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Opposite Transcriptional Regulation in Skeletal Muscle of AMP-activated Protein Kinase γ3 R225Q Transgenic *Versus* Knock-out Mice*[S]

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AMP-activated protein kinase (AMPK) is an evolutionarily conserved heterotrimer important for metabolic sensing in all eukaryotes. The muscle-specific isoform of the regulatory γ-subunit of the kinase, AMPK γ3, has an important role in glucose uptake, glycogen synthesis, and fat oxidation in white skeletal muscle, as previously demonstrated by physiological characterization of AMPK γ3 mutant (R225Q) transgenic (*TgPrkag3*^{225Q}) and γ3 knock-out (*Prkag3*$^{-/-}$) mice. We determined AMPK γ3-dependent regulation of gene expression by analyzing global transcription profiles in glycolytic skeletal muscle from γ3 mutant transgenic and knock-out mice using oligonucleotide microarray technology. Evidence is provided for coordinated and reciprocal regulation of multiple key components in glucose and fat metabolism, as well as skeletal muscle ergogenics in *TgPrkag3*^{225Q} and *Prkag3*$^{-/-}$ mice. The differential gene expression profile was consistent with the physiological differences between the models, providing a molecular mechanism for the observed phenotype. The striking pattern of opposing transcriptional changes between *TgPrkag3*^{225Q} and *Prkag3*$^{-/-}$ mice identifies differentially expressed targets being truly regulated by AMPK and is consistent with the view that R225Q is an activating mutation, in terms of its downstream effects. Additionally, we identified a wide array of novel targets and regulatory pathways for AMPK in skeletal muscle.

AMP-activated protein kinase (AMPK)[2] is a critical regulator of carbohydrate and fat metabolism in eukaryotic cells (reviewed in Refs. 1 and 2). AMPK is a heterotrimer that consists of α-, β-, and γ-subunits, all of which are required for its activity. The catalytic α-subunit contains a conventional serine/threonine protein kinase domain, and phosphorylation of Thr-172 residue within the activation loop of the α-subunit by upstream kinases is essential for the activity of the heterotrimer (3–6). Once phosphorylated at Thr-172, AMPK can be further activated by allosteric binding of AMP to the evolutionary conserved cystathionine β-synthase domains in the regulatory γ-subunit (7). The AMPK β-subunit acts as a scaffold for binding of the α- and γ-subunits (8). The β-subunit also contains a glycogen-binding domain, and recent findings provide evidence that this motif is involved in targeting the AMPK complex to cellular glycogen stores (9, 10). The mammalian genome contains seven AMPK genes encoding for two α-, two β-, and three γ-isoforms. Thus, there are 12 possible combinations of heterotrimeric AMPK, and the physiological function of the AMPK holoenzyme depends on the particular isoforms present in the complex.

We have provided evidence that AMPK γ3 is the predominant γ-isoform expressed in glycolytic (white, fast-twitch type II) skeletal muscle (11). In contrast, it is expressed at low levels in oxidative (red, slow-twitch type I) skeletal muscle and is undetectable in brain, liver, heart, or white adipose tissue (11). Thus, the AMPK γ3-subunit is the only isoform exhibiting tissue-specific expression. Furthermore, the AMPK γ3-subunit primarily forms heterotrimers with the α2- and β2-isoforms in glycolytic skeletal muscle (11).

The functional significance of the AMPK γ3-subunit has been demonstrated by phenotypic analysis of animal models carrying a mutated form of the gene. The dominant Rendement Napole (RN) phenotype identified in Hampshire pigs is caused by a single missense mutation (R225Q) in the AMPK γ3-subunit (12). RN pigs have a 70% increase in glycogen content in skeletal muscle, whereas liver and heart glycogen content remains unchanged (13). Furthermore, RN carriers are also characterized by a higher oxidative capacity in white skeletal muscle fibers (14, 15). Conversely, a second mutation (V224I) identified in pigs at the neighboring amino acid residue of the γ3-protein is associated with an opposite phenotype compared with the RN allele, resulting in reduced skeletal muscle glycogen content (16). Characterization of transgenic mice with skeletal muscle-specific expression of the mutant (R225Q) form of the mouse AMPK γ3-subunit, as well as AMPK γ3-subunit knock-out mice, provided further evidence that the γ3-subunit plays a key role in skeletal muscle carbohydrate and lipid metabolism. Glycogen resynthesis after exercise was impaired in AMPK γ3 knock-out mice but was markedly enhanced in transgenic mutant mice. An AMPK-activator failed to increase skeletal muscle glucose uptake in knock-out mice, whereas insulin-mediated glucose uptake was unaltered. When fed with a high fat diet, γ3 R225Q transgenic mice were protected against excessive triglyceride accumulation and insulin resistance in skeletal muscle, presumably due to an increase in fat oxidation (17). Additionally, skeletal muscle from γ3 R225Q mutant mice is characterized by enhanced work performance, whereas knock-out mice are fatigue-prone (18).

To further characterize the role of AMPK γ3 in skeletal muscle and to uncover molecular mechanisms explaining phenotypic consequences

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental text, Table SI, and Fig. S1.

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[2] The abbreviations used are: AMPK, AMP-activated protein kinase; RN, Rendement Napole; qRT-PCR, quantitative real-time PCR; EST, expressed sequence tag; MAPK, mitogen-activate protein kinase; AICAR, 5-amino-4-imidazole-carboxamide riboside.

of the mutations in this isoform, we have studied AMPK γ3-dependent gene transcription by a systematic approach, using global analysis of the mRNA expression pattern in the skeletal muscle of γ3 R225Q mutant and γ3 knock-out mice. Here we describe distinct biomarker patterns, comprising AMPK γ3-dependent transcriptional changes of genes involved in glucose and lipid metabolism and skeletal muscle ergogenics.

EXPERIMENTAL PROCEDURES

AMPK Knock-out (Prkag3$^{-/-}$) and R225Q Transgenic (TgPrkag3^{225Q}) Mice—The *Prkag3*$^{-/-}$ and *TgPrkag3*^{225Q} mice used in this study have been previously described (17). *Prkag3*$^{-/-}$ mice were created by conventional gene targeting techniques. *TgPrkag3*^{225Q} mice express the mutant γ3 R225Q subunit under the control of mouse myosin-light chain promoter and enhancer elements. Mice used in the study were bred into the C57BL/6 genetic background. Mice were maintained in a 12-h light-dark cycle and were cared for in accordance with regulations for the protection of laboratory animals. The study was performed after prior approval from the local ethical committee. Gene expression profiles were characterized in male mice fasted overnight (food was removed 16 h prior to study). The white portion of the gastrocnemius muscle was dissected from anesthetized mice, cleaned of fat and blood, and quickly frozen in liquid nitrogen as described (17).

Preparation of Total RNA—Total RNA was isolated from the white portion of the gastrocnemius muscle using the RNeasy Fibrous Mini Kit (Qiagen) applying Mixer Mill MM 301 (Retsch) followed by a DNase digestion step using RNase-Free DNase Set (Qiagen) according to the manufacturer's instructions. The RNA yield was quantified by spectrophotometric analysis and the RNA purity was determined based on the A_{260}/A_{280} ratio. The quality of the RNA was confirmed by Agilent 2100 Bioanalyzer analysis (Agilent Technologies) using the RNA 6000 Nano Assay Kit (Agilent Technologies).

