



Sequencing of PRKAG3 gene revealed several novel amino acid substitutions in the Finnish Yorkshire

Pekka Uimari and Anu Sironen

MTT

Finland

Introduction

PRKAG3



- Protein kinase, AMP-activated, gamma 3 non-catalytic subunit
- On chromosome 15
- AMPK is an enzyme that plays a key role in cellular energy homeostasis including muscle glucose uptake
- AMPK is formed by α , β , and γ subunits with several isoforms
- PRKAG3 encodes the γ 3 subunit of AMPK
- AMPK γ 3 isoform is expressed mainly in white skeletal muscle cells
- Mutations in PRKAG3 (namely 200Q or RN⁻ allele) result in high stored glycogen content in white skeletal muscles
 - Lower muscle pH 24h post mortem
 - Poor water-holding capacity
 - Low processing yield

Introduction

PRKAG3

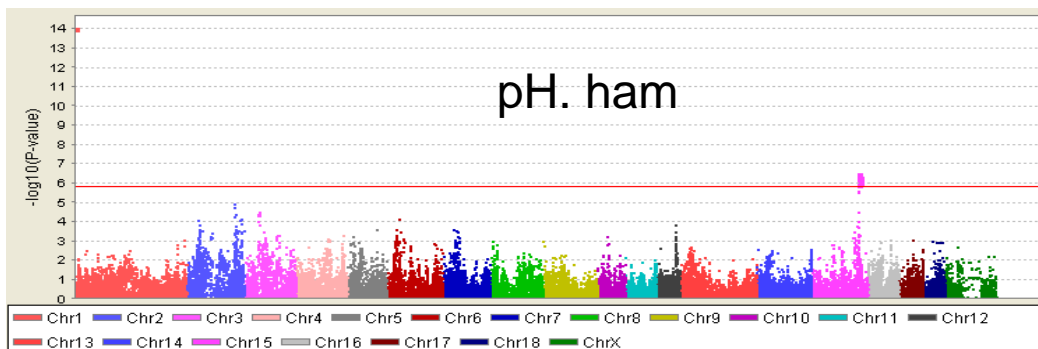
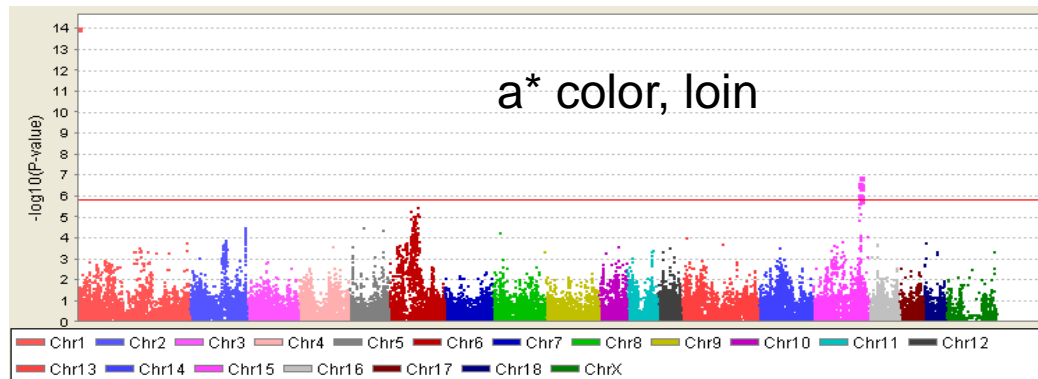
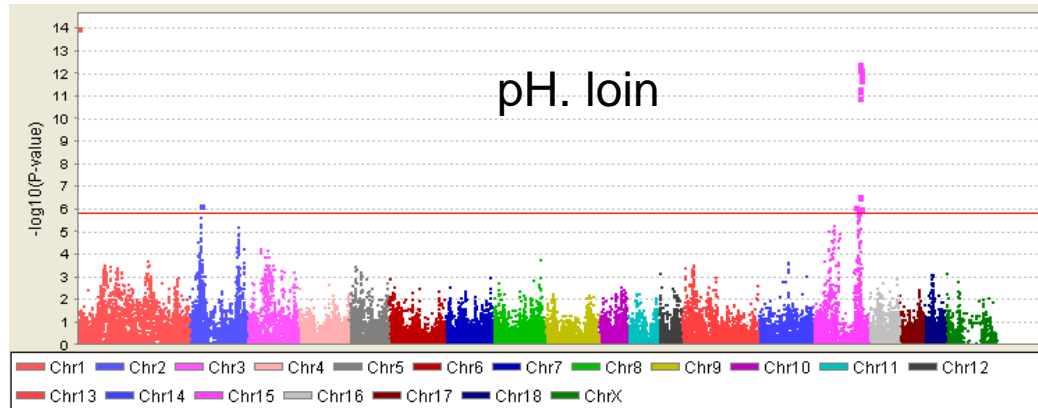


