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Two Novel Mutations in *ABHD12*: Expansion of the Mutation Spectrum in PHARC and Assessment of their Functional Effects

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Abstract

PHARC (Polyneuropathy, Hearing loss, Ataxia, Retinitis pigmentosa, and Cataracts) is a recently described autosomal recessive neurodegenerative disease caused by mutations in the α - β -hydrolase domain-containing 12 gene (*ABHD12*). Only five homozygous *ABHD12* mutations have been reported and the pathogenesis of PHARC remains unclear. We evaluated a woman who manifested short stature as well as the typical features of PHARC. Sequence analysis of *ABHD12* revealed a novel heterozygous c.1129A>T (p.Lys377X) mutation. Targeted comparative genomic hybridization detected a 59 kb deletion that encompasses exon 1 of *ABHD12* and exons 1–4 of an adjacent gene, *GINS1*, and includes the promoters of both genes. The heterozygous deletion was also carried by the patient's asymptomatic mother. qRT-PCR demonstrated ~50% decreased expression of *ABHD12* RNA in lymphoblastoid cell lines from both individuals. Activity-based protein profiling of serine hydrolases revealed absence of *ABHD12* hydrolase activity in the patient and 50% reduction in her mother. This is the first report of compound heterozygosity in

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PHARC and the first study to describe how a mutation might affect ABHD12 expression and function. The possible involvement of haploinsufficiency for GINS1, a DNA replication complex protein, in the short stature of the patient and her mother requires further studies.

Keywords

PHARC; ABHD12; GINS1; short stature; endocannabinoid; hydrolase activity

Introduction

PHARC is a recently described autosomal recessive neurodegenerative disease of Polyneuropathy, Hearing loss, Ataxia, Retinitis pigmentosa and Cataract (PHARC; MIM# 612674) [Fiskerstrand et al., 2009] that is caused by mutations in the α - β -hydrolase domain-containing 12 gene (*ABHD12*; Gene ID 26090; MIM# 613599). [Fiskerstrand et al., 2010]. The first study reported four homozygous mutations in nine families and subsequently a different homozygous mutation in *ABHD12* was detected in a family with progressive hearing loss, RP and cataracts, initially clinically diagnosed with Usher syndrome [Eisenberger et al., 2012]. Reexamination of one affected member of this family revealed ataxia but not polyneuropathy, a demonstration of both the phenotypic heterogeneity in PHARC and the need for careful neurological assessments to distinguish this disease from other neuropathic disorders.

All five published PHARC mutations result in premature stop codons and are presumed, but not corroborated, to cause loss of function. Heterozygous carriers are all clinically asymptomatic, consistent with recessive inheritance and implying that a single functional copy of *ABHD12* produces sufficient enzyme activity to prevent each feature of PHARC. We now report molecular genetic and functional studies of new *ABHD12* mutations in a patient with PHARC.

Materials and Methods

Subjects

Under a protocol approved by the University of Washington's (UW) Institutional Review Board, informed consent was obtained, clinical evaluations were performed in the UW Genetic Medicine Clinic and blood samples were obtained from a 29-year-old woman with slowly progressive peripheral neuropathy, her mother and her maternal half-brother. The pedigree is shown in Figure 1A.

Mutation Detection in *ABHD12*

DNA was extracted from peripheral blood by standard methods. Fragments encompassing each of the 13 coding exons of *ABHD12* (RefSeq NM_001042472.1) and their corresponding splice junctions were amplified using forward and reverse primer pairs shown in Supp. Table S1 and sequenced with the same forward primers as in the PCR amplification as previously described [Chen et al. 2010]. To confirm the mutation, exon 12 was also sequenced in reverse.

