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Presenilin-dependent expression of STIM proteins and dysregulation of capacitative Ca^{2+} entry in familial Alzheimer's disease

Lukasz Bojarski [a], Pawel Pomorski [b], Aleksandra Szybinska [a], Mirosław Drab [a], Anna Skibinska-Kijek [a], Joanna Gruszczynska-Biegala [a], Jacek Kuznicki [a,b,*]

[a] Laboratory of Neurodegeneration, International Institute of Molecular and Cell Biology, 4 Ks. Trojdena Street, 02-109 Warsaw, Poland
[b] Nencki Institute of Experimental Biology, 3 Pasteur Street, 02-093 Warsaw, Poland

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ABSTRACT

Mutations in presenilin 1 (PS1), which are the major cause of familial Alzheimer's disease (FAD), are involved in perturbations of cellular Ca^{2+} homeostasis. Attenuation of capacitative Ca^{2+} entry (CCE) is the most often observed alteration of Ca^{2+} homeostasis in cells bearing FAD PS1 mutations. However, molecular mechanisms underlying this CCE impairment remains elusive. We demonstrate that cellular levels of STIM1 and STIM2 proteins, which are key players in CCE, depend on presenilins. We found increased level of STIM1 and decreased level of STIM2 proteins in mouse embryonic fibroblasts lacking presenilins. Fura-2 ratiometric assays revealed that CCE is enhanced in these cells after Ca^{2+} stores depletion by thapsigargin treatment. In turn, overexpression of PS1 with FAD mutations in HEK293 cells led to an attenuation of CCE. Although, no changes in STIM protein levels were observed in these HEK293 cells, FAD mutations in endogenous PS1 in human B lymphocytes resulted in a decreased expression of STIM2 in parallel to an attenuation of CCE. Our experiments showing that knock-out of presenilins in MEF cells and FAD mutations in endogenous PS1 in lymphocytes affect both CCE and the cellular level of STIM proteins open new perspectives for studies on CCE in FAD.

1. Introduction

Alzheimer's disease (AD) is a chronic disorder that leads to the death of neurons and causes severe cognitive disability and dementia. Mutations in presenilin1 (PS1), presenilin2 (PS2) and amyloid precursor protein (APP) lead to familial Alzheimer's disease (FAD), reviewed in [1]. The neuropathological hallmarks of AD include a progressive loss of neurons, synaptic degeneration, as well as a deposition of β-amyloid (Aβ) plaques and of neurofibrillary tangles in specific brain regions. Additionally, disturbances in Ca^{2+} homeostasis have been demonstrated as one of the early pathological changes in cells of AD patients [2,3], reviewed in [4] and [5].

Endoplasmic reticulum (ER) is the main Ca^{2+} store in the cell. After cell activation, Ca^{2+} is released from ER in a pattern specific for time, space and dynamics [6]. The Ca^{2+} release results in a depletion of ER Ca^{2+} stores, which have to be replenished by influx of Ca^{2+} by store-operated Ca^{2+} channels (SOCs) [7]. This process of Ca^{2+} influx via channels activated by the depletion of ER Ca^{2+} stores, is called capacitative Ca^{2+} entry (CCE) [7]. CCE is considered to be ubiquitous in non-excitable cells where it has been studied extensively, reviewed in [7]. There are, however, reports demonstrating that CCE also occurs in excitable cells, such as smooth muscle cells [8] and neuronal cells [9,10].

Attenuation of CCE was shown in several cell types expressing different FAD mutations in PS1 [11–13], reviewed in [5]. This alteration of Ca^{2+} homeostasis may contribute to AD pathogenesis, as the PS1 mutation-driven inhibition of CCE was shown to influence APP processing leading to an increased generation of neurotoxic Aβ1-42 [12].

To date, the molecular mechanism underlying FAD-related disturbances in CCE has not been clarified. However, the recent discovery that STIM1 and STIM2 (Stromal Interacting Molecules 1 and 2) are regulators of CCE, shed a new light on this issue [14,15]. STIM1 and STIM2 are type I ER membrane proteins, containing an unpaired EF-hand motifs that act as Ca^{2+} sensors in the ER lumen. These proteins transduce information on the ER Ca^{2+} level to the plasma membrane. Upon ER Ca^{2+} store depletion, STIM1 and STIM2 redistribute into punctuate structures, move towards the plasma membrane and activate SOC channels [14,16]. Orai1 (CRACM1) is a plasma membrane protein that was shown to be a pore-forming subunit of the SOC channel, activated by STIM1 and STIM2 [17–22].

The aim of our work was to assess whether the expression of STIM proteins depends on presenilins and, if so, whether STIM1 and STIM2

* Corresponding author. International Institute of Molecular and Cell Biology, 4 Ks. Trojdena Street, 02-109 Warsaw, Poland. Tel.: +48225970700; fax: +48225970715.
E-mail address: jacek.kuznicki@iimcb.gov.pl (J. Kuznicki).

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are involved in CCE alterations observed in FAD. Our experiments show that both cellular levels of STIM proteins and CCE are influenced by presenilins, which suggest that STIM1 and STIM2 may play a role in presenilin-dependent alterations of CCE in Alzheimer's disease.

