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An independent validation association study of carcass quality, shear force, intramuscular fat percentage and omega-3 polyunsaturated fatty acid content with gene markers in Australian lamb ☆

Matthew I. Knight [a,b,c,*], Hans D. Daetwyler [a,b], Ben J. Hayes [a,b,d], Matthew J. Hayden [b], Alex J. Ball [a,e], David W. Pethick [a,f], Matthew B. McDonagh [a,c]

[a] Cooperative Research Centre for Sheep Industry Innovation, CJ Hawkins Homestead Building, University of New England, Armidale, NSW, 2351, Australia
[b] Biosciences Research Division, Department of Primary Industries, AgriBio Building, 5 Ring Road, Bundoora, Vic, 3083, Australia
[c] Future Farming Systems Research Division, Department of Primary Industries, 915 Mt Napier Road, Hamilton, Vic, 3300, Australia
[d] La Trobe University, 3086 Plenty Road, Bundoora, Vic., 3083, Australia
[e] Meat and Livestock Australia, CJ Hawkins Homestead Building, University of New England, Armidale, NSW, 2351, Australia
[f] School of Veterinary and Biomedical Science, Murdoch University, Murdoch, WA 6150, Australia

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ABSTRACT

Previous association studies revealed several single nucleotide polymorphisms (SNPs) that explained the observed phenotypic variation for meat tenderness and long-chain omega-3 polyunsaturated fatty acid (PUFA) content of Australian lamb. To confirm the validity of these associated SNPs at predicting meat tenderness and omega-3 PUFA content, an independent validation study was designed. The OvineSNP50 genotypes of these animals were used to impute the 192 SNP Meat Quality Research (MQR) panel genotypes on nearly 6200 animals from the Cooperative Research Centre for Sheep Industry Innovation Information Nucleus Flock and Sheep Genomics Falkiner Memorial Field Station flock. Association analysis revealed numerous SNP from the 192 SNP MQR panel that were associated with carcass quality – fat depth at the C-site and eye muscle depth; shear force at day 1 and day 5 after slaughter (SF1 and SF5); and omega-3 PUFA content at $P < 0.01$. However, 1 SNP was independently validated for SF5 (i.e. CAST_101781475). The magnitude of the effect of each significant SNP and the relative allele frequencies across Merino-, Maternal- and Terminal-sired progeny was determined. The independently validated SNP for SF5 and the associated SNP with omega-3 PUFA content will accelerate efforts to improve these phenotypic traits in Australian lamb.

1. Introduction

The efficient production of meat that is of consistent quality and value is necessary for the sheep meat industry to remain competitive (Pethick, Warner, & Banks, 2006). The Australian sheep meat industry is focussed on appropriate management strategies (pre- and post-slaughter) incorporated with genome assisted breeding programs (i.e. genomic selection) to meet these expectations (Rowe, 2010). Thus, identifying gene markers that can predict carcass quality, shear force at day 1 and day 5 after slaughter (SF1 and SF5), intramuscular fat (IMF) percentage and omega-3 polyunsaturated fatty acid (PUFA) content in lamb are of great interest to the Sheep Meat Industry.

Carcass quality traits can assist in predicting the overall saleable meat yield or proportions of fat, lean and bone on carcass (Gardner et al., 2010; Hopkins, 1994; Hopkins & Fogarty, 1998; Stanford, Jones, & Price, 1998). Saleable meat yield is an important financial determinant of the overall value of the carcass (Hopkins, Wotton, Gamble, Atkinson, Slack-Smith, & Hal, 1995). Carcass quality traits targeted by genetic breeding programs include: post-weaning weight (PWWT), fat depth at the C-site (PFAT) and eye muscle depth (PEMD). Studies investigating the potential of Australian Sire Breeding Values (ASBVs) for high PWWT showed that available nutrition significantly affected the sire's genetic potential for growth (Hegarty et al., 2006). Gardner et al. (2010) showed that progeny from Merino-, Maternal- and Terminal-sires with high ASBVs for PWWT had increased weights at slaughter and hot carcass weights (HCWT). Further studies revealed that sires with high ASBVs for PEMD (i.e. 1 mm) showed increases in loin depth on carcass of 0.6 mm (Hegarty et al., 2006). This increase

* Corresponding author at: Future Farming Systems Research Division, Department of Primary Industries, 915 Mt Napier Road, Hamilton, Vic, 3300, Australia. Tel.: +61 3 5573 0903; fax: +61 3 5571 1523.
E-mail address: Matthew.Knight@depi.vic.gov.au (M.I. Knight).

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in loin depth was not affected by available nutrition (Hegarty et al., 2006).

Meat tenderness is the principal desirable attribute associated with meat eating quality and can be measured objectively by shear force (Hopkins, Toohey, Warner, Kerr, & van de Ven, 2010), trained panellists (Safari, Fogarty, Ferrier, Hopkins, & Gilmour, 2001) or consumers (Hopkins, Walker, Thompson & Pethick, 2005). Numerous studies have shown that the greatest biological factor which contributes to the meat tenderisation process, post slaughter are calcium-activated proteases (i.e. the calpains) and their activity. To date, 14 different calpain gene family members have been identified (Goll, Thompson, Li, Wei, & Cong, 2003). Three members of the calpain family are ubiquitously expressed in skeletal muscle, μ-calpain (CAPN1), *m*-calpain (CAPN2) and calpain 3 (CAPN3). Associated with the calcium-activated proteases is the calpain specific inhibitor, calpastatin (CAST).

IMF percentage has also been shown to affect meat tenderness and the overall sheep meat eating quality of lamb (i.e. flavour, juiciness, tenderness and overall likeability). In beef cattle, there is a genetic correlation between IMF percentage and meat tenderness (Reverter, Johnston, Perry, Goddard, & Burrow, 2003). For Australian lamb to achieve consumer satisfaction – "good every day" score (MSA grade – 3 out of 5); lamb meat must contain an IMF percentage between 4 – 5% (Hopkins, Hegarty, Walker, & Pethick, 2006a).

In human diets, red meat is an important source of omega-3 PUFAs (Howe, Meyer, Record, & Baghurst, 2006). Numerous studies have identified the health benefits of omega-3 PUFAs, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Psota, Gebauer, & Kris-Etherton, 2006). In humans, both EPA and DHA have cardiovascular and anti-inflammatory health benefits (Marik & Varon, 2009; Psota et al., 2006; Swanson, Block, & Mousa, 2012). Docosapentaenoic acid (DPA) is another omega-3 PUFA, however the nutritional content of DPA cannot currently be claimed as an Omega-3 under the Food Standards Code (Howe, Buckley, & Meyer, 2007). Thus, achieving levels of omega-3 PUFAs that comply with recommended dietary guidelines (i.e. "good source) has been proposed as a goal for lamb production systems (Pethick et al., 2006). In mammals, the omega-3 PUFA biosynthetic pathway is controlled by fatty acid desaturases (i.e. FADS1, FADS2, FADS3) and elongases (for example, ELOVL2) (Sprecher, Luthria, Mohammed, & Baykousheva, 1995).

