DATA NOTE

REVISED The identification of high-performing antibodies for Coiled-coil-helix-coiled-coil-helix domain containing protein 10 (CHCHD10) for use in Western Blot, immunoprecipitation and immunofluorescence [version 2; peer review: 2 approved, 1 approved with reservations]

Riham Ayoubi[1], Walaa Alshafie[1], Kathleen Southern[1], Peter S. McPherson[1], Carl Laflamme[1], NeuroSGC/YCharOS/EDDU collaborative group

[1]Department of Neurology and Neurosurgery, Structural Genomics Consortium, The Montreal Neurological Institute, McGill University, Montreal, Québec, H3A 2B4, Canada

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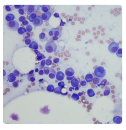
Abstract

CHCHD10 is a mitochondrial protein, implicated in the regulation of mitochondrial morphology and cristae structure, as well as the maintenance of mitochondrial DNA integrity. Recently discovered to be associated with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) in its mutant form, the scientific community would benefit from the availability of validated anti-CHCHD10 antibodies. In this study, we characterized four CHCHD10 commercial antibodies for Western Blot, immunoprecipitation, and immunofluorescence using a standardized experimental protocol based on comparing read-outs in knockout cell lines and isogenic parental controls. As this study highlights high-performing antibodies for CHCHD10, we encourage readers to use it as a guide to select the most appropriate antibody for their specific needs.

Keywords

Uniprot ID Q8WYQ3, CHCHD10, Coiled-coil-helix-coiled-coil-helix domain-containing protein 10, antibody characterization, antibody validation, Western Blot, immunoprecipitation, immunofluorescence



This article is included in the Cell & Molecular Biology gateway.

Open Peer Review

Approval Status ✓ ? ✓

	1	2	3
version 2 (revision) 26 Jul 2023		? view	✓ view
version 1 14 Apr 2023	✓ view	? view	

1. **Yang Liu**, University of the Chinese Academy of Sciences, Beijing, China
2. **Derek P. Narendra**, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, USA
3. **Andrew D. Nguyen**, Saint Louis University, St. Louis, USA

Any reports and responses or comments on the article can be found at the end of the article.

This article is included in the YCharOS (Antibody Characterization through Open Science) gateway.

Corresponding author: Carl Laflamme (carl.laflamme@mcgill.ca)

Author roles: Ayoubi R: Investigation, Methodology, Visualization, Writing – Review & Editing; **Alshafie W**: Investigation, Methodology; **Southern K**: Writing – Original Draft Preparation, Writing – Review & Editing; **McPherson PS**: Conceptualization, Funding Acquisition, Resources, Supervision, Validation; **Laflamme C**: Conceptualization, Data Curation, Funding Acquisition, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Review & Editing;

Competing interests: For this project, the laboratory of Peter McPherson developed partnerships with high-quality antibody manufacturers and knockout cell line providers. The partners provide antibodies and knockout cell lines to the McPherson laboratory at no cost. These partners include: - Abcam- Aviva Systems Biology -Bio Techne -Cell Signalling Technology -Developmental Studies Hybridoma Bank -GeneTex – Horizon Discovery – Proteintech – Synaptic Systems –Thermo Fisher Scientific.

Grant information: This work was supported in part by the ALS-Reproducible Antibody Platform (ALS-RAP). ALS-RAP is a private-public partnership created by the ALS Association (USA), the Motor Neurone Disease Association (UK), and the ALS Society of Canada. The grant was from a Canadian Institutes of Health Research Foundation Grant (FDN154305) and by the Government of Canada through Genome Canada, Genome Quebec and Ontario Genomics (OGI-210). The Structural Genomics Consortium is a registered charity (no. 1097737) that receives funds from Bayer AG, Boehringer Ingelheim, Bristol-Myers Squibb, Genentech, Genome Canada through Ontario Genomics Institute (grant no. OGI-196), the EU and EFPIA through the Innovative Medicines Initiative 2 Joint Undertaking (EUbOPEN grant no. 875510), Janssen, Merck KGaA (also known as EMD in Canada and the United States), Pfizer and Takeda. RA and WA were supported by a Mitacs fellowship. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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First published: 14 Apr 2023, **12**:403 https://doi.org/10.12688/f1000research.133479.1

REVISED **Amendments from Version 1**

The newly submitted version of the article clarifies in the introduction that CHCHD10 protein contains a single CHCH domain formed by two CX_9C motifs.

