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RUNNING HEAD: Nonsense mutation in the pig *ASS1* gene

**Detection of homozygous genotypes for a putatively lethal
recessive mutation in the porcine argininosuccinate synthase 1
(*ASS1*) gene**

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Summary

The sequencing of the pig genome revealed the existence of homozygous individuals for a nonsense mutation in the argininosuccinate synthase 1 (*ASS1*) gene (rs81212146, c.944T>A, L315X). Paradoxically, an AA homozygous genotype for this polymorphism is expected to abolish the function of the *ASS1* enzyme that participates in the urea cycle, leading to citrullinemia, hyperammonemia, coma and death. Sequencing of five Duroc boars that sired a population of 350 Duroc barrows revealed the segregation of the c.944T>A polymorphism, so we aimed to investigate its phenotypic consequences. Genotyping of this mutation in the 350 Duroc barrows revealed the existence of 7 individuals homozygous (AA) for the nonsense mutation. These AA pigs had a normal weight despite the fact that mild citrullinemia often involves an impaired growth. Sequencing of the region surrounding the mutation in TT, TA and AA individuals revealed that the A substitution in the second position of the codon (c.944T>A) is in complete linkage disequilibrium with a C replacement (c.943T>C) in the first position of the codon. This second mutation would compensate the potentially damaging effect of the c.944T>A replacement. In fact, this is the most probable reason why pigs with homozygous AA genotypes at the 944 site of the *ASS1* coding region are alive. Our results illustrate the complexities of predicting the consequences of nonsense mutations on gene function and phenotypes, not only because of annotation issues but also due to the existence of genetic mechanisms that sometimes limit the penetrance of highly harmful mutations.

Keywords: pig, single nucleotide polymorphism, nonsense mutation, citrullinemia, premature stop codon.

The sequencing of the pig genome led to the discovery of 157 nonsense mutations mapping to 142 genes, and 11 of them were reported to have pathological effects in humans (Groenen *et al.* 2012). Although most of these 11 damaging nonsense variants were found in a heterozygous state, two mutations mapping to the argininosuccinate synthase 1 (*ASS1*, rs81212146, c.944T>A, L315X) and to the RBBP8 binding protein 8 endonuclease (*RBBP8*) genes displayed homozygous genotypes. The inactivation of the *RBBP8* gene causes embryonic lethality (Polato *et al.* 2014), but it should be noticed that the current release of the Ensembl database (<https://www.ensembl.org>) does not report any stop gained mutation in the porcine *RBBP8* gene. With regard to the *ASS1* enzyme, its inactivation leads to the disruption of the urea cycle and to citrullinemia, a disease characterized by increased ammonia levels in blood, stupor, convulsions, coma and death (Endo *et al.* 2004). Groenen *et al.* (2012) argued that homozygosity for the nonsense *ASS1* mutation might be associated with a milder form of citrullinemia. However, the mild course of this disease is usually, but not always, explained by mutations causing only a partial abolishment of the function of the *ASS1* enzyme (Häberle *et al.* 2003). In some cases, the mild form of the disease involves the development of symptoms such as poor growth, liver failure, cerebral infarction, or spasticity, while in other occasions patients remain asymptomatic (Häberle *et al.* 2003).

Whole-genome sequencing (our unpublished data) of the five Duroc boars that sired a purebred population of 350 Duroc barrows (Gallardo *et al.* 2008, 2009) revealed the segregation of the rs81212146 *ASS1* nonsense polymorphism, providing an opportunity to investigate its phenotypic effects. Indeed, the consequences of this polymorphism were just predicted by NCBI automated computational analysis, but no experiment has been previously made to assess the accuracy of such prediction. By

using a QuantStudio 12K flex Real-Time PCR System available at the Servei Veterinari de Genètica Molecular at the Universitat Autònoma de Barcelona (<http://sct.uab.cat/svgm/en>), we genotyped the 350 offspring of the five boars with a dedicated TaqMan Open Array multiplex assay. In total, 323 pigs were successfully genotyped for the rs81212146 polymorphism, which led to the identification of 239 TT, 77 TA and 7 AA pigs, hence confirming the existence of homozygous individuals for this mutation in the population under study. Since one of the potential symptoms of mild citrullinemia is retarded growth, we inspected the final weight of the AA pigs compared with their TT and TA counterparts. Live weights were measured before slaughtering and carcass weights were also collected after evisceration at the abattoir. The average live weights at 190 days of TT, TA and AA pigs were 122.55 ± 12.18 kg, 121.26 ± 16.66 kg and 119.92 ± 21.91 kg respectively. Moreover, carcass weights of TT, TA and AA pigs were 94.47 ± 10.18 kg, 95.09 ± 11.78 kg and 93.67 ± 11.67 kg, respectively (**Supplementary Figure 1**). An analysis of variance (ANOVA) performed with the *aov* R function and contrasting *ASS1* genotypic means for both live (P -value = 0.724) and carcass (P -value = 0.893) weights did not reveal any significant difference. In summary, we did not find evidence of a significantly decreased weight, before or after slaughter, in AA pigs.

