Biochemical and Biophysical Research Communications 437 (2013) 140–145

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Effects of the gout-causing Q141K polymorphism and a CFTR ΔF508 mimicking mutation on the processing and stability of the ABCG2 protein

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ARTICLE INFO

Article history:
Received 12 June 2013
Available online 22 June 2013

Keywords:
ABCG2
Polymorphism
Gout
Cystic fibrosis
Stability
Structure

ABSTRACT

ABCG2 is an important multidrug transporter involved also in urate transport, thus its mutations can lead to the development of gout and may also alter general drug absorption, distribution and excretion. The frequent ABCG2 polymorphism, Q141K, is associated with an elevated risk of gout and has been controversially reported to reduce the plasma membrane expression and/or the transport function of the protein. In the present work we examined the stability and cellular processing of the Q141K ABCG2 variant, as well as that of the ΔF142 ABCG2, corresponding to the ΔF508 mutation in the CFTR (ABCC7) protein, causing cystic fibrosis. The processing and localization of full length ABCG2 variants were investigated in mammalian cells, followed by Western blotting and confocal microscopy, respectively. Folding and stability were examined by limited proteolysis of Sf9 insect cell membranes expressing these ABCG2 constructs. Stability of isolated nucleotide binding domains, expressed in and purified from bacteria, was studied by CD spectroscopy. We find that the Q141K variant has a mild processing defect which can be rescued by low temperature, a slightly reduced activity, and a mild folding defect, especially affecting the NBD. In contrast, the ΔF142 mutant has major processing and folding defects, and no ATPase function. We suggest that although these mutations are both localized within the NBD, based on molecular modeling their contribution to the ABCG2 structure and function is different, thus rescue strategies may be devised accordingly.

1. Introduction

ABCG2 is a member of the ABC (ATP-Binding Cassette) superfamily, containing membrane transporters that have important roles in cellular transport processes by extruding endo- and xenobiotics from the cells [1]. It has been shown that ABCG2 plays a significant protective role at physiological barriers (e.g. the blood–brain, blood–testis and maternal–fetus barriers) by exporting xenobiotics with different chemical properties. ABCG2 also takes part in the elimination of different metabolic products, including porphyrin derivatives and urate [2]. The catalytic cycle of the transport process is powered by ATP. The cytosolic nucleotide binding domains (NBD) bind and hydrolyze ATP, while the transmembrane domains (TMD) provide the pathway through the membrane bilayer and are important determinants of substrate recognition [3]. Small α-helical regions in the intracellular segments of the transmembrane domain, called coupling helices, connect the TMDs with NBDs, physically permitting the effects of ATP binding and hydrolysis at the NBD to be transmitted to the TMDs [3]. ABCG2 contains one NBD, located at the N-terminus, and one TMD. A functional ABCG2 transporter needs homo-dimerization [4], however, no reassuring molecular model is available for this protein as yet.

Functional expression, substrate specificity, as well as cellular processing of membrane transporters can be significantly

Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator, ABCC7; HEK, human embryonic kidney 293 cells; NBD, nucleotide binding domain; MBP, maltose binding protein; TMD, transmembrane domain; PBA, 4-phenylbutyrate; Sf9, *Spodoptera frugiperda* cells; SNP, single nucleotide polymorphism; 3R, G188E, R191Q, and R193K rescue mutations in ABCG2-NBD.

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http://dx.doi.org/10.1016/j.bbrc.2013.06.054

influenced by mutations and polymorphisms. A frequent SNP in the ABCG2 gene produces a Q141K variant [5] of the protein. This amino acid change is located in the NBD, and the polymorphism was shown to be associated with gout by genome-wide association studies [2]. The Q141K variation has been reported to result in altered processing and a less efficient substrate transport by ABCG2 [6–8]. Expression of ABCG2 variants in single gene copy systems suggested that the Q141K variant has a reduced plasma membrane expression level and increased intracellular concentration [9,10].

In order to rescue the Q141K variant phenotype, which may be relevant in preventing gout and increasing protection against xenobiotics, the molecular details associated with this amino acid change have to be explored. The Q141K change is located in the NBD of ABCG2, adjacent to F142, which has an analogous position to F508 of the CFTR (ABCC7) protein (Figs. S1 and S2). The most frequent and studied mutation in CFTR, causing cystic fibrosis, is the deletion of F508. Therefore it has been suggested that the ABCG2 Q141K variant may cause similar changes to the ΔF508 mutation, disrupting the interface between the NBD and the TMD [2,11]. F508 in CFTR is located in a surface loop of NBD1, interacting with other hydrophobic amino acids in the coupling helix [12]. The ΔF508 in CFTR affects not only the integrity of this interface but also the folding and stability of NBD1 [13]. Therefore the mutant CFTR is promptly degraded after synthesis and does not reach the plasma membrane. Although ΔF508 CFTR folding and plasma membrane expression can be facilitated by small molecules, called correctors, the mutant protein is not functional at a physiological temperature [14]. Other types of drugs, called stimulators are needed to restore the function.

