Journal of Molecular Graphics and Modelling 80 (2018) 52–58

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM

Exploring the association of rs10490924 polymorphism with age-related macular degeneration: An *in silico* approach

Farhad Jahanfar[a], Hamed Hamishehkar[b,*]

[a] *Biotechnology Research Center and Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran*
[b] *Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

ARTICLE INFO

Article history:
Available online 4 January 2018

Keywords:
ARMS2
Age-related macular degeneration
Rs10490924
In silico
Molecular dynamics
Ab initio

ABSTRACT

The polymorphism rs10490924 (A69S) in the age-related maculopathy susceptibility 2 (ARMS2) gene is highly associated with age-related macular degeneration, which is the leading cause of blindness among the elderly population. ARMS2 gene encodes a putative small (11 kDa) protein, which the function and localization of the ARMS2 protein remain under debate. For a better understanding of functional impacts of A69S mutation, we performed a detailed analysis of an ARMS2 sequence with a broad set of bioinformatics tools. *In silico* analysis was followed to predict the tertiary structure, putative binding site regions, and binding site residues. Also, the effects of this mutation on protein stability, aggregation propensity, and homodimerization were analyzed. Next, a molecular dynamic simulation was carried out to understand the dynamic behavior of wild-type, A69S, and phosphorylated A69S structures. The results showed alterations in the putative post-translational modification sites on the ARMS2 protein, due to the mutation. Furthermore, the stability of protein and putative homodimer conformations were affected by the mutation. Molecular dynamic simulation results revealed that A69S mutation enhances the rigidity of the ARMS2 structure and residue serine at position 69 is buried and may not be phosphorylated; however, phosphorylated serine enhances the flexibility of the ARMS2 structure. In conclusion, our study provides new insights into the deleterious effects of A69S mutation on the ARMS2 structure.

1. Introduction

Age-related Macular Degeneration (AMD) is the leading cause of irreversible blindness in the elderly population [1]. Due to the growing number of older population, the prevalence of individuals with AMD is estimated to increase to approximately 196 million by 2020 and 288 million by 2040 [2]. AMD is a multifactorial disease associated with various genetic variants and environmental risk factors and mainly affects the photoreceptors, retinal pigment epithelium, Bruch's membrane, and choriocapillaris. Considering the list of environmental factors, aging and cigarette smoking are regarded as the most significant risks for AMD [3]. Several genetic linkage studies have identified numerous genetic variants [4–6].

Five highly correlated genetic variants, namely rs1061170 and rs800292 in the complement factor H (CFH) gene, rs11200638 in the HtrA serine peptidase 1 (HTRA1) promoter, rs2230199 in the complement component 3 (C3) gene, and rs10490924 in ARMS2 gene have indicated the strongest association with AMD. The rs10490924 (G/T) SNP is responsible for nonsynonymous alteration of the amino acid from alanine (A) to serine (S) at position 69. In this regard, substitution creates a putative phosphorylation site [7]. While CFH, C3, and HTRA1 are considered as highly conserved proteins, ARMS2 is regarded as an evolutionary new gene and only presents in human, Old World monkeys, and apes. ARMS2 gene has two exons encoding a putative protein of 107 amino acids. Moreover, mRNA transcript of ARMS2 was detected in human placental, retinal pigment epithelial cells, and neural retinal tissues [8].

Structural, functional, and biological properties of the ARMS2 protein are still unknown. In this connection, Kanda et al. (2007) and Fritsche et al. (2008) proposed that ARMS2 is localized in the mitochondrial outer membrane and addressed the functional role of ARMS2 protein in mitochondrial homeostasis. Based on these studies, the mitochondrial pathway is regarded as the underlying mechanism of ARSM2 in the pathogenesis of AMD [7,9]. In contrast, Wang et al. (2009) claimed that ARMS2 is mainly localized in the cytosol [10] while Kortvely et al. (2010) suggested that ARMS2 is localized in the extracellular matrix (ECM), where it interacts with ECM proteins [11]. Furthermore, the relationship between ARMS2

DOI of original article: http://dx.doi.org/10.1016/j.jmgm.2017.09.003.
* Corresponding author.
E-mail addresses: hamishehkarh@tbzmed.ac.ir, hamishehkar.hamed@gmail.com (H. Hamishehkar).

https://doi.org/10.1016/j.jmgm.2017.12.023
1093-3263/

and inflammatory mechanisms was reported to be another underlying mechanism for the pathogenesis of AMD [12]. Moreover, rs10490924 and rs11200638 are in a strong linkage disequilibrium, which makes it hard to distinguish the effects of each SNPs on AMD progression [3,13]. Despite the conducted studies, most aspects of ARMS2 protein remain unclear.

In the present study, the structure of ARMS2 was generated using *ab initio* modeling, and then a compilation of the computational tools was employed to study ARMS2 sequence and examine the effects of A69S mutation on protein post-translational modifications, stability, aggregation, and homodimerization. Finally, comparative molecular dynamics simulations were performed to study the possible structural effects on ARMS2 protein resulted from A69S mutation and also from phosphorylation of Ser69 in A69S model. The findings of this study may open new horizons to personalized medicine and drug discovery in AMD therapy and may present interpretations of the underlying AMD progression mechanisms.

