final report

Project code: B.SGP.0100

Prepared by: Hutton Oddy, Greg Harper Jason White, James Kijas, Ross Tellam

Date published: 23 March 2009

ISBN: 9781741919646

PUBLISHED BY
Meat and Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

sheepGENOMICS Muscle subprogram

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Executive summary

The sheepGENOMICS Muscle sub-program was established as part of the sheepGENOMICS strategic initiative in late 2003.

The Muscle subprogram employed a Systems Biology approach to using the tools of “new biology” (including genomic technologies) for improving understanding of the biology of muscle and meat related traits.

The Muscle subprogram engaged with an independent Scientific Advisory Committee, comprised of experts in the field, to select projects for those submitted, guide investment into more promising areas and to ensure quality of the science conducted was best in the field. This ensured that although “high risk” studies were entered into they were monitored in a timely and appropriate manner.

It was anticipated that three important outcomes would be achieved during the life of the Muscle subprogram. They were:-

1. Understand how to discover the function of genes that affected muscle and meat development of sheep
2. Discover and exploit genes that influence novel muscle phenotypes
3. Develop the capability to conduct genomics research on production and quality of sheep meat in Australia

Significant components of each of these outcomes has been achieved. They are summarized below.

1. Discovery of the function of genes that influence muscle:-
Key outcomes included:-

- Development of a list of potential candidate genes and metabolic pathways that influence muscle growth and development. This was achieved through studies which obtained extensive gene expression data in selected muscles of Callipyge and normal lambs (both microarray and RT-PCR procedures), animals with and without the putative Carwell haplotype marker and with high and low EBVs for muscle growth. This work included the first use of MPSS to explore gene expression in muscle of CLPG and normal sheep, and new data from a survey of proteins differentially regulated in 4 organelles of muscle from Callipyge and normal sheep.

- Testing of potential candidates from the list of differentially expressed genes and pathways. Testing ranged from study of follistatin like 1 (FSTL1) as a candidate gene that involved discovery of markers within the genomic region and subsequent association studies, to the use of Notch inhibitors (DAPT) and inhibitors of HDAC9 (specific siRNAs) tested in muscle cell lines, DLK1 and SLC22A3 transfection systems tested in immortalised sheep muscle cell lines. Two naturally occurring potential modulatory compounds (Benfotiamine and Resveratrol) were administered in feed to lambs and found to affect the rate of fat and possibly lean deposition.

- Development of the only immortalized sheep muscle cell lines in the world, and use of these cells to evaluate the effect of candidate genes

2. Discovery of genes that influence muscle phenotypes**.**

- Discovery and evaluation of the effects of g+6723G->A and other polymorphisms in the myostatin region on production, carcass and meat eating quality traits in sheep.

- Discovery and evaluation of the effects of a putative Carwell haplotype on production, carcass and meat eating quality traits.

- Discovery and evaluation of the effects of polymorphisms in the calpastatin and calpain 1 and Follistatin Like 1 gene regions on production, carcass and meat eating quality attributes in sheep

- Development and application (in more than 2350 lambs) of new phenotyping procedures to better understand the effects of genes on biology of sheep meat production.
    - New high throughput methods were developed, and applied, for measuring number, cross section and potential aerobicity of muscle fibres
    - Use of Dual X –ray absorption spectroscopy (DXA) on primals and half carcasses to measure chemical composition and estimate retail yield on large numbers of lambs
    - Measurement of carcass, meat yield, meat quality and muscle characteristics on more than 2350 lambs from the sires used at FMFS, and within other industry based populations for evaluation of the effects of gene markers.
    - Measurements of industry growth, fat and muscle phenotypes on more than 4800 lambs from the sires used at FMFS, and more than 1000 other lambs from subsequent FMFS and industry joinings.

The samples obtained from the FMFS lambs have been genotyped with the ovine SNP50 beadchip. Association analysis (between genotype and phenotypes) is currently underway.

3. Development of capability to conduct genomics research on meat sheep

- The Muscle subprogram contributed to the development of new sheep specific genomic resources. These include:-
    - linkage and physical maps,
    - virtual sheep genome,
    - many SNPs and their location on the genome,
    - use of Affymetrix Bovine array for sheep gene expression studies,
    - unique immortalized ovine muscle cell lines.

- The Muscle subprogram developed cross disciplinary (and organization) teams to carry out fundamental muscle R & D for the meat & livestock industry. Some of the expertise in these teams was derived from a much wider range of disciplines and backgrounds than usually associated with meat research.
    - Eight new scientists, post-doctoral fellows and PhD students were exposed to the Australian meat and livestock Industry

- Publication (to date) of more than 10 full papers in refereed journals and over 39 papers / posters / conference communications from participants in the Muscle sub-program

- This capability still remains to be fully engaged with the sheep industry. For example, few of the human and genomics resources developed with the Muscle subprogram are currently engaged with the Sheep CRC.

A number of technical tasks remain to be completed. Those of most immediate interest are listed in the conclusions and recommendations section of this report. There are other tasks that remain to be completed to set up the landscape for the future, these include:-

- The Benfotiamine and Resveratrol trial should be repeated with more and younger lambs. Body composition changes should be measured more thoroughly than in the preliminary study. It is likely that the combination of the two natural compounds may reduce fatness. This may be valuable information for use of these compounds in applications other than manipulation of body composition of sheep (e.g. as additives for human diets to reduce obesity).

- Lists of gene pathways and differentially expressed genes that may be candidates for differential muscle development should be compared with outcomes from the ovine SNP50 beadchip with a view to rapidly locating high priority candidates for gene discovery.

- All the data from evaluation of myostatin, Carwell and other DNA / gene markers should be combined into a single analysis to determine the “best bet” estimate of effects. This information needs to be passed to industry as quickly as possible.

- The shgen.003 DNA should be secured in a safe and readily accessible place and a process to ensure unencumbered access for the future established. It is anticipated that this resource will become more valuable as the need to confirm associations between markers and traits in independent populations is recognized.

- An inventory of samples (DNA / blood / tissue) and all phenotype data from all studies conducted within the Muscle subprogram should be established. This is especially important because to date most of these studies have been used for validation of several markers. They provide an opportunity for evaluation of new markers and new panels of SNPs when they become available.

Given that results from the ovine SNP50 beadchip will become available before July 2009, the following should also be given high priority.

- Key scientists involved with development of FMFS phenotyped resources should remain engaged with the analysis of associations between ovine SNP50 beadchip and meat traits. The purpose of this recommendation is to ensure ongoing engagement of those doing the analysis of genotypic effects with those who have been instrumental in generating the phenotype data and developing hypotheses about the basis of different phenotypes. It is anticipated this will lead to more rapid discovery of underlying mutations. This work will not be undertaken within the Sheep CRC.

- Close collaboration between the ISGC Haplotype Mapping project and sheepGENOMICS muscle subprogram scientists should continue to ensure lessons learned about selection sweeps in the “global” sheep population inform future work on understanding genetic mechanisms that impact on growth and development of muscle. It is not planned that this work will be undertaken by the Sheep CRC.

- Close collaboration between key scientists in sheepGENOMICS and others in the field (SheepCRC and independently) be encouraged to develop DNA / gene

markers that work across sheep breeds (i.e. almost certainly causal mutations). It is anticipated that knowledge of gene function and effect on phenotype will be required to do this, rather than simply associations between markers and traits. It is anticipated that this information will ultimately provide more robust solutions to industry than reliance on associations alone.

