

NIH Public Access

Author Manuscript

FEBS Lett. Author manuscript; available in PMC 2013 August 14.

Published in final edited form as:

FEBS Lett. 2012 August 14; 586(17): 2795–2799. doi:10.1016/j.febslet.2012.03.041.

Molecular Insights into the WW domain of the Golabi-Ito-Hall Syndrome Protein PQBP1

Marius Sudol^{a,b,*}, Caleb B. McDonald^{c,d}, and Amjad Farooq^{c,d}

^aLaboratory of Signal Transduction and Proteomic Profiling, Weis Center for Research, Geisinger Clinic, Danville, PA 17821, USA

^bDepartment of Medicine, Mount Sinai School of Medicine, New York, NY 10029, USA

^cDepartment of Biochemistry and Molecular Biology, Leonard Miller School of Medicine, University of Miami, Miami, FL 33136, USA

^dBraman Family Breast Cancer Institute, Sylvester Comprehensive Cancer Center, Leonard Miller School of Medicine, University of Miami, Miami, FL 33136, USA

Abstract

The WW domain-containing PQBP1 (polyglutamine tract-binding protein 1) protein regulates mRNA processing and gene transcription. Mutations in the PQBP1 gene were reported in several X chromosome-linked intellectual disability (XLID) disorders, including Golabi-Ito-Hall (GIH) syndrome. The missense mutation in the GIH syndrome maps within a functional region of the PQBP1 protein known as the WW domain. The causative mutation of PQBP1 replaces the conserved tyrosine (Y) at position 65 within the aromatic core of the WW domain to cysteine (C), which is a chemically significant change. In this short review, we analyze structural models of the Y65C mutated and wild type WW domains of PQBP1 in order to infer potential molecular mechanisms that render the mutated PQBP1 protein inactive in terms of ligand binding and its function as a regulator of mRNA splicing.

Keywords

WW domain; Intellectual disability; Cysteine oxidation; disulfide bridge; mRNA processing

1. Introduction

The WW domain-containing PQBP1 (polyglutamine tract-binding protein 1) gene encodes a protein that regulates mRNA processing and RNA Polymerase II-driven transcription [1–5]. The PQBP1 gene is highly conserved in evolution and its orthologs in plants, worms and mammals share a high degree of sequence similarity. In mammals, the PQBP1 gene is widely expressed, but its transcript is enriched in the brain [6]. Originally, the PQBP1 protein was identified as a protein partner of poly-glutamine-(poly-Q)-containing proteins, including huntingtin, ataxin-1 and neuronal transcription factor Brn-2. Mutations in the PQBP1 gene were reported in several X chromosome-linked intellectual disability (XLID)

^{© 2012} Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

^{*}Corresponding Author: Fax: +1 570-271-6701, msudol1@geisinger.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

disorders, such as Renpenning, Sutherland-Haan, Hamel, Porteous, and Golabi-Ito-Hall (GIH) syndromes [7,8]. Interestingly, although caused by different mutations that affect the coding sequence of the PQBP1 gene - frame shifts that result in truncated protein products or a missense point mutation - these syndromes share similar clinical features [7]. In addition to severe intellectual disability, the patients also have a lean body, short stature, small head, and are frequently diagnosed with cardiac abnormalities [9,10]. The missense mutation in the GIH syndrome maps within a functional region of the PQBP1 protein known as the WW domain (Figure 1) [8,11–13]. In contrast to other mutations reported for PQBP1, the genetic lesion of GIH syndrome does not affect the size of the mutated protein, which normally migrates on SDS-PAGE as a singlet of 38 kilo-Daltons [14].

The WW domain is one of the smallest protein modules mediating specific protein-protein interactions with short proline-rich or proline-containing motifs [11–13]. The WW domain mutation implicated in the GIH syndrome replaces the conserved tyrosine (Y) at position 65 within the aromatic core of the domain to cysteine (C), which is a chemically significant change to the protein that we would like to discuss here in detail [14].

Recently, we have documented that the binding of the Y65C mutant of PQBP1 protein to its cognate ligand, WBP11 (also known as SIPP1), which regulates mRNA processing, was abrogated compared to that of the control wild-type protein [14]. More importantly, we have verified that in lymphoblasts derived from a GIH patient, the complex between the endogenous PQBP1 and endogenous WBP11 proteins was compromised compared with the intact complex between these two proteins found in lymphoblasts derived from a normal individual who was matched for race and sex with the GIH patient [14]. Using a cell culture model and a reporter gene construct that monitors efficiency of mRNA splicing, we have shown that the missense mutation in the PQBP1 WW domain resulted in a substantial decrease in pre-mRNA splicing [14].