- Commonly known amino acid substitutions in PRKAG3 (Milan et al., 2000; Ciobanu et al., 2001)
 - T30N
 - G52S: L* (Ciobanu et al., 2001)
 - L53P
 - I199V: pH (Ciobanu et al., 2001; Cherel et al., 2010); drip loss (Otto et al., 2007) etc...
 - R200Q
- SNP variation in the promoter region
 - Association with PRKAG3 gene expression and meat quality (Ryan et al., 2012)
 - Association with a particular combination of promoter SNPs and I199V allele (Ryan et al., 2012)

Introduction



Previous findings based on GWA



529 Finnish Yorkshire AI boars (on average 24 progeny) were genotyped with PorcineSNP60 BeadChip

Deregressed EBVs and single SNP genome wide association analysis

Traits analyzed: meat color and pH measured from loin or ham 24h post mortem

Uimari et al., 2013, Journal of Animal Science, 91:2001-2011.

Introduction



Previous findings based on GWA

P-values of the known amino acid substitutions of PRKAG3 and ASGA0070634, Finnish Yorkshire

	Amino acid substitution					
	T30N	G52S	L53P	I199V	R200Q	ASGA 0070634
Loin pH	NA	0.36	NA	0.02	NA	2.18E-10
Loin a*	NA	0.21	NA	0.04	NA	4.43E-04
Loin L*	NA	0.58	NA	0.28	NA	1.03E-04
Ham pH	NA	0.78	NA	0.12	NA	2.45E-04
Ham a*	NA	0.81	NA	0.05	NA	0.02
Ham L*	NA	0.91	NA	0.62	NA	0.35

Material and Methods

Animal material



- AI-boars
- Two breeds (Finnish)
 - Yorkshire (Large White) (total 220)
 - Landrace (total 230)
- Boars were related within breed
- Boars have BLUP-based EBVs for meat quality traits
- Phenotypic measurements were taken from test animals
- Main information comes from sibs and progeny
- Test animals were raised in test-stations from 30 kg to 100 kg of live weight

Material and Methods

Meat quality measurements



- Meat quality measurements were taken 24 h post-mortem using Knick 752 pH meter and an Ingold 406 electrode
- Measurements were taken from loin (longissimus) and ham (semimembranosus) muscles
- Recorded measurements (traits)
 - pH
 - L* color
 - a* color
 - (b* color)

Material and Methods

Sequencing



- Primer pairs for genomic sequence analysis were designed
- DNA fragments were amplified with the gene-specific primers
- PCR amplicons were purified using ExoSAP-IT
- PCR fragments were sequenced in both directions with the same primers as in the amplification procedures
- Sequencing was performed on a 3500xL Genetic Analyzer

