International Journal of Computational Bioinformatics and In Silico Modeling
Vol. 3, No. 3 (2014): 374-380
Research Article
Open Access

ISSN: 2320-0634

In silico analysis show incidence of very virulent Marek's Disease Virus (MDV)

G.Sathish[1], Kurunchi C.Divya[2], M.Parthiban[1]* and A. Wilson Aruni[3]

[1] *Department of Animal Biotechnology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, INDIA*
[2] *Department of Biotechnology, Faculty of Allied Health Sciences, Chettinad Academy of Research and Education, Chennai, Tamil Nadu, INDIA.*
[3] *Division of Microbiology and Molecular Genetics, School of Medicine, Loma Linda University, CA-92354 , USA*

*Corresponding author: M.Parthiban; e-mail: drparthiban66@gmail.com

Received: 21 April 2014 Accepted: 07 May 2014 Online: 12 May 2014

ABSTRACT

The phylogenetic analysis indicated that Tamil Nadu isolates of Marek's disease viruses formed a cluster with very virulent MDV strains. Multiple sequence alignment of amino acid sequences of Meq protein showed variation among the Tamil Nadu isolates at position 71st (threonine instead of isoleucine) and was found to be important for transactivation of the Meq promoter. Due to the non-availability of Meq protein structure in protein databases, *In silico* modeling of the Meq protein of Tamil Nadu strain , RB-1B (vvMEQ), 648A (vv+MEQ) strains was successfully carried out using the Modeller9.10 software. The generated models were evaluated using *PROSA*, *PROCHECK*, *PROMOTIF*. Ramachandran plot analysis, z-score statistical analysis and energy distribution of protein structure models were enumerated. The amino acid variations in modeled structures as motif may be useful to evaluate epitope vaccines to protect oncogenic MDV.

Keywords: Marek's disease virus, *meq* gene, phylogenetic analysis, multiple sequence alignment, protein structure modeling, epitope prediction

INTRODUCTION

Marek's disease virus (MDV) is a double stranded DNA avian herpesvirus, placed under genus *Mardivirus* that causes tumors in chickens and turkeys. It is one of the most economically important pathogen that severely affects the poultry industry [1]. Marek's disease (MD) continue to be a most important area of scientific interest, both because of its importance as a major disease affecting poultry health as well as through its status as an excellent model of herpesvirus induced T-cell lymphomas in their natural avian hosts [2]. Previously, natural MDV isolates of variable virulence have been isolated, including very virulent plus, very virulent, virulent, mild, belonging to serotype 1 and avirulent strains, belonging to serotypes 1, 2 and 3 [3] (MDV-1, MDV-2, HVT currently classified as Gallid herpesvirus-2(GaHV-2), GaHV-3, Meleagrid herpesvirus-1 (MeHV-1) respectively).

MDV-1 is an evolving pathogen. Mild/Virulent MDV-1 that could have been prevented by HVT underwent changes that resulted in the occurrence of very virulent MDV-1 strains resistant to HVT vaccination [4]. After the introduction of bivalent vaccines (avirulent HVT and MDV-2) viruses of MDV-1 breaking the vaccine protection were isolated in 1990s (vv+MDV) [3]. The MDV-1 viruses breaking the immunity of CVI988 vaccine (attenuated MDV-1 strain) were also isolated (vv++MDV) [5]. It is clear that serial *in vitro* passage of MDV-1 causes attenuation of the virus with dynamic changes in its genome. Although there is still a general feeling that we do not fully understand or have undermined the impact of these genetic changes on the biological properties of different MDV-1 isolates and their passage history [6].

The genome sequences of representatives of MDV-1 (GA, Md-5 strains), MDV-2 (HPRS24 strain) and HVT were completed ([7], [8], [9]). Among the most

important genes of the virus, the MDV encoding leucine zipper protein Meq which structurally resemble the Jun/Fos family of transcriptional activators have been shown to be important for the MDV-associated oncogene expression [10], it was found that the *meq* gene contained polymorphisms and point mutations that seemed to directly correlate with MDV virulence [11]. Recent studies have shown that meq deleted vaccine for Marek's disease is potent in stimulating the maternal antibody response to the disease [12].

