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Full Length Research Paper

Structural analysis of deafness associated E216V mutation in human myosin VI protein

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Deafness is the most common form of sensory impairment in humans. Several mutations have been reported in the myosin VI proteins that are responsible for autosomal dominant and recessive deafness. One such missense mutation is that which changes glutamate (E) to valine (V) at amino acid 216 (E216V). The mutation resides in the myosin VI head domain; however, detailed insights regarding the impact of this mutation on structural stability are not known. To characterize the potential impact of this mutation on protein stability and its association with deafness, we performed computational sequence and structural analysis of normal and mutated myosin VI. Overall, protein stability declined when the mutation was introduced, as predicted by I-mutant and Cologne University Protein Stability Analysis Tool (CUPSAT). Similarly, a decline in hydrophobicity around position 216 was observed. Phosphorylation of myosin VI was also observed to be influenced by the E216V mutation. Furthermore, a three-dimensional structure of myosin VI was studied using Swiss-PdbViewer to identify structural variations in the protein that may occur as a result of the E216V mutation. Distortion in the hydrogen bonding between Val216 and Ser449 in the mutated protein structure was observed, exposing the Ser449 hydroxyl group and reducing overall protein stability.

Key words: Myosin VI, genetics, deafness, hereditary mutations, structure analysis.

INTRODUCTION

Deafness is the most common form of sensory impairment in humans. Mutations in unconventional myosins cause deafness in humans and mice. The mouse recessive deafness mutation, Snell's waltzer, contains an intragenic deletion in an unconventional myosin, myosin VI (locus designation, Myo6). The requirement of Myo6 for proper hearing in mice makes this gene an excellent candidate for a human deafness disorder (Avraham et al., 1997). The structure and development of the inner ear and the pathology leading to hearing impairment are very similar between mouse and human (Steel and Brown, 1994). The expression of the *MYO6* gene in the human fetal cochlea demonstrates the importance of myosin VI in the mammalian inner ear and supports a potential role in human inner ear pathology. Within hair cells, myosin VI protein is enriched in the cuticular plate, an actin-rich structure at the base of the stereocilia. To carry out mechanical transduction, the hair cell consumes an actin-rich hair bundle made of stereocilia, which is inserted rigidly into the cuticular plate. The arrangement and number of stereocilia at the surface of the hair cell are very precise and are formed in a series of steps to generate a complex actin cytoskeleton (Tilney et al., 1992).

Myosin VI is encoded by the mouse Snell's waltzer deafness gene. The protein is thought to be necessary for anchoring the stereocilia membrane in the cuticular plate of the inner ear (Avraham et al., 1995; Steel and Brown, 1994). This region is active in transporting vesicles to the apical surface of cells. It seems that without myosin VI, the structural integrity of hair cells is lost, leading to hearing loss. The connection between hearing loss and myosin VI has been observed in humans as well, where patients suffering from a non-

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syndromic dominant form of deafness were discovered to have a missense mutation in the myosin VI gene, MYO6 (Self et al., 1999). Previously, a missense mutation glutamate (E) to valine (V) at position 216 in myosin VI protein had been reported; however, the functional significance and potential impact of this mutation on the structure of myosin VI protein is not yet known (Ahmed et al., 2003). In this study, we pursued a bioinformatics approach to perform the sequence and structural analysis of human myosin VI protein. Our goal was to characterize the potential impact of E216V mutation on the three-dimensional (3D) structure of this multi-functional protein that may play role in the onset and progression of hereditary deafness.

MATERIALS AND METHODS

Sequences

The amino acid sequence of human myosin VI protein was retrieved from the National Center for Biotechnology Information (NCBI) database using the primary accession number NP_004990.3. A BLASTP (Altschul et al., 1997) sequence homology search was performed using the National Center for Biotechnology Information (NCBI) database with default parameters for all known sequences. The sequences selected from various organisms were based on higher bits score, and E-values ≤0. The selected myosin VI protein target sequences originated from *Mus musculus* (NP_001034635.2), *Sus scrofa* (NP_999186.1), *Bos taurus* (NP_001193001.1), *Macaca mulatta* (NP_001098006.1), *Gallus gallus* (NP_990066.1), and *Danio rerio* (NP_001004110.1). The selected sequences, including the query, were aligned using ClustalW2 (Larkin et al., 2007) and alignment visualization, calculation of conserved residues, and secondary structure analysis was performed using Jalview (Waterhouse et al., 2009). The FASTA sequence of human myosin VI containing the E216V mutation was generated using MUTATER (Butt and Ahmed, 2009).