2. Y65 of the WW domain of PQBP1 is conserved among the family of WW domains

In an effort to understand the importance of tyrosine at position 65 (Y65) in the function of PQBP1, we performed amino acid sequence alignment of the entire human family of WW domains (Figure 2). Our analysis reveals that Y65 is predominantly conserved across all WW domains and that it is frequently substituted by its chemical analogue phenylalanine, further implicating its central role in the function of WW domains. In addition to Y65, a number of other residues in the WW domain of PQBP1 are also predominantly conserved across the entire human family of WW domains. These include the signature tryptophan residues at positions 52 and 75 (W52 and W75), an asparagine at position 67 (N67) and a proline at position 78 (P78). It is notable that while W52 is substituted by tyrosine in only a few WW domains, W75 displays more versatility and is replaced by amino acids as diverse as tyrosine, phenylalanine, isoleucine, glutamine and also cysteine. In a manner akin to the structural versatility of W75, the N67 residue can also be replaced by amino acids as diverse as aspartate, serine, tyrosine and histidine. In contrast, WW domains appear to have an obligate requirement for P78 as it is only absent from the WW2 domain of ARHGAP27 GTPase. The highly conserved nature of W52, Y65, N67, W75 and P78 residues in PQBP1 across the entire family of WW domains and their key role in the structural and functional integrity of WW domains is further corroborated by site-directed mutagenesis studies on the WW domain of Pin1 [15]. The mutagenesis of the WW domain of Pin1 revealed that W52, N67, Y65 and P78 form a hydrophobic core of the domain, which may be important in stabilizing the structure [15].

3. Y65C PQBP1 mutant and intramolecular disulfide bonds

In order to gain better insight about the effect of Y65C mutation on the WW domain fold, we generated 3D atomic models of wt and Y65C-mutant WW domains of POBP1 (Figure 3). As discussed in our recent study, the hydrophobic core that is located beneath the ligandbinding pocket of the wt WW domain is composed of a highly conserved quartet of hydrophobic residues W52, Y65, N67 and P78 (and perhaps to a lesser extent by W75). While Y65 constitutes a key component of the hydrophobic core within the wild type domain, the placement of cysteine at this position within the Y65C-mutant WW domain may not only result in the collapse of the triple-stranded β -sheet "roof" supporting the ligand-binding "cradle" [16,17], but under oxidative conditions, the formation of an intramolecular disulfide bond between C60 and C65 residues within the Y65C-mutant WW domain may also be favored (Figure 4). This scenario is conceivable due to the inherent flexibility of the β 1- β 2 loop allowing it to curve away from the ligand-binding "cradle" toward the hydrophobic core of the WW domain, so as to bring the C60 and C65 residues in close proximity and thereby drive their covalent linkage via a disulfide bond. As we have shown recently in an *in vitro* assay, the binding of Y65C-mutant WW domain to its cognate peptide ligands is indeed compromised, but perhaps not as dramatically as would be expected from the replacement of a conserved tyrosine with a cysteine within its hydrophobic core. This salient observation argues in favor of an alternative mechanism by which the Y65C mutation could lead to loss-of-function in PQBP1. Given that the in vitro binding studies, which we recently reported, were conducted under reducing conditions in the presence of a reducing agent, 5mM β -mercaptoethanol [14], it is thus conceivable that the Y65C-mutant WW domain may behave differently toward its cognate ligands within the milieu of the cell, than in an in vitro binding assay. Thus, under non-reducing conditions in response to cellular oxidative stress, for instance, it is conceivable that the Y65C-mutant WW domain may undergo intramolecular disulfide bond formation as proposed above. Accordingly, such intramolecular covalent modification would destroy the "cradle" optimized for ligand binding and the resulting Y65C-mutant domain would not be expected to bind to its cognate ligands in a physiologically-relevant manner, leading to loss-offunction in PQBP1, as we observed in co-immunoprecipitation assays performed on full length proteins expressed in cultured cells. It is also important to note that C60 occupies a unique position in the WW domain of PQBP1 and is largely absent from other WW domains. Moreover, it is intriguing that the position of C60 in the WW domain of PQBP1 is either absent in other WW domains or substituted for by other amino acids, with the exception of cysteine (Figure 2). Of particular interest is the observation that the occurrence of more than one cysteine in the WW domains is a rare event. Of ~100 human WW domains, only four contain more than one cysteine. These include the WW domains of ARHGAP9, CEP164, MLH3 and PLEKHA2 (Figure 2). Notably, these cysteine residues are located in the following areas: within the β 2 and β 3 strands (ARHGAP9), the β 1 strand and the C-terminus following the β 3 strand (CEP164), within the N-terminus preceding the β 1 strand (MLH3), and within the β 1- β 2 loop and β 3 strand (PLEKHA2). The markedly differential location of cysteine residues with the WW domain family suggests that they contribute to WW domain structure in distinct manners and may modulate their function via distinct mechanisms. Although the presence of more than one cysteine within a protein opens up the possibility of intramolecular disulfide bond formation, it should be noted that this remains an unlikely scenario in the case of WW domains of ARHGAP9, CEP164 and PLEKHA2 proteins on structural grounds. Thus, unlike the Y65C-mutant WW domain of PQBP1, the cysteine residues are either located within one of the three rigid β -strands or away from the flexible β 1- β 2 loop in the WW domains of ARHGAP9, CEP164 and PLEKHA2. Taken together, it seems that the Y65C mutation may predispose the WW domain of PQBP1 to loss-of-function via the formation of an intramolecular disulfide bond. However, it is important to mention that in some cases intra-molecular disulfide bond