Meq is a 339 amino-acid protein, characterized by an N-terminal basic leucine zipper (bZIP) domain (1-120) and a proline-rich C-terminal transactivation domain (121-339). Meq also contains both nucleus and nucleolus localization signals [13] and co-localizes with Cdk2 in coiled bodies [14]. Like other bZIP proteins, Meq posses transactivation activity and is capable of forming homodimers with itself as well as heterodimers with other bZIP proteins such as c-Jun. Meq homodimers have been shown to repress MDV early promoters such as pp38 and pp14 while Meq/c-Jun heterodimers have been reported to activate the meq promoter [15]. Meq, like v-Jun, is involved in MDV transformation by increased transcription of antiapoptosis genes [16] and has been shown to interact with a cellular co-repressor, c-terminal binding protein (CtBP), essential for MDV oncogenesis [17].

The C-terminal domain contains two and one-half repeats of proline-rich sequences with several PPPP and PXXP motifs, known to be involved in protein-protein interaction modules (especially for SH3-containing proteins) [18]. The very virulent plus (vv+MDV) strains carry mutations at the second position of the PPPP motif (threonine, alanine) [5]. However, 59-111 amino acids structure (bZIP domain) is only available for predicted Meq protein structure in molecular modeling database (MMDB). MicroRNAS related to MDV-1 pathogenesis, flank to the *meq* oncogene and/or map to latency associated transcripts that are antisense to the immediate early ICP4 gene were identified [19]. Stik *et al.* [20] proposed that gga-miR-21 is up regulated during MDV-1 infection, indicating that Meq oncoprotein map to the relevant gga-miR-21 promoter of the TMEM49 gene.

MDV evolution is an enormous problem for the poultry industry because developing novel more efficacious vaccines is extremely hard. Despite of numerous attempts to develop vaccines that exceed protection conferred by CVI988, none of them have been successful [5]. Current methods of control will not be adequate in the future if MDV continue its evolution towards greater virulence. More efficacious MD vaccines and sustainable strategies will be needed, if the evolution of MDV towards greater virulence is to be understood [2]. Hence this study envisages understanding the evolution of MDV towards greater virulence, an extensive study from sequence to protein structure is aimed through analysis of Meq protein and it's *in silico* modeling using sequencing and phylogenetic analysis, multiple amino acid sequence alignment, protein structure modeling, stability by Ramachandran plot analysis respectively.

MATERIALS AND METHODS

Sequencing of meq gene

The three TN strains tn-n1, tn-n2 and tn-n3 were isolated in Chicken Kidney Culture (CKC) and further grown in Chicken Embryo Fibroblast (CEF) culture were used in this study [21]. The cell culture isolated viruses were injected in 3-day-old chickens and infection was confirmed by PCR [22]. The full length of *meq* gene sequences was amplified by PCR (amplicon size of 1020bp). The purified PCR products were cloned in TA vector (GeNei Instant TA cloning kit, GeNei, India) and sequenced. The sequences were submitted in NCBI database (Accession number: Genbank HM749324, Genbank HM749325, Genbank HM749326) [23].

Multiple sequence alignment

The amino acid sequences of Meq protein from different strains mMDV (CU-12 strain), vMDV (GA strain), vvMDV (Md5 and RB-1B), vv+MDV (648A strain) with TN (India) strains (tn-n1, n2, and n3) were used for multiple sequence alignment using CLUSTAL W 2.1 software (www.ebi.ac.uk/Tools/msa/clustalw2/). The representatives of mild, virulent, very virulent (Md5 and RB-1B), very virulent plus strains are taken based on [11], [13], [7], [4], [6] respectively. Short Meq (s-Meq*)* and very short Meq (vs-Meq) in MDV transformed cell lines were also included in alignment for analysis.

Phylogenetic analysis

A phylogenetic tree of *meq* gene (1020 bp) was constructed using MEGA 5.0 software by comparing the *meq* gene of different virus strains based on pathotype taken from already published sequence data of [11].

Protein structure modeling

Due to absence of meq protein structure in NCBI database, meq protein of tn-n3, RB-1B (vvMEQ), 648A (vv+MEQ) strains were structurally modeled using modeller9.10 software (salilab.org/modeler/). mGenThreader online software (bioinf:cs.ucl.ac.uk/psipred/) was used to align the protein sequence data as input to modeller9.10 utilizing the difficult modeling protocol. The output Meq protein structure models were also evaluated by deriving DOPE and GA341 scores. The best model for each strain was selected based on minimum DOPE score and maximum GA341 score. The template protein used was c-myc structure (1mv3) which has c-myc/Bin1interaction domain, act as transformation and repressor [24]. These functional domains are also conserved in SH3-containing Meq oncoprotein.

Protein structure evaluation

Modeled protein structures were validated using *PROSA* [25], *PROCHECK* [26] and *PROMOTIF* [27] for motif prediction.