Stability changes in human myosin VI

We employed both sequence and structural level approaches to assess the impact of the E216V mutation. For this purpose, two different algorithms were used to predict the change in protein stability. I-mutant (Capriotti et al., 2005) was used to calculate protein stability based on protein sequence. Results were obtained via email and analyzed further. To calculate the effect of the E216V mutation on protein structural stability, we used the Cologne University Protein Stability Analysis Tool (CUPSAT) (Parthiban et al., 2006).

Phosphorylation and hydrophobicity profile of human myosin VI

NetPhos 2.0 was used to predict the phosphorylation potential of wild-type and mutated human myosin VI (Blom et al., 1999). This program calculates a score for potential phosphorylation sites on each Ser, Thr, and Tyr residue. It uses a threshold value of 0.5 for any Ser, Thr, and Tyr to indicate a potential phosphorylation site. Potential changes in overall hydrophobicity due to E216V were calculated by the Kyte and Doolittle (1982) method via BioEdit v7.0.9 (Hall, 1999).

Three-dimensional structural analysis of human myosin VI

A Basic Local Alignment Search Tool Programme (BLASTp) search for protein sequences was performed using the NCBI database to identify experimentally determined 3D structures of human myosin VI. We found that the 3D structure of human myosin VI has not been published. The closest match came from the myosin VI protein of *S. scrofa* (PDB id: 2bki). The sequence identity between human and *S. scrofa* was 843/858 (98%). Sequence alignment between human and *S. scrofa* showed that E216 is a conserved residue. We therefore used this structure to analyze protein stability changes using Swissprot-PdbViewer (Deepviewer) (Guex and Peitsch, 1997). Wild-type human myosin VI was uploaded and compared with the myosin VI *S. Scrofa* PDB file. The sequence and structure were superimposed, and the E216V mutation was introduced into the 3D structure. Comparative analysis of normal and mutated structures was then performed to search for possible changes in bond orientations among amino acids and any overall change in 3D structure.

RESULTS AND DISCUSSION

Recent studies have reported that myosin VI has an important role in hearing loss in animals as well as humans. Two mutations, 36-37insT in the motor domain, and R1166X in the globular tail domain, constitute genetic evidence of mutations that disable the *MYO6* gene and cause autosomal recessive deafness in humans. In addition to these mutations, there is another reported mutation, E216V, in the motor domain of the myosin VI protein. This mutation was first identified in a Pakistani family (Ahmed et al., 2003) but it has not been directly linked to deafness. Therefore, the focus of our study was to determine whether this missense mutation contributes to deafness as a result of decreased structural stability of the myosin VI protein. For this purpose, we employed several bioinformatics tools and various structural parameters were calculated. We first performed multiple sequence alignment between closely related species to see if E216 is a conserved residue. This analysis shows that Glu 216 is common among closely related species. No species contained a Val at this position (Figure 1). We then used a sequence- and structure-based approach to predict protein stability following introduction of the E216V mutation. The analysis was performed by I-mutant and CUPSAT, and the combined results suggest structural instability, an unfavorable torsion angle, and a decrease in energy level (Tables 1 and 2).