FEBS Lett. Author manuscript; available in PMC 2013 August 14.

formation could lead to a gain of protein function, as exemplified by the bridge between cysteines 377 and 330 in Cdc25C phosphatase, which results in enhanced binding to 14-3-3 protein and the cytoplasmic sequestration of the protein [18]. Fittingly, in our *in vitro* binding studies, we did observe a small number of peptide ligands that bound more tightly to the Y65C PQBP1 WW domain compared to the wild type control. Therefore, it is possible, that in the network or systems view, the Y65C mutations may result in both loss and gain of function changes that in concert deregulate mRNA processing.

4. Y65C PQBP1 regulation via oxidation

Although in general, proteins that signal in the cytoplasm are embedded in a reducing environment, the reversible oxidation of cysteines of cytoplasmic proteins is a wellestablished regulatory mechanism for various signaling proteins, including protein-tyrosine phosphatases [19]. The general cellular redox state and the extracellular-ligand stimulated production of reactive oxygen or reactive nitrogen species (ROS or RNS) affects oxidation of cysteines in proteins, in a reversible manner. Reaction of H_2O_2 oxidizes cysteine thiols at first to sulphenic acid (SOH), and at higher concentrations of the oxidant, to sulphinic (SO₂H) or sulphonic acid (SO₃H) derivatives [19]. The sulphenic acid (SOH) derivative of C65 of PQBP1 could form sulphenylamides by reaction with other amino acids of the PQBP1 that are located in the proximity of the WW domain, in addition to forming an intramolecular disulfide bridge with C60. Interestingly, the wt PQBP1 protein does not contain any other cysteine residues except for the WW domain-located C60.

We cannot exclude other possibilities by which oxidation of C65 could regulate the function of PQBP1 protein. The sulphinic (SO₂H) or sulphonic acid (SO₃H) derivatives of C65 in PQBP1 may lead to conformational changes that also render the domain inactive in terms of ligand binding. Moreover, the cysteine thiols in a local environment with pKa below 6 could form a reactive thiolate anion that is permissive for posttranslational modifications such as phosphorylation, glutathionylation or even adduct formation with other endogenous electrophilic molecules [19]. Such modifications could also render the mutated WW domain inactive in terms of ligand binding and signaling of the PQBP1 complex in mRNA processing.

5. Concluding remarks

The atomic details of the Y65C lesion of the PQBP1 protein will hopefully come soon from crystal structures of the wild type and mutant proteins. Apart from the intrinsic changes that the mutation causes to the WW domain itself, it will be very important to see the relative location of the carboxy-terminal tail of the protein with its well-conserved motif (*CM*) (Figure 1). Since most of the PQBP1 mutations that result in XLID syndromes have the carboxy-terminal region of the protein missing, including the very *CM* sequence, we presume that somehow the WW domain and the *CM* region collaborate together to regulate mRNA splicing.

Apart from X-ray crystallography, two other approaches represent attractive ways to study the effect of the Y65C mutation on the molecular architecture of the PQBP1 protein. These are protein folding assays in the periplasm of bacteria [20] and atomic force microscopy [21]. Both of these approaches are able to interrogate the PQBP1-Y65C mutant and wild type proteins under reducing and non-reducing conditions. These two approaches could quickly validate several regulatory scenarios proposed here.