2. Materials and methods

2.1. Cell cultures and antibodies

Human peripheral blood B lymphocytes were isolated from healthy 65 year-old individuals (control group) and from FAD patients with identified mutations in PS1: M139V, H163R, S170F (two patients), E318G, I213F and P117R. In most of the experiments the control group consisted of 8 cell lines isolated from 8 individuals. For the measurements of free intracellular Ca^{2+}, 4 control cell lines were used. Cells were immortalized with Epstein–Barr virus according to a previously described protocol [23] and cultured in RPMI 1640 medium in 1% HEPES buffer and 1% penicillin-streptomycin, supplemented with 10% fetal bovine serum and 2% L-glutamine (all from Sigma). Experimental procedures were approved by the Ethical Committee at the Central Clinical Hospital of the Ministry of Internal Affairs and Administration in Warsaw, Poland. Wild type mouse embryonic fibroblasts (MEF) and PS1−/−, PS2−/− and double knockout cell lines (DKO, lacking both PS1 and PS2) were a kind gift of Bart De Strooper (Catholic University, Leuven, Belgium). MEF as well as HEK293 cells were cultured in DMEM F12 HAM medium with 1% penicillin-streptomycin, supplemented with 10% fetal bovine serum. We used rabbit anti-STIM1 (ProteinTech Group Inc.) and anti-STIM2 (CT) (ProSci Incorporated) polyclonal antibodies as well as mouse anti-actin monoclonal antibody (Sigma).

2.2. Site directed mutagenesis and generation of HEK293 cell lines stably transfected with PS1 FAD mutants

To obtain FAD mutants (P117R, S170F, I213F, L226F, E318G) of human PS1, site directed mutagenesis was performed with PCR in which Turbo Pfu polymerase (Stratagene) and pcDNA3.1(−)/wtPS1 vector (a kind gift of Dr. Jessie Theuns from The University of Antwerp, Belgium) were used as a template. PCR was conducted with the following pairs of primers: P117R F: 5′ GCAGCTAATCTATACCC(C→G)ATTCACA GAAGATACCGA and R: 5′TCGGTATCTTCTGTGAATCGGGTATAGATTAGCTGC, S170F F: 5′ C→TTCTATTGTTGCTGTTCTTTTTTTCATTCATTTAC and R: 5′ ATG ATATAATAAGCCAGGCATGGATGACC, I213F F: 5′ GGTGGGAATGATTTC C(A→T)TTCACTGGAAAGGTCC and R: 5′ TGGACCTTTCCAGTGAAAGGAAATCA TTCCCACC, L226F F: 5′ CGACTCCAGCAGGCATAT(T→A) TCATTATGATTAGTG CC and R: 5′ GGCACTAATCATATGAAATATGCCTGCTGGAGTCG, E318G F: 5′A→GAAGCACAGAAAGGGGTACAAGACAC and R: 5′ CTGCATTATACTTG GAATTTTTGGATACTCTCC. After the reaction the PCR products were incubated with *DpnI* restrictase to eliminate the methylated template. HEK293 cells were transfected with PEI (New England Biolabs) according to manufacturer's protocol. Selection of stable transfectants with G418 (Promega) was started after the cells in culture reached 80% confluency.

2.3. Intracellular Ca^{2+} measurements

Fura-2 digital fluorescence microscopy was used to determine the changes in intracellular Ca^{2+} levels ($[Ca2^{+}]_i$) [24]. Briefly, MEF and HEK293 cells were plated on poly-L-lysine (Sigma) coated coverslips the day before, and human B lymphocytes 3 h before the start of the experiment. 30 min before the Ca^{2+} measurements, the culture medium was replaced by 2 μM Fura-2 AM (Molecular Probes) in a solution containing 137 mM NaCl, 2.7 mM KCl, 1 mM Na_2HPO_4, 25 mM glucose, 20 mM HEPES (pH 7.4), 1 mM $MgCl_2$, 1% bovine serum albumin (standard buffer) supplemented with 2 mM $CaCl_2$ at 37 °C. The coverslips were then mounted in a chamber on a Nikon Diaphot inverted-stage microscope equipped with a fluo×40/1.3 NA oil-immersion objective lens. Ludl Lep MAC 5000 filter wheel system loaded with a Chroma Fura-2 filter set was used for illumination of specimens. Images were acquired using a Retiga 1300 chilled digital CCD camera (QImaging). Measurements were started in a standard buffer supplemented with 0.5 mM EGTA, which after 1 min was replaced by standard buffer supplemented with 0.5 mM EGTA and 1 μM thapsigargin (SIGMA). Afterwards ER Ca^{2+} stores depletion medium was replaced by standard buffer supplemented with 2 mM $CaCl_2$. Data processing was carried out using AQM Advance 6 (Kinetic Imaging Inc) and the MS Excel software. The number of cells measured in at least 3 independent experiments ranged from 52 to 149 for each human B cell line, 89–136 cells for each HEK293 cell line and 125–175 cells for each MEF cell line. $\Delta [Ca^{2+}]$ was calculated as a ratio of the maximum Fura-2 340/380 nm fluorescence intensity after cell treatment with thapsigargin and $CaCl_2$ re-addition, to the value of basal Fura-2 340/380 nm fluorescence intensity, averaged for the first minute of the experiment.