The Sheep Industries OvineSNP50 chip (Illumina, CA, USA) which includes 54,977 genome wide SNP includes 6 SNP in close proximity to genes that influence shear force: CAPN1 (OAR21:47225725), CAPN2 (OAR12:28694194) and CAPN3 (OAR7:39000331) and CAST (OAR5:101742566, OAR5:101792466 and OAR5:101853472) and four SNP in close proximity to genes that potentially influence omega-3 PUFA content; FADS1 (OAR21:43637798 and OAR21:43646542) and FADS3 (OAR21:43754091and OAR21:43748475). Preliminary genome wide association studies (GWAS) have shown that these SNP in close proximity are not capable of predicting shear force in lamb. However, 10 SNP on the OvineSNP50 chip (Illumina, CA, USA) were associated with omega-3 PUFA levels in lamb (B.J. Hayes, pers. comm.).

Thus, identifying SNP in closer proximity to or within these genes could improve genomic predictions for shear force and omega-3 PUFA content in sheep. Recent findings reported by Knight et al. (2012) identified 182 SNP is close proximity to or within the genes previously associated with SF1, SF5 and omega-3 PUFA content in lamb. Association studies revealed 3 SNP in CAST and CAPN2 genes that were associated with SF5 (i.e. meat tenderness) and no SNP within the FADS1/2/3, ELOVL2 and SLC26A10 gene regions associated with long-chain omega-3 fatty acid content of Australian lamb (Knight et al., 2012). Thus, a 192 SNP Meat Quality Research (MQR) panel was designed containing the 182 new SNP identified by Knight et al. (2012) and the 10 SNP on the OvineSNP50 chip (Illumina, CA, USA) that were associated with omega-3 PUFA levels in lamb.

The aim of this study was to validate the associated SNPs at predicting SF5 and long-chain omega-3 PUFA content in lamb in an independent sheep population. The OvineSNP50 genotypes of the independent sheep population were used to impute the 192 SNP MQR genotypes on nearly 6200 animals from the Sheep CRC INF and Sheep Genomics Falkiner Memorial Field Station (FMFS) flock. Using the imputed genotypes of nearly 6200 animals, this paper reports the associations of each SNP on the MQR panel with carcass quality, SF1, SF5, IMF percentage and omega-3 PUFA content. Linear mixed model analysis was then performed to estimate the magnitude of effect of all significant SNP. The relative allele frequencies of all significant SNP is reported across Merino-, Maternal- and Terminal-sired progeny.

2. Materials and methods

2.1. Phenotypic data

Phenotypic data used in the association study was collected from the Sheep CRC INF flock (van der Werf, Kinghorn, & Banks, 2010) and Sheep Genomics FMFS flock (White et al., 2012). The INF animals were located at eight geographically different sites across Australia and the FMFS animals were raised in Deniliquin, NSW, Australia. The eight geographical sites for the INF included: Kirby Research Station, University of New England, Armidale, NSW; Trangie Agricultural Research Centre, NSW; Cowra Agricultural Research and Advisory Station, NSW; DPI Hamilton Centre, Vic.; DPI Rutherglen Centre, Vic., Struan Research Station, SA; Turretfield Research Station, SA and Great Southern Agricultural Research Institute, Katanning, WA. All dams used in the INF and FMFS flocks had a Merino background that ranged from fine wool to strong wool. The sires were either from Terminal, Maternal (i.e. Border Leicester, Coopworth and East Friesian), or Merino breeds. Whilst the Merino sheep were mostly purebred, the remaining breeds this study represented were mainly crossbred animals because of their crosses with Merino ewes. Therefore both research flocks (i.e. the INF and FMFS) represented the majority of flock structures seen in the Australian Sheep Industry. Table 1 represents all the breeds used in this study.

Table 1
Number of progeny by breed of sire and dam for the Cooperative Research Centre for Sheep Industry Innovation Information Nucleus flock (before backslash) and the Sheep Genomics Falkiner Memorial Research Station flock (after backslash) (BL – Border Leicester, BL x EF – Border Leicester x East Friesian, MER – Merino, PD – Poll Dorset, PD x WS – Poll Dorset x White Suffolk, PM – Poll Merino, EF – East Friesian, WD – White Dorper, WS – White Suffolk, XB – cross breed and BL x MER – Border Leicester x Merino).

SIRE Breed	DAM Breed						
	MER	PD	PM	WS	XB	BL X MER	Totals
BOND	6/0	0/0	0/0	0/0	0/0	0/0	6/0
BOOROOLA	44/0	0/0	0/0	0/0	0/0	0/0	44/0
BL	405/124	0/10	5/0	0/7	0/0	0/49	587/190
BL X EF	0/66	0/2	0/0	0/2	0/0	0/25	0/95
MER	415/1274	0/0	10/0	0/2	0/0	0/0	491/1276
PD	518/103	0/9	13/0	0/7	638/0	0/44	1511/163
PD X WS	0/143	0/12	0/0	0/13	0/0	0/45	0/213
PM	247/0	0/0	6/0	0/0	0/0	0/0	297/0
COOPWORTH	64/66	0/5	5/0	0/5	0/0	0/22	69/98
CORRIEDALE	61/0	0/0	6/0	0/0	0/0	0/0	67/0
DOHNE MERINOS	69/0	0/0	8/0	0/0	0/0	0/0	77/0
DORPER	27/0	0/0	0/0	0/0	0/0	0/0	27/0
EF	3/0	0/0	0/0	0/0	0/0	0/0	3/0
HAMPSHIRE DOWN	5/0	0/0	0/0	0/0	0/0	0/0	5/0
ILE DE FRANCE	12/0	0/0	0/0	0/0	2/0	0/0	14/0
PRIME SAMM	83/0	0/0	9/0	0/0	0/0	0/0	92/0
RESEARCH	2/0	0/0	0/0	0/0	0/0	0/0	2/0
SOUTHDOWN	16/0	0/0	0/0	0/0	9/0	0/0	25/0
SUFFOLK	92/0	0/0	2/0	0/0	129/0	0/0	293/0
TEXEL	91/0	0/0	1/0	0/0	130/0	0/0	328/0
WD	53/0	0/0	0/0	0/0	0/0	0/0	53/0
WS	337/49	0/5	17/0	0/8	526/0	0/28	1179/90
Totals	2550/1825	0/43	82/0	0/44	1434/0	0/213	4066/2125

All lambs were slaughtered and trimmed at commercial abattoirs according the AUS-MEAT specifications (Anon., 1992). Carcasses were then subjected to medium voltage electrical stimulation (Pearce et al., 2010). The average target carcass weight was 22 kg for wethers and 21.5 kg for ewes. All post-slaughter measurements and muscle samples for carcass quality were collected according to (Gardner et al., 2010); shear force was performed according to (Warner, Greenwood, Pethick, & Ferguson, 2010) and omega-3 PUFA concentration was performed according to (Ponnampalam et al., 2009).