Copy number analysis was carried out in genomic DNA of the patient, her mother and two normal controls using the TaqMan Copy Number Assay (Applied Biosystems, Carlsbad, CA) with primer/probe sets in intron 1 (Hs07223109_cn), exon 6 (Hs01071795_cn), exon 9 (Hs00901943_cn) and exon 12 (Hs01593340_cn). We also designed the assay targeting the known region in intron 1 that has only one allele, evidenced by a SNP in this fragment that is not shared by the patient and her mother. Primers and the probe of this custom assay are given in Supp. Table S2. All the assays included an internal reference probe RNase P and were performed per the manufacturer's protocol. The reactions were run on an ABI 7300 Real-Time PCR system and the data were analyzed using CopyCaller software (Applied Biosystems).

The *ABHD12* deletion boundaries were determined by array comparative genomic hybridization (CGH) using DNA from the patient and her mother. Briefly, subject DNA was labeled using Cy3-tagged random primers and DNA from a single male reference individual (Coriell NA15724) was labeled using Cy5-tagged random primers. Equal amounts of labeled subject and reference DNA were co-hybridized to a commercially available oligonucleotide array (SurePrint G3 1M Human CGH Microarray, Agilent Technologies, Santa Clara, CA) containing one million probes with mean probe spacing ~3 kb. Data were analyzed using Agilent's Genomics Workbench software per the manufacturer's instructions.

To define the breakpoints of the deletion, nested PCR was performed using primers located outside the estimated deletion ranges given by CGH array. TaKaRa Ex Taq polymerase (Millipore, Billerica, MA) was used in a first round of PCR using an outside primer set, followed by a second round of PCR using 1 µl of the product from the initial PCR and an internal primer pair set. The products were directly sequenced using a series of primers to successively approach the breakpoints. Primer sequences and conditions are given in Supp. Table S1. The identified mutations have been submitted to a LSDB at <http://databases.lovd.nl/shared/genes>

RNA transcription of mutant *ABHD12*

RNA from 1×10^6 lymphoblastoid cells of each subject in the PHARC family and three unrelated normal controls was extracted using a Qiagen RNeasy kit (Qiagen, Venlo, Netherlands) and cDNA was synthesized using a Superscript III first-strand synthesis kit (Life Technology, Carlsbad, CA) with 500 ng RNA and oligo(dT) primer. PCR amplification of a cDNA fragment of exons 10–13 encompassing the c.1129A>T mutation was performed with the primers and the conditions listed in Supp. Table S1. The PCR product was subjected to direct sequencing.

ABHD12 RNA expression by real-time reverse transcription-PCR (qRT-PCR)

RNA (100 ng) from three subjects in the PHARC family and three unrelated normal controls was used for one-step TaqMan® qRT-PCR reactions with a RNA Master Hydrolysis Probes system (Roche, Basel, Switzerland). To increase the likelihood of obtaining robust results, two sets of primers-probe, designed from Universal Probe Library by Roche, were used to amplify the gene fragments. Primers and probes sequences and conditions for qPCR analysis

are given in Supp. Table S3. Data were normalized to a geometric mean of three housekeeping genes (*GAPDH*, *HPRT*, *YWHAZ*), and analyzed using a 2-*c*(t) method. A total of 4–5 replicates were analyzed from each lymphoblastoid line.

Detection of ABHD12 protein by immunoblotting

Lymphoblastoid cells (10×10^6) from the PHARC family and homogenized brain tissue (~30 g) from an ABHD12 knockout mouse (from Dr. Cravatt's laboratory [Blankman et al. 2013]) were lysed in 2% SDS followed by sonication to obtain whole-cell lysate. Aliquots of the supernatant were fractionated on 4–12% Bis-Tris polyacrylamide gels (Life Technologies) under reducing condition and transferred to PDVF membranes (Life Technologies). We tested several anti-ABHD12 antibodies to evaluate their specificity. After treatment for 30 min in SuperBlock Buffers (Thermo Scientific, Waltham, MA), the membranes were separately probed overnight at 4°C with 1:1000 goat anti-ABHD12 (Thermo Scientific), 1:1000 goat anti-ABHD12 (abcam, Cambridge, MA) and 1:500 rabbit anti-ABHD12 (Santa Cruz Biotechnology, Santa Cruz, CA) in SuperBlock Buffer. GAPDH antibody (Sigma Aldrich, St Louis, MO) was also probed at 1:10,000 as an internal control. The membranes then were incubated with 1:2000 HRP-conjugated secondary antibodies (Santa Cruz Biotechnology) against the species corresponding to each primary antibody. The protein bands were visualized by enhanced chemiluminescence (Thermo Scientific) on a BioChemi digital imaging system (UVP, Upland, CA) and quantitated by densitometry using Labworks software (UVP).