2. Methods

2.1. Sequence analysis

The ARMS2 sequence was sourced from National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/protein/). Phosphorylation, sumoylation, ubiquitylation, and glycosylation sites of ARMS2 protein were predicted by NetPhos [14], SUMOplot (http://www.abgent.com/sumoplot), Ubpred [15], NetGlycate [16], NetOGlyc [17], and GlycoEP [18] servers, respectively. To gain new insight into ARMS2 function, protein motif and domain predictions were obtained using Eukaryotic Linear Motif (ELM) resource [19].

2.2. Protein structure prediction

The secondary and tertiary structures of the ARMS2 protein were constructed using the QUARK server (http://zhanglab.ccmb.med.umich.edu/QUARK/) [20]. QUARK 3D structures were constructed from the amino acid sequence using the template free "*ab initio*" folding method. Furthermore, QUARK was specifically designed to predict the structure of proteins containing with less than 200 amino acids. QUARK provided 10 models of the 3D structure, which were refined using ModRefiner [21]. All the models were analyzed using Procheck-Ramachandran plot [22], Verify3D [23], and ProSA [24] programs. The best 3D model was selected by evaluating the 3D profile, energetic feature, and stereochemical quality using the Verify3D program, ProSA program, and Ramachandran plot, respectively.

2.3. Identification of binding sites using FTSite server

To investigate the relationship between mutation and putative binding sites, the FTSite server was used to predict the binding sites of the ARMS2 protein. FTSite could precisely identify the binding sites with an experimental accuracy of 94% [25]. The protein data bank (PDB) file of wild-type ARMS2 model was given as input to the FTSite server. The figure was prepared using PyMol software (http://pymol.sourceforge.net/).

2.4. The effects of mutation on protein stability, aggregation, and homodimerization

To analyze the destabilizing effects of A69S mutation on protein stability, mCSM-Stability [26], SDM [27], DUET [28], and I-Mutant 2.0 [29] servers were used. In this regard, the changes in thermodynamic stability ($\Delta\Delta G$) determine the effect of the mutation. If A69S mutation destabilizes ARMS2 protein, the $\Delta\Delta G$ value would be negative while stabilization increases the $\Delta\Delta G$ value. As the number of mutations promotes the aggregation when they destabilize the native state of the protein, Aggrescan [30], Aggrescan 3D [31], and Tango [32] servers were used to analyze the effects of mutation on ARMS2 aggregation. Furthermore, proteins often assemble into multimeric structures to perform specific biological functions and mutations could affect these structures. Hence, M-ZDOCK server [33] was used to find possible homodimer states of ARMS2 and determine how mutation and phosphorylation could affect the homodimers using a rigid-body docking. The PDB files of wild-type, A69S, and phosphorylated A69S (A69SP2) models of ARMS2 were imported to the M-ZDOCK server. Conformation of the complex protein with the highest ZDOCK score was considered and the associated figures were prepared using PyMol software (http://pymol.sourceforge.net/).

2.5. Molecular dynamics (MD) simulation

The best 3D model of the ARMS2 protein was subjected to molecular dynamics (MD) simulation using Nanoscale Molecular Dynamics (NAMD) [34] version 2.11 and CHARMM22 force field with the φ/Ψ angle cross-term map (CMAP) correction [35]. Mutation of alanine to serine at position 69 was performed using the Visual molecular dynamics (VMD) plugin mutator [36]. Then, dianionic phosphorylation of serine was modeled using the SP2 phosphoserine patch [37] in VMD psfgen. Each of the wild-type ARMS2, mutated A69S, and phosphorylated mutant models were solvated in transferable intermolecular potential with 3 points (TIP3P) water box with 14 Å (Angstrom) padding in all directions. Each system was neutralized by adding 150 millimolar (mM) of sodium chloride (NaCl). A 10,000-step minimization was performed at 0 K (K), followed by heating each system to 310 K in 80,000 steps. Then, each system was equilibrated at 310 K and 1 atmosphere (atm) for 2.5 nanosecond (ns). Finally, production MD simulations were carried out for 5 ns at 310 K for all systems. All simulations were performed under the constant-number, constant-pressure and constant-temperature (NPT) ensemble with a time step of 2.0 femtosecond (fs). Periodic boundary conditions were used, and the long-range electrostatic interactions were treated by the particle mesh Ewald method [38]. Besides, the short range van der Waals interactions were adjusted at 10 A°. Next, the SHAKE algorithm [39] was applied to constrain the bonds of all hydrogen atoms. All trajectories were stored every 1 ps for further analysis. Analyses of MD trajectories were performed using VMD, its plugins, and Excel 2007. The root mean square deviation (RMSD), root mean square fluctuation (RMSF), solvent accessible surface area (SASA), radius of gyration (Rg), salt bridges, and hydrogen bond variations were determined for all simulations.

3. Results and discussion

3.1. Sequence analysis

As presented in Fig. 1, analysis of ARMS2 sequence reveals the presence of 15 putative phosphorylation sites according to NetPhos. The predicted number is more than nine phosphorylation sites, which were previously predicted [7]. Prediction of glycation sites using NetGlycate and NetOGlyc indicated four putative glycation sites of ε-amino groups of lysines and two putative O-linked glycation sites, respectively. However, applying GlycoEP-O with the prediction based on position-specific scoring matrix profile of patterns (PPP) and default threshold, seven putative O-linked glycation sites were predicted. Moreover, one site was predicted with SUMOplot to take into account the possible chance of sumoylation.