPs on the 3D structure of bat Sho: The structural effects of non-synonymous SNPs in bat Sho were analyzed using Swiss-Pdb Viewer by examining changes in hydrogen bonding between wild-type and mutant alleles. Most substitutions, including G27D, R28C, R44C, R50L, G68E, V74A, G76E, V114A and Y115H did not significantly disrupt the overall structural framework (Fig. 5B-J). However, W9R and P130S altered local interactions, particularly hydrogen bonding patterns. At codon 9, R9 was predicted to form two hydrogen bonds with A5, whereas W9 formed only one (Fig. 5A). At codon 130, S130 formed a hydrogen bond with P126, while P130 did not (Fig. 5K). Overall, these variants induced local structural changes without affecting the global fold.

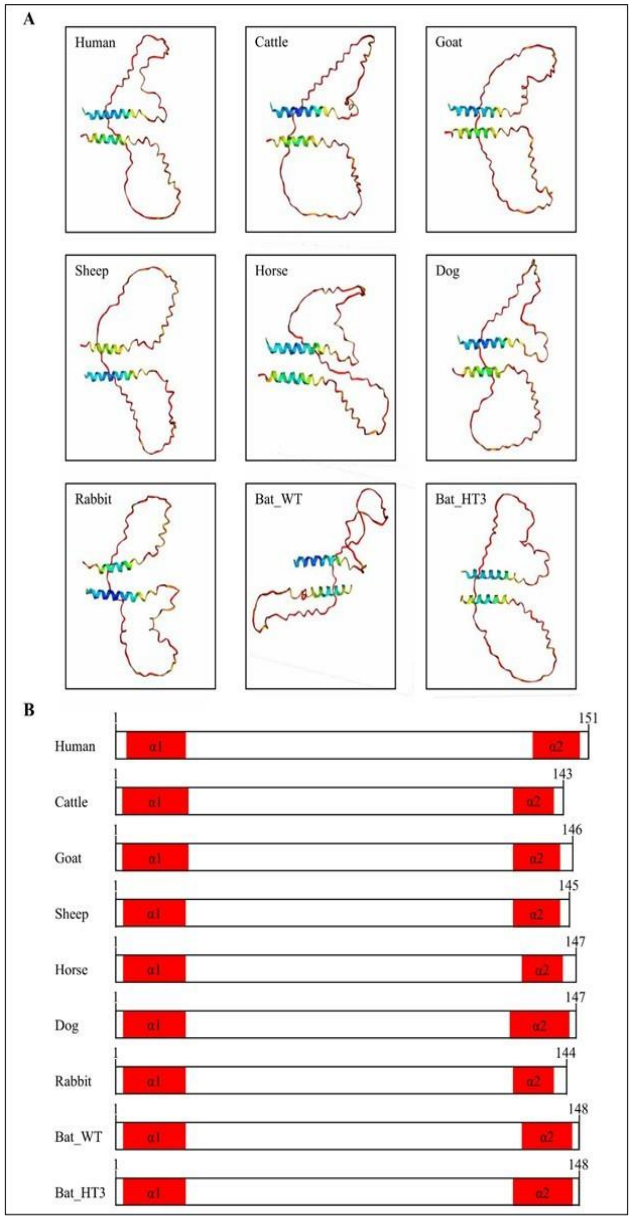

Fig. 4: The tertiary and secondary structures of the shadow of prion protein (Sho). (**A**) The tertiary structural modeling of Sho predicted by AlphaFold2. (**B**) The secondary structural modeling of Sho. Red regions indicate *α*-helical structures, and white regions represent coil regions. Bat_WT: wild-type bat Sho. Bat_HT3: bat Sho carrying the HT3 allele

Computational prediction of functional impact and stability changes of *SPRN* polymorphisms in bats: The effects of 11 non-synonymous SNPs in the bat *SPRN* gene were predicted using multiple *in-silico* tools, including MutPred2, MUpro, SIFT, and SNPs&GO (Table 6). SIFT predicted most variants as deleterious, except for V74A and P130S. MutPred2 indicated potentially harmful effects for several variants (score > 0.6), including W9R, G27D,

G68E, G76E, V114A, and Y115H. In contrast, SNPs&GO predicted most variants as neutral, with W9R and R50L identified as disease-associated. MUpro suggested that most variants decrease protein stability, although R44C, R50L, and G68E were predicted to slightly increase stability.

