DATA SUPPLEMENT

Embryonic expression of AMPK γ subunits and the identification of a novel γ2 transcript variant in adult heart

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Key words: AMP-activated protein kinase; PRKAG2 transcripts; cardiomyopathy.

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1. PRKAG2 – human mouse; transcript variants

Fig. S1
The PRKAG2 locus and the alignment of the γ2 transcript variants
A: Alignment of the human and mouse exon 3b; from the start codon the coding sequence is boxed in gray. Nucleotide sequence homology is marked. Amino acids, identical in both sequences are shown in red above or below the corresponding codons. B: Alignment of the human γ2 proteins. The unique N-terminal segment of the γ2-3B is underlined.

2. Expression of γ2c in human and mouse tissues
The γ2c was cloned originally from a human hippocampus cDNA library and is also annotated in Ensembl. It has not yet been annotated in mouse Ensembl. We have tested cDNAs from a human tissue panel and could not detect reasonable amount of γ2c in any tissue; even the brain sample did not yield a PCR product. Expression of γ2c might be restricted to the hippocampus. However, we have found γ2c in mouse cDNA, but we only detected reasonable expression in brain (Fig. S2), therefore we have ruled out any cardiac specificity in mouse or in human. The protein would be 59 kDa, close to the mass of γ2-long (63 kDa). The γ2c protein does not contain nuclear localization signal (NLS) which is encoded by exon 1 as we have described it earlier [1]. The γ2-long antibody we have used in our immuno-histochemistry study would react with both γ2a and γ2c.

Fig. S2
Expression of γ2c in adult mouse tissues – real-time PCR
CATCATCTCAACACAGCAACACAG – forward primer (anneals in the 5’ UTR);
GGCAAGGAACGTCAAGTC – reverse primer (QuantiTect SYBR Green reagents).

3. Amplification and cloning of the γ2-3B coding sequence from mouse tissues and from human cardiac cDNA
Total RNA was extracted with TRI-REAGENT (Sigma) from mouse tissues and cDNA was synthesized with SuperScript III (Invitrogen). Primers for amplifying the entire coding sequences anneal in the alternative exon 3b and in the 3’ UTR: TGGGAGCCTCAGGAGCAACAAGA (forward) and CATGAGGCAAAACGTGACCCAGAGA (reverse) for human γ2-3B; CACCAGGGGCACAGCTCGGAACC (forward) and GCAGAGCGCTTAGAGGCATCACATTT (reverse) for the mouse γ2-3B. The PCR reaction was performed with the Expand High Fidelity PCR System (Roche) and the product was cloned into pGEM-T Easy (Promega) for sequence analysis. GAPDH fragment was amplified with primers: GTTTCTTACTCCTTGGAGGCCAT (forward) and TGATGACATCAAGAAGTGGTGAA (reverse) from mouse cDNA.

**Fig. S3** Expression of γ2-3B in mouse tissues and in human heart
Mouse tissues: B – brain; K – kidney; L – liver; Sk – skeletal muscle; H – heart and cardiac cDNA from a human cDNA panel.

**4. Validating the γ2-3B antibody**

We expressed γ2-3B in bacteria after cloning it into the pET-41a expression vector. The coding sequence for the full-length protein were inserted at the start codon immediately after the ribosome-binding site and fused to the C-terminal His-tag. Expression was induced in BL21pLysS cells with 0.4 mM IPTG. Samples were harvested before and after induction, and the cells lysed with SDS-PAGE loading buffer. Equal volumes of bacterial cultures were sampled before (0) or after induction (1, 3 h). Equal volumes of samples were loaded on SDS-PAGE for Western blot analysis. The molecular weight is the predicted 50 kDa (plus the C-terminal His-tag, approximately 1 kDa). The proteins appear to be stable, no degradation has been observed after 3 hours induction (Fig. S4).

