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Molecular characterization of VP2 and VP3 proteins of chicken anemia virus isolates in Turkey

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Abstract: Chicken anemia virus (CAV) has a circular 2.3 kb DNA genome encoding VP1, VP2, and VP3 proteins. VP1 protein is assembled into viral capsid. VP2 is a nonstructural protein required for the correct assembly of VP1. Also, there is an interaction between VP2 and VP3 protein, known as apoptin. Mostly, VP1 protein of CAV isolates have been investigated in previous studies but the information about VP2 and VP3 proteins is insufficient. The aim of this study is molecular characterization of VP2 and VP3 proteins from 20 CAV isolates previously collected in different regions of Turkey using in silico tools. The open reading frames (ORFs) for VP2 and VP3 were determined to be 651 and 366 base pairs (bp) encoding 216 and 121 amino acids, respectively. Nucleotide substitutions were identified at 21 and 14 positions for VP2 and VP3, respectively. Three isolates had one apiece amino acid substitutions in VP2 sequences. The MW and pI values for VP2 were predicted as 24.08–24.138 kDa and 6.96–8.35, respectively. Moreover, seven isolates had one to three amino acid substitutions for VP3 protein. The MW and pI values for VP3 were calculated as 13.188–13.372 kDa and 9.44–9.95, respectively. The secondary structures were predicted and phylogenetic trees were constructed for VP2 and VP3 proteins. As a conclusion, molecular characterization of VP2 and VP3 proteins from 20 CAV isolates contributes to the current knowledge on these proteins having diverse functions and immunological potentials.

Key words: Bioinformatics, chicken anemia virus, VP2, VP3

1. Introduction

Chicken anemia virus (CAV) was first reported in 1979 by Yuasa et al. [1] as an agent inducing anemia in chicks. Later, CAV was defined as an icosahedral and nonenveloped virus in the *Gyrovirus* genus of Anelloviridae family, and the causative agent ofthe disease chicken infectious anemia (CIA) [2]. CAV causes immune suppression, anemia, bone marrow aplasia, weight loss and intramuscular hemorrhage especiallyin young chicken lacking maternal antibodies, resulting in important economic losses worldwide in poultry industry [3]. Mortality and morbidity rates of the disease can be up to 55% and 80%, respectively [4]. CAV can transmit both horizontally and vertically, also the presence of this virus has been reported in feces samples of human, stray cat, dog and mouse [5]. Recently, it was reported that ducks can be carriers of CAV, causing cross-species transmission of the virus in the farms where mixed-species of poultry are reared [6].

Commercial vaccines against CAV are produced via serial passage of wild strains in chicken embryos or in some lymphoblastoid cell lines such as MDCC-MSB1 cell to attenuate viruses [7]. However, complete attenuation may not be ensured and young chicks may be infected with the live viruses from the feces of vaccinated animals [7]. Also, chicken-to-chicken transmission of attenuated viruses may cause appearance of virulent phenotypes. Therefore, vaccination against CAV is not recommended for chickens younger than six week-old, and before four weeks to lay [7]. Healthy breeder flocks are vaccinated to prevent vertical transmission and to protect young chicks by maternal antibodies [8,9]. However, titer of protective maternal antibodies may decrease after two weeks of age and CAV positivity may be detected as early as three weeks of age [10,11].

The genome of CAV is composed of circular 2.3 kb single-stranded ambisense or negative-sense DNA producing a single polycistronic messenger RNA (mRNA). There are three open reading frames (ORFs), partially overlapped, encoding viral proteins VP1, VP2, and VP3. VP1 is a 51 kDa structural protein assembled into viral capsid. It has roles in replication and virulence of CAV but the presence of glutamines at the positions 139 and

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144 results in the weakness of these roles [12]. VP2 is a nonstructural protein required for the correct assembly of VP1, and neutralizing antibodies can be induced by both VP1 and VP2 [4]. VP2 is a dual-specificity phosphatase (DSP) associated with viral replication, virulence, and cytopathology [13]. VP3, named as apoptin, is also a nonstructural protein with apoptotic activity in chicken lymphoblastoid T cells and thymocytes as well as in human tumor cell lines [3,7] without damaging primary and nontransformed cells, which makes it a promising candidate for cancer therapy [13]. A direct interaction between VP2 and VP3 in the nucleus was reported to decrease the rate of apoptosis via dephosphorylation of the threonine 108 of apoptin by VP2 [14]. VP2 protein also acts as a scaffold for VP1 protein. Genotyping of CAV strains is performed according to VP1 sequences. In addition to the genotypes I, II, III (IIIa, IIIb, IIIc), and IV, Van Dong et al. [15] defined genotype V as a result of recombination between genotypes II and IIIc.

