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The ANKK1 Protein Associated with Addictions has Nuclear and Cytoplasmic Localization and Shows a Differential Response of Ala239Thr to Apomorphine

E. Garrido · T. Palomo · G. Ponce · I. García-Consuegra · M. A. Jiménez-Arriero · J. Hoenicka

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Abstract The *Taq*IA single-nucleotide polymorphism (SNP), which is the most widely studied genetic polymorphism in addictions, is located at the gene that encodes the RIP kinase ANKK1 near the gene for dopamine receptor D2. The *Taq*IA SNP is in strong linkage disequilibrium with the SNP rs7118900, which changes the alanine at position 239 to threonine in the ANKK1 protein (Ala239/A2; Thr239/A1). In silico analysis has predicted that this polymorphic substitution creates an additional phosphorylation site in the kinase domain of ANKK1. To investigate the contribution of ANKK1 to the pathophysiology of *Taq*IA-associated phenotypes, we analyzed transfected HEK293T cells with the human ANKK1-kinase^{Ala239} and ANKK1-kinase^{Thr239} variants tagged with GFP. We observed that the ANKK1-kinase is located in both the nucleus and the cytoplasm, suggesting that there is nucleocytoplasmic shuttling of this putative signal transducer. In addition, we found that the Ala239Thr ANKK1-kinase polymorphism exhibited strong expression differences in both the nucleus and the cytoplasm at basal level and when stimulated with the dopamine agonist apomorphine. Specifically, the ANKK1-kinase^{Thr239} variant showed the highest level of basal protein expression, while ANKK1-kinase^{Ala239} was 0.64-fold lower. After treatment with apomorphine, ANKK1-kinase^{Ala239} showed a 2.4-fold increment in protein levels, whereas a 0.67-fold reduction was observed in ANKK1-kinase^{Thr239}. Thus, here we provide the first evidence of functional ANKK1 differences that are marked by *Taq*IA and could be associated with vulnerability to addiction.

Keywords ANKK1 · *Taq*IA · Ala239Thr · Addictions · Nucleus · RIP5

E. Garrido · T. Palomo · G. Ponce · M. A. Jiménez-Arriero · J. Hoenicka
Laboratory of Neurosciences, Instituto de Investigación, Hospital Universitario 12 de Octubre, Avda. de Córdoba s/n, 28041 Madrid, Spain

E. Garrido · T. Palomo · G. Ponce · M. A. Jiménez-Arriero · J. Hoenicka
Department of Psychiatry, Hospital Universitario 12 de Octubre, Avda. de Córdoba s/n, 28041 Madrid, Spain

E. Garrido · T. Palomo · G. Ponce · M. A. Jiménez-Arriero · J. Hoenicka (✉)
Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Madrid, Spain
e-mail: jhoenicka@gmail.com

I. García-Consuegra
Proteomics Unit, Instituto de Investigación, Hospital Universitario 12 de Octubre, Avda. de Córdoba s/n, 28041 Madrid, Spain

I. García-Consuegra
Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain

Introduction

The *Taq*IA single nucleotide polymorphism (SNP, rs1800 497), which is the most widely analyzed genetic substitution in addictions (Noble 2003; Munafo et al. 2007, 2009), is located at the gene that encodes the kinase ANKK1 (ankyrin repeat and kinase domain 1, *ANKK1*, chromosome 11q22–q23), near the dopamine receptor D2 gene (*DRD2*). *Taq*IA consists of a single-nucleotide C to T change; the two alleles are referred to as A2 (C) and A1 (T). The first study on the relationship between *Taq*IA and alcoholism appeared in 1990 (Blum et al. 1990). Since then, this SNP has been widely associated with different psychiatric disorders, neuropsychological endophenotypes, dopaminergic traits, and

pharmacological response (David et al. 2007; Ponce et al. 2009; Shen et al. 2009).