RESULTS AND DISCUSSION

Multiple sequence alignment

The Multiple sequence alignment results using clustal W 2.1 were listed in Table-1. tn-n3 strain showed the least variation at 194thleucine instead of proline, tn-n2 strain at 194, 209, 263th positions showed leucine, proline, glycine instead of proline, leucine, glutamate respectively and in tn-n1 strain showed proline, leucine, tryptophan, threonine at 209, 286, 313, 339th positions instead of leucine, phenylalanine, leucine, proline.

Table1. Amino acid variations in TN strains comparing with MDV reference strains using multiple sequence alignment clustal w2 results

amino acid position	Common amino acid in these strains	Amino acid variations in multiple sequence alignment results									
		mMDV CU-12 strain ACF94907	vMDV GA strain AAF67210	vvMDV Md-5 strain AAG14278	vvMDV RB-1B Strain ABR13046	TN-N1 strain ADN52666	TN-N2 strain ADN52667	TN-N3 strain ADN52668	vv+MDV 648A strain AAM00005	s-meq BAC02923	vs-meq BAC02924
70	Alanine									Serine	Serine
71	Alanine	Serine									
77	Lysine	Glutamate									
100	Arginine									Histidine	Histidine
119	Cysteine								Arginine		
153	Proline								Glutamine		
180	Threonine								Alanine		
194	Proline						Leucine	Leucine			
209	Leucine					Proline	Proline				
217	Proline			Alanine					Alanine		
263	Glutamate						Glycine				
277	Leucine								Proline		
283	Alanine			Valine							
286	Phenyl alanine					Leucine					
313	Leucine					Tryptophan					
320	Isoleucine			Threonine							
326	Threonine									Isoleucine	Isoleucine
339	Proline					Threonine					

mMDV- mild Marek's Disease Virus; vMDV- virulent Marek's Disease Virus; vvMDV- very virulent Marek's Disease Virus; vv+MDV- very virulent plus Marek's Disease Virus.
sMeq, vsMeq – Meq protein in MDV transformed cell lines (MSB1 and MTB1).

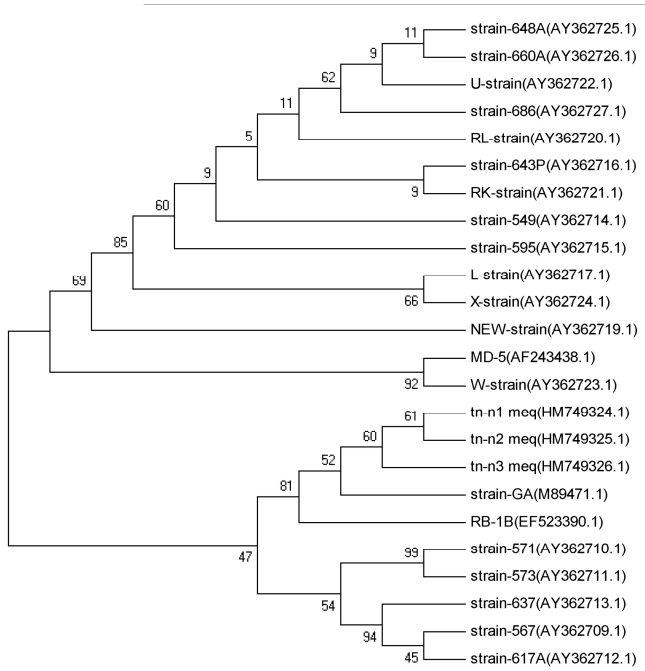
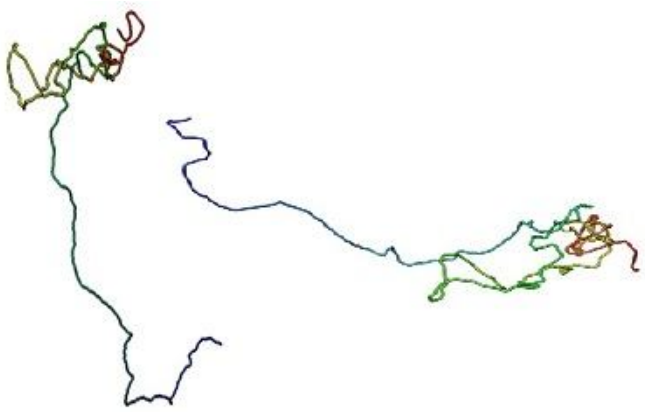