Fostering of ongoing collaboration between the International Sheep Genomics Consortium (ISGC), the remnants of sheepGENOMICS, Sheep CRC and Sheep Genetics is of utmost importance. It is recommended that an “integrated pipeline” be established to co-ordinate strategic investment in development of resources in the field, exploration of the possibilities that can come from the application of the technologies that depend of those resources, and delivery of technical solutions for industry.

Contents

1. Background

SheepGENOMICS was designed as a strategic investment to build capability and resources in the field. It was developed with the expectation that significant outcomes would arise from global investments in mammalian genomics (human, model and production animals). It was considered that for the sheep industry to have some control over its destiny in this space it required development of genomics resources, people with skills in the technology, and its application to sheep industry opportunities, within Australia.

Industry targets (improve meat and wool yield and quality, increase resistance to intestinal parasites, improve reproductive rate) were set and used to provide focus within components of the program. The expectation was that new knowledge would come from this program. The methods for delivery were considered at high level only, with the expectation that these would be fine tuned as practical outcomes became apparent.

The context in which the Muscle subprogram was developed is described below from an industry, investment landscape and science implementation perspective.

1.1. Sheep industry context

Demand for sheep meats outstrips worldwide supply. Within the category of animal derived protein foods there is intense competition with alternate food sources (chicken, pork, beef, lamb, fish). Thus, it is imperative that the nature of sheep meat, the efficiency of its production and its quality attributes continue to improve their fit with consumer expectations. Since the initial design of sheepGENOMICS issues which impact the livestock industries other than production and product quality, such as animal welfare, environmental impact and greenhouse gas abatement have increasingly become community concerns.

In the domestic market lamb is positioned away from the low cost product of the 1990's to a high value, highly sought product. It has achieved this through concerted marketing and research efforts to ensure that lamb meets consumer expectations as to size and type of cut, limits to fat content and consistently high eating quality. Recent activities include a Sheep Meat Eating Quality research program, followed by delivery of sheep meat eating quality standards throughout industry. In so doing, sheep meat has positioned itself to export into other high value markets around the world (notably the USA), many of which have similar expectations of product quality as the Australian domestic market.

The Australian lamb industry has grown from a lamb kill of approx 16 million in the late 1990's (of which 90% was sold on the domestic market) to the present kill of 19 million of which 40% is exported. **In value terms this equates to growth from $900 m p.a. to more than $2 bn p.a. over the past decade**. During this transition the proportion of lambs from improved (terminal and specialist maternal) breeds have increased at the expense of Merino, although Merino's constitute the majority of the maternal genetics. The industry continues to strive for increased carcass yield with reduced fatness and improved eating quality, along with reduced cost of feed and increased reproductive performance.

In this context the key industry targets addressed by the Muscle Subprogram of sheepGENOMICS were:-

- Development of gene markers for high value hard to measure traits (lean meat yield, fat content, eating quality, efficiency of feed utilization);
- Development of therapeutic approaches to manipulating lamb growth and composition of the carcass through developing an understanding of the function of major genes affecting meat yield, efficiency of feed utilisation and product quality;

- Development of capability or maintenance of the capacity to achieve the above industry targets over the medium to long term.

1.2. The R&D investment landscape

The Sheep Genomics Program (ShG) was established to foster an investment landscape that facilitated development of genomic resources (tools for gene discovery including BAC library, sequence, improved genetic maps, expression tools) with the intent of enabling researchers to efficiently conduct leading edge R&D for the benefit of the industry and consumers. The ShG Program also sought to coordinate the investments of other research organizations so that there was critical mass and reduced technical risk. It was clear that Australia and New Zealand were best placed globally to capture benefits from these investments and were the most likely countries to invest. Both Australian and New Zealand entities commenced investment in the field in the late 1980's, and while some New Zealand entities continued to invest, Australian entities had ceased investment by the early 1990s. Moreover, predecessors of Meat & Livestock Australia and Australian Wool Innovation Limited had to some extent fragmented the research community, with consequent lack of co-ordination between research agencies. It was clear that a coordinated investment on behalf of all sheep producers, rather than solely for meat sheep and wool sheep was required. Accordingly, sheepGENOMICS was designed to achieve high level co-ordination across investment and research agencies. The model chosen was similar to the original idea of the Co-operative Research Centres program, but with a simpler structure.

Publicly available genomic resources for sheep were limited (the sheep linkage map had less than1000 markers, there were a low number of ESTs, a poor coverage BAC library) and were distributed across many research agencies around the world. For ShG to succeed, it needed to rapidly develop access to sheep specific genomic information. ShG did this primarily through engagement with the international community and by playing a leadership role in development of the International Sheep Genomic Consortium (ISGC). Without the resources developed collaboratively within the ISGC many of the objectives of the industry subprograms of sheepGENOMICS could not have been met. A description of the resources developed through the ISGC is provided in the Core Technologies sub-program report.

1.3. Design considerations for implementation of meat science and sheep production within sheepGENOMICS

The "path to impact" that underpinned the design of the muscle sub-program was to:-

- find specific genes that lead to marked change in phenotype;
- study the mechanism by which these genes and genetic polymorphisms influence phenotype, using the developing tools of Systems Biology;
- discover ways to manipulate the causative pathways to replicate the desirable phenotypes preferably without transgenesis or use of hormonal growth promotants.

There were several research agencies within Australia and New Zealand who were already working on aspects of this path to impact around the time ShG started, but these efforts were not specifically focused on sheep industry issues, or were not well coordinated. To activate this process required specific resources, including animal genotypes and phenotypes but most importantly scientists and research agencies with capability and capacity to develop and exploit gene expression, proteomic, muscle cell biological and phenotypic data. Many of the skills required lay outside those traditionally engaged with animal science, and were more typically found amongst muscle biologists engaged in understanding human diseases. One of the challenges presented to the muscle sub-program leader at the commencement of the sub-program was to engage with a community outside that normally involved in animal production research (such as conducted within the

Beef and Sheep CRCs) and to forge a new team with different but complementary skills. In building a new team the intent was to grow a new capacity to conduct research in muscle biology for the meat industry, and to expand the scope of such research.

In the context of this report, the single major prior investment in resources for gene discovery in sheep meat animals in Australia (MLA, shgen.003, initiated in 2000) took place in an environment where there was insufficient knowledge of the sheep genome (and accordingly access to genomic resources) to be successful. Nonetheless, this well phenotyped resource of greater than 1280 progeny from 15 industry sires became an important first point for exploration of the Carwell and later the Myostatin loci. More recently, the shgen.003 resource has been used to test other candidate genes identified within the Muscle Subprogram. As better knowledge of the sheep genome and more precise physical maps become available the value of the shgen.003 resource will come to complement resources subsequently developed by sheepGENOMICS (described in more detail in this report) and in the future, the Sheep CRC.

To implement the Sub-program's path, the team needed to access animals with a known major gene of large effect on muscle phenotypes. Lambs with the Callipyge and Carwell mutations were chosen as the initial experimental models. The former is a single point mutation in an intergenic region that has a large effect (~40% increase in mass) on specific muscles. Carwell on the other hand, has only a small effect (<10% increase in mass of a specific muscle) and the responsible mutation has not been elucidated. It was anticipated that during the course of the sub-program's research that new genes of moderate to large effect would be discovered (by genome association scans) to allow the methods developed for investigation of the Callipyge mutation to be applied to identify causal mechanisms. That was unable to be realized, because the first results from genome wide association studies on the progeny from FMFS were not available until mid 2009.