**Fig. S4**
Expression of γ2-3B in bacteria – validation of the γ2-3B antibody, G2I
The size of the protein is 51 kDa with the C-terminal His-tag.

**5. Cell fractionation – Western blots and SDS-PAGE stained for proteins**
Fig. S5

Cell fractionation of cardiac tissue
A: Proteins in four fractions are separated in 9 % SDS-PAGE and stained for protein or the western blot was probed with the PRKAG2 antibody (Atlas Antibodies) that recognizes all γ2 proteins. CS – cytosol, M/P – membrane/particulates, N – nuclear and CSk – cytoskeletal fraction. Equal volumes of the fractions were applied (1x); more was loaded of the cytoskeletal fraction (4x) on a separate gel.
B: Western blot – γ2 proteins detected with the C-terminal γ2 antibody (gift from D. Carling) in the cytoskeletal fraction; 4-12 % gradient gel.

6. Transcription of γ2 variants in mouse embryonic heart
To compare the contribution of the different γ2 transcripts to the γ2 pool in mouse, we amplified fragments of each transcript variant with 5'-specific primers. Total γ2 transcription was estimated by amplifying a fragment from the nucleotide-binding region of γ2. As a reference, GAPDH was used. PCR reactions were carried out under the same conditions by using equal amount of cDNA; equal volumes of PCR reactions were loaded on 1.2 % agarose gel containing ethidium bromide (Fig. S2). The γ2-long is the minority γ2 component in embryonic heart. The short form appears to be the major γ2 at 13.5 dpc; however, the increasingly expressed γ2-3B probably equals γ2-short by birth.
In adult mouse tissues the ratio of γ2-short and γ2-long varies (not shown). There is much more γ2-short in brain, cardiac and skeletal muscle than in kidney for example and γ2-short is hardly detectable in liver as shown in Fig. 1.

Fig. S6

Transcription of the different γ2 variants in the developing mouse heart – RT-PCR
The primers for γ2-3B: AGGCCGGCGAGGCTGACACTGC (forward) with and AAAAAAGGCCATGGGGTTCCGACTG (reverse) Equal amounts of cDNA were used in the reactions and equal volumes of the reactions were loaded on agarose gel. The band intensities were normalized to the GAPDH content in
each sample (Primers as in Fig. S3). Primers for the other \( \gamma_2 \) transcripts were:

\( \gamma_2 \)-long – CGGGCGGAGCAGTGCAAAAGGAAC (forward) and GAGAAGAACCAGGTGCCAGGAGG (reverse);

\( \gamma_2 \)-short – CGCGGCCCATGCTGATCGGTGTCC (forward) and ATAGGGTCAATAACTGCAATCTGTGG (reverse);

total \( \gamma_2 \) – TGACATCGTAAAACAGGCAGTGA (forward) – ATAGGGTCAATAACTGCAATCTGTGG (reverse).

**Table S1**

Primers and probes for qPCR

| Oligonucleotides |
|------------------|
| **\( \gamma_2 \) total** |
| Forward: GACATTCTGCAAGCCCTGATC |
| Reverse: CAAAAGGAGACAGAAACGGAGTG |
| Probe: CACCAGCAGGTGCAA |
| Location: exon 16; **amplicon**: 65 |
| **\( \gamma_2 \) a** |
| Forward: CGCCATGCCGCTCTCT |
| Reverse: CTCTGGAGAAGAACCCTTTGGA |
| Probe: TCGAAGGTGGGAGCGCC |
| Location: exon 1-2; **amplicon**: 110 |
| **\( \gamma_2 \) b** |
| Forward: TCATGCTGATCGGTGTTC |
| Reverse: AACTCCAGCTTCTCCAGCATG |
| Probe: CCCCTCAAGCAGGC |
| Location: 5' UTR – exon 5; **amplicon**: 93 |
| **\( \gamma_2 \) c** |
| Forward: TCGCATCCCTGCCCATCCA |
| Reverse: GAGGTCTTCTTCCAGATGCTAATA |
| Probe: CAGCTTACAAGCCTGC |
| Location: 5' UTR; **amplicon**: 66 |
| **\( \gamma_2 \)-3B** |
| Forward: AGAGGGCCCAGTCCGGAG |
| Reverse: CGTGAAACACCCCAACCC |
| Probe: CTTTTCGCCCTCCGCCCT |
| Location: exon 3b; **amplicon**: 71 |
| **\( \gamma_3 \)** |
| Applied Biosystems design, No. Hs00179660_m1 |