The following meat eating quality phenotypic traits were evaluated on the INF and the FMFS flock: HCWT, EMD, FatC, SF1 and SF5. SF1 and SF5 phenotypic data were standardised to ensure there was consistency in the phenotypic data between the INF and FMFS flocks. IMF percentage, EPA + DPA + DHA and EPA + DHA concentration was measured on the INF flock. A summary of the phenotype data is shown in Table 2. Trait phenotypes and covariates were removed if they were greater than four SD from the means for the INF and FMFS flock.

2.2. Genomic DNA and genotypic information

All animals were genotyped using the Illumina Ovine50 SNP chip (Illumina Inc., CA, USA). The Ovine50 SNP chip contains 54 977 SNP. SNP were removed from the analysis if the following quality control measures were not met – SNP required a call rate of > 95%, a genotype call (GC) score of < 0.6, and a minor allele frequency of < 0.01, were out of the Hardy-Weinberg equilibrium (a *P*-value cut-off of 1^{-15}), had no genome location or were in > 0.99 linkage disequilibrium (LD) with another SNP on the array (Hill & Robertson, 1968). After these quality control measures were applied, 48 599 SNP remained and were used for further analysis. Data for genotyped animals were removed if their genotype call rate was < 0.9. Genotyping was performed over a number of years, earlier years were imputed using fastPHASE (Scheet & Stephens, 2006) and more recently with Beagle (Browning & Browning, 2009).

2.3. Genotypic data for 192 SNP MQR panel

Genomic DNA from 1440 animals from the INF were genotyped using the 192 SNP MQR panel. The 192 SNP MQR panel contained 182 new SNP that were identified by re-sequencing candidate gene regions for improving shear force and omega-3 PUFA content of lamb (Knight et al., 2012) and 10 SNP from the OvineSNP50 chip (Illumina, CA, USA) that have been shown to be associated with EPA + DPA + DHA and EPA + DHA concentration in lamb (s45673, OAR1_204757734, OAR3_166417678, OAR3_144434162, OAR3_115443384, s50854, OAR9_48598227, s65382, OAR16_43758108 and OAR18_30003500) (Hayes, B.J., unpub data).

Of the 1440 animals genotyped from the Sheep CRC INF using the 192 SNP MQR panel, 1212 animals also had OvineSNP50 genotypes. These data were then used to impute the new SNP on 6191 animals from the INF and FMFS flock which had OvineSNP50 genotypes and HCWT phenotypic data. Thus, the final number of animals genotyped/imputed for the 192 SNP MQR panel was 6191. Twenty iterations of the Beagle program were required to impute the unknown genotypes (Browning & Browning, 2009).

2.4. Association model

Association studies are regressions of phenotypes onto single locus genotypes. The following mixed model was fitted in ASReml (Gilmour, Gogel, Cullis, & Thompson, 2009):

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e},$$

where **y** is the phenotype, **X** and **Z** are design matrices for fixed and random effects, respectively; **b** is a vector of fixed effects, **u** is a polygenic animal effects and **e** is a vector of residuals. Random effects u and e had assumed distributions $N(0, \sigma_a^2\mathbf{A})$ and $N(0, \sigma_e^2\mathbf{I})$, where σ_a^2 is the additive genetic variance, **A** is the numerator relationship matrix calculated from the pedigree, σ_e^2 is the residual variance and **I** is an identity matrix. All models included fixed effects for year, slaughter group, location, sex, birth and rear type, sire type (merino, terminal, maternal), and fixed covariates hot carcass weight and age at slaughter (days). In addition, omega-3 fatty acid analyses included intra-muscular fat percentage as a covariate, and shear force and compression included ultimate meat pH as a covariate. In traits where Sheep Genomics data was used, dam breed information was inferred using the program STRUCTURE and was fitted as the proportion Merino, Border Leicester cross, Polled Dorset or White Suffolk (Pritchard, Stephens, & Donnelly, 2000). The effect of a SNP was fitted as a fixed effect and was coded 0, 1, or 2 corresponding to the number of allele copies an animal carried.

False discovery rates were calculated as expected number of significant tests at a significance level of $P = 0.001$ divided by the number of tests found to be significant (N test * *P* threshold/N < *P*).

2.5. Phenotypic and genetic variance explained

The data of Knight et al. (2012) and the data reported in this paper were combined to get the best estimate of additional genetic variance explained by the 182 new SNP in a genomic BLUP (GBLUP) model. Genomic prediction models, such as GBLUP, fit all SNP simultaneously, in contrast to association studies. This model was also fitted in ASReml (Gilmour et al., 2009):

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zg} + \mathbf{e}$$

Table 2
Phenotypic data, number of records (N-Ref) and heritabilities (h^2) with standard error (s.e.) for eight traits for the reference population of the Sheep CRC Cooperative Research Centre Information Nucleus flock (INF) and the Sheep Genomics Falkiner Memorial Field Station flock (FMFS) (HCWT – hot carcass weight, EMD – carcass eye muscle depth, FatC - carcass C-site fat depth, SF1 – shear force at day 1 after slaughter, SF-5 – shear force at day 5 after slaughter, IMF – intramuscular fat percentage, EPA + DPA + DHA - eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid, EPA + DHA - eicosapentaenoic acid and docosahexaenoic acid).