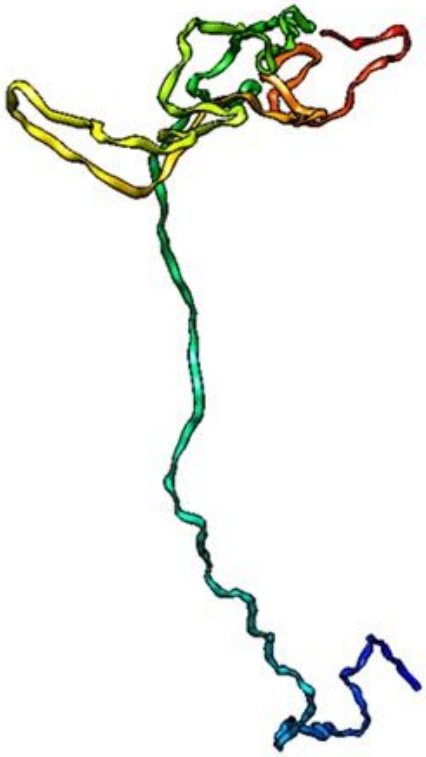
Figure 1. Phylogenetic analysis of TN (India) strains with MDV reference strains available in the NCBI database-*Meq gene* sequence (1020 bp) by Neighbor joining method using Tamura-nei statistical model. (bootstrap samples n=1000).

Phylogenetic analysis

Phylogenetic analysis of the TN (India) strains using rooted Neighbour joining method and statistical models of Tajima Nei and tamura, formed clusters with thefirst showing a minimal evolutionary strains of vvMDV, however, a cluster with the boot strap value of 81 showed the tn-n strains and GA strain formed a close group with vvMDV RB-1B strain (Fig. 1).The TN strains showed the closest relationship with RB-1B strain of which tn-n3 being the closest followed by tn-n2 and tn-n1. The bootstrapping values are shown on the node of bifurcation.



(A) **(B)**



(C)

Figure 2. MEQ protein structure models of (A) 648A strain (vv+), (B) RB-1B strain (vv), (C) tn-n3 strain using 1mv3 (c-myc) as template alignment in mGen Threader online software by modeller9.10 difficult method.

Colour range	: based on B-factor (stability)	
B-factor value-3	------	BLUE
B-factor value-30	-------	RED
B-factor value-50	-------	YELLOW

Protein structure modeling and evaluation

The best fit model for each MDV strains were selected based on minimum DOPE score and maximum GA341 score (DOPE=-10725.44922; GA341=0.00043 for 648A Meq protein, DOPE=-12297.21592; GA341=0.00005 for RB-1B Meq protein, DOPE=-9811.44824; GA341=0.00076 for TN-N3 Meq protein) (Fig. 2). UCSF chimera 1.8 software [28] was used for forming protein images. Geometrical analysis of protein structure models was done using PROCHECK software. PROSA software was used for Z score statistical analysis and energy distribution of protein structure models. The results are listed in table 2.

Even though the emerging newer vaccines have proven extremely efficacious in controlling the disease, such prophylaxis doesn't hinder MDV evolution in the closely knit poultry population [29]. HVT and bivalent vaccines (HVT and MDV-2) are the common vaccination programme in Namakkal poultry farms. The reemergence of MDV outbreak in vaccinated flocks [30] clearly imply the prevalence of vvMDV strains through molecular evolution of *meq* gene sequence that was confirmed by bioinformatics analysis. Sequence analysis of predominant MDV strains of Chinese origin was found to form a separate clade to the MDV reference strains by *meq* gene (1020 bp) analysis [31]. But, from our study based on the phylogenetic analysis [32], it was evident that the TN (India) strains formed the clade within the vvMDV reference strains.