In sum, our recent studies on PQBP1 and various plausible mechanisms discussed here for the inactivation of PQBP1 provide the framework for conducting future studies to unlock the molecular basis of XILD disorders at atomic level with important consequences on the rationale design of therapeutic approaches.

Acknowledgments

We thank Virginia Mazack for valuable comments on the first version of the manuscript.

This work was supported by the National Institutes of Health Grants R01-GM083897 and funds from the USylvester Braman Family Breast Cancer Institute (to AF), and by PA Breast Cancer Coalition Grants (#60707 an #920093) and by the Geisinger Clinic (to MS)

REFERENCES

- Komuro A, Saeki M, Kato S. Association of two nuclear proteins, Npw38 and NpwBP, via the interaction between the WW domain and a novel proline-rich motif containing glycine and arginine. J Biol Chem. 1999; 274:36513–36519. [PubMed: 10593949]
- Komuro A, Saeki M, Kato S. Npw38, a novel nuclear protein possessing a WW domain capable of activating basal transcription. Nucleic Acids Res. 1999; 27:1957–1965. [PubMed: 10198427]
- Waragai M, Lammers CH, Takeuchi S, Imafuku I, Udagawa Y, Kanazawa I, Kawabata M, Mouradian MM, Okazawa H. PQBP-1, a novel polyglutamine tract-binding protein, inhibits transcription activation by Brn-2 and affects cell survival. Hum Mol Genet. 1999; 8:977–987. [PubMed: 10332029]
- 4. Okazawa H, Rich T, Chang A, Lin X, Waragai M, Kajikawa M, Enokido Y, Komuro A, Kato S, Shibata M, Hatanaka H, Mouradian MM, Sudol M, Kanazawa I. Interaction between mutant ataxin-1 and PQBP-1 affects transcription and cell death. Neuron. 2002; 34:701–713. [PubMed: 12062018]
- Sudol M, Sliwa K, Russo T. Functions of WW domains in the nucleus. FEBS Lett. 2001; 490:190– 195. [PubMed: 11223034]
- 6. Okazawa H, Sudol M, Rich T. PQBP-1 (Np/PQ): a polyglutamine tract-binding and nuclear inclusion-forming protein. Brain Res Bull. 2001; 56:273–280. [PubMed: 11719261]
- Kalscheuer VM, Freude K, Musante L, Jensen LR, Yntema HG, Gecz J, Sefiani A, Hoffmann K, Moser B, Haas S, Gurok U, Haesler S, Aranda B, Nshedjan A, Tzschach A, Hartmann N, Roloff TC, Shoichet S, Hagens O, Tao J, Van Bokhoven H, Turner G, Chelly J, Moraine C, Fryns JP, Nuber U, Hoeltzenbein M, Scharff C, Scherthan H, Lenzner S, Hamel BC, Schweiger S, Ropers HH. Mutations in the polyglutamine binding protein 1 gene cause X-linked mental retardation. Nat Genet. 2003; 35:313–315. [PubMed: 14634649]
- Lubs H, Abidi FE, Echeverri R, Holloway L, Meindl A, Stevenson RE, Schwartz CE. Golabi-Ito-Hall syndrome results from a missense mutation in the WW domain of the PQBP1 gene. J Med Genet. 2006; 43:e30. [PubMed: 16740914]
- Golabi M, Ito M, Hall BD. A new X-linked multiple congenital/mental retardation syndrome. Am J Med Genet. 1984; 17:367–374. [PubMed: 6711604]
- Kunde SA, Musante L, Grimme A, Fischer U, Muller E, Wanker EE, Kalscheuer VM. The Xchromosome-linked intellectual disability protein PQBP1 is a component of neuronal RNA granules and regulates the appearance of stress granules. Hum Mol Genet. 2011; 20:4916–4931. [PubMed: 21933836]
- Bork P, Sudol M. The WW domain: a signalling site in dystrophin? Trends Biochem Sci. 1994; 19:531–533. [PubMed: 7846762]
- Chen HI, Sudol M. The WW domain of Yes-associated protein binds a proline-rich ligand that differs from the consensus established for Src homology 3-binding modules. Proc Natl Acad Sci U S A. 1995; 92:7819–7823. [PubMed: 7644498]
- Sudol M, Bork P, Einbond A, Kastury K, Druck T, Negrini M, Huebner K, Lehman D. Characterization of the mammalian YAP (Yes-associated protein) gene and its role in defining a novel protein module, the WW domain. J Biol Chem. 1995; 270:14733–14741. [PubMed: 7782338]