Activity-based protein profiling analysis

Lymphoblastoid cells from three subjects in PHARC family and two controls were lysed in PBS by sonication. The lysates were centrifuged at $100,000 \times g$ for 45 min at 4°C and the supernatant was saved as the soluble proteome. The pellet was washed and resuspended in PBS, sonicated, and saved as the membrane proteome. Protein concentration was determined with the Dc Protein Assay kit (Bio-Rad, Hercules, CA). Activity-based protein profiling (ABPP) was performed essentially as described [Blankman et al., 2007]. Soluble and membrane proteomes (50 µg in PBS buffer) were pre-treated with 20 µM tetrahydropyridine (THL, Orlistat®, Sigma-Aldrich) or DMSO vehicle for 30 min at 37°C and then incubated with 2 µM FP-rhodamine [Patricelli et al., 2011] for 1 hr at 25°C. Deglycosylation was achieved with PNGaseF (New England Biolabs, Ipswich, MA) treatment for 45 min and reactions were quenched with 2× SDS/PAGE reducing loading buffer. Products were separated by SDS/PAGE (10% acrylamide) and visualized in-gel with a Bio-Rad ChemiDoc™ XRS fluorescence imager. ABHD12 band intensity was quantified with ImageJ software (NIH). Results represent the background-corrected average band intensity minus the corresponding signal in the THL-treated samples $\pm$ SEM for three independent replicates per cell line. Statistical analysis was by Student's *t*-test.

Results

Clinical phenotypes and evaluation

A 29-year-old woman was born with bilateral foot deformities requiring corrective casts and braces during infancy and early childhood. She underwent bilateral pes cavus arch and

tendon transfer surgery at age 12 years. She has a bilateral steppage gait with decreased temperature and vibratory sense in a stocking/glove distribution and absent deep tendon reflexes with flexor plantar response. Her peripheral neuropathy slowly progressed but she remains ambulatory. At age 27 needle EMG was normal but nerve conduction studies revealed severe demyelinating motor and sensory peripheral neuropathy with slow conduction velocities in upper and lower extremities (17–33 meters per second in the motor peroneal and ulnar nerves and 28–33 meters per second in the sensory sural and ulnar nerves).

She was found to have 25% bilateral hearing loss during a routine screen in kindergarten. The hearing loss was severe by age 18 and she had bilateral cochlear transplants at 21. Bilateral cataract surgery was performed at age 21. An electroretinogram at age 22 revealed mild bilateral rod dysfunction with preserved cone function, suggestive of Usher's syndrome. Pattern reversal evoked potentials were normal. Ophthalmologic exam revealed stippled changes in the retina, as well as bilateral chronic cystoid macular edema on optical coherence tomogram (OCT) that has not responded to steroid treatment (Supp. Figure S1). Visual acuity is 20/80 in the right eye and 20/70 in the left eye. She has full extra ocular movements with smooth gaze pursuits and no nystagmus. Mild dysmetria is elicited with finger to nose and heel to shin testing. There is no bradykinesia or dysidiadochokinesia with rapid alternating movements. She has a stable wide-based gait and a negative Romberg test. CT and MRI brain imaging were normal. Despite her physical and sensory limitations, she completed high school and attended community college.