Preparation of cRNA, Gene Chip Hybridization—10 μg of total RNA spiked with poly-A controls (pGIBS-TRP, -THR, and -LYS, American Type Culture Collection) was converted to cDNA utilizing a T7 promoter-polyT primer (Affymetrix) and the reverse transcriptase Superscript II (Invitrogen), followed by a second strand cDNA synthesis (Invitrogen). Double-stranded cDNA was *in vitro* transcribed to biotinylated cRNA (Enzo) and then fragmented (Invitrogen). The fragmented cRNA was mixed with control oligonucleotide B2 (Affymetrix) and a hybridization control cRNA mixture (BioB, BioC, BioD, and Cre, Affymetrix). Aliquots of each sample were hybridized (16 h at 45 °C) to GeneChip Mouse Expression Set 430A arrays (Affymetrix). The arrays were subsequently washed, stained, and scanned according the manufacturer's instructions (GeneChip Expression Analysis Technical Manual, Affymetrix).

Data Analysis—Data were analyzed using GeneTraffic UNO 3.2–11 (Iobion Informatics) and Spotfire DecisionSite 8.1 (Spotfire Inc.). The *TgPrkag3*^{225Q} dataset was analyzed separately from the *Prkag3*$^{-/-}$ dataset. For further details see the supplemental information.

Quantitative Real-time PCR—Quantification of mRNA levels for selected genes was performed by qRT-PCR as described (19) using acidic ribosomal phosphoprotein P0 (*Arbp*) as endogenous control (see supplemental Table SI for primer information). qRT-PCR was performed on extended set of samples including 7 *Prkag3*$^{-/-}$ mice with 8 wild-type littermates and 13 *TgPrkag3*^{225Q} mice with 10 wild-type littermates, while RNA from 6 animals in each group was used in gene array analysis.

Histochemistry—Enzyme activity staining for succinate dehydrogenase and cytochrome *c* oxidase was done on serial cross-sections (10-μm thickness) of frozen gastrocnemius muscle as described previously (20, 21). For succinate dehydrogenase activity staining, sections were incubated for 4 min in a 0.1 M phosphate buffer (pH 7.6) containing 5 mM EDTA, 45 mM disodium succinate, 1.2 mM nitro blue tetrazolium, 1 mM potassium cyanide, and 1 mM phenazine methosulfate. Cytochrome *c* oxidase activity staining was performed by incubating sections for 1 h in a 50 mM phosphate buffer (pH 7.6) containing 0.22 M sucrose, 2.3 mM 3,3′-diaminobenzidine tetrahydrochloride, 1 mM cytochrome *c*, and 1300 units of catalase.

RESULTS

Microarray Analysis of the mRNA Expression in the Skeletal Muscle of AMPK γ3 Knock-out (Prkag3$^{-/-}$) and R225Q Transgenic (TgPrkag3^{225Q}) Mice—To determine the role of γ3-containing AMPK complexes in regulation of gene expression in the skeletal muscle, we utilized mouse models that either lack the AMPK γ3-protein (*Prkag3*$^{-/-}$) or express a R225Q mutant form of this protein in skeletal muscle (*TgPrkag3*^{225Q}) (17). In *Prkag3*$^{-/-}$mice, AMPK γ3-protein expression is completely ablated, and importantly, no compensatory increase in γ1- or γ2-isoform is detected (17). Equally important, AMPK expression in *TgPrkag3*^{225Q} mice resembles the expression pattern in wild-type mice, both with regard to tissue distribution and protein expression, with the mutant (R225Q) form replacing the endogenous AMPK γ3-protein (17). Global gene expression profiles in the white portion of gastrocnemius muscle of *Prkag3*$^{-/-}$ and *TgPrkag3*^{225Q} mice were compared with the corresponding wild-type littermates using oligonucleotide microarrays. The expression of 167 genes was significantly ($p \leq 0.05$) changed by a factor of 20% or more, in *TgPrkag3*^{225Q} and/or *Prkag3*$^{-/-}$ mice, relative to the wild-type controls (Table 1). Applying the same filtering criteria on randomly created groups within the *Prkag3*$^{-/-}$ dataset and *TgPrkag3*^{225Q} dataset resulted in six genes determined as differentially expressed. This indicates that the rate of false positives is low. Consequently, the vast majority of the genes appearing differently expressed in *Prkag3*$^{-/-}$ and/or *TgPrkag3*^{225Q} mice can be considered as truly regulated.

Of the 167 differentially expressed transcripts, the identity of 148 genes is known and represents proteins of different functional classes, whereas 19 transcripts only show homology to sequences in the EST or genomic databases. Interestingly, the expression level of 16 genes was significantly changed in both AMPK γ3 R225Q transgenic and AMPK γ3 knock-out mice, compared with their respective wild-type littermates (Table 1). For these 16 transcripts, the direction of the observed change was the opposite in knock-out *versus* mutant transgenic mice. Furthermore, most of the genes that were significantly changed exclusively in *Prkag3*$^{-/-}$ mice tended to be regulated in an opposite manner in *TgPrkag3*^{225Q} mice, even though this difference did not reach statistical significance and/or meet the -fold change criteria. Correspondingly, the vast majority of transcripts, which were differentially regulated exclusively in R225Q transgenic mice, exhibited an opposite trend in knock-out mice. The striking pattern of opposing transcriptional changes in the AMPK γ3 R225Q transgenic *versus* knock-out mice, as compared with their wild-type littermates, is illustrated (Fig. 1).

Many of the genes, which were found to be differentially expressed in *Prkag3*$^{-/-}$ and/or *TgPrkag3*^{225Q} mice, are previously undescribed as being regulated by AMPK. Full functional significance of these changes in global transcriptional profile remains to be addressed in further experiments. To determine the possible mechanistic explanations for previously described physiological differences between *Prkag3*$^{-/-}$ and *TgPrkag3*^{225Q} mice (17, 18), we performed a more detailed analysis of gene expression changes for targets that are known to be involved in lipid and carbohydrate metabolism and muscle ergogenics. The expression of several genes involved in these functions depends on the skeletal

TABLE 1

Differentially expressed genes in *TgPrkag3*^{225Q} and/or *Prkag3*$^{-/-}$ mice compared with wild-type littermates

Global mRNA expression pattern was characterized in the white portion of the gastrocnemius skeletal muscle in male mice of C57BL/6 genetic background. The filtering criteria were set to a mean absolute -fold change >1.2 and a *p* value ≤0.05. In addition the mean intensity in the group showing highest expression should be >75.