2.4. Real time PCR analysis

RNA was isolated from human B lymphocytes or from MEF cells using the RNeasy Mini Kit (Qiagen). First-strand cDNA (prepared with SuperScript III, Invitrogen) was examined by quantitative Real time PCR with one pair of the following gene- and species-specific primers: human STIM1 (5′AGGCTCTCAATGCCSTGACTTCCA, 5′GTTCAGCGCCAGTAATGCCTTCTT), human STIM2 (5′AGGATAGCAGTGACG AACCTTA, 5′ TTCATCCAGTTATGAGGTGGGCGT), mouse STIM1 (5′GCTCTCA ATGCCATGCCTTCCAAT, 5′TCTAGGCCATGGTTCAACGCCATA) and mouse STIM2 (5′AGCAGTAGTTTATGCCGCTCTCGT, 5′AGGGCAACTTGACACAGACAG GAT), using SYBR Green dye (Applied Biosystems). The samples were analyzed using 7000 Sequence Detection System hardware and software (Applied Biosystems). 18S RNA for normalization was used with the following pair of primers: 5′AACGAACGAGACTC TGGCATG, 5′ CGGACATCTAAGGGCATCACA.

2.5. Immunoblotting

Total protein extracts from B lymphocytes were prepared in a lysis buffer containing 50 mM Tris pH 7.5, 1% NP-40, 0.5% NaDOC and Complete EDTA-Free Protease Inhibitors Cocktail (Roche). Cells were homogenized on ice with a motorized pellet pestle (Sigma) for 5 min and cleared by centrifugation at 12,000 ×g for 10 min. Protein extracts were separated on a 10% SDS-PAGE gel and transferred to Hybond-ECL nitrocellulose membranes (Amersham). The membranes were then immunostained with the appropriate antibodies. The intensity of the bands was measured using a GS-800 Calibrated densitometer and the Quantity One software (Biorad). As a double band was seen on immunoblots on human B lymphocytes and HEK293 cells developed with anti-STIM2 antibody (ProSci Incorporated), we analyzed the lower band which, according to antibody manufacturer information, represents STIM2 protein.

2.6. Statistical analysis

Statistical significance of results of intracellular Ca^{2+} measurements in human B cells was calculated with non-parametric Mann–Whitney *U* test. Statistical significance of results of calcium measurements in MEF and HEK293 cell lines, protein level analysis in HEK293 and MEF cell lines as well as quantification of mRNA for STIM proteins in MEF cell lines were calculated with analysis of variance (ANOVA). Normal distribution of results of all other experiments was indicated with Shapiro–Wilk normality test and

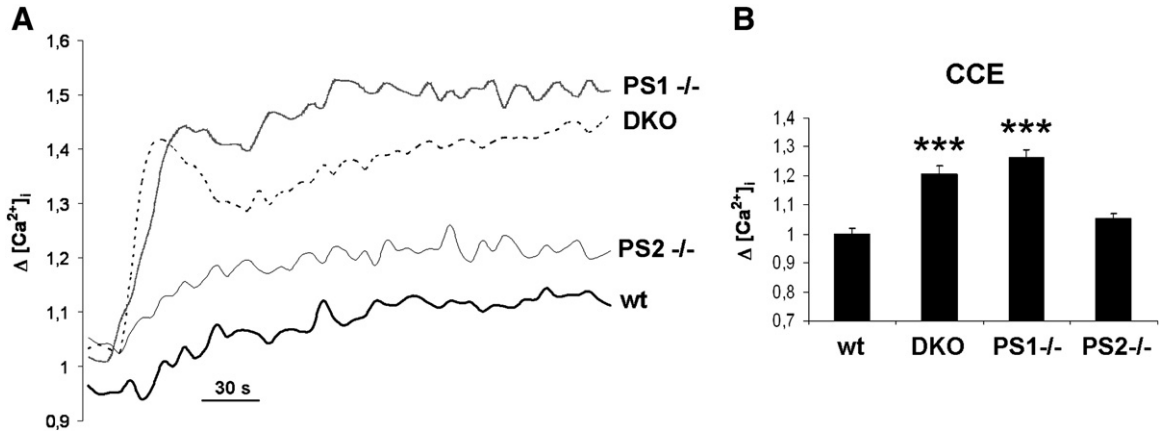

Fig. 1. CCE is enhanced in MEF cells lacking presenilins. (A) Averaged traces obtained by ratiometric Fura-2 analysis of MEF cell lines derived from wt, PS1−/−, PS2−/− or DKO (lacking both PS1 and PS2) animals. (B) Results of quantification of CCE in 4 MEF cell lines (***P<0,001).

statistical significance of normally distributed data was calculated with *T*-test.

3. Results

3.1. Capacitative Ca^{2+} entry is enhanced in cells lacking presenilins

To establish the extent of CCE changes caused by a loss of presenilins, we first analyzed wt, PS1−/−, PS2−/− and DKO (double knock-out, lacking both presenilins) MEF cell lines. Fura-2 assay was used to monitor changes of cytosolic Ca^{2+} concentrations. MEF cells were treated with the SERCA pump inhibitor thapsigargin in Ca^{2+}-depleted medium to empty ER Ca^{2+} stores. After stores depletion, the bath solution was replaced by Ca^{2+} rich buffer in order to facilitate CCE.