In the present study we compared the biochemical properties of the wild-type ABCG2 to those of the Q141K variant and the ΔF142 mutation. The full length ABCG2 constructs were expressed in mammalian cell lines to study their expression and localization, and in insect cells to investigate conformational alterations by limited proteolysis. Isolated NBD constructs were expressed and purified from bacterial expression, to compare changes in the NBD structures at a higher resolution. Our results indicate that the Q141K polymorphism reduces the efficient processing and membrane targeting, while has only a mild effect on the structure and function of ABCG2. This is in contrast to the major structural and functional effects of the ΔF142 mutation.

2. Methods

2.1. Vectors and cell lines

ABCG2 constructs for mammalian expression were generated in a vector for the *Sleeping Beauty* transposase system [15]. The CMV promoter-ABCG2-polyA fragment from pcDNA3.1 containing the cDNA of WT ABCG2 [8] was inserted into the pSB-CAG-puromycin vector (kindly provided by O. Kolacsek and T. Orban, RCNS, Budapest, Hungary). This pSB-CMV-ABCG2-CAG-puromycin vector was used as a template for mutagenesis. Stable cell lines were obtained by cotransfection with 100 ng of the SB100x *Sleeping Beauty* transposase and selection with 1.2 μg/ml puromycin for 2 weeks. HEK 293 cell lines were transfected using FuGENE® HD (Promega). Cells were grown under standard condition.

For expression in insect cells the pAcUW21-L/ABCG2 vector was used [16], to which the mutations were introduced by cassette exchange. Recombinant baculoviruses were generated with the BaculoGold Transfection Kit (BD Biosciences) according to the manufacturer's instructions. Sf9 cells were infected and cultured as described [17]. Individual virus clones, expressing high levels of the different human ABCG2 variants, were obtained by end point dilution and subsequent amplification.

2.2. Western blotting

HEK cells or Sf9 membranes were supplemented with Laemmli buffer in the presence of 5% β-mercaptoethanol and subjected to SDS–PAGE. Western blot analysis was performed with the BXP-21 anti-ABCG2 monoclonal antibody (kindly provided by Drs. George Scheffer and Rik Scheper, Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands) in a 500× (HEK cells) or 2000× (Sf9 cells) dilution and a goat anti-mouseCy5 (Amersham) fluorescent secondary antibody (2500× dilution). Blots were scanned by a Typhoon 9410 scanner and images were quantified with ImageJ 1.44p.

2.3. Confocal microscopy

Transiently transfected HEK cells were grown for 48 h on 8-well Nunc Lab-Tek II chambered coverglasses (Nalge Nunc International). Immunostaining of permeabilized cells was performed using BXP-21 plus anti-mouseAlexa488 (Molecular Probes), anti-pan-cadherin (Abcam) plus anti-rabbit-Alexa594 (Molecular Probes), and nuclei were stained with DAPI (Molecular Probes) [18].

2.4. Cell surface labeling using FACS

Cell surface labeling was performed as described in the Supplementary methods. Briefly, HEK 293 cells were transfected with ABCG2 constructs and EGFP, the cells cultured for two days and then collected. Aliquots were incubated with the 5D3 antibody or IgG2b isotype control in the presence or absence of Ko143. Cells were washed then incubated with a goat anti-mouse Alexa647-conjugated secondary antibody and labeling was measured using a FACSCalibur flow cytometer.

2.5. Membrane preparation and limited proteolysis

Limited proteolysis experiments were performed on Sf9 membranes which were prepared as described earlier [19]. The levels of the ABCG2 variants in the membrane were determined by modified Lowry method and Western blotting [17]. To achieve similar level of the ABCG2 variants, membranes with higher expression levels were supplemented with non-ABCG2 expressing membranes to have the same ABCG2/total membrane protein ratio. Reaction mixtures (125 μg protein, 0.5 mM EGTA, 40 mM Tris, 50 mM KCl, 0.5 mM DTT, pH 7.4) were pre-incubated for 1 h at 37 °C then trypsin was added to start the proteolysis (enzyme to total protein ratio was 1:250 w/w). The reaction was stopped at 2, 4, 6, 8, 10, 20 min with a protease inhibitor mixture (Laemmli buffer supplemented with 8 μg/ml aprotinin, 10 μg/ml leupeptin, and 50 μM PMSF), and 5 μg protein was loaded for SDS–PAGE. The ABCG2 fragments were visualized by Western blotting.

2.6. NBD purification and CD spectroscopy

The ABCG2 NBD (aa. 1–288) was expressed and purified from bacteria as an MBP fusion protein (Fig. S3) and CD spectra were measured as described in the Supplementary material (Fig. S3).