The patient is of German and British ancestry and her family history is negative for neuropathy or other PHARC related symptoms. Both the patient and her mother have short stature, 4'11" and 5'0" tall respectively, with normal proportions. The maternal half-brother is 5'11" tall. The patient's father was not available for examination but was reported to be 5'5" tall.

Genetic testing was negative for dominant and recessive forms of CMT, including *PM22*, *GAP1*, *PRX*, *EGR2*, *SH3TC2*, *NDRG1* and *connexin32*, for a UW mitochondrial panel, including NARP, MELAS, MERRF, Leigh syndrome and LHON, and for the Harvard Genetics Lab Otochip 19-genes hearing loss panel. Peroxisome function testing for Refsum disease was normal.

The combination of typical neurologic features and negative biochemical and molecular testing for alternative disorders was strongly suggestive of PHARC. To confirm this clinical diagnosis, we screened the patient's DNA for mutations in *ABHD12*, the gene for PHARC.

Identification of mutations in *ABHD12*

PCR-based sequencing of all 13 coding exons of *ABHD12* identified a heterozygous A to T transversion at nucleotide 1129 in exon 12 that predicts substitution of a stop codon for lysine in residue 377 (c.1129 A>T; p.Lys377*) (Supp. Figure S2A). This nonsense mutation was not present in dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP/>) or the 1000 Genome Project database (<http://www.1000genomes.org/>). The p.Lys377* mutation was not found in the patient's mother or brother. No other pathologic change was found in the patient.

Because PHARC is autosomal recessive, we presumed that a second *ABHD12* mutation was missed by exon sequencing. Further analysis of sequencing results revealed lack of heterozygosity in exons 1–6 of the patient and her mother, and at least one SNP genotype in exon 1 was not shared by the patient and her mother. We assumed that the patient only carries the paternal allele in this region and that there is a large deletion in the maternal allele. TaqMan Copy Number Assays with probes located in intron 1 revealed a copy number less than 2 in the patient and her mother (0.9 and 1.2 respectively), while assays using probes in exons 6, 9, and 12 showed copy numbers of 2 in both subjects. This confirmed a deletion at least encompassing exon 1 and intron 1.

To determine the deletion boundaries, we used array CGH to detect CNVs in *ABHD12*. Compared to controls, the patient and her mother both had loss of one copy in a region of ~ 62 Kb between intron 1 of *ABHD12* and exon 4 of the neighboring gene *GINS1* (Figure 1B). The orientation of *ABHD12* is 3′–5′, while *GINS1* is 5′–3′. The 5′ breakpoint of the deletion lies between nucleotide positions g. 20:25,338,399 and 20:25,340,800 (based on UCSC hg19) corresponding to intron 1 of *ABHD12*, while the 3′ breakpoint lies between g. 20:25,397,825 and 20:25,402,367 in intron 4 of *GINS1*. Finer resolution by long-range PCR and successive sequencing placed the breakpoints at 20:25,340,670 and 20:25,399,884 for a precise deletion size of 59,214 bp (Figure 1B; Supp. Figure S2B). In place of the deletion, there is a 97 bp insertion. Blast analysis of the fragment disclosed it consisted of 62 bp and 21 bp fragments of *GINS1* intron 1 and 14 bp of unspecific DNA (Supp. Fig S2B). The mutational mechanism of this complex genomic rearrangement is unknown, but the presence of a long interspersed element (LINE) close to the end of the deletion raises the possibility of LINE-mediated recombination. The deletion removes exon 1 of *ABHD12*, exons 1–4 of *GINS1*, and both promoters.

The RT-PCR amplicon of the exon 10–13 cDNA fragment encompassing the c.1129A>T mutation contained only T at nucleotide 1129 (Supp. Figure S2C), indicating that the paternal allele with this nonsense mutation can be transcribed, whereas the gross deletion containing promoters of *ABHD12* and *GINS1* on the maternal chromosome disrupts RNA expression.