Analysis of Fura-2 340 nm/380 nm fluorescence ratio revealed enhanced CCE in PS1−/− and DKO cell lines compared to wt control (Fig. 1). These results confirmed earlier data on the effects of presenilin loss on CCE [12,25,26] and proved the usefulness of MEF cells for further studies on mechanisms of presenilin dependent CCE alterations.

3.2. Cellular levels of STIM1 and STIM2 are presenilin dependent

To determine whether the expression of STIM1 and STIM2 proteins depended on presenilins, we measured the levels of both mRNA and protein of STIMs in MEF cells lacking PS1 or PS2 or both genes. Immunoblotting with anti-STIM1 antibody showed elevated level of STIM1 in MEF DKO cells in comparison to the wt cell line (Fig. 2A, Supplementary Table 1.). This result correlated with trends observed in several RT-PCR experiments in which increased level of *Stim1* mRNA was detected in MEF DKO cells (Fig. 2B).

In turn, the level of *Stim2* mRNA was decreased in MEF DKO cells in comparison to wt MEF, however this change was not statistically significant (Fig. 2C). Densitometric analysis of immunoblots of protein extracts obtained from MEF cells, probed with anti-STIM2 antibody, showed decreased STIM2 protein levels in both DKO and PS1−/− cell lines (Fig. 2D).

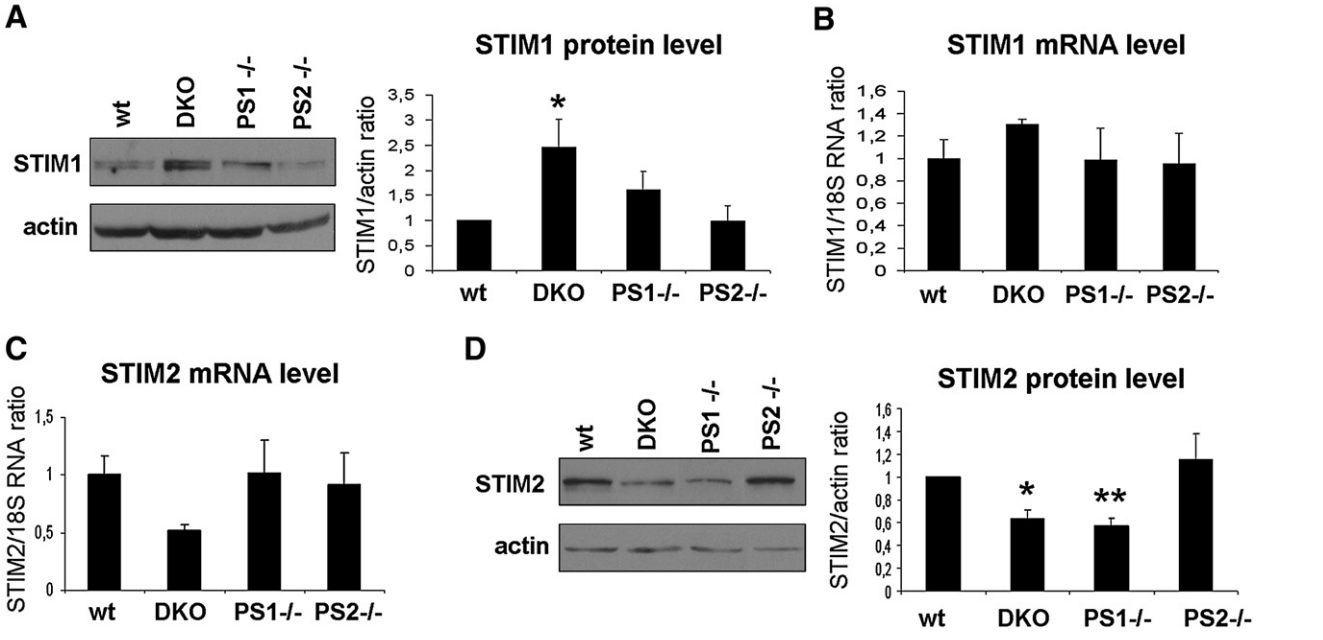

Fig. 2. Cellular level of STIM1 protein is elevated and that of STIM2 is decreased in MEF DKO cells. (A,D) Representative immunoblots of protein extracts from B cell lines developed with anti-STIM1 and anti-STIM2 antibodies and results of densitometric analysis of five independent experiments. (B,C) Results of RT-PCR quantification of STIM1 and STIM2 mRNA levels in MEF cell lines derived from wt, PS1−/−, PS2−/− or DKO (lacking both PS1 and PS2) animals.