3. Results and discussion

3.1. The Q141K variant localizes to the plasma membrane, in contrast to the ΔF142 mutant

In order to examine the effects of Q141K and ΔF142 alterations on the ABCG2 protein expression and localization, we transiently

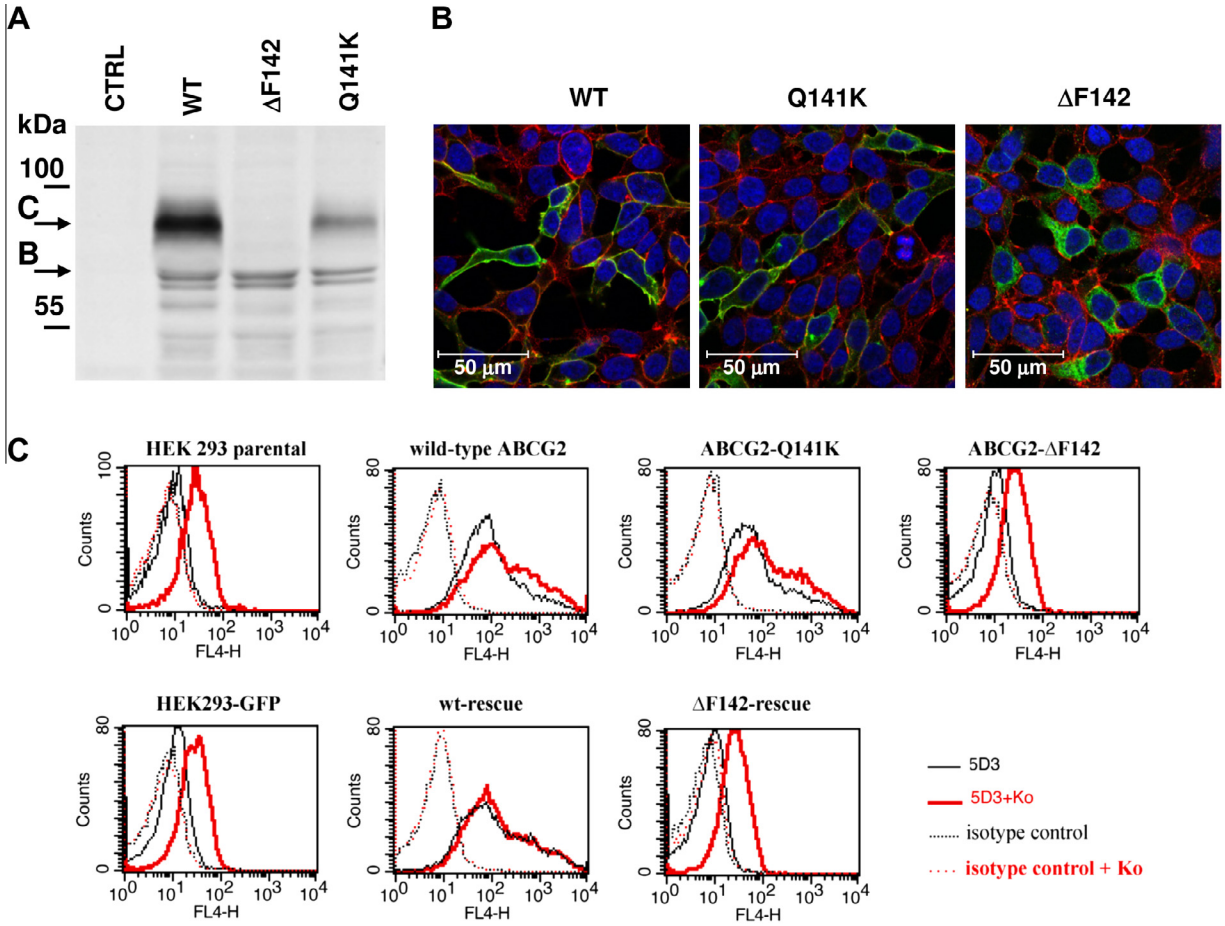

Fig. 1. Expression of ABCG2 WT, Q141K, and ΔF142 constructs in HEK cells. (A) Transient expression of the variants in HEK cells. Western blotting was performed with BXP-21 antibody. Fully glycosylated ABCG2 is detected at 72–75 kDa. Arrows indicate the fully and not glycosylated bands C and B, respectively. (B) Cellular localization of the ABCG2 proteins in transiently transfected HEK cells. BXP-21 anti-ABCG2: green, membrane marker anti-cadherin: red, nucleus stain DAPI: blue. (C) Flow cytometry measurement of the cell surface expression of the ABCG2 constructs, labeled by the 5D3 antibody recognizing an epitope in the third extracellular loop of ABCG2. Quantitation of 5D3 labeling is provided in Table 1.

expressed these constructs in HEK cells. Since the correctly folded and mature ABCG2 protein is fully glycosylated and exhibits higher molecular weight (72 kDa) as compared to the immature form, the expression and maturation of the constructs were assessed by Western blotting, using the BXP-21 antibody. As shown in Fig. 1A, the Q141K variant exhibited significant level of maturation, although the amount of the fully glycosylated form was significantly lower, compared to that of the wild type ABCG2. In contrast, the ΔF142 mutant was expressed at a low level and only in a core-glycosylated form, suggesting that this mutant is degraded before reaching the Golgi compartment. The transfection efficiency was the same in the case of all constructs, as indicated by the equal levels of the co-expressed EGFP (see Fig. S4).