- 14. Tapia VE, Nicolaescu E, McDonald CB, Musi V, Oka T, Inayoshi Y, Satteson AC, Mazack V, Humbert J, Gaffney CJ, Beullens M, Schwartz CE, Landgraf C, Volkmer R, Pastore A, Farooq A, Bollen M, Sudol M. Y65C missense mutation in the WW domain of the Golabi-Ito-Hall syndrome protein PQBP1 affects its binding activity and deregulates pre-mRNA splicing. J Biol Chem. 2010; 285:19391–19401. [PubMed: 20410308]
- Jager M, Zhang Y, Bieschke J, Nguyen H, Dendle M, Bowman ME, Noel JP, Gruebele M, Kelly JW. Structure-function-folding relationship in a WW domain. Proc Natl Acad Sci U S A. 2006; 103:10648–10653. [PubMed: 16807295]
- Macias MJ, Hyvonen M, Baraldi E, Schultz J, Sudol M, Saraste M, Oschkinat H. Structure of the WW domain of a kinase-associated protein complexed with a proline-rich peptide. Nature. 1996; 382:646–649. [PubMed: 8757138]
- Huang X, Poy F, Zhang R, Joachimiak A, Sudol M, Eck MJ. Structure of a WW domain containing fragment of dystrophin in complex with beta-dystroglycan. Nat Struct Biol. 2000; 7:634–638. [PubMed: 10932245]
- Savitsky PA, Finkel T. Redox regulation of Cdc25C. J Biol Chem. 2002; 277:20535–20540. [PubMed: 11925443]
- Ostman A, Frijhoff J, Sandin A, Bohmer FD. Regulation of protein tyrosine phosphatases by reversible oxidation. J Biochem. 2011; 150:345–356. [PubMed: 21856739]
- Mansell TJ, Linderman SW, Fisher AC, DeLisa MP. A rapid protein folding assay for the bacterial periplasm. Protein Sci. 2010; 19:1079–1090. [PubMed: 20440843]
- Baclayon M, Roos WH, Wuite GJ. Sampling protein form and function with the atomic force microscope. Mol Cell Proteomics. 2010; 9:1678–1688. [PubMed: 20562411]

NIH-PA Author Manuscript

NIH-PA Author Manuscript



Figure 1.

Schematic of the organization of the PQBP1 protein. PQBP1 contains only one discernable modular domain, the WW domain located at the N-(amino)-terminus. The causative Y-C PQBP1 mutation of the GIH syndrome is located within the middle of the WW domain sequence and it is indicated with a red arrow. There are multiple copies of three distinct amino acid repeats found in PQBP1: five consecutive copies of DRXH(D/E)KX and ten copies of (D/E)R, which occur consecutively in repeats of three and seven separated by a small stretch of intervening amino acids. At the C-(carboxy)-terminal region there is a stretch of highly conserved amino acids –GPLFQQRPYPSPG-, which are identical among PQBP1 proteins of man (*Homo sapiens*), a sweet water polyp (*Hydra magnipapilata*) and a small flowering plant (*Arabidopsis thaliana*). We indicate the relative location of this sequence as a green box with *CM*, after "conserved motif". An indentation on the box is to suggest a possibility that the *CM* motif interacts with the WW domain, shown as a gray triangle.

PQBP1_WW APBB1_WW

APBB2 WW APBB3_WW ARHGAP9_WW ARHGAP12 WW1 ARHGAP12_WW2 ARHGAP27_WW1 ARHGAP27 WW2 ARHGAP27_WW3 BAG3 WW CEP164 WW DIGEORGE WW DYSTROPHIN WW DRP2_WW FLJ22029 WW1 FLJ22029 WW2 FNBP3_WW1 FNBP3_WW2 FNBP4 WW1 FNBP4 WW2 FTSJD2 WW GAS7 WW HECW1_WW1 HECW1 WW2 HECW2_WW1

HECW2 WW2 HLADQA2_WW HYPB_WW HYPC WW1 HYPC WW2 IQGAP1_WW IQGAP2_WW IQGAP3 WW ITCH WW1 ITCH WW2 ITCH_WW3 ITCH WW4 KIAA1688 WW1 KIAA1688_WW2 KIBRA WW1 KIBRA WW2 MAGI1_WW1 MAGI1 WW2 MAGI2 WW1 MAGI2_WW2 MAGI3 WW1 MAGI3_WW2 MLH3 WW

