Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 360 (2007) 381–387
BBRC
www.elsevier.com/locate/ybbrc

A *PRKAG2* mutation causes biphasic changes in myocardial AMPK activity and does not protect against ischemia

Sanjay K. Banerjee [a], Ravi Ramani [a], Samir Saba [a], Jennifer Rager [a], Rong Tian [b], Michael A. Mathier [a], Ferhaan Ahmad [a,c,*]

[a] *Cardiovascular Institute, Department of Medicine, University of Pittsburgh, 200 Lothrop Street, Scaife Hall, S558, Pittsburgh, PA 15213, USA*
[b] *Cardiovascular Division, Brigham and Women's Hospital, Boston, MA 02115, USA*
[c] *Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA 15213, USA*

Received 6 June 2007
Available online 19 June 2007

Abstract

Dominant mutations in the γ2 regulatory subunit of AMP-activated protein kinase (AMPK), encoded by the gene *PRKAG2*, cause glycogen storage cardiomyopathy. We sought to elucidate the effect of the Thr400Asn (T400N) human mutation in a transgenic mouse (TG^{T400N}) on AMPK activity, and its ability to protect the heart against ischemia–reperfusion injury. TG^{T400N} hearts had markedly vacuolated myocytes, excessive accumulation of glycogen, hypertrophy, and preexcitation. Early activation of myocardial AMPK, followed by depression, and then recovery to wild-type levels was observed. AMPK activity correlated inversely with glycogen content. Partial rescue of the phenotype was observed when TG^{T400N} mice were crossbred with $TG^{\alpha 2DN}$ mice, which overexpress a dominant negative mutant of the AMPK α2 catalytic subunit. TG^{T400N} hearts had greater infarct sizes and apoptosis when subjected to ischemia–reperfusion. Increased AMPK activity is responsible for glycogen storage cardiomyopathy. Despite high glycogen content, the TG^{T400N} heart is not protected against ischemia–reperfusion injury.

Keywords: Glycogen cardiomyopathy; AMPK; Transgenic mouse model; Cardiac hypertrophy; Myocardial ischemia–reperfusion

AMP-activated protein kinase (AMPK), a heterotrimeric protein composed of a catalytic α subunit and regulatory β and γ subunits, is activated under conditions of energy depletion manifested by increased cellular AMP levels [1,2]. AMPK modulates glucose uptake and glycolysis [1,3]. We and others have found dominant mutations in the γ2 regulatory subunit, encoded by the gene *PRKAG2*, to cause cardiac hypertrophy and increased risk of sudden cardiac death [4–6]. *PRKAG2* mutations produce a distinctive cardiac histopathology characterized by enlarged myocytes with vacuoles containing glycogen derivatives [6]. In addition, patients frequently manifest electrophysiological abnormalities such as ventricular preexcitation, atrial fibrillation, and progressive development of atrioventricular conduction block [4–6].

The association between *PRKAG2* mutations and glycogen cardiomyopathy has been confirmed in three transgenic (TG) mouse models [7–9]. However the effect of these mutations on AMPK activity remains controversial. Whereas the TG^{N488I} mouse was reported to have an increase in AMPK activity [7], other investigators found a decrease in activity in TG^{R302Q} [8] and TG^{R531G} [9] mice. To resolve this discrepancy and to uncover the mechanisms by which *PRKAG2* defects cause disease, we constructed a transgenic mouse with the *PRKAG2* Thr400Asn (T400N) mutation (TG^{T400N}), previously identified in humans [6]. Because of our demonstration that stored glycogen in

* Corresponding author. Address: Cardiovascular Institute, Department of Medicine, University of Pittsburgh, 200 Lothrop Street, Scaife Hall, S558, Pittsburgh, PA 15213, USA. Fax: +1 412 647 4227.
E-mail address: ahmadf@upmc.edu (F. Ahmad).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.06.067

TG^{N488I} hearts can be utilized during exercise stress [10], we also tested whether glycogen in TG^{T400N} hearts confers protection against ischemia–reperfusion injury.