The cellular localization of the ABCG2 protein variants was studied by confocal microscopy. Transiently transfected HEK cells were fixed, permeabilized, and labeled by the BXP-21 antibody. Both the wild type and Q141K ABCG2 were found to be expressed mostly in the plasma membrane, although the Q141K variant exhibited a higher level of intracellular staining (Fig. 1B). In contrast, the ΔF142 ABCG2 protein was not present in the plasma membrane and showed strong intracellular staining. ABCG2 cell surface expression was quantified by measuring the binding of 5D3 antibody, which recognizes an extracellular epitope, thus marking the cell surface ABCG2 when studying non-permeabilized cells by flow cytometry (Fig. 1C and Table 1). These experiments confirmed lower cell surface expression of the Q141K variant compared to the wild type, whereas cells expressing the ΔF142 mutant did not bind the 5D3 antibody. Our results demonstrate that the effect of the Q141K variation causes a milder phenotype in ABCG2 than the ΔF142 mutation, while this latter mutation results in an expression deficiency similar to that of ΔF508 CFTR.

3.2. Phenylbutyrate treatment increases the level of mature WT and Q141K ABCG2, while do not rescue ΔF142 ABCG2

To evaluate the potential folding and trafficking deficiency of the ABCG2 Q141K and ΔF142 proteins, their expression was characterized under more permissive conditions. Cells were grown at physiological and low temperature in the absence or presence of phenylbutyrate to promote protein maturation. It has been shown that mutant membrane proteins, including ΔF508 CFTR can be rescued by culturing cells at low temperature, when the quality control is less stringent. Phenylbutyrate (PBA) has been suggested to increase the expression of chaperons that most likely aid the folding of mutant proteins [20].

As shown in Fig. 2, in transiently transfected HEK cells the level of the wild type and the Q141K proteins slightly decreased at low temperature (Fig. 2), indicating the adverse effect of low temperature on protein synthesis. Phenylbutyrate increased the protein expression of both the WT and the Q142K ABCG2 similarly (1.6–1.7×). The ratio of the fully and non-glycosylated proteins remained constant at all temperature with and without PBA

Table 1
Cell surface expression of the ABCG2 constructs quantitated by flow cytometry.

	Geometric mean values						
	HEK parent	EGFP only	ABCG2 WT	ABCG2 Q141K	ABCG2 ΔF142	ABCG2 3R	ABCG2ΔF142 3R
Isotype control	7.5	7.6	7.3	7.2	7	8.4	7
Isotype control + Ko143	6.99	7.3	7.3	7.1	7	8.3	7.44
5D3	9.45	12	110	78	10	146	8.4
5D3 + Ko143	30	29.5	210	150	27	152	27.4
Transfection efficiency EGFP FL1	0%	85%	93%	89%	89%	83%	89%

treatment (Fig. 2, numbers in brackets). This observation suggest that both the low temperature and PBA generally increase protein expression (enhance folding, diminish quality control and degradation) and do not act specifically on ABCG2 maturation. Neither the expression nor the maturation of the ΔF142 ABCG2 mutant was affected by lower temperature or PBA.

The ΔF508 CFTR was reported to be rescued by second site mutations, reversing the kinetic and structural changes caused by the deletion. The most effective ones in CFTR NBD1 are the G550E, R553Q, and R555K mutations, located in the highly conserved ABC signature region [13,21]. Therefore all these three mutations were introduced into the corresponding regions of the ABCG2 ΔF142 construct (3R: G188E, R191Q, and R193K). However, these rescue mutations were ineffective in the case of ABCG2 (Fig. S5). These observations may stress the different structural features of ABCG2 as compared to the members of the B or C subfamily of ABC proteins. First, ABCG2 is a "half-transporter" and has to dimerize to be functional, thus the co-translational and post-translational processes may be different from those in the "full transporters". Second, the domain order of the NBD and TMD is reversed, that may also determine a distinct architecture for ABCG2. Third, the size of the ABCG2 TMD domain is significantly smaller than in other exporter type ABC proteins. This results in shorter intracellular parts of the transmembrane helices, thus the coupling helix and NBD are most likely located closer to the membrane bilayer. Interestingly, one of the rescue mutations, G188E has been reported to promote Q141K maturation [11]. Most likely, similarly to the homologous CFTR G550E [13,21], this mutation increases the thermostability of NBD in an extent that is sufficient to counteract the effect of the mild Q141K mutation, but not that of the ΔF142. While this G188E mutation does not increase the maturation of WT ABCG2 [11], the analogous G550E mutation promotes the maturation of WT CFTR [21] that again suggest fundamental differences between the NBDs of the two proteins.