W52 C60 Y65 N67 ↓ ↓ ↓ ↓	w75 ↓	Р78 ↓		w52 ↓	C60 ↓	¥65 ↓	N67 ↓	w75 ↓	Р78 ↓
LEGLPPS W YKVFDPS C GLPY Y WNADI	DLVS <mark>W</mark> LS	PHDPN	PQBP1_WW	LEGLPPS W YKV	FDPS C G	LPY <mark>Y</mark> W	, Nadte	LVS W L	S P HDPN
DSDLPAGWMRVQDTS-G-TYYWHIP1	'GTTQ <mark>₩</mark> EP	PGRAS	NEDD4_WW1	PSPLPPGWEER	QDIL-G	RTY y v	NHESF	RTQ W K	R <mark>P</mark> TPQD
DPDLPPGWKRVSDIA-G-TYYWHIPT	'GTTQ w er	P VSIP	NEDD4_WW2	SSGLPPGWEEK	QDER-G	rsy <mark>y</mark> v	DHNSF	TTT W T	K P TVQA
ETGLPPGWRKIHDAA-G-TYYWHVPS	SGSTQ W QR	PTWEL	NEDD4 WW3	QGFLPKG <mark>W</mark> EVR	HAPN-G	RPF f i	DHNTF	TTT W E	D P RLKI
LLQRPDAWEQHLDPNSGRCFYINSL1	IG C KS W KP	PRRSR	NEDD4 WW4	LGPLPPG <mark>W</mark> EER	THTD-G	RIF <mark>Y</mark> I	NHNIK	RTQ W E	DPRLEN
AIQINGEWETHKDSS-GRCYYYNRGI	'QERT w kp	PRWTR	NEDD4L WW1	PPPLPPG <mark>W</mark> EEK	VDNL-G	rty y v	N HNNF	RTTQ W H	R <mark>P</mark> SLMD
SDYTNEKWLKHVDDQ-GRQYYYSADO	GSRSEWEL	P KYNA	NEDD4L WW2	TPGLPSG W EER	KDAK-G	rty y v	N HNNF	TTT W T	R P IMQL
APLPSPVWETHTDAGTGRPYYYNPD1	GVTTWES	P FEAA	NEDD4L WW3	QSFLPPG <mark>W</mark> EMR	IAPN-G	RPF F I	DHNTF	TTT W E	D P RLKF
HVSLETEWGQYWDEESRRVFFYNPL1	IGETA <mark>w</mark> ed	EAENE	NEDD4L WW4	lgplppg <mark>w</mark> eer	IHLD-G	RTF Y I	DHNSK	ITQ W E	DPRLQN
NHFTQEQ W VRLEDPH-GKPY F YNPEI)SSVR <mark>W</mark> EL	P QVPV	PCIF1 WW	EELVHAG W EK C	WSRREN	RPY y F	NRFTN	IQSL <mark>W</mark> E	M P VLGQ
RDPLPPGWEIKIDPQTGWPFFVDHNS	SRTTT W ND	PRVPS	PDZK10 WW	SQVPPYG <mark>W</mark> EMT.	ANRD-G	RDY f i	NHMTÇ	AIPFD	DPRLES
VAPLPGEWKPCQDIT-GDIYYFNFAN	IGQSM W DH	I PC DEH	PIN1 WW	EEKLPPG <mark>W</mark> EKRI	MSRSSG	rvy y f	NHITN	iasq w e	RPSGNS
TEPLPDGWIMTFHNS-GVPVYLHRES	SRVVT W SR	P YFLG	PIN1L WW	EEKLPPG <mark>W</mark> EKRI	MSRPSG	RGY <mark>y</mark> f	NHITN	ipsq w e	RPSGNS
STSVQGPWERAISPN-KVPYYINHET	IQTT CW DH	P KMTE	PLEKHA2 WW1	RDTLPEH W SYG	V C RD-G	RVF f i	NDQLF	CTTWL	H P RTGE
SSSVQVPWERAISPN-KVPYYINHQA	AQTT CW DH	I P KMTE	PLEKHA2 WW2	RSDLPRG <mark>W</mark> EEG	FTEE-G	ASYFI	DHNQC	TTAFR	H P VTGQ
QLPLPRGWEEARDYD-GKVFYIDHNI	RRTSWID	PRDRL	PLEKHA5 WW1	WISLPRSWTYG	ITRG-G	RVF F I	NEEAK	STTWL	H P VTGG
GDELPWGWEAGFDPQIG-VYYIDHIN	IKTTQ i ed	PRKQW	PLEKHA5 WW2	STDLPTG W EEA	YTFE-G	ARY <mark>y</mark> i	NHNEF	KVT C K	H P VTGQ
ASGAKSMWTEHKSPD-GRTYYYNTET	KOSTWEK	PDDLK	SAV1 WW1	DLPLPPG <mark>W</mark> SVD	WTMR-G	rky y i	DHNTN	ITTH <mark>W</mark> S	HPLERE
QLLSKCPWKEYKSDS-GKPYYYNSQ7	KESRWAK	(P KELE	SAV1 WW2	REGLPPGWERV	ESSEFG	-TY y v	DHTNF	KAQYR	H PC APS
VGIEMGDWOEVWDENTGCYYYWNTOT	NEVT W EL	POYLA	SMURF1 WW1	SPELPEG <mark>Y</mark> EOR	TTVO-G	ovy f i	HTOTO	vst w h	DPRIPS
INATPKGWSCHWDRDHRRYFYVNEOS	SGESOWEF	PDGEE	SMURF1 WW2	LGPLPPGWEVR	STVS-G	RIY F V	- DHNNF	TTOFT	DPRLHH
VRTVNEPWTMGFSKSFKKKF F Y N KKT	KDST F DL	PADSI	SMURF2 WW1	DNDLPDGWEER	RTAS-G	RIO <mark>Y</mark> I	NHITF	TTOWE	RPTRPA