Recently, the genome and the VP1 characteristics of CAV isolates from Turkey have been reported [12]; however, the information about the VP2 and VP3 sequences of these isolates was lacking. In this study, the aim was to characterize the VP2 and VP3 proteins of CAV isolates from Turkey using the nucleotide and amino acid sequences. Mutations in the nucleotide sequences and amino acid substitutions were identified, phylogenetic relationships were determined, and the characteristics of these proteins were predicted.

2. Materials and methods

2.1. The sequences of VP2 and VP3

Nucleotide and amino acid sequences of VP2 and VP3 from 20 Chicken Anemia Virus (CAV) isolates, obtained from the chicken farms in five different geographical regions of Turkey, were used for the characterization (Table 1). Previously, Aşkar [12] obtained these CAV isolates from the thymus tissues of broilers, and DNA samples purified using QIAamp MinElute Virus Spin Kit were utilized for PCR-amplification of genomes. The genome sequences were obtained via next-generation sequencing (MiSeq, Illumina), and submitted to GenBank[1] with the accession numbers of MT259306-MT259325. The reference strain Cuxhaven-1 (accession number M55918) and the vaccine strain 3711/chicken/Australia (accession number EF683159) were included in the analyses.

2.2. Mutational and phylogenetic analyses

Nucleotide and amino acid sequences of VP2 and VP3 from CAV isolates were aligned using ClustalW algorithm in MEGA X software [16]. The settings for pairwise and multiple alignment were 15.00 for gap opening penalty and 6.66 for gap extension penalty. For matrix options, DNA weight matrix was IUB, transition weight was 0.50, and delay divergent cutoff was 30%. The consensus sequences were disregarded and the shifted nucleotides or amino acids were recorded.

The aligned nucleotide sequences for VP2 and VP3 proteins of CAV isolates from Turkey and other countries were used for the construction of phylogenetic trees using Neighbor-Joining method [17] in MEGA X. The maximum composite likelihood method was used to compute evolutionary distances [18]. They phylogeny was tested using bootstrap test (1000 replicates).

2.3. Characterization of VP2 and VP3 proteins

The secondary structures of VP2 and VP3 proteins were predicted using PSIPRED 4.0 tool[2] [19] using amino acid sequences. The molecular weight (MW) and isoelectric point (pI) values of the VP2 and VP3 proteins were predicted using ProtParam tool [20] in ExPASy bioinformatics resource portal[3].

3. Results

3.1. Mutational and phylogenetic analyses for the genes encoding VP2 and VP3

The number of nucleotides for all VP2 and VP3 proteins of 20 CAV isolates from Turkey was determined to be 651 and 366, respectively.

Alignment of the nucleotide sequences using ClustalW algorithm revealed base-pair substitutions at 21 positions (from 9 to 650) for VP2 and 14 positions (from 11 to 352) for VP3 (Table 2). Mutations were identified in 13 isolates while seven isolates had consensus sequences. The highest number of mutation was five observed in the isolate AB1K, and four mutations were identified in the isolate GDA5K. The nucleotide sequence of the vaccine strain contained eight nucleotide substitutions compared to the consensus sequences.

The phylogenetic analysis using the nucleotide sequences encoding VP2 and VP3 proteins of the 20 CAV isolates from Turkey as well as from different countries showed that 17 isolates from Turkey were grouped together, where EB10K (MT259318) and AB1K (MT259319) were on a separate branch, segregating from the isolates of

[1] International Nucleotide Sequence Database Collaboration (2020). GenBank [online]. Website https://www.ncbi.nlm.nih.gov/genbank [accessed 12 March 2020].

[2] UCL Department of Computer Science: Bioinformatics Group (2021). PSIPRED 4.0 [online]. Website http://bioinf.cs.ucl.ac.uk/psipred [accessed 24 April 2020].

[3] Swiss Institute of Bioinformatics (2020). ExPASy Bioinformatics Resource (ProtParam tool) [online]. Website https://www.expasy.org [accessed 11 May 2020].