In parallel to this approach, comparison of the hydrophobicity of normal and mutated protein sequences showed a decrease in the overall hydrophobicity at the site of mutation, suggesting the protein structure unstability (Figure 2). Intermolecular hydrogen bonds (H-bonds) provide an important contribution to protein stability (Rose et al., 2006). The strength of hydrogen bonds depends on the environment. H-bonds enveloped in a hydrophobic core contribute more than H-bonds exposed to the aqueous environment and to the stability of the native state (Deechongkit et al., 2004). We were

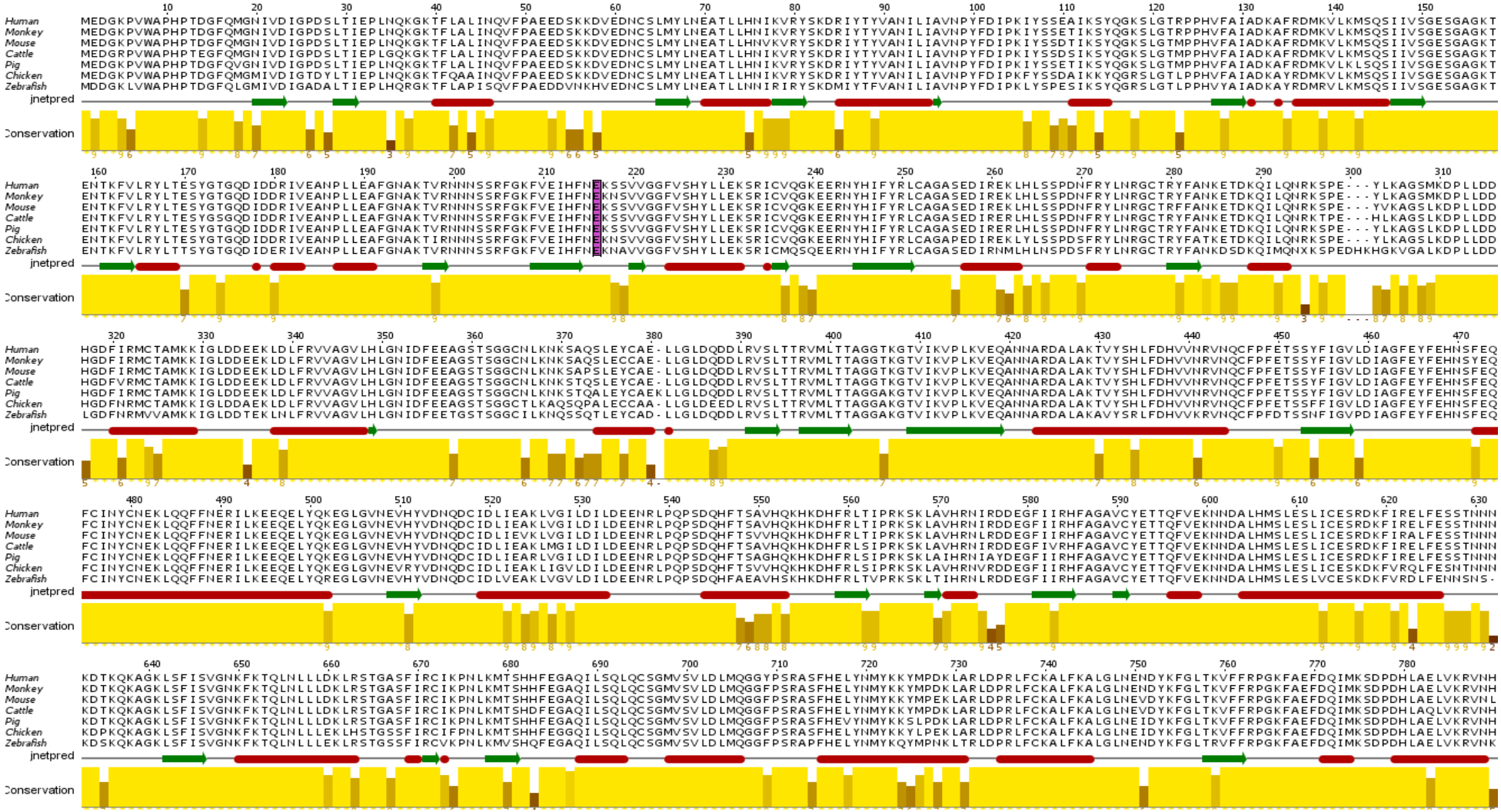

Figure 1. Multiple sequence alignment and secondary structure analysis of the myosin VI protein from humans and closely related species.