TVILPPGWOSYLSPO-GRRYYVNTT	INETTWER	PSSSP	SMURF2 WW2	PPDLPEG Y EOR	TTOO-G	OVYFI	HTOTO	VST W H	DPRVPR
DEPLPPNWEARIDSH-GRVFYVDHVN	JRTTT W OR	PTAAA	SMURF2 WW3	LGPLPPGWEIR	NTAT-G	RVYFV	DHNNF	TTOFT	DPRLSA
RLELPRGWEIKTDOO-GKSFFVDHNS	SRATTFID	PRIPL	STXBP4 WW	MDCLPYGWEEA	YTAD-G	IKYFI	NHVTC	TTSWI	HPVMSV
DEALPPNWEARIDSH-GRIFYVDHVN	JRTTT W OR	PTAPP	TCERG1 WW1	LPPTEEIWVEN	KTPD-G	KVYYY	NARTE	ESAWT	KPDGVK
OLELPRGWEMKHDHO-GKAFFVDHNS	SRTTT F ID	PRLPL	TCERG1 WW2	GATAVSEWTEY	KTAD-G	ΚΤΥΥ	NRTI	ESTWE	KPOELK
SHGPSGOYTHEFDGDEEFYVDLET	KETVWOL	PMFSK	TCERG1 WW3	APTPGTPWCVV	WTGD-E	RVFFY	NPTTE	LSMWD	RPDDIT
TIVI.PPNWKTARDPE-GK-IYYYHVI	TROTOWD	PTWE	TCERG1L WW1	ATPIGKSWIDK	RIPN-C	KTFF	INSFAT	DSTWT	HPEESR
TGPPRALWSEHVAPD-GRIYYYNADD	KOSVWEK	PSVLK	TCERG1L WW2	TPVPGSPWCVV	WTGD-D	RVFFF	NPTME	USVWE	KPMDLK
LLLSOCPWKEYKSDT-GKPYYYNNOS	SKESRWTR	PKDLD	USP8 WW	VPGLPSGWAKE	LDPTTG	TFRYY	HSPTN	TVHMY	PPEMAP
VGDNNSKWVKHWVKG-GYYYYHNLET	OEGGWDE	PPNEV	UTROPHIN WW	STSVOLPWORS	TSHN-K	VPYYI	NHOTO	TTCWD	HPKMTE
RVSSDGSWLKLNLHK-KYDYYNTDS	SKESSWVT	PESCE	WAC WW	PYDSADDWSEH	ISSS-G	KKYYY	NCRTE	VSOWE	KPKEWL
RPADTAFWVOHDMKD-GTAYYFHLOT	FOGIWEO	PPGCP	WRP4 WW1	KDPSKGRWVEG	TTSE-G	YHYYY	DLTSG	ASOWE	KPEGEO
OAPLPPGWEORVDOH-GRVYYVDHVE	KRTT W DR	PEPLP	WBP4_WW2	KTAVKTVWVEG	LSED-G	FTYYY	NTETO	ESRWE	KPDDFT
		DTIFS	WWOX WW1	FDFL PPCWFFP	TTKD-C		NHTEE	KTONE	HDKTCK
LGPLPPGWEKRTDSN-GRVYFVNHNT	RTTOWED	PRSOG	WWOX_WW2	AGDI.PYGWEOE	TDEN-G	OVET	THINK	RTTYL	DPRIAF
EKPLPEGWEMBETVD-GIPYFVDHNE	RTTTTT	PRTCK	WWD1 WW1	TETLPSGWEOR	KDDH-G	RTY Y V	DHNTE	TTTT	RPOPLP
CONTRI FWVFI I FORTOFOMYANI VI	CFCVMDD	DACUD	WWII_WWI WWD1_WW2	DODI DDCWEDD	VDDD_D	DUVV	DHNTE		DDUMES
KRTSENOWWELEDPNTSREYYYNASI	OBTWWHR		WWP1 WW3	YGPL PPGWEKR	VDST-D	RVYEV	NHNTK	TTOWE	DPRTOG
FI DI DECMEEADDED-CKUVVIDHTM		VDDDD	WWI1_WWS	FEDI DECMETO	VTDF-C	VDVEN	DUNTE	TTTTT TTTTTT	DDDNCK
SDEL BLOWEEANDPOUC-DYFIDHNT	VINT I O T ED		WWF1_WW4 WWF2_WW1	DDAT DACWEOD	FIDN_C	DUVV	DUNTE	TTTTT TTTTT	
I CPI DENWEMAYTEN_CEVYEIDUNI	rktiQ⊥⊡D rktrtQuiD		WWF2_WWT	FDDI DDCWEKD		DEVV			
FIFIDACWERTEDDUVC_IVVDUIN	IDKTOVEN		WWFZ_WWZ	I CDI DDCWEKD	ODNC		NUNTE		DPRTOC
DDDI.DDNINEMAYTEK-CEVVETDUNG	νιαιτ Ω⊥ ΕΙΝ	DBTVK	WW12_WW3	FDALDDCWEMK	VTCF-C		DHNTE	······································	DPRPCF
ENELDYCWEKIDDDIVC-TVVDUIN	IDDU(UDDU)			FI.DI.DDCWEMT		ORVET	NHTFE		DPRXM
TEDI DKNINEMAVDDTTIG-TITVDHID	יועדעע ד נע אועדעע ד נוע		VAD1 WW	DUDIDACMEMA	KLGG-0	OBA B i Övit i t	NHIDO	.⊥⊥⊥ ™ Q	DDDRAM
DGET DAGMERTEDDOAC-MAAADDALK	IOKAO B eni IOKAO B eni	DVFIC	VAD2 WW		KLCC-C	ODALL ODALL	NHIDO	י⊥⊥1 ₩ ע שתית ינו י	DDDRAM
SNTCCSDWQRHFDVALGRMVYVNKLI	GLST F IA	PTEDI	YAP2_WW1 YAP2_WW2	SGPLPDGWEQA	MTQD-G	EIY <mark>y</mark> i	NHKNF	TTS <mark>W</mark> L	DPRLDP
β1 β2	β3			β1]	β2]—	β3	