2. Planned outputs from the Muscle Subprogram

The muscle sub-program was established to build capacity to deliver upon the following technical and industry objectives

2.1 Scientific outcomes

To discover gene markers and use these to develop tools for improved rates of selective breeding

- Gene markers that account for significant variation in key traits
- Panels of gene markers that account for significant variation in a number of key traits
- Panels of gene markers derived from genome wide screens that contribute an increase in accuracy for existing traits and new breeding values for novel traits

To use knowledge of effects of major genes and a Systems Biology approach to important meat industry traits to develop novel therapeutics and diagnostics:

- New targets to modulate key meat related traits
- New therapeutics / modulators that can alter meat related traits without the need for genetic selection
- New diagnostic and phenotyping procedures to aid both phenotypic and genetic selection of sheep for improved meat and production efficiency related traits

2.2 To develop or maintain Australian R&D capacity to use genomic tools effectively and deliver novel information for the benefit of the sheep industry and the community

- Gather and support a team of researchers with a strong focus on delivering to industry new technologies that utilise an understanding of the function of genes for meat production and quality;
- Propose and test an effective gene discovery system;
- Develop an understanding of how to combine data from variation in genome structure, gene expression, protein profiles, muscle cellular phenotypes (behaviour of cells, location of protein, location of expressed genes), and industrial phenotype to provide a biological basis for improvement in meat related traits in production animals;
- Provide the skills to implement genomic tools for industry development

3. Structure and Focus of the Muscle sub-program

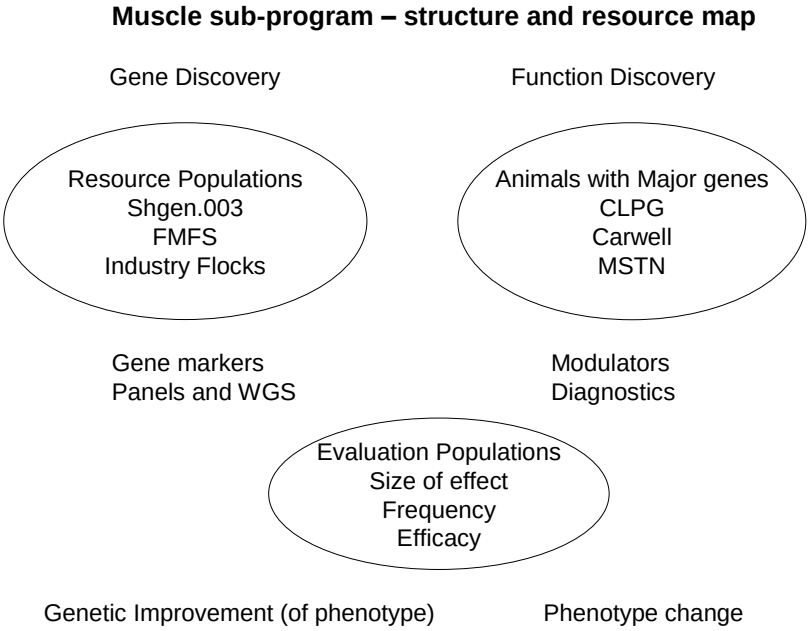

Figure 1. The muscle sub-program had two major themes; gene discovery and discovery of function of genes. Outcomes at industry level are methods for further genetic improvement and methods for modulating phenotype without necessarily changing the underlying genotype. To achieve these outcomes requires discovery of new or expansion of knowledge on existing gene markers, and from knowledge of how the genes work to develop modulators or diagnostic tests that improve productivity and / or product quality. This map provides an overview of the program intent, and shows animal resources used throughout the sub-program.

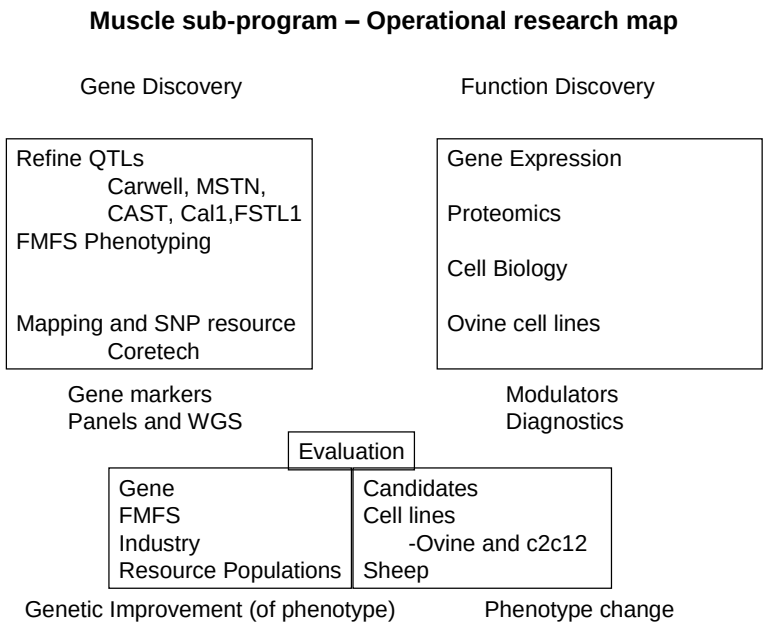

Figure 2. Research activities within the muscle sub-program. This map shows the breakdown of sub-program activities within the gene discovery and function discovery streams. They have come together during the evaluation phase, and during the research phase there was continual cross-talk between the two themes.

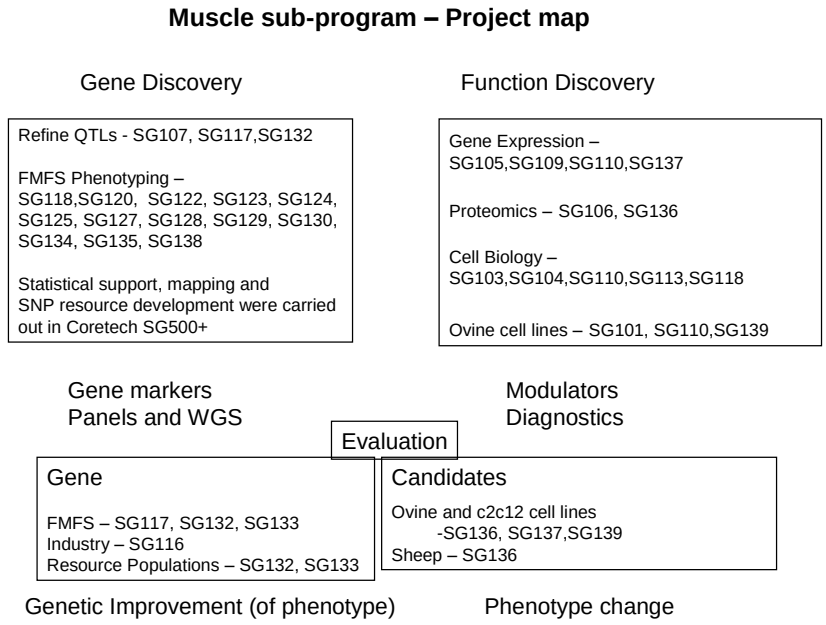

Figure 3. Project map for the Muscle sub-program. This shows the projects implemented during the sub-program and their interdependencies. The body of this report refers to outputs from each project and the way they provided information for
other projects to develop further.