Trait	INF			FMFS			N-Ref	h^2 ± s.e.[a]
	# Animals	Mean	SD	# Animals	Mean	SD		
HCWT (kg)	4066	22.8	3.7	2125	18.3	2.7	6191	0.35 ± 0.06
EMD (mm)	4020	29.5	3.7	2118	24.7	3.4	6138	0.25 ± 0.05
FatC (mm)	3973	3.9	2.2	2098	2.7	1.4	6071	0.23 ± 0.04
SF1 (N)	2205	37.7	13.1	2099	48.0	14.3	4304	0.27 ± 0.07
SF5 (N)	3406	27.5	9.3	2076	34.8	12.3	5482	0.38 ± 0.08
IMF (%)	3547	4.2	1.0	–	–	–	3547	0.39 ± 0.05
EPA + DPA + DHA (mg/100 g of meat)	2416	49.1	15.0	–	–	–	2416	0.29 ± 0.07 0.11 ± 0.05 0.25 ± 0.06
EPA + DHA (mg/100 g of meat)	2412	24.1	8.9	–	–	–	2412	0.29 ± 0.07 0.25 ± 0.06

[a] (Mortimer et al., 2010).

where **g** is a vector of random genomic animal effects assumed to be from $N(0, \sigma_g^2\mathbf{G})$ and **G** was a genomic relationship matrix, calculated using the method of (Yang et al., 2010). Fixed effects were the same as the association model. **G**s were calculated using all Ovine50 SNP chip, Ovine50 SNP chip plus 182 SNP MQR SNP panel, and only the 182 SNP MRQ panel ($\mathbf{G_{182}}$). The matrix **G** can contain a large sampling error (magnitude ~ 1/N SNP) when the number of SNP used is small, hence we regressed the off-diagonals of $\mathbf{G}_{182}$ towards the mean as shown in (Yang et al., 2010). In our case, the sampling error may be even larger than 1/N SNP, because the SNP are clustered.

2.6. Estimation of effects when fitting significant SNP jointly

An issue in association studies is that the allele substitution effects are overestimated due to fitting single locus regressions and the Beavis effect (Beavis, 1998). We partially accounted for this by jointly fitting all significant SNP in a gene region. However, the SNP effects are likely to be overestimated. All significant SNP with $P < 0.01$, were simultaneously fitted using REML in GenStat (13th Edition, VSN International Ltd., Hemel Hempstead, UK) in traits FatC, EMD, SF1, SF5, IMF percentage, EPA + DPA + DHA and EPA + DHA concentration. The starting models were the same as in the association study, but non-significant fixed effects were removed. The final effects presented are adjusted means ± the standard error of the difference between means.

3. Results

3.1. Carcass quality

Three carcass quality traits (i.e HCWT, EMD and FatC) were investigated by the described association study in the combined INF and FMFS flock. The association study revealed that no SNP were associated with HCWT, EMD and FatC at a significance level of $P < 0.001$. Further investigation revealed two SNP for FatC (i.e. CAPN2_28672486 and CAPN3_38942291) and one SNP for EMD (i.e. CAPN2_28714323) on the 192 SNP MQR panel were significant at $P < 0.01$ (Fig. 1a). After fitting both significant SNP for FatC to a REML model neither SNP remained significant (Supplementary Table 3). When fitting the REML model to the one SNP that was significant for EMD at $P < 0.01$, the data did not converge.

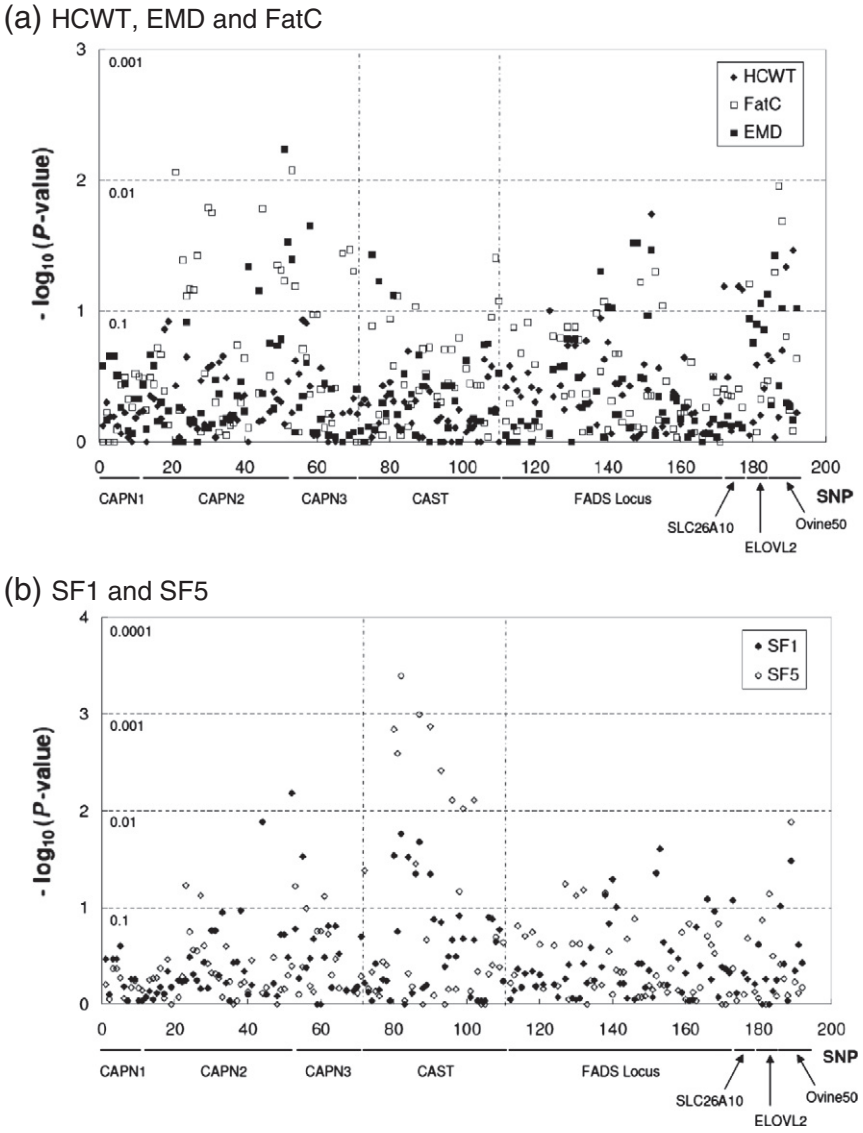

Fig. 1. Association of (a) HCWT, EMD and FatC and (b) SF1 and SF5 determined for the 192 single nucleotide polymorphism on the MQR panel across the calpains (CAPN1/2/3), calpastatin (CAST1), the fatty acid desaturase gene locus (FADS1/2/3), elongase of very long fatty acid gene 2 (ELOVL2), solute carrier family 26 member 10 gene (SLC26A10) and 10 SNP from the OvineSNP50 chip. Each genomic association was determined using the genotypes and the phenotypes of approximately 7000 animals from the Australian Sheep Industry CRC Information Nucleus flock and Sheep Genomic's Falkiner Memorial Field Station flock. Values above $-\log_{10}$ (*P*-value) > 3 are equivalent to *P*-value < 0.001.