ANKK1, also known as receptor interacting protein 5 (RIP5), belongs to the RIP serine/threonine kinases, which constitute a family of seven members containing a highly similar kinase domain. RIP members are important regulators of cell proliferation and differentiation, and initiate responses to various environmental factors by activating transcription factors such as NF-κB or JNK AP-1 (Meylan and Tschopp 2005). Recently, in silico evidence has become available for at least three ANKK1 isoforms with putative different functions: ANKK1 (with RIP kinase and ankyrin repeat domains), ANKK1-kinase (only the RIP kinase domain), and ANKK1-ankyrin (only the ankyrin repeat domain) (Hoenicka et al. 2010). The RIP kinase domain of RIP4, the protein most closely related to ANKK1 (Meylan and Tschopp 2005), activates NF-κB signaling as efficiently as the full-length protein (Meylan et al. 2002). Nonetheless, an inhibitory effect has been proposed for the RIP4 ankyrin repeat domain in kinase activity during apoptosis (Meylan et al. 2002). Although less is known about the function of ANKK1, its expression in the astrocytes of adults and in the radial glia of the developing central nervous system (Hoenicka et al. 2010) suggests that this protein could be involved in the coordinated regulation of diverse processes.

ANKK1 has recently been shown to be activated by apomorphine, a dopaminergic agonist, thus indicating a potential connection between *ANKK1* and the dopaminergic system (Hoenicka et al. 2010). This relationship is also suggested by in silico analyses of the *ANKK1* and *DRD2* gene locus (Hoenicka et al. 2010). Indeed, *Taq*IA is a marker of functional variants of both the *DRD2* and *ANKK1* genes, and in healthy humans, it is linked to *DRD2* polymorphisms that modify mRNA expression and splicing, and working memory pathways (Zhang et al. 2007). On the *ANKK1* side, *Taq*IA causes the change of a glutamic in amino acid position (aa) 713 to a lysine of the longest ANKK1 isoform (Neville et al. 2004). Moreover, *Taq*IA is in strong linkage disequilibrium ($r^2 = 0.9$) with *ANKK1* SNP rs7118900, which changes alanine in position 239 to threonine (Ala239Thr), thus creating an additional phosphorylation site (Hoenicka et al. 2010). Although *Taq*IA and Ala239Thr could modify ANKK1 function, no study has analyzed their impact on protein function in living systems.

To investigate the contribution of ANKK1 to *Taq*IA-associated phenotypes, we analyzed transfected human embryonic kidney (HEK) 293T cells with the human ANKK1-kinase^{Ala239} and ANKK1-kinase^{Thr239} variants using green fluorescent protein (GFP) as a tag. We also studied ANKK1-kinase subcellular localization and the response to apomorphine.

Methods

Cloning and Mutagenesis

The cDNA of human ANKK1-kinase containing an alanine residue at position 239 (ANKK1-kinase^{Ala239}) was synthesized by Geneart AG (Regensburg, Germany). The isoform ANKK1-kinase^{Thr239} and the mutant ANKK1-kinase^{Arg51} with no kinase activity were obtained by site-directed mutagenesis using the QuikChange XL Site-Directed Mutagenesis Kit (Stratagene, Santa Clara, California, USA) according to the manufacturer's protocol and verified by sequencing. The following primers were used for mutagenesis: Ala239Thr, 5′-ATTATTATTCGT GTTACGGCGGGTATGCGTCCG-3′ (forward) and 5′-C GGACGCATACCCGCCGTAACACGAATAATAATC-3′ (reverse), and Lys51Arg, 5′-CCGAATATGCGATTAGA TGCGCGCCGTGCC-3′ (forward) and 5′-GGCACGGC GCGCATCTAATCGCATATTCGG-3′ (reverse).

The GFP-tagged versions of these ANKK1-kinase proteins were obtained by cloning the ANKK1 cDNAs in the same reading frame as the pEGFP-N1 expression vector (Clontech, Mountain View, California, USA) using *Hin*dIII and *Bam*HI restriction sites. The tag was generated at the C-terminal position of the ANKK1-kinase peptide to reduce targeting interferences.

Cell Culture, Transfection, and Treatment

HEK293T cells were grown in a humidified incubator with 5% CO_2 at 37°C in DMEM containing 10% (v/v) fetal bovine serum supplemented with 1% penicillin–streptomycin, 2-mM L-glutamine, and 1-mM sodium pyruvate (all from Lonza, Basel, Switzerland). Transfection was performed with lipofectamine 2000 reagent (Invitrogen, Carlsbad, California, USA) following the manufacturer's instructions. In some cases, 24-h post-transfection, cells were treated with the dopamine receptor agonist (R)-(−)-apomorphine (Sigma, Saint Louis, Missouri, USA) at 10 μM in complete medium with 0.5% serum for 8 h. Cells were then trypsinized and proteins were isolated.