4. The participants in the Muscle Subprogram

The following research organisations and personnel were involved in the subprogram.

Research Organisation	Researcher	Technical staff	Postdoctoral Fellows	PhD students
University of Melbourne	Prof Eleanor Mackie Dr Jason White Dr Charlie Pagel	Su Toulson	Dr Lopeti Lavulo	Chris Gorman
Department of Primary Industries, Victoria	Dr Matthew McDonagh Dr Brendan Tatham Dr Robyn Warner	Daniel Huylett Matt Kerr	Dr Mathew Knight Dr Eva Ostrovska	
CSIRO LI	Dr Ross Tellam Tony Vuocolo Dr James Kijas Dr Greg Harper Dr Peter Allingham Dr Chris Bidwell (on leave from Purdue University)	Keren Byrne Russell McCulloch Vicki Whan		Jennifer Meadows Thu Nguyen Jolena Fleming (Purdue University)
University of Western Australia	Dr Jason White Prof Miranda Grounds (Member Scientific Advisory Committee)	Joseph Laundy Marilyn Davies Melissa Berg		Thu Nguyen
University of New England	Prof Julius van der Werf Prof Hutton Oddy	Jason Siddell	Dr Cedric Gondro Dr Hong Lee	
Liggins Institute (Auckland University)	Dr John Bass (Member Scientific Advisory Committee)			

5. Research activities and outcomes from the Muscle sub-program

5.1 Discovery of gene function

The discovery of gene function component of the muscle sub-program utilised single mutations of large effect on muscle phenotype to increase the chances of determining the underpinning mechanisms that affect muscle development and its consequences.

The Callipyge (CLPG) mutation was selected because of the magnitude of enhanced muscle development of the hindquarters - up to 40% increase in affected muscles. Affected animals exhibited site specific muscle hypertrophy (Koohmaraie et al, 1995), most of which is associated with an increase in the proportion of fast-twitch glycolytic myofibres (Carpenter et al, 1996; Vuocolo et al, 2007). The causative mutation is in an imprinted region at the distal end of oar18 (Freking et al, 2002). Animals with the CLPG mutation display an unusual mode of inheritance (polar over-dominance) in which only those animals carrying the paternally derived allele express the phenotype (Cockett et al, 1996).

In addition to animals with the CLPG mutation, animals with the Carwell phenotype (which results in ~ 10% increase in cross-sectional area and weight of loin muscle) were also chosen for further study. Carwell was considered a complementary model to CLPG, because the muscle phenotype was also site specific (loin but not other muscles) and although not allelic to CLPG it is located near the same imprinted region at the distal end of oar18. Unfortunately the causative mutation for Carwell was not known and it was hoped that during the course of the work, that it would become known (at the time of writing -March 2009 - this had yet to happen). Moreover, at the time of choosing Carwell as a model, the available evidence suggested that the effect was additive (i.e. quite different mechanism to the polar over-dominant mode of inheritance of Callipyge). There was an expectation that because the putative Carwell mutation region was close (within 1 cM) to Callipyge, and the phenotype effect was similar (localized rather than general muscle hypertophy) that knowledge of site specific regulation of muscle development would apply generally and assist in location of the causative mutation for Carwell.

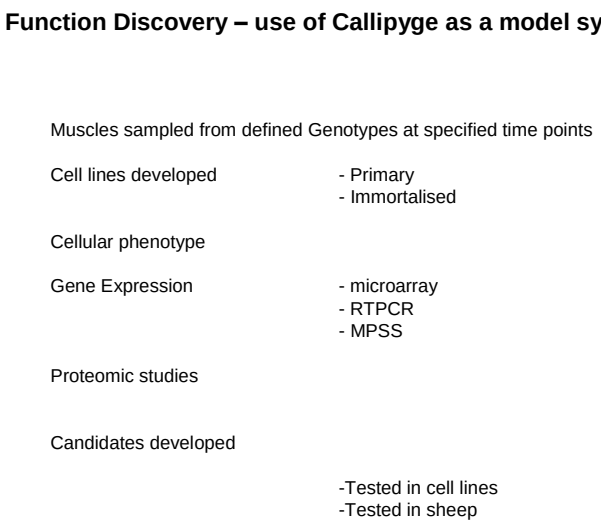

Figure 4. Overview of use of Callipyge as a model system for understanding how to discover gene function and develop and test novel candidate genes and pathways.

5.1.1 Projects that utilized Callipyge (CLPG) animals

5.1.2 Characterisation of CLPG cellular phenotype

Projects that addressed this goal:- SG103, SG104, SG110, SG113, SG118, SG130 Principle investigators – Jason White, Greg Harper, Eleanor Mackie, Matthew McDonagh, Paul Greenwood

Samples from lambs from the four possible CLPG genotypes (CN, NN, CC and NC) and from affected and unaffected muscles were collected at 80, 100, 120d PC and 1d and ~70d PN. These samples correspond with secondary to tertiary fetal muscle development (80-100d PC) and hypertrophy (120d PC to 1, 70 d PN). The samples were collected in the USA, in collaboration with Prof. Noelle Cockett's team at Utah State University.

Results confirm the switch to type IIX fibres in affected muscles. By 72d PN more than 90% of fibre cross-sectional area (CSA) in ST and SM of CN (phenotypically affected) animals are type IIX fibres, compared with less than 75% in NN (phenotypically normal) animals. Associated with the increases in CSA attributed to type IIX fibres was a small reduction in contribution of type I muscle fibres to cross-sectional area of CN cf NN animals (White et al, 2008), and a significant reduction in the proportion of type IIA fibres in CN lambs (Greenwood 2008 SG130). All type II fibres in CN lambs had larger CSA than in NN lambs. Unfortunately the published data refer mainly to the LD muscle, which although a muscle of major industry concern, is less responsive than ST and SM in CN cf NN lambs. (Note LD = m. longisimus dorsi, ST = m. semitendonosus SM = m. semimembranosus, SS = m. supraspinalis)

By 120 d post conception (PC) expression of Delta-like 1 (Dlk1), a protein encoding gene immediately adjacent to the intergenic callipyge mutation, in affected muscles of CN animals was significantly higher than in either unaffected muscles of CN animals or muscles of NN

animals generally. Elevated Dlk1 expression continues until at least 72 d after birth (PN) (White et al, 2008). In an independent study it was demonstrated that enhanced muscling and elevated Dlk1 expression was present in callipyge sheep at least 18 months PN (Tellam and Vuocolo, unpublished observation).

Primary cells lines isolated from ST and LD muscles of 120d fetal lambs of each CLPG genotype (CN, NN) demonstrated that myoblasts from CN ST proliferated faster than from NN ST, and that cells isolated from ST and LD CN muscle were more resistant to serum deprivation induced apoptosis than equivalent cells isolated from NN animals (Lavulo et al, 2008). These observations suggest that there are intrinsic differences in the behaviour of isolated myoblasts associated with their muscle and genotype of origin.