Effect of non-synonymous polymorphisms on the interaction of bat Sho with bat PrP: Protein–protein interactions between bat Sho and PrP were predicted using HawkDock. Wild-type bat Sho and variants carrying the G68E, V74A, and G76E SNPs exhibited distinct binding conformations in complex with bat PrP. More negative binding free energy values were interpreted as stronger binding affinity and increased complex stability. Variants carrying E68 and E76 exhibited less negative binding free energies (-35.43 and -30.6 kcal/mol, respectively) than the wild type (-40.97 kcal/mol), indicating reduced binding affinity and decreased complex stability (Fig. 6A, B, and D). In contrast, the V74A variant showed a more negative binding free energy (-50.21kcal/mol), suggesting slightly increased binding affinity and enhanced complex stability (Fig. 6C).

Table 6: *In-silico* evaluation on the effect of non-synonymous single nucleotide polymorphisms (SNPs) of the *SPRN* gene in bats

Variations	MutPred2	MUpro	SIFT	SNPs&GO
W9R	Deleterious (0.771)	Decrease (-0.318)	Deleterious (0.000)	Disease (0.753)
G27D	Deleterious (0.721)	Decrease (-1.159)	Deleterious (0.000)	Neutral (0.378)
R28C	Benign (0.497)	Decrease (-0.522)	Deleterious (0.000)	Neutral (0.372)
R44C	Benign (0.157)	Increase (0.097)	Deleterious (0.000)	Neutral (0.463)
R50L	Benign (0.245)	Increase (0.016)	Deleterious (0.000)	Disease (0.661)
G68E	Deleterious (0.694)	Increase (0.110)	Deleterious (0.030)	Neutral (0.307)
V74A	Benign (0.099)	Decrease (-0.281)	Tolerated (1.000)	Neutral (0.005)
G76E	Deleterious (0.807)	Decrease (-0.027)	Deleterious (0.030)	Neutral (0.202)
V114A	Deleterious (0.631)	Decrease (-1.241)	Deleterious (0.020)	Neutral (0.273)
Y115H	Deleterious (0.820)	Decrease (-0.336)	Deleterious (0.000)	Neutral (0.402)
P130S	Benign (0.100)	Decrease (-0.645)	Tolerated (0.330)	Neutral (0.105)

In the prediction categories generated by SNPs&GO, variants with a probability score > 0.5 were classified as "Disease", whereas variants with a score ≤ 0.5 were classified as "Neutral". Higher probability scores indicate a greater likelihood of deleterious effects.