3.2. Shear force – SF1 and SF5

The independent validation association study identified a SNP that was significant at $P < 0.001$ for SF5 in the combined INF and FMFS flock (Table 3). This SNP was located in the CAST gene at position CAST_101783060 (Fig. 1b) and is located between Exon 1 - 2 of the CAST gene. The false discovery rate calculated for SF5 was 0.175 (Table 3). At the significance level of $P < 0.001$, no associations were identified between SNP within CAPN1, CAPN2 and CAPN3 for SF1 and SF5. Further investigation revealed another 1 SNP and 9 SNP significant at $P < 0.01$ for SF1 and SF5, respectively (Supplementary Table 3). Using REML, all significant SNP at $P < 0.01$ were then fitted jointly to re-estimate the allele substitution effects of individual SNP. For SF1, the 1 significant SNP (i.e. CAPN2_28714686) at $P < 0.01$ did not remain significant when fitted to the REML model (Supplementary Table 3). For the 9 SNP that were significant at $P < 0.01$ for SF5, 3 SNP remained significant at $P < 0.05$ when all SNP were fitted simultaneously to the REML model: CAST_101781475, CAST_101783060 and CAST_101829736 (Supplementary Table 3). CAST_101781475 and CAST_101783060 are located between Exon 1 – 2 and CAST_101829736 is located between Exon 2 – 3 of the CAST gene. The magnitude of effect of each of the 3 significant SNP in the CAST gene ranged from -0.4 N to +2.1 N, with the summed effect of the 3 SNP equivalent to 3.6 N SF5 (Table 4). The MAF for each of these 3 SNP in the CAST gene is shown on Fig. 3. This figure shows that over 74% of Maternal (i.e. Border Leceister), Terminal and Merino sires contain 2 copies of the A allele at CAST_101781475. The size of effect for this allele was +2.1 N (Table 4). For SF5, CAST_101781475 is the only SNP that validates the previous findings reported by Knight et al. (2012) in this independent population.

3.3. IMF percentage

The independent validation association study revealed that no SNP on the 192 SNP MQR panel that were associated with IMF percentage at $P < 0.01$ (Fig. 2).

3.4. Omega-3 PUFA content

In the INF flock, the independent validation association study identified 5 SNP that where significant at $P < 0.001$ for EPA + DPA + DHA concentration (i.e. long-chain fatty acid content of Australian lamb). The 5 significant SNP for EPA + DPA + DHA concentration were s45673, OAR3_144434162, OAR3_115443384, OAR9_48598227 and OAR18_30003500 (Fig. 2 and Supplementary Table 2). Four SNP from the 192 SNP MQR panel were significant at $P < 0.001$ for EPA + DHA concentration. The 4 significant SNP were OAR3_115443384, OAR9_48598227, OAR16_43758108 and OAR18_30003500 (Supplementary Table 2). All significant SNP for EPA + DPA + DHA and EPA + DHA concentration were SNP that were present on the Illumina OvineSNP50 chip. The false iscovery rate calculated for EPA + DPA + DHA and EPA + DHA concentration was 0.035 and 0.044, respectively (Table 3).

Using REML, all 8 significant SNP at $P < 0.01$ were simultaneously fitted to re-estimate the effects for EPA + DPA + DHA concentration (Supplementary Table 4). Only 4 SNP remained significant at $P < 0.05$ when fitting them jointly for EPA + DPA + DHA long chain fatty acid concentration. The effect of each of the 4 significant SNP ranged from -1.67 mg/100 g to 1.28 mg/100 g, with the summed effect of all 4 SNP equivalent to 5.3 mg/100 g EPA + DPA + DHA content of meat (Table 5). For EPA + DHA concentration, the association study revealed 7 SNP that were significant at $P < 0.01$ (Supplementary Table 4). Of the 7 significant SNP for EPA + DHA concentration at $P < 0.01$, only 4 SNP remained significant at $P < 0.05$ when fitted simultaneously – OAR3_115443384, OAR3_144434162, OAR3_166417678 and OAR16_43758108 (Supplementary Table 4). The overall magnitude of each of the 4 significant SNP ranged from -11.0 mg/100 g to 0.84 mg/100 g of meat (Table 5). However, the allelic variation observed for OAR3_166417678 was minimal (Fig. 4), thus we have removed this SNP for the summed effect of these SNPs on EPA + DHA concentration. Thus, the summed effect of the remaining 3 significant SNP is equivalent to 2.4 mg/100 g of meat (Table 5).

Table 3
Number of loci below significance level $P < 0.001$ for traits in the association study for 192 Meat Quality Research panel. False discovery rates were calculated as expected number of significant associations divided by actual number found significant.

Trait	N < P 0.001 in INF	False Discovery Rate (FDR)	N < P 0.001 in INF + FMFS	False Discovery Rate (FDR)
HCWT	0	–	0	–
FatC	0	–	0	–
EMD	0	–	0	–
SF1	0	–	0	–
SF5	1	0.175	1	0.175
IMF	0	–	N/A	
EPA + DPA + DHA	5	0.035	N/A	
EPA + DHA	4	0.044	N/A	

Table 4
Estimated effects on shear force at day 5 (SF5) after slaughter (in Newtons) for 0, 1 and 2 copies of each allele of each single nucleotide polymorphism (SNP) when fitted simultaneously in a linear mixed model analysis.

Trait	SNP	0	1	2	SED	P-value
SF5 (N)	CAST_101781475 G > A	0	1.2	2.1	1.4	<0.001
	CAST_101783060 A > G	0	−0.4	0.9	0.4	0.008
	CAST_101829736 T > C	0	−0.2	0.6	1.2	0.032

3.5. Phenotypic and genetic variance

Using previous data reported by Knight et al. (2012) and along with the data reported in this paper, the phenotypic and genetic variance explained for SF5, IMF percentage, EPA + DPA + DHA and EPA + DHA concentration was calculated (Table 6). Genomic relationship matrices were calculated from the OvineSNP50 chip, OvineSNP50 chip plus the 182 new SNP and the 182 SNP alone. The phenotypic variance explained (h^2) increased slightly for SF5 when the OvineSNP50 chip plus 182 new SNP were used. The proportion of the genetic variance explained by the 182 new SNP was greatest for SF5 (0.10). Nonetheless, these results show that there could be value in adding more SNP to routine analyses of SF5.

4. Discussion

The main objective of this study was to validate SNP on the 192 SNP MQR panel that are associated with improving carcass quality, SF1, SF5, IMF percentage and omega-3 PUFA content of Australian lamb in an independent population.

