

## Association analysis of *PPARGC1A* mutations with meat quality parameters in a commercial hybrid pig population

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**ABSTRACT:** *Peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$*  (*PPARGC1A*) is a promising candidate gene for selection on meat and carcass quality traits in the pig industry. In the pig, several SNPs have been reported in both coding and regulatory regions of this gene, some of which were associated with fat characteristics, but none of these associations have been confirmed and many SNPs have not yet been evaluated. Therefore, 18 *PPARGC1A* SNPs were genotyped in 65 slaughter pigs of a commercial hybrid population and used in an association analysis with multiple muscle and carcass traits. Several SNPs located in the 3'UTR and exon 8 and 9 of *PPARGC1A* were significantly ( $P < 0.05$ – $0.001$ ) associated with various carcass composition, tenderness and muscle fibre traits. They could potentially serve as DNA selection markers, if their impact was to be confirmed by a functional analysis.

**Keywords:** association analysis; carcass composition; meat quality; pig; *PPARGC1A*; SNP

Meat quality is of a great economic importance to the pork industry, but it is a complex trait comprising multiple objectively and subjectively assessed parameters which directly or indirectly influence this trait. It consists of several traits such as tenderness, juiciness and flavour (Davoli and Braglia, 2007). Besides environmental factors, the genetic composition of the animal (strongly) influences many meat quality parameters, which makes identification of the genes involved crucial (Cameron, 1990; Rohrer and Keele, 1998).

*Peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$*  (*PPARGC1A*) is a particularly interesting

candidate gene for meat and carcass quality traits, for several reasons. It is an important transcriptional coactivator with a vital role in energy and fat metabolism, and is involved in adipogenesis, muscle fibre type formation, mitochondrial biogenesis/respiration and adaptive thermogenesis (Puigserver et al., 1998; Wu et al., 1999; Medina-Gomez et al., 2007). *PPARGC1A* has a strong impact on body weight composition and regulation, and is expected to have a significant influence on important meat quality characteristics like fat distribution, intramuscular fat (IMF) content and muscle fibre type composition (Dulloo and Samec, 2001; Lin et al., 2002). Furthermore, porcine

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*PPARGC1A* is the only gene so far that has been mapped to the QTL-region for leaf fat weight and backfat on chromosome 8p21 (Rohrer and Keele, 1998; Jacobs et al., 2006). *PPARGC1A* gene expression was shown to vary between and within porcine backfat and *longissimus dorsi* muscle (*MLD*), which are tissues with different demands regarding technological and eating quality (Erkens et al., 2006).

Different single nucleotide polymorphisms (SNPs), in both coding and regulatory regions of *PPARGC1A*, have been independently associated with several fat characteristics in pigs, with milk fat yield in cattle and with obesity/type II diabetes in humans (Ek et al., 2001; Weikard et al., 2005; Jacobs et al., 2006; Vimalaswaran et al., 2006; Stachowiak et al., 2007). However, the associations found in pigs were only breed specific and could not be confirmed in other pig populations. Furthermore, several SNPs in regulatory and coding regions of porcine *PPARGC1A* have not yet been evaluated in an association study (Jacobs et al., 2006; Erkens et al., 2009). To determine the possible use of all these porcine SNPs as selection markers for meat or carcass quality, 18 *PPARGC1A* SNPs were geno-

typed in a commercial hybrid pig population and evaluated in an association analysis with multiple muscle and carcass traits.

## MATERIAL AND METHODS

### Animals

The pigs used in the association analysis were the offspring of a cross between 11 Pietrain commercial hybrid sows (crossbred of a Pietrain sire line and a commercial three-way cross of Landrace and Large White) and a sire of a synthetic dam line based on Landrace and Large White (Rattlerow-Seghers, Lokeren, Belgium). The sows were heterozygous for a g.1843C>T mutation in *ryanodine receptor 1* (*RYR1*; Fujii et al., 1991) and the sire was heterozygous for a g.3072G>A mutation in *insulin-like growth factor 2* (*IGF2*; Van Laere et al., 2003). A total of 65 animals (barrows and gilts) were used in the experiment, and consisted of complete litters and randomly selected piglets from incomplete litters (3–14 piglets were used