Material and Methods

Statistical analysis



- Deregressed EBVs were used as observations (y)
- Relatedness of the boars were taken into account using extended pedigree information in the analysis (polygenic effect, a with a full additive relationship matrix A)
- Each SNP and sequence variant were analyzed separately for both breeds
- Allelic substitution effect of the variants were estimated using the number of minor alleles as a covariant (x)
- $y_i = \mu + b \cdot x_i + a_i + e_i$,
- Substitution effect of the haplotypes were tested with the same model were x indicates the number of haplotypes the boar carries (either 0, 1 or 2)
- DMU variance/covariance estimation package was used

Results

Sequence variants



Exon	Variation	Alleles	Yorkshire	Landrace
	ASGA0070 625	A/G	20.6	27.9
	PRO388	G/A	19.1	13.0
	PRO298	A/G	19.1	12.8
	PRO234	G/C	20.1	26.2
	PRO58	G/A	20.1	25.8
2	K36E	A/G	16.9	20.0
3	T30N	A/ C	1.2	13.2
3	I41V	A/ G	19.5	15.7
3	G52S	A/ G	42.5	11.8
4	L53P	C/ C	0.0	0.0
4	K131R	G/ A	19.4	15.4
4	P134L	T/ C	19.4	15.6
5	193	C/ T	3.2	0.5
5	194	C/ T	3.2	0.5
5	I199V	A/ G	45.2	29.8
5	R200Q	T/ T	0.0	0.0

The well known amino acid substitutions are in bold

In the "Alleles" – column the minor allele is given first

Wild alleles are given in bold (based on the genotypes of the wild boars)

Frequency of the minor allele

Results

Allele effects, Yorkshire



Allele A: -0.059 ± 0.008 (pH “)

Variation	Loin			Ham		
	pH	L*	a*	pH	L*	a*
ASGA00706						
25	7.27E-13	5.52E-06	4.38E-05	3.47E-06	7.28E-01	8.85E-03
PRO388	6.83E-10	4.22E-05	6.28E-04	1.44E-04	7.38E-01	5.13E-02
PRO298	6.83E-10	4.22E-05	6.28E-04	1.44E-04	7.38E-01	5.13E-02
PRO234	7.94E-11	2.79E-05	4.29E-04	2.68E-05	5.62E-01	3.66E-02
PRO58	7.94E-11	2.79E-05	4.29E-04	2.68E-05	5.62E-01	3.66E-02
K36E	5.80E-10	3.83E-05	3.16E-03	7.83E-05	3.78E-01	3.64E-02
T30N	1.32E-01	3.94E-01	4.63E-01	1.29E-01	6.17E-01	3.62E-01
I41V	1.41E-11	1.43E-05	9.20E-05	1.96E-05	8.19E-01	1.64E-02
G52S	3.58E-01	5.80E-01	2.12E-01	7.79E-01	9.11E-01	8.08E-01
L53P	NA	NA	NA	NA	NA	NA
K131R	1.41E-11	1.43E-05	9.20E-05	1.96E-05	8.19E-01	1.64E-02
P134L	1.41E-11	1.43E-05	9.20E-05	1.96E-05	8.19E-01	1.64E-02
193	4.72E-02	1.36E-01	4.54E-01	1.20E-01	4.41E-01	5.03E-01
194	4.72E-02	1.36E-01	4.54E-01	1.20E-01	4.41E-01	5.03E-01
I199V	1.26E-02	1.89E-01	1.17E-01	9.09E-02	9.26E-01	1.04E-01
R200Q	NA	NA	NA	NA	NA	1NA