Table 1. The GenBank ID, name, location, collection year, and host of 20 CAV isolates from Turkey.

GeneBank ID	Isolate name	Location	Collection year	Host
MT259306	KB1K	Black Sea Region	2015	Broiler
MT259307	GDA8K	Southeastern Anatolia Region	2015	Broiler
MT259308	DAB2K	Eastern Anatolia Region	2015	Broiler
MT259309	DAB3K	Eastern Anatolia Region	2015	Broiler
MT259310	DAB4K	Eastern Anatolia Region	2015	Broiler
MT259311	DAB5K	Eastern Anatolia Region	2015	Broiler
MT259312	DAB6K	Eastern Anatolia Region	2015	Broiler
MT259313	DAB8K	Eastern Anatolia Region	2015	Broiler
MT259314	EB1K	Aegean Region	2016	Broiler
MT259315	KB3K	Black Sea Region	2015	Broiler
MT259316	EB7K	Aegean Region	2016	Broiler
MT259317	EB8K	Aegean Region	2016	Broiler
MT259318	EB10K	Aegean Region	2016	Broiler
MT259319	AB1K	Mediterranean Region	2016	Broiler
MT259320	KB6K	Black Sea Region	2015	Broiler
MT259321	KB9K	Black Sea Region	2015	Broiler
MT259322	KB10K	Black Sea Region	2015	Broiler
MT259323	GDA2K	Southeastern Anatolia Region	2015	Broiler
MT259324	GDA5K	Southeastern Anatolia Region	2015	Broiler
MT259325	GDA6K	Southeastern Anatolia Region	2015	Broiler

other countries. However, KB1K (MT259306) and EB7K (MT259316) isolates were grouped together with the isolates mainly from the East Asia. The GDA5K isolate, having a unique T to C mutation, was grouped with the isolates from Tunisia (Figure 1). The phylogenetic analysis also revealed that all the isolates from the same location in Turkey were not grouped together. Some of the isolates from different locations showed the same mutations and grouped together.

3.2. Sequence analysis and characteristics of VP2 proteins

Majority of the isolates from Turkey had a consensus sequence for VP2 protein but DAB3K, DAB6K, and AB1K had R166Q, Y43F, and D169G changes, respectively. Additionally, Cuxhaven-1 and 3711/Australia strains had V153A and R126L changes for VP2, respectively (Table 3).

The characteristics of VP2 proteins in terms of number of amino acids, MW, and pI values were also predicted. All of the VP2 proteins were consisted of 216 amino acids. The predicted MW and pI for the VP2 proteins of isolates having consensus sequences were 24.138 kDa and 7.70, respectively. The MW values of VP2 proteins for DAB3K, DAB6K, AB1K, Cuxhaven-1, and 3711/Australia were predicted as 24.11, 24.12, 24.08, 24.11, and 24.09kDa, respectively. Additionally, the predicted pI values for VP2 proteins of DAB3K, DAB6K, AB1K, Cuxhaven-1, and 3711/Australia were 6.96, 7.71, 8.35, 7.70, and 6.96, respectively (Table 2).

The secondary structures of VP2 proteins were analyzed in silico. The length and location of α-helix and β-strand structures were very similar. All of the VP2 proteins contained six α-helix structures except Cuxhaven-1 having an additional helix composed of H142 and Y143 residues. Also, DAB3K and AB1K included D161 in the fifth α-helix structure. All VP2 proteins had four β-strand structures at the same positions. For DAB6K, shifted F43 was excluded from the β-strand as well as DAB3K, AB1K, and 3711/Australia included Y154 and K155 to the fourth β-strand structures (Figure 2).

3.3. Sequence analysis and characteristics of VP3 proteins

Thirteen isolates from Turkey showed a consensus sequence for VP3 protein. The isolates KB1K, DAB6K, EB1K, and EB7K had one amino acid change. GDA5K and EB10K had two, and AB1K had three shifted amino acids. Cuxhaven-1 and 3711/Australia strains had three and five amino acids substitutions, respectively (Table 4).

All VP3 proteins were composed of 121 amino acids. The MW and pI values for consensus sequences were 13.217 kDa and 9.65, respectively. The mutated VP3

Table 2. Mutations in nucleotide sequences encoding VP2 and VP3 proteins. The ORF of VP3 is inside the ORF of VP2 with different start and stop codons. Only the nucleotide positions affected were shown.