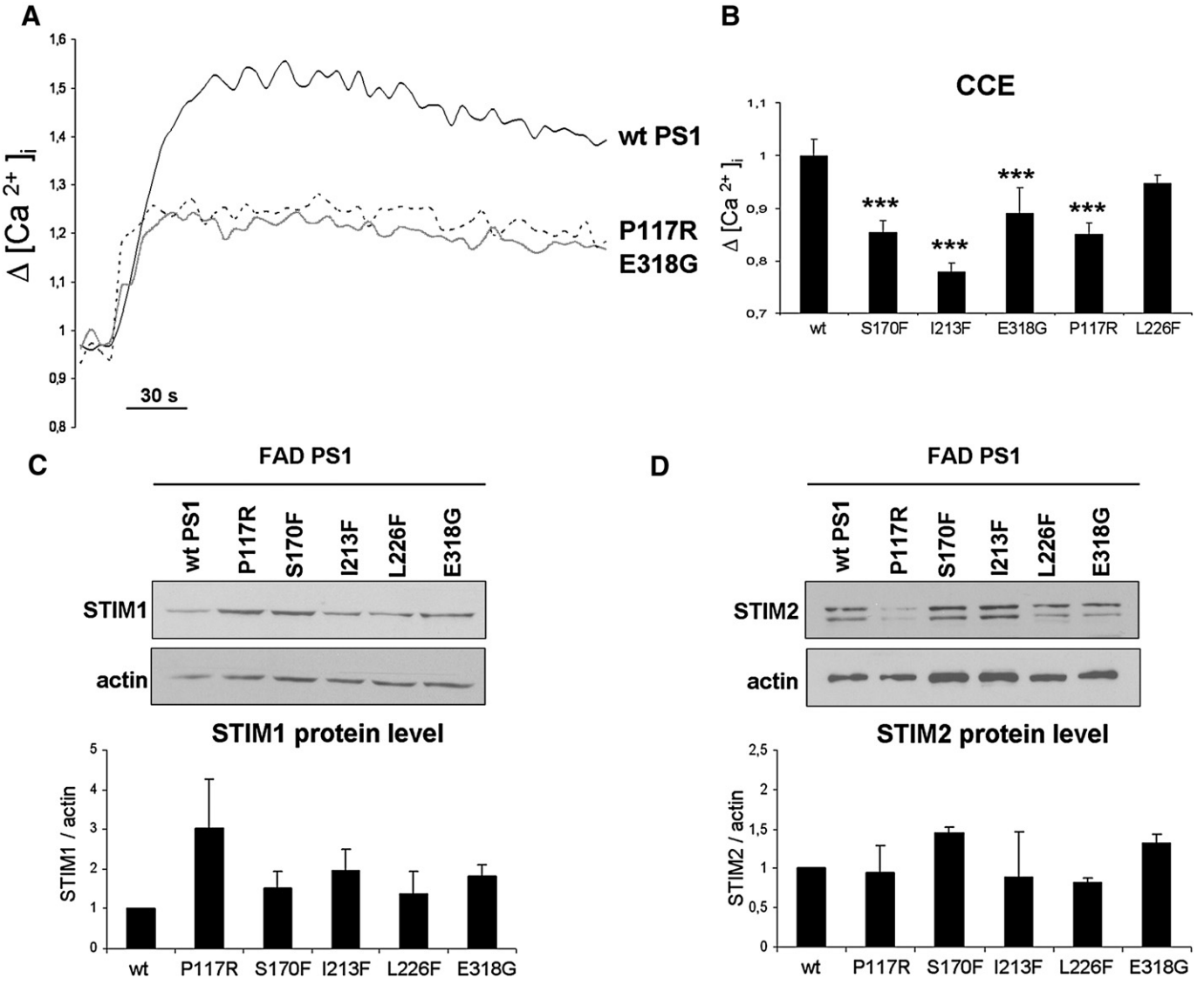

Fig. 3. CCE is attenuated but STIM proteins' levels are not affected by overexpression of PS1 FAD mutants in HEK293 cells. (A) Averaged traces obtained by ratiometric Fura-2 analysis of HEK293 cell lines overexpressing wt PS1 and PS1 P117R and PS1 E318G mutants. (B) Results of quantification of CCE in analyzed HEK293 cell lines (***$P<0{,}001$). (C) Representative immunoblot of protein extracts from HEK293 cell lines developed with anti-STIM1 and anti-STIM2 (D) antibodies and results of densitometric analysis of three independent experiments.

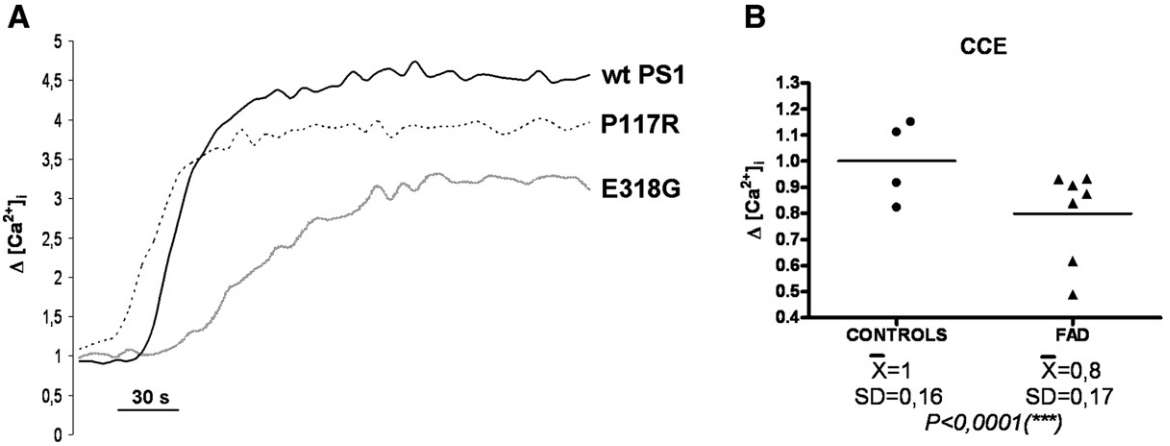

Fig. 4. CCE is attenuated in B cells isolated from FAD patients with PS1 mutations. (A) Averaged traces obtained by ratiometric Fura-2 analysis of B cells isolated from healthy individual (wtPS1) and FAD patients (P117R and E318G). (B) Results of quantification of averaged CCE in cell populations of healthy controls and FAD patients. Each symbol represents the mean value of three independent experiments on each cell line bearing one of analyzed FAD mutations in PS1. Horizontal lines represent mean values of results within a group.