Any further responses from the reviewers can be found at the end of the article

Introduction

Coiled-coil-helix-coiled-coil-helix domain containing protein 10 (CHCHD10) is a protein localized to the mitochondrial intermembrane space, and is postulated to be involved in the maintenance of mitochondrial organization and cristae structure.[1] Twin CX_9C motifs make up the single coiled-coil-helix-coiled-coil-helix domain, which stabilizes the helix-turn-helix fold.[2]

Recent studies have demonstrated that CHCHD10 is important for neuronal health.[1] As such, *CHCHD10* gene variants have been reported in patients with ALS, FTD, Parkinson's disease, motor neuron disease, and mitochondrial myopathy, suggesting that they contribute to neurodegenerative disease progression.[1,3,4] Additional work is needed to understand the underlying function and regulation of CHCHD10, in its native and mutant conformation, to advance the development of therapeutic strategies for targeting these deteriorating diseases. Mechanistic studies would be greatly facilitated with the availability of high-quality CHCHD10 antibodies.

Here, we compared the performance of a range of commercially available CHCHD10 antibodies for Western Blot, immunoprecipitation and immunofluorescence, enabling biochemical and cellular assessment of CHCHD10 properties and function.

Results and discussion

Our standard protocol involves comparing readouts from wild-type and knockout cells.[5–9] The first step was to identify a cell line(s) that expresses sufficient levels of CHCHD10 to generate a measurable signal to noise. To this end, we examined the DepMap transcriptomics databases to identify all cell lines that express the target at levels greater than 2.5 $\log_2$ (transcripts per million "TPM" +1), which we have found to be a suitable cut-off (Cancer Dependency Map Portal, RRID:SCR_017655). Commercially available HAP1 cells expressed the *CHCHD10* transcript at RNA levels above the average range of cancer cells analyzed. Parental and *CHCHD10* knockout HAP1 cells were obtained from Horizon Discovery. Parental HCT116 cells were obtained from Abcam for immunoprecipitation experiments (Table 1).

For Western Blot experiments, we resolved proteins from WT and *CHCHD10* KO cell extracts and probed them side-by-side with all antibodies in parallel (Figure 1).[6–12]

For immunoprecipitation experiments, we used the antibodies to immunopurify CHCHD10 from cell extracts. The performance of each antibody was evaluated using Western Blot by detecting the CHCHD10 protein in extracts, in the immunodepleted extracts and in the immunoprecipitates (Figure 2).[6–12]

For immunofluorescence, as described previously, antibodies were screened using a mosaic strategy.[13] In brief, we plated WT and KO cells together in the same well and imaged both cell types in the same field of view to reduce staining, imaging and image analysis bias (Figure 3).

In conclusion, we have screened CHCHD10 commercial antibodies by Western Blot, immunoprecipitation and immunofluorescence and identified high-quality antibodies under our standardized experimental conditions. The underlying data can be found on Zenodo.[14,15]

Table 1. Summary of the cell lines used.

Institution	Catalog number	RRID (Cellosaurus)	Cell line	Genotype
Horizon Discovery	C631	CVCL_Y019	HAP1	WT
Horizon Discovery	HZGHC005043c003	CVCL_SI77	HAP1	*CHCHD10* KO
Abcam	ab255451	CVCL_0291	HCT116	WT

