nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Only commercially available software used. All relevant software is indicated in the figure legends and/or Methods section.
Illumina HiSeq4000, Illumina HiSeq2500, Illumina HiSeq2000 from Illumina, San Diego, CA, USA, sequencers were used to generate 100 bp or 150-bp paired-end reads.
Computed tomography data were collected on
Magnetic resonance imaging data were collected on Prisma scanner (Siemens, Erlangen, Germany, TR = 2000 ms, TI = 850 ms, TE = 2·93 ms, acquisition matrix = 256 × 256, spatial resolution = 1·1 mm).
Western blot data were collected on ChemiDoc™ XRS+.
Inorganic phosphate concentration was read in an iMarkTM Microplate Absorbance Reader (Bio-Rad Laboratories) at excitation/emission wavelengths of 544/590 nm.
Microscopy images were acquired with Leica Application Suite X software applying the Lightening module.

Data analysis

All relevant software is indicated in the figure legends and/or Methods section.
Alignment was performed using BWA 2.1 (http://bio-bwa.sourceforge.net/).
Variants were called using the GATK v1.6 Unified Genotyper-based pipeline.
All variants were annotated using ANNOVAR (https://annovar.openbioinformatics.org/en/latest/)
SAMtools v.0.1.18
Genome Aggregation Database v2.1 (gnomAD)
MySQL database of variants called by Manta (Structural variant and indel caller for mapped sequencing data https://github.com/Illumina/manta) and CANVAS (Copy number variant (CNV) calling from DNA sequencing data https://github.com/Illumina/canvas) from Illumina.
Shared regions of homozygosity were identified using HomozygosityMapper (https://www.homozygositymapper.org).

Kinship and relatedness were inspected with Peddy (https://github.com/brentp/peddy) and Automap (https://automap.iob.ch)
Bioinformatical analysis of missense mutants was performed with DynaMut (https://biosig.lab.uq.edu.au/dynamut/).
NAA60 structure was visualized in Edu PyMOL v4.6.0.
Western blots were analyzed with Image lab 6.1.
Microscopy images were processed in ImageJ.
Weighted Gene Co-expression Network Analyses (WGCNA) R package
GTEx V6 bundle
gProfileR (https://biit.cs.ut.ee/gprofiler/gost)
The network diagrams were generated with Cytoscape 3.10
CoExpGTEx (https://github.com/juanbot/CoExpGTEx)
CoExpWeb software tool (https://rytenlab.com/coexp)
We corrected for batch effect using ComBat (https://rdrr.io/bioc/sva/man/ComBat.html)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data generated or analysed during this study and used to produce the plots are included in this published article (and its supplementary information files). Source data are provided with this paper. The raw DNA sequencing data from the cases reported here, computed tomography (CT) and brain magnetic resonance imaging (MRI) are protected and are not available due to data privacy laws. The immunofluorescence and fluorescence microscopy, surface biotinylation, DTNB in vitro peptide Nt-acetylation assay for NAA60, immunoprecipitation and [14-C]-Nt-acetylation assay, quantification of inorganic phosphate and co-expression networks analyses data generated in this study are provided in the Supplementary Information and Source Data file.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	We used the term sex (biological attribute) in the manuscript for reporting the findings. Sex was the only information that was collected and was determined on self-report. we report only the number of male versus female. No disaggregated sex analysis was performed as the sample size was too low to enable meaningful conclusion. Sex was not considered in the study design.
Reporting on race, ethnicity, or other socially relevant groupings	We did not report on race or ethnicity. We only report the country of origin where the case was identified.
Population characteristics	Age was defined by the data reported by patient or their carer. Age was used to establish disease onset, disease duration and time to brain imaging. Sex was determined on self-report. No disaggregated sex analysis was performed as the sample size was too small. Genotypic information was used to correlate with clinical and brain neuroimaging features Neurological examination was described including motor features, cognitive features, psychiatric features.
Recruitment	All patients were recruited by neurologists specialized in rare diseases after assessing them in clinic. Patients with genetically unsolved diagnosis that matched the clinical phenotype where recruited. There is no self-selection bias. All participants signed informed consent after receiving all information regarding the research project and having had extended opportunities to consider and ask questions about the research. We first screened with whole exome sequencing a cohort at UCL, comprising 78 cases from 53 families with PFBC who were negative for pathogenic variants in genes already linked to PFBC (SLC20A2, PDGFB, PDGFRβ, XPR1, MYORG, JAM2). We identified NAA60 biallelic variants co-segregating with the disease in one family (F1) through a combination of whole-exome sequencing (WES) and homozygosity mapping. Then we screened for NAA60 variants in WES data from two French brain calcification series, the pan-European SolveRD project, unsolved rare disease cohorts from Turkey and Saudi Arabia. Finally we screened the rare diseases cohort from the 100,000 Genomes Project22 that had whole genome sequencing (WGS) performed and data filtered as per protocol. As a result of this extensive screening, we found deleterious bi-allelic variants in NAA60 associated with PFBC in 8 more patients from 6 unrelated families with autosomal recessive inheritance (F2-F7).
Ethics oversight	The individuals included in this study were recruited along with unaffected family members with informed consent under ethics-approved research protocols (UCLH: 04/N034; Rouen: RBM-0259 approved by the CPP Ile de France II ethics committee, and CERDE (notification E2023-40, Rouen University Hospital) approved the retrospective analysis of genomic and phenotypic data; Comité de Protection des Personnes ID-RCB/EUDRACT: 2014-A01017-40); Saudi Arabia: KFSHRC IRB (RAC# 2121053)). The 100,000 Genomes Project Protocol has ethical approval from the HRA Committee East of England – Cambridge South (REC Ref 14/EE/1112).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size is indicated in the figure legend and /or in Methods. No statistical method was used to pre-determine samples size since the reported NAA60-related condition has never been reported before we could not estimate the size in advance because of lack of prior data.. The sample size is sufficient as we report 10 patients from 7 unrelated families with a unifying clinical feature- brain calcification and a similar molecular impairement- reduced NAA60 N-terminal-acetylation.
Data exclusions	All technically sound data were included.
Replication	We reported biological repeats as "n" values and technical repeats as " in duplicates or triplicates" in the Figure legends.
Randomization	Due to the nature of experiments/analysis, samples were not randomized.
Blinding	We did not blind the operators during the analysis since they analyzed their own data .To perform the analysis, we had to know the phenotype of individuals (i.e. whether an individual is affected or not).

