Shaker1 mice carrying a Q720X mutation have been shown to be bonafide null mutations, with no evidence of a truncated product in western blots (Liu et al., 1999). It is reasonable to assume, therefore, that the following MYO7A mutations in this study also result in null mutations: C31X (P20 heterozygous), R159fs (P29 heterozygous), Y333X (P7,P9,P11,P12 homozygous), E495X (P20 heterozygous), R634X (P26,P28 heterozygous), and R669X (P29 heterozygous). By this prediction, P7, P9, P11, P12, P20 and P29 would have two null MYO7A mutations. The following mutations might allow for the production of a major part of the MYO7A molecule, but lack at least a complete second FERM domain, which appears to be critical for MYO7A function: Q1178fs (P16 heterozygous), K1255fs (P21,P27 heterozygous), H1355fs (P5 heterozygous), E1716X (P32 heterozygous), Q1798X (P33 heterozygous), K1737fs (P6,P15 heterozygous), G1942X (P2,P4,P21,P27 heterozygous), E1917X (P33 heterozygous), F1963fs (P2,P4 heterozygous), and R2024X (P5 heterozygous). In this case, P2, P4, P5, P21, P2 and P33 would have two alleles that might result in mutant protein that lacks a complete second FERM domain. Although frameshift mutations that lead to the presence of nonsense sequence in addition to premature termination may be degraded by nonsense-mediated decay mechanisms, a previous report (Schwander et al., 2009) identified a splicing mutation in mouse Myo7a that resulted in stable mutant MYO7A in the retina, despite the presence of nonsense sequence. This mutation results in premature truncation after the first 56 amino acids of the C-terminal FERM domain, and 33 aberrant missense amino acids added to the C-terminus.
Splice site mutations were analyzed using the NNSPLICE0.9 server, which identifies consensus splice donor and acceptor sites based on a training dataset of human genomic splice sites (Reese et al., 1997). The 592 +1 G>T mutation (P18) would be expected to be a functional null, as the G>T transition inactivates the canonical exon 6/intron 7 splice donor site, and most likely leads to transcriptional read through with premature transcriptional termination occurring three codons into intron 7 due to an in-frame opal stop codon.

Three point mutations fall within the motor domain, which has close homology to the motor domain of MYO5A, for which a crystal structure in the ADP-bound state has been described (Coureux et al., 2004). Based on this structure, we have used homology modeling to predict the effect of the R212H (P10 homozygous), G163R (P18 heterozygous), K164N (P19 heterozygous), and G519D (P23 heterozygous) mutations on the motor domain of MYO7A.

Modeling of the R212H mutation leads to the prediction that it will abrogate the salt bridge that forms between switch one and switch two in the MYO7A closed position. The histidine, unlike the wild type arginine, no longer projects into the nucleotide binding pocket, and the position of the co-ordinating glutamic acid 442 in switch 2 is altered slightly. The main effect of this mutation is expected to be very similar to what happens to the function of *Dictyostelium discoideum* myosin II upon loss of the salt bridge between Switch I and Switch II. Namely, there would be marked defects in nucleotide-binding, reduced rates of ATP hydrolysis and a significant reduction in actin affinity. The switch regions are highly conserved across myosins and kinesins, and the orthologous residues to MYO7A R212 and G442 seem to be completely conserved.

The G163R mutation exchanges a polar glycine for a bulky basic arginine at position 163 (Supplement Fig. 2A-C) and is predicted to inhibit ATP binding within the conserved P-loop region. The presence of the large basic sidechain also resulted in a
moderate displacement of the non-polar valine at position 105 within this critical region. The K164N mutation was predicted to have little or no stearic effect on the structure of this highly conserved region in the model (Supplement Fig. 2D-F), but does represent a net loss of charge, and may alter the functionality of the ATP-binding loop. Finally, the G519D mutation occurs adjacent to the switch II and relay loop, altering the net charge of this region and was predicted to moderately displace phenylalanine at position 516. The switch II and relay loop regions undergo large conformation changes during ATP hydrolysis (Rayment et al., 1993; Dominguez et al., 1998; Kollmar et al., 2002; Coureux et al., 2004) moving from a closed to an open state, and alterations in the charge of these regions are known to result in dominant negative inhibition of motor function in myosin II from Dictyostelium (Tsiavaliaris et al., 2002).

The other point mutations affect regions outside of the motor domain of MYO7A and appear to interfere with the structure of described motifs found in the MYO7A tail (Supplement Fig. 1). Biochemical evidence indicates that these tail domains interact with each other to regulate MYO7A cargo binding and motor function. Wu et al. (2011) recently solved the structure of the MYO7A MyTH4-FERM1-SH3 tail region bound to the CEN1/2 interaction sites of the SANS protein, a known binding partner of MYO7A. From this study, together with previous biochemical studies, the following consequences can be predicted.

The G955S and E968D point mutations occur just after the single alpha helical domain (SAH) and would be expected to interfere with the structure of this region (Yang et al., 2009). The E1170K, R1240Q, and A1288P point mutations occur within the first MyTH4-FERM region and are expected, based on the crystal structure of this region, to disrupt the correct folding of the MyTH4 or FERM domains. The R1743W point mutation resides within a relatively unstructured loop between the alpha 1 and alpha 2 helices of the second Myth4 domain and it is unclear from the crystal structure alone what
functional effect this mutation would cause. The A1770D point mutation also resides within the second MyTH4 domain, and mutates a highly conserved arginine residue at the beginning of the alpha 2 helix, one of the helical domains that form the Myth4 core; it would be predicted to affect protein-protein interactions. The R1883Q point mutation occurs within this second MyTH4 domain and would be predicted to disrupt the interface between the MyTH4 and FERM3 domain. The G1982E point mutation resides within a relatively unstructured loop between the alpha B and alpha E helices of the F3 lobe of the second FERM domain. It is unclear from the crystal structure how this would affect the folding of this region, but replacing the zwitterionic glycine with a large polar glutamic acid significantly affects the charge of this region, especially if it is solvent exposed, and could detrimentally affect the folding of the neighboring helices. Both the D2010N and Y2015H point mutations reside within the alpha Af2 region of the F3 lobe of the second FERM domain, a region important for forming the cargo binding interaction pocket, and would be predicted to detrimentally affect protein-protein interactions. The L1858P point mutation is in the second MyTH4 domain and would be predicted to disrupt the correct conformational folding of the final Myth4-FERM3 supramolecule.

Supplementary references (alphabetical order)


**Supplementary Figure Legends**

**Supplementary Figure 1.** Diagram indicating domains based on primary structure.

**Supplementary Figure 2.** Molecular modeling of the P-loop ATP binding region of wild-type (A, D), G163R (B) and K164N (E) mutant MYO7A, and of the Switch II and relay loop regions from wild-type (G) or G519D mutant (H) MYO7A. Structural overlays of the wild-type and mutant regions for G163R, K164N and G519D are shown in panels C, F and I respectively. Mutant residues are colored pink, wild-type residues and coordinating residues affected by the mutations are colored by CPK. Panel J. Homology modeling of the wild type MYO7A switch 1 and switch 2 regions showing the position of R212 in switch 1 and G442 in switch two. Panel K. Homology model of H212 mutant MYO7A with H212 highlighted in purple and G442 highlighted in green. Panel L. Merged image of MYO7A R212 and MYO7A H212 structures showing displacement of the G442 sidechain.