In order to further investigate the potential consequences of the rs81212146 polymorphism, we sequenced the region of the *ASS1* gene containing the putative nonsense mutation by making use of both genomic DNA and complementary DNA (cDNA) as templates. A total of sixteen liver samples belonging to each of five TT and AA and six TA animals were selected at random. Genomic DNA extraction was performed by digestion of 30 mg of liver tissue in 0.5 mL lysis buffer (50 mM Tris-HCl, pH = 8; 20 mM EDTA, pH = 8; 2% SDS) plus 15 μ L 1 μ g/ μ L proteinase K and

incubated overnight at 56 °C. Subsequently, 500 µL of the lysate were deproteinized with 0.5 mL of a mixture of phenol: chloroform: isoamyl alcohol (25:24:1). The resulting supernatant was mixed with 1 mL ice-cold pure ethanol plus 50 µL NaCl (2 M) and centrifuged for 30 minutes at maximum speed. The DNA pellets obtained in this way were washed with 500 µL of ethanol 70% and resuspended in 50 µL of ultrapure water. We also extracted RNA from the same selected liver samples corresponding to TT, AA and TA pigs. In brief, liver samples were pulverized in liquid nitrogen with a mortar and a pestle and subsequently submerged and homogenized in 1 mL of TRI Reagent (Thermo Fisher Scientific, Barcelona, Spain). Total RNA was then purified with the RiboPure kit (Ambion, Austin, TX) in accordance with the instructions of the manufacturer. The concentration and purity of DNA and RNA samples were assessed with a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Barcelona, Spain). A Bioanalyzer-2100 equipment (Agilent Technologies Inc., Santa Clara, CA) was employed for determining RNA integrity (RIN) with the Agilent RNA 6000 Nano Kit (Agilent Technologies, Inc., Santa Clara, CA). All RNA samples had RIN values > 7. The average RIN values of RNA preparations corresponding to TT, TA and AA pigs were 7.46, 7.24 and 7.52, respectively. Reverse transcription (RT) was carried out with the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Barcelona, Spain). Each reverse transcription reaction contained 2 µL 10 × RT Buffer, 0.8 µL 25 × dNTP Mix (100 mM), 2 µL 10 × RT Random Primers, 1 µL MultiScribe Reverse Transcriptase (50 U/µL) and 10 µL total RNA (~ 100 ng/ µL). Ultrapure water was added until reaching a final volume of 20 µL. The RT thermal profile included an incubation step at 25 °C for 10 minutes, followed by 120 minutes at 37 °C and an inactivation step at 95 °C for 5 minutes.

Genomic DNA and cDNA samples were then subjected to polymerase chain reaction (PCR) amplification. Primers (**Supplementary Table 1**) were designed with the Primer3 software (Untergasser *et al.* 2012) to span contiguous exon-intron and exon-exon junctions for genomic DNA and cDNA amplicons, respectively. Expected sizes were 278 bp and 221 bp for PCR products amplified from genomic DNA and cDNA templates, respectively. The relative position of genotyped rs81212146 polymorphism in genomic and cDNA amplicons is depicted in **Supplementary Figure 2**. Amplification reactions contained 2 μ L of 10 $\times$ PCR buffer, 0.2 μ L dNTPs (25 mM), 0.6 μ L of each primer (10 μ M), 2 μ L of MgCl₂ (25 mM), 2.5 μ L of genomic DNA (10 ng/ μ L) or 2.5 μ L of a 5-fold dilution of the RT-reaction, and 0.2 μ L AMPLITAQ GOLD DNA Polymerase (5 U/ μ L) (Thermo Fisher Scientific, Barcelona, Spain). Ultrapure water was added until reaching a 20 μ L final volume. The thermal profile included a denaturation step at 95 °C for 10 minutes, followed by 35 cycles of denaturation at 95 °C for 1 minute, annealing at 60 °C for 1 minute and extension at 72 °C for 1 minute, plus a final extension step at 72 °C for 7 minutes. Amplicons with the expected size were purified with the ExoSAP-IT PCR Clean-up kit (Thermo Fisher Scientific, Barcelona, Spain). They were subsequently sequenced with the BigDye Terminator Cycle Sequencing Kit v1.1 (Applied Biosystems, Foster City, CA) and primers listed in **Supplementary Table 1**. Sequencing reactions were electrophoresed in an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA). The Mega software v.6.0 (Tamura *et al.* 2013) was employed to visualize the results of the sequencing experiment. Partial *ASS1* sequences obtained from genomic DNA (accession numbers: MN296492-MN296493) and cDNA (accession numbers: MN296494-MN296495) were submitted to Genbank database.

The predicted consequence of the replacement of T by A at the second position of codon 315 would be the introduction of a premature stop codon (TTG>TAG) completely abolishing the function of the ASS1 enzyme. However, sequencing of ASS1 DNA and cDNA amplicons revealed that the A allele in the second position of the codon is linked to a C replacement (rs81212145, c.943T>C) in the first position of the codon, both at genomic and transcriptomic level (**Figure 2, Supplementary Figure 3**), leading to the generation of a benign missense mutation. As revealed by PolyPhen-2 algorithm (Adzhubei *et al.* 2010) the substitution of leucine (TTG) by glutamine (CAG) is predicted to be tolerated (PolyPhen-2 score = 0.012). Moreover, this second mutation is expected to compensate the potentially damaging effect of the c.944T>A replacement. Indeed, homozygosity for the TAG codon at position 315 should be lethal in pigs, and in consequence, it might have been strongly selected against. Interestingly, all sequenced animals displaying an AA genotype for the second position of codon 315 were also homozygous CC for the first position (rs81212145), *i.e.* all of them were CAG for codon 315, suggesting the existence of complete linkage disequilibrium between both polymorphisms. By using a previously generated liver microarray data set from the same Duroc population analyzed herewith (Manunza *et al.* 2014), we compared the levels of ASS1 mRNA expression between two c.944T>A genotypes *i.e.* TA (N = 18) vs TT (N = 67). A t-test analysis performed with *t.test* R function did not reveal any significant difference between these two genotypes (*P*-value = 0.346), suggesting that the c.944T>A polymorphism does not have any effect on the transcriptional rate of the ASS1 gene.