Public ID	Gene symbol	Gene title	*TgPrkag3*^{225Q} *versus* wild-type littermates		*Prkag3*$^{-/-}$ *versus* wild-type littermates	
			-Fold change	*p* value	-Fold change	*p* value
Genes differentially expressed in both *TgPrkag3*^{225Q} and *Prkag3*$^{-/-}$ compared with wild-type littermates						
AU017649	*Gdap1*[a]	Ganglioside-induced differentiation-associated-protein 1	−1.82	0.0004	1.27	0.003
BB336256	*Mafa*	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein A	−1.58	0.000008	1.29	0.002
BC026377	*Rasd2*[a]	RASD family, member 2	1.53	0.00005	−1.21	0.048
NM_010016	*Daf1*[a]	Decay accelerating factor 1	1.50	0.002	−1.20	0.018
M62838	*Slc7a2*[a]	Solute carrier family 7, member 2	1.46	0.006	−1.35	0.023
BB414515	*Slc2a3*[a]	Solute carrier family 2, member 3	−1.42	0.002	1.22	0.035
BB326929	*Sh3kbp1*	SH3-domain kinase binding protein 1	1.41	0.010	−1.23	0.011
NM_028803	*Gbe1*	Glucan branching enzyme 1	1.40	0.012	−1.28	0.011
AI788759	*Ugp2*[a]	UDP-glucose pyrophosphorylase 2	1.39	0.0001	−1.21	0.008
AF226613	*Slc40a1*	Solute carrier family 40, member 1	1.38	0.006	−1.26	0.019
NM_009876	*Cdkn1c*	Cyclin-dependent kinase inhibitor 1C	−1.32	0.0005	1.20	0.022
AV337591	*Xpr1*	Xenotropic and polytropic retrovirus receptor 1	−1.26	0.009	1.21	0.005
BM114165	*Rpl5*	Ribosomal protein L5	−1.24	0.015	1.21	0.024
NM_009079	*Rpl22*	Ribosomal protein L22	−1.24	0.007	1.20	0.00004
AV030603	*2010109N14Rik*	RIKEN clone 2010109N14	−1.22	0.010	1.21	0.037
BC011152	*Golph2*	Golgi phosphoprotein 2	−1.22	0.003	1.25	0.002
Genes differentially expressed in *TgPrkag3*^{225Q} compared with wild-type littermates						
NM_007913	*Egr1*	*Mus musculus* early growth response 1	2.36	0.021	−1.05	0.887
BC024613	*Tmem37*	*M. musculus* transmembrane protein 37	2.03	0.000007	1.16	0.375
NM_008161	*Gpx3*	Glutathione peroxidase 3	1.90	0.000004	−1.14	0.030
NM_007570	*Btg2*[a]	B-cell translocation gene 2, anti-proliferative	1.76	0.023	1.11	0.535
NM_026433	*1810057C19Rik*	Integral membrane protein	−1.64	0.0004	1.09	0.607
NM_008357	*Il15*	Interleukin 15	1.57	0.00007	−1.06	0.059
AI315015	*Ces3*[a]	Carboxylesterase 3	1.57	0.018	1.10	0.513
NM_008416	*Junb*	Jun-B oncogene	1.56	0.043	−1.03	0.864
AK011596	*Tfrc*	Transferrin receptor	−1.54	0.040	1.09	0.315
X14678	*Zfp36*[a]	Zinc finger protein 36	1.49	0.041	−1.03	0.824
BG065702	*D230025D16Rik*[a]	RIKEN cDNA D230025D16 gene	1.47	0.023	1.01	0.823
NM_025427	*RGC-32*[a]	Response gene to complement 32 (*Homo sapiens*)	1.47	0.009	−1.05	0.686
NM_023065	*Ifi30*	Interferon γ-inducible protein 30	1.46	0.00007	−1.10	0.094
C81193	*Odc1*[a]	Ornithine decarboxylase, structural 1	−1.46	0.002	1.17	0.052
BC010758	*Cbr2*	Carbonyl reductase 2	1.45	0.003	−1.10	0.179
AK012825	*2810026P18Rik*	RIKEN cDNA 2810026P18 gene	−1.45	0.016	1.18	0.066
BC024118	*9430059P22Rik*[a]	Transmembrane protein 46	−1.44	0.006	1.16	0.178
U18812	*Lep*	Leptin	−1.43	0.019	1.11	0.158
NM_010858	*Myl4*	Myosin, light polypeptide 4	−1.43	0.0002	1.18	0.039
BB221402	*Cidec*	Cell death-inducing DFFA-like effector c	−1.43	0.040	1.20	0.058
BB794641	*Nos1*	Nitric-oxide synthase 1, neuronal	1.39	0.00007	−1.17	0.009
NM_008452	*Klf2*	Kruppel-like factor 2	1.38	0.027	1.00	0.968
AF378088	*Rhou*[a]	Ras homolog gene family, member U	1.37	0.004	−1.00	0.945
BM200248	*Peg3*	Paternally expressed 3	−1.37	0.015	−1.02	0.790
NM_013525	*Gas5*[a]	Growth arrest-specific 5	−1.36	0.004	1.17	0.043
NM_026524	*Mid1ip1*	Mid1 interacting protein 1	1.34	0.004	1.20	0.197
AJ132394	*Rorc*	RAR-related orphan receptor gamma	1.34	0.003	−1.11	0.041
BC026450	*Cova1*	Cytosolic ovarian carcinoma antigen 1	−1.34	0.006	1.15	0.148
BB039269	*Gja1*	Gap junction membrane channel protein alpha 1	1.34	0.030	−1.01	0.873
BG069413	*Klf4*	Kruppel-like factor 4	1.33	0.023	−1.03	0.739
BB644164	*Cugbp2*[a]	CUG triplet repeat, RNA binding protein 2	−1.32	0.011	1.08	0.437
BC012405	*2310001H12Rik*	Gonadotropin-regulated transcription factor	1.32	0.022	1.04	0.436
BB144704	*Abca1*	ATP-binding cassette, sub-family A, member 1	−1.32	0.006	1.16	0.023
BM120823	*Eif4el3*	Eukaryotic translation initiation factor 4E-like 3	1.32	0.004	−1.06	0.485
AF176524	*Fbxl10*	F-box and leucine-rich repeat protein 10	−1.32	0.002	1.13	0.094
BC011116	*M6prbp1*	Mannose-6-phosphate receptor binding protein 1	1.30	0.013	−1.05	0.327
NM_010866	*Myod1*	Myogenic differentiation 1	−1.30	0.0009	1.00	0.978
AI326423	*Srebf1*	Sterol regulatory element binding factor 1	−1.29	0.0009	1.17	0.014
BB475271	*Luc7l2*	LUC7-like 2 (*S. cerevisiae*)	−1.29	0.038	1.02	0.783
NM_022019	*Dusp10*	Dual specificity phosphatase 10	−1.29	0.034	1.18	0.193
BC010564	*Hist2h2aa1*	Histone 2, H2aa1	−1.29	0.0007	1.14	0.010
NM_007394	*Acvr1*	Activin A receptor, type 1	−1.28	0.009	1.11	0.122
BC025461	*Tm4sf3*	Transmembrane 4 superfamily member 3	1.28	0.019	−1.15	0.100
NM_010761	*Ccndbp1*	Cyclin D-type binding protein 1	1.28	0.007	−1.09	0.232
BC022110	*Alas1*	Aminolevulinic acid synthase 1	1.28	0.00005	−1.09	0.154
BF467211	*Cdc42*	Cell division cycle 42 homolog (*S. cerevisiae*)	1.28	0.001	−1.13	0.036
AJ306425	*Hfe*	Hemochromatosis	1.27	0.021	−1.17	0.004
NM_009665	*Amd1*	*S*-Adenosylmethionine decarboxylase 1	−1.27	0.021	1.08	0.363
NM_008735	*Nrip1*	Nuclear receptor interacting protein 1	−1.27	0.024	1.00	0.990
NM_015753	*Zfhx1b*	Zinc finger homeobox 1b	−1.27	0.012	−1.06	0.545
NM_026481	*2700055K07Rik*	CGI-38	−1.27	0.00002	1.16	0.108
BC015254	*Cmkor1*	Chemokine orphan receptor 1	1.27	0.022	−1.06	0.567
NM_020604	*Jph1*	Junctophilin 1	−1.26	0.019	1.05	0.663
BF577544	*Pole4*	Polymerase epsilon 4	−1.26	0.021	1.04	0.375