Figure 2.

Amino acid sequence alignment of the human family of WW domains. Alignment is divided into two columns for clarity and each column is headed by the WW domain of PQBP1. Highly conserved amino acid residues across the entire family of WW domains are colored red and green, and the equivalent residues in the WW domain of PQBP1 are indicated by vertical arrows. Cysteines are in bold font.

Sudol et al.



Figure 3.

3D atomic models of wild type (A) and Y65C-mutant (B) WW domains of human PQBP1. The triple-stranded β -sheet of the WW domains is shown in blue and the intervening loops in gray. The sidechains of residues W52, Y65/C65, N67, W75 and P78 that constitute the hydrophobic core of the domains are colored red.



Figure 4.

A hypothetical model for the loss-of-function in the Y65C mutant form of human PQBP1 in Golabi-Ito-Hall syndrome. Under oxidative conditions, the formation of an intra-molecular disulfide bond between C60 and C65 residues within the Y65C-mutant WW domain may be favoured. This scenario is conceivable due to the inherent flexibility of the β 1- β 2 loop, allowing it to curve away from the ligand-binding "cradle" toward the hydrophobic core of the WW domain, so as to bring the C60 and C65 residues in close proximity and thereby drive their covalent linkage via an S-S bond. Note that the S-S bond between the sidechain SG atoms of C60 and C65 residues is omitted for clarity.

FEBS Lett. Author manuscript; available in PMC 2013 August 14.