Table 1. Primers used for genotyping of *PPARGC1A* mutations<sup>a</sup>

Primer name	Primer sequence (5'→3')	Detected SNP	Amplicon length	Ta <sup>b</sup>	Location
PGC1A+1	TCTTTGTGGGGAGTGCAGAGT	SNP1–4	1 039 bp	61°C	promoter region
PGC1A-1	CTTTGCCCAGATCAGCTTAGTTTC				
PGC1A+2	TGCCCCGTTCTCTGCTTT	SNP5	563 bp	64°C	
PGC1A-2	AACCAGCCCCTTACTGAGAGTG				
PGC1A+3	GAAGCCAGTATTTCTTTCCCTTG	SNP7–8	1 302 bp	57°C	3'UTR
PGC1A-3	GCACAGCTTACATTTTGATGG				
PGC1A+4	ATCCTTCCAGTGCAACAAAA	SNP9–11	489 bp	60°C	exon 8
PGC1A-4	TCATACAATGAATAAAACCACAACA				
PGC1A+5	CCTCCTCATAAAGCCAACCA	SNP12, 14–18	728 bp	64°C	
PGC1A-5	GAAGAACAAGAAGGCGACACA				
PGC1A+6	ACGGTCACTGGGAAACCTC	SNP13	554 bp	59°C	exon 9
PGC1A-6	GAAACCCCTCCTGTAAAACGAAA				

<sup>a</sup>primers 1 and 2 were based on conserved regions of a consensus sequence consisting of human ENSG00000109819 and AY382576, bovine ENSBTAG00000017024, rat ENSRNOG00000004473 and AY382577, murine ENSMUSG000000029167, and porcine AY484494. Primers 3 and 4 were based on a consensus sequence consisting of porcine AB106108, AY346132 and NM\_213963, and human NM\_013261

<sup>b</sup>Ta = annealing temperature

per litter). Pigs were slaughtered at a target live weight of 110 kg. All procedures concerning feeding, housing, slaughtering and the measurement of all muscle and carcass parameters were performed as described in Van den Maagdenberg et al. (2008a,b).

### DNA isolation, primers and sequencing

Genomic DNA was isolated from tissue samples with the GenElute Mammalian Genomic DNA

Miniprep Kit (Sigma-Aldrich) according to the manufacturer's protocol.

Primers PGC1A+/-1, 2, 3 and 4 were used from Erkens et al. (2009), primers PGC1A+/-5 and 6 were used from Jacobs et al. (2006; Table 1). All PCRs were performed with FastStart Taq DNA Polymerase (Roche) and included a no-template control. Genotyping was conducted by direct sequencing of the amplicons of the primer pairs listed in Table 1, on an Applied Biosystems 3730xl DNA Analyser with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), according to the instructions manual.

Table 2. Genotypes of the samples used in the association analysis

	Mutation <sup>a</sup>	Amino acid change	Genotype		
SNP1	g.237C>G		65 C/C		
SNP2	g.471G>A		65 G/G		
	g.479G>A		65 G/G		
SNP3	g.654A>G		28 A/A	36 A/G	1 G/G
SNP4	g.698C>G		28 C/C	36 C/G	1 G/G
SNP5	g.1487G>A		28 G/G	36 G/A	1 A/A
	g.2885C>T				
	g.2886T>C				
	g.2888C>T				
	g.2889T>C				
SNP6 <sup>b</sup>	g.2895C>T		–	–	–
SNP7	g.4844T>C		53 T/T	12 T/C	
SNP8	g.5018C>T		12 C/C	33 C/T	20 T/T
SNP9	g.5452G>T		57 G/G	8 G/T	
SNP10	g.5460C>T		53 C/C	12 C/T	
SNP11	g.5842T>G		53 T/T	12 T/G	
SNP12	g.678T>A	Cys430Ser	47 T/T	17 T/A	1 A/A
SNP13	g.1747C>A	Pro615Thr	59 C/C	6 C/A	
SNP14	g.330C>G	Leu314Val	65 C/C		
SNP15	g.857C>T	Asn489Asn	57 C/C	8 C/T	
SNP16	g.495C>A	Arg369Arg	21 C/C	33 C/A	11 A/A
SNP17	g.404C>G	His338Gln	65 C/C		
SNP18	g.467G>A	Thr359Thr	65 G/G		