TABLE 1—*CONTINUED*

Public ID	Gene symbol	Gene title	*TgPrkag3*^{225Q} *versus* wild-type littermates		*Prkag3*$^{-/-}$ *versus* wild-type littermates	
			-Fold change	*p* value	-Fold change	*p* value
NM_009523	*Wnt4*	Wingless-related MMTV integration site 4	−1.26	0.0001	1.13	0.018
BB114336	*Bace1*	β-Site APP cleaving enzyme 1	−1.26	0.036	1.04	0.550
AK004781	*Sox17*	SRY-box containing gene 17	1.26	0.003	−1.01	0.950
BC014718	*Dnase1*	Deoxyribonuclease I	−1.25	0.001	1.16	0.115
AW988981	*1110008H02Rik*	RIKEN cDNA 1110008H02 gene	−1.25	0.006	1.19	0.166
NM_011430	*Sncg*	Synuclein, gamma	−1.25	0.021	1.03	0.638
NM_010240	*Ftl1*	Ferritin light chain 1	1.25	0.015	1.00	0.959
BC009165	*Thrsp*	Thyroid hormone-responsive SPOT14 homolog (*Rattus*)	1.25	0.027	1.01	0.931
NM_008393	*Irx3*	Iroquois-related homeobox 3 (*Drosophila*)	1.25	0.012	1.00	0.953
NM_009379	*Thpo*	Thrombopoietin	1.25	0.004	−1.11	0.024
NM_008258	*Hn1*	Hematological and neurological expressed sequence 1	1.25	0.009	1.02	0.782
BG070255	*Pde7a*[a]	Phosphodiesterase 7A	1.25	0.028	−1.10	0.141
NM_015797	*Fbxo6b*	F-box only protein 6b	1.24	0.010	−1.07	0.080
BC010712	*Cri1*	CREBBP/EP300 inhibitory protein 1	−1.24	0.026	−1.02	0.763
BB261602	*Map2k6*	Mitogen-activated protein kinase kinase 6	1.24	0.030	−1.07	0.107
U43884	*Idb1*	Inhibitor of DNA binding 1	1.24	0.033	−1.05	0.473
BB278286	*1810073P09Rik*	mKIAA1760	1.24	0.013	−1.02	0.659
AK004847	*Rnf128*[a]	Ring finger protein 128	1.24	0.015	−1.07	0.176
AK018605	*4631408O11Rik*	RIKEN cDNA 4631408O11 gene	−1.24	0.039	−1.01	0.883
BB039247	*C1qr1*	Complement component 1, q subcomponent, receptor 1	1.24	0.007	1.00	0.991
AV276428	*BC043118*	cDNA sequence BC043118	1.24	0.002	−1.10	0.036
AK018482	*Fbxo9*	F-box only protein 9	−1.24	0.031	1.01	0.866
BB066232	*Catna1*	Catenin α 1	1.24	0.047	1.01	0.913
BI143942	*Sdh1*[a]	Sorbitol dehydrogenase 1	1.24	0.0007	−1.01	0.903
BB359043	*1700007D05Rik*	Transcription termination factor-like protein	1.23	0.016	−1.09	0.060
BC021914	*Mmd*	Monocyte to macrophage differentiation-associated	−1.23	0.042	1.00	0.983
AK020120	*Hrmt1l2*	Heterogeneous nuclear ribonucleoproteins methyltransferase-like 2 (*S. cerevisiae*)	−1.23	0.043	1.10	0.652
AK010029	*Oxct1*[a]	3-Oxoacid-CoA transferase 1	1.23	0.001	−1.10	0.024
BM935811	*Itga6*	Integrin α 6	1.23	0.016	1.02	0.786
BB818702	*4933439F18Rik*	RIKEN cDNA 4933439F18 gene	−1.23	0.015	1.07	0.069
BE986849	*Ppp1r14b*	Protein phosphatase 1, regulatory subunit 14B	−1.23	0.00002	1.11	0.005
BB145101	*1110028E10Rik*	Choline transporter-like properties	−1.23	0.003	1.09	0.221
BC019757	*Hist1h4i*	Histone 1, H4i	−1.22	0.016	1.12	0.231
AV336908	*Dlat*	Dihydrolipoamide *S*-acetyltransferase	1.22	0.019	−1.12	0.080
AB031049	*Rev3l*	REV3-like	−1.22	0.002	1.05	0.569
BB033733	*Trim16*	Tripartite motif protein 16	1.22	0.021	−1.02	0.565
AB032010	*Fxyd6*	FXYD domain-containing ion transport regulator 6	−1.22	0.020	−1.04	0.362
NM_009214	*Sms*	Spermine synthase	−1.22	0.026	1.10	0.199
AW543698	*Cdh5*	Cadherin 5	1.22	0.009	−1.04	0.299
BC023112	*Galnact2*	Chondroitin sulfate GalNAcT-2	−1.21	0.041	1.13	0.058
NM_024439	*H47*	Histocompatibility 47	−1.21	0.026	1.14	0.022
NM_020581	*Angptl4*	Angiopoietin-like 4	−1.21	0.011	1.05	0.287
AF289490	*Asph*[a]	Aspartate-β-hydroxylase	−1.21	0.023	1.18	0.001
BC008105	*Polk*	Polymerase, kappa	−1.21	0.0005	1.08	0.166
NM_018832	*Pdzx*	PDZ domain containing, X chromosome	1.21	0.016	−1.05	0.328
AF276917	*Glrx1*	Glutaredoxin 1 (thioltransferase)	1.21	0.018	−1.17	0.016
NM_007472	*Aqp1*	Aquaporin 1	1.21	0.010	−1.04	0.294
AW741459	*Eif4b*	Eukaryotic translation initiation factor 4B	−1.21	0.005	1.07	0.130
NM_009076	*Rpl12*	Ribosomal protein L12	−1.21	0.015	1.10	0.224
C78422	*Coq3*	Coenzyme Q3 homolog, methyltransferase (yeast)	1.21	0.013	−1.08	0.036
BI739053	*Clcn3*	Chloride channel 3	1.21	0.039	−1.02	0.866
NM_008173	*Nr3c1*	Nuclear receptor subfamily 3, group C, member 1	−1.21	0.009	1.14	0.191
NM_022310	*Hspa5*	Heat shock 70-kDa protein 5 (glucose-regulated protein)	−1.21	0.007	1.14	0.024
BG801851	*Actr1b*	ARP1 actin-related protein 1 homolog B (yeast)	1.20	0.040	1.01	0.946
AW989410	*Pbef1*	Pre-B-cell colony-enhancing factor 1	1.20	0.005	−1.13	0.016
NM_138953	*Ell2*	Elongation factor RNA polymerase II 2	−1.20	0.023	1.15	0.297
BB667778	*Neo1*	Neogenin	−1.20	0.00003	1.08	0.043
NM_010437	*Hivep2*	Human immunodeficiency virus type I enhancer binding protein 2	−1.20	0.027	−1.01	0.877
NM_016959	*Rps3a*	Ribosomal protein S3a	−1.20	0.00009	1.06	0.067
NM_007508	*Atp6v1a1*	ATPase, H+ transporting, V1 subunit A, isoform 1	1.20	0.002	−1.01	0.894
BC022959	*Acsl6*	Acyl-CoA synthetase long-chain family member 6	1.20	0.025	−1.01	0.735
AK003350	*5730454B08Rik*	Zinc finger CCCH-type domain containing 11A	−1.20	0.007	1.06	0.067
BC003451	*Mat2a*	Methionine adenosyltransferase II, α	−1.20	0.037	1.15	0.225
Genes differentially expressed in *Prkag3*$^{-/-}$ compared with wild-type littermates						
NM_021537	*Stk25*	Serine/threonine kinase 25 (yeast)	−1.00	0.913	−1.88	0.00003
AK009959	*Ankrd1*[a]	Ankyrin repeat domain 1	1.23	0.141	−1.48	0.030
D87867	*Ugt1a12*[a]	UDP-glycosyltransferase 1 family polypeptide members A12, A10, A5, A6, A1, and A2	−1.10	0.328	1.42	0.009
AK009828	*Neu2*	Neuraminidase 2	1.17	0.002	−1.42	0.000004
BC019124	*Lmcd1*	LIM and cysteine-rich domains 1	1.09	0.356	−1.40	0.010