In order to estimate the co-association between the two mutations in the first and second positions of codon 315, 120 whole-genome sequences belonging to European and Asian domestic pigs and wild boars were retrieved from the NCBI Sequence Read

Archive (SRA, <https://www.ncbi.nlm.nih.gov/sra>). Detailed information about these whole-genome sequences is available in **Supplementary Table 2**. All raw SRA files were converted into FASTQ format by using the fastq-dump 2.8.2 tool from the SRA-toolkit package (<https://www.ncbi.nlm.nih.gov/sra/docs/toolkitsoft>). The FASTQ files were subsequently filtered for any sequencing adaptors with the Trimmomatic v. 0.36 software (Bolger *et al.* 2014). Paired-end filtered sequences were then aligned to the porcine reference genome (Sscrofa11.1, Warr *et al.* 2019) with the BWA MEM algorithm (Li 2013). Alignment files were sorted and binarized and PCR duplicates were marked and removed with the PICARD tool (<https://broadinstitute.github.io/picard>). INDEL realignment and base recalibration were performed and the HaplotypeCaller function from the GATK 3.8 tool (McKenna *et al.*, 2010) with default parameters was used to generate variant call format (vcf) files and hard filtering was applied according to GATK best practices (<https://software.broadinstitute.org/gatk/best-practices/>). The rs81212145 and rs81212146 contiguous polymorphisms were retrieved and their co-segregation in European and Asian domestic pigs as well as in European and Asian wild boars was investigated by estimating the r^2 parameter which defines the amount of linkage disequilibrium between two markers. (Hill & Robertson 1968).

This analysis supported the notion that rs81212145 and rs81212146 polymorphism are in complete linkage disequilibrium (**Table 1**), implying the existence of two potential sequences CAG and TTG at codon 315. In contrast, the TAG sequence, which would imply severe deleterious effects on ASS1 enzyme activity, was not detected in our whole-genome sequence data set. The frequency of the CAG haplotype was much higher in pigs and wild boars from Asia than in those with a European origin

(**Table 1**). This result is probably due to the high genetic divergence between Asian and European pigs, which separated 1 million years ago (Frantz *et al.* 2015).

There is an increasing interest in characterizing nonsense mutations associated with lethality because they can have a negative effect on the profitability of pig farms. For instance, Derks *et al.* (2017) analyzed, with an 80K SNP array, 24,000 pigs from commercial farms and found 35 haplotypes with complete absence or depletion of homozygous genotypes and showing adverse effects on reproduction traits. Moreover, Derks *et al.* (2019) detected five relatively frequent recessive lethal haplotypes in two commercial Norwegian Landrace and Duroc purebred populations which cause important reductions (15.1 to 21.6%) of litter size due to the embryonic death of homozygous individuals. Interestingly, these recessive lethal haplotypes increase litter size in crossbred individuals due to a positive heterotic effect on fertility.

The results of our study reflect the difficulties of predicting the outcome of putative loss-of-function mutations, either because problems in their correct annotation (Narasimhan *et al.* 2016) or due to the existence of mechanisms of genetic compensation that prevent lethality. Indeed, the rs81212145 and rs81212146 SNPs are annotated as synonymous and stop gained substitutions in the Sscrofa10.2 and Sscrofa11.1 assemblies of the pig genome, respectively, but according to our analysis they should be jointly considered as a dinucleotide polymorphism in codon 315 with a missense effect. With regard to genetic compensation, an analysis of 589,306 human genomes led to the identification of 13 individuals with homozygous (autosomal recessive disease) or heterozygous (autosomal dominant disease) genotypes for eight severe Mendelian childhood diseases (Chen *et al.* 2016). These individuals should have manifested serious clinical symptoms before the age of 18 years but, apparently, they were perfectly healthy (Chen *et al.* 2016). The only explanation for such paradoxical

result is that there are mechanisms at play that decrease the penetrance of nonsense mutations, including suppressor mutations able to change the sequence of the affected codon or to induce splicing events eliminating the exon carrying the nonsense mutation (MacArthur *et al.* 2012). Alternatively, the readthrough of the premature stop codon during ribosomal translation might also prevent its truncating effect on protein synthesis (Rausell *et al.* 2014). In conclusion, our data indicate that the c.944T>A mutation reported by Groenen *et al.* (2012) probably does not have pathological consequences on pigs due to the existence of an adjacent mutation that prevents the formation of a premature stop codon. The considerable amount of deleterious variation segregating in domestic animals (Makino *et al.* 2018) offers an unparalleled opportunity to explore the effects of loss-of-function mutations on phenotypes of economic interest, as well as to elucidate the genetic mechanisms that, in some occasions, counteract their harmful consequences.

Conflict of interest

The authors declare no conflict of interest.

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Data availability

Partial *ASS1* sequences obtained from genomic DNA (accession numbers: MN296492-MN296493) and cDNA (accession numbers: MN296494-MN296495) have been submitted to Genbank.

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Table 1. Frequency of the CAG *ASS1* haplotype in a total of 120 sequenced pigs and wild boars and measurement of the r^2 parameter between polymorphisms c.943T>C and c.944T>A.

Parameter	European domestic pigs (N = 40)	European wild boars (N = 20)	Asian domestic pigs (N = 40)	Asian wild boars (N = 20)
Missing ^a	0.375	1	0.05	0.15
CAG frequency ^b	0.08	-	0.89	0.47
r^2 LD ^c	1	-	1	1

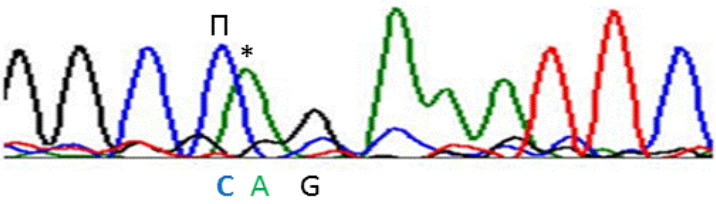
^aMissing: percentage of pigs with missing genotypes for codon 315 of the *ASS1* gene;

^bCAG Freq: CAG haplotype frequency; ^c r^2 LD: magnitude of the linkage disequilibrium between polymorphisms c.943T>C and c.944T>A expressed as r^2 (Hill & Robertson 1968).