<sup>a</sup>location number of SNP1–11 is based on the position in the *PPARGC1A* promoter region and 3'UTR sequence of GenBank accession number (acc. No.) EU088376; location number of SNP12–18 is based on the position in the *PPARGC1A* coding sequence acc. No. AY484500

<sup>b</sup>SNP6 was not genotyped in this study, because it was shown to be in complete linkage disequilibrium with SNP7, 10 and 11 in a previous report (Erkens et al., 2009)

Table 3. Results of the association analysis with animal and carcass traits

	Mean	SD <sup>f</sup>	SNP	P	Least square mean per genotype <sup>g</sup>	
Birth weight (kg)	1.53	0.29	9	0.003	G/G (57): 1.49	G/T (8): 1.81
Live weight at slaughter (kg)	108.40	7.06	7	0.018	T/T (47): 107.20	T/C (12): 112.90
ADG (g/day) <sup>a</sup>	584.80	55.18	7	0.000	T/T (47): 573.80	T/C (12): 632.30
Carcass length (cm)	84.87	2.48	9	0.002	G/G (57): 84.50	G/T (8): 87.39
Muscle thickness (mm) <sup>b</sup>	58.49	7.00	13	0.023	C/C (56): 58.12	C/A (6): 64.30
Lean meat (%) <sup>c</sup>	54.35	6.02	8	0.052	T/T (20): 52.76	T/C (33): 55.91
ADLMG (g/day) <sup>a</sup>	252.60	33.08	7	0.057	T/T (53): 248.90	T/C (12): 269.40
Loin <i>MLD</i> (%) <sup>c</sup>	2.91	0.34	13	0.002	C/C (53): 2.88	C/A (5): 3.23
Right ham width (mm)	184.90	7.64	13	0.052	C/C (59): 184.70	C/A (6): 190.70
Left ham width (mm)	185.20	9.43	13	0.001	C/C (59): 184.70	C/A (6): 195.30
Left ham angle	49.49	7.06	12	0.048	T/T (43): 50.76	T/A (15): 46.34
Backfat thickness 7 <sup>th</sup> rib (mm)	30.01	6.36	13	0.008	C/C (59): 30.25	C/A (6): 24.50
Backfat thickness LV (mm) <sup>d</sup>	28.19	5.98	13	0.042	C/C (59): 28.47	C/A (6): 23.82
Ham (%) <sup>c</sup>	21.33	1.08	13	0.035	C/C (58): 21.34	C/A (6): 22.10
Shoulder (%) <sup>c</sup>	16.28	0.99	5	0.018	G/G (28): 15.92	G/A (35): 16.52
			12	0.018	T/T (47): 16.11	T/A (16): 16.83
Belly (%) <sup>c</sup>	16.68	1.14	16	0.000	C/C (20): 17.03 <sup>x</sup>	C/A (33): 16.05 <sup>y</sup>
			12	0.002	T/T (47): 16.89	T/A (17): 15.93
Loin (%) <sup>c</sup>	17.25	1.30	13	0.000	C/C (59): 16.81	C/A (6): 15.11
Area <i>MLD</i> (cm <sup>2</sup> ) <sup>e</sup>	54.27	7.36	9	0.023	G/G (51): 17.02	G/T (8): 18.16
			9	0.051	G/G (57): 53.89	G/T (8): 58.42
			12	0.050	T/T (47): 53.66	T/A (17): 56.95
			13	0.000	C/C (59): 53.61	C/A (6): 62.87

<sup>a</sup>ADG = average daily gain; ADLMG = average daily carcass lean meat growth; <sup>b</sup>muscle thickness measured with the CGM apparatus between the third and fourth last rib; <sup>c</sup>cut according to a commercial Belgian protocol and expressed as percentage relative to carcass weight; <sup>d</sup>backfat thickness recorded at the loin vertebra; <sup>e</sup>cross-sectional area of the *MLD*; <sup>f</sup>SD = standard deviation; <sup>g</sup>number in parenthesis indicates the number of the respective genotype used in the analysis; <sup>x,y</sup>means with different superscripts are significantly different at  $P < 0.005$

Table 4. Results of the association analysis with meat quality traits in *MLD* and *MTB*