Results

Allele effects, Landrace



Variation	Loin			Ham		
	pH	L*	a*	pH	L*	a*
ASGA00706						
25	1.51E-02	3.48E-02	3.09E-02	2.17E-02	3.48E-01	7.95E-01
PRO388	2.88E-01	3.59E-01	3.60E-01	6.04E-01	9.32E-01	7.95E-01
PRO298	3.28E-01	6.15E-01	3.66E-01	6.38E-01	9.85E-01	7.71E-01
PRO234	2.18E-02	5.84E-02	3.68E-01	5.23E-02	3.80E-01	8.36E-01
PRO58	2.92E-02	8.62E-02	3.56E-01	6.18E-02	4.13E-01	9.26E-01
K36E	2.33E-01	2.74E-01	1.58E-01	1.51E-01	5.78E-01	5.40E-01
T30N	1.58E-02	6.51E-03	3.24E-01	1.34E-02	1.61E-01	5.05E-01
I41V	4.56E-01	9.20E-01	4.33E-02	6.79E-01	5.32E-01	5.99E-01
G52S	4.11E-01	7.92E-01	8.37E-01	7.76E-01	5.34E-01	6.49E-01
L53P	NA	NA	NA	NA	NA	NA
K131R	4.15E-01	9.25E-01	7.62E-02	5.44E-01	6.02E-01	6.23E-01
P134L	4.31E-01	8.51E-01	3.51E-02	6.95E-01	5.51E-01	6.70E-01
193	3.81E-01	5.04E-01	7.75E-01	3.93E-01	4.46E-01	2.67E-01
194	3.81E-01	5.04E-01	7.75E-01	3.93E-01	4.46E-01	2.67E-01
I199V	1.62E-05	9.29E-02	3.81E-02	6.44E-07	1.71E-01	6.78E-01
R200Q	NA	NA	NA	NA	NA	NA

aa I or allele A: 0.030 ± 0.006 (pH ')

Results

Observed haplotypes



Haplo	1	2	3	4	5	6	7	8	9	10	11	12	13
ASGA007 0625	G	G	G	G	A	A	A	A	A	A	A	A	A
PRO388	A	A	A	A	A	A	A	G	G	G	G	G	G
PRO298	G	G	G	G	G	G	G	A	A	A	A	A	A
PRO234	C	C	C	C	G	G	G	G	G	G	G	G	G
PRO58	A	A	A	A	G	G	G	G	G	G	G	G	G
K36E	G	G	G	G	A	G	G	A	A	A	A	A	A
T30N	C	C	C	C	A	A	A	C	C	C	C	A	A
I41V	G	G	G	G	G	G	G	A	A	A	G	G	G
G52S	A	G	G	A	G	G	G	G	G	G	G	G	G
L53P	C	C	C	C	C	C	C	C	C	C	C	C	C
K131R	A	A	A	A	A	A	A	G	G	G	A	A	A
P134L	C	C	C	C	C	C	C	T	T	T	C	C	C
193	T	T	T	T	T	T	T	T	T	C	C	T	C
194	T	T	T	T	T	T	T	T	T	C	C	T	C
I199V	G	A	G	A	G	A	G	A	G	G	G	G	G
Freq.			WILD										
Yorkshire	0.42	0.36	0.01	0.01	0.00	0.00	0.01	0.08	0.09	0.03	0.00	0.00	0.00
Landrace	0.12	0.25	0.34	0.00	0.12	0.00	0.00	0.04	0.12	0.00	0.00	0.00	0.00

Results

Haplotype effects, Yorkshire



0.039±0.007 (pH ')

Variation	Loin			Ham		
	pH	L*	a*	pH	L*	a*
Haplo 1	4.73E-01	9.00E-01	1.44E-01	7.58E-01	6.91E-01	6.61E-01
Haplo 2	6.35E-08	6.00E-04	1.37E-02	1.72E-04	9.67E-01	1.09E-02
Haplo 3	1.45E-01	5.14E-01	1.67E-01	4.03E-01	2.96E-02	5.98E-03
Haplo 4	4.21E-01	5.75E-02	2.44E-01	8.83E-01	2.28E-01	3.78E-01
Haplo 5	3.22E-02	7.19E-01	3.66E-01	4.99E-01	2.97E-01	8.26E-01
Haplo 7	7.33E-01	4.38E-01	7.83E-01	1.63E-01	8.04E-01	3.23E-01
Haplo 8	2.25E-04	3.24E-03	1.12E-01	3.03E-03	8.18E-01	3.52E-01
Haplo 9	2.29E-04	5.35E-02	4.98E-04	1.25E-01	4.80E-01	7.84E-02
Haplo 10	3.80E-02	1.68E-01	4.98E-01	1.11E-01	3.76E-01	4.15E-01