TABLE 1—*CONTINUED*

Public ID	Gene symbol	Gene title	*TgPrkag3*^{225Q} *versus* wild-type littermates		*Prkag3*$^{-/-}$ *versus* wild-type littermates	
			-Fold change	*p* value	-Fold change	*p* value
AV152334	*Atp1b1*[a]	ATPase, Na^+/K^+ transporting, β1 polypeptide	−1.14	0.395	−1.37	0.004
NM_009208	*Slc4a3*	Solute carrier family 4, member 3	−1.04	0.653	1.34	0.010
BB534670	*Cd36*	CD36 antigen	1.08	0.658	−1.33	0.008
AJ288061	*Clasp1*	CLIP associating protein 1	1.00	0.935	1.32	0.006
NM_024264	*Cyp27a1*	Cytochrome P450, family 27, subfamily a, polypeptide 1	−1.17	0.102	1.30	0.001
AK004757	*Stk11ip*	Serine/threonine kinase 11 interacting protein	1.02	0.815	−1.29	0.00003
AK007410	*Gadd45g*	Growth arrest and DNA-damage-inducible 45γ	−1.13	0.617	1.27	0.010
NM_016894	*Ramp1*	Receptor activity modifying protein 1	1.02	0.780	−1.26	0.005
NM_013626	*Pam*	Peptidylglycine α-amidating monooxygenase	−1.04	0.272	1.26	0.001
NM_013750	*Phlda3*	Pleckstrin homology-like domain, family A, member 3	1.16	0.003	−1.25	0.026
BM207588	*Slc2a1*	Solute carrier family 2 (facilitated glucose transporter), member 1	−1.13	0.203	1.25	0.022
BB085604	*2610031L17Rik*	putative mitochondrial outer membrane protein import receptor	1.01	0.965	1.25	0.008
AA792094	*Got1*	Glutamate oxaloacetate transaminase 1, soluble	1.17	0.016	−1.24	0.011
BC013271	*Anxa8*	Annexin A8	1.07	0.211	−1.24	0.009
AF335325	*Ddit4l*	DNA-damage-inducible transcript 4-like	−1.21	0.087	1.23	0.013
BF225802	*Igfbp5*	Insulin-like growth factor-binding protein 5	−1.17	0.211	1.22	0.045
AV337619	*Ppargc1a*	PPARγ, coactivator 1α	−1.06	0.531	−1.22	0.007
M64279	*Bmi1*	B lymphoma Mo-MLV insertion region 1	−1.27	0.072	1.22	0.043
BG060909	*Scd2*	Stearoyl-Coenzyme A desaturase 2	1.00	0.964	1.22	0.003
M65053	*Fgfr1*	Fibroblast growth factor receptor 1	−1.20	0.096	1.22	0.008
BB503267	*C330005L02Rik*	Hypothetical mitochondrial energy transfer proteins	1.10	0.210	−1.21	0.015
BB044517	*5730551F12Rik*	RIKEN cDNA 5730551F12 gene	1.02	0.830	1.21	0.024
AK004359	*Tmcc2*	Transmembrane and coiled-coil domains 2	−1.19	0.003	1.21	0.00002
AV171622	*3300001H21Rik*	RIKEN cDNA 3300001H21 gene	−1.14	0.281	1.20	0.033
BC024809	*Apbb3*	Amyloid β precursor protein-binding, family B, member 3	1.08	0.072	−1.20	0.026
AV023018	*Rpe*[a]	Ribulose-5-phosphate-3-epimerase	−1.23	0.058	1.20	0.049
AI324936	*Rpl13a*	Ribosomal protein L13a	−1.13	0.002	1.20	0.005
BB000894	*Mtap4*	Microtubule-associated protein 4	−1.19	0.006	1.20	0.003
BG064031	*Nap1l1*[a]	Nucleosome assembly protein 1-like 1	−1.17	0.041	1.20	0.014

[a] Genes having several probe sets supporting the regulation.

muscle fiber type, and, correspondingly, any alterations in skeletal muscle fiber type composition might contribute to expression differences. However, relative expression of slow and fast myosin and troponin isoforms remained unchanged in *TgPrkag3*^{225Q} or *Prkag3*$^{-/-}$ mice *versus* their respective wild-type littermates (data not shown). Moreover, enzyme activity staining for succinate dehydrogenase and cytochrome *c* oxidase (markers for oxidative energy metabolism that stain red muscle fibers containing high levels of mitochondria more intensively than white fibers with fewer mitochondria) did not show any clear alteration in fiber type composition between the different genotypes (supplementary Fig. S1). Taken together, these data indicate that differences in the transcriptional profile we describe are independent of skeletal muscle fiber type changes.

qRT-PCR Validation of Differentially Expressed Genes—To minimize erroneous conclusions due to technical variability of the microarray technology, qRT-PCR analysis was applied to validate expression profiles of 13 genes selected on the basis of biological relevance (Fig. 2). For all the transcripts examined, qRT-PCR data verified the significant differences in gene expression (-fold change >1.2; $p \leq 0.05$) detected by gene array analysis. Furthermore, individual animal-to-animal comparison of the expression profiles for these genes showed close to perfect correlation comparing the two techniques (data not shown). The high level of correlation between the expression profiles generated by microarray *versus* qRT-PCR approach illustrates the reliability of the gene array results. However, for 11 of 13 transcripts examined the microarray data tended to underestimate the expression change compared with qRT-PCR results.

Altered Expression of Components of the Glycogen Synthesis Pathway in AMPK γ3 Knock-out and R225Q Transgenic Mice—AMPK function is closely connected to glycogen storage. In human and rat skeletal muscle, high glycogen content represses AMPK activity (22, 23). Concomitantly, there is also genetic evidence that AMPK regulates glycogen levels, because mutations of the γ3- or γ2-subunit affect glycogen storage in skeletal muscle of RN pigs or in human cardiac muscle, respectively (12, 16, 24). Furthermore, AMPK γ3 R225Q transgenic and γ3 knock-out mice have a respective increase or decrease in the rate of glycogenesis in the recovery phase following exercise (17). Notably, the expression of two transcripts encoding proteins involved in glycogen synthesis, *Ugp2* and *Gbe1*, was significantly up-regulated in *TgPrkag3*^{225Q} mice, while being significantly down-regulated in *Prkag3*$^{-/-}$ mice, as determined by the microarray analysis (Table 1). The observed change in the expression pattern was further confirmed by a qRT-PCR approach (Fig. 2). *Ugp2* codes for UDP-glucose pyrophosphorylase 2 (EC 2.7.7.9), an enzyme catalyzing the synthesis of UDP-glucose, a common substrate for glycogenin and glycogen synthase. Glycogenin catalyzes the first step in glycogen synthesis: a self-glycosylation reaction to form an oligosaccharide chain of around eight residues in length (25). Secondly, glycogen synthase (EC 2.4.1.11), with the participation of the glycogen branching enzyme, Gbe1 (EC 2.4.1.18), elongates the oligosaccharide chain to form a mature glycogen molecule. Thus, coordinated changes in *Ugp2* and *Gbe1* expression are likely to contribute to the differences in the glycogen synthesis rate observed comparing *Prkag3*$^{-/-}$ and *TgPrkag3*^{225Q} muscle.