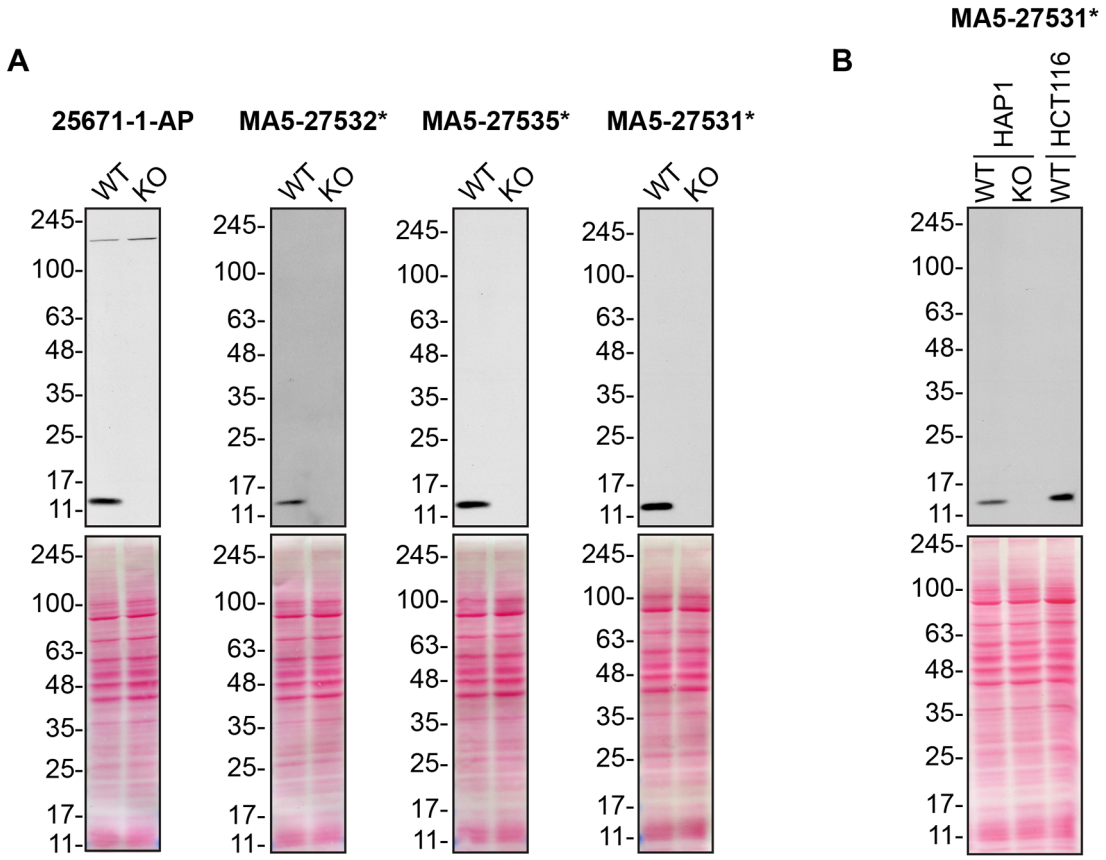

Figure 1. CHCHD10 antibody screening by Western Blot. A) Lysates of HAP1 (WT and *CHCHD10* KO) were prepared, and 50 µg of protein were processed for Western Blot with the indicated CHCHD10 antibodies. The Ponceau stained transfers of each blot are presented to show equal loading of WT and KO lysates and protein transfer efficiency from the polyacrylamide gels to the nitrocellulose membrane. Antibody dilutions were chosen according to the recommendations of the antibody supplier. Antibody dilution used: 25671-1-AP at 1/1000, MA5-27532* at 1/500, MA5-27535* at 1/500, MA5-27531* at 1/500. Predicted band size: 14 kDa. *= monoclonal antibody. B) Lysates of HAP1 (WT and *CHCHD10* KO) and HCT116 were prepared as in A). MA5-27531* was used at 1/500. *= monoclonal antibody.

Methods

Antibodies

All CHCHD10 antibodies are listed in Table 2, together with their corresponding Research Resource Identifiers (RRID), to ensure the antibodies are cited properly.[16] Peroxidase-conjugated goat anti-rabbit and anti-mouse antibodies are from Thermo Fisher Scientific (cat. number 65-6120 and 62-6520). Alexa-555-conjugated goat anti-rabbit and anti-mouse secondary antibodies are from Thermo Fisher Scientific (cat. number A21429 and A21424).

Cell culture

Both HAP1 WT and *CHCHD10* KO cell lines used are listed in Table 1, together with their corresponding RRID, to ensure the cell lines are cited properly.[17] Cells were cultured in DMEM high-glucose (GE Healthcare cat. number SH30081.01) containing 10% fetal bovine serum (Wisent, cat. number 080450), 2 mM L-glutamate (Wisent cat. number 609065), 100 IU penicillin and 100 µg/mL streptomycin (Wisent cat. number 450201).

Antibody screening by Western Blot

Western Blots were performed as described in our standard operating procedure.[18] HAP1 WT and *CHCHD10* KO were collected in RIPA buffer (50 mM Tris pH 8.0, 150 mM NaCl, 1.0 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with 1x protease inhibitor cocktail mix (MilliporeSigma, cat. number 78429). Lysates were sonicated briefly and incubated for 30 min on ice. Lysates were spun at ~110,000 x g for 15 min at 4°C and equal protein aliquots of the supernatants were analyzed by SDS-PAGE and Western Blot. BLUelf prestained protein ladder from GeneDireX (cat. number PM008-0500) was used.