Confocal Microscopy

For intracellular localization studies, cells were grown on L-lysinated coverslips, transfected for 24 h, and treated as described above. The cells were then fixed with 4% (w/v) paraformaldehyde and methanol (−20°C) for 20 min each. After the nuclei were stained with DAPI (1:10000), cells were mounted and visualized with a Zeiss LSM 510 Meta scanning laser confocal microscope with the 63× oil immersion objective. Three hundred cells from two independent experiments were counted for each condition to calculate an average of the observed fluorescence patterns.

Western Blotting

Western blot was performed on pooled samples from two independent transfection experiments. Total protein extracts were obtained after cell homogenization in lysis buffer (150-mM NaCl, 20-mM Tris HCl, 5-mM EDTA, 10% glycerol, 1% NP-40) containing a protease inhibitor cocktail (Complete Mini-Protease Inhibitor Cocktail, Roche, Basel, Switzerland) and mechanical breaking with an embolus. Cells were centrifuged at 13000 rpm for 30 min at 4°C to separate the cytoplasmic and nuclear enriched fractions. The nuclear pellet was resuspended in lysis buffer containing 0.5% SDS and 0.2 mg/ml DNAsaI (Roche). Genomic DNA was sheared by sonication for 5 s with a Branson Sonifier 150 at output 3 and centrifuged. Supernatants of the cytoplasmic-enriched fractions were quantified by the RC DC method (BioRad, Hercules, California, USA). Samples of the same characteristics were pooled together to represent one particular cellular compartment/condition in 50 μg of protein in the same volume, resolved in a 7% SDS-PAGE and electroblotted from the gels onto Immobilon-P polyvinylidene difluoride membranes (Millipore, Billerica, Massachusetts, USA). After overnight blocking (5% nonfat dry milk and 0.1% Tween20 in TBS), membranes were incubated for 1 h with the appropriate primary antibodies: monoclonal anti-GFP antibody (1:2000, Abcam, Cambridge, UK), anti-βIII tubulin as a cytoplasmic marker (1:7000, Promega, Madison, Wisconsin, USA), and anti-β-actin as a reference to quantify the relative protein expression level (1:20000, Sigma). All washing steps were performed with TBS-0.1% Tween20. Incubation with goat anti-rabbit peroxidase-conjugated IgG (1:8000–1:10000, Abcam) was performed at room temperature for 1 h. Proteins were processed for chemiluminescence with the ECL Plus Detection System (GE Healthcare, Little Chalfont, UK).

Membranes were scanned and band intensity was measured by taking into account the total area of the peaks using the Image J 1.38v software from the National Institutes of Health (NIH).

Results

ANKK1-Kinase is Located in Both the Cytoplasm and the Nucleus

We first performed an in silico analysis of the ANKK1-kinase amino acid (aa) sequence using WoLF PSORT (http://wolfpsort.org/). This program predicts the subcellular localization sites of proteins, including those that appear in two localizations, such as proteins shuttling between the cytosol and the nucleus (Horton et al. 2007). ANKK1-kinase was predicted to be in both the nucleus and the cytosol (score: 18.0). In support of this dual location, we also found in silico evidence for nuclear localization signals (NLS) and nuclear export sequences (NES) on ANKK1. The NUCDISC program (from the PSORT II server, http://psort.ims.u-tokyo.ac.jp) identified two NLSs: NLS-1 (RHRR, aa 40–44) and NLS-2 (PKKRPCF, aa 272–276), encoded in exons 1 and 5, respectively (Fig. 1a). These putative NLS are present in the ANKK1-kinase and ANKK1 full-length isoforms, but not in the ANKK1-ankyrin isoform. In addition, NLS-1 is unique to the human peptide, while NLS-2 is 100% homologous to the ANKK1 of phylogenetically distant organisms (for instance *Monodelphis domestica*, *Canis familiaris*, *Mus musculus*). The ANKK1-kinase NLS-2 sequence is also very similar in all RIP family members (Fig. 1b).