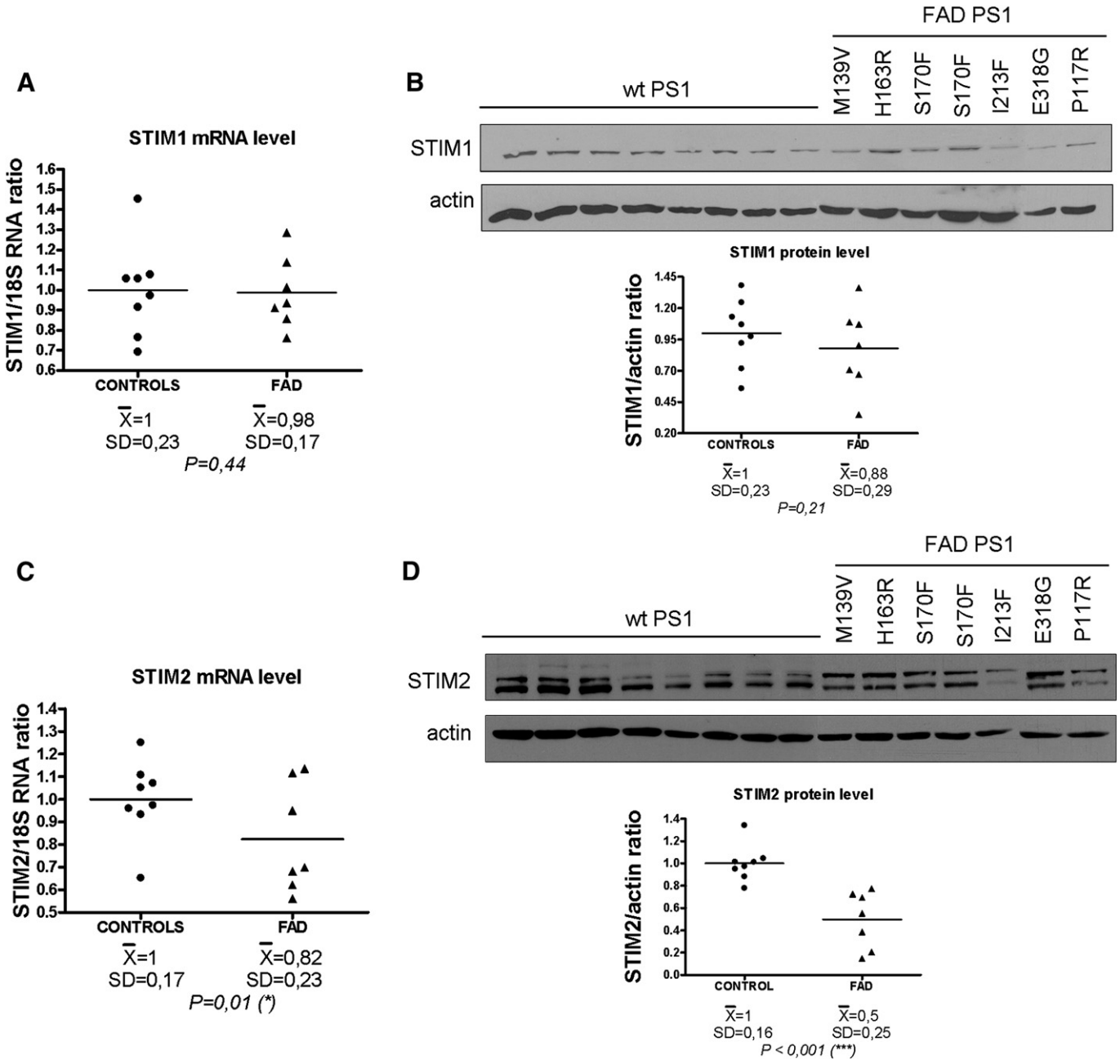

Fig. 5. *STIM2* mRNA and protein levels are decreased in B lymphocytes isolated from FAD patients. (A,C) Results of RT-PCR quantification of *STIM1* and *STIM2* mRNA levels in cell populations from healthy controls and FAD patients. (B,D) Representative immunoblot of protein extracts from B lymphocytes lines developed with anti-STIM1 and anti-STIM2 (D) antibodies and results of densitometric analysis of three independent experiments.

Taken together, these results indicate that cellular levels of STIM proteins depend on presenilins, and that STIM1 and STIM2 are differently affected by presenilins' loss. Lack of both PS1 and PS2 results in an increased cellular level of STIM1 and in a decreased amount of STIM2 in MEF cells.