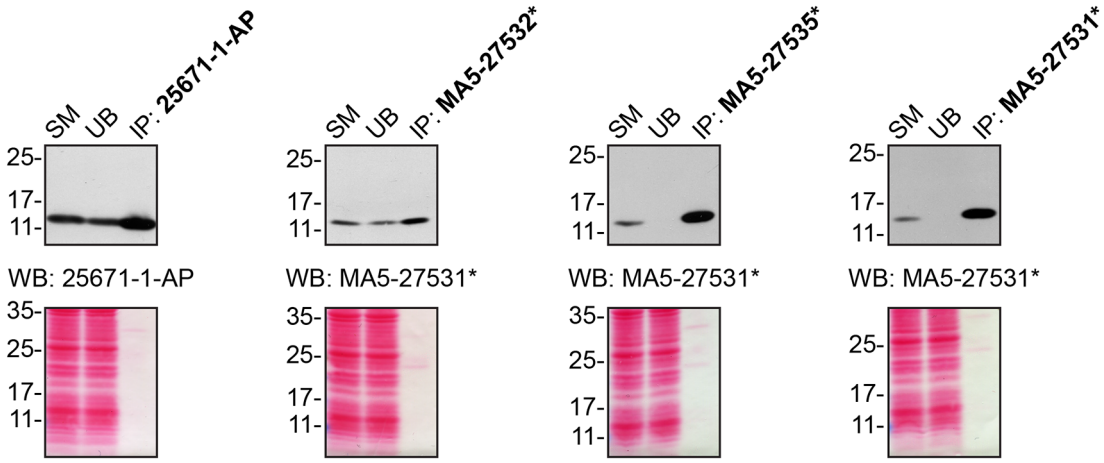

Figure 2. CHCHD10 antibody screening by immunoprecipitation. HCT116 lysates were prepared, and IP was performed using 1.0 µg of the indicated CHCHD10 antibodies pre-coupled to protein A or protein G Sepharose beads. Samples were washed and processed for Western Blot with the indicated CHCHD10 antibody. For Western Blot, 25671-1-AP and MA5-27531* were used at 1/1000. The Ponceau stained transfers of each blot are shown for similar reasons as in Figure 1. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate, *= monoclonal antibody.

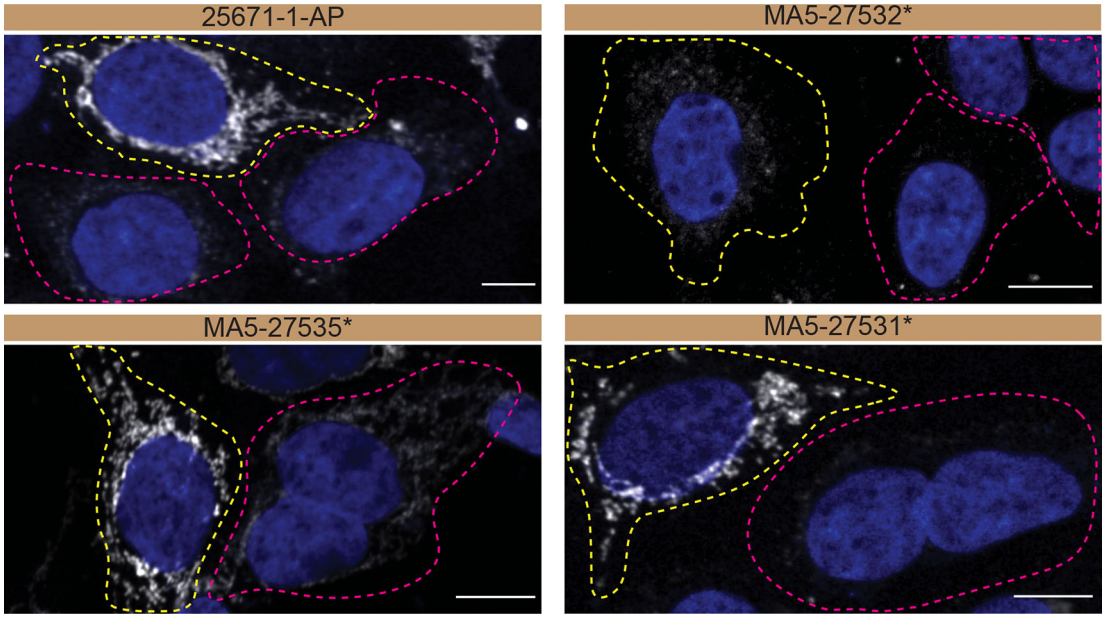

Figure 3. CHCHD10 antibody screening by immunofluorescence. HAP1 WT and *CHCHD10* KO cells were labelled with a green or a far-red fluorescent dye, respectively. WT and KO cells were mixed and plated to a 1:1 ratio on coverslips. Cells were stained with the indicated CHCHD10 antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody including DAPI. Acquisition of the blue (nucleus-DAPI), green (WT), red (antibody staining) and far-red (KO) channels was performed. Representative images of the merged blue and red (grayscale) channels are shown. WT and KO cells are outlined with yellow and magenta dashed line, respectively. Antibody dilutions were chosen according to the recommendations of the antibody supplier. When the concentration was not indicated by the supplier, which was the case for antibodies MA5-27532* and MA5-27535*, we tested antibodies at 1/100 and 1/1000, respectively. At this concentration, the signal from each antibody was in the range of detection of the microscope used. Antibody dilution used: 25671-1-AP at 1/300, MA5-27532* at 1/100, MA5-27535* at 1/1000, MA5-27531* at 1/100. Bars = 10 µm. *= monoclonal antibody.