We found in silico evidence of one leucine-rich NES in the ANKK1 peptide. The program NetNES v.1.1 (la Cour et al. 2004) identified the motif IKPPLLHLDL (Bogerd et al. 1996) (aa 137–146, exon 3) (Fig. 1a). This ANKK1 NES fits with the (LIVFM)-XX-(LIVFM)-X-(LIVFM) consensus sequence and is located in the α-helix, close to the transition between two elements of the secondary structure of the protein (la Cour et al. 2004). Within the ANKK1 NES region, where hydrophobic residues are well conserved, acidic residues are conserved both across species and between ANKK1 and RIP2 and RIP4 (Fig. 1b). Besides, we identified five putative NESs that fulfilled the less astringent pattern and the secondary structure limits defined by la Cour et al. (2004). They were LGSLPV (aa 10–16), MKKIKF (aa 75–80), ISDFGL (aa 161–166), VYSFAI (aa 209–214), and MIIIRV (aa 233–238).

To determine the subcellular ANKK1-kinase localization in vivo, a series of transient transfection assays in HEK293T cells were performed using GFP as a tag. The predicted ANKK1-kinase isoforms (Ala239Thr) were cloned into a GFP-fusion vector to produce C-terminal fusion proteins. We observed that the transient expression of the ANKK1-kinase^{Ala239} and ANKK1-kinase^{Thr239} fusion proteins did not alter cell growth or morphology. Fluorescence was localized diffusely in the cytoplasm and the nucleus (Fig. 2). More than half of the ANKK1-kinase^{Ala239}-transfected cells exhibited a punctate structure in the cytoplasm. This pattern was also observed in ANKK1-kinase^{Thr239} cells, although the punctate structure was less frequent. To examine whether these two different localizations (cytoplasm and nucleus) could be attributed to ANKK1 kinase activity, we generated the point mutant ANKK1-kinase^{Arg51} (K51R), which abrogates kinase activity. ANKK1-kinase^{Arg51} also showed a cytoplasmic/nuclear localization, although the punctate structures appeared in only 2% of the ANKK1-kinase^{Arg51} cells. Furthermore, 12% of the cells exhibited protein aggregations within the cytoplasm, together with a reduction