**Table S2**

Ct values

|          | Adult heart | Skeletal muscle |
|----------|-------------|-----------------|
| **\( \gamma_2 \) total** | 24          | 28.5            |
| **\( \gamma_2 \) a**     | 28.8        | 31.7            |
| **\( \gamma_2 \) b**     | 25.4        | 33.9            |
|   |   |   |
|---|---|---|
| $\gamma^2c$ | 34.5 | -* |
| $\gamma^2$-3B | 24.8 | 29.8 |
| $\gamma^3$ | 35.9 | 23.5 |

*not tested

**References**

[1] Pinter K, Jefferson A, Czibik G, Watkins H, Redwood C. Subunit composition of AMPK trimers present in the cytokinetic apparatus: Implications for drug target identification. Cell Cycle 2012; 11: 917 - 21.
Exon 3b - alignment of the human and mouse sequences

**HUMAN**

GCTGATGCTCTCTTGCTCCCGGCCACCTGGGGCTGAC

**MOUSE**

TCCTTCTGGTGAGCCCGCTTTCCCCGCCTAGACTCAC

Exon 3b - alignment of the human and mouse sequences

**HUMAN**

ATGAAGCGCTTTGGGAGCCTCAGGAGCAACAAGAAACACAAGGACCAAAATCGAAGCACG

**MOUSE**

ATGAAGCGCTTTGGAAGTCTGAGGGGCACCAAGAAACCCAAGGACCAAAACCGAAGCACC

**Fig. S1**
B

PRKAG2 ENSG00000106617 – human
http://www.ensembl.org/index.html

Colour code:
Alternating exons, Alternating exons, Residue overlap splice site

γ2a
MGSAVM0T7KKKDVSSP0GSGGKNNASQKR3RLRVHIPDLSSFAMP1LDDGLEGSGKHSS
MPLLGDLEGSGKHSS

γ2c
RKVDSPFGP0GSGGKS0RFSRGP0FRPSP0PMASPVRFKTSGPSFKTFSP0YQSSFP0SPRPR
RKVDSPFGP0GSGGKS0RFSRGP0FRPSP0PMASPVRFKTSGPSFKTFSP0YQSSFP0SPRPR

γ2α
M0FSGF0R6SS0KKE0S0NPS0ATSP0GIRFFS0RFS0RTSGLSS0P0STP0QVTQ0HTPHLESY
M0FSGF0R6SS0KKE0S0NPS0ATSP0GIRFFS0RFS0RTSGLSS0P0STP0QVTQ0HTPHLESY
MKFRGSL0NKKH0KQRN0STERQ0SPHGLFA0LGS0S0P0STP0QVTQ0HTPHLESY

γ2c
KNEFRL0N0YASSS0P0DT0QFC0P0S0F0SPF0PLAS0THY0PKAA0A0A0A0GPAEA
KNEFRL0N0YASSS0P0DT0QFC0P0S0F0SPF0PLAS0THY0PKAA0A0A0A0GPAEA

γ2β
GM0L0E0L0FE0DE0A0VED0S0G0V0MRF0S0RK0CY0D0VTF0SS0KLV0VF0D0T0QL0V0K0A0FA0L0V0ANG
GM0L0E0L0FE0DE0A0VED0S0G0V0MRF0S0RK0CY0D0VTF0SS0KLV0VF0D0T0QL0V0K0A0FA0L0V0ANG
GM0L0E0L0FE0DE0A0VED0S0G0V0MRF0S0RK0CY0D0VTF0SS0KLV0VF0D0T0QL0V0K0A0FA0L0V0ANG