able to clearly identify structural distortions due to the E216V mutation. The E216V substitution affects Ser 449. Under normal conditions, two hydrogen bonds are present between Glu216 and Ser449, as inferred from the myosin VI protein structure (Figure 3). However, after introducing the E216V mutation, one of the two hydrogen bonds was broken (Figure 4), since the Glu and Val side chains are not identical. Val was able to

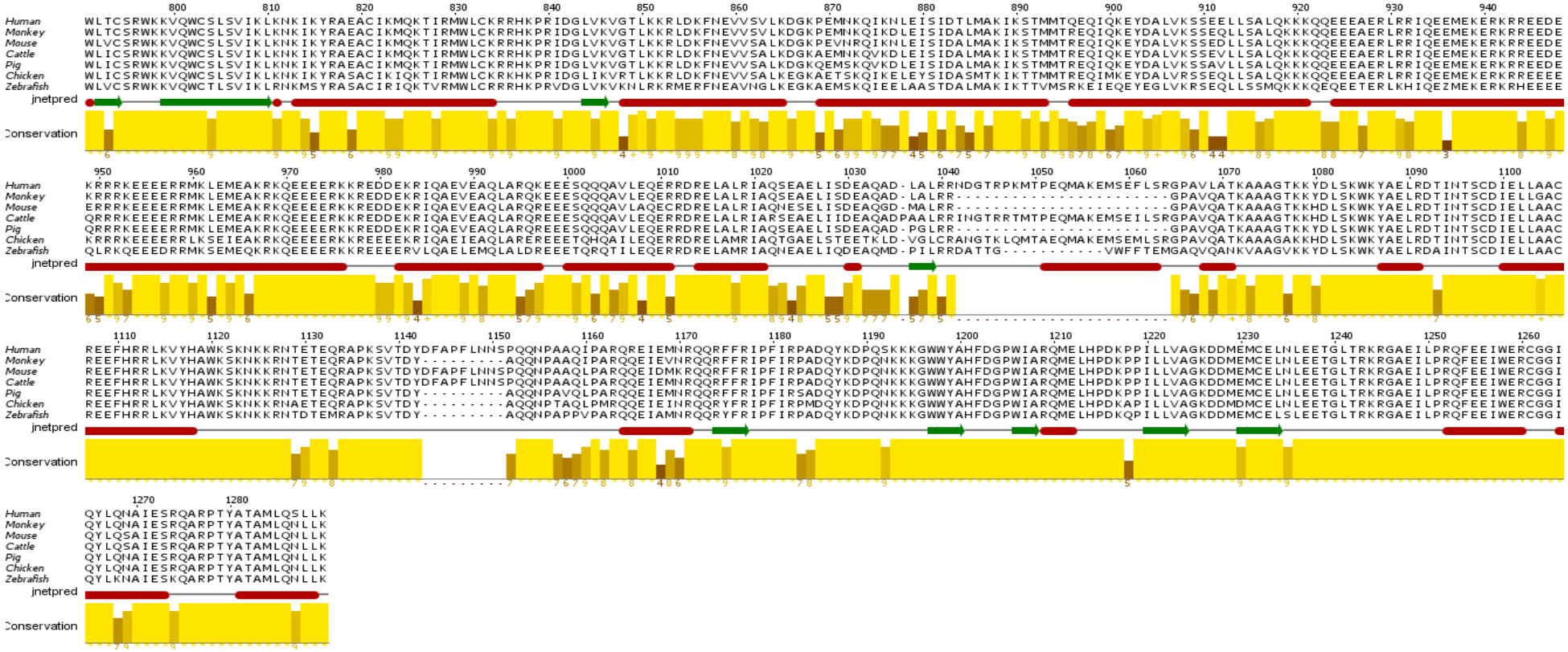

Figure 1. Contd.

Table 1. Sequence-based stability analysis of human myosin VI protein using I-mutant.