	Mean	SD <sup>b</sup>	SNP	P	Least square mean per genotype <sup>c</sup>		
Drip losses (%)							
<i>MLD</i>	4.00	1.83	13	0.017	C/C (59): 3.91	C/A (6): 5.41	
<i>MTB</i>	1.05	0.38	5	0.050	G/G (28): 1.14	G/A (36): 0.96	
Thawing losses (%)							
<i>MLD</i>	11.44	3.55	12	0.001	T/T (47): 10.55	T/A (17): 13.76	
<i>MTB</i>	27.31	2.70	9	0.010	G/G (57): 27.66	G/T (8): 25.72	
Cooking losses (%)							
<i>MLD</i>	48.46	24.18	5	0.057	G/G (28): 54.55	G/A (36): 45.53	
<i>MLD</i>	32.45	4.98	13	0.026	C/C (59): 32.89	C/A (6): 28.11	
<i>MTB</i>	31.91	4.29	16	0.038	C/C (21): 30.07 <sup>x</sup>	C/A (33): 33.10 <sup>y</sup>	A/A (11): 31.77 <sup>x,y</sup>
Shear force raw (N)							
<i>MLD</i>	18.24	3.51	13	0.002	C/C (59): 18.65	C/A (6): 14.12	
<i>MTB</i>	31.03	4.47	16	0.043	C/C (21): 29.12 <sup>x</sup>	C/A (33): 32.19 <sup>y</sup>	A/A (11): 31.08 <sup>x,y</sup>
First peak shear force (N)							
<i>MLD</i>	276.30	38.08	13	0.013	C/C (59): 280.10	C/A (6): 239.50	
<i>MTB</i>	245.30	37.24	7	0.013	T/T (53): 240.10	T/C (12): 268.80	
Total work (N.mm)							
<i>MLD</i>	159.40	30.34	13	0.044	C/C (59): 161.80	C/A (6): 135.30	
<i>MTB</i>	156.40	26.48	16	0.042	C/C (21): 144.80 <sup>x</sup>	C/A (33): 163.30 <sup>y</sup>	A/A (11): 156.70 <sup>x,y</sup>
Collagen work (N.mm)							
<i>MLD</i>	116.90	14.66	13	0.025	C/C (59): 118.30	C/A (6): 104.20	
<i>MTB</i>	88.88	17.15	7	0.009	T/T (53): 86.59	T/C (12): 100.20	
			9	0.005	G/G (57): 91.26	G/T (8): 74.05	

<sup>a</sup>FPM = water-holding capacity measured with the filter paper method<sup>b</sup>SD = standard deviation<sup>c</sup>number in parenthesis indicates the number of the respective genotype used in the analysis<sup>x,y</sup> means with different superscripts are significantly different at  $P < 0.05$

## Data analysis

Muscle and meat quality traits were analysed using a univariate general linear model with the respective SNP (2 or 3 genotypes depending on the SNP), gender (castrated males and females) and *RYR1* genotype (*N/n* or *N/N*; Fujii et al., 1991; De Smet et al., 1996) as fixed factors. For the analysis of carcass traits, the *IGF2* genotype (positive or negative) was also included as a fixed factor because a mutation in this gene is known to affect carcass composition but not meat quality (Van den Maagdenberg et al., 2008a). Two-way interactions between the respective fixed factors were evaluated during preliminary analyses, but these were omitted from the model because they were not mostly significant. In the case of a significant SNP effect, the Bonferroni post hoc test was performed to separate the means. For both SNP5 and SNP12 the A/A genotype was detected only once, and this observation was omitted from the calculations yielding 2 genotypes for these SNPs. All analyses were performed with SPSS 15.0 for Windows (SPSS Inc.).

## RESULTS

### Genotyping

The genotypes of the 65 samples are listed in Table 2. For SNP1, 2, 14, 17 and 18 only 1 allele was detected, and therefore it was not possible to evaluate their phenotypic effect in the association analysis. For several SNPs only 2 out of 3 possible genotypes were present in the population (SNP7, 9 and 13) or a genotype was only observed once (SNP3, 4, 5 and 12). Some SNPs were linked with each other, resulting in an identical outcome of the association analysis (SNP3, 4 and 5; SNP7, 10 and 11; SNP9 and 15). Therefore, only 1 of these respective SNPs is mentioned in the results tables. Because SNP6 was shown to be in complete linkage disequilibrium with SNP7, 10 and 11 in a previous report (Erkens et al., 2009), it was not genotyped in this study. It was included in Table 2 to maintain the same SNP numbering order as in the previous report.