Results



Haplotype effects, Landrace

0.031±0.007 (pH ')

0.039±0.007 (pH ')

Variation	Loin			Ham		
	pH	L*	a*	pH	L*	a*
Haplo 1	4.13E-01	8.93E-01	9.23E-01	6.47E-01	6.05E-01	5.74E-01
Haplo 2	8.69E-06	1.53E-01	3.99E-03	1.80E-08	3.49E-01	4.09E-01
Haplo 3	4.20E-02	4.12E-01	1.69E-01	1.71E-02	7.37E-01	1.32E-01
Haplo 4	1.81E-02	1.30E-02	2.69E-01	2.40E-02	1.71E-01	5.97E-01
Haplo 5	1.17E-01	4.87E-02	8.33E-01	1.20E-01	8.04E-02	4.84E-01
Haplo 7	7.44E-02	2.32E-01	1.22E-01	1.58E-01	6.31E-01	9.42E-01
Haplo 8	4.50E-01	4.94E-01	8.38E-01	3.51E-01	5.63E-01	3.33E-01
Haplo 9	1.88E-01	7.63E-02	5.72E-01	5.41E-01	4.26E-01	9.83E-01
Haplo 10	4.50E-01	4.94E-01	8.38E-01	3.51E-01	5.63E-01	3.33E-01

Results

Observed haplotypes



Haplo	1	2	3	4	5	6	7	8	9	10	11	12	13
ASGA007 0625	G	G	G	G	A	A	A	A	A	A	A	A	A
PRO388	A	A	A	A	A	A	A	G	G	G	G	G	G
PRO298	G	G	G	G	G	G	G	A	A	A	A	A	A
PRO234	C	C	C	C	G	G	G	G	G	G	G	G	G
PRO58	A	A	A	A	G	G	G	G	G	G	G	G	G
K36E	G	G	G	G	A	G	G	A	A	A	A	A	A
T30N	C	C	C	C	A	A	A	C	C	C	C	A	A
I41V	G	G	G	G	G	G	G	A	A	A	G	G	G
G52S	A	G	G	A	G	G	G	G	G	G	G	G	G
L53P	C	C	C	C	C	C	C	C	C	C	C	C	C
K131R	A	A	A	A	A	A	A	G	G	G	A	A	A
P134L	C	C	C	C	C	C	C	T	T	T	C	C	C
193	T	T	T	T	T	T	T	T	T	C	C	T	C
194	T	T	T	T	T	T	T	T	T	C	C	T	C
I199V	G	A	G	A	G	A	G	A	G	G	G	G	G
Freq.			WILD										
Yorkshire	0.42	0.36	0.01	0.01	0.00	0.00	0.01	0.08	0.09	0.03	0.00	0.00	0.00
Landrace	0.12	0.25	0.34	0.00	0.12	0.00	0.00	0.04	0.12	0.00	0.00	0.00	0.00

Conclusions

- Several new PRKAG3 amino acid substitutions found in Finnish Yorkshire and Landrace
- Strong linkage disequilibrium between the variants
- Previously known I199V does not explain alone the differences in pH (Yorkshire)
- Smallest P-values were obtained for variants located either in the promoter region of PRKAG3 (Yorkshire) or for I199V (Landrace)

Conclusions

- 13 haplotypes were obtained
- In both breeds the same haplotype gave the strongest association with pH
- Hypothesis 1: animals that have the wild type promoter are superior for pH
- Hypothesis 2: animals that have the wild type promoter and 199I (nucleotide A) are superior for pH

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