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Do *PACS1* variants impeding adaptor protein binding predispose to syndromic intellectual disability?

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Abstract

To date, *PACS1*-neurodevelopmental disorder (*PACS1*-NDD) has been associated with recurrent variation of Arg203 and is considered diagnostic of *PACS1*-NDD, an autosomal dominant syndromic intellectual disability disorder. Although incompletely defined, the proposed disease mechanism for this variant is altered PACS1 affinity for its client proteins. Given this proposed mechanism, we hypothesized that *PACS1* variants that interfere with binding of adaptor proteins might also give rise to syndromic intellectual disability. Herein we report a proposita and her mother with phenotypic features overlapping *PACS1*-NDD and a novel *PACS1* variant (NM_018026.3:c.[755C>T];[=], p.(Ser252Phe)) that impedes binding of the adaptor protein GGA3 (Golgi-associated, gamma-adaptin ear-containing, ARF-binding protein 3). We hypothesize that attenuating PACS1 binding of GGA3 also gives rise to a disorder with features overlapping those of PACS1-NDD. This observation better delineates the mechanism by which *PACS1* variation predisposes to syndromic intellectual disability.

Keywords

PACS-1; Learning disability; CK2 (casein kinase 2); protein trafficking; membrane trafficking

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Author contribution statement: CFB, H-KL, and DH phenotyped the proposita and arranged testing. LL provided Virtual Geneticist analysis. AM-H, DH, and CFB collaboratively created the concept and content of the manuscript. GT, JBL, YY, and KC performed the co-immunoprecipitation. AM-H, DH, CFB, GT, H-KL, , LL, and YY contributed to editing of the manuscript.

Conflict of Interests: There are no conflicts of interest to declare.

INTRODUCTION

Intellectual disability (ID), a genetically and phenotypically heterogeneous group of conditions, affects 1–3% of the population. Despite recent progress, the pathogenicity of novel variants, even within established ID genes, is difficult to interpret in the absence of robust functional studies (Ilyas et al., 2020)

Among the homeostatic disturbances contributing to ID are perturbations of protein and membrane trafficking. A player in such trafficking is *PACS1* (Figure 1A), which encodes the phosphofurin acidic cluster sorting protein-1 (OMIM #607492) that is involved in the localization of trans-Golgi network (TGN) membrane proteins that contain acidic cluster sorting motifs (Thomas et al., 2017). PACS1, which is expressed at high levels in the brain during the embryonic period and down regulated in the postnatal period (Liu et al., 2021), mediates protein trafficking from endosomes to the TGN or to the cell surface (Scott et al., 2006). It also has roles in cellular apoptosis, genomic stability, and calcium flux in the endoplasmic reticulum (Arnedo et al., 2022; Thomas et al., 2017). Interacting with acidic clusters that can be phosphorylated by CK2 (casein kinase 2), PACS1 binds to CK2, client proteins, and adaptor proteins, facilitates client and adaptor protein phosphorylation by CK2, and activates CK2 through binding of the CK2 beta subunit (Scott et al., 2006; Thomas et al., 2017). PACS1 binding affinity is regulated by CK2 phosphorylation of an autoregulatory site Ser^{278} within the PACS1 acidic domain (Scott et al., 2006).

PACS1 modulates protein and membrane trafficking through interaction with the clathrin adaptors AP (adaptor-related protein complexes)-1 and AP-3 and the monomeric adaptor GGA3 (Golgi-associated, gamma-adaptin ear-containing, ARF-binding protein 3) (Thomas et al., 2017). Through these interactions, it mediates localization of furin and other client proteins to the TGN and targets a subset of client proteins to the primary cilium, including the adaptor protein nephrocystin and the olfactory cyclic-nucleotide-gated ion channel (Thomas et al., 2017).

Alteration of a single amino acid within PACS1 (NP_060496.2:p.Arg203) has been associated with the autosomal dominant syndromic intellectual disability disorder, *PACS1*-neurodevelopmental disorder (PACS1 neurodevelopmental disorder -NDD, OMIM #615009) (Schuurs-Hoeijmakers et al., 2012; Seto et al., 2021; Stern et al., 2017; Tenorio-Castano et al., 2021; Van Nuland et al., 2021). Residing adjacent to the CK2 binding site (Arg196_Tyr200, Figure 1A), Arg203Trp alters PACS1 affinity for client proteins (GT, unpublished data) and likely alters CK2 phosphorylation of affected client proteins and PACS1-facilitated interaction of affected client proteins with adaptor proteins. The mechanism by which Arg203Trp causes disease is a gain-of-function rather than a loss-of-function such as haploinsufficiency (Liu et al., 2021; Schuurs-Hoeijmakers et al., 2012).