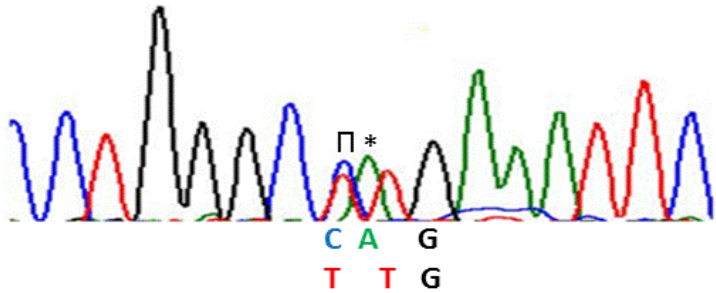
LEGENDS TO FIGURES

Figure 1: Sequencing of codon 315 of the porcine *ASS1* gene and its surrounding region using genomic DNA as a template. The upper (A), central (B) and lower (C) electropherograms display the three codon 315 genotypes (CAG/CAG CAG/TTG and TTG/TTG) detected by Sanger sequencing in a sample of sixteen pigs. The c.943T>C and c.944T>A polymorphisms are indicated with the (II) and (*) symbols, respectively.

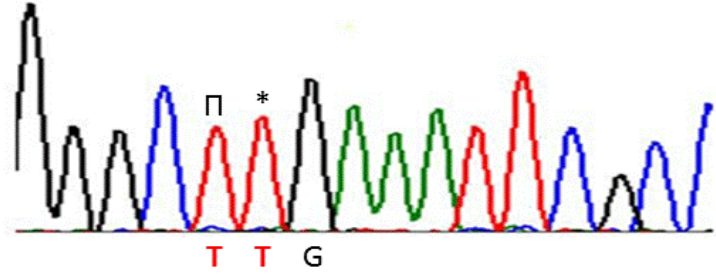
A. Homozygous CAG/CAG individual for codon 315



B. Heterozygous CAG/TTG individual for codon 315



C. Homozygous TTG/TTG for codon 315



Supplementary Table 1. Primers employed in the PCR amplification and partial sequencing of the porcine argininosuccinate synthase 1 (*ASS1*) gene¹.

Template	Primer ID	Primer sequence	T _m (°C)	GC (%)
gDNA	Primer-F	5'-GGA CGA TTC TTT ACC ACG CTC-3'	57	52
	Primer-R	5'-TTA TGA ACG AGG CAG GTC CC-3'	59	55
cDNA	Primer-F	5'-ATG AAG TCC CGA GGT ATC TAC GA-3'	58	48
	Primer-R	5'-ACC TTC CCT TCC ACA CGC-3'	57	61

¹gDNA: genomic DNA template, cDNA: complementary DNA template, Primer-F: forward primer, Primer-R: reverse primer, T_m (°C): melting temperature, GC%: GC content of the primers.

416 **Supplementary Table 2.** List of the porcine whole-genome sequences used in the current study and genotype of codon 315 in Asian domestic
417 (ADM), Asian wild boar (AWB), European domestic (EDM) and European wild boar (EWB) pigs.

Run	BioSample	Sample	SRA Study	Experiment	Group	Breed/Location	Center Name	ReleaseDate	Genotype
ERR173202	SAMEA1557410	ERS177334	ERP001813	ERX149165	ADM	Meishan	WUR-PBR	2012-10-12	TTG
SRR1172563	SAMN02646543	SRS559499	SRP038221	SRX473146	ADM	Tongchen	HUAZHONG AGRICULTURAL UNIVERSITY	2014-08-22	CAG
SRR1172577	SAMN02646545	SRS559500	SRP038221	SRX473147	ADM	Tongchen	HUAZHONG AGRICULTURAL UNIVERSITY	2014-08-22	CAG
SRR1216635	SAMN02646544	SRS559501	SRP038221	SRX473148	ADM	Tongchen	HUAZHONG AGRICULTURAL UNIVERSITY	2014-08-22	-
SRR1216636	SAMN02646546	SRS559502	SRP038221	SRX473149	ADM	Tongchen	HUAZHONG AGRICULTURAL UNIVERSITY	2014-08-22	-
SRR3123375	SAMN04440480	SRS1261811	SRP068560	SRX1544453	ADM	Jinhua	NOVOGENE	2016-07-06	CAG
SRR3123391	SAMN04440482	SRS1261812	SRP068560	SRX1544455	ADM	Rongchang	NOVOGENE	2016-07-06	CAG
SRR3123398	SAMN04440481	SRS1261813	SRP068560	SRX1544454	ADM	Meishan	NOVOGENE	2016-07-06	CAG
SRR448588	SAMN02953814	SRS688476	SRP011937	SRX131601	ADM	Wuzhishan	BGI	2012-11-26	CAG
SRR5088931	SAMN06115553	SRS1844647	SRP075519	SRX2405926	ADM	Rongchang	CHINA AGRICULTURAL UNIVERSTIY	2017-01-01	CAG
SRR5088932	SAMN06115552	SRS1844648	SRP075519	SRX2405927	ADM	Rongchang	CHINA AGRICULTURAL UNIVERSTIY	2017-01-01	CAG
SRR949626	SAMN02298073	SRS465708	SRP028348	SRX329710	ADM	Wuzhishan	BGI	2015-07-22	CAG
SRR949628	SAMN02298074	SRS465709	SRP028348	SRX329712	ADM	Wuzhishan	BGI	2015-07-22	CAG
SRR949630	SAMN02298075	SRS465710	SRP028348	SRX329714	ADM	Wuzhishan	BGI	2015-07-22	CAG
SRR949678	SAMN02298096	SRS465731	SRP028348	SRX329762	ADM	Tibetan	BGI	2015-07-22	CAG
SRR949680	SAMN02298097	SRS465732	SRP028348	SRX329764	ADM	Tibetan	BGI	2015-07-22	TTG/CAG
SRR949681	SAMN02298098	SRS465733	SRP028348	SRX329765	ADM	Tibetan	BGI	2015-07-22	CAG
SRR949697	SAMN02298106	SRS465741	SRP028348	SRX329781	ADM	Sichuan	BGI	2015-07-22	CAG
SRR949702	SAMN02298108	SRS465743	SRP028348	SRX329786	ADM	Sichuan	BGI	2015-07-22	CAG
SRR949703	SAMN02298109	SRS465744	SRP028348	SRX329787	ADM	Sichuan	BGI	2015-07-22	CAG
SRR949715	SAMN02298115	SRS465750	SRP028348	SRX329799	ADM	Hetao	BGI	2015-07-22	CAG
SRR949717	SAMN02298116	SRS465751	SRP028348	SRX329801	ADM	Hetao	BGI	2015-07-22	TTG/CAG