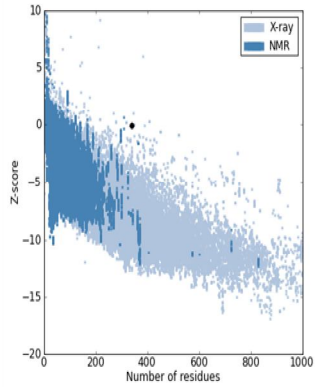
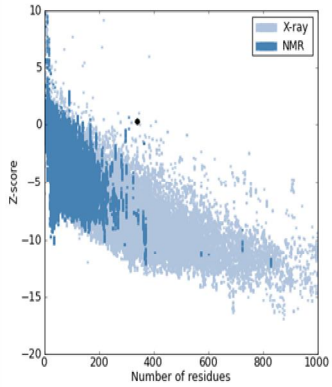
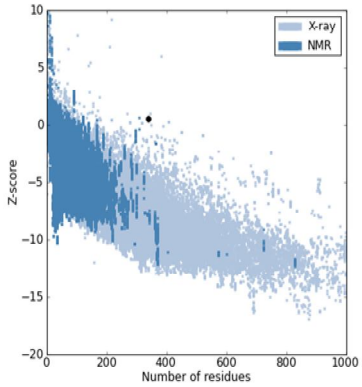
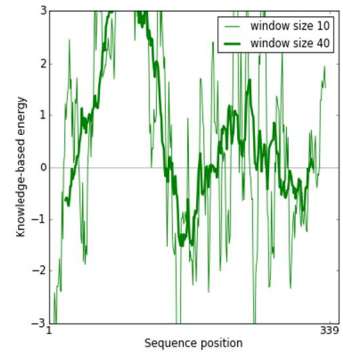
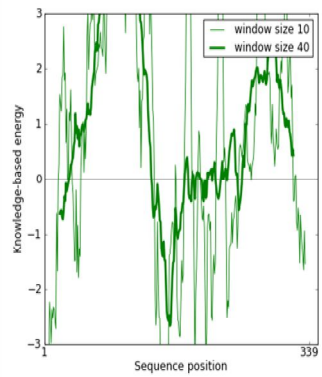
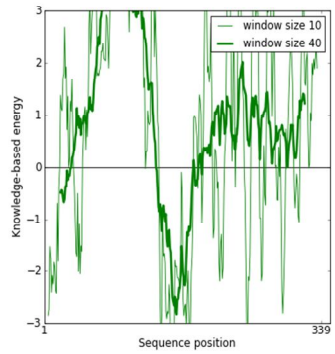
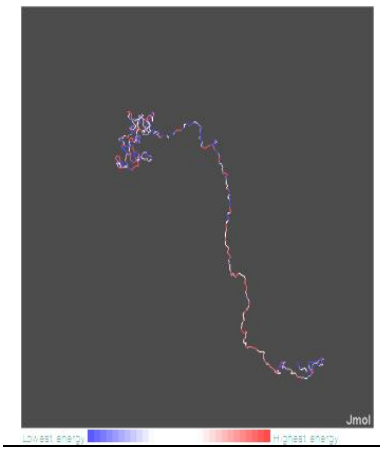
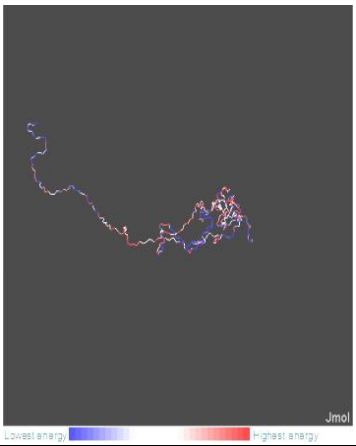
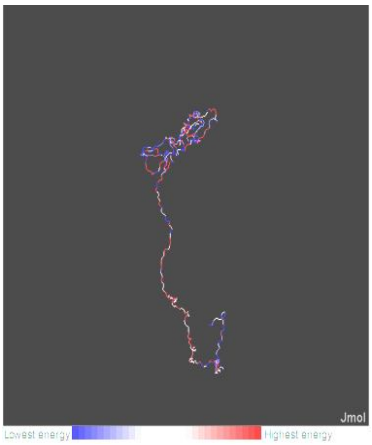
Meq suppresses pp38/pp14 bidirectional promoter (latency) but activates MDV-1 gB late promoter which might promote virus replication after latency (reactivation). Thus, the role of Meq in the biology of MDV-1 is very complex and Meq may promote latency or reactivation depending upon its dimerization partner, phosphorylation status, level of expression of splice variants, and microRNAs [15]. Amino acid residues at positions 71st and 320th of the very virulent Md5 Meq protein (alanine, threonine instead of serine, isoleucine respectively) were found to be important for transactivation of the Meq promoter comparing with CVI988 vaccine strain [33]. Even though TN strains showed variations at 71st amino acid in like Md5 strain, there was no variation observed at 320th position which was similar to the very virulent RB-1B strain.

All the three models generated showed sequence similarity above 80% in the most favoured regions of the Meq protein. On comparison with NMR and X ray diffraction data of related models, all the three generated models showed the Z-score just above the normal (-0.22, 0.25, 0.02). Also, the energy distribution showed low energy (0-50, 125-339) except for 50-125 amino acids region which is responsible in the stimulation of bZIP basic leucine zipper domain. However, similar protein structure 1H8A with the bZIP domain showed low energy distribution. Multiple sequence alignment study using CLUSTALW 2 of Meq protein showed amino acid variations between positions 59-111 and this area was found to exhibit a high energy distribution compared to other regions in the protein. This unstable basic region could correspond to nuclear localization function of the protein. On the contrary, the amino acids spanning positions 130-339 residues showed low energy distribution and this domain could be involved in transformation/transactivation function of the protein. This stable C-terminal low energy distribution domain is important in molecular pathogenesis of the virus facilitating tumour formation this is in consonant with earlier reports [10]. Based on structural evaluation, MEQ protein from the 648A strain showed higher stability and low energy level (Z-test, energy distribution) comparing with other 2 structures.