Variation deleterious to components of the PACS1 complex also cause disease (Table S1). Pathogenic CK2 subunit variants cause autosomal dominant ID disorders (Asif et al., 2022; Caefer et al., 2022). Pathogenic variation in the adaptor proteins nephrocystin, AP-1, and AP-3 cause autosomal recessive ID disorders (Huizing et al., 2002; Konig et al., 2017; Martinelli et al., 2013; Usmani et al., 2021).

We hypothesized that *PACS1* variants impeding the binding of adaptor proteins to PACS1 give rise to intellectual disability. We report a proband and mother with cognitive dysfunction and a novel *PACS1* variant (NM_018026.3:c.[755C>T];[=], p.(Ser252Phe)) that impedes GGA3 binding (Figure 1A).

MATERIALS AND METHODS

Clinical Report

The proposita, age 5 years, presented with global developmental delay, short stature, bilateral hip dysplasia, feeding difficulties, and behavioral problems (Individual III-1, Figure 1A). Her mother (Individual II-4, Figure 1A), age 43 years, presented with historical and ongoing learning difficulties, anxiety, and depression. The proposita and her mother were of Chinese extraction. No other family members had a learning disability or intellectual disability.

Individual III-1—The proposita was born at 38 weeks gestational age via caesarean section for breech presentation, oligohydramnios, and mild preeclampsia. She had prenatal exposure to fluoxetine and quetiapine prescribed for maternal mental health. Her birth measures were weight 2460g (−1.86 standard deviations (SD)), length 49 cm ((−0.17 SD), and head circumference 34 cm (−0.75 SD). She had no anomalies except for bilateral hip dysplasia. She was discharged on day 6 of life following resolution of hypoglycemia and hyperbilirubinemia. Because of inadequate caloric intake and growth failure (Supplementary Figure S1), she had a gastric tube placed at age 25 months. At age 6 years 6 months, she still received G-tube feeds for adequate caloric consumption.

The proposita had slow skill acquisition in all domains. She walked independently by age 23 months. Evaluation at 36 months confirmed the delays and excluded autism spectrum disorder. Evaluation at 64 months showed gross motor delay, hypotonia, and decreased muscle strength; also, she was unable to feed herself with a spoon, was not yet toilet trained, and manifested multiple behavioral problems (difficulty playing with others, separation anxiety, and poor emotional regulation). On examination at age 64 and 78 months, she had normocephaly, a rounded face, telecanthus, broad nasal bridge, bilateral epicanthus, deep philtrum, short distal phalanges, normal palmar creases, and hypotonia posture (Figure 1C–E, Table S2).

Initial genetic, biochemical, and endocrine testing as well as imaging did not identify an etiology for the proposita's multiple problems (Supplementary Material).

Individual II-4—As a child, the mother of the proposita had growth failure, a learning disability resulting in academic challenges, and anxiety leading to social withdrawal. Despite requiring learning assistance, she completed a two-year college diploma and found employment. Following the death of her mother and familial turmoil, she was hospitalized at age 28 years for major depressive disorder and social anxiety disorder. At age 32 years, work stress and motor vehicle accidents precipitated hospitalization for a relapse and attempted suicide. She was diagnosed with bipolar affective disorder.

On examination at age 44 years, she had a round face, bilateral epicanthus, mild retrognathia, low-set and posteriorly rotated ears, broad nasal bridge and bulbous nose, deep philtrum, palmar eczema, normal palmar creases, and normal fingernails. (Figure 1F–I).

Human Subjects

As part of a research protocol approved by the University of British Columbia Research Ethics Board (H09-01228, Vancouver, BC Canada), the proposita's parents gave informed written consent to the study, data analysis, and publication of findings.

Reagents and Co-immunoprecipitation (Co-IP)

These were developed and performed as previously described (Scott et al., 2006). Please see Supplementary Material and Methods for details.

RESULTS

Singleton clinical exome sequencing reports several rare genomic variants

To screen for genetic etiologies, the proposita had singleton clinical exome sequencing, which reported five variants in four genes (Figure 2A). Consideration of phenotype, variant characteristics, and parent of origin diminished suspicion for the deleteriousness of the reported variants in *DPP6, USP9X,* and *SNX14* (Figure 2B).

In contrast, these considerations increased suspicion of the *PACS1* variant (NM_018026.3:c.[755C>T];[=]) (Table S3). This variant segregates with disease, is absent from GnomAD, alters a highly conserved nucleotide (phastCons: 1.00, phyloP: 7.40) and amino acid (Figure 2C), and is predicted deleterious by multiple in silico algorithms (Figure 2A–C). Consistent with this, Virtual Geneticist prioritized the *PACS1* variant as first among all the exome variants (data not shown).