RNA expression of *ABHD12* is decreased in the subjects

To determine if the *ABHD12* mutations affect its transcription, *ABHD12* mRNA from lymphoblastoid cells of the patient, her mother and her half-brother was amplified by qRT-PCR. We used two primer-probe sets in *ABHD12* to verify the consistency of the qRT-PCR results. *ABHD12* RNA levels were decreased by approximately 50% in the patient and her mother, but not in the half-brother, compared with controls when analyzed by one-way ANOVA ($F_{(3,16)}=3.72$, $p=0.03$; $F_{(3,15)}=9.30$, $p=0.001$ for F31 and F71 primers, respectively) and by Fisher's *t*-test (Figure 2). Similarly, using two primers-probe sets in *GINS1*, we found that *GINS1* RNA was also reduced by approximately 50% in the patient and the mother, but not the brother when analyzed by one-way ANOVA ($F_{(2,12)}=3.94$, $p=0.04$ and $F_{(3,16)}=4.59$, $p=0.02$, for F46 and F52 primer sets, respectively) and by *t*-test (Figure 2). Remarkably RNA levels in the patient and her mother reached similar levels, indicating that the reductions of RNA are a result of the gross deletion that is carried by both, and that the

paternal allele with the nonsense mutation carried only by the patient but not her mother produces stable RNA. To determine if ABHD12 protein expression was affected, we performed western blot analysis using several available ABHD12 antibodies. Bands at the expected size of ABHD12 were observed with proteins from lymphoblastoid cells but were also present in proteins from ABHD12 knockout-mice brain, suggesting that these antibodies lack specificity (data not shown).

Activity-based protein profiling (ABPP) reveals absence of ABHD12 activity in the patient

To assess the effect of the *ABHD12* mutations on ABHD12 activity, we utilized the functional proteomic strategy ABPP with the fluorophosphonate (FP)-rhodamine probe that reacts selectively with serine hydrolase enzymes [Simon and Cravatt 2010]. ABPP analysis on proteomes prepared from lymphoblastoid cells derived from the patient, her mother, her half-brother and two controls identified an activity that migrated at ~45 kDa (Figure 3A), was present only in the membrane proteome, and was sensitive to PNGaseF digestion, matching the previous characterization of ABHD12 [Blankman et al., 2007]. Importantly, this activity was blocked by pretreatment with the lipase inhibitor THL, which inhibits ABHD12 [Blankman et al., 2007; Hoover et al., 2008], supporting the annotation of this activity as ABHD12. Qualitative and quantitative assessment of the ABPP gel profiles revealed a clear lack of ABHD12 activity in the patient-derived lymphoblastoid proteomes and a marked reduction to 49.9% of the normal level in the proteomes obtained from her mother (Figure 3A–B).

Discussion

In this study, we describe a young woman with many clinical features of PHARC. Given the observed phenotypic variability, even within a family, in other PHARC cases [Fiskerstrand et al., 2009; 2010; Eisenberger et al., 2012], the lack of cerebellar atrophy on MRI in our patient is not surprising and, in fact, the onset of ataxia is the most highly variable among the symptoms in this syndrome. Her gait ataxia likely results from her peripheral neuropathy. Our identification of two novel *ABHD12* mutations in this patient is the first report of compound heterozygosity causing PHARC. Disease onset, severity and progression in our patient are similar to the five previously reported cases with homozygous mutations. The clinically asymptomatic mother is a heterozygous carrier of the gross deletion. We show that RNA expression, as predicted, was eliminated from the maternal allele with the deletion of the promoter, while RNA expressed from the allele containing the nonsense mutation remained at a close to normal level. This absence of nonsense-mediated decay (NMD) of the mRNA is likely due to the location of the mutation close to the C-terminus of the gene. NMD is a surveillance mechanism for degradation of transcripts carrying premature termination codons (PTC) to limit production of truncated and potentially harmful proteins [Hentze and Kulozik 1999]. Although the majority of PTCs are processed to NMD, those locate near exon junctions or are at the 3' end of the coding region will not be recognized and will evade NMD [Amrani et al 2004; Singh et al., 2008]. These NMD resistant mutations may produce truncated proteins that have dominant-negative or gain-of-function effects [reviewed in Bhuvanagiri et al., 2010]. Alternatively, truncated proteins may be highly unstable and functionally identical to NMD [Khan et al., 2006;