Emergence of new pathotypes that can break through the protective efficacy of the widely used CVI988 (Rispens) vaccine will have serious effects on the poultry industry worldwide. Hence the major effort in the immediate future will be towards the development of new generations of vaccines that can stop evolutionary trend of MDV [2]. Comparative to CVI988, Md-5 strain (vvMDV) showed the amino acid variations in 71, 77, 217, 283, 320 and 326 positions [33]. Comparative to vMDV (GA strain), Md-5 strain (vvMDV) showed the amino acid variations in 217, 283, 320. RB-1B strain (vvMDV) showed no amino acid variations with vMDV (GA strain). Comparative to closely related

RB-1B strain, tn-n1, tn-n2, tn-n3 showed variations in 209, 286, 313, 339 amino acid positions, 194, 209, 263 amino acid positions, 194th amino acid position respectively. vv+MDV (648A strain) showed variation in 119, 153, 180, 277, 283, 320 amino acid positions with vvMDV (Md-5 strain) and 119, 153, 180, 217, and 277 amino acid positions with vMDV (GA strain). These amino acid positions are driven forces to predict active motifs both *in vivo* and *in vitro* experimental analysis. *In vitro* s-meq, vs-meq already submitted in NCBI database also showed amino acid variations in 70, 100, and 326 amino acid positions with vMDV (GA strain) need to be considered in experiment.

Table 2. Evaluation results of modeled protein structures by *PROSA* and *PROCHECK* softwares

648A	RB-1B	TN-N3
Residues in most favoured region 85.1% (RAMACHANDRAN PLOT)	Residues in most favoured region 84.6% (RAMACHANDRAN PLOT)	Residues in most favoured region 84.3% (RAMACHANDRAN PLOT)
		
Z score: -0.22	Z score: 0.25	Z score: 0.02
		
ENERGY distribution of protein structure models		
		

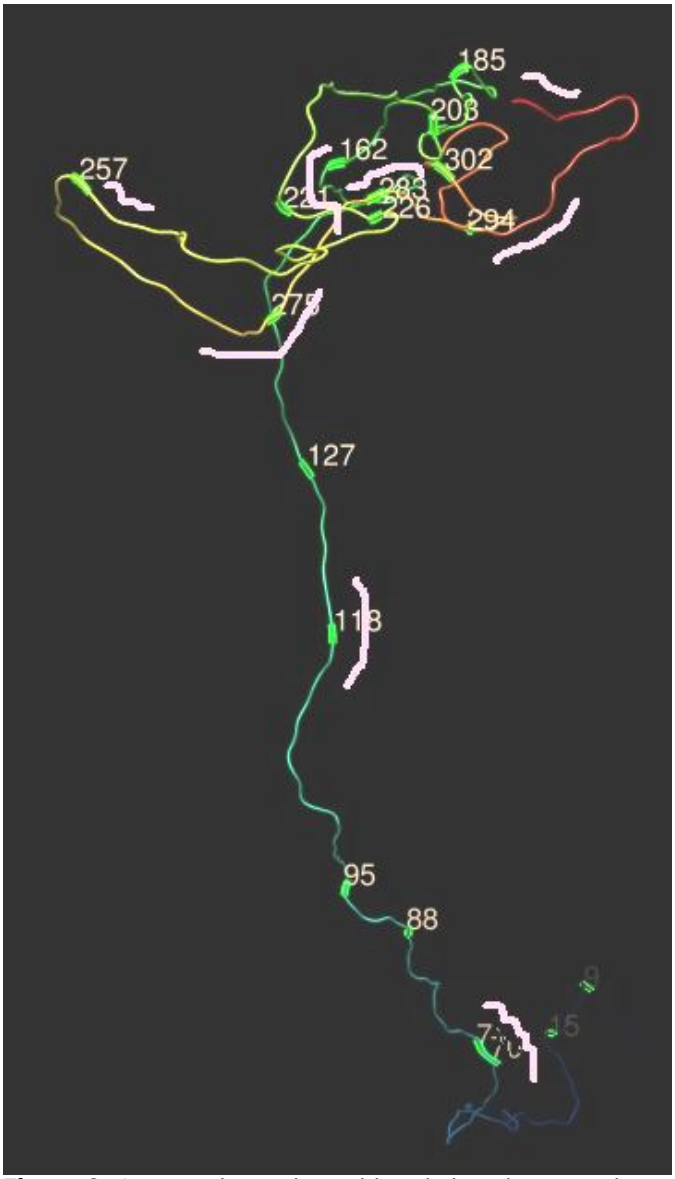


Figure 3. Comparative amino acid variations between the strains were used to predict active motifs (violet colour lines) (numbers represent alanine amino acid residues in protein structure for indication)