VP2	9	117	128	136	150	174	180	267	306	324	377	385	414	453	458	497	506	513	537	579	650
VP3	-	11	22	30	44	68	74	161	200	218	271	279	308	347	352	-	-	-	-	-	-
KB1K	G	T	A	C	T	G	T	C	G	T	G	C	G	G	T	G	A	G	C	A	G
KB3K	G	T	A	C	T	G	T	C	G	C	G	C	G	G	T	G	A	G	A	C	G
KB6K	G	T	A	C	T	G	T	C	G	C	G	A	G	G	T	G	A	G	A	A	G
KB9K	G	T	A	C	T	G	T	C	G	C	G	C	G	G	T	G	A	G	A	A	G
KB10K	G	T	A	C	T	G	T	C	G	C	G	C	G	G	T	G	A	G	A	A	G
GDA2K	G	T	A	C	T	G	T	C	G	C	G	C	G	G	T	G	A	G	A	A	G
GDA5K	G	T	A	A	T	G	C	C	G	T	G	C	G	G	T	G	A	G	C	A	G
GDA6K	G	T	A	C	T	G	T	C	G	C	G	C	G	G	T	G	A	G	A	A	G
GDA8K	G	T	A	C	T	G	T	C	G	C	G	C	G	G	T	G	A	G	A	C	G
DAB2K	G	T	A	C	T	G	T	C	G	C	G	C	G	G	T	G	A	G	A	C	G
DAB3K	G	T	A	C	T	G	T	C	G	C	G	C	G	G	T	A	A	G	A	A	G
DAB4K	G	T	A	C	T	G	T	C	G	C	G	C	G	G	T	G	A	G	A	A	G
DAB5K	G	T	A	C	T	G	T	C	G	C	G	C	G	G	T	G	A	G	A	A	G
DAB6K	G	T	T	C	T	G	T	C	G	C	G	C	G	G	T	G	A	G	A	A	G
DAB8K	G	T	A	C	T	G	T	C	G	C	G	C	G	G	T	G	A	G	A	C	G
EB1K	G	T	A	C	C	G	T	C	G	C	G	C	G	G	T	G	A	G	A	A	A
EB7K	G	T	A	C	T	G	T	C	G	T	G	C	G	G	T	G	A	G	C	A	G
EB8K	G	T	A	C	T	G	T	C	G	C	G	C	G	G	T	G	A	G	A	A	G
EB10K	G	T	A	C	T	G	T	C	G	T	G	C	A	G	T	G	A	G	C	A	G
AB1K	G	C	A	C	T	A	T	C	G	C	G	C	A	G	T	G	G	G	C	A	G
Cuxhaven-1	G	T	A	C	T	G	T	C	G	T	G	C	G	A	C	G	A	T	C	A	G
3711/Australia	A	A	A	A	T	G	T	T	A	T	T	C	G	G	T	G	A	G	C	A	G

A: adenine, C: cytosine, G: guanine, T: thymine. The shifted nucleotides at each position were shown in different colors, i.e. pink for A, green for C, purple for G, and blue for T.

sequences had MW values ranging from13.188 to 13.372 kDa. The pI value for the majority of VP3 proteins was predicted as 9.65 except for AB1K, Cuxhaven-1, and 3711/Australia, which were 9.44, 9.95, and 9.78, respectively (Table 4).

The secondary structure analysis for VP3 proteins showed that the consensus sequences, represented by the KB3K, shared the same α-helix and β-strand structures with VP3 of KB1K, DAB6K, and EB1K. The VP3 protein of GDA5K differed from them with exclusion of H29 from the β-strand structure. Moreover, VP3 of EB10K and AB1K had the same secondary structure with an additional β-strand composed of I105 and T106 as well as S98 was excluded and E102 was included in the α-helix. The VP3 of Cuxhaven-1 had a longer α-helix structure including V97, E102, and S103. 3711/Australia was added P55 to β-strand and excluded S98 from the α-helix structure of VP3 (Figure 3).