Table 2. Summary of the CHCHD10 antibodies tested.

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µL)	Vendors recommended applications
Proteintech	25671-1-AP	53318	AB_2880187	polyclonal	-	rabbit	0.30	Wb, IP, IF
Thermo Fisher Scientific	MA5-27532*	VL3152361A	AB_2724131	monoclonal	OTI2B6	mouse	1.00	Wb
Thermo Fisher Scientific	MA5-27535*	VL3152362A	AB_2724132	monoclonal	OTI3B8	mouse	1.00	Wb
Thermo Fisher Scientific	MA5-27531*	VL3152369	AB_2724133	monoclonal	OTI4C12	mouse	1.00	Wb

Wb=Western Blot; IF= immunofluorescence; IP=immunoprecipitation.
*= monoclonal antibody.

Western Blots were performed with large 8-16% gradient polyacrylamide gels and transferred on nitrocellulose membranes. Proteins on the blots were visualized with Ponceau S staining (Thermo Fisher Scientific, cat. number BP103-10) which is scanned to show together with individual Western Blot. Blots were blocked with 5% milk for 1 hr, and antibodies were incubated overnight at 4°C with 5% bovine serum albumin (BSA) (Wisent, cat. number 800-095) in TBS with 0,1% Tween 20 (TBST) (Cell Signaling Technology, cat. number 9997). Following three washes with TBST, the peroxidase conjugated secondary antibody was incubated at a dilution of ~0.2 µg/mL in TBST with 5% milk for 1 hr at room temperature followed by three washes with TBST. Membranes were incubated with Pierce ECL from Thermo Fisher Scientific (cat. number 32106) prior to detection with the HyBlot CL autoradiography films from Denville (cat. number 1159T41).

Antibody screening by immunoprecipitation

Immunoprecipitation was performed as described in our standard operating procedure.[19] Antibody-bead conjugates were prepared by adding 1.0 µg of antibody to 500 µL of phosphate-buffered saline (PBS) (Wisent, cat. number 311-010-CL) with 0,01% triton X-100 (Thermo Fisher Scientific, cat. number BP151-500) in a 1.5 mL microcentrifuge tube, together with 30 µL of protein A- (for rabbit antibodies) or protein G- (for mouse antibodies) Sepharose beads. Tubes were rocked overnight at 4°C followed by two washes to remove unbound antibodies.

HCT116 WT were collected in HEPES buffer (20 mM HEPES, 100 mM sodium chloride, 1 mM EDTA, 1% Triton X-100, pH 7.4) supplemented with protease inhibitor. Lysates were rocked for 30 min at 4°C and spun at 110,000 x g for 15 min at 4°C. One mL aliquots at 1.0 mg/mL of lysate were incubated with an antibody-bead conjugate for ~2 hrs at 4°C. The unbound fractions were collected, and beads were subsequently washed three times with 1.0 mL of HEPES lysis buffer and processed for SDS-PAGE and Western Blot on 8-16% polyacrylamide gels. As secondary detections systems, the Veriblot for immunoprecipitation detection reagent and the anti-mouse IgG for immunoprecipitation (HRP) from Abcam (cat. number ab131366 and ab131368, respectively) were used.

Antibody screening by immunofluorescence

Immunofluorescence was performed as described in our standard operating procedure.[6–13] HAP1 WT and *CHCHD10* KO were labelled with a green and a far-red fluorescence dye, respectively. The fluorescent dyes used are from Thermo Fisher Scientific (cat. number C2925 and C34565). The nuclei were labelled with DAPI (Thermo Fisher Scientific, cat. Number D3571) fluorescent stain. WT and KO cells were plated on glass coverslips as a mosaic and incubated for 24 hrs in a cell culture incubator at 37°C, 5% CO_2. Cells were fixed in 4% paraformaldehyde (PFA) (Beantown chemical, cat. number 140770-10ml) in PBS for 15 min at room temperature and then washed 3 times with PBS. Cells were permeabilized in PBS with 0,1% Triton X-100 for 10 min at room temperature and blocked with PBS with 5% BSA, 5% goat serum (Gibco, cat. number 16210-064) and 0.01% Triton X-100 for 30 min at room temperature. Cells were incubated with IF buffer (PBS, 5% BSA, 0,01% Triton X-100) containing the primary CHCHD10 antibodies overnight at 4°C. Cells were then washed 3 × 10 min with IF buffer and incubated with corresponding Alexa Fluor 555-conjugated secondary antibodies in IF buffer at a dilution of 1.0 µg/mL for 1 hr at room temperature with DAPI. Cells were washed 3 × 10 min with IF buffer and once with PBS. Coverslips were mounted on a microscopic slide using fluorescence mounting media (DAKO).

