LETTER

Little evidence that FAM65B belongs to the family of phox homology (PX) and bin/amphiphysin/rvs (BAR) domain-containing proteins

Diaz-Horta et al. (1) report the characterization of FAM65B (family with sequence similarity 65, member B), including its mutation in families with recessive hearing loss, its expression and localization in hair cell stereocilia, and the sensorineural hearing loss in a FAM65B knockout zebrafish model. The authors also provide a structural model of the N-terminal 300 amino acids of this 1,068 amino acid protein. This model suggests a homology to the sorting nexin (SNX) family members possessing phox homology (PX) and bin/amphiphysin/rvs (BAR) domains, which promote membrane binding and remodeling, respectively (2, 3). However, our analyses of the FAM65B sequence indicate it is unlikely that it belongs to this protein family.

Because no sequence alignment of FAM65B to PX–BAR proteins was presented, we first performed BLAST searches using the FAM65B sequence (NP_055537.2) against human protein sequences. None of the algorithms (blastp, psi-blast, and delta-blast) identified any homology with PX domain-containing proteins. Next, given that Diaz-Horta et al. used the SNX33 PX–BAR domain structure (PDB ID code 4AKV) as a template for homology modeling of the first 300 amino acids of the FAM65B protein (1), we performed a similar BLAST search against the PDB expecting to identify SNX33 as a hit. Again, no proteins containing a PX or BAR domain were identified. Pairwise alignments with full-length FAM65B and SNX33 failed to identify the claimed region of alignment in which 44% of residues within it displayed strong sequence similarity.

We next attempted to replicate the homology modeling of FAM65B N-terminal residues 1–300. SwissModel (http://swissmodel.expasy.org) in its default mode did not identify a suitable starting template. We then specified SNX33 as the structural template but the program was unable to identify a sufficiently aligned segment to initiate modeling. Using the Phyre2 server (www.sbg.bio.ic.ac.uk/phyre2), no PX–BAR proteins were found in the top 100 hits, and the best result modeled only 63 residues of FAM65B (135–198) in a helical hairpin, based on a low sequence identity (22%) to a fragment of protein kinase PRK1 (PDB ID code 1URF).

The final analysis we performed was a secondary structure prediction of FAM65B using Jpred (www.compbio.dundee.ac.uk/www-jpred). In previous studies we have found that all members of the PX–BAR protein family have predicted secondary structures that match closely to their actual secondary structures determined by NMR and X-ray crystallography (2). That is, the PX domain has three predicted β-strands followed by three α-helices, and the BAR domain has three predicted extended α-helices with a strong propensity for coiled-coil formation. Secondary structure predictions of FAM65 do not reveal any similarity to the PX–BAR family of molecules. To conclude, we believe that although the authors show that FAM65B plays an important role in hearing, they do not provide sufficient evidence to show a relationship to the PX–BAR proteins. The mutation in FAM65B resulting in hearing loss, Δ34–86, is proposed to affect the structural and functional properties of a putative PX domain. However, our analyses indicate that this is unlikely the case.

Rohan D. Teasdale and Brett M. Collins[1]
Institute for Molecular Bioscience, University of Queensland, St. Lucia, QLD 4072, Australia

1 Diaz-Horta O, et al. (2014) FAM65B is a membrane-associated protein of hair cell stereocilia required for hearing. *Proc Natl Acad Sci USA* 111(27):9864–9868.
2 Teasdale RD, Collins BM (2012) Insights into the PX (phox-homology) domain and SNX (sorting nexin) protein families: Structures, functions and roles in disease. *Biochem J* 441(1):39–59.
3 Cullen PJ (2008) Endosomal sorting and signalling: An emerging role for sorting nexins. *Nat Rev Mol Cell Biol* 9(7):574–582.

Author contributions: R.D.T. and B.M.C. designed research, performed research, analyzed data, and wrote the paper.

The authors declare no conflict of interest.

[1]To whom correspondence should be addressed. Email: b.collins@imb.uq.edu.au.