4. Discussion

The genome of CAV includes three ORFs for VP1, VP2, and VP3 proteins [21,22]. The nucleotide and amino acid sequences for VP2 and VP3 proteins belonging to isolates from Turkey were analyzed in this study. VP2 and VP3 proteins interact with each other to induce apoptosis in infected cells [13,14]. Therefore, increased knowledge on these proteins would have a contribution to improve the understanding of CAV infections. Moreover, VP2 and VP3 proteins have a potential to be used as a target antigen for production of monoclonal antibodies [3]. Although many studies characterized VP1 protein [12,21,23], the information about the characteristics of VP2 and VP3 proteins is scarce.

The ORFs for all VP2 and VP3 proteins investigated in this study were constituted of 651 and 366 bp in length, respectively, showing the absence of an insertion or a deletion. The lengths for VP2 and VP3 ORFs of isolates

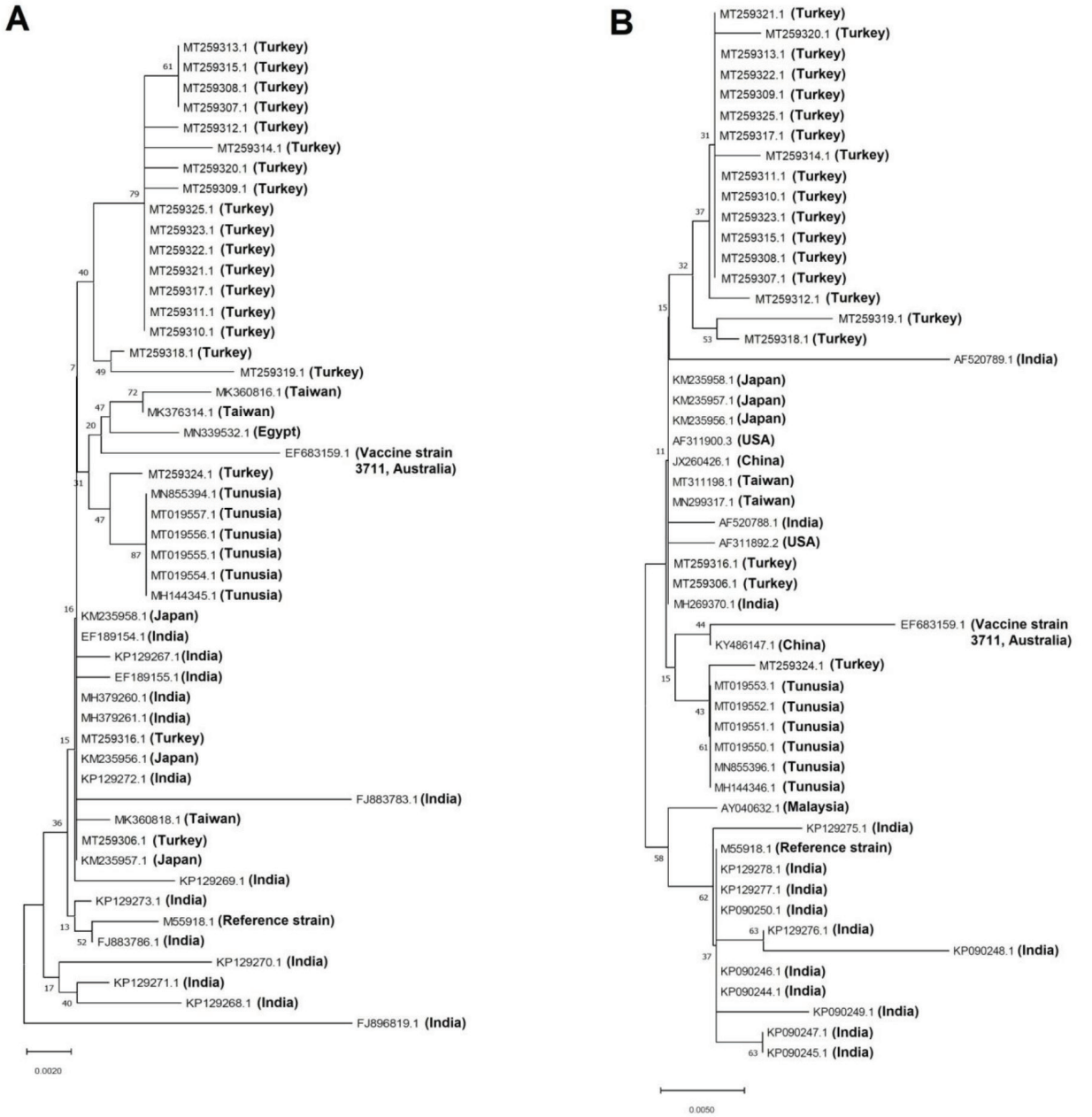

Figure 1. Phylogenetic relationships of the isolates based on the nucleotide sequences encoding (A) VP2 and (B) VP3 proteins of isolates from Turkey and other countries. GenBank accession numbers and source countries of the sequences were indicated.

from China were reported same [4,21]. However, Hiremath et al. [24] reported the number of nucleotides for VP2 and VP3 ORFs of the CAV isolates from India as 721 and 349, respectively.