We have also introduced potential rescue mutations into the putative coupling helix, but with no successful rescue of the ΔF142 ABCG2 (Fig. S5B). Using bioinformatics tools we identified the same region as a putative coupling helix that is also suggested by Woodward *et al.*, and includes aa. 473 as a potential amino acid interacting with F142 [11]. We found that second site mutations at these positions did not rescue ΔF142 ABCG2 (Fig. S5B). Based on these observations, we either incorrectly predict the coupling helix, or rescuing via the coupling helix works for CFTR, but not for the structurally different ABCG2.

3.3. The Q141K NBD is more stable than the ΔF142 NBD, indicated by limited proteolysis experiments

Since the quality control system in insect cells is less stringent than in mammalian cells, Sf9 cells were used to express the Q141K, ΔF142, and WT ABCG2 for functional and biochemical studies. In Sf9 cells the expression levels of the wild type and the Q141K ABCG2 variant were similar, while the expression of the deletion mutant was significantly lower (Fig. 3A). The vanadate sensitive ATPase activity of the Q141K variant in isolated Sf9 membrane was approximately 70% of that of the wild type protein ([8] and Fig. S6A). In contrast, the ΔF142 mutant had no measurable ATPase activity.

In order to examine the changes in stability and conformation of the ABCG2 variants, the isolated membranes were subjected to limited enzymatic proteolysis. In this case different cleavage sites may be accessible in the mutants than in the wild type protein, resulting in different cleavage patterns or rate of digestion [22]. Membranes expressing Q141K, ΔF142, and WT ABCG2 were

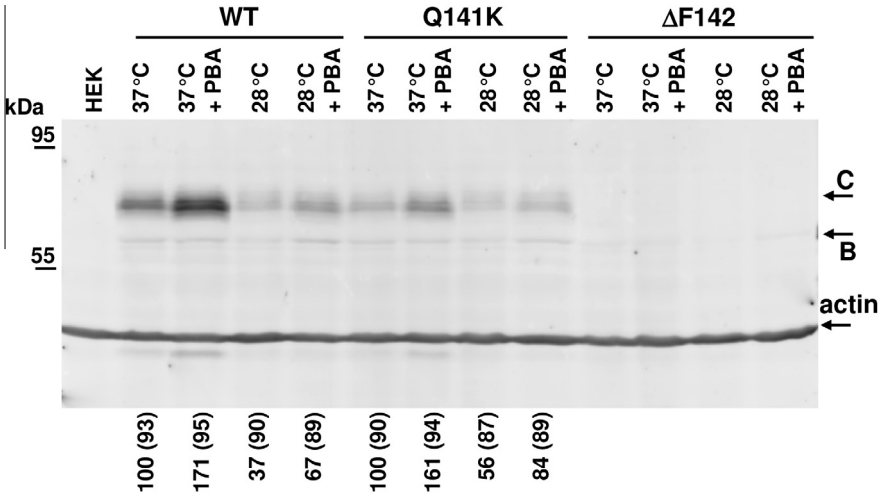

Fig. 2. Rescuing ABCG2 variants by low temperature and phenylbutyrate. HEK cells were transiently transfected and grown at 37 °C and 28 °C in the absence or presence of 2 mM phenylbutyrate to rescue the expression of the variants. After two days cells were collected and subjected to Western blotting with the BXP-21 and anti-beta actin antibodies. The same amount (50 μg total protein) of samples was loaded. A representative blot is shown. Changes were quantified and normalized to the cells grown at 37 °C and the ratio of the intensity of the fully glycosylated band C to that of total (bands B and C) were calculated and shown in brackets. Numbers are percentage values, PBA: phenylbutyrate.