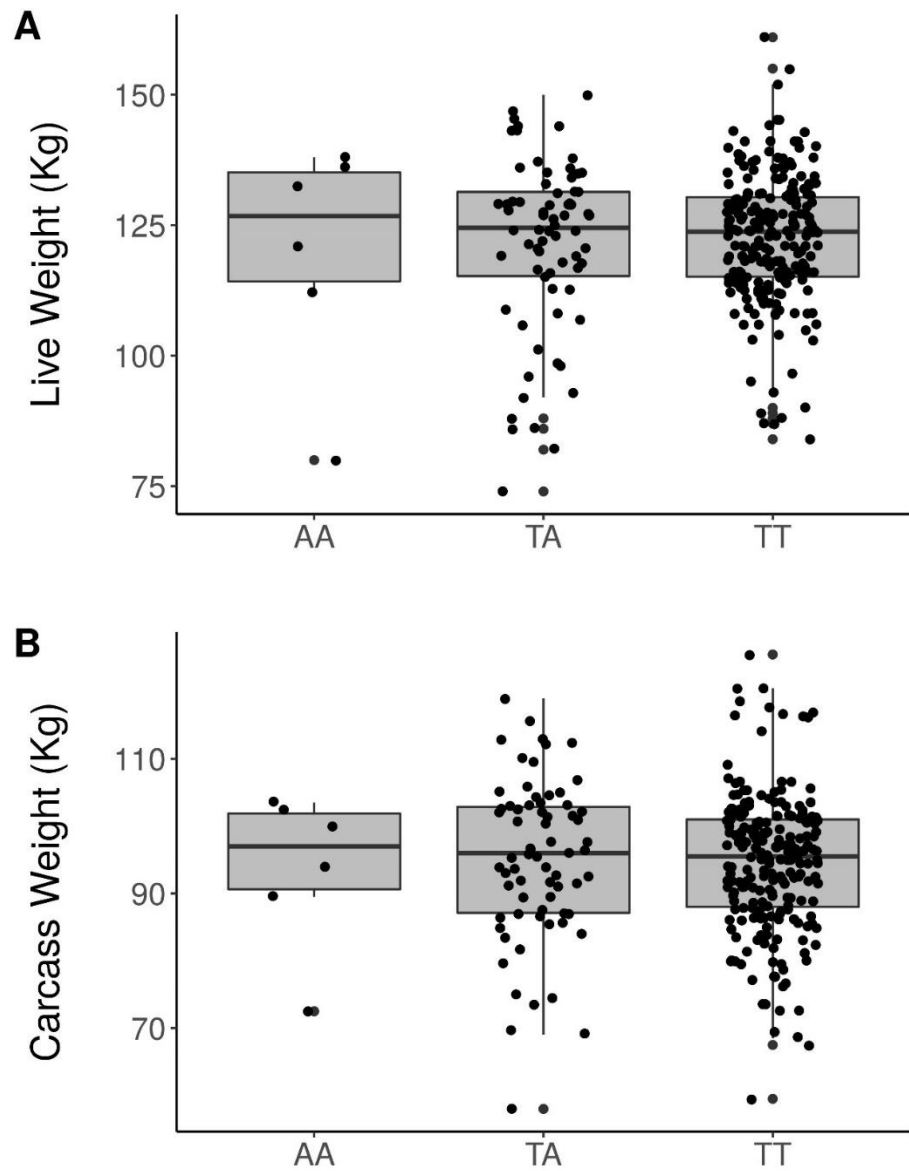
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SRR949726	SAMN02298119	SRS465754	SRP028348	SRX329810	ADM	Hetao	BGI	2015-07-22	CAG
SRR949727	SAMN02298120	SRS465755	SRP028348	SRX329811	ADM	Hetao	BGI	2015-07-22	TTG
SRR949732	SAMN02298122	SRS465757	SRP028348	SRX329816	ADM	Minzhu	BGI	2015-07-22	CAG
SRR949733	SAMN02298123	SRS465758	SRP028348	SRX329817	ADM	Minzhu	BGI	2015-07-22	CAG
SRR949736	SAMN02298124	SRS465759	SRP028348	SRX329820	ADM	Minzhu	BGI	2015-07-22	CAG
SRR949738	SAMN02298125	SRS465760	SRP028348	SRX329822	ADM	Minzhu	BGI	2015-07-22	CAG
SRR949740	SAMN02298126	SRS465761	SRP028348	SRX329824	ADM	Minzhu	BGI	2015-07-22	CAG
SRR949742	SAMN02298127	SRS465762	SRP028348	SRX329826	ADM	Bamaxiang	BGI	2015-07-22	CAG
SRR949743	SAMN02298128	SRS465763	SRP028348	SRX329827	ADM	Bamaxiang	BGI	2015-07-22	CAG
SRR949746	SAMN02298129	SRS465764	SRP028348	SRX329830	ADM	Bamaxiang	BGI	2015-07-22	CAG
SRR949747	SAMN02298130	SRS465765	SRP028348	SRX329831	ADM	Bamaxiang	BGI	2015-07-22	CAG
SRR949749	SAMN02298131	SRS465766	SRP028348	SRX329833	ADM	Bamaxiang	BGI	2015-07-22	CAG
SRR949753	SAMN02298133	SRS465768	SRP028348	SRX329837	ADM	Laiwu	BGI	2015-07-22	CAG
SRR949756	SAMN02298134	SRS465769	SRP028348	SRX329840	ADM	Laiwu	BGI	2015-07-22	CAG
SRR949757	SAMN02298135	SRS465770	SRP028348	SRX329841	ADM	Laiwu	BGI	2015-07-22	TTG
SRR949760	SAMN02298136	SRS465771	SRP028348	SRX329844	ADM	Laiwu	BGI	2015-07-22	CAG
SRR949762	SAMN02298137	SRS465772	SRP028348	SRX329846	ADM	Laiwu	BGI	2015-07-22	CAG
ERR173212	SAMEA1557411	ERS177344	ERP001813	ERX149175	AWB	Japan	WUR-PBR	2012-10-11	TTG
ERR173220	SAMEA1557396	ERS177352	ERP001813	ERX149183	AWB	South China	WUR-PBR	2012-10-11	TTG
ERR173222	SAMEA1557421	ERS177354	ERP001813	ERX149185	AWB	North China	WUR-PBR	2012-10-11	TTG
ERR977151	SAMEA3497815	ERS804964	ERP011076	ERX1054134	AWB		WAGENINGEN UNIVERSITY	2015-08-28	CAG
ERR977165	SAMEA3497818	ERS804967	ERP011076	ERX1054148	AWB		WAGENINGEN UNIVERSITY	2015-08-28	CAG
ERR977168	SAMEA3497819	ERS804968	ERP011076	ERX1054151	AWB		WAGENINGEN UNIVERSITY	2015-08-28	CAG
ERR977180	SAMEA3497822	ERS804971	ERP011076	ERX1054163	AWB		WAGENINGEN UNIVERSITY	2015-08-28	TTG/CAG
SRR1581061	SAMN03031172	SRS703308	SRP047260	SRX703567	AWB	Korea	SEOUL NATIONAL UNIVERSITY	2015-03-16	TTG
SRR1581063	SAMN03031173	SRS703309	SRP047260	SRX703568	AWB	Korea	SEOUL NATIONAL UNIVERSITY	2015-03-16	-
SRR1581067	SAMN03031174	SRS703310	SRP047260	SRX703569	AWB	Korea	SEOUL NATIONAL UNIVERSITY	2015-03-16	CAG