4.1. Carcass quality

The first carcass quality trait investigated was HCWT. HCWT is an important phenotype for lamb producers as this is the primary character in which payment is made on lambs sold over the hooks. HCWT is highly correlated to cold carcass weight (r ~ 0.98) (Lambe et al., 2009) and is used routinely to predict many other carcass quality traits such as primal cut weights, wholesale and tray-ready cuts expressed as a percentage of carcass weight (Berg, Forrest, Thomas, Nusbaum, & Kauffman, 1994; Garrett, Savell, Cross, & Johnson, 1992; Hopkins, Wotton, Gamble,

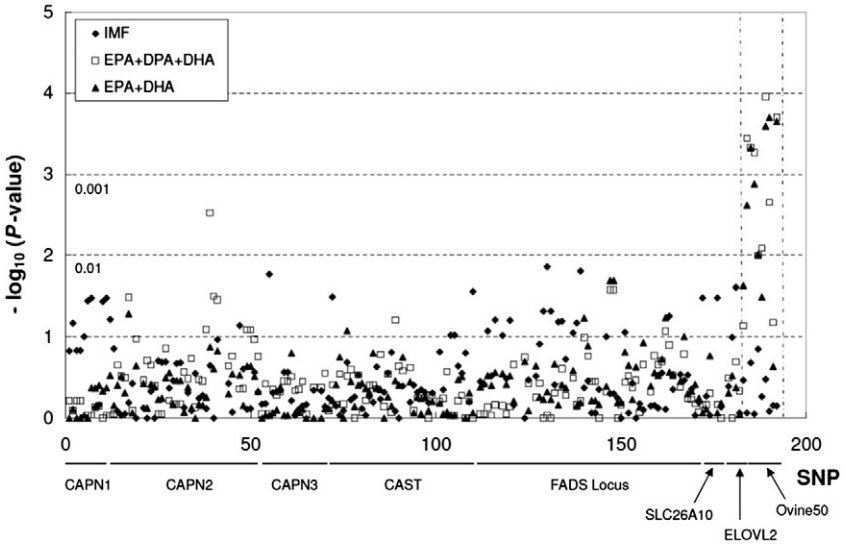

Fig. 2. Association of IMF percentage and omega-3 long chain PUFA content (EPA + DPA + DHA and EPA + DHA) determined for the 192 single nucleotide polymorphism on the Meat Quality Research chip across the calpains (CAPN1/2/3), calpastatin (CAST1), the fatty acid desaturase gene locus (FADS1/2/3), elongase of very long fatty acid gene 2 (ELOVL2), solute carrier family 26 member 10 gene (SLC26A10) and 10 SNP from the OvineSNP50 chip. Each genomic association was determined using the genotypes and the phenotypes of approximately 4000 animals from the Australian Sheep Industry CRC Information Nucleus flock. Values above -$\log_{10}$ (*P*-value) > 3 are equivalent to *P*-value < 0.001.

Atkinson, Slack-Smith, et al., 1995). This study identified no SNP on the 192 SNP MQR panel were associated with HCWT at $P < 0.01$ (Fig. 1a).

It is well documented in the literature that lambs sired by rams with high breeding values for fatness had better tenderness sensory scores than lambs sired by rams with lower breeding values for fatness (Hopkins, Hegarty, & Farrell, 2005; Hopkins et al., 2007). Thus, any knowledge that improves our understanding of how fat is deposited across the carcass or our ability to genomically predict carcass fatness will be valuable to lamb producers. FatC is one carcass quality trait that fits this category. FatC has been shown to influence lean meat yield and GR tissue depth (Gardner et al., 2010; Hopkins, Wotton, Gamble, & Atkinson, 1995). This trait is a measure of fat depth taken at the C-site in a quartered carcass and is adjusted to constant weight. FatC is reported in millimetres and correlates with the ultrasonic measure of C-fat (PFAT) in the live animal (Hopkins, Wotton, Gamble, Atkinson, 1995). Two SNP – CAPN2_28672486 and CAPN3_38942291 had $P < 0.01$ for FatC. However, when both SNP were fitted to a REML model, both SNP were not significant at $P < 0.05$ (Supplementary Table 3).

EMD is another important carcass quality trait. Rams with higher breeding values for EMD produce lambs with more muscle independent of weight and have a higher lean meat yield (Gardner et al., 2010; Gilmour, Luff, Fogarty, & Banks, 1994; Hopkins, 1990). Only 1 SNP (i.e. CAPN2_28714323) on the 192 SNP MQR panel was able to predict EMD at $P < 0.01$.

Table 5
Estimated effects for omega-3 fatty acid content (in mg/100 g of meat) and intra-muscular fat (IMF) percentage (in %) for 0, 1 and 2 copies of each of each significant single nucleotide polymorphism (SNP) when fitted simultaneously in a mixed model analysis.

Trait	SNP	0	1	2	SED	*P*-value
EPA + DPA + DHA (mg/100 g)	s45673 T > C	0	0.02	1.23	0.415	0.013
	OAR3_115443384 T > G	0	0.04	1.15	0.456	0.039
	OAR3_144434162 T > C	0	0.24	1.28	0.447	0.005
	s50854 C > T	0	-0.55	-1.67	0.466	0.010
EPA + DHA (mg/100 g)	OAR3_115443384 T > G	0	0.36	0.84	0.267	0.006
	OAR3_144434162 T > C	0	0.17	0.82	0.262	0.003
	OAR3_166417678 A > G	0	-0.17	-11.0	3.085	0.039
	OAR16_43758108 A > G	0	0.58	0.73	0.270	0.049

4.2. Shear force – SF1 and SF5

Genomic association studies in cattle have identified SNP in the CAPN1, CAPN2 and CAPN3 genes that correlate with meat tenderisation (i.e. shear force). Several of these quantitative trait loci are located in the vicinity of the CAPN1 gene on chromosome 29 (Casas et al., 2003; Smith, Casas, Rexroad, Kappes, & Keele, 2000). Two non-synonymous SNP and two intronic SNP in CAPN1 have been significantly associated with meat tenderness in *Bos indicus* and *Bos taurus* (Page et al., 2004; White et al., 2005). In cattle, several SNP in the CAST gene have also been associated with improving shear force (i.e. meat tenderness). The most successful being in the 3′ UTR region of the CAST gene (Barendse, 2002). Several studies have shown that this gene marker is significantly associated with meat tenderness in *Bos taurus* and *Bos taurus* x *Bos indicus* beef cattle (Casas et al., 2006; Curi et al., 2009; Morris et al., 2006). Another example in cattle, is a single SNP in the CAST gene that is responsible for a 0.37 kg ± 0.17 kg (i.e 3.7 N ± 1.7 N) change in shear force for the longissimus muscle at day 7 (Schenkel et al., 2006).