Imaging was performed using a Zeiss LSM 880 laser scanning confocal microscope equipped with a Plan-Apo 40x oil objective (NA = 1.40). Analysis was done using the Zen navigation software (Zeiss). All cell images represent a single

focal plane. Figures were assembled with Adobe Photoshop (version 24.1.2) to adjust contrast then assembled with Adobe Illustrator (version 27.3.1).

Data availability

Underlying data

Zenodo: Antibody Characterization Report for CHCHD10, https://doi.org/10.5281/zenodo.5259992.[14]

Zenodo: Dataset for the CHCHD10 antibody screening study, https://doi.org/10.5281/zenodo.7779321.[15]

Acknowledgment

We would like to thank the NeuroSGC/YCharOS/EDDU collaborative group for their important contribution to the creation of an open scientific ecosystem of antibody manufacturers and knockout cell line suppliers, for the development of community-agreed protocols, and for their shared ideas, resources and collaboration. Members of the group can be found below.

NeuroSGC/YCharOS/EDDU collaborative group: Riham Ayoubi, Aled M. Edwards, Carl Laflamme, Peter S. McPherson, Chetan Raina, and Kathleen Southern

An earlier version of this of this article can be found on Zenodo (doi: 10.5281/zenodo.5259992)

References

1. Bannwarth S, Ait-El-Mkadem S, Chaussenot A, *et al.*: **A mitochondrial origin for frontotemporal dementia and amyotrophic lateral sclerosis through CHCHD10 involvement.** *Brain.* 2014; **137**(Pt 8): 2329–2345.
PubMed Abstract | Publisher Full Text | Free Full Text

2. Imai Y, Meng H, Shiba-Fukushima K, *et al.*: **Twin CHCH Proteins, CHCHD2, and CHCHD10: Key Molecules of Parkinson's Disease, Amyotrophic Lateral Sclerosis, and Frontotemporal Dementia.** *Int. J. Mol. Sci.* 2019; **20**(4): 908.
PubMed Abstract | Publisher Full Text | Free Full Text

3. Ikeda A, Imai Y, Hattori N: **Neurodegeneration-associated mitochondrial proteins, CHCHD2 and CHCHD10-what distinguishes the two?** *Front. Cell Dev. Biol.* 2022; **10**: 996061.
PubMed Abstract | Publisher Full Text | Free Full Text

4. Harjuhaahto S, Rasila TS, Molchanova SM, *et al.*: **ALS and Parkinson's disease genes CHCHD10 and CHCHD2 modify synaptic transcriptomes in human iPSC-derived motor neurons.** *Neurobiol. Dis.* 2020; **141**: 104940.
PubMed Abstract | Publisher Full Text

5. Laflamme C, McKeever PM, Kumar R, *et al.*: **Implementation of an antibody characterization procedure and application to the major ALS/FTD disease gene C9ORF72.** *elife.* 2019; **8**: 8.
Publisher Full Text

6. Alshafie W, Fotouhi M, Shlaifer I, *et al.*: **Identification of highly specific antibodies for Serine/threonine-protein kinase TBK1 for use in immunoblot, immunoprecipitation and immunofluorescence.** *F1000Res.* 2022; **11**: 977.
Publisher Full Text

7. Alshafie W, Ayoubi R, Fotouhi M, *et al.*: **The identification of high-performing antibodies for Moesin for use in Western Blot, immunoprecipitation, and immunofluorescence [version 1; peer review: awaiting peer review].** *F1000Res.* 2023; **2023**(12): 172.

8. Worrall D, Ayoubi R, Fotouhi M, *et al.*: **The identification of high-performing antibodies for TDP-43 for use in Western Blot, immunoprecipitation and immunofluorescence [version 1; peer review: 1 approved].** *F1000Res.* 2023; **12**: 277.
Publisher Full Text

9. Ayoubi R, Fotouhi M, Southern K, *et al.*: **The identification of high-performing antibodies for transmembrane protein 106B (TMEM106B) for use in Western blot, immunoprecipitation, and immunofluorescence [version 1; peer review: awaiting peer review].** *F1000Res.* 2023; **12**: 308.
Publisher Full Text

10. Ayoubi R, Alshafie W, Shlaifer I, *et al.*: **The identification of high-performing antibodies for Sequestosome-1 for use in Western blot, immunoprecipitation and immunofluorescence [version 1; peer review: awaiting peer review].** *F1000Res.* 2023; **12**: 324.
Publisher Full Text