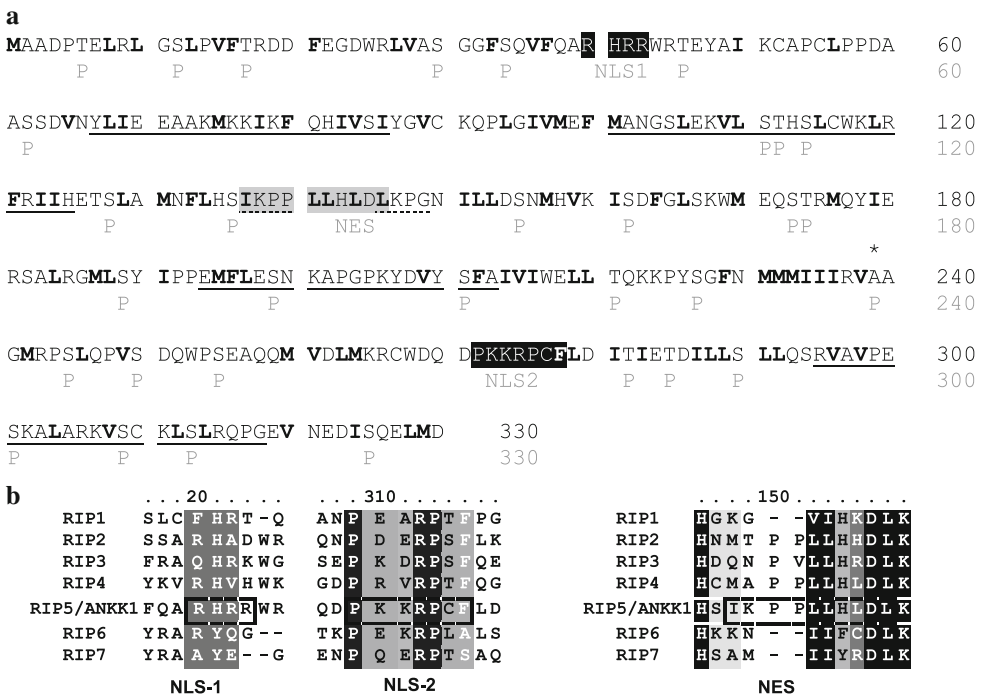


Fig. 1 Amino acid sequence of the human ANKK1-kinase isoform, as cloned in this study. The kinase domain comprises residues 22–289. **a** Identification of nuclear targeting motifs and post-translational modification sites with prediction software. *P* putative phosphorylation site, as predicted by one or more of the prediction programs (PhosphoMotif Finder, Kinasephos, ScanProsite, PredPhospho v.1). * Position of the Ala239Thr polymorphism (in linkage disequilibrium with the *Taq*IA polymorphism). *Black* basic amino acid-rich nuclear localization signals (NLS), discriminated by NUCDISC software. *Gray* putative nuclear export signal (NES) according to the most abundant consensus motif (LIVFM)-X2-3-(LIVFM)-X2-3-(LIVFM)-X-(LIVFM) as found by the NetNES v1.1 server. Highly hydrophobic amino acids that can take part in NESs (V, I, L, M, F) are marked in *bold*. *Underlined with a continuous line* potential ubiquitination sites (BDM-PUB prediction software). *Underlined with a dotted line* sumoylation motifs detected by SUMOplot software. **b** Identity alignment between all the members of the RIP kinase family with respect to the putative ANKK1 NLS and NES (*boxed*). The *darker* the *gray*, the more conserved is the residue

in the nuclear ANKK1-kinase^{Arg51}. This pattern seemed to be associated with the loss of ANKK1 kinase activity, as the presence of these aggregated structures was reduced in cells transfected with ANKK1-kinase^{Ala239} or ANKK1-kinase^{Thr239} constructs. The GFP-tagged ANKK1-kinase dual location was not exclusive to HEK293T cells, as we found the same cytoplasmic/nuclear pattern in COS-7 and CHO cells when these were transfected with the same constructs (data not shown). Furthermore, to rule out the possibility that the GFP tag caused an artifactual localization of ANKK1-kinase, the polyclonal antibody STk2-Ab (Hoenicka et al. 2010) was used to detect the native protein in nontransfected HEK293T cells. ANKK1 was detected in both the cytoplasm and the nucleus (data not shown).

ANKK1-Kinase Variants Show a Different Response to Apomorphine

Since there is evidence of a relationship between ANKK1 and the dopaminergic system, we also studied the effect of apomorphine on our transfected HEK293T cells. After treatment with apomorphine, we did not observe any effect on cell morphology, growth rate, or recombinant protein distribution in any of the ANKK1-kinase variants (data not shown).

To further study ANKK1-kinase expression and localization, we performed western blot analysis of cytosol- and nuclei-enriched fractions of these transfected HEK293T cells. The fusion protein with GFP and ANKK1-kinase was detected in both the nucleus and the cytoplasm of all the samples at the predicted molecular weight of 65 kDa (Fig. 3). The ANKK1-kinase^{Thr239} variant showed the highest level of basal protein expression (cytoplasm [C], 5.83; nucleus [N], 1.40), while expression of the ANKK1-kinase^{Ala239} was 0.64-fold lower (C, 1.64; N, 0.45). The absence of kinase activity did not affect protein levels, as ANKK1-kinase^{Arg51} had similar values to those of the ANKK1-kinase^{Thr239} (C, 5.03; N, 1.45) (Fig. 3).

We also found differences in the response to treatment with apomorphine among the ANKK1-kinase variants. With respect to the expression levels, ANKK1-kinase^{Ala239} showed a 2.4-fold increment with apomorphine (0.91–2.24). In contrast, a reduction in protein was observed in both the ANKK1-kinase^{Thr239} (0.67-fold, 2.51–1.70) and the mutant (0.82-fold, 2.34–1.93) (Fig. 3).