Coordinated and Reciprocal Changes in the Expression of Map2k6 and Dusp10 Genes in Prkag3$^{-/-}$ *and TgPrkag3*^{225Q} *Muscle*—Based on correlative evidence, stimulation of glucose uptake has been reported to be partly regulated by Mapk14 (also known as p38 MAPK), a downstream target of AMPK (26–30). However, the exact mechanism of how the activation of AMPK would lead to an increase

in Mapk14 phosphorylation remains obscure. Interestingly, two transcripts encoding proteins involved in regulation of Mapk14 activity, *Map2k6* and *Dusp10*, were coordinately regulated in γ3 R225Q transgenic and knock-out mice, as shown by microarray analysis as well as qRT-PCR (Fig. 2). A protein encoded by *Map2k6* (mitogen-activated protein kinase kinase 6) is known to activate Mapk14 by dual phosphorylation of specific threonine and tyrosine residues (31). Dusp10 (dual specificity phosphatase 10), on the other hand, down-regulates the enzymatic activity of MAPKs by dephosphorylating the threonine and tyrosine residues, with selectivity toward Mapk14 (32). Thus, the finding of an up-regulation of *Map2k6* in combination with a suppression of the *Dusp10* transcript in $TgPrkag3^{225Q}$ mice, and the reversed pattern of changes seen in $Prkag3^{-/-}$ mice, suggests that Mapk14 is a target of AMPK γ3-containing trimers in the skeletal muscle. An up-regulation of Map2k6 mRNA in $TgPrkag3^{225Q}$ muscle was accompanied by an increase in the protein level, as seen by Western blot analysis (data not shown).

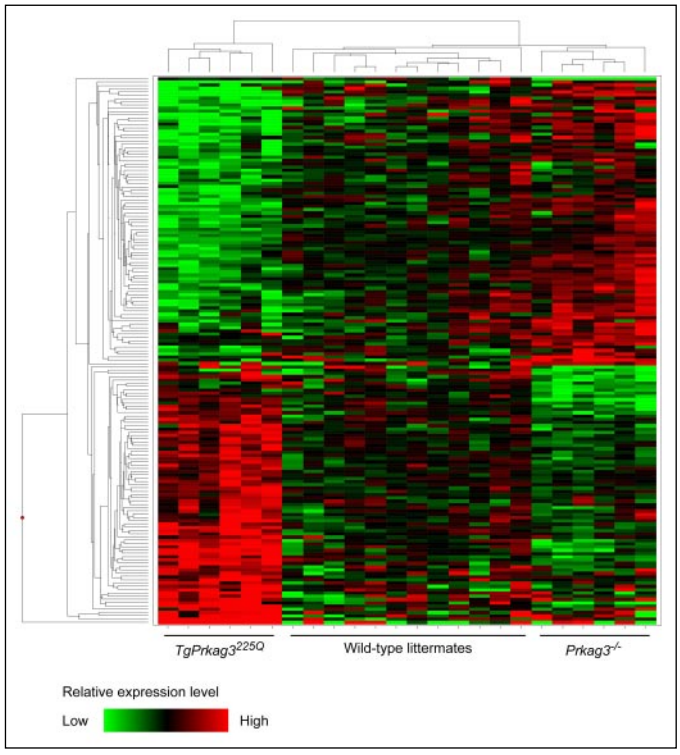

FIGURE 1. **Hierarchical clustering of 167 genes differentially expressed in AMPK γ3 R225Q transgenic and/or knock-out mice, compared with the corresponding wild-type littermates.** Euclidean distance is used as similarity measure. Each *lane* represents one individual mouse, and each *horizontal stripe* represents a single gene transcript. The *colors* represent the gene expression level in each individual mouse relative the average expression of their corresponding wild-type group, with *green* indicating down-regulation and *red* indicating up-regulation.

γ3-Containing AMPK Heterotrimers Regulate Lipid Metabolism Gene Expression in Skeletal Muscle—The AMPK γ3-subunit has previously been shown to be involved in regulation of fat oxidation. Pigs and mice carrying the R225Q mutation in AMPK γ3-gene are characterized by increased lipid oxidation in skeletal muscle (14, 15, 17). In the microarray and qRT-PCR analysis, several genes involved in fat metabolism were differentially expressed in $TgPrkag3^{225Q}$ and $Prkag3^{-/-}$ mice, suggesting that γ3-containing AMPK complexes are involved in the regulation of lipid oxidation in skeletal muscle at the transcriptional level. mRNA for Srebf1 (sterol regulatory element binding factor 1), implicated in lipogenic gene expression (33), was down-regulated in γ3 R225Q mutant mice, whereas mRNA encoding for Ppargc1a (peroxisome proliferative-activated receptor, γ coactivator 1 α), known to increase the expression of both nuclear and mitochondrial-encoded genes of oxidative metabolism (34), was up-regulated. Additionally, a key gene integral to free fatty acid uptake (*Cd36* (35)), as well as genes involved in use of fat-derived energy (3-oxoacid-CoA transferase 1, *Oxct1*, EC 2.8.3.5 and carboxylesterase 3, *Ces3*, EC 3.1.1.1) were up-regulated in $TgPrkag3^{225Q}$ mice. The opposite pattern of changes was observed in γ3 knock-out mice, with differential expression of mRNA for Cd36, Oxct1, and Ppargc1a reaching statistical significance (Fig. 2).

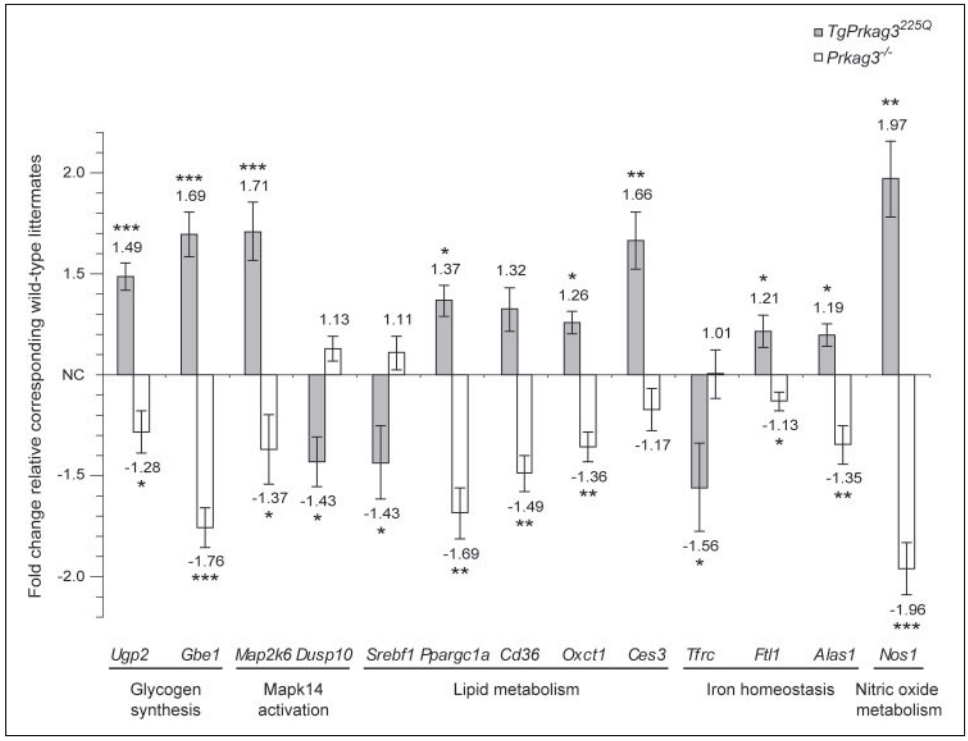

FIGURE 2. **Expression analysis by qRT-PCR.** The *number* associated with each *bar* indicates the -fold change in the AMPK γ3 R225Q transgenic or knock-out mice relative their wild-type littermates. *NC* indicates no change and corresponds to a -fold change of 1 or −1. Statistical differences among genotypes were determined by two-sided Student's *t* test (*, $p \leq 0.05$; **, $p \leq 0.01$; and ***, $p \leq 0.001$).