CONCLUSION

The variation in amino acid residues of the Meq protein domains of Marek's Disease Virus (MDV) studied were analysed for epitope prediction using SYFPEITHI software [34] in order to arrive at a suitable epitope vaccine candidates. In recent years, *in silico* structure based epitopes were predicted by using programs like Discotope, Seppa, Epitopia, BEPro, and Ellipro with analyzing specificity and sensitivity algorithms [35]. However, both *in vivo* and *in vitro* studies are needed in the future to evaluate efficiency of such epitope vaccine candidates. As a model of virus induced oncogenesis, MD continues to provide excellent insights into various molecular mechanisms of neoplastic transformation. This molecular modeling will not only open up new areas and scenarios in prevention of oncogenic MDV but also openup new dimensions in tumour research because MDV is considered as an efficient virus induced tumour model.

REFERENCES

1. Osterrieder N, Kamil JP, Schumacher D, *et al.* (2006). Marek's disease virus: from miasma to model. *Nat. Rev. Microbiol.* 4:283-294.
2. Biggs, PM and Nair V (2012). The long view: 40 years of Marek's disease research and AvianPathology. *Avian pathol.* 41(1):3-9.
3. Witter RL (1997). Increased virulence of Marek's disease virus field isolates. *Avian Dis*, 41:149.
4. Schat, K.A., B. W. Calnek and J. Fabricant (1982). Characterization of two highly oncogenic strains of Marek's disease virus. *Avian Pathol.* 11:593–605.
5. Gimeno IM (2008). Marek's disease vaccines: A solution for today but a worry for tomorrow? *Vaccine*, 26S:C31-C41.
6. Spatz, SJ, Volkening JD, Gimeno IM et al. (2012). Dynamic equilibrium of Marek's disease genomes during in vitro serial passage. *Virus Genes.* 45(3):526-536.
7. Tulman, E. R., Afonso, C. L., Lu, Z., *et al.* (2000).The genome of a very virulent Marek's disease virus. *J Virol.* 74:7980–7988.
8. Izumiya Y, Jang HK, Ono M, *et al.* **(**2001**)**. A complete genomic DNA sequence of Marek's disease virus type 2, strain HPRS24. *Current Topics in Microbiology and Immunology.* 255:191-221.
9. Afonso CL, Tulman ER, Lu Z, *et al.* (2001). The genome of turkey herpesvirus. *J virol.*75:971-978.
10. Lupiani B, Lee L.F, Cui X, GimenoI,Anderson A, *et al.* (2004). Marek's disease virus encoided *Meq* gene is involved in transformation of lymphocytes but is dispensable for replication. PNAS 101(32): 11815-11820.
11. Shamblin CE, Greene N, Arumagaswami V, *et al.* (2004). Comparative analysis of Marek's disease virus (MDV) glycoprotein-, lytic antigen pp38- and transformation antigen *Meq*-encoding genes: association of meq mutations with MDVs of high virulence. *Vet Microbiol.* 102:147-167.
12. Lee LF, Kreager K, Heidari M, et *al.* (2013). Properties of a *meq*-Deleted rMd5 Marek's Disease Vaccine: Protection Against Virulent MDV Challenge and Induction of Lymphoid Organ Atrophy Are Simultaneously Attenuated by Serial Passage *In Vitro*. *Avian Dis.* 57(2s1): 491-497.
13. Liu JL, Lee LF, Ye Y, *et al.* (1997). Nucleolar and nuclear localization properties of a herpesvirus bZIP oncoprotein MEQ. *J Virol.* 71(4):3188-3196.
14. Liu JL, Ye Y, Qian Z, *et al.* (1999). Functional interactions between herpesvirus oncoprotein MEQ and cell cycle regulator CDK2. *J Virol.* 73(5):4208-4219.
15. Levy AM, Izumiya Y, Brunovskis P, *et al.* (2003). Characterization of the chromosomal binding sites and dimerization patterns of the viral oncoprotein Meq in Marek's disease virus transformed T cells. *J. Virol.* 77(23):12841-12851.
16. Levy AM, Gliad O, Xia L, Izumiya *et al.* (2005). Marek's disease virus Meq transforms chicken cells via the v-Jun s transcriptional cascade: a converging transforming pathway for avian oncoviruses. *Proc. Natl. Acad. Sci. U.S.A.* 102(41):14831-14836.
17. Brown AC, Baigent SJ, Smith LP, *et al.* (2006). Interaction of MEQ protein and C-terminal-binding protein is critical for induction of lymphomas by Marek's disease virus. *Proc. Natl. Acad. Sci. U.S.A.* 103(6):1687-1692.
18. Nair V and Kung HJ: Marek's disease virus oncogenicity: molecular mechanisms. In Davison F and Nair V (Ed), Marek's disease: an evolving problem, Elsevier publication, 2004. p.32-48.
19. Burnside, J., Bernberg, E., Anderson, A., *et al.* (2006). Marek's disease virus encodes microRNAs that map to meq and the latency-associated transcript. *J Virol.* 80, 8778–8786.
20. Stik G, Dambrine G, Pfeffer S, *et al.* (2013). The Oncogenic MicroRNA OncomiR-21 overexpressed during Marek's diseae Lymphomagenesis is transactivated by the viral oncoprotein Meq. *J Virol.* 87(1):80-93.
21. Sathish Gopal, Parthiban Manoharan, Kumanan Kathaperumal, *et al.* (2012). Differential detection of avian oncogenic viruses in poultry layer farms and turkeys by use of multiplex PCR. *J. Clin. Microbiol,* 50(8):2668-2673.
22. Murata S, Chang KS, Lee SI, *et al.* (2007). Development of a nested polymerase chain reaction method to detect oncogenic Marek's disease virus from feather tips. *Journal of Veterinary Diagnostic Investigation* 19, 471-478.

