

Is ABCC6 a genuine mitochondrial protein?

Marc Ferré, Pascal Reynier, Arnaud Chevrollier, Delphine Prunier-Mirebeau, Georges Lefthériotis, Daniel Henrion, Dominique Bonneau, Vincent Procaccio, Ludovic Martin

► **To cite this version:**

Marc Ferré, Pascal Reynier, Arnaud Chevrollier, Delphine Prunier-Mirebeau, Georges Lefthériotis, et al.. Is ABCC6 a genuine mitochondrial protein?. BMC Research Notes, BioMed Central, 2013, 6 (1), pp.427. <10.1186/1756-0500-6-427>. <inserm-00877607>

HAL Id: inserm-00877607

<http://www.hal.inserm.fr/inserm-00877607>

Submitted on 29 Oct 2013

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

CORRESPONDENCE

Open Access

Is ABCC6 a genuine mitochondrial protein?

Marc Ferré^{1,2*}, Pascal Reynier^{1,2}, Arnaud Chevrollier^{1,2}, Delphine Prunier-Mirebeau^{1,2}, Georges Lefthériotis^{1,3}, Daniel Henrion¹, Dominique Bonneau^{1,2}, Vincent Procaccio^{1,2} and Ludovic Martin^{1,3}

Abstract

Background: A recent article in *Circulation Research* suggests that the protein ABCC6, which when defective is responsible for pseudoxanthoma elasticum, an inherited condition with skin, eye and cardiovascular manifestations, is associated with dysfunction in mitochondria – Martin et al.: **ABCC6 Localizes to the Mitochondria-Associated Membrane**. *Circ Res* 2012, **111**:516–520. We present complementary information based on a bioinformatics analysis, which was not performed in the article cited, to examine the suggestion that ABCC6 is localized to mitochondria.

Results: All the computational strategies and integrative approaches that constitute references in the field indicate that ABCC6 is localized outside of mitochondria.

Conclusion: Our computational and integrative results, including both experimental and predictive data, show that there is no support in favor of the localization of ABCC6 in mitochondria.

Keywords: Mitochondria, ABCC6/MRP6, Pseudoxanthoma elasticum (PXE), Mitochondria-associated membrane (MAM)

Discussion

Pseudoxanthoma elasticum (PXE), an inherited condition with skin, eye and cardiovascular manifestations resulting from dystrophic calcification, is caused by a defect in the ATP-binding cassette, subfamily C, member 6 protein (ABCC6) [1]. ABCC6 and the murine ortholog *Abcc6*, which have been repeatedly located to the plasma membrane of the liver and proximal tubule kidney cells, are virtually absent in affected tissues in PXE and in related murine conditions [1-3]. These findings are of major pathophysiological significance since they indicate that ABCC6 mediates remote calcification *via* the circulation and that PXE is a systemic condition [4]. However, a recent article in *Circulation Research* claiming that ABCC6 localizes to the mitochondria-associated membrane (MAM) [5] has given rise to some debate [6,7]. MAMs have been defined as the contact sites between mitochondria and the ER with no involvement of any direct membrane fusion [8]. In their article, the authors describe various structural mitochondrial changes in *Abcc6*^{-/-} kidney and heart cells, though not

in liver cells, as well as changes in the endoplasmic reticulum (ER) bordering mitochondria and decreased reserve respiration capacity in liver cells. Taken together, these findings may suggest dysfunction in mitochondria rather than in MAM. We therefore set out to test the possibility of ABCC6 being localized to mitochondria, using the computational and integrative approaches that are now references in the field.

Computational analysis

Mitochondrial proteins may be targeted to the mitochondrial matrix, inner membrane, outer membrane, or the intermembrane space [9]. Proteins destined for import into the organelle *via* a cleavable peptide presequence, typically 15–50 residues long, contain an α -helix with positively charged residues on one side, and uncharged hydrophobic residues on the other. Some of these proteins are subsequently directed to the inner membrane and the intermembrane space according to the consecutive peptide sequences they carry.

The identification of the targeting sequence of mitochondrial proteins by means of a purely computational strategy is now well established and commonly used. Several algorithms have been developed for the detection of mitochondrial targeting sequences on proteins translated in the cytosol. Although these tools suffer

* Correspondence: MaFerre@chu-angers.fr

¹CNRS 6214/INSERM 1083, Angers University, Angers, France

²Department of Biochemistry and Genetics, University Hospital, Angers, France

Full list of author information is available at the end of the article

from the limitation of leading to false-positive predictions of mitochondrial localization, they all predict the presence of ABCC6 outside mitochondria and the ER. Thus:

- Predotar [10] localizes ABCC6 neither in mitochondria nor the endoplasmic reticulum ($p=0.99$);
- MitoProt [11] finds a low probability of the protein being exported to mitochondria ($p=0.0304$);
- TargetP [12] indicates a location other than mitochondria and the secretory pathway (score: 0.845);
- Prowler [13] localizes the protein neither in mitochondria, the secretory pathway, nor the peroxisomal matrix (score: 0.59);
- CELLO [14], WoLF PSORT [15] and PSORT-II [16] localize the protein in the plasma membrane; and
- iPSORT [17] predicts the protein has no signaling or mitochondrial targeting peptide.