Early association studies in sheep were not as successful at identifying any SNP in the CAST gene or CAPN3 gene associated with improving meat tenderness (Byun, Zhou, & Hickford, 2009; Zhou, Byun, Frampton, Bickerstaffe, & Hickford, 2008; Zhou, Hickford, & Fang, 2007). However, recent studies by Knight et al. (2012) identified two SNP in CAST (CAST_101781475 and CAST_101841509) and one SNP in CAPN2 (CAPN2_28667683) that were associated with improving SF5. The summed magnitude of effect for all 3 SNP associated with improving SF5 was estimated to be 4.1 N (Knight et al., 2012).

This paper identified 3 SNP in the CAST gene (CAST_101781475, CAST_101783060 and CAST_101829736) that predict SF5 at $P < 0.05$ (Table 4). The summed magnitude of effect of all 3 significant SNP for SF5 was 3.6 N. The size of this effect for is meaningful from an industry perspective considering the estimate cut-off level for consumer acceptability of lamb tenderness is approximately 27 N shear force (Hopkins, Hegarty, Walker, & Pethick, 2006b). In the INF and the FMFS flock, the mean SF5 was 27.5 N ± 9 N and 34.8 N ± 12.3 N, respectively (Table 2). Based on the magnitude of effect on SF5 (i.e. 3.6 N); and the distribution of allele frequencies ranging from 15% to 60% for all significant SNP genotypes across breed types (Fig. 3); these findings suggest that the significant SNP are potentially useful to the Sheep Industry to improve meat tenderness.

Table 6
Additive (Var(A)) and phenotypic (Var(P)) variance, and proportion of additive (Prop(A)) and phenotypic (Prop(P)) variance explained by GBLUP models using Gs calculated using only the SNP on the Ovine50 SNP chip, Ovine50 SNP chip plus 182 new SNP from the 192 MQR panel, and 182 new SNP from the 192 MQR panel regressed to account for sampling.

SNP used	Variable	SF5	IMF	EPA + DPA + DHA	EPA + DHA
OvineSNP50	Var(A)	0.15	0.34	11.3	4.2
	Var(P)	0.66	0.76	60.1	20.3
	Prop(P) (h^2)	0.22	0.44	0.19	0.21
OvineSNP50 + 182 MQR panel	Var(A)	0.15	0.33	11.08	4.21
	Var(P)	0.66	0.76	60.1	20.3
	Prop(P) (h^2)	0.22	0.44	0.18	0.21
182_{Reg}	Var(A)	0.02	0.01	0.60E-05*	0.82E-06*
	Var(P)	0.65	0.73	59.1	20.0
	Prop(P) (h^2)	0.02	0.01	0.0	0.0
	Prop(A) = $Var(A_{182})/Var(A_{50k+182})$	0.11	0.02	0.0	0.0

* Fixed at boundary.

4.3. Omega-3 PUFA content

GWAS have shown there is a strong relationship between SNP located at the FADS locus (FADS1/2/3), ELOVL2 and SLC26A10 genes and omega-3 levels in human plasma (Tanaka et al., 2009). Nutritional studies in humans, other monogastrics and ruminant livestock have shown that there is poor conversion of precursor short-chain omega-3 PUFAs such as linolenic acid (n-3 C18:3, rich in green forage), into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). This paper identifies several SNP on the OvineSNP50 chip that were able to improve the prediction power for omega-3 PUFA content at $P < 0.01$. Based on the magnitude of effect on EPA + DPA + DHA concentration (i.e. 5.3 mg/100 g of meat) and EPA + DHA concentration (i.e. 2.4 mg/100 g of meat) and the allele distribution frequencies ranging from 8% to 37% for all significant SNP genotypes across Merino-, Maternal- and Terminal-sired progeny, with the exception of OAR3_166417678 which has a poor allelic distribution (Fig. 4); all significant SNP identified have good potential utility to improve omega-3 PUFA content in Australian lamb. However, the biological relationship between these significant SNP and omega-3 PUFA metabolism (i.e. omega-3 fatty acid content in muscle) remains unknown. Further investigation is required to determine the potential biological link between these SNP and omega-3 PUFA metabolism. It is interesting to note that no SNP located in the FADS1, FADS2, FADS3, SLC26A10 and ELOVL2 genes were associated with omega-3 PUFA content in lamb meat. This observation was not consistent with the findings reported by Tanaka et al. (2009) who reported several SNP in these genes that associated with omega-3 PUFA levels in human plasma. However, our result is not that surprising because of the species variation between humans and sheep and also a difference in the tissue measured, meat versus plasma.