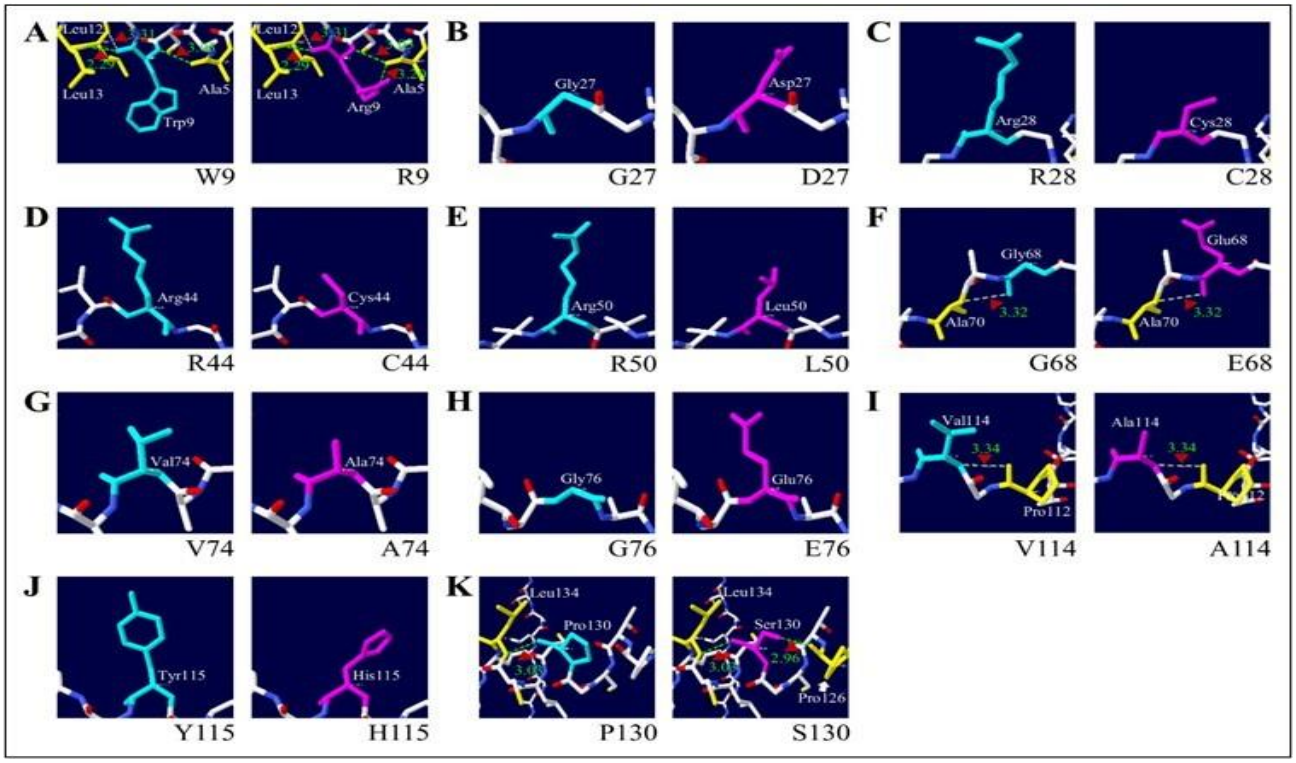

Fig. 5: The 3D structural models and hydrogen-bonding patterns of non-synonymous polymorphisms in bat Sho. (**A-K**) Comparative analysis of structural changes and hydrogen-bonding patterns for the W9R, G27D, R28C, R44C, R50L, G68E, V74A, G76E, V114A, Y115H, and P130S variants.

DISCUSSION

In this study, we identified 20 novel polymorphisms within the coding region of the *SPRN* gene in bats, including 11 non-synonymous SNPs (Fig. 1; Table 2). However, the HWE deviations, LD patterns, and haplotype distributions may have been influenced by population structure originating from multiple bat species with distinct genetic backgrounds and differences in sample origins. Therefore, these genetic analyses should be interpreted cautiously. Although prion diseases have never been reported in bats, they exhibit a high number of SNPs, with a distribution pattern distinct from that of other species. Notably, these variants were unique to bats and were not detected in any other species included in the comparative analysis (Fig. 3), suggesting a species-specific genetic variation profile of the *SPRN* gene. This may reflect distinct evolutionary pressures allowing the accumulation of genetic variation without apparent deleterious effects. However, cross-species comparisons of SNP distributions

should be interpreted cautiously because the sample sizes, sequenced regions, and detection methods differed among studies and species. This interpretation is based on comparative observations and does not establish a causal relationship. Therefore, future studies are required to elucidate how *SPRN* genetic variation contributes to prion pathogenesis.

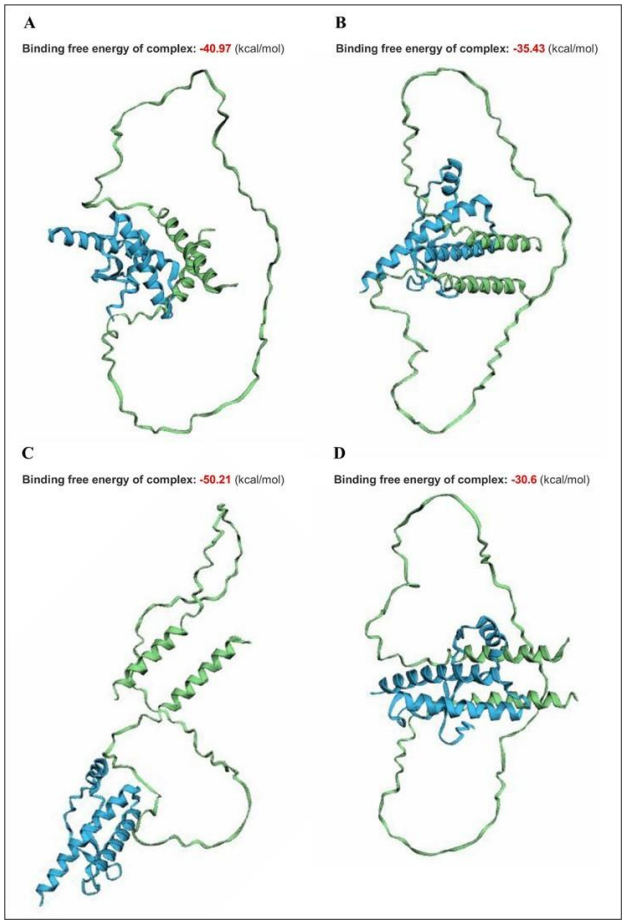

Fig. 6: Structural comparison of protein–protein interactions between bat PrP and bat Sho variants. (**A-D**) The interaction complexes of bat PrP with bat Sho carrying the wild-type, E68, A74 and E76 alleles, respectively. Bat PrP is shown in blue (receptor) and bat Sho is shown in green (ligand).

Furthermore, no frameshift variants were observed within the coding region of the bat *SPRN* gene. Similar findings have been reported in *SPRN* studies of putatively prion-resistant species such as horses, rabbits, chickens, ducks, quail, and pheasants (Fig. 3). In contrast, several indel polymorphisms have been identified in prion-susceptible species, including humans, cattle, and sheep (Fig. 3). This pattern may suggest a potential association between indel variants and prion susceptibility. Accordingly, the absence of indels may represent a feature of *SPRN* in species considered relatively resistant to prion infection. However, direct functional evidence linking indel polymorphisms to prion susceptibility remains limited, and further studies are required to clarify their impact on prion disease.