Anczukow et al., 2008]. Unfortunately, because the lack of specific antibodies to ABHD12 protein hampered western blot analysis, we were not able to characterize the expression of the presumably truncated ABHD12 protein. However, the similarity of symptoms in our patient and other PHARC patients argues against a gain-of-function effect of the truncated protein, if these fragments are indeed produced and stable. One limitation to the interpretation is that we do not know the neurologic status of our patient's father who is the presumptive carrier of the NMD-resistant mutation. Our study provides the first quantification of RNA expression of *ABHD12* mutants. It would be interesting to carry out similar studies of RNA expression and NMD, and – when possible – protein expression for other mutations in *ABHD12* identified in patients with PHARC.

We demonstrated absence of detectable ABHD12 activity in the patient-derived lymphoblastoid cells and reduced ABHD12 activity in cells from the mother. The heterozygous carrier of the deletion that diminishes RNA expression from one allele and retains only about 50% activity does not manifest an obvious phenotype related to PHARC, supporting haplo-sufficient function of ABHD12, in agreement with the recessive inheritance pattern of PHARC. This is the first report of the effect of PHARC-related mutations on ABHD12 function. Our results support an essential role for ABHD12 catalytic activity in the maintenance of peripheral and central nervous system function. ABHD12 knockout (*ABHD12^{-/-}*) mice develop several PHARC-related phenotypes at an advanced age, including defective auditory function and motor behavior [Blankman et al., 2013]. Metabolomic characterization of the *ABHD12^{-/-}* mouse brain revealed that lysophosphatidylserine (LPS) is an endogenous substrate for ABHD12 as levels of several LPS species were dramatically elevated following genetic ablation of ABHD12, suggesting that deregulation of LPS pathways may contribute to the pathogenesis of PHARC. ABHD12 hydrolyzes 2-arachidonoylglycerol (2-AG) *in vitro* [Blankman et al., 2007; Navia-Paldanius et al., 2012] and is responsible for a small fraction of the 2-AG hydrolase activity in mouse brain membrane homogenates [Blankman et al., 2007]. In brain, two other serine hydrolases, monoacylglycerol lipase (MAGL) and α - β -hydrolase domain-containing 6 (ABHD6) account for the remaining brain 2-AG hydrolase activity [Blankman et al., 2007; Marrs et al., 2010]. Although total brain 2-AG levels are unaltered in *ABHD12^{-/-}* mice [Blankman et al., 2013], it remains possible that altered 2-AG metabolism and signaling in specific brain regions or cell types may also contribute to PHARC. One cell type of interest is microglia, which constitute only a small fraction of the total brain mass but highly express ABHD12 [Fiskerstrand et al., 2010]. Accumulating lines of evidence support the role of microglial activation and neuroinflammation in neurodegenerative diseases [reviewed in Colton 2013]. Endocannabinoids have been implicated in microglia-mediated neuroinflammation [Stella 2009; Nomura et al., 2011] and loss of ABHD12 activity in these immune cells could contribute to this process [Blankman et al., 2013]. Future research aimed at elucidating which activities of ABHD12 are required for normal nervous system function and how loss of that function results in PHARC may reveal potential therapeutic strategies for treatment of the disease.