SRR1581070	SAMN03031175	SRS703311	SRP047260	SRX703570	AWB	Korea	SEOUL NATIONAL UNIVERSITY	2015-03-16	CAG
SRR1581073	SAMN03031176	SRS703312	SRP047260	SRX703571	AWB	Korea	SEOUL NATIONAL UNIVERSITY	2015-03-16	TTG
SRR1581075	SAMN03031177	SRS703313	SRP047260	SRX703572	AWB	Korea	SEOUL NATIONAL UNIVERSITY	2015-03-16	TTG
SRR1581080	SAMN03031179	SRS703315	SRP047260	SRX703574	AWB	Korea	SEOUL NATIONAL UNIVERSITY	2015-03-16	TTG
SRR3745076	SAMN05362551	SRS1541811	SRP074357	SRX1898989	AWB	Russia	CRAG	2016-09-05	-
SRR949641	SAMN02298081	SRS465716	SRP028348	SRX329725	AWB	South China	BGI	2015-07-22	TTG/CAG
SRR949643	SAMN02298082	SRS465717	SRP028348	SRX329727	AWB	South China	BGI	2015-07-22	CAG
SRR949650	SAMN02298084	SRS465719	SRP028348	SRX329734	AWB	South China	BGI	2015-07-22	CAG
SRR949653	SAMN02298085	SRS465720	SRP028348	SRX329737	AWB	South China	BGI	2015-07-22	TTG
SRR949654	SAMN02298086	SRS465721	SRP028348	SRX329738	AWB	South China	BGI	2015-07-22	-
ERR875316	SAMEA3376937	ERS718609	ERP010412	ERX954919	EDM	Pietrain	WAGENINGEN UNIVERSITY	2015-05-15	-
ERR875318	SAMEA3376938	ERS718610	ERP010412	ERX954921	EDM	Pietrain	WAGENINGEN UNIVERSITY	2015-05-15	-
ERR875320	SAMEA3376939	ERS718611	ERP010412	ERX954923	EDM	Pietrain	WAGENINGEN UNIVERSITY	2015-05-15	-
SRR1178916	SAMN02665306	SRS562609	SRP039012	SRX476909	EDM	Mangalitza	AGRICULTURAL BIOTECHNOLOGY CENTER	2014-08-15	TTG
SRR1178923	SAMN02665304	SRS562610	SRP039012	SRX476910	EDM	Mangalitza	AGRICULTURAL BIOTECHNOLOGY CENTER	2014-08-15	TTG
SRR1178925	SAMN02665305	SRS562611	SRP039012	SRX476911	EDM	Mangalitza	AGRICULTURAL BIOTECHNOLOGY CENTER	2014-08-15	TTG
SRR1513307	SAMN02904857	SRS655955	SRP044261	SRX648632	EDM	Iberian	CRAG	2014-08-08	-
SRR1577877	SAMN03031142	SRS703276	SRP047260	SRX703536	EDM	Duroc	SEOUL NATIONAL UNIVERSITY	2015-03-16	-
SRR1577880	SAMN03031145	SRS703279	SRP047260	SRX703539	EDM	Duroc	SEOUL NATIONAL UNIVERSITY	2015-03-16	-
SRR1581045	SAMN03031159	SRS703294	SRP047260	SRX703553	EDM	Yucatan	SEOUL NATIONAL UNIVERSITY	2015-03-16	TTG
SRR1581046	SAMN03031160	SRS703295	SRP047260	SRX703554	EDM	Yucatan	SEOUL NATIONAL UNIVERSITY	2015-03-16	TTG
SRR1581047	SAMN03031161	SRS703296	SRP047260	SRX703555	EDM	Yucatan	SEOUL NATIONAL UNIVERSITY	2015-03-16	-
SRR1581048	SAMN03031162	SRS703298	SRP047260	SRX703557	EDM	Yucatan	SEOUL NATIONAL UNIVERSITY	2015-03-16	TTG/CAG
SRR1581052	SAMN03031166	SRS703302	SRP047260	SRX703561	EDM	Yucatan	SEOUL NATIONAL UNIVERSITY	2015-03-16	TTG
SRR1581055	SAMN03031169	SRS703305	SRP047260	SRX703564	EDM	Yucatan	SEOUL NATIONAL UNIVERSITY	2015-03-16	TTG
SRR1581056	SAMN03031170	SRS703306	SRP047260	SRX703565	EDM	Yucatan	SEOUL NATIONAL UNIVERSITY	2015-03-16	TTG