Possible Role for the AMPK γ3-Subunit in the Regulation of Cellular Iron Homeostasis—Expression of several genes involved in iron metabolism was coordinately changed in AMPK γ3 R225Q transgenic mice, compared with the wild-type littermates. The transferrin receptor, ferritin, and aminolevulinic acid synthase (a key enzyme for heme synthesis) regulate the uptake, storage, and use of iron in cells, respectively. Expression of these markers is known to be coordinately controlled by cellular iron supply such that, under conditions of iron starvation, the transferrin receptor is up-regulated, whereas ferritin and aminolevulinic acid synthase are down-regulated (36). In *TgPrkag3*^{225Q} mice, the transcription of the transferrin receptor, *Tfrc* was decreased, while mRNAs for ferritin light chain 1 and aminolevulinic acid synthase 1 were increased, suggesting the possibility of improved iron status compared with the wild-type mice (Fig. 2). Improved iron status may increase skeletal muscle capacity for aerobic metabolism (37, 38). In line with this, the level of skeletal muscle myoglobin, a heme-carrying protein, transporting oxygen to the mitochondria, was non-significantly increased in *TgPrkag3*^{225Q} mice, as determined by Western blot analysis (data not shown).

AMPK γ3-Containing Complexes Regulate Transcription of the Nos1 Gene—Nitric oxide synthase 1 (*Nos1* also known as *nNos*) was significantly up- and down-regulated by ~2-fold in γ3 R225Q transgenic and knock-out mice, respectively (Fig. 2). The family of Nos enzymes catalyzes formation of endogenous nitric oxide, a mediator implicated in regulation of skeletal muscle contractility, mitochondrial function, as well as glucose uptake (39, 40). Thus, the direction of differential expression of this gene was in complete agreement with the observed phenotype in the mouse models. Although Nos1 is considered a predominant isoform expressed in skeletal muscle fibers, endothelial Nos3 (Nos3 or eNos) and inducible Nos2 (Nos2 or iNos) are also expressed in this tissue (41–43). Interestingly, *Nos2* and *Nos3* were represented on oligonucleotide microarray and were detected in the skeletal muscle samples. However, mRNA levels for these two genes were unaltered.

DISCUSSION

The present study provides the first systematic characterization of the role of the γ3-containing AMPK heterotrimers in transcriptional regulation in skeletal muscle. An oligonucleotide microarray technology was used to compare global transcriptional profiles in white skeletal muscle from AMPK γ3 R225Q transgenic (*TgPrkag3*^{225Q}) and knock-out (*Prkag3*$^{-/-}$) mice and their respective wild-type littermates. Collectively, evidence is provided for an important role of the AMPK γ3-subunit in the transcriptional regulation of divergent groups of genes in glycolytic skeletal muscle. The expression of 167 genes was significantly changed (-fold change > 1.2 and $p \leq 0.05$) in skeletal muscle from γ3 R225Q transgenic and/or knock-out mice. A number of genes from the same biological pathways was coordinately regulated. Changes in levels of mRNA of particular interest, including genes involved in glycogen and lipid metabolism, as well as iron homeostasis and Mapk14 signaling, were selected to be further verified by qRT-PCR (Fig. 2). For all of 13 selected genes, the qRT-PCR results were consistent with the gene array data, which emphasizes the quality of the microarray results. Of note is that most of the genes differentially regulated in *TgPrkag3*^{225Q} mice were changed in an opposing manner in AMPK γ3 knock-out mice (Fig. 1). The reciprocal and coordinated expression pattern observed increases the confidence in the gene-array data and demonstrates that the majority of differentially expressed genes are true positives.

This reverse transcription pattern seen in *TgPrkag3*^{225Q} *versus* *Prkag3*$^{-/-}$ mice is intriguing, considering that the mechanism of action of R225Q substitution in AMPK γ3-subunit is still unresolved. A previous report provided evidence that the kinase activity of AMPK was reduced by ~3-fold in skeletal muscle of γ3 R225Q mutant pigs when measured in the presence and absence of the allosteric activator, AMP (12). Nevertheless, in resting muscle from *TgPrkag3*^{225Q} mice, AMPK activity was unaltered (18). Excessive glycogen content characterizing skeletal muscle of mice and pigs carrying the γ3 R225Q mutation may inhibit AMPK activation by a feedback mechanism. Consistent with this hypothesis, AMPK activity and phosphorylation of the Thr^{172} residue in the α-subunit was elevated in Cos cells transiently transfected with plasmids encoding α2β2γ3 R225Q, compared with the cells transfected with plasmids encoding wild-type trimers (17). Interestingly, the introduction of the R225Q mutation at the corresponding site of the AMPK γ1 (R70Q) or γ2 (R302Q) leads to increased or decreased kinase activity, respectively, when measured in the presence of AMP (44, 45). Our data support a role for the γ3 R225Q as an activating mutation, as judged by its biological effects, because this substitution rendered the opposing changes in the gene expression profile compared with the changes resulting from genetic ablation of the AMPK γ3-subunit (Fig. 1).

The wide array of differentially expressed genes reported in this study has not previously been identified to be regulated by AMPK. Moreover, the biological function of a number of the regulated transcripts is poorly described in skeletal muscle and 19 of the differentially expressed genes remain unknown. Therefore, additional molecular and functional studies will be required to understand the full biological significance of the transcriptional changes described here. Importantly, the expression of several genes with known function in fat and carbohydrate metabolism and skeletal muscle ergogenics was coordinately and reciprocally changed in AMPK γ3 R225Q transgenic and knock-out mice. Thus, the transcriptional regulation by AMPK γ3-containing complexes could lead to at least some of the physiological differences observed in skeletal muscle from *TgPrkag3*^{225Q} and *Prkag3*$^{-/-}$ mice (Fig. 3).