23. Divya KC, Parthiban M, Sathish G, *et al.* (2011). Detection of oncogenic strains of MDV serotype 1 in Tamil Nadu by duplex PCR. *Indian J Anim Sci.* 81(1): 6-8.
24. Pineda-Lucena A, Ho CS, Mao DY, *et al*. (2005). A structure-based model of the c-Myc/Bin1 protein interaction shows alternative splicing of Bin1 and c-Myc phosphorylation are key binding determinants. *J Mol Biol.* 351(1):182-94.
25. Wiederstein M and Sippl M J (2007).ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic acid Res.* 35:407-410.
26. Laskowski RA, MacArthur MW, Moss DS, *et al.* (1993). PROCHECK - a program to check the stereochemical quality of protein structures. *Journal of Applied Crystallography*, 26, 283-291.
27. Hutchinson E G and Thornton J M (1996). PROMOTIF: A program to identify and analyze structural motifs in proteins. *Protein Sci.* 5:212-220.
28. Pettersen EF, Goddard TD, Huang CC, et al. (2004). UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem.* 25(13):1605-12.
29. Sridhar A, Shoba K, Saminathan P, *et al*. (1997). Pattern of mortality in commercial layer farms at Namakkal. *Ind. Vet. J.* 74:996-997.
30. Balachandran C, Pazhanivel N, Vairamuthu S, *et al*. (2009). Marek's Disease and Lymphoid Leucosis in chicken – A histopathological survey. *Tamilnadu J. Veterinary & Animal Sciences.*5:167-170.
31. Zhang Y, Liu C, Zhang F, *et al.* (2011). Sequence analysis of the *Meq* gene in the predominant Marek's disease virus strains isolated in china during 2006-2008. *Virus Genes*, 43:353-357.
32. Hassanin O, Abdallah F and. El-Araby IE, (2013). Molecular Characterization and Phylogenetic Analysis of Marek's Disease Virus from Clinical Cases of Marek's Disease in Egypt. *Avian Dis.* 57:555–561.
33. Ajithdoss DK, Reddy SM, Suchodolski PF, *et al.* (2009). In vitro characterization of the Meq proteins of Marek's disease virus vaccine strain CVI988. *Virus Research,* 142:57-67.
34. Rammensee H, Bachmann J, Emmerich NN, *et al.* (1999). SYFPEITHI: database for MHC ligands and peptide motifs. *Immunogenetics*, 50: 213-219.
35. Borley DW, Mahapatra M, Paton DJ, *et al*. (2013). Evaluation and use of *In-Silico* structure based epitope prediction with Foot and Mouth Disease Virus. *Plos one.* 8(5):1-14.

*****