Letter to the Editor

Response to Pomozi et al’s Research Commentary

To the Editor

In the course of studying the effects of ABCC6 deficiency in mice, we observed an enrichment of mitochondrial gene expression signatures. In subsequent studies, we found that ABCC6 null mice exhibited abnormal mitochondrial morphology and functional mitochondrial deficiencies. We then carried out subcellular fractionation studies, indicating that ABCC6 colocalized with markers of the mitochondria-associated membranes in mouse liver and kidney. Our results differed from Le Saux et al from the Varadi group, who had concluded in a recent publication that ABCC6 resided in the plasma membrane. To test whether ABCC6 was localized in plasma membrane, we performed cell surface protein biotin labeling experiments, which were negative for ABCC6.

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Pomozi et al of the Varadi group have now challenged our conclusions based, as in their previous report, on immunofluorescent imaging of frozen liver sections and cells in culture showing peripheral cellular localization of antibody binding. They argue that cell disruption and subcellular fractionation in our study may have resulted in artifactual associations of membrane proteins. However, this seems improbable, given that the plasma membrane markers that we examined did not fractionate with ABCC6, and mitochondria-associated membranes constitute a very small fraction of the total membranes. Cellular fractionation techniques have been used almost universally to provide definitive evidence of subcellular localization. Pomozi et al also argue that ABCC6 lacks sufficient amine groups on the extracellular surface to allow biotin labeling. This possibility cannot be excluded, although the manufacturer of the surface protein biotin labeling assay (Thermo Pierce) indicates that it is sensitive to even a single amine group, which would be present in the N terminus or in one of the predicted available lysines.

In our studies, we used the N-terminal binding (S-20) antibody from the same commercial supplier (Santa Cruz Biotechnology) as reported by Pomozi et al. In our hands, this antibody exhibited significant nonspecific binding in ABCC6 null mouse tissue sections (not shown) and in Western blots. We note that in their publication, the methods state that the S-20 antibody is made in rabbit and blocking was performed using goat serum. In fact, the antibody is made in goat and blocking with goat serum would produce nonspecific labeling (per Santa Cruz Biotechnology). An advantage of our results obtained by subcellular fractionation compared with immunofluorescence of tissue sections is that, in the former, the protein is detected on Western blots after separation by gel electrophoresis, thus allowing better separation from cross-reacting proteins. A lack of accompanying Western data in the current challenge, in our opinion, significantly weakens the ability to ascertain the specificity of the signal.

In conclusion, we recognize that localization of proteins can be challenging and is highly dependent on the quality of the antibody reagents. Although we cannot exclude the possibility that some of the ABCC6 protein resides on the plasma membrane, low-resolution imaging studies of frozen liver sections and cells in culture do not provide convincing evidence against the localization of ABCC6 in the mitochondria-associated membranes.

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References


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