In addition, some localization software techniques [14-16], which rely not only on the N-terminal targeting sequence but apply to whole-cell localizations, predicts the localization of ABCC6 in the plasma membrane.

Integrative analysis

Currently, the most successful experimental strategy for establishing mitochondrial protein localization combines in-depth protein mass spectrometry of 14 mouse tissue samples, large-scale green fluorescent protein-tagging microscopy, machine learning, literature curation, the presence of mitochondrial-specific protein domains, mRNA coexpression, targeting-signal prediction, transcriptional induction during mitochondrial proliferation, homology to yeast mitochondrial proteins, and protein homology to *Rickettsia*, a relative of the mitochondrion's bacterial ancestor [18]. The results of all these integrative approaches, compiled in the online databases MitoP2 [19], MitoCarta [20], MitoProteome [21], MitoMiner [22], and HMPDb [23], indicate that ABCC6 is not localized in mitochondria.

The most comprehensive mitochondrial database is now estimated to be 85 % complete, containing about 1,100 mitochondrial proteins [20]. The incompleteness may be explained by the fact that the protein composition of mitochondria varies from one tissue to another. Approximately half of the mitochondrial proteins are ubiquitous, whereas the expression of the remainder is tissue-specific expression [20,24]. However, Martin *et al.* [5] found ABCC6 localized to mitochondria in the liver as well as the kidney, both organs that were studied in the integrative works cited above.

Conclusions

As emphasized by Martin *et al.* [5], the determination of the subcellular location of ABCC6 is critical in deciphering

the exact role of the transporter in physiology, in general, and in the pathophysiology of PXE, in particular, when ABCC6 is absent or defective. Biochemical and immunological approaches have led to the description of ABCC6 as a cytoplasmic membrane protein. Our computational and integrative results show that there is no support in favor of the localization of ABCC6 either in mitochondria or in the ER membrane.

We hypothesize that the MAM localization of ABCC6 may be transitory. It would be interesting to study the vesicular traffic through the sites of contact between mitochondria and the ER, especially as it has been recently shown that the plasma membrane contributes in part to the lipid bilayers of the autophagosome, creating a possible pathway requiring the close contact between ABCC6 and mitochondria during mitophagy [25].

Abbreviations

ER: Endoplasmic reticulum; MAM: Mitochondria-associated membrane; PXE: Pseudoxanthoma elasticum.

Competing interests

The authors state no conflict of interest.

Authors' contribution

MF, PR, & LM initiated the study. MF conducted the experiments. MF & AC drafted the article. MF wrote the final manuscript. AC, PR, DPM, GL, DH, DB, VP, & LM provided critical revision. All authors read and approved the final manuscript.

Authors' information

MF obtained his Ph. D. (2009) with the bioinformatics analysis of the mitochondrial proteome. MF, AC, PR, DPM, DB, & VP belong to Mitolab (<http://mitolab.eu>), a French academic team of the CNRS 6214/INSERM 1083 research unit specialized in disorders involving mitochondrial dynamics and bioenergetics. The Department of Integrated Neurovascular and Mitochondrial Biology (CNRS 6214/INSERM 1083) is headed by DH. PR, DB, & VP are members of the French Reference Centre for Mitochondrial Diseases in children and adults. GL & LM are members of the PXE Consultation Center of the University Hospital of Angers, France.

Acknowledgments

We are grateful to Kanaya Malkani for critical reading and comments on the manuscript.

Author details

¹CNRS 6214/INSERM 1083, Angers University, Angers, France. ²Department of Biochemistry and Genetics, University Hospital, Angers, France. ³PXE Consultation Center, University Hospital, Angers, France.

Received: 15 February 2013 Accepted: 11 October 2013

Published: 23 October 2013

References

1. Bergen AA, Plomp AS, Schuurman EJ, Terry S, Breuning M, Dauwerse H, Swart J, Kool M, Van Soest S, Baas F, *et al*: **Mutations in ABCC6 cause pseudoxanthoma elasticum.** *Nat Genet* 2000, **25**:228–231.
2. Le Saux O, Fulop K, Yamaguchi Y, Ilias A, Szabo Z, Brampton CN, Pomozi V, Huszar K, Aranyi T, Varadi A: **Expression and in vivo rescue of human ABCC6 disease-causing mutants in mouse liver.** *PLoS one* 2011, **6**:e24738.
3. Madon J, Hagenbuch B, Landmann L, Meier PJ, Stieger B: **Transport function and hepatocellular localization of mrp6 in rat liver.** *Mol Pharmacol* 2000, **57**:634–641.