SRR1581135	SAMN03031189	SRS703326	SRP047260	SRX703586	EDM	Yorkshire	SEOUL NATIONAL UNIVERSITY	2015-03-16	-
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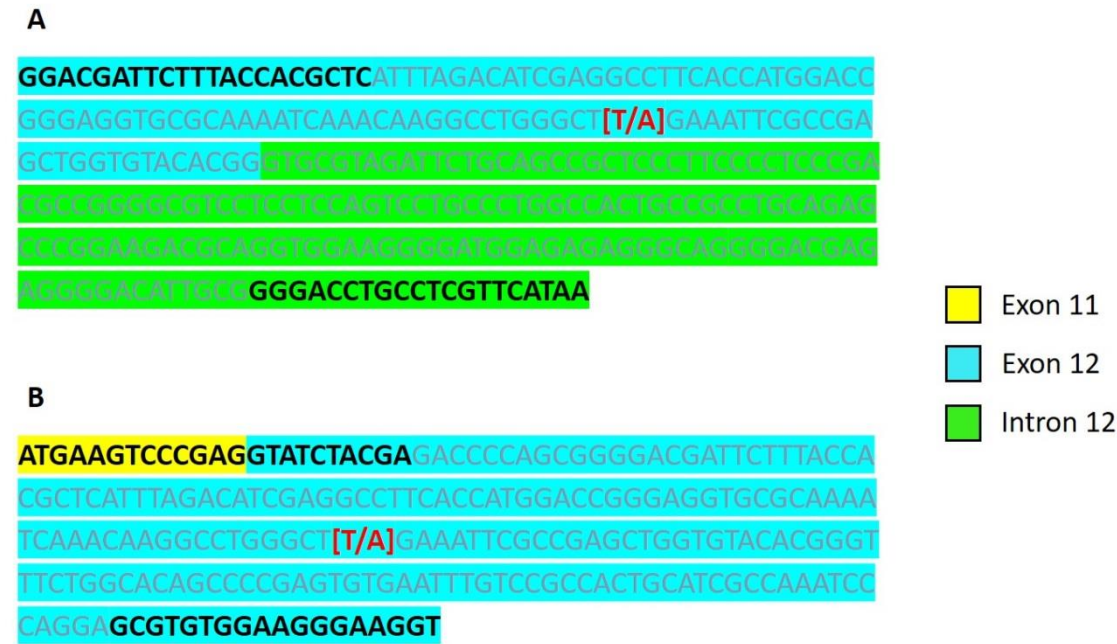
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SRR1917381	SAMN03421607	SRS875335	SRP074357	SRX958019	EDM	Iberian	UNIVERSITAT AUTONOMA BARCELONA	2015-04-27	TTG
SRR3118615	SAMN04440474	SRS1261795	SRP068560	SRX1544456	EDM	Hampshire	NOVOGENE	2016-07-05	TTG
SRR3123333	SAMN04440477	SRS1261808	SRP068560	SRX1544450	EDM	Pietrain	NOVOGENE	2016-07-06	TTG/CAG
SRR3123347	SAMN04440478	SRS1261809	SRP068560	SRX1544451	EDM	Large-White	NOVOGENE	2016-07-06	TTG
SRR4341272	SAMN05791662	SRS1725713	SRP090776	SRX2208350	EDM	Landrace	USDA-ARS-USMARC	2016-11-01	TTG
SRR4341276	SAMN05791666	SRS1725717	SRP090776	SRX2208354	EDM	Duroc	USDA-ARS-USMARC	2016-11-01	TTG
SRR4341277	SAMN05791667	SRS1725718	SRP090776	SRX2208355	EDM	Duroc	USDA-ARS-USMARC	2016-11-01	TTG
SRR4341312	SAMN05791653	SRS1725753	SRP090776	SRX2208390	EDM	Landrace	USDA-ARS-USMARC	2016-11-01	TTG
SRR4341337	SAMN05791657	SRS1725778	SRP090776	SRX2208415	EDM	Landrace	USDA-ARS-USMARC	2016-11-01	TTG
SRR5131497	SAMN06115550	SRS1879087	SRP075519	SRX2445320	EDM	Duroc	CHINA AGRICULTURAL UNIVERSTIY	2016-12-31	TTG
SRR5513124	SAMN06895011	SRS2168992	SRP044261	SRX2787051	EDM	Large-White	CENTRE FOR RESEARCH IN AGRIGENOMICS	2017-05-10	-
SRR5515065	SAMN06895012	SRS2170012	SRP044261	SRX2788443	EDM	Iberian	CENTRE FOR RESEARCH IN AGRIGENOMICS	2017-05-10	TTG
SRR5518325	SAMN06917569	SRS2173160	SRP106658	SRX2791684	EDM	Large-White	GEO	2017-09-07	-
SRR5818327	SAMN07344468	SRS2347775	SRP111615	SRX2996556	EDM	Pietrain	INRA	2017-07-12	-
SRR6251675	SAMN05791648	SRS1725708	SRP090776	SRX2208345	EDM	Landrace	USDA-ARS-USMARC	2017-11-05	TTG
SRR6252601	SAMN05791652	SRS1725743	SRP090776	SRX2208379	EDM	Landrace	USDA-ARS-USMARC	2017-11-08	TTG
SRR6252608	SAMN05791660	SRS1725711	SRP090776	SRX2208348	EDM	Landrace	USDA-ARS-USMARC	2017-11-07	TTG
SRR6261392	SAMN05791663	SRS1725714	SRP090776	SRX2208351	EDM	Duroc	USDA-ARS-USMARC	2017-11-10	TTG
SRR6261493	SAMN05791665	SRS1725716	SRP090776	SRX2208353	EDM	Duroc	USDA-ARS-USMARC	2017-11-09	CAG
ERR173214	SAMEA1557433	ERS177346	ERP001813	ERX149177	EWB	Netherlands	WUR-PBR	2012-10-11	-
ERR173217	SAMEA1557401	ERS177349	ERP001813	ERX149180	EWB	France	WUR-PBR	2012-10-11	-
ERR173218	SAMEA1557403	ERS177350	ERP001813	ERX149181	EWB	Switzerland	WUR-PBR	2012-10-11	-
ERR977316	SAMEA3497864	ERS805013	ERP011076	ERX1054299	EWB	Netherlands	WAGENINGEN UNIVERSITY	2015-08-28	-
ERR977317	SAMEA3497865	ERS805014	ERP011076	ERX1054300	EWB	Netherlands	WAGENINGEN UNIVERSITY	2015-08-28	-
ERR977326	SAMEA3497867	ERS805016	ERP011076	ERX1054309	EWB	Netherlands	WAGENINGEN UNIVERSITY	2015-08-28	-