Coimmunoprecipitation shows reduced affinity of PACS1 S252F for GGA3

Because Ser252 resides in the GGA3 binding motif (Ala246_Ser253, Figure 1A) we hypothesized that it alters GGA3 binding to PACS1. To test this, we expressed Flag-tagged PACS1^{S252F} and wild type Flag-tagged PACS1 in HCT116 cells co-expressing 3xHA tagged GGA3. As assessed by co-IP, Flag-tagged PACS1^{S252F} bound approximately 40% less 3xHA-GGA3 by than did wild type Flag-tagged PACS1 (Figure2E).

DISCUSSION

We describe a mother and daughter with cognitive, behavioral, and feeding problems segregating with the a novel *PACS1* variant (NM_018026.3:c.[755C>T];[=], p.(Ser252Phe)) that impedes binding of the GGA3 adaptor protein to PACS1. The facial, cognitive, social, and behavioral characteristics of the proposita and her mother resemble those of *PACS1*-NDD (Table S2).

Previous studies using a PACS1 mutant that is unable to bind GGA3 have shown that the lack of PACS1 binding to GGA3 selectively disrupts the trafficking of some client proteins similar to inhibition of CK2 binding to PACS1 and impedes the release of GGA3

from endosomal membranes (Scott et al., 2006). Although human disorders associated with GGA3 and its interactors largely remain to be described (Table S3), we hypothesize that deleterious variation in these predispose to ID.

Although the features of the proposita and her mother are milder than those of many *PACS1*-NDD individuals (Bruno et al., 2023; Seto et al., 2021), a later report proposes a phenotypic spectrum and underreporting of milder presentations (Martinez-Monseny et al., 2018). The psychiatric features of the proposita's mother have not been previously described in association with *PACS1*-NDD; however, few reported individuals are adults, and all carry a variant of Arg203. Although a recent study suggests a link between PACS1 and bipolar disorder (Chen et al., 2022), further study is needed to understand the full spectrum of *PACS1*-related disorders and whether variation in different domains of PACS1 give rise to different diseases or a continuum of *PACS1*-NDD.

Little published evidence delineates the mechanism of disease in *PACS1*-NDD. *In vivo* functional assays found that overexpression of *PACS1* mRNA encoding Arg203Trp in zebrafish embryos generated craniofacial defects through a presumed dominant-negative mechanism (Schuurs-Hoeijmakers et al., 2012). We postulate a similar mechanism for Ser252Phe.

In conclusion, we describe a proposita and her mother with phenotypic features consistent with *PACS1*-NDD (Table S2) and a novel *PACS1* variant (NM_018026.3:c.[755C>T];[=], p.(Ser252Phe)) that impedes binding of the GGA3 adaptor protein. Further functional studies are needed to determine to what extent the reduced interaction between $PACS1^{S252F}$ and GGA contribute to the overall disease phenotype in the proposita as well as define the mechanism of disease for other deleterious *PACS1* variants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Sharing and Data Accessibility:

The family has not consented to the public release of genomic data. The *PACS1* variant is submitted to ClinVar (accession number: SCV002605330).