Independent analysis of the primary cell lines (by Thu Nguyen, Sheep CRC PhD scholar) has shown that proliferation of CN primary myoblasts is ~20% faster than NN primary myoblasts (confirming the observations above by Lopeti Lavulo). CN myotubes are consistently larger (almost 2x wider) and have more myonucleii (approx 50% more) than NN myotubes.

Together, these results suggest that there are intrinsic differences in behaviour of primary myoblasts and myotubes derived from CN and NN muscles. The most striking is that myotubes from primary cell lines from muscle of CN lambs are larger and have more nuclei than myotubes from NN lambs.

Gene expression analysis of muscle tissue (this project, see below, and Davis et al, 2004) consistently show over expression of Dlk1 mRNA in effected muscle from adult animals of the CN genotype (transgenic Dlk1 over-expression in mice also causes muscle hypertrophy but not to the same extent as in callipyge sheep). It was initially surprising that we were unable to show any expression of Dlk1 in primary or immortalized ovine muscle cells grown in culture, however, this has been a repeatable observation in all studies involving cultured muscle cells from CLPG animals.

These observations raise a question about the cellular origin of Dlk1 RNA and protein. Polyclonal antibodies show localization of Foetal Antigen 1 (FA1, soluble component of Dlk1 corresponding to its extracellular domain) to muscle cell membranes, and in particular to Type II muscle fibres and to Pax-7 staining cells (muscle satellite cells). Dlk1 is primarily a membrane bound protein with an extracellular EGF domain that is thought to be a Notch ligand. The protein also undergoes proteolytic processing to produce a circulating soluble form analogous to FA1. It would be expected that Dlk1 (FA1) protein should be found in locations where Notch is present, but where is Dlk1 made?

Yevtodiyenko and Schmidt (2006) have demonstrated that significant quantities of Dlk1 mRNA are present in developing skeletal muscle of the mouse. *In situ* hybridisation for Dlk1 mRNA in sheep muscle indicates that the cellular source of Dlk1, at 120 days post conception (30 days before birth) is fibroblasts in connective tissue not muscle fibres (Gorman and White, Pers Comm). This is consistent with Yevtodiyenko and Schmidt's (2006) suggestion that Dlk1 is more highly expressed in proliferating rather than differentiating cells of the embryo, and has a role in regulation of branching morphogenesis. The high tissue levels of Dlk1 mRNA suggests that CN sheep might also differ in wool follicle morphology, consistent with anecdotal evidence that CN sheep have different fleece characteristics (finer, tighter) to NN sheep (Matt McDonagh, pers comm.). Skin samples are available from CN and NN lambs and will be used to determine if there are differences in wool follicle patterning consistent with increased branching of follicles.

Unpublished observations from Chris Bidwell suggest that Dlk1 is expressed in primary muscle cultures but this disappears during passaging indicating that as a cell becomes less

"stem" like after extraction it loses Dlk1 expression, which is similar to what is seen with Pax7. The latter is a marker for myogenic stem cells. This is consistent with observations that Dlk1 localises with Pax7 positive cells during development but the association is lost over time. This points to an extracellular cue which seems to be required to recapitulate the genotype specific gene expression program and is involved in development of the callipyge phenotype. These observations also suggest a limitation to the usefulness of tissue culture as a way of studying the callipyge mutation. There are three pieces of evidence that implicate some level of extracellular regulation of the callipyge muscle phenotype:

1. A soluble serum derived signal which has been implicated in the study by Rodiguez et al (2001) that shows serum from callipyge animals induces myoblast proliferation irrespective of the genotype of the myoblasts; this may not be IGF1 as there is no increase in serum IGF1 in callipyge (Whisnant, 1998). Preliminary evidence suggests that callipyge cells may be more responsive through constitutive phosphorylation of Akt1.
2. Another environmental cue could be from the connective tissue itself (where some Dlk1 is located); Chris Gorman has generated data which shows that if you use laminin (a protein of the connective tissue matrix) as a matrix substrate then C2C12 cells show evidence of hypertrophy.
3. Innervation has been shown to have very large effects on muscle cells and their gene expression programs. The lack of innervation in tissue culture may underlie the inability of these cells to duplicate the genotype specific effects of the callipyge mutation.

Site specificity of Dlk1 expression may be modulated by regulation of expression through different methylation status of the genomic region around the Callipyge mutation. Vuocolo et al (SG109) have demonstrated hypomethylation of CpG islands of the paternal allele around the CLPG locus on oar18 in CN cf NN sheep. The pattern of methylation between normal (NN) and Callipyge (CN) lambs differs between affected (LD) and unaffected (SS) muscle at 12 weeks of age, but not in the foetus, consistent with tissue specific regulation of DLK1 expression.

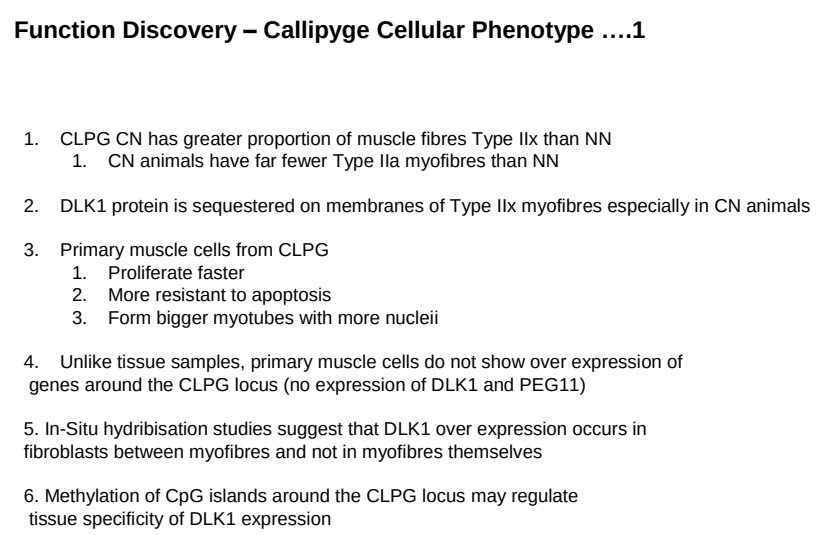

Figure 5. Summary of differences in Cellular Phenotype between normal (NN) and paternal Callipyge (CN) animals expressing the Callipyge phenotype.

5.1.3 Transcriptional analysis of tissues from normal and Callipyge lambs – development of candidate genes and pathways

Projects:- SG105, SG109, SG110, SG137
Principal Investigators :- Tony Vuocolo, Ross Tellam

The samples collected from CLPG animals were used as described in Section 7.1.2 above. A developmental time series analysis using the Affymetrix Bovine Expression array indicated a major switch in patterns of gene expression between 120d PC and 1 d PN (Figure 6, below) .