ERR977330	SAMEA3497869	ERS805018	ERP011076	ERX1054313	EWB	Netherlands	WAGENINGEN UNIVERSITY	2015-08-28	-
ERR977340	SAMEA3497872	ERS805021	ERP011076	ERX1054323	EWB	Netherlands	WAGENINGEN UNIVERSITY	2015-08-28	-
ERR977341	SAMEA3497873	ERS805022	ERP011076	ERX1054324	EWB	Netherlands	WAGENINGEN UNIVERSITY	2015-08-28	-
ERR977342	SAMEA3497874	ERS805023	ERP011076	ERX1054325	EWB	Netherlands	WAGENINGEN UNIVERSITY	2015-08-28	-
ERR977343	SAMEA3497875	ERS805024	ERP011076	ERX1054326	EWB	Netherlands	WAGENINGEN UNIVERSITY	2015-08-28	-
ERR977352	SAMEA3497877	ERS805026	ERP011076	ERX1054335	EWB	Switzerland	WAGENINGEN UNIVERSITY	2015-08-28	-
ERR977356	SAMEA3497879	ERS805028	ERP011076	ERX1054339	EWB	Italy	WAGENINGEN UNIVERSITY	2015-08-28	-
ERR977359	SAMEA3497880	ERS805029	ERP011076	ERX1054342	EWB	Greece	WAGENINGEN UNIVERSITY	2015-08-28	-
ERR977374	SAMEA3497885	ERS805034	ERP011076	ERX1054357	EWB	Middle East	WAGENINGEN UNIVERSITY	2015-08-28	-
ERR977377	SAMEA3497886	ERS805035	ERP011076	ERX1054360	EWB	Italy	WAGENINGEN UNIVERSITY	2015-08-28	-
ERR977380	SAMEA3497887	ERS805036	ERP011076	ERX1054363	EWB	Italy	WAGENINGEN UNIVERSITY	2015-08-28	-
ERR977386	SAMEA3497890	ERS805039	ERP011076	ERX1054369	EWB	Ukraine	WAGENINGEN UNIVERSITY	2015-08-28	-
SRR1513306	SAMN02904855	SRS655622	SRP044261	SRX648328	EWB	Spain	CRAG	2014-08-08	-
SRR3745077	SAMN05362552	SRS1541812	SRP074357	SRX1898990	EWB	Spain	CRAG	2016-09-05	-

Supplementary Figure 1: Boxplots depicting the distribution of **(A)** live weight and **(B)** carcass weight for pigs with TT (N = 239), TA (N = 77) and AA (N = 7) rs81212146 genotypes.



Supplementary Figure 2. Primer binding regions (highlighted in bold) in the ASS1 amplicons generated from **(A)** genomic DNA and **(B)** cDNA. The position of the rs81212146 polymorphism is depicted in red. Exon-intron structure of the two amplicons is also indicated.



Supplementary Figure 3. Sequencing of codon 315 of the porcine *ASS1* gene and its surrounding region using cDNA as a template. The upper and lower electropherograms display two codon 315 genotypes (CAG/CAG and TTG/TTG) detected by Sanger sequencing in a sample of sixteen pigs. The c.943T>C and c.944T>A polymorphisms are indicated with the (Π) and (*) symbols, respectively.