### Association analysis

The results of the association analysis with animal and carcass traits are presented in Table 3. No sig-

Table 5. Results of the association analysis with muscle fibre characteristics in *MLD*

	Mean	SD <sup>c</sup>	SNP	P	Least square mean per genotype <sup>d</sup>			
					T/T (47):	T/A (17):	T/C (28):	C/C (10):
Type IIb muscle fibre (%)	75.56	3.76	12	0.032	76.32	74.14	74.14	
CSA (cm <sup>2</sup> ) <sup>a</sup>	4 300.00	814.30	8	0.057	4 338.00	4 479.00	4 479.00	3 820
Cytoplasm/nucleus (cm <sup>2</sup> ) <sup>b</sup>	2 213.00	483.10	8	0.012	2 142.00 <sup>x,y</sup>	2 373.00 <sup>x</sup>	2 373.00 <sup>x</sup>	1 868 <sup>y</sup>
			9	0.058	2 167.00	G/T (6):	2 574.00	

<sup>a</sup>CSA = mean fibre cross-sectional area

<sup>b</sup>amount of cytoplasm per nucleus

<sup>c</sup>SD = standard deviation

<sup>d</sup>number in parenthesis indicates the number of the respective genotype used in the analysis

<sup>x,y</sup>means with different superscripts are significantly different at  $P < 0.05$

nificant association was found for carcass weight, carcass yield, backfat thickness at the first rib, fat thickness between the third and fourth last rib, or right ham angle. A distinct difference in growth rate (average daily gain: 574–632 g/day;  $P < 0.001$ ) and live weight at slaughter (107–113 kg;  $P = 0.018$ ) was detected between the T/T and T/C genotype of SNP7, but no significant effect on any of the other carcass parameters was found. For SNP9, the mean carcass length was 2.89 cm longer for the G/T compared to the G/G genotype ( $P = 0.002$ ), accompanied by a higher loin percentage ( $P = 0.023$ ) and a borderline significant association with the cross-sectional area of the *MLD* ( $P = 0.051$ ). Significant associations ( $P < 0.05$ ) with the proportion of several carcass cuts were also found for several other SNPs. Especially for SNP13 some very significant associations were detected with the loin percentage and cross-sectional area of the *MLD* ( $P < 0.005$ ). In addition, backfat thickness at the 7<sup>th</sup> rib ( $P = 0.008$ ) and loin vertebra ( $P = 0.042$ ) was significantly reduced in the C/A (24.50 and 23.82 mm, respectively) compared to the C/C genotype (30.25 and 28.47 mm, respectively) of this SNP.

Data of the association analysis with meat quality characteristics of *MLD* and *triceps brachii* muscle (*MTB*) are presented in Table 4. Muscle fibre characteristics were determined only for *MLD*, and significant associations ( $P < 0.05$ ) were detected with the amount of cytoplasm per nucleus and the % of type IIB muscle fibres (Table 5). No significant associations were observed with other types of muscle fibre (Ia, IIa, IIX), the oxidative/glycolytic fibre ratio, the number of nuclei per cell, or energetic enzyme activity (lactate dehydrogenase, citrate synthase,  $\beta$ -hydroxyacyl-CoA dehydrogenase;  $P > 0.1$ ). In both muscles, significant associations of several SNPs were detected with various water-holding characteristics ( $P < 0.05$ ). For *MLD*, only SNP13 showed significant associations ( $P < 0.05$ ) with meat tenderness characteristics (shear force and work). In *MTB* on the other hand, several SNPs were significantly ( $P < 0.05$ ) associated with tenderness characteristics. No significant associations were found with various colour parameters (CIELAB  $L^*/a^*/b^*$ , or Japanese pork colour scale) described in Van den Maagdenberg et al. (2008a).

## DISCUSSION

Genotyping showed that for some SNPs of *PPARGC1A* no variation was detected in the stud-

ied hybrid pig population (Table 2), which is in accordance with previous reports (Jacobs et al., 2006; Erkens et al., 2009). Hence, even if they had an impact on meat quality, their practical use in pig selection would be questionable because of the extremely low prevalence.