Mutations were identified at 21 positions for VP2 and 14 positions for VP3 in 13 isolates. The highest number of mutation was five observed in the isolate AB1K. Li et al. [4] reported that the sequences encoding VP2 and VP3 proteins from the 24 CAV isolates from chickens in China had 27 and 12 nucleotide substitutions, respectively, showing a similar rate of mutations in CAV.

Although the number of mutations in nucleotide sequences was higher, it was not reflected to the amino acid sequences for VP2. All VP2 proteins were composed of 216 amino acids, and the majority of the isolates had a consensus sequence with one apiece substitutions for three isolates. MW and pI values for VP2 were predicted as 24.08–24.138 kDa and 6.96–8.35, respectively. The MW of VP2 was mentioned as 24 kDa previously [4,14,23].

The VP3 protein is known as apoptin due to its apoptotic properties in infected cells where its localization can be in different compartments [22]. In

Table 3. Substitutions in amino acid sequences, and characteristics of VP2 proteins.

	Changes in amino acids in peptide sequences					Protein parameters		
aa position / Isolate	43	126	153	166	169	#aa	MW (kDa)	pI
KB1K	Y	R	V	R	D	216	24.138	7.70
KB3K	Y	R	V	R	D	216	24.138	7.70
KB6K	Y	R	V	R	D	216	24.138	7.70
KB9K	Y	R	V	R	D	216	24.138	7.70
KB10K	Y	R	V	R	D	216	24.138	7.70
GDA2K	Y	R	V	R	D	216	24.138	7.70
GDA5K	Y	R	V	R	D	216	24.138	7.70
GDA6K	Y	R	V	R	D	216	24.138	7.70
GDA8K	Y	R	V	R	D	216	24.138	7.70
DAB2K	Y	R	V	R	D	216	24.138	7.70
DAB3K	Y	R	V	Q	D	216	24.110	6.96
DAB4K	Y	R	V	R	D	216	24.138	7.70
DAB5K	Y	R	V	R	D	216	24.138	7.70
DAB6K	F	R	V	R	D	216	24.122	7.71
DAB8K	Y	R	V	R	D	216	24.138	7.70
EB1K	Y	R	V	R	D	216	24.138	7.70
EB7K	Y	R	V	R	D	216	24.138	7.70
EB8K	Y	R	V	R	D	216	24.138	7.70
EB10K	Y	R	V	R	D	216	24.138	7.70
AB1K	Y	R	V	R	G	216	24.080	8.35
Cuxhaven-1	Y	R	A	R	D	216	24.110	7.70
3711/Australia	Y	L	V	R	D	216	24.095	6.96

aa: amino acid, MW: molecular weight, pI: isoelectric point, A: alanine, D: aspartate, F: phenylalanine, G: glycine, L: leucine, Q: glutamine, R: arginine, V: valine, Y: tyrosine. The shifted amino acids were shown in different colors, i.e. yellow for A, pink for F, purple for G, blue for L, and green for Q.

our study, a consensus sequence was identified in 13 isolates while seven isolates had one to three amino acid substitutions. The number of changed amino acids was higher compared to VP2 proteins showing that the high number of nucleotide mutations affected VP3 proteins more. The VP2 protein interacts with VP3 causing decreased phosphorylation of T108 of VP3 and suppressing apoptosis [14]. The T108 residue of VP3 was not substituted in the isolates covered in this study. The MW and pI values for VP3 proteins, composed of 121 amino acids, were predicted as 13.188–13.372 kDa and 9.44–9.95, respectively. Previously, the MW of VP3 protein was reported as 13.6 kDa [4,21] or 13 kDa [14,23]. The variety in MW values shows the high number of changes in amino acid sequences.