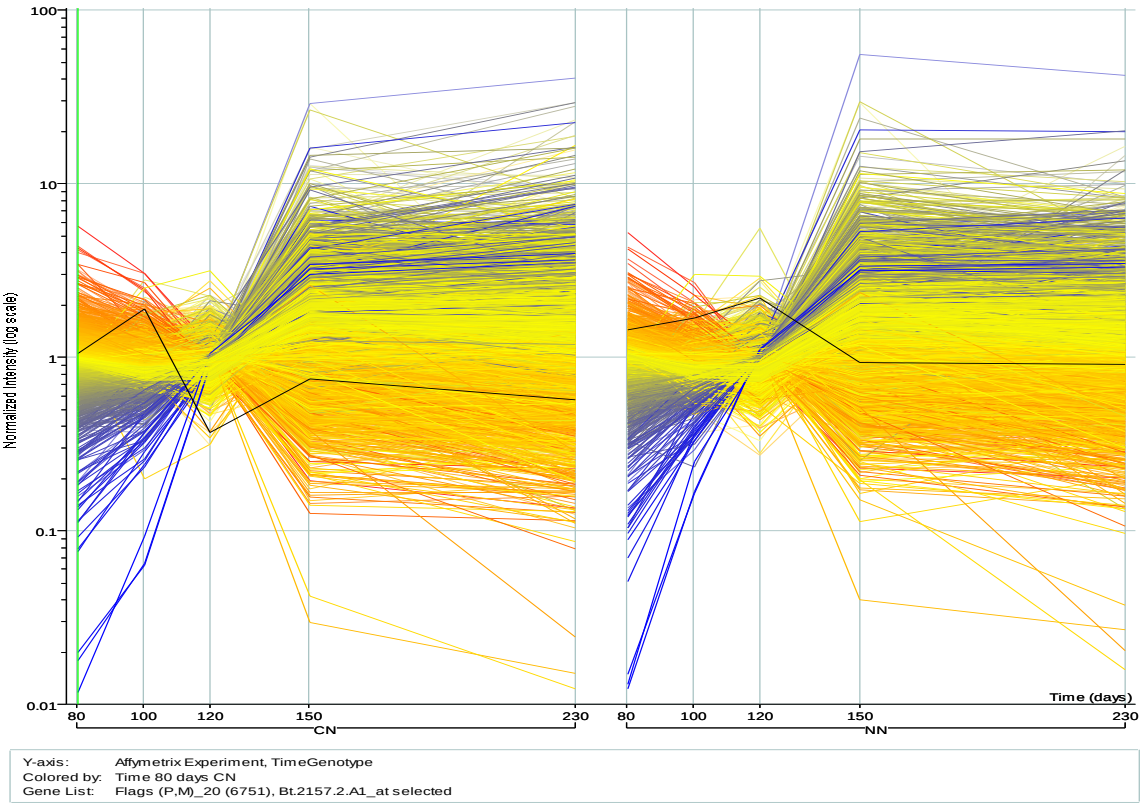

Figure 6. Developmental Switch in Gene Expression in muscle of Callipyge (CN) and normal (NN) lambs. Left hand panel =CN, Right hand panel = NN lambs. Data generated using m RNA extracted from muscle samples and applied to Affymetrix Bovine gene expression array.

Summary

- There is a substantial change in the pattern of gene expression of skeletal muscle just prior to birth of NN animals. This could be interpreted as a time / development related switch in gene expression that ensures that the muscle and therefore the newborn animal is prepared for immediate use after birth.
- The CLPG mutation over-rides a major developmental switch (in sofar as Dlk1 expression continues at a fetal level into post-natal life), in affected muscles thereby producing a fetal like gene expression program in post-natal samples.
- Dlk1 is the probable ‘effector’ of the Callipyge phenotype but the involvement of Peg11, or both genes, cannot be ruled out.
- Peg11 is expressed as a protein in Callipyge muscle. Until this work, it was thought that PEG11 did not code for a protein and that no PEG11 protein was present in mammals.

- Dlk1 up-regulation is present before birth and this probably orchestrates gene expression changes that underpin the post-natal phenotype.
- Several hundred genes on chromosomes other than where the mutation has been reported are affected by the CLPG mutation (trans-affected gene expression changes). These include for example SLc22A3, HDAC9 and an anonymous, methyl transferase. All gene expression data and the genes that are differentially expressed have been deposited with the sheepGENOMICS bioinformatics core database facility. These genes were prioritised for future functional analyses using an extensive set of scientific and industry selection criteria.
- Epigenetic mechanisms underpin Callipyge-induced muscle hypertrophy. Time and genotype dependent differences in DNA methylation around the CLPG locus suggest there is a difference in access of transcriptional machinery to that region in CLPG (CN) vs normal (NN) lambs.
- The use of transcription profiling to identify causal genetic polymorphisms is clearly demonstrated with the callipyge mutation, in which the two most differentially expressed genes according to genotype and affected muscle type were the two genes flanking each side of the mutation i.e. Dlk1 and Gtl2.

A number of different approaches were used to search for an expressed transcript from, and for specific protein binding to, the CLPG locus. Evidence for a transcript at low abundance was confirmed in SG105 (Hulet et al, 2004) and found only in foetal samples (at low abundance) by Vuocolo et al, 2005, that a protein binds to this locus has previously been reported (Freking et al, 2002). We have been unable to obtain the sequence for a protein product from this transcript. It remains unclear if a transcription factor binds to the CLPG locus (see SG110, SG136).

The CpG islands around the CLPG locus of affected muscles from paternally derived animals are less methylated after birth than in the same muscles in normal animals (SG105). This is consistent with the observations of Murphy et al (2006) who also showed that in LD muscle there is even less CpG island methylation around the CLPG locus of animals with two mutant alleles than animals with one mutant allele. Thus, epigenetic mechanisms are likely to be involved in translating the mutation into function.

Function Discovery – Callipyge: Gene Expression ….2

1. Pattern of gene expression in muscle is time and tissue dependent. There is a major shift in gene expression in muscle around birth.
2. Affected muscles of CLPG have greater expression of DLK1 and PEG11 with associated changes in expression of genes on other chromosomes including HDAC9, SLC22a3, RNA methyl transferases, notch1.
3. First to show that PEG11 protein was produced from the PEG11 transcript in muscle
4. Testing systems developed for most likely candidates – DLK1, SLC22a3, Notch1, HDAC9.
5. Methylation status of CpG islands, especially on the paternal allele, surrounding the CLPG locus associated with tissue specificity of DLK1 expression
6. List of candidate genes for function discovery generated. DLK1, SLC22a3, Notch1,HDAC9 tested, many other potential candidates available for testing

Figure 7. Key outcomes from Callipyge gene expression profiling.

5.1.4 Proteomic analysis – development of candidate functional pathways

Projects :- SG106, SG136
Principal Investigators:- Matthew McDonagh, Matthew Knight

Proteomics is the measurement and identification of protein products. This open platform technology has the potential to identify new proteins and pathways of protein and metabolite interactions (by identification of enzymes in particular pathways) without the constraint of prior assumptions about the presence and identity of genes that hamper microarray analysis of gene expression.

In the muscle sub-program, a study of protein abundance and location in normal and callipyge lambs at two time points was carried out. The purpose of this study was to complement the gene expression studies conducted using the same samples, and to help inform the synthesis of information from in-vivo samples from Callipyge and normal siblings (tissue and cell histology), gene expression (qRT-PCR, microarray and MPSS - Solexa) to build a comprehensive picture of the effect of the CLPG mutation on muscle growth and development.

There were a substantial number of technical difficulties during start up which were largely overcome within 18 months. These included issues to do with development of techniques, equipment and support tools. In proteomics, standard methods for spot picking from two dimensional electrophoresis gels, software for identifying differential amounts and quantities of protein in 2DE gels and MALDI TOF/TOF mass spectrophotometric techniques for generating MS signatures of proteins and peptides are still under development. Without an annotated sheep genome sequence it was necessary to use gene sequence and hence putative protein sequence from other species. Work on building a protein database that includes proteins specific to sheep is still underway. The identification of differentially present proteins has been conducted using annotated sequence from other species.