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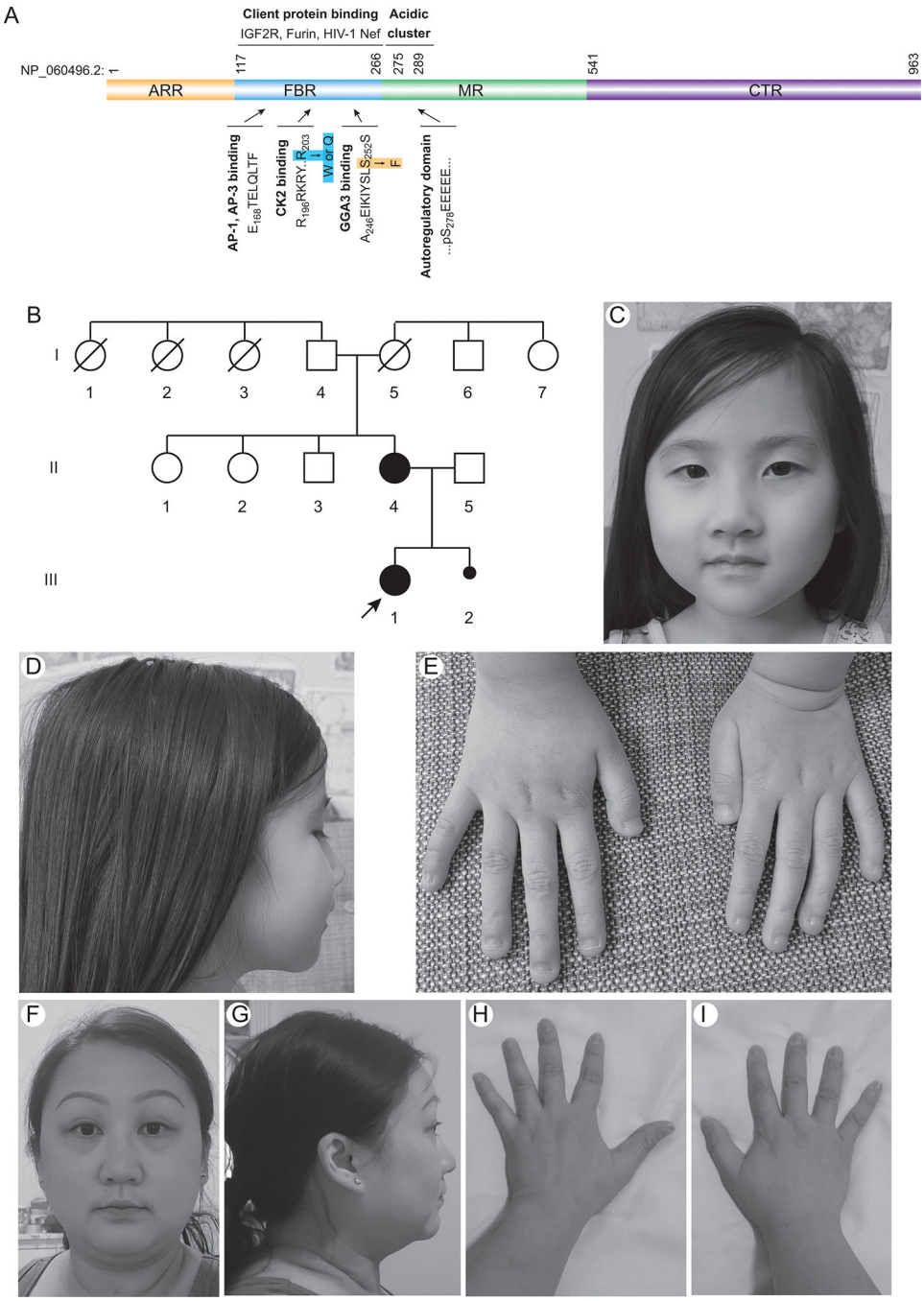

Figure 1.

Diagram of the PACS1 protein and the pedigree and features of the proposita and her mother. (A) Diagram of the PACS1 protein showing the atropin-1-related region (ARR), furin binding region (FBR), middle region (MR), and C-terminal region (CTR). The FBR binds client proteins (e.g., IGF2R, furin, HIV-1 Nef) and has motifs binding CK2 (casein kinase 2) and the clathrin adaptors AP (adaptor-related protein complexes)-1, AP-3 and GGA3 (Golgi-associated, gamma-adaptin ear-containing, ARF-binding protein 3). The MR contains an acidic cluster recognized by CK2, which phosphorylates serine (S278) to

regulate GGA3. R203 (blue), which is associated with *PACS1*-NDD, is adjacent to the CK2 binding motif. S252 (orange), which is altered in the proposita and her mother, is in the GGA3 binding motif. (B) Pedigree of the described family. Open and filled symbols represent affected and unaffected individuals, respectively. The parents of the mother were unavailable for testing or examination. (C, D) Frontal and profile photographs of the proposita. (E) Photograph of the proposita's hands. (F, G) Frontal and profile photographs of the proposita's mother. (H, I) Photograph of the hands of the proposita's mother.

A

Gene	Transcript	Variant	Zygosity	Amino acid change	Inheritance	MAF in gnomAD frequency	CADD score	LIST-S2 score
DPP6	NM_130797.3	c.169G>T	Het	p.(Gly57Cys)	paternal	0	23.8	0.462454
USP9X	NM_001039590.2	c.3983T>G	Het	p.(Val1328Gly)	paternal	0	26.7	0.862114
SNX14	NM_153816.5	c.1933A>T	Het	p.(Ile645Phe)	paternal	0	23.9	0.935706
SNX14	NM_153816.5	c.511C>T	Het	p.(Arg171Cys)	maternal	0.011%	29.6	0.980702
PACS1	NM_018026.3	c.755C>T	Het	p.(Ser252Phe)	maternal	0	27.7	0.961904