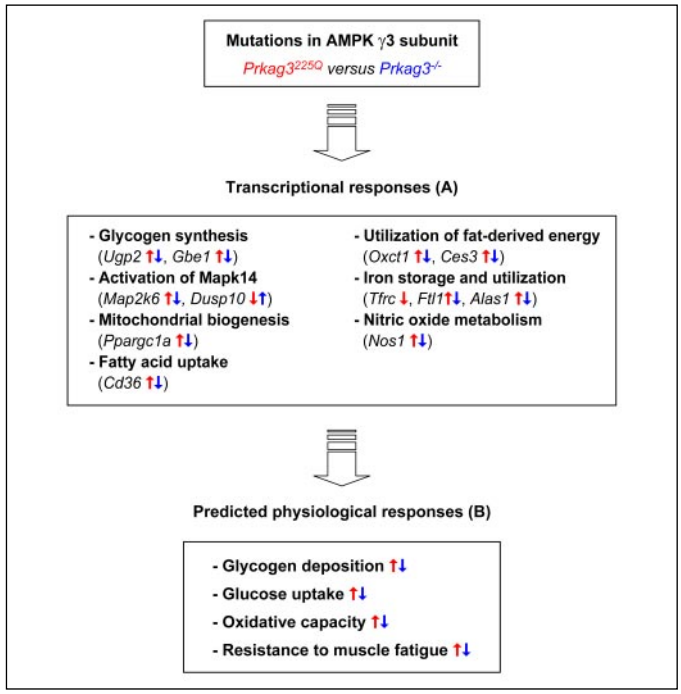

FIGURE 3. **Contrasting transcriptional and physiological responses in the skeletal muscle from AMPK γ3 R225Q transgenic and knock-out mice.** A schematic illustration of the selected transcriptional changes (*A*) in *TgPrkag3*^{225Q} (indicated by *red arrows*) and *Prkag3*$^{-/-}$ mice (indicated by *blue arrows*), compared with wild-type littermates, and the predicted physiological response (*B*). The role of AMPKγ3 in regulation of functions like glycogen deposition, glucose uptake, oxidative metabolism, and muscle fatigue has previously been described (17, 18). The current study provides a transcriptional mechanism for the physiological differences between the genotypes.

One of the most distinct physiological differences between skeletal muscle from AMPK γ3 R225Q transgenic and knock-out mice is the change in glycogen metabolism (17). Glycogen content in skeletal muscle from *TgPrkag3*^{225Q} mice is 2-fold higher under either fed or fasted conditions. Furthermore, skeletal muscle glycogen re-synthesis after exercise is markedly enhanced in *TgPrkag3*^{225Q} mice. In contrast, glycogenesis after exercise was impaired in *Prkag3*$^{-/-}$ mice (17). Glycogen synthase catalyzes a rate-determining step in the glycogen biosynthesis pathway. However, at least in situations where glycogen synthase is activated, the reactions mediated by other enzymes in synthesis pathway can become rate-limiting (46). Interestingly, the microarray data and qRT-PCR analysis revealed a significant up- and down-regulation of *Ugp2* and *Gbe1* mRNAs (encoding two key enzymes in glyocogen synthesis) in skeletal muscle from AMPK γ3 R225Q transgenic and knock-out mice, respectively. In agreement with these results, the protein level and enzyme activity of Ugp2 and Gbe1 is increased in RN pigs carrying an R225Q mutant form of the AMPK γ3-subunit (14, 47). Based on these observations, we hypothesize that the differences in the skeletal muscle glycogen re-synthesis rate displayed by γ3 R225Q transgenic and knock-out mice may be at least partly explained by coordinated differential transcription of *Ugp2* and *Gbe1*.

Activation of AMPK either by physiological stimulation such as muscle contraction or by the pharmacological activator 5-amino-4-imidazole-carboxamide riboside (AICAR) leads to an increase in skeletal muscle glucose transport by promoting Glut4 translocation to the cell surface (48), as well as increasing *Glut4* gene transcription (49). Importantly, the AICAR effect on glucose uptake is completely abolished in AMPK γ3 knock-out mice, suggesting that the γ3-subunit is essential for AICAR-induced glucose transport in skeletal muscle (17). Nonetheless, our microarray data did not detect any difference in the expression of the *Glut4* gene when comparing skeletal muscle from *TgPrkag3*^{225Q} and *Prkag3*$^{-/-}$ mice. Mapk14 (also known as p38 MAPK) has been implicated as a downstream mediator of AMPK signaling to glucose transport in response to AICAR. In this context, it is interesting to note that mRNAs encoding two proteins involved in regulation of the phosphorylation pattern of Mapk14, Map2k6 and Dusp10, were coordinately and reciprocally regulated in AMPK γ3 R225Q and knock-out mice. Another mediator implicated in AMPK-regulated glucose transport is nitric-oxide synthase, Nos (50). The microarray and qRT-PCR analyses revealed the mRNA encoding Nos1, the predominant nitric-oxide synthase isoform in skeletal muscle, was significantly up- and down-regulated by ~2-fold in *TgPrkag3*^{225Q} and *Prkag3*$^{-/-}$ muscle, respectively. Thus, the present study provides the first evidence of *Nos1* being a transcriptional target of AMPK signaling.

Previously, the AMPK γ3-subunit has been shown to influence muscle ergogenics (18). In response to electrically stimulated muscle contraction, isolated EDL muscle from *TgPrkag3*^{225Q} mice is resistant to fatigue. Conversely, skeletal muscle from *Prkag3*$^{-/-}$ mice is fatigue prone (18). The increase in glycogen content in skeletal muscle of *TgPrkag3*^{225Q} mice may have a direct positive effect on muscle performance. Additionally, skeletal muscle is known to adapt to endurance exercise by increasing the oxygen carrying capacity. Our microarray data demonstrate a coordinated up-regulation of genes involved in the storage (*Ftl1*) and use (*Alas1*) of cellular iron in *TgPrkag3*^{225Q} mice, which may indicate improved iron status and correspondingly, increased oxidative capacity in skeletal muscle. Accordingly, the level of skeletal muscle oxygen carrying protein, myoglobin, was increased in *TgPrkag3*^{225Q} mice, compared with the wild-type mice.

Endurance exercise is dependent upon oxidation of fatty acids as a major source of ATP. Two transcription factors implicated in the regulation of fatty acid homeostasis, *Ppargc1a* and *Srebf1*, as well as several genes necessary for the transport (*Cd36*) and utilization (*Ces3* and *Oxct1*) of fatty acids were differentially expressed in skeletal muscle from *TgPrkag3*^{225Q} mice, suggesting increased fatty acid availability and oxidation. In agreement with these observations, elevated reliance of fat-derived energy has been described in γ3 R225Q transgenic mice during exercise, as well as after challenge with high fat diet (17, 18).

We have applied global transcriptome analysis by oligonucleotide microarrays to systematically characterize the role of the AMPK γ3-subunit in the regulation of gene expression. Recently, a number of studies have used qRT-PCR analysis to characterize the role of AMPK complexes in transcription regulation (51, 52). However, gene array approaches allow for concurrent analysis of global mRNA profiles and offer an advantage of reducing bias in data collection, compared with the candidate gene-based approaches. Taken together, our data indicate that a number of genes involved in carbohydrate and fat metabolism, as well as skeletal muscle ergogenics are coordinately and reciprocally regulated in the skeletal muscle from AMPK γ3 R225Q transgenic and knock-out mice and provide a molecular mechanism for the previously described physiological differences between the models. Furthermore, the current study identifies that AMPK γ3-containing complexes play an important role in regulation of novel downstream targets and pathways in skeletal muscle.

AMPK has been identified as an attractive therapeutic target in the treatment of obesity and type II diabetes (53). However, the ideal therapeutic small molecule should modify the activity of AMPK heterotrimers in metabolic tissues such as skeletal muscle to increase fatty acid oxidation and glucose uptake without exerting any effect on organs including central nervous system, heart, pancreas, or lung, where AMPK activation potentially would lead to harmful consequences (54–57). Therefore, therapeutic agents that selectively modulate the activity of γ3-containing AMPK heterotrimers could potentially provide a way to specifically target skeletal muscle and thereby prevent any adverse effects in other organ types. The current study contributes to the understanding of the role of the AMPK γ3-subunit in the regulation of gene transcription and defines several sets of potential biomarkers to characterize the molecular effects in response to the administration of lead substances ideally mimicking the *TgPrkag3*^{225Q} phenotypes in mice and pigs.

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