There was a significantly higher average daily gain and live weight at slaughter in T/C compared to T/T pigs for SNP7, but no effect on lean meat percentage or other carcass cuts was observed. This suggests that this SNP induces faster growth without affecting carcass composition, which is possibly of great economic interest. Because of its central and vital role in energy metabolism, it is likely that *PPARGC1A* has an influence on growth rate, and SNP7 is located in the important 3'UTR regulatory region and could possibly affect its expression (Handschin and Spiegelman, 2006). However, the C allele was also associated with less tender meat in *MTB* and a larger contribution of the collagen component to this lower tenderness. Further evaluation and the analysis of the homozygous C genotype will have to prove whether or not this SNP can be used as a selection marker.

The mean carcass length was increased by 2.9 cm in the G/T genotype of SNP9, which might be related to the higher loin percentage, although no effect on the percentage of loin *MLD* in respect to carcass weight was detected. In addition, a significantly higher birth weight was associated with this same genotype, suggesting an influence on prenatal growth rate. SNP9 (located in the 3'UTR) was linked with SNP15 (a silent mutation located in exon 8). Although SNP15 does not change the amino acid (AA) sequence, it could have some kind of influence on the mRNA level, and it is therefore impossible to determine whether only 1 or both these SNPs have an effect on *PPARGC1A* expression.

The SNP12 mutation results in a Cys430Ser AA change in the myocyte enhancer factor 2C (MEF2C) binding domain of the *PPARGC1A* protein (Wu et al., 1999). MEF2C is involved in the induction of proteins typical of oxidative muscle fibres (Lin et al., 2002). The significantly lower percentage of type IIB glycolytic muscle fibres in the T/A compared to the T/T genotype suggests that the AA change could have a positive impact on *PPARGC1A* protein stability and/or MEF2C binding and functioning. A breed specific association with feed conversion has been previously reported for this SNP, while no relation with carcass composition was found in another study (Jacobs et al., 2006; Stachowiak et

al., 2007). Our results showed significant associations with a few carcass composition parameters, but no consistent conclusions could be drawn from these data.

SNP13 was (very) significantly associated with multiple carcass composition and muscle parameters, which is in correspondence with previous findings in a Meishan research population where it was associated with average backfat depth, belly weight and leaf fat weight (Jacobs et al., 2006). However, our results have to be evaluated with caution because only a limited number of pigs possessed the A allele. An interesting finding is that the C/A genotype was associated with a higher percentage and increased cross-sectional area of loin *MLD*, while several backfat thickness parameters were significantly reduced. Although no significant association with lean meat percentage was observed, this percentage appeared to be higher in the C/A animals, as would be expected. At the same time a significant positive association was found with several tenderness parameters in *MLD*. These data suggested that this genotype had a positive impact on meat quality without negatively influencing carcass composition. Fewer associations were found in the more oxidative *MTB*, which is possibly related to their difference in fibre type composition and *PPARGC1A* expression. SNP13 is located in exon 9 and results in a Pro615Thr AA change in the RNA processing domain of the *PPARGC1A* protein. This region also contributes to the coactivation function of *PPARGC1A* on several transcription factors (Knutti et al., 2000; Handschin and Spiegelman, 2006). It can therefore be expected that SNP13 has an important effect on *PPARGC1A* functioning and possibly influences meat quality in this way.

Only a small effect on shoulder percentage and a few tenderness parameters was observed for SNP16. Another study reported a breed specific association with backfat, but our results could not confirm this (Stachowiak et al., 2007). SNP16 is a silent mutation located in exon 8 and is not therefore expected to have a major impact on *PPARGC1A* expression or functioning, although it could have an influence on the mRNA level.

## CONCLUSIONS

In conclusion, the results from the association analysis indicate that several SNPs in both regulatory and coding regions of the *PPARGC1A* gene

are significantly associated with various muscle, carcass and meat quality traits, and could potentially serve as DNA selection markers. However, the prevalence of some SNPs was low or only 2 out of 3 possible genotypes could be evaluated. It is therefore necessary to verify these results in other and larger populations and to exclude possible accompanying unwanted effects. Their impact should also be confirmed by a functional analysis.

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