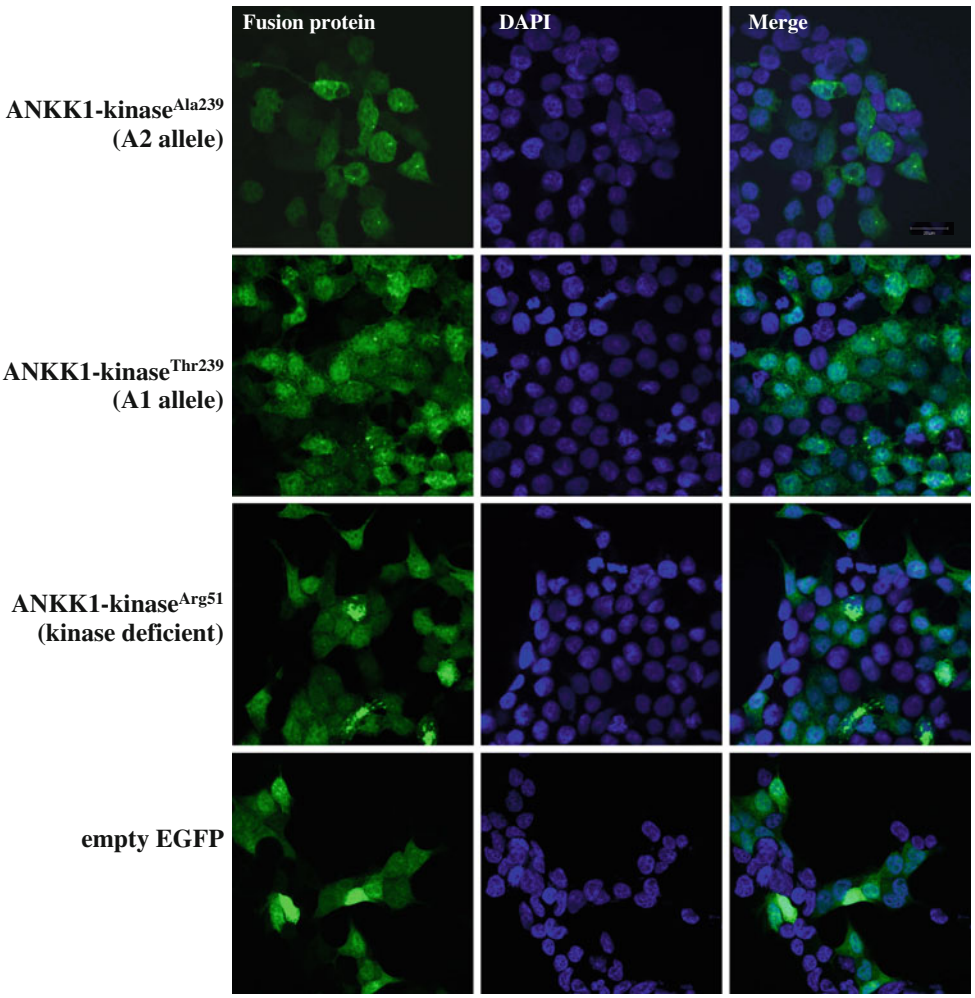


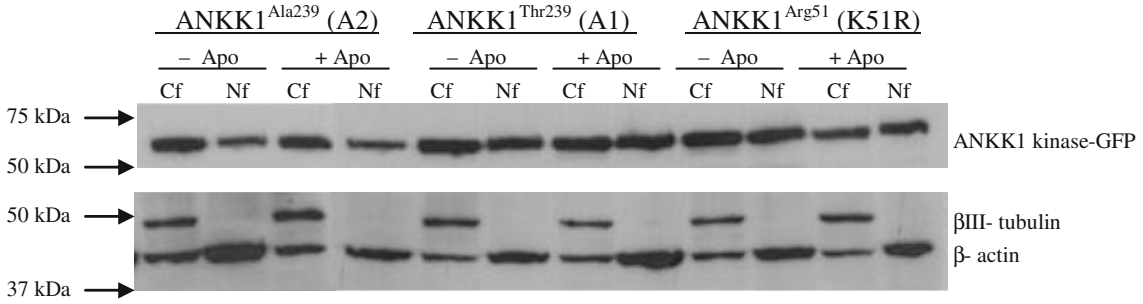
Fig. 2 Sub-cellular localization of the human ANKK1-kinase^{Ala239} and ANKK1-kinase^{Thr239} polymorphic variants. Confocal optical sections showing the GFP-tagged ANKK1 polymorphic variants, mutant fusion proteins (*left*), and DAPI (*center*) in transfected HEK293T cells (×630)