11. McDowell I, Ayoubi R, Fotouhi M, *et al.*: **The identification of high-preforming antibodies for Ubiquilin-2 for use in Western Blot, immunoprecipitation, and immunofluorescence [version 1; peer review: awaiting peer review].** *F1000Res.* 2023; **12**: 355.
Publisher Full Text

12. Ayoubi R, McDowell I, Fotouhi M, *et al.*: **The identification of high-performing antibodies for Profilin-1 for use in Western blot, immunoprecipitation and immunofluorescence [version 1; peer review: awaiting peer review].** *F1000Res.* 2023; **12**: 348.
Publisher Full Text

13. Alshafie W, McPherson P, Laflamme C: **Antibody screening by Immunofluorescence.** 2021.

14. Ayoubi R, Alshafie W, Straub I, *et al.*: **Antibody Characterization Report for Coiled-coil-helix-coiled-coil-helix domain-containing protein 10, mitochondrial (CHCHD10).** 2021.

15. Laflamme C: Dataset for the CHCHD10 antibody screening study. [Data set]. *Zenodo.* 2023.

16. Bandrowski A, Pairish M, Eckmann P, *et al.*: **The Antibody Registry: ten years of registering antibodies.** *Nucleic Acids Res.* 2023; **51**(D1): D358–D367.
PubMed Abstract | Publisher Full Text | Free Full Text

17. Bairoch A: **The Cellosaurus, a Cell-Line Knowledge Resource.** *J. Biomol. Tech.* 2018; **29**(2): 25–38.
PubMed Abstract | Publisher Full Text | Free Full Text

18. Ayoubi R, McPherson PS, Laflamme C: **Antibody Screening by Immunoblot.** 2021.

19. Ayoubi R, Fotouhi M, McPherson P, *et al.*: **Antibody screening by Immunoprecitation.** 2021.

Open Peer Review

Current Peer Review Status: ✔ ? ✔

Version 2

Reviewer Report 14 September 2023

https://doi.org/10.5256/f1000research.153618.r188088

✔ **Andrew D. Nguyen**
Saint Louis University, St. Louis, Missouri, USA

In this Data Note, the authors report the characterization of four commercially available antibodies for CHCHD10 in several different applications. Their approach was methodical, and the results are clear. The manuscript is well written and the data are presented clearly. Overall, I believe this is a valuable resource to the research community.

I have a few minor suggestions below to improve the manuscript:

- The authors may consider including a brief discussion and/or interpretation of their results.
- For references 13, 14, 18, 19: including additional information (such as indicating Zenodo and providing DOIs) would help readers find these references.

Is the rationale for creating the dataset(s) clearly described?
Yes

Are the protocols appropriate and is the work technically sound?
Yes

Are sufficient details of methods and materials provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Frontotemporal dementia, lipid metabolism, antisense oligonucleotides

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 14 September 2023

https://doi.org/10.5256/f1000research.153618.r190842

?

Derek P. Narendra
National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA

The authors have not addressed my concerns.

Most importantly, they have not addressed why specific antibodies that have been well-validated in prior publications, such as the anti-CHCHD10 antibody developed by Atlas (Sigma, Cat#HPA003440), were not included in their comparison. The referenced antibody was validated using human KO cells and mouse lines in our publications and those of others (see for instance, Burstein *et al.*, 2018[1], Huang *et al.*, 2018[2] and Liu *et al.*, 2020[3]).

It is important that the authors acknowledge that other antibodies not included in their analysis have been previously validated in other publications. Their conclusions about what antibody is optimal is biased by the exclusion of these antibodies in their evaluation.

References

1. Burstein SR, Valsecchi F, Kawamata H, Bourens M, et al.: In vitro and in vivo studies of the ALS-FTLD protein CHCHD10 reveal novel mitochondrial topology and protein interactions.*Hum Mol Genet*. 2018; **27** (1): 160-177 PubMed Abstract | Publisher Full Text
2. Huang X, Wu BP, Nguyen D, Liu YT, et al.: CHCHD2 accumulates in distressed mitochondria and facilitates oligomerization of CHCHD10.*Hum Mol Genet*. 2018; **27** (22): 3881-3900 PubMed Abstract | Publisher Full Text
3. Liu YT, Huang X, Nguyen D, Shammas MK, et al.: Loss of CHCHD2 and CHCHD10 activates OMA1 peptidase to disrupt mitochondrial cristae phenocopying patient mutations.*Hum Mol Genet*. 2020; **29** (9): 1547-1567 PubMed Abstract | Publisher Full Text

Is the rationale for creating the dataset(s) clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of methods and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: neurogenetics, mitochondrial biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 1

Reviewer Report 19 July 2023

https://doi.org/10.5256/f1000research.146471.r184169

?

Derek P. Narendra
[1] National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA
[2] National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA

In general, the paper was well-written, and the data included are of high quality.