5. Conclusion

In the genetic characterization of CAV isolates, mostly the sequence of VP1 protein has been used in previous studies, and knowledge on VP2 and VP3 proteins is scarce. An in silico characterization was performed in this study using nucleotide and amino acid sequences belonging to VP2 and VP3 proteins of 20 CAV isolates from five different regions of Turkey. The number of amino acid substitutions was found higher in VP3 than VP2, which was reflected to the variety of MW and PI values as well as secondary structures. Two isolates, GDA5K and AB1K, had the highest amount of diversity. These determined differentiations might affect the apoptotic capability of VP3 proteins as well as virulence of the isolates, which needs to be investigated in further studies.

Figure 2. Secondary structures of the VP2 proteins. Only the proteins having substituted amino acid in sequence were included. KB1K represents the consensus sequences. A: alanine, C: cysteine, D: aspartate, E: glutamate, F: phenylalanine, G: glycine, H: histidine, I: isoleucine, K: lysine, L: leucine, M: methionine, N: asparagine, P: proline, Q: glutamine, R: arginine, S: serine, T: threonine, V: valine, W: tryptophan, Y: tyrosine.

Table 4. Substitutions in amino acid sequences, and characteristics of VP3 proteins.

	Changes in amino acids in peptide sequences												Protein parameters		
aa position / Isolate	4	8	15	23	25	54	67	73	91	103	116	118	#aa	MW	pI
KB1K	L	T	V	R	L	A	S	V	D	S	R	C	121	13.245	9.65
KB3K	L	T	V	R	L	A	S	A	D	S	R	C	121	13.217	9.65
KB6K	L	T	V	R	L	A	S	A	D	S	R	C	121	13.217	9.65
KB9K	L	T	V	R	L	A	S	A	D	S	R	C	121	13.217	9.65
KB10K	L	T	V	R	L	A	S	A	D	S	R	C	121	13.217	9.65
GDA2K	L	T	V	R	L	A	S	A	D	S	R	C	121	13.217	9.65
GDA5K	L	T	V	R	S	A	S	V	D	S	R	C	121	13.218	9.65
GDA6K	L	T	V	R	L	A	S	A	D	S	R	C	121	13.217	9.65
GDA8K	L	T	V	R	L	A	S	A	D	S	R	C	121	13.217	9.65
DAB2K	L	T	V	R	L	A	S	A	D	S	R	C	121	13.217	9.65
DAB3K	L	T	V	R	L	A	S	A	D	S	R	C	121	13.217	9.65
DAB4K	L	T	V	R	L	A	S	A	D	S	R	C	121	13.217	9.65
DAB5K	L	T	V	R	L	A	S	A	D	S	R	C	121	13.217	9.65
DAB6K	L	S	V	R	L	A	S	A	D	S	R	C	121	13.202	9.65
DAB8K	L	T	V	R	L	A	S	A	D	S	R	C	121	13.217	9.65
EB1K	L	T	A	R	L	A	S	A	D	S	R	C	121	13.188	9.65
EB7K	L	T	V	R	L	A	S	V	D	S	R	C	121	13.245	9.65
EB8K	L	T	V	R	L	A	S	A	D	S	R	C	121	13.217	9.65
EB10K	L	T	V	R	L	A	S	V	D	N	R	C	121	13.272	9.65
AB1K	P	T	V	Q	L	A	S	A	D	N	R	C	121	13.199	9.44
Cuxhaven-1	L	T	V	R	L	A	S	V	D	S	K	R	121	13.270	9.95
3711/Australia	H	T	V	R	L	V	N	V	Y	S	R	C	121	13.372	9.78

aa: amino acid, MW: molecular weight, pI: isoelectric point, A: alanine, C: cysteine, D: aspartate, H: histidine, K: lysine, L: leucine, N: asparagine, P: proline, Q: glutamine, R: arginine, S: serine, T: threonine, V: valine, Y: tyrosine. The shifted amino acids were shown in different colors, i.e. yellow for A, turquoise for H, orange for K, red for N, purple for P, green for Q, grey for R, pink for S, blue for V, and brown for Y.