Position	Wild type	Mutated type	Stability	Reliability index	pH	Temperature (C)	DDG (ΔΔG)
216	E	V	Decrease	4	7.4	37	-0.44

DDG is calculated using the formula: DG (mutated type) – DG (wild type). A value of DDG less than zero corresponds to decrease in stability whereas, DDG value greater than zero indicates stable protein. E, Glutamate; V, valine.

maintain only a single hydrogen bond with Ser449. This event also led to exposure of the Ser449 hydroxyl group. Whether this exposed hydroxyl group has any impact on myosin VI binding properties is not known and will require further study.

Phosphorylation plays an important role in activating myosin VI (Yoshimura et al., 2001). Myosin VI is phosphorylated by p21, the Rac-

Table 2. Structure-based stability analysis of human myosin VI protein using CUPSAT

Position	Wild type	Mutated type	Structure stability	Torsion	Torsion angle (Phi, Psi)	Solvent accessibility	DDG (ΔΔG)
216	E	V	Decrease	Unfavorable	-54.0 φ; -31.9 ψ	74.41%	-2.58

E, Glutamate; V, valine; CUPSAT, Cologne University y analysis tool.

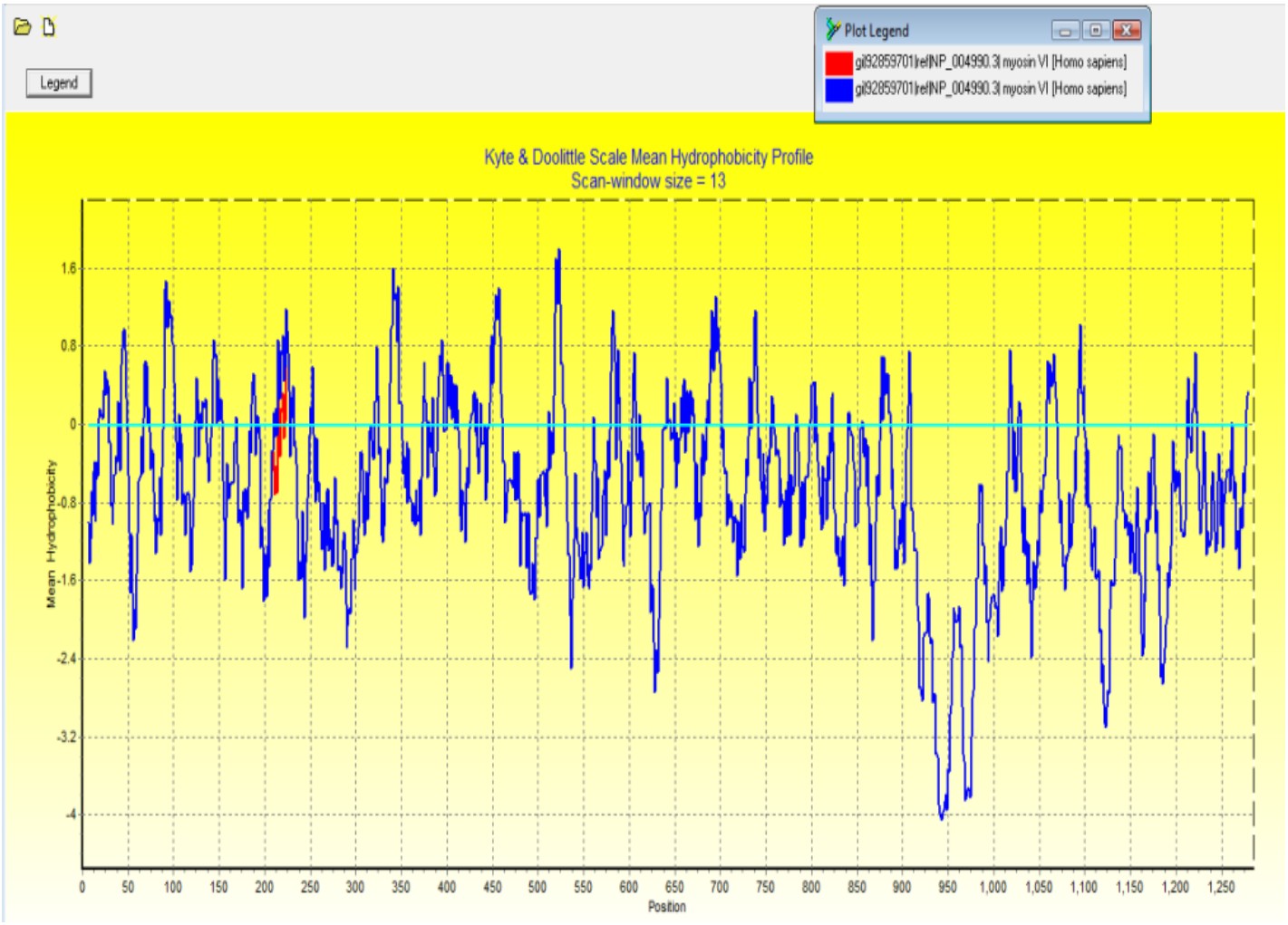

Figure 2. Hydrophobicity plot of wild type and mutated human myosin VI protein. Blue color represents wild type structure and red color represents myosin VI protein with E216V mutation.