A major concern is that the choice of antibodies excludes certain well validated commercial antibodies, which may skew the findings to favor those of the author's commercial partners.

There are few suggestions that would make the manuscript stronger.

1. The rationale for choosing the commercial antibodies tested is not clear. The four come from only two suppliers. A notable omission is the the HPA003440 Atlas Antibodies / Sigma-Aldrich antibody, which has been used in several publications, including those that validated in KO cell lines and mouse models. It is not clear why this was not chosen in the head-to-head comparisons. How the antibodies tested were chosen should be discussed as well as the omission of several that have appeared in prior publications.

2. In the second sentence of the introduction, the authors should clarify that CHCHD10 has a

single CHCH domain, which is composed of two CX9C motifs.

3. In the first sentence of the second paragraph, stating that CHCHD10 is important for neuronal survival implies that loss of its function results in neuronal death. This is not clear from the literature. CHCHD10 KO appears to be well-tolerated in mammals, and the mutations are thought to cause disease by a toxic gain-of-function (GoF) mechanism. This is perhaps best demonstrated in studies that have compared CHCHD10 KO mice to those with patient mutations knocked in (PMID: 30877432 and 35700042)[1,2]. This is also discussed in a recent review (PMID: 37021679). The introduction could be modified to clarify this point.

4. Figure 1. It would be helpful to state the concentration of antibody used. A more informative head-to-head comparison might be to keep the concentration of antibody constant rather or in addition to using the supplier's recommended concentration.

5. In Figure 2 the authors should comment on whether it is possible the antibodies would also co-immunoprecipitate CHCHD2. Ideally this would also be assessed experimentally on CHCHD10 KO cells. We have observed cross-reactivity of antibodies for CHCHD2 and CHCHD10 in immunoprecipitation experiments. This affects the interpretation of results as a binding partner attributed to CHCHD10 may actually be binding CHCHD2.

6. For Figure 3, in addition to the shown images it would be helpful to see those windowed for the low intensity signal in the KO cells. This would allow better assessment of what appears to be a mitochondrial signal with some of the antibodies (e.g., MA5-27535 but maybe others). We have observed some cross-reactivity with its paralog CHCHD2. Whether this could be an explanation for the residual mitochondrial signal should be discussed and perhaps evaluated by assessment of double knockout cells.

References

1. Anderson CJ, Bredvik K, Burstein SR, Davis C, et al.: ALS/FTD mutant CHCHD10 mice reveal a tissue-specific toxic gain-of-function and mitochondrial stress response.*Acta Neuropathol*. 2019; **138** (1): 103-121 PubMed Abstract | Publisher Full Text
2. Shammas MK, Huang X, Wu BP, Fessler E, et al.: OMA1 mediates local and global stress responses against protein misfolding in CHCHD10 mitochondrial myopathy.*J Clin Invest*. 2022; **132** (14). PubMed Abstract | Publisher Full Text
3. Shammas MK, Huang TH, Narendra DP: CHCHD2 and CHCHD10-related neurodegeneration: molecular pathogenesis and the path to precision therapy.*Biochem Soc Trans*. 2023; **51** (2): 797-809 PubMed Abstract | Publisher Full Text

Is the rationale for creating the dataset(s) clearly described?

Partly

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of methods and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: neurogenetics, mitochondrial biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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Yang Liu
[1] Chengdu Institute of Biology, University of the Chinese Academy of Sciences, Beijing, Beijing, China
[2] Chengdu Institute of Biology, University of the Chinese Academy of Sciences, Beijing, Beijing, China

Thank you for the opportunity to review the manuscript entitled "The identification of high-performing antibodies for Coiled-coil-helix-coiled-coil-helix domain containing protein 10 (CHCHD10) for use in Western Blot, immunoprecipitation and immunofluorescence ."

Four commercial antibodies for CHCHD10, a mitochondrial protein that affects mitochondrial morphology and DNA integrity, are profiled in the study. Mutations in CHCHD10 have been associated to ALS and FTD. Using a consistent experimental procedure based on comparing read-outs in knockout cell lines and isogenic parental controls, the researchers characterized four CHCHD10 commercial antibodies for Western Blot, immunoprecipitation, and immunofluorescence. The study discovered high-performing antibodies for CHCHD10 and recommends that readers use it as a guide to select the appropriate antibody for their specific needs.

Overall, I find the paper to be well-written and informative. However, I recommend providing more discussion and interpretation of the results. It is best to evaluate the advantages and disadvantages of the following types of antibodies. Thank you for considering my feedback.

Is the rationale for creating the dataset(s) clearly described?
Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of methods and materials provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular mechanisms for infrared sensing in snakes and function & potential medical applications of snake venom.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.