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	(1) We listed the detailed information of the primary and secondary antibodies in the method section (2) The information of antibody used is also listed below Primary antibodies: rabbit anti-GPP130 (Covance, PRB144C), rabbit anti-GM130 [EP892Y] (Abcam, ab52649) used at 1:100, mouse anti-FLAG M2 (Sigma, F1804) used at 1:1000 for WB and 1:100 for IF, mouse anti-GAPDH (Santa Cruz Biotechnology, Sc-47724) used at 1:5000, rabbit anti-NAA60 polyclonal (BioGenes custom made Arnesenlab) used at 1:500, rabbit anti-actin (Sigma, A2066), rabbit anti-SLC20A2 (Abcam, ab191182). As the immunogen used to generate the SLC20A2 antibody maps to a region of SLC20A2 that has some sequence match with SLC20A1, it cannot be excluded that this antibody could also have reactivity towards SLC20A1 which has a very similar molecular weight. Secondary antibodies: All secondary antibodies conjugated to fluorescent dyes were purchased from Molecular Probes (Eugene, OR, USA) and used at 1:100. Secondary antibodies for western blot were ECL™ Anti-Rabbit IgG HRP Linked Whole antibody (Cytiva Life science, NA934) used at 1:3000 and ECL™ Anti-Mouse IgG HRP Linked Whole antibody (Cytiva Lifescience, NA931V) used at 1:3000.
Validation	We validated antibodies according to their molecular weight on our western blots and their correct subcellular localization in immunofluorescence assays. Additionally, some antibodies were KO-validated on WB (anti-NAA60, Figure 3d). Moreover we refer to the validation statements on the manufacturer's websites: - rabbit anti-GM130 [EP892Y] https://www.abcam.com/en-no/products/primary-antibodies/gm130-antibody-ep892y-cis-golgi-marker-ab52649 - mouse anti-FLAG M2 https://www.sigmaaldrich.com/NO/en/product/sigma/f1804?gclid=CjwKCAiAmsurBhBvEiwA6e-WPAc-eBfBDBUHud9N5J2v-DDx9SgbrtUV3tF0IaFG2eooeEXrXLiIRRoCsQsQAvD_BwE - mouse anti-GAPDH https://www.scbt.com/p/gapdh-antibody-0411 - rabbit anti-NAA60 polyclonal (BioGenes custom made Arnesenlab, KO-validated in Fig 3d)

- rabbit anti-actin https://www.sigmaaldrich.com/NO/en/product/sigma/a2066
- rabbit anti-SLC20A2 https://www.abcam.com/en-no/products/primary-antibodies/slc20a2-pit2-antibody-ab191182

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HeLa CCL-2 (sex: female) were obtained from ATCC. HAP1 WT cells (clone C631; sex: male; RRID:CVCL Y019) and the HAP1 gene-KO cell lines NAA60 KO (HZGHC003172c010) and NAA80 KO (HZGHC003171c012) were obtained from Horizon Discovery. RPE1 cells (CRL-4000; sex: female; hTERT immortalized) were obtained from ATCC. HEK293T used for protein production were obtained from Sigma (12022001).
Authentication	HAP1 cell lines were verified for the relevant protein knockout by Sanger sequencing and Western blot. All HAP1 cells were quality controlled according to PMID: 33184093. Primary cells were validated for their genotype by western blot (Fig 3d). All cell lines were inspected for morphology being stable, heterogenous and in agreement with literature and images on manufacturer websites. No further authentication was performed and was not deemed neccessary.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination by DAPI staining.
Commonly misidentified lines (See ICLAC register)	HeLa cells were used for initial investigation of localization of ectopically expressed recombinant proteins, and results were subsequently verified in RPE-1 cells.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*