3.3. In HEK293 cells overexpressing PS1 with FAD mutations CCE is attenuated, but level of STIM proteins is not changed

Attenuation of CCE is the most often observed alteration of Ca^{2+} homeostasis in cells bearing FAD PS1 mutations as reviewed in [4] and [5]. To test, if FAD mutations in PS1 affect the expression of STIM proteins, we examined HEK293 cell lines stably transfected with wt PS1 or with different PS1 mutants, namely: S170F, I213F, E318G, P117R and L226F. First, we analyzed CCE in these 6 cell lines with the Fura-2 assay as described for MEF cells. We found that overexpression of any of the analyzed PS1 FAD mutants resulted in CCE attenuation in HEK293 cells in comparison with a cell line transfected with wt PS1 (Fig. 3A,B, Supplementary Table 2.). In all cases of analyzed mutations except one (L226F), the decrease of CCE was statistically significant. These results confirm that attenuation of CCE is a characteristic feature of cells bearing PS1 FAD mutations.

Knowing that CCE is altered in HEK293 cells overexpressing PS1 with FAD mutations, we next measured the levels of STIM1 and STIM2 proteins in these cells. Analysis of immunoblots probed with specific antibodies showed no statistically significant differences in STIM protein levels between cells overexpressing PS1 with FAD mutations and cells transfected with wt PS1 (Fig. 3C and D). This demonstrates that despite alterations of CCE by overexpression of PS1 FAD mutants,

cellular levels of STIM1 and STIM2 proteins are not changed in transfected cells.

3.4. In human B lymphocytes isolated from FAD patients with mutations in PS1, CCE is attenuated and expression of STIM2 is decreased

Taking into consideration that overexpression of PS1 FAD mutants on the background of endogenous wt PS1 did not reveal changes in STIM protein's levels in HEK293 cells, we examined immortalized B lymphocytes isolated from FAD patients with identified mutations in endogenous PS1, namely: M139V, H163R, S170F, E318G, I213F and P117R [27]. Human B lymphocytes were assayed with Fura-2 in the same way as the MEF and HEK293 cells. Mean CCE was reduced in a group of PS1 FAD B lymphocytes compared with controls (Fig. 4).

To investigate the putative link between attenuated CCE and expression of STIM proteins in the population of human B lymphocytes bearing PS1 FAD mutations, we measured the levels of both mRNA and protein of STIMs in these cells. The mRNA level for STIM1 protein was not affected in cells bearing PS1 FAD mutations (Fig. 5A). Similarly, the densitometric analysis of immunoblots of protein extracts obtained from B lymphocytes, probed with anti-STIM1 antibody, showed no difference in STIM1 protein levels between two analyzed cell populations (Fig. 5B). However, a significantly lower level of *STIM2* mRNA was detected in the group of cells with PS1 FAD mutations compared to cells from healthy individuals (Fig. 5C). Similarly, a decrease in the STIM2 protein level in cells bearing PS1 FAD mutations was observed (Fig. 5D and Supplementary Table 3.) Cellular localization of STIM proteins was not altered in B cells with PS1 FAD mutations (data not shown).

These results indicate that FAD mutations in PS1 lead to a decrease of average STIM2 mRNA and protein levels in analyzed group of human B cells. A decreased level of STIM2 and attenuated CCE occurred in parallel in this group of cells, suggesting a possible link between the level of this protein and alterations of Ca^{2+} stores refilling associated with PS1 FAD mutations.

4. Discussion

Disturbances of CCE as well as other alterations of Ca^{2+} homeostasis in AD had been reported both in neurons and in peripheral cells [2,28,29]. PS1 and PS2, which are key proteins involved in AD pathogenesis, had been shown to modulate CCE [12,30]. CCE attenuation was demonstrated to influence proteolysis of APP and to lead to increase of neurotoxic Aβ-42 generation in cells bearing PS1 FAD mutation [12]. Furthermore, STIM1 and STIM2 were recently identified as key regulators of CCE in non-excitable cells [14,15]. We have found that STIM1 and STIM2 interact with Orai1 upon thapsigargin treatment to form puncta-like structures in neurons [31]. Here, we showed that presenilins are involved in the regulation of expression of STIM proteins.

We demonstrated that loss of PS1 or both presenilins results in enhanced CCE, while PS1 FAD mutations exert the opposite effect on Ca^{2+} influx. Our results corroborate earlier reports on presenilins and CCE [11–13,30] and thus further document the usefulness of our cellular models in studies on mechanisms of presenilin-related CCE alterations. These data also show that the inhibition of CCE is one of the biological activities of presenilins, as their loss causes CCE enhancement in comparison to cells expressing wt PS1. Conversely, expression of PS1 FAD mutants leads to the attenuation of CCE. This indicates that FAD mutations in PS1 display a gain of function phenotype in terms of CCE inhibition. However, the molecular mechanism underlying CCE modulation mediated by presenilins has not yet been clarified.

We hypothesized that presenilins may affect the expression of STIM1 and STIM2, and that presenilin-related disturbances in CCE, observed in FAD, may result from altered STIM protein levels. We found an increased protein level of STIM1 and a decreased protein level of STIM2 in MEF DKO cells. These alterations correlated with trends observed for *Stim1* and *Stim2* mRNA levels. This shows that PS1 together with PS2 take part in the regulation of cellular levels of STIM proteins and demonstrates that STIM1 and STIM2 are differently affected by the loss of presenilins.