It is worth commenting on the possibility of a GINS-haploinsufficiency effect in the patient and her mother. The GINS complex, a heterotetramer of GINS1, GINS2, GINS3 and

GIN54, plays an essential role in the initiation of DNA replication and progression of DNA replication forks in eukaryotic genomes [Boos et al., 2012; Onesti and Macneill 2013]. The gene and its function are poorly characterized; it has been hypothesized that it or its subunit PSF1 may play a role in tumor growth [Ryu et al., 2007; Nagahama et al., 2010]. No mutations of this gene have been associated with human disease. Our finding that the patient and her mother only have 50% of *GIN51* RNA expression compared to normal controls implies that there is no expression from the allele that contains the promoter deletion. To investigate a possible phenotype related to the deletion, we reviewed the clinical manifestations in the family and the only remarkable finding common to the patient and her mother and not present in other PHARC cases is their short stature. Both women are below the 5th percentile for height in Caucasian women while the brother who does not have the *GIN51* deletion has normal height. The possible association of *GIN51* haploinsufficiency and short stature of the patient and her mother requires further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

ABHD12 mutations in patients with PHARC. A. Pedigree of a patient with PHARC. Ages, heights and mutations of ABHD12 are indicated under the pedigree symbols for three tested individuals. ★: DNA was screened for ABHD12 mutations. ca = death from cancer. B. Identification and precise delineation of a 59kb deletion in the patient and her mother by CGH array and PCR-sequencing. The deletion removes exon 1 of ABHD12 and exons 1–4 of GINS1 and both promoters. A 97 bp fragment comprised of 2 small pieces of intron 1 of GINS1 and an unspecific segment of DNA is inserted, the sequencing chromatograph shown

in Supp. Figure S2B. C. Exonic organization of ABHD12 and mutations in PHARC. ❖: Compound heterozygous mutations identified in this study. The detection of the nonsense mutation p.Lys377* is shown in Supp. Figure S2A. Others are published mutations. Nucleotide numbering reflects cDNA with +1 corresponding to the A of the ATG translation initiation codon in the ABHD12 NM_001042472.1, and GINS1 NM_021067.3. The mutations nomenclature follows the instruction given in HGVS (<http://www.hgvs.org/mutnomen>).

A

B

Figure 2.

Decreased RNA expression of ABHD12 in the lymphoblastoid line in a family with PHARC. Quantitative RT-PCR (qRT-PCR) analysis of ABHD12 (A, with two independent PCR primers-probe sets) and GINS1 (B, with two independent PCR primers-probe sets) on RNA isolated from the lymphoblastoid cell lines of the patient, her mother, her half-brother, and controls. RNA transcripts of both ABHD12 and GINS1 were significantly reduced in the patient and the mother, but not the brother. No difference in RNA expression of these two genes was identified between the patient and her mother. Data were the average of N = 4 replicates per cell line. Error bars depict standard error. Post-hoc tests were conducted using Fisher's t-test, and are indicated as either * or **, for $p < 0.05$ and $p < 0.01$, respectively.

Figure 3.

ABHD12 activity is absent in PHARC patient-derived lymphoblastoid cells as determined by activity-based protein profiling. A. Soluble and membrane proteomes prepared from lymphoblastoid cells from the patient with PHARC (Pt), the mother (Mo), the brother (Bro) and two normal controls (C1 and C2) were labeled with the activity-based fluorophosphonate (FP)-rhodamine probe, deglycosylated by PNGaseF treatment, and analyzed by SDS-PAGE. Membrane proteomes pre-treated with 20 μ M tetrahydrolipstatin (THL) were subjected to the same procedure. Fluorescence was visualized with a Bio-Rad ChemiDoc gel scanner and is shown in gray scale. B. Quantitation of the ABHD12 band fluorescence intensity was performed with the ImageJ software (NIH). Data represent background-corrected average values of $N = 3$ replicates per cell line $\pm$ SEM. *** $P < 0.001$; ** $P < 0.01$; ns, not significant, as compared to control sample C1 by the Student's t-test.