In this study, we evaluated the effects of non-synonymous SNPs using multiple *in-silico* prediction tools. Most variants were predicted to be deleterious by MutPred2 or SIFT, including W9R, G27D, R28C, R44C, R50L, G68E, G76E, V114A, and Y115H, suggesting potential impacts on protein structure and function. In contrast, SNPs&GO classified most variants as neutral, with only W9R and R50L predicted to be disease-associated (Table 6). In terms of protein stability, MUpro predicted G27D and V114A to cause the greatest decrease (score <−1). These inconsistencies highlight the limitations of *in-silico* predictions in accurately determining functional consequences. However, many of these variants were detected at low frequencies and were present in only a small number of individuals. Therefore, the biological significance of these variants in prion pathogenesis remains unclear and requires further validation using larger sample sizes and functional experiments.

As Sho is highly conserved among vertebrates, multiple sequence alignment (Fig. 2) revealed that bat Sho preserves the key structural features, including the N-terminal signal peptide, the positively charged RG-rich region, the hydrophobic domain, the C-terminal region containing the N-X-T motif, and a potential GPI-anchor site (Premzl *et al.*, 2003). Meanwhile, non-synonymous substitutions were distributed across these conserved or functionally important regions, suggesting potential functional relevance. The W9R mutation is located within the N-terminal signal peptide and may affect hydrophobicity and protein translocation into the endoplasmic reticulum (Stewart *et al.*, 2001). Conformational analysis also predicted that the W9R variant forms an additional hydrogen bond (Fig. 5A), leading to local interaction changes that may influence the stability of the α1 helix. In addition, the P130S mutation is located within the α2 helix near the C-terminus, adjacent to the GPI-anchor site, and may affect helix stability and the spatial positioning of Sho relative to the cell membrane (Gu *et al.*, 2008). Interestingly, the alanine-rich region of Sho, analogous to the hydrophobic core of PrP (Watts *et al.*, 2007), shows substantial differences between species susceptible to prion disease and species without reported natural prion disease (Fig. 2). Previous studies using yeast two-hybrid systems and biochemical assays have demonstrated that amino acid residues 61–77 of Sho constitute the minimal interaction region required for binding to PrP, and deletion of this region results in loss of protective activity against stress (Jiayu *et al.*, 2010). In bats, the Sho–PrP interaction region contains multiple mutations, including G68E, V74A, and G76E. Structural and binding free energy analyses were performed to evaluate their potential effects on protein–protein interactions. Docking analysis suggested that G68E and G76E may reduce complex stability compared to the wild-type, whereas V74A was predicted to exhibit a slightly stabilizing effect. However, these interaction analyses are based on computational docking and should be interpreted with caution. The functional roles of these polymorphisms remain to be confirmed through further bioassays in prion-infected bats carrying wild-type and variant alleles.

Taken together, the accumulation of non-synonymous SNPs in bat *SPRN*, despite the absence of prion disease, may reflect potential evolutionary adaptations. Some rare variants, such as W9R, G68E, and G76E, were predicted to potentially influence the structure and biological function of bat Sho. However, these interpretations remain speculative, as protein expression and functional effects

were not experimentally validated. In addition, the relatively limited sample size and species diversity included in this study may constrain the generalizability of these findings.

Conclusions: To summarize, we identified a total of 20 SNPs in the ORF of the *SPRN* gene from 100 individual bats and observed distinct patterns of amino acid substitution compared to other mammals. Our results provide initial insights into the unique pattern of *SPRN* variation in bats and contribute to a better understanding of prion susceptibility and resistance. Furthermore, these findings provide a useful genetic basis for future functional studies of Sho and host-specific prion biology. To our knowledge, this is the first study to report genetic polymorphisms in the *SPRN* gene in bats.

Ethical statements: The Institutional Animal Care and Use Committee (IACUC) at Jeonbuk National University (NON2023-227) approved the totality of the experimental techniques in this study.

Author contributions: CGT, DIC, BHJ conceived and designed the experiments; CGT, DIC performed the experiments; CGT, DIC, EJN, JKO, BHJ analyzed the data; CGT drafted the manuscript; CGT, DIC, EJN, JKO, BHJ revised and edited the manuscript; All authors read and approved the final manuscript.

Data Availability Statement: Data are available from the corresponding author upon reasonable request. The nucleotide sequence data generated in this study have been deposited in GenBank under accession number PZ238804.
Declaration of competing interest: The authors declare no competing interests.

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