4. Uitto J, Bercovitch L, Terry SF, Terry PF: **Pseudoxanthoma elasticum: progress in diagnostics and research towards treatment : Summary of the 2010 PXE International Research Meeting.** *Am J Med Genet Part A* 2011, **155A**:1517–1526.
5. Martin LJ, Lau E, Singh H, Vergnes L, Tarling EJ, Mehrabian M, Mungrue I, Xiao S, Shih D, Castellani L, et al: **ABCC6 Localizes to the Mitochondria-Associated Membrane.** *Circ Res* 2012, **111**:516–520.
6. Pomozi V, Le Saux O, Brampton C, Apana A, Ilias A, Szeri F, Martin L, Monostory K, Paku S, Sarkadi B, et al: **ABCC6 is a basolateral plasma membrane protein.** *Circ Res* 2013, **112**:e148–e151.
7. Martin LJ, Lau E, Singh H, Vergnes L, Tarling EJ, Mehrabian M, Mungrue I, Xiao S, Shih D, Castellani L, et al: **Response to Pomozi et al's research commentary.** *Circ Res* 2013, **112**:e152–e153.
8. Wieckowski MR, Giorgi C, Lebiecinska M, Duszynski J, Pinton P: **Isolation of mitochondria-associated membranes and mitochondria from animal tissues and cells.** *Nat protocols* 2009, **4**:1582–1590.
9. Chacinska A, Koehler CM, Milenkovic D, Lithgow T, Pfanner N: **Importing mitochondrial proteins: machineries and mechanisms.** *Cell* 2009, **138**:628–644.
10. Small I, Peeters N, Legeai F, Lurin C: **Predotar: A tool for rapidly screening proteomes for N-terminal targeting sequences.** *Proteomics* 2004, **4**:1581–1590.
11. Claros MG, Vincens P: **Computational method to predict mitochondrially imported proteins and their targeting sequences.** *Eur J Biochem / FEBS* 1996, **241**:779–786.
12. Emanuelsson O, Nielsen H, Brunak S, von Heijne G: **Predicting subcellular localization of proteins based on their N-terminal amino acid sequence.** *J Mol Biol* 2000, **300**:1005–1016.
13. Boden M, Hawkins J: **Prediction of subcellular localization using sequence-biased recurrent networks.** *Bioinformatics* 2005, **21**:2279–2286.
14. Yu CS, Chen YC, Lu CH, Hwang JK: **Prediction of protein subcellular localization.** *Proteins* 2006, **64**:643–651.
15. Horton P, Park KJ, Obayashi T, Fujita N, Harada H, Adams-Collier CJ, Nakai K: **WoLF PSORT: protein localization predictor.** *Nucleic Acids Res* 2007, **35**:W585–W587.
16. Nakai K, Horton P: **PSORT: a program for detecting sorting signals in proteins and predicting their subcellular localization.** *TIBS* 1999, **24**:34–36.
17. Bannai H, Tamada Y, Maruyama O, Nakai K, Miyano S: **Extensive feature detection of N-terminal protein sorting signals.** *Bioinformatics* 2002, **18**:298–305.
18. Calvo SE, Mootha VK: **The mitochondrial proteome and human disease.** *An rev genom human genet* 2010, **11**:25–44.
19. Elstner M, Andreoli C, Ahting U, Tetko I, Klopstock T, Meitinger T, Prokisch H: **MitoP2: an integrative tool for the analysis of the mitochondrial proteome.** *Mol Biotechnol* 2008, **40**:306–315.
20. Pagliarini DJ, Calvo SE, Chang B, Sheth SA, Vafai SB, Ong SE, Walford GA, Sugiana C, Boneh A, Chen WK, et al: **A mitochondrial protein compendium elucidates complex I disease biology.** *Cell* 2008, **134**:112–123.
21. Cotter D, Guda P, Fahy E, Subramaniam S: **MitoProteome: mitochondrial protein sequence database and annotation system.** *Nucleic Acids Res* 2004, **32**:D463–D467.
22. Smith AC, Robinson AJ: **MitoMiner, an integrated database for the storage and analysis of mitochondrial proteomics data.** *Mol cell proteomics : MCP* 2009, **8**:1324–1337.
23. *Human Mitochondrial Protein Database.* <http://bioinfo.nist.gov>.
24. Mootha VK, Bunkenborg J, Olsen JV, Hjerrild M, Wisniewski JR, Stahl E, Bolouri MS, Ray HN, Sihag S, Kamal M, et al: **Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria.** *Cell* 2003, **115**:629–640.
25. Ravikumar B, Moreau K, Jahreiss L, Puri C, Rubinsztein DC: **Plasma membrane contributes to the formation of pre-autophagosomal structures.** *Nat cell biol* 2010, **12**:747–757.

doi:10.1186/1756-0500-6-427

Cite this article as: Ferré et al.: Is ABCC6 a genuine mitochondrial protein?. *BMC Research Notes* 2013 **6**:427.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