Despite the original intent of combining all the information from animal phenotype, cell biology, protein abundance and gene expression into a comprehensive model of transactions within muscle, this objective is not complete. In fact attempts to make it happen have failed due to lack of tools to incorporate information across the many scales and organelles within muscle. Ashley Waardenburgh a PhD student in Brian Dalrymple's lab is developing a model of muscle at the gene expression level outside the domain of the muscle sub-program. This may eventually provide a vehicle for integration of knowledge about muscle growth and development across the domains that relate gene expression to functional and structural components of muscle.

Nonetheless, some outstanding new insights into regulation of muscle growth in CLPG cf normal sheep have emerged from the proteomics work in sheepGENOMICS.

To quote from the executive summary of the final report SG.106-SFG.011
"Major cellular pathways controlling energy and protein metabolism are altered in the affected muscles of *Callipyge* animals compared to normal animals. Hypertrophic muscle from *Callipyge* animals shows a greater reliance on energy supply via glycolysis within cytosolic and mitochondrial fractions, the major locations for metabolic processes associated with energy supply within muscle. Down regulation of proteins associated with cytoskeletal formation suggests decreased myofibrillar protein degradation of these proteins in *Callipyge* muscle, indicating a shift towards energy in muscle being used for growth and not for protein turnover. Increased expression of translational regulators, including elongation factors suggests that elevated protein synthesis is also supporting muscle hypertrophy in *Callipyge* animals. Down regulation of G protein, GTPase and cAMP mediated signalling pathways

may be involved in propagation of the hypertrophic phenotype in Callipyge muscle and for changes in cellular metabolism."

Three pathways that could have potential for modulation of muscle growth and efficiency were proposed.

A. Changes in Proteasome complex proteins indicate that manipulation of protein degradation, possibly through NFKB and PIF provides one means of reducing energy expenditure and increasing protein accretion in muscle.
B. Changes in energy utilization within the glycolysis / glyogenolysis pathways were identified in CLPG cf normal animals. These suggest that modulation of the activity of Akt1 and associated pathways may provide a means to affecting muscle growth and energy expenditure
C. Ca2+ and cAMP signaling pathways differed in CLPG and normal animals. Calcineurin is responsible for NFAT mediated changes in muscle specific transcription factors such as MEF-2.

Strategies to manipulate these pathways were devised and in several cases investigated using *in-vitro* models. In the case of Akt1 an *in-vivo* study was conducted to ascertain if manipulation of lambs using naturally occurring modulators of the proteasome and Akt1 pathway (Resveratrol and Benfotiamine) could change body composition, eating quality and efficiency of energy retention. See below.

Identification of protein(s) that bind to the Callipyge locus.

Understanding how the Callipyge mutation was regulated was a key target of proteomics research. It has been postulated for some time that although the CLPG mutation lies in a non-coding region of oar18, it has a motif that looks similar to a target for a transcription factor. Indeed Smit and Cockett (M. Smit, PhD Thesis, Utah State University) have demonstrated using EMSA (electrophoretic shift mobility assay) that a protein does bind to the CLPG. In an earlier sheepGENOMICS project (SG105, Tatham et al, 2004) suggested that transcripts from that locus are expressed (although other evidence suggested, if they are, it is at very low levels and restricted to fetal samples, Vuocolo, Pers Comm). Proteomics has the ability to sequence and identify proteins if they can be isolated in sufficient quantities and if the protein has been reported previously and sequence has been lodged in a database.

Extensive studies using EMSA, a novel CLPG pull-down assay developed by Primary Industries Victoria and a commercial ForteBio Octet assay system were unable to identify the protein bound to the CLPG locus. The results suggested that a protein did bind to the CLPG locus, but the mass spectrophotometric procedure was unable to identify the protein in any mammalian database. It may be that the protein binding to the locus was a transcription factor (which are normally present at low concentrations) as postulated by Smit and by Tatham (above). The data do not support differential protein binding to the wild type and mutant CLPG allele at the CLPG locus, but this may be because the amounts of novel protein present are at very low levels and beyond the resolution of the methods used.

Function Discovery – Callipyge: Proteomics ….3

1. Energy metabolism in affected muscles from CLPG CN animals shifted to greater reliance on glycolysis in cytosol and mitochondria than in NN animals
    1. Major change protein that regulates cytosol / mitrochondria C3 shuttle
    2. Suggests changes in Akt1 pathway may be used to modulate energy use in muscle
2. Protein degradation pathway reduced in CLPG CN vs NN animals suggested by changes in proteasome complex proteins
    1. Indicates multiple pathways associated with reduced energy expenditure in muscle (mediated through variation in protein synthesis / degradation, possible NFKB and PIF as well as C6-C3 pathways)
    2. Elevated protein synthesis in CLPG CN cf NN suggested by increased quantities of translational regulators
3. Calcium and cAMP signalling pathways differed in CN cf NN animals
    1. Evidence that differential regulation of Calcineurin may be involved
4. Evidence that a protein does bind to the CLPG locus, but unable to identify it.
5. Proteomics provided a different insight into regulation of the CLPG phenotype than gene expression. Unable to reconcile many of the differences, although proteomics seems to provide more comprehensive data on downstream biochemical effects than gene expression. Both methodologies support complex changes in regulation associated with CLPG phenotype.
6. Modulators of the Akt1 pathway have now been tested in sheep.

Figure 8. Key outcomes from proteomics studies to understand the CLPG phenotype

5.1.5 Development of immortalized ovine cell lines

Project SG101, SG110, SG139
Principal Investigators:- Lopeti Lavulo, Charlie Pagel, Eleanor Mackie

Characterisation of primary cell lines and effects of genotype on cellular phenotype

In vitro models of myogenesis such as primary myogenic cells and myogenic cell lines have contributed greatly to the understanding of the molecular and cellular processes involved in the formation of skeletal muscle. However, both the available established rodent myogenic cell lines and primary muscle cell cultures have limitations in the context of the study of myogenesis in commercially relevant species such as sheep and cattle. Whilst primary myogenic cells are readily isolated from bovine, porcine and avian muscles they have a limited proliferative lifespan in culture and commonly contain variable and high proportions of contaminating non-myogenic cells such as fibroblastic and adipogenic cells. In contrast, immortalised myogenic cell lines have an indefinite proliferative lifespan *in vitro* and are clonal in nature, representing a pure population of myogenic cells. Prior to this project, myogenic cell lines from commercially relevant species such as sheep and cattle were not available. All available myogenic cell lines, such as L6 and C2C12, were originally established by prolonged cultures of senescent primary rodent myogenic cells. In such cultures random genetic events accumulated by cells ultimately led to the clonal expansion of spontaneous immortalised myogenic cells. However, the nature of the genetic changes resulting in immortalisation of these cell lines is uncharacterised and studies have demonstrated that many of these cell lines exhibit a transformed phenotype *in vitro* and *in vivo*. Therefore, along with the species of origin, the transformed nature of the available myogenic cell lines raised serious questions as to their suitability with which to study the control of myogenesis in developing ovine muscles.