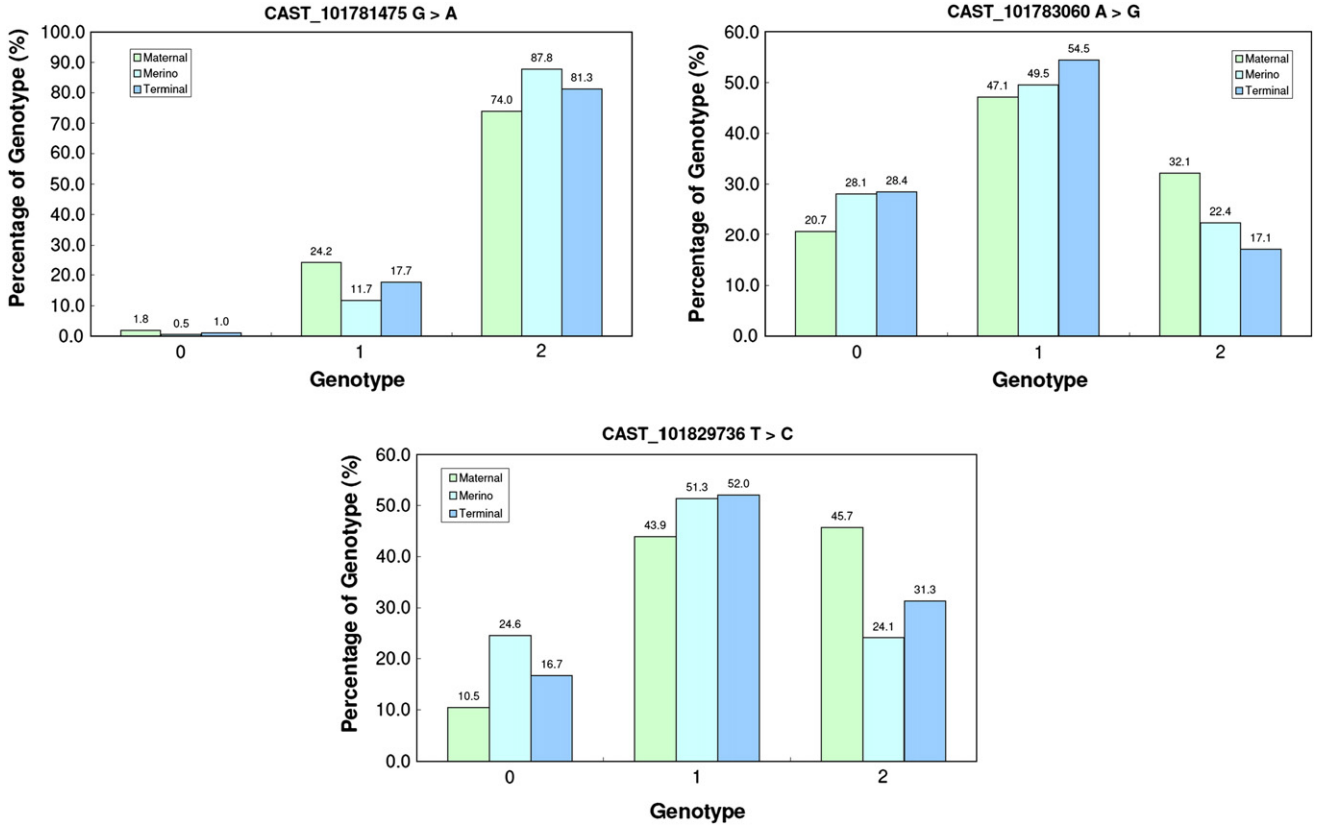

Fig. 3. Proportional distribution of genotypes with 0, 1 or 2 copies of each allele for SF5 (CAST_101781475, CAST_101783060, and CAST_101829736) in progeny from Maternal-, Merino- and Terminal-sired animals from the Information Nucleus Flock and the Falkiner Memorial Field Station flock.

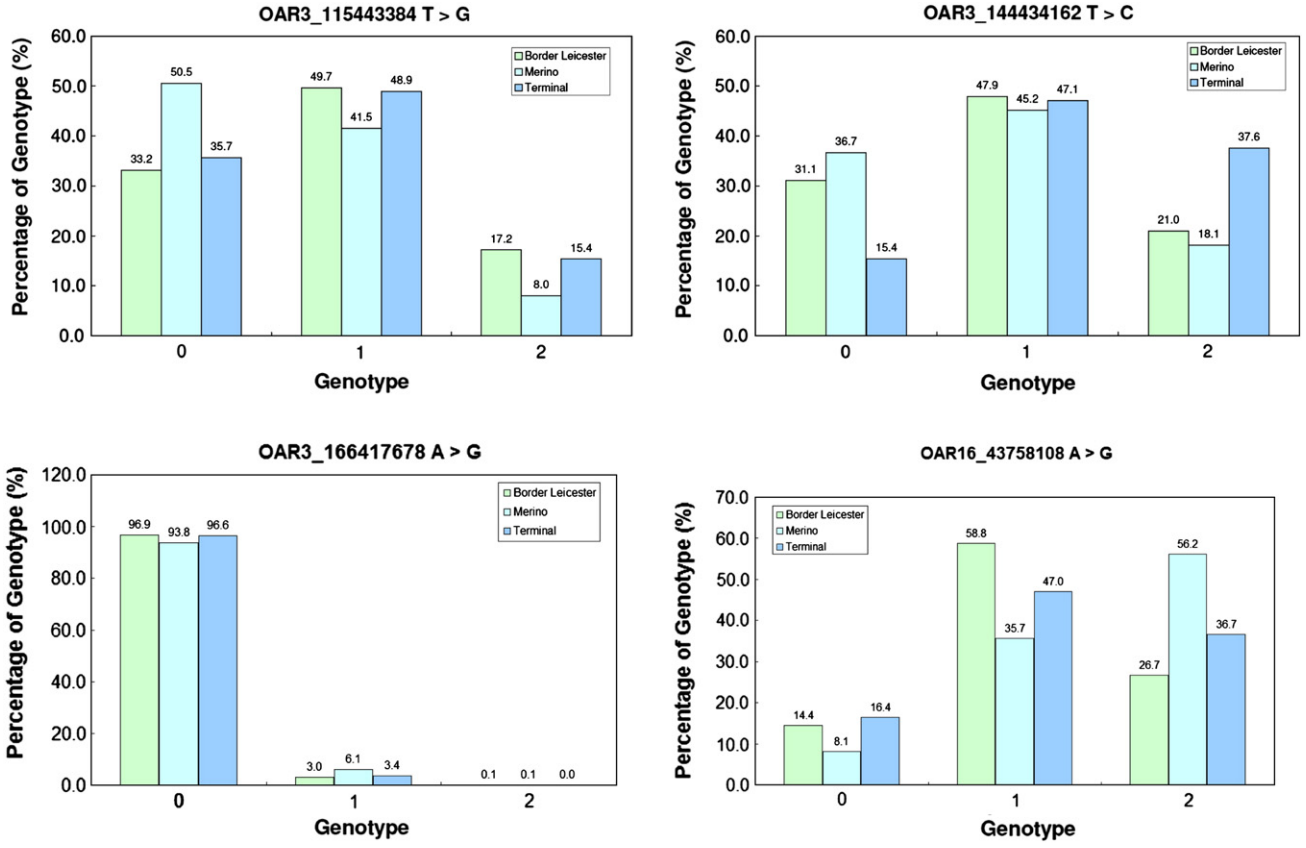

Fig. 4. Proportional distribution of genotypes with 0, 1 or 2 copies of each allele for EPA + DPA + DHA concentration (OAR3_115443384, OAR3_144434162, s45673 and s50854) and EPA + DHA concentration (OAR3_115443384, OAR3_144434162, OAR3_166417678 and OAR16_43758108) in progeny from Maternal-, Merino- and Terminal-sired animals from the Information Nucleus Flock.

5. Conclusions

One SNP (i.e. CAST_101781475) of the 3 SNP that were associated with SF5 reported by Knight et al. (2012) was independently validated in this population of sheep. We also report that several SNP on the OvineSNP50 chip were associated with omega-3 PUFA content in Australian lamb. Considering the size of the effects on each phenotype, while realising that they are likely to be overestimated, and the relative allele frequencies across breed types (i.e. Maternal-, Terminal- and Merino-sired progeny), these SNP will provide an additional resource for the management of SF5 and omega-3 PUFA content in Australian lamb. We also conclude that including the significant SNP for SF5 on the Sheep Industries OvineSNP50 chip could improve current genomic predictions for SF5.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.meatsci.2013.07.008.

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