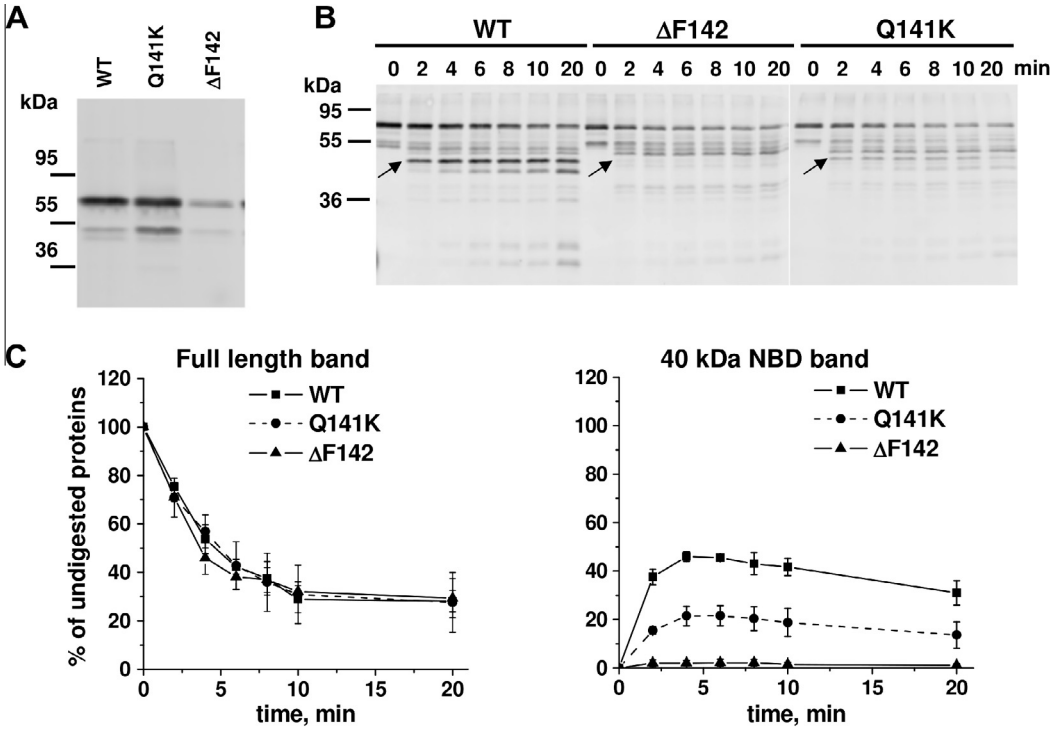

Fig. 3. Instability of NBD is tested by limited proteolytic experiments with full length proteins. (A) WT and Q141K ABCG2 are expressed at similar levels in membranes isolated from Sf9 insect cells, as detected by Western blotting using the BXP-21 antibody at ~60 kDa. (B) Limited proteolytic experiments were performed on Sf9 membranes with similar levels of the ABCG2 variants. Reaction mixtures were pre-incubated for 1 h at 37 °C then trypsin was added to initiate the reaction, and then proteolysis was stopped with a protease inhibitor mixture. The ABCG2 fragments containing the antibody epitope were visualized after Western blotting with the BXP-21 antibody. The ~40 kDa NBD containing fragment is indicated by arrows. (C) The signal of the fluorescent secondary antibody was quantified by densitometry and values normalized to the full length band at 0 min were plotted. The figures show the mean values with standard errors of three independent experiments.

treated by low concentrations of trypsin for various time periods. The samples were applied to SDS–PAGE and the ABCG2 fragments were visualized by Western blotting, using anti-ABCG2 antibodies (Fig. 3B and Fig. S7). The full length ABCG2 protein and a 40 kDa fragment were both recognized by the pAb405 and BXP-21 antibodies, generated against epitopes located around the N- and C-terminal ends of NBD, respectively.

The degradation rate of the full length ABCG2 (band 72 KDa) was relatively slow and similar for all ABCG2 variants (Fig. 3C) indicating that dimers of the protein variants are formed and relatively stable in contrast to ΔF508 CFTR which degraded at a high rate [22,23]. Interestingly, the full length ΔF142 protein was as resistant to digestion even at 37 °C, and in the presence of mild detergents (1% Igepal CA-630; not shown) as the wild type, suggesting homodimer formation also by this mutant (Fig. S6B). When the NBD-containing 40 kDa fragments were released from the full length proteins, they exhibited significant differences in their sensitivity to trypsin (Fig. 3C). The ΔF142 NBD was degraded at a high rate (similar to that reported for ΔF508 NBD1 in the CFTR). Although the rate of Q141K degradation was somewhat greater than that of the WT, it was clearly smaller than the rate of the ΔF142 fragment. It should be noted that the sensitivity of the

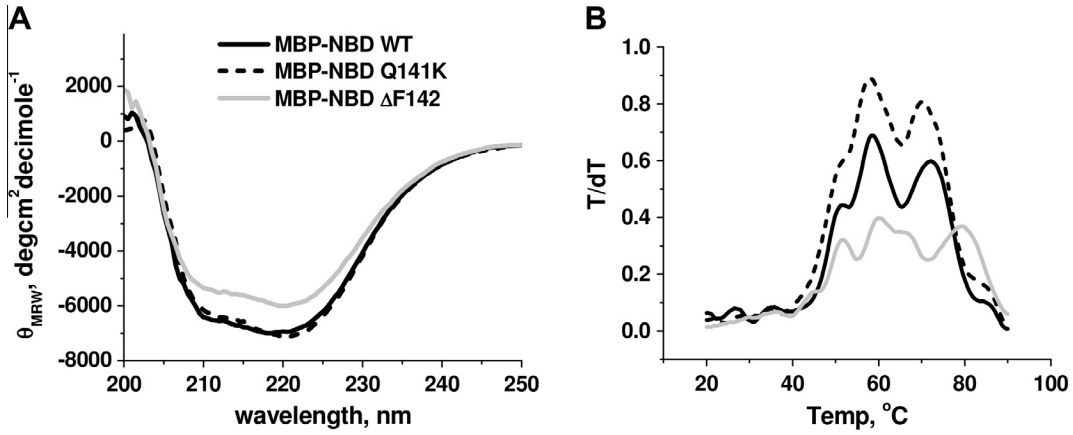

Fig. 4. Stability of the isolated NBD constructs characterized by CD spectroscopy. (A) Far UV CD spectra of WT, Q141K, and ΔF142 NBD fused to MBP, expressed in and isolated from *E. coli*. Spectra were obtained in 10 mM Tris–HCl, 100 mM NaCl, 10% glycerol, 5 mM β-mercaptoethanol at 25 °C and pH 8.0. (B) Thermal unfolding of isolated MBP-fused NBD constructs was followed by measuring CD at 222 nm between 20 °C and 90 °C.