γ2c γ2β
ML0K0E0L0FE0DE0A0VED0S0G0V0MRF0S0RK0CY0D0VTF0SS0KLV0VF0D0T0QL0V0K0A0FA0L0V0ANG

γ2α
VRAA0PL0NE0K0Q0SF0V0ML0TI0DF0IN0L0HR0Y0KG0PM0Q0Y0E0L0HE0K0I0TE0RE0LY0Q0ET0FP
VRAA0PL0NE0K0Q0SF0V0ML0TI0DF0IN0L0HR0Y0KG0PM0Q0Y0E0L0HE0K0I0TE0RE0LY0Q0ET0FP
VRAA0PL0NE0K0Q0SF0V0ML0TI0DF0IN0L0HR0Y0LG0PM0Q0Y0E0L0HE0K0I0TE0RE0LY0Q0ET0FP

γ2a γ2β
LV0NISP0D0A0LF0D0V0YS0L0N0K0IH0RL0PV0D0PS0NAL0Y0L0TH0R0K0L0F0Q0LF0MS0D0MP0FA0
LV0NISP0D0A0LF0D0V0YS0L0N0K0IH0RL0PV0D0PS0NAL0Y0L0TH0R0K0L0F0Q0LF0MS0D0MP0FA0
LV0NISP0D0A0LF0D0V0YS0L0N0K0IH0RL0PV0D0PS0NAL0Y0L0TH0R0K0L0F0Q0LF0MS0D0MP0FA0

γ2α γ2β
MKQN0L0DE0L0GI0TH0N0IA0FH0PD0TP0I0K0AL0N0F0V0R0R0IS0AL0PV0DES0KV0D0I0Y0SK0FD0VIN
MKQN0L0DE0L0GI0TH0N0IA0FH0PD0TP0I0K0AL0N0F0V0R0R0IS0AL0PV0DES0KV0D0I0Y0SK0FD0VIN
MKQN0L0DE0L0GI0TH0N0IA0FH0PD0TP0I0K0AL0N0F0V0R0R0IS0AL0PV0DES0KV0D0I0Y0SK0FD0VIN

γ2α γ2β
LA0AE0K0YN0L0DI0IT0V0QL0HR0Q0F0G0V0CK0L0E0L0I0T0I0V0D0RI0V0RA0EH0RL0V0V0NE0ADS
LA0AE0K0YN0L0DI0IT0V0QL0HR0Q0F0G0V0CK0L0E0L0I0T0I0V0D0RI0V0RA0EH0RL0V0V0NE0ADS
LA0AE0K0YN0L0DI0IT0V0QL0HR0Q0F0G0V0CK0L0E0L0I0T0I0V0D0RI0V0RA0EH0RL0V0V0NE0ADS

γ2α γ2β
IV0G10L0S0L0D0IL0Q0AL0I0L0T0P0G0A0Q0K0ET0TE0
IV0G10L0S0L0D0IL0Q0AL0I0L0T0P0G0A0Q0K0ET0TE0
IV0G10L0S0L0D0IL0Q0AL0I0L0T0P0G0A0Q0K0ET0TE0

((PRKAG2 ENSMUSG00000028944 – mouse)
Fig. S3
Fig. S4
Fig. S5

A

SDS-PAGE – protein staining

Western blot

kDa
225
150
102
76
52
38
31

CS
M/P
N
CSk

B

kDa
64
50
38

γ-long
γ-3B
γ-short
Fig. S6

**γ2-3B expression**

| Time  | 13.5 | 19.5 | birth |
|-------|------|------|-------|

- **γ2-3B**
- **GAPDH**
- **γ2-long**
- **γ2-short**
- **γ2(all)**