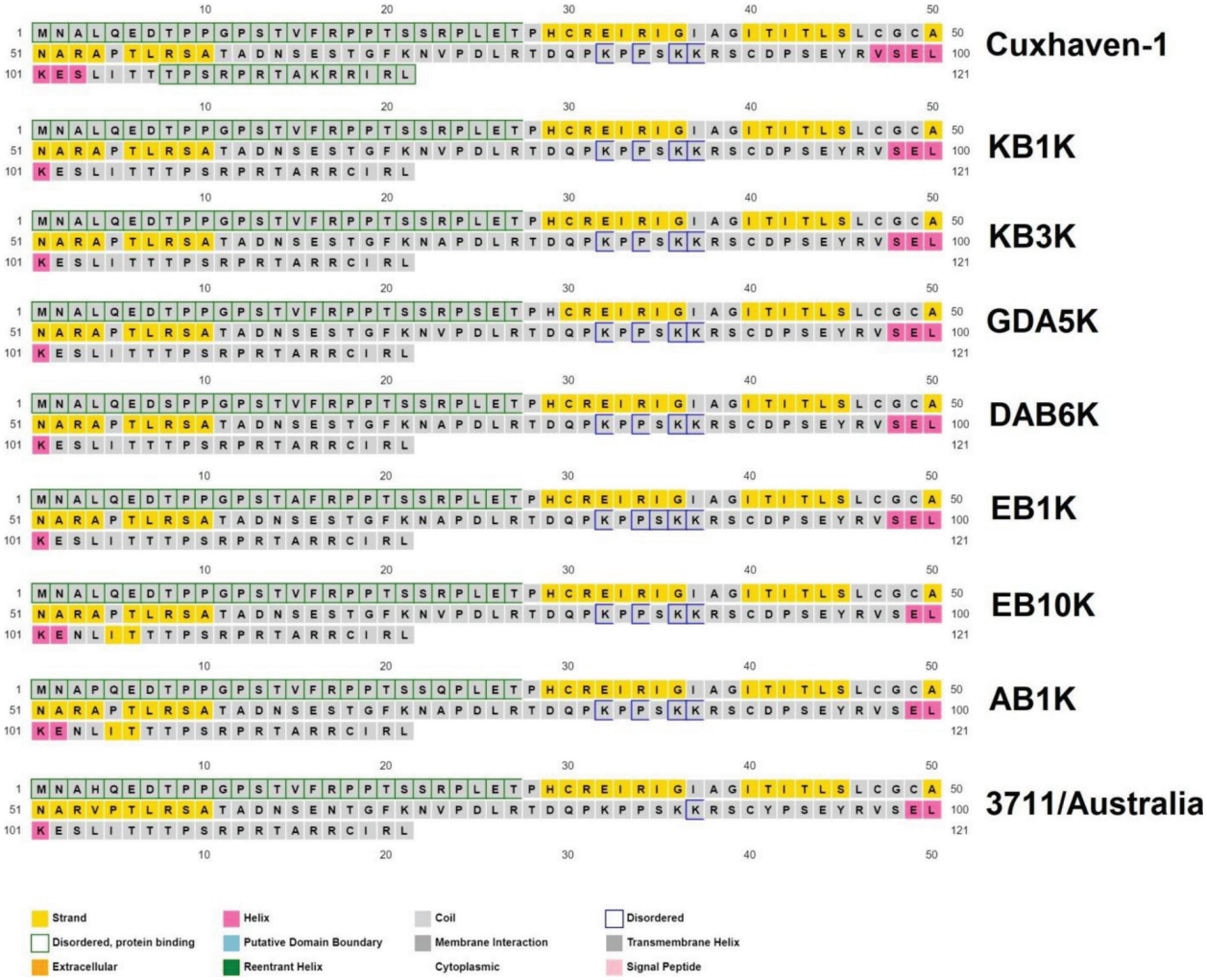

Figure 3. Secondary structures of the VP3 proteins. Only the proteins having substituted amino acid in sequence were included. KB3K represents the consensus sequences. A: alanine, C: cysteine, D: aspartate, E: glutamate, F: phenylalanine, G: glycine, H: histidine, I: isoleucine, K: lysine, L: leucine, M: methionine, N: asparagine, P: proline, Q: glutamine, R: arginine, S: serine, T: threonine, V: valine, Y: tyrosine.

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Ethical statement

All applicable international, national, and institutional guidelines for the care and use of animals were followed. Collection of the virus samples was approved by the Animal Welfare Committee of Kırıkkale University (Approval Number: R.N.:15/01).

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