activated Ser/Thr kinase (PAK) (Baker and Titus, 1997) and is phosphorylated at Thr406 (in the motor domain) by PAK3 (Wells et al., 1999). This phosphorylation activates its motility, whereas calcium inhibits it (Wells et al., 1999). To determine the potential impact of E216V on myosin VI phosphorylation, we used NetPhos 2.0. A change in phosphorylation score from 0.968 to 0.951 in normal and mutated myosin VI, respectively, at position 219, indicated that this transformation could possibly be due to the E216V mutation or to a relevant change in hydrophobicity (Table 3). Therefore, it is proposed that the exposed hydroxyl group of Ser449 after mutation may be involved in making another phosphorylation site that destabilizes the myosin VI protein, owing to formation of an extra reactive site.

In summary, our analysis highlights the importance of the functional characterization of pathogenic mutations at a structural level. Our results derived using various bioinformatics tools, suggest that the E216V mutation may have an indirect role in hearing disorders owing to structural abnormalities in myosin VI 3D structure. We are currently performing large scale analysis of other

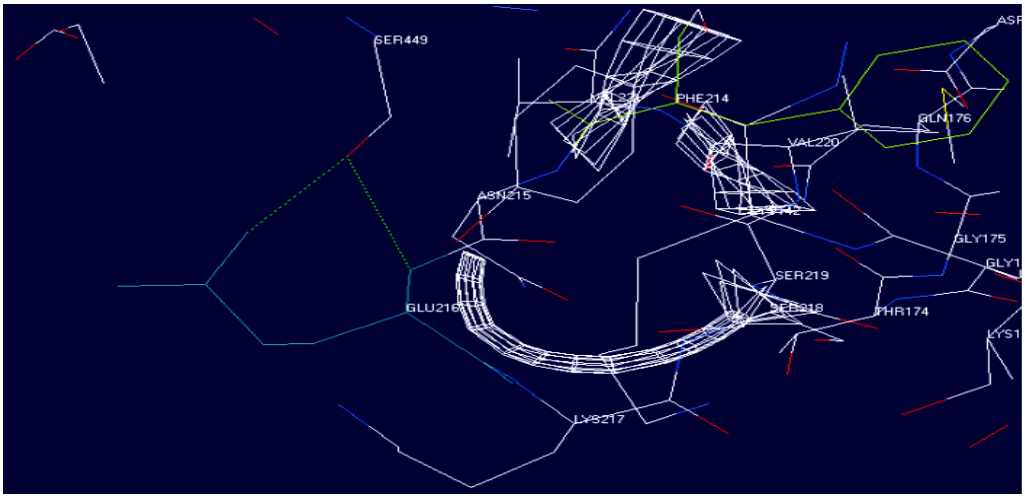

Figure 3. 3D structure of wild type myosin VI protein showing two hydrogen bonds between Glu216 and Ser449. The dotted green line indicated hydrogen bonds. The structure was prepared and visualized in deepview.