To get an insight into the putative contribution of alterations in expression of STIM proteins to the pathogenesis of AD, we examined cells bearing FAD mutations in PS1. Despite attenuated CCE, we found no difference in STIM protein levels between HEK293 cells overexpressing PS1 with FAD mutations and cell line transfected with wt PS1. This may suggest that PS1 FAD dependent attenuation of CCE results from other mechanisms than alteration of cellular levels of STIM proteins. However, one cannot exclude that overexpression of PS1 FAD mutants on the background of endogenous wt PS1 results in subtle changes of STIM protein levels, which are however sufficient to cause CCE attenuation. This explanation seems to be reinforced by results of experiments conducted on human B lymphocytes isolated from patients bearing FAD mutations in endogenous PS1. We found that *STIM2* mRNA and protein level are down-regulated in the population of B cells bearing PS1 FAD mutations. Decreased level of STIM2 occurs in parallel to the attenuated CCE in the analyzed group of B lymphocytes with PS1 FAD mutations.

FAD mutations in PS1 exert the opposite effect on CCE than do presenilins knock-out. Moreover, the expression of STIM proteins is differently affected by PS1 FAD mutants than by the loss of presenilins. We hypothesize that an elevated level of STIM1 in MEF DKO cell line may mask the effect of a decrease in STIM2 and thus finally result in CCE enhancement. This may be also the case for MEF PS1−/− cell line, however increase of STIM1 protein level did not reach statistical significance in these cells. Such an explanation may be supported by previously published observations that STIM1 is a more potent CCE regulator than STIM2. Indeed, the expression of STIM1 in HeLa cells facilitated higher CCE than the expression of the same concentration of STIM2 protein [32]. Conversely, down-regulation of STIM2 with specific siRNA caused CCE attenuation to a smaller degree than in STIM1 depleted cells [14,32]. Such a masking effect of STIM1 protein is not found in human B lymphocytes in which STIM1 is not elevated above physiological level by FAD mutations in PS1. Hence, STIM2 down-regulation is sufficient to attenuate CCE in these cells.

The observed trends of mRNA levels of STIM proteins may indicate that presenilins exert their effects on STIM1 and STIM2 expressions at

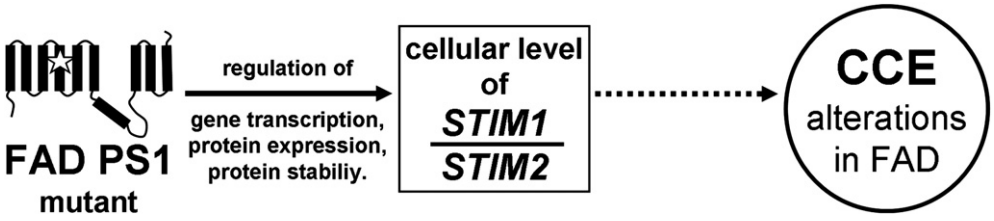

Fig. 6. Model of putative relationship between alterations in expression of STIM proteins triggered by mutations in PS1 and dysregulation of CCE in cells of FAD patients. FAD mutations in PS1 affect cellular levels of STIM proteins by a yet unknown mechanism, which may result in disturbances of CCE.

the transcription stage. However, one cannot exclude that PS1 and PS2 may affect expression and stability of STIM proteins. The effect of presenilins on the expression of STIM genes is likely indirect. Presenilins, as an active constituents of the γ-secretase complex, take part in the proteolytic liberation of amyloid protein intracellular domain (AICD), reviewed in [33]. This domain was shown to play a role in the regulation of transcription of several genes [34–36]. Therefore, its involvement in regulating the expression of STIM proteins by presenilins should be taken into consideration. Presenilins were also demonstrated to interact with several cellular signaling cascades including phosphatidyloinositol-3 kinase/Akt, ERK1/ERK2 and Wnt, which possibly involve these pathways in the regulation of transcription of STIM genes [37–39].

Our results confirm previous observations that presenilins are negative regulators of CCE and that FAD mutations in PS1 result in a gain of function phenotype displayed as an attenuation of CCE. We demonstrate here for the first time that presenilins' loss affect the mutual ratio of cellular levels of STIM proteins. Since presenilins are known to be involved in multiple cellular processes (reviewed in [40]) the mechanism of their influence on STIM1 and STIM2 may be indirect and sophisticated. No direct correlation between the extent of alterations of CCE and STIM2 expression was found when B lymphocyte cell lines with different PS1 FAD mutations were analyzed separately. However, group analysis revealed the decrease of both mean mRNA and protein level of STIM2 occurring in parallel to the attenuation of CCE in the population of B cells with mutations. This suggests possible involvement of STIM proteins in SOCE alterations in FAD. We propose a model according to which mutations in presenilins alter cellular levels of STIM proteins (either by regulation of their genes' expression or by influence on STIM proteins expression and stability) and therefore may affect CCE in cells of FAD patients (Fig. 6).

Our observation opens a new perspective for research on CCE alterations in FAD. Alterations of Ca^{2+} homeostasis have been reported not only for neurons, but also for peripheral cells of AD patients such as lymphocytes [13,41,42]. Nevertheless, further studies on the role of STIM proteins in PS1 FAD dependent attenuation of CCE in neurons are required to confirm the putative role of STIM1 and STIM2 in neurodegeneration observed in FAD.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbamcr.2008.11.008.

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