We then analyzed whether treatment with apomorphine affected the ratio between the nuclear fraction protein and the cytosolic fraction protein (NC ratio) of the ANKK1-kinase variants. Although there were no NC ratio differences before stimulation with apomorphine (0.28, 0.24, 0.29), we found different responses to this dopaminergic agonist among the ANKK1-kinase variants (Fig. 3). Specifically, ANKK1-kinase^{Ala239} showed no differences in the NC ratio (No Apo 0.28 vs Apo 0.30), ANKK1-kinase^{Thr239} exhibited a significant decrease in the nuclear fraction (No Apo 0.24 vs. Apo 0.16) (Fig. 3), and ANKK1-kinase^{Arg51} showed a decrease in the cytosolic fraction (No Apo 0.29 vs. Apo 0.39) (Fig. 3).

Discussion

We recently reported on the location of ANKK1 in the brain, thus giving a new perspective on the understanding of the physiology underlying *Taq*IA-associated phenotypes (Hoenicka et al. 2010). Of the three different peptides resulting from *ANKK1* transcription, we focused on ANKK1-kinase (only the RIP kinase domain), which was the isoform found in adult mouse brain and in other tissues (Hoenicka et al. 2010). Here, we show for the first time that the ANKK1-kinase has a dual subcellular localization and responds differently to treatment with apomorphine depending which of the two polymorphic isoforms is considered.

We found that GFP-tagged ANKK1-kinase Ala239Thr polymorphic variants in cultured HEK293T cells were localized not only in the cytoplasm but also in the nucleus. This result was also observed in cells transfected with the mutant ANKK1-kinase^{Arg51}. The ANKK1-kinase nuclear localization cannot be explained by passive diffusion, as our fusion constructs are larger than the limit size of the nuclear pore (40–60 kDa). Instead, we propose active nucleocytoplasmic transport. Our in silico analyses revealed two potential nuclear localization signals (NLS1 and NLS2) and one canonical nuclear export sequence (NES) on ANKK1. The close similarity of the ANKK1 NLS-2 and NES motifs across species and among RIP family members suggests that these sequences have played important roles during the evolution of the dual nuclear–cytoplasmic localization of the protein. Although RIP kinases are mostly cytoplasmic, there is evidence for their



	Fraction	ANKK1/β-actin	NC Ratio
ANKK1-kinase$^{Ala\ 239}$ (A2)	Cf	1.64	
	Nf	0.45	0.28
	Total	0.91	
(Apomorphine 10 μM)	Cf	3.96	
	Nf	1.19	0.30
	Total	2.24	
ANKK1-kinase^{Thr239} (A1)	Cf	5.83	
	Nf	1.40	0.24
	Total	2.51	
(Apomorphine 10 μM)	Cf	6.40	
	Nf	1.00	0.16
	Total	1.70	
ANKK1-kinase^{Arg51} (K51R)	Cf	5.03	
	Nf	1.45	0.29
	Total	2.34	
(Apomorphine 10 μM)	Cf	3.62	
	Nf	1.39	0.39
	Total	1.93	

Fig. 3 Western blot analysis of cytosol (Cf)- and nuclei (Nf)-enriched fractions of HEK 293T cells transfected with the human ANKK1 kinase variants ANKK1^{Ala239}, ANKK1^{Thr239}, and ANKK1^{Arg51}, fused with a GFP tag. The sub-cellular distribution of the protein was evaluated using the ratio between the nuclear fraction protein and the cytosolic fraction protein (NC ratio). Band intensity was measured through total area of the peaks using the Image J 1.38v software. *β*-Actin was used as a reference to quantify relative protein expression level and *β*III tubulin as a cytoplasmic marker. Ratios are shown in the table