Within the muscle sub-program clonal myogenic cell lines from normal sheep and sheep carrying a paternally inherited copy of the *callipyge* single nucleotide polymorphism were developed. Initially primary cultures of myogenic cells were established from a variety of hypertrophy-responsive and non-responsive muscles from both callipyge and normal animals. The growth characteristics of these cultures were studied and significant differences in the proliferation rates and resistance to serum-deprivation induced cell death between the callipyge and normal cultures were observed (Lavulo 2008). Following *in vitro* expansion, the primary-derived cultures were frozen in liquid nitrogen to provide a resource for further experimentation and collaborations with groups outside the sheepGENOMICS program. Cultures from selected muscle isolates were then immortalised by transfection with a construct consisting of a thermolabile mutant of the simian virus 40 (SV40) large tumour (Large T) antigen (tsA58) linked to a zinc ion inducible promoter element (metallothionein-1 promoter).

Immortalisation of cells using viral oncogenes such as SV40 Large T antigen does not require extended periods of culture of senescent cells and thus avoids the problems associated with spontaneous immortalisation of cells detailed above. However, most immortalising agents, including SV40 Large T antigen, act to prevent withdrawal of cells from the cell cycle. As withdrawal of myogenic cells from the cell cycle is an essential step in myogenesis immortalising agents have the potential to inhibit terminal myogenic differentiation. In the system we chose, expression of SV40 Large T antigen, and therefore immortalisation of the cells, is dependent upon the culture conditions under which cells are maintained. Under conditions permissive for the expression of Large T antigen (33°C with Zn^{2+}) cells are immortalised. However, under non-permissive conditions (39°C without Zn^{2+}) cells are released from the immortalising effects of the Large T antigen and can be induced to undergo normal terminal myogenic differentiation.

Following immortalisation, transfected cells were selected by their antibiotic resistance under permissive conditions. Clones of conditionally immortalised cells were then isolated and expanded under permissive conditions. The expression pattern of Large T antigen and myogenic markers such as myogenin, myosin heavy chain and desmin of individual clones under both permissive conditions and non-permissive conditions was determined to ensure that clones were myogenic and to confirm that control of Large T antigen expression had been established. One myogenic clone from the *semitendinosus* muscle of a normal sheep (5005ST-1) was then selected for use in subsequent studies to determine the functional effects of candidate gene expression on terminal myogenic differentiation

5.1.6 Techniques for insertion of candidate genes

Project :- SG139
Principal Investigators:- Lopeti Lavulo, Charlie Pagel, Eleanor Mackie, Tony Vuocolo, Ross Tellam

A candidate gene testing system based on the commercially available T-Rex system (Invitrogen) was developed and optimised. The conditionally immortalised ovine cell line was transfected with a DNA construct that constitutively expresses the Tet repressor (TR) protein. Clones in which the construct was stably integrated were isolated on the basis of antibiotic resistance. One such clone (5005ST-1 TR) was then used to transfect in the genes of interest under control of a tetracycline-responsive promoter element. Using this system the expression of the gene of interest is tightly controlled by the TR protein which binds the Tet operator sequence and stops transcription of the transgene. When tetracycline is added to the growth media, tetracycline binds the Tet repressor protein and prevents it from binding the Tet operator, therefore, allowing the transcription of the gene of interest. Using this system transfected conditionally immortalised cells carrying genes of interest can be induced to differentiate through manipulation of the culture conditions. At specific times during the

resulting terminal myogenic differentiation expression of the gene of interest can be induced by the addition of tetracycline to the culture medium. Thus the effect of expression of the gene of interest on myoblast proliferation, differentiation and recruitment can be determined.

The aim of the second phase of this project was to determine the functional effects of a number of hypertrophy-associated genes on terminal myogenic differentiation. Candidate genes were identified within the muscle sub-program based upon microarray analysis of callipyge and normal muscles and included Delta-like 1 (*Drosophila delta* homolog-like 1, Dlk1), solute carrier family 22 (extraneuronal monoamine transporter), member 3 (SLC22a3) and Notch, a transmembrane protein believed to be a receptor for DLK1.

Development of a unique immortalised ovine cell line resource has provided a tool for studies into the regulation of myogenesis in the hypertrophic muscles of callipyge sheep, and it is hoped they will form the basis for new strategies to improve and increase the growth of skeletal muscle in commercial sheep breeds.

5.1.7 Candidate gene/pathway testing

Projects:- SG137, SG139, SG136
Principal Investigators :- Lopeti Lavulo, Charlie Pagel, Eleanor Mackie, Jason White, Tony Vuoloco, Ross Tellam, Matthew McDonagh, Matthew Knight

Cell lines

Immortalised ovine muscle cell lines

DLK1

Three constructs of Dlk1 (corresponding to the entire protein, C-terminal intracellular domain and extracellular domain) were transfected into immortalised ovine cell lines. Dlk1 (Fetal Antigen- 1) was extracted from amniotic fluid and purified. It was anticipated that it would be added to cell lines to determine the nature of the effect of exogenous DLK-1 addition to the cells as a precursor to the transfection studies (but has not yet been completed)). The three variants of DLK-1 were over-expressed in the ovine cell lines. Two of the DLK-1 isoforms (entire protein and extracellular domain) resulted in more myoblasts but did not permit the cell lines to differentiate and form myotubes. The C-terminal intracellular domain construct of Dlk1 consistently deranged the cell lines, interfering with proliferation and preventing differentiation.

SLC22a3

SLC22a3 was engineered into an over-expression vector and inserted into immortalized ovine cells. There was no expression of SLC22a3 (and therefore no effect) suggesting that the SLC22a3 construct did not properly incorporate into the cells.

C2C12 (immortal murine muscle cell lines)

HDAC9 – siRNA modulation

A molecular model of the effects of the callipyge mutation based on gene expression data suggests that HDAC9 is a negative regulator of myogenesis (Figure 9). It was thought that inhibition of HDAC9 or reduction in its expression levels would accelerate myogenesis. A full length cDNA encoding ovine Hdac9 (isoform 3) was cloned, sequenced and validated. This gene has been analysed for splice variants and siRNA target sequences identified and

designed for gene suppression experiments in ovine muscle cells. In addition, similar work has been undertaken to identify a murine Hdac9 (isoform 3) mRNA sequence and the design and production of siRNAs undertaken for gene expression suppression in a murine muscle cell line model. Murine assays were developed for monitoring and analysis of gene suppression effects. This has enabled subsequent studies to be undertaken which investigated the effects of Hdac9 suppression and its effect on myogenesis in cell line models. Class II histone deacetylase inhibitors were also identified from the literature and a list of these and sources has been produced and made available to collaborating scientists at DPI-Victoria for studies in cell lines and/or animals.

Four independent siRNAs targeting Hdac9 were engineered and along with two matched siRNA negative controls, successfully transfected into C2C12 cells, a murine myogenic cell line. Successful transfection was demonstrated by localisation of control fluorescently labelled oligos in cells and by specific demonstration of Hdac9 gene expression as measured by quantitative gene expression assays. In addition, creatine kinase activity assays, performed as an indicator of myogenic differentiation, demonstrated enhanced levels of differentiated myoblasts in the Hdac9 siRNA treated cells compared to control cells. Histology of the cells also revealed that specific Hdac9 siRNAs had a marked effect on promoting myogenesis. Thus, suppression of HDAC9 greatly accelerated myogenesis in this cell culture model.