Materials and methods

Generation of transgenic mice (TG^{T400N}). A T400N cDNA was generated from human cardiac RNA by PCR mutagenesis, and inserted into a pBluescript based vector with the mouse α-myosin heavy chain (αMHC) promoter [11], a highly active cardiac myocyte specific promoter (Fig. 1A). The transgenic vector was linearized with Bam HI, size-fractionated, purified, and microinjected into fertilized FVB mouse oocytes at the University of Pittsburgh Transgenic and Chimeric Mouse Facility. Transgenic founders were identified by Southern blot analyses. Offspring of founders were genotyped by PCR amplification of the transgene using two sets of primer pairs—SPKG2 (5′-CCGCTCCTCCTCCAAAGAGT-3′) and ASPKG3 (5′-GCAATGTTGTGGTACGTTCC-3′) both within the *PRKAG2* cDNA; and MHC F1 (5′-CGGCACTCTTAGCAAACCTC-3′) within the vector backbone 5′ of the cDNA, and MHC R1 (5′-TTCT GGCTGGCATTTTTCTT-3′) within the cDNA.

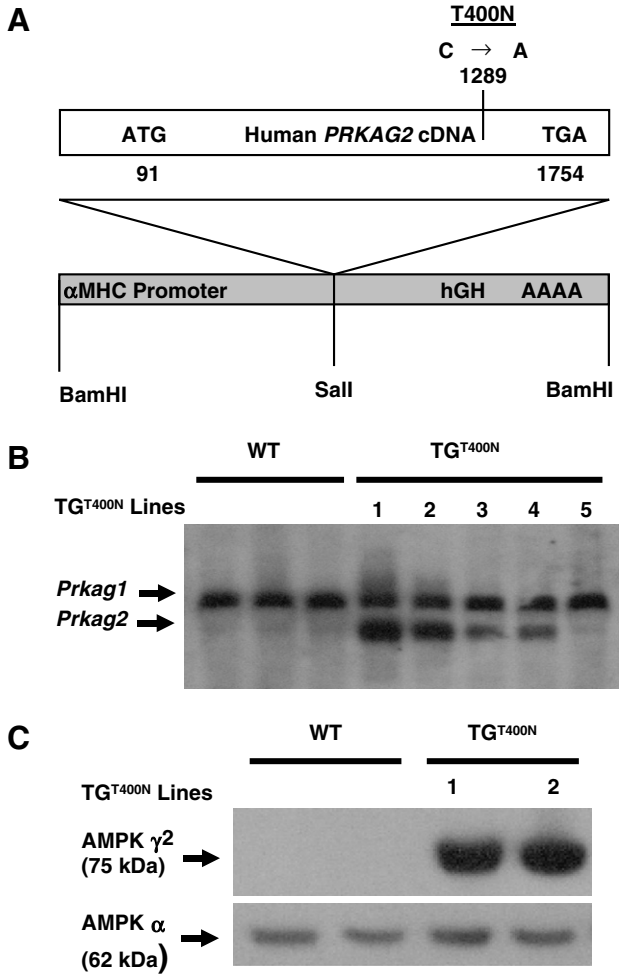

Fig. 1. Generation of transgenic mouse. (A) Transgene construct containing human *PRKAG2* cDNA with the T400N missense mutation at nucleotide 1289, under the control of the αMHC promoter and terminated by the human growth hormone 3′UTR (hGH) and polyadenylation signal (AAAA). (B) Northern blots using *Prkag1* and *Prkag2 cRNA* probes to assess expression in WT and TG^{T400N} hearts. *Prkag1* expression was similar in both WT and TG^{T400N} hearts. (C) Western blots using AMPK α and γ2 subunit antibodies to assess protein expression in WT and TG^{T400N} hearts. *PRKAG2* RNA and the γ2 subunit protein were expressed only in TG^{T400N} hearts.