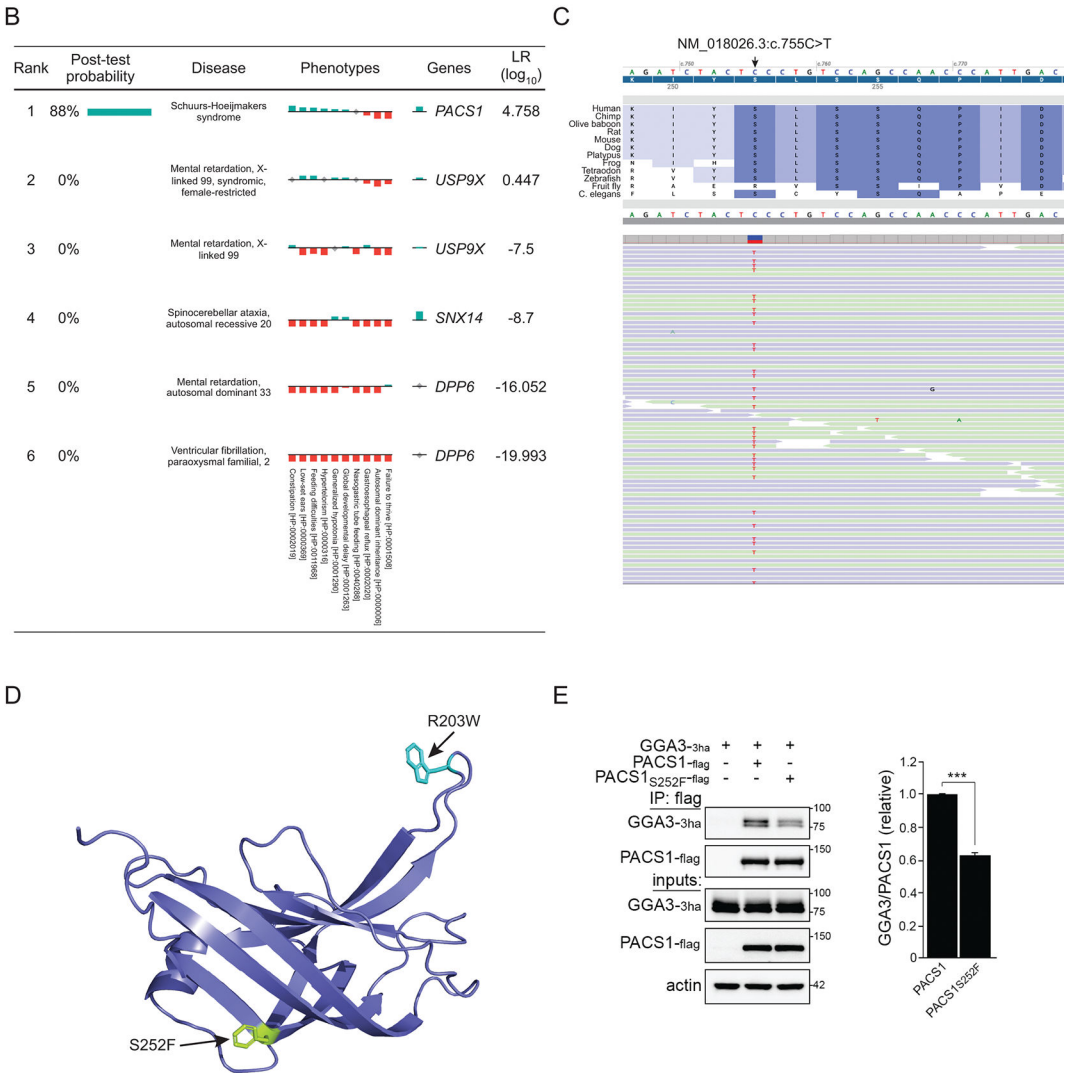

Figure 2.

Characterization of PACS1 S252F. (A) Variants reported on by clinical exome for the proposita. (B) LIRICAL graphical representation of phenotype in the context of genotype (Robinson et al., 2020). The green bars represent phenotypic features consistent with the disorder. The red bars represent phenotypic features not consistent with the disorder. (C) Screen shot from Alamut Visual showing amino acid conservation for S252 and the sequence reads for NM_018026.3:c.[755C>T];[=] (bottom). (D) Predicted crystal structure of PACS1 FBR showing the locations of R203 and of S252. (E) S252F reduces

PACS1 binding of GGA3. Left: HA-tagged GGA3 was expressed alone or together with either FLAG-tagged PACS1 or PACS1^{S252F}. Flag-tagged PACS1 proteins were immunoprecipitated and co-precipitating GGA3 was detected by western blot. Error bars = 1 standard deviation. n =3, $p = 0.00045$.