Q141K variant may also be caused by a potential extra trypsin cleavage site introduced by the Q141K variation [11].

3.4. The thermal stability of the isolated Q141K NBD is similar to the WT, in contrast to the ΔF142

To identify the effects of the amino acid changes on the NBD conformation and dynamics, isolated NBD constructs fused to MBP, were purified from bacteria (the MBP could not be removed because of a rapid precipitation of the isolated NBDs). Similarly to other isolated ABC transporter NBD constructs, no significant ATPase activity was observed (not shown). The CD spectra of the NBD constructs indicated a significant level of stable α-helices and β-sheets, thus they were likely folded (Fig. 4A).

In order to investigate the stability of these constructs, CD spectra were recorded at 222 nm between 20 °C and 90 °C. The curves from derivative processing of the CD spectra changes are plotted in Fig. 4B, where peaks indicate melting points. For the NBD constructs several melting points were observed, as expected for fused proteins and also for ABC-NBDs, which consist of two subdomains. We found that the melting curve of the Q141K variant was similar to that of the wild type, while the melting profile of the ΔF142 differed significantly.

As a summary, we found that the ABCG2 Q141K variant is expressed in the plasma membrane at a lower level and with a somewhat decreased function, as compared to the wild type protein. The summation of these two moderate effects may result in insufficient transport activity, thus in pathological consequences. While the Q141K variation is located in a position adjacent to the F508 in CFTR, their structural roles and functions are significantly different based on our experiments and *in silico* analysis (Fig. S2). These differences should be taken into account when designing specific rescue strategies. Moreover, studies focusing on positions similar to the ABCG2 Q141K variant in other ABC transporters may help to understand alterations at the protein level in various diseases, and promote the development of successful treatments.

Acknowledgments

We gratefully thank Géza Antallfy for his help in confocal microscopy, Gergely Szakács for suggestions to this manuscript and the technical help of Éva Krizsán is gratefully acknowledged. This work was supported by MB08C-80039 and OTKA 83533.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.06.054.