FVB background $TG^{\alpha 2DN}$ mice, which overexpress a dominant negative mutant of the AMPK α2 catalytic subunit and have low myocardial AMPK activity, have been previously described [12]. Double transgenic mice ($TG^{T400N}/TG^{\alpha 2DN}$) were obtained by crossbreeding.

Transgenic mouse lines with the wildtype *PRKAG2* have been generated previously by three laboratories [7–9] and demonstrate only a mild phenotype intermediate between wild-type (WT) and mutant lines. Because our focus was on the mechanisms of glycogen cardiomyopathy, which is not seen by mere overexpression of a cardiac protein, and on the effect of excess glycogen on response to ischemia, we did not use these mouse lines in the current study.

RNA and protein analyses. Northern blots and quantitative analysis of *PRKAG1* and *PRKAG2* RNA expression were performed as described [7]. Western blots for α and γ2 subunits of AMPK were performed using 30 μg total cardiac protein from WT and TG^{T400N} mice with antibodies specific for the α and γ2 subunits (Cell Signaling).

Glycogen content, AMPK activity, and AMPK phosphorylation. Glycogen content was determined by the amyloglucosidase digestion method [10] and total basal AMPK activity was assayed as described [7,10]. We measured myocardial AMPK activity and glycogen levels in TG^{T400N} and WT hearts at ages 2 days, and 1, 2, 4, 8, 12, and 20 weeks. Levels of Thr172 phosphorylated AMPK α subunit (P-AMPK) were determined by Western blotting using 10 μg total cardiac protein with antibody specific for P-AMPK (Cell Signaling).

Echocardiography and electrocardiography (ECG). Transthoracic echocardiography was performed every 4 weeks at ages 4–20 weeks after sedation with tribromoethanol (125 mg/kg IP) using a VisualSonics Vevo 770 machine with a 45 MHz transducer [10]. Left ventricular end diastolic (LVEDD) and end systolic (LVESD) chamber dimensions and anterior wall thickness (LVAWT) were obtained from M-mode tracings. LV fractional shortening (FS) was calculated as (LVEDD-LVESD)/LVEDD × 100%. ECGs were performed as described [7,10].

Histopathology. Hearts were excised, washed in PBS, and weighed. Cardiac tissue was treated as described previously [7] for staining with either hematoxylin and eosin (H&E) or periodic acid–Schiff (PAS) for glycogen.

Cardiac ischemia and reperfusion. Six to eight week old mice were anesthetized with tribromoethanol (125 mg/kg IP) and subjected to *in vivo* ischemia for 30 min by ligation of the left anterior descending coronary artery (LAD), followed by 48 h reperfusion [13]. The LAD was then reoccluded and injected with 1 ml 1.0% Evans blue (Sigma) through the jugular vein to delineate nonischemic tissue. The heart was excised and cut into four transverse slices. Slices were stained for 15 min with 1.5% 2,3,5-triphenyltetrazolium chloride (TTC) (Sigma) and placed in 2.5% formaldehyde to determine infarct area under a microscope. Left ventricular (LV) area, area at risk (AAR), and infarct area (IA) were determined by computerized planimetry using Image J software. Five micrometer heart sections were assessed for apoptosis by TUNEL staining using ApopTag Peroxidase Kits (Millipore).

Data analysis. Results are expressed as means ± SEM. Differences between pairs of mouse genotypes were assessed by Student's *t*-test. A $p < 0.05$ was considered significant.

Results

PRKAG2 expression

PRKAG2 RNA expression was evident at different levels in four of five TG^{T400N} lines (Fig. 1B), whereas similar expression of *Prkag1* RNA was observed in both WT and TG^{T400N} lines. Initial characterization demonstrated glycogen cardiomyopathy in transgenic lines 1 through 4. Line 1, demonstrating the highest levels of expression,