presence in the nucleus. Such is the case of RIP1, which interacts with the transcription factor Sp1 (Ramnarain et al. 2008). RIP3, which acts as a nucleocytoplasmic shuttling protein, causes apoptosis-induced activity in the nucleus (Yang et al. 2004). With respect to RIP2 and RIP4, our in silico analyses also point to a dual nuclear/cytoplasmic localization for these kinases (data not shown). Given that RIP kinases are crucial regulators of cell survival and death (Meylan and Tschopp 2005), their presence in the nucleus is not surprising. Nuclear translocation of signal transduction proteins is an essential step in the regulation of gene expression by extracellular signals. Indeed, some signalling molecules of the NF-κB pathway that are functionally related to RIP kinases shuttle constitutively between the cytoplasm and the nucleus (Birbach et al. 2002). Further studies with leptomycin B, which blocks the nuclear export of many proteins, would make it possible to analyze the kinetics of ANKK1-kinase localization in the nucleus.

In addition, we found that Ala239Thr GFP-tagged ANKK1-kinase polymorphic variants exhibited strong differences in HEK293T-transfected cells. First, they have different expression levels at baseline. ANKK1-kinase^{Thr239} is expressed 2.8-fold more than that of the ANKK1-kinase^{Ala239}. Second, after dopaminergic stimulation with apomorphine, these variants differed in the magnitude and direction of response. Third, a different effect of

apomorphine on the NC ratio was also evident among ANKK1-kinase variants. Given that phosphorylation of proteins modulates their activity and the threonine 239 of ANKK1 is predicted to create a new phosphorylation site, it is plausible that different phosphorylation patterns could explain our results. Thus, analyses of threonine 239 phosphorylation in the ANKK1 peptide may help us to better understand the differences observed among their variants.

In clinical terms, these results provide for the first time evidence for the functional differences of ANKK1 that are marked by the A2 and A1 alleles of *Taq*IA. This SNP, which is in linkage disequilibrium with the Ala239Thr studied in this study (Ala 239/A2; Thr239/A1), has been associated mostly with addictions, a group of disorders that develop after continued use of substances affecting dopamine transmission. It has been reported, a *Taq*IA effect on striatal dopamine synthesis, independent of the C957T *DRD2* genotype (Laakso et al. 2005). Extensive genotyping of *DRD2* and *ANKK1* genes suggests that the association between the locus 11q22–q23 and substance dependence is also due to *ANKK1* variants (Gelernter et al. 2006; Dick et al. 2007; Yang et al. 2007). Psychopathic traits in alcoholic patients are related to an epistatic interaction between the *ANKK1 Taq*IA and the *DRD2* C957T SNP genotypes (Ponce et al. 2008). Here, we present functional evidence for the differential dopaminergic response of *ANKK1* polymorphisms, which strengthens the ANKK1-related explanation for previously reported *Taq*IA-associated phenotypes. The different response of ANKK1 and other dopamine-related proteins after drug consumption could be considered a condition of vulnerability. The influence of the *Taq*IA A1 allele on reduced apomorphine-induced growth hormone response, a test that indicates a different response after dopaminergic stimulation, has been identified in alcoholic patients (Samochowiec et al. 2000; Lucht et al. 2010). Moreover, this reduced growth hormone response to apomorphine stimulation has been found in severe alcohol dependence (Schmidt et al. 1996), thus suggesting the influence of biological factors on the neuropharmacological effects of chronic alcohol exposure and the clinical course of alcoholism. In any case, our results are consistent with the vast amount of genetic data suggesting that carriers of the *Taq*IA A1 allele have a particular condition and/or differential response to dopaminergic stimulation. Future studies will clarify how these ANKK1 differences are related to the neurobiological processes that make carriers of *Taq*IA A1 more prone to addictive disorders.

Conclusion

This study opens new avenues in the study of the role of ANKK1 in the pathophysiology of *Taq*IA-associated disorders. The ANKK1-kinase was found in both the nucleus and the cytoplasm, thus suggesting the involvement of this protein in controlling the expression of certain genes. In addition, we found that Ala239Thr ANKK1-kinase polymorphic variants exhibited strong expression differences in both basal conditions and after dopaminergic stimulation with apomorphine. Given that *Taq*IA is a marker of the Ala239Thr ANKK1 SNP, this study presents for the first time functional differences in ANKK1 that could affect vulnerability to addictions and other phenotypes associated with *Taq*IA.

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