References

[1] B. Sarkadi, L. Homolya, G. Szakacs, A. Varadi, Human multidrug resistance ABCB and ABCG transporters: participation in a chemoimmunity defense system, Physiol. Rev. 86 (2006) 1179–1236.
[2] O.M. Woodward, A. Kottgen, J. Coresh, E. Boerwinkle, W.B. Guggino, M. Kottgen, Identification of a urate transporter, ABCG2, with a common functional polymorphism causing gout, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 10338–10342.
[3] I.D. Kerr, P.M. Jones, A.M. George, Multidrug efflux pumps: the structures of prokaryotic ATP-binding cassette transporter efflux pumps and implications for our understanding of eukaryotic P-glycoproteins and homologues, FEBS J. 277 (2010) 550–563.
[4] C. Ozvegy, T. Litman, G. Szakacs, Z. Nagy, S. Bates, A. Varadi, B. Sarkadi, Functional characterization of the human multidrug transporter, ABCG2, expressed in insect cells, Biochem. Biophys. Res. Commun. 285 (2001) 111–117.
[5] Y. Imai, M. Nakane, K. Kage, S. Tsukahara, E. Ishikawa, T. Tsuruo, Y. Miki, Y. Sugimoto, C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141K protein and low-level drug resistance, Mol. Cancer Ther. 1 (2002) 611–616.
[6] C. Kondo, H. Suzuki, M. Itoda, S. Ozawa, J. Sawada, D. Kobayashi, I. Ieiri, K. Mine, K. Ohtsubo, Y. Sugiyama, Functional analysis of SNPs variants of BCRP/ABCG2, Pharm. Res. 21 (2004) 1895–1903.
[7] S. Mizuarai, N. Aozasa, H. Kotani, Single nucleotide polymorphisms result in impaired membrane localization and reduced atpase activity in multidrug transporter ABCG2, Int. J. Cancer 109 (2004) 238–246.
[8] K. Morisaki, R.W. Robey, C. Ozvegy-Laczka, Y. Honjo, O. Polgar, K. Steadman, B. Sarkadi, S.E. Bates, Single nucleotide polymorphisms modify the transporter activity of ABCG2, Cancer Chemother. Pharmacol. 56 (2005) 161–172.
[9] A. Basseville, A. Tamaki, C. Ierano, S. Trostel, Y. Ward, R.W. Robey, R.S. Hegde, S.E. Bates, Histone deacetylase inhibitors influence chemotherapy transport by modulating expression and trafficking of a common polymorphic variant of the ABCG2 efflux transporter, Cancer Res. 72 (2012) 3642–3651.
[10] K. Wakabayashi, H. Nakagawa, T. Adachi, I. Kii, E. Kobatake, A. Kudo, T. Ishikawa, Identification of cysteine residues critically involved in homodimer formation and protein expression of human ATP-binding cassette transporter ABCG2: a new approach using the flp recombinase system, J. Exp. Ther. Oncol. 5 (2006) 205–222.
[11] O.M. Woodward, D.N. Tukaye, J. Cui, P. Greenwell, L.M. Constantoulakis, B.S. Parker, A. Rao, M. Kottgen, P.C. Maloney, W.B. Guggino, Gout-causing Q141K mutation in ABCG2 leads to instability of the nucleotide-binding domain and can be corrected with small molecules, Proc. Natl. Acad. Sci. U.S.A. 110 (2013) 5223–5228.
[12] A.W. Serohijos, T. Hegedus, A.A. Aleksandrov, L. He, L. Cui, N.V. Dokholyan, J.R. Riordan, Phenylalanine-508 mediates a cytoplasmic-membrane domain contact in the CFTR 3D structure crucial to assembly and channel function, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 3256–3261.
[13] W.M. Rabeh, F. Bossard, H. Xu, T. Okiyoneda, M. Bagdany, C.M. Mulvihill, K. Du, S. di Bernardo, Y. Liu, L. Konermann, A. Roldan, G.L. Lukacs, Correction of both NBD1 energetics and domain interface is required to restore DeltaF508 CFTR folding and function, Cell 148 (2012) 150–163.
[14] K. Varga, R.F. Goldstein, A. Jurkuvenaite, L. Chen, S. Matalon, E.J. Sorscher, Z. Bebok, J.F. Collawn, Enhanced cell-surface stability of rescued DeltaF508 cystic fibrosis transmembrane conductance regulator (CFTR) by pharmacological chaperones, Biochem. J. 410 (2008) 555–564.
[15] Z. Izsvak, Z. Ivics, R.H. Plasterk, Sleeping beauty, a wide host-range transposon vector for genetic transformation in vertebrates, J. Mol. Biol. 302 (2000) 93–102.
[16] C. Ozvegy, A. Varadi, B. Sarkadi, Characterization of drug transport, ATP hydrolysis, and nucleotide trapping by the human ABCG2 multidrug transporter. Modulation of substrate specificity by a point mutation, J. Biol. Chem. 277 (2002) 47980–47990.
[17] M. Muller, E. Bakos, E. Welker, A. Varadi, U.A. Germann, M.M. Gottesman, B.S. Morse, I.B. Roninson, B. Sarkadi, Altered drug-stimulated ATPase activity in mutants of the human multidrug resistance protein, J. Biol. Chem. 271 (1996) 1877–1883.
[18] C. Ozvegy-Laczka, R. Laczko, C. Hegedus, T. Litman, G. Varady, K. Goda, T. Hegedus, N.V. Dokholyan, B.P. Sorrentino, A. Varadi, B. Sarkadi, Interaction with the 5D3 monoclonal antibody is regulated by intramolecular rearrangements but not by covalent dimer formation of the human ABCG2 multidrug transporter, J. Biol. Chem. 283 (2008) 26059–26070.
[19] B. Sarkadi, E.M. Price, R.C. Boucher, U.A. Germann, G.A. Scarborough, Expression of the human multidrug resistance cDNA in insect cells generates a high activity drug-stimulated membrane ATPase, J. Biol. Chem. 267 (1992) 4854–4858.
[20] M. Lim, K. McKenzie, A.D. Floyd, E. Kwon, P.L. Zeitlin, Modulation of deltaF508 cystic fibrosis transmembrane regulator trafficking and function with 4-phenylbutyrate and flavonoids, Am. J. Respir. Cell Mol. Biol. 31 (2004) 351–357.
[21] A.C. DeCarvalho, L.J. Gansheroff, J.L. Teem, Mutations in the nucleotide binding domain 1 signature motif region rescue processing and functional defects of cystic fibrosis transmembrane conductance regulator delta f508, J. Biol. Chem. 277 (2002) 35896–35905.
[22] F. Zhang, N. Kartner, G.L. Lukacs, Limited proteolysis as a probe for arrested conformational maturation of delta F508 CFTR, Nat. Struct. Biol. 5 (1998) 180–183.
[23] T. Hegedus, A. Aleksandrov, L. Cui, M. Gentzsch, X.B. Chang, J.R. Riordan, F508del CFTR with two altered RXR motifs escapes from ER quality control but its channel activity is thermally sensitive, Biochim. Biophys. Acta 1758 (2006) 565–572.