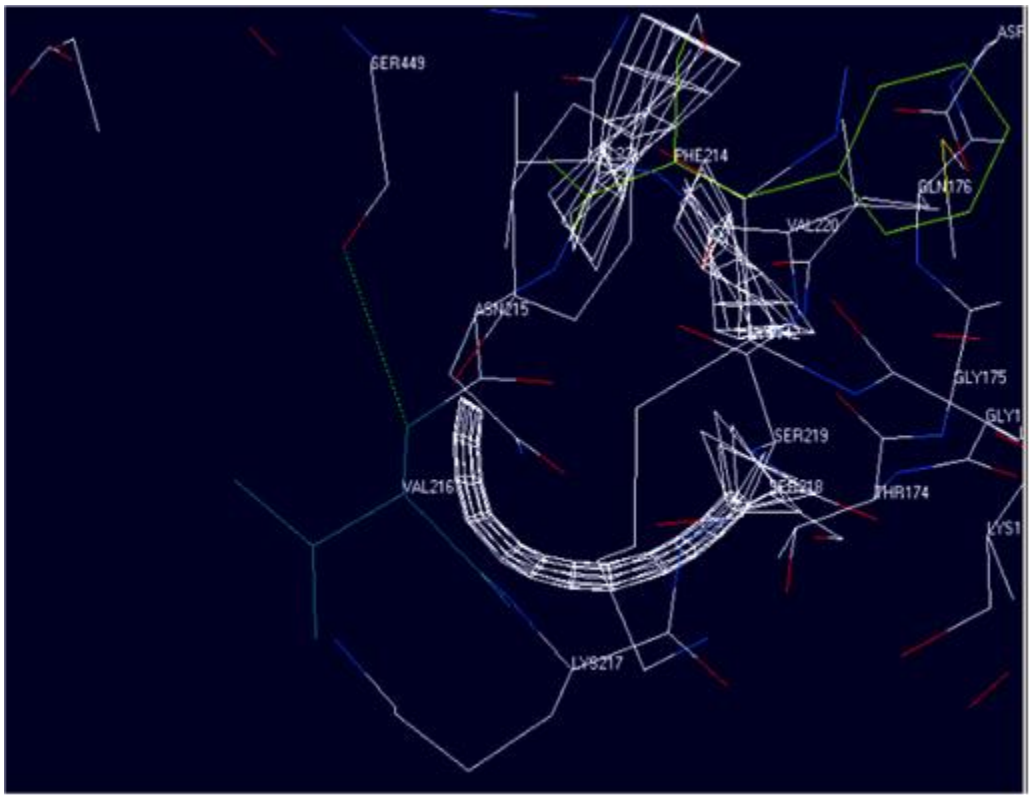

Figure 4. 3D structure of mutated myosin VI protein showing single hydrogen bond between Val216 and Ser449. The dotted green line indicated hydrogen bonds. The structure was prepared and visualized in deepview.

deafness-associated mutations in myosin VI protein, in order to further characterize correlation between structural abnormalities and hereditary deafness.

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Table 3. Comparative analysis of wild type and mutated myosin VI protein at serine residues.

Position	Context	Wild type myosin VI score	Context	Mutated myosin VI score
55	AEEDSKKDV	0.997	AEEDSKKDV	0.997
82	KVRYSKDRI	0.995	KVRYSKDRI	0.995
108	PKIYSSEAI	0.865	PKIYSSEAI	0.865
144	VLKMSQSII	0.524	VLKMSQSII	0.524
150	SIIVSGESG	0.614	SIIVSGESG	0.614
153	VSGESGAGK	0.518	VSGESGAGK	0.518
219	N**E**KSSVVGG	0.968	N**V**KSSVVGG	0.951
266	KLHLSSPDN	0.954	KLHLSSPDN	0.954
267	LHLSSPDNF	0.957	LHLSSPDNF	0.957
297	QNRKSPEYL	0.997	QNRKSPEYL	0.997
305	LKAGSMKDP	0.997	LKAGSMKDP	0.997
357	EEAGSTSGG	0.962	EEAGSTSGG	0.962
359	AGSTSGGCN	0.610	AGSTSGGCN	0.610

The potential phosphorylation scores were calculated using NetPhos 2.0 on the basis of default threshold of 0.5. **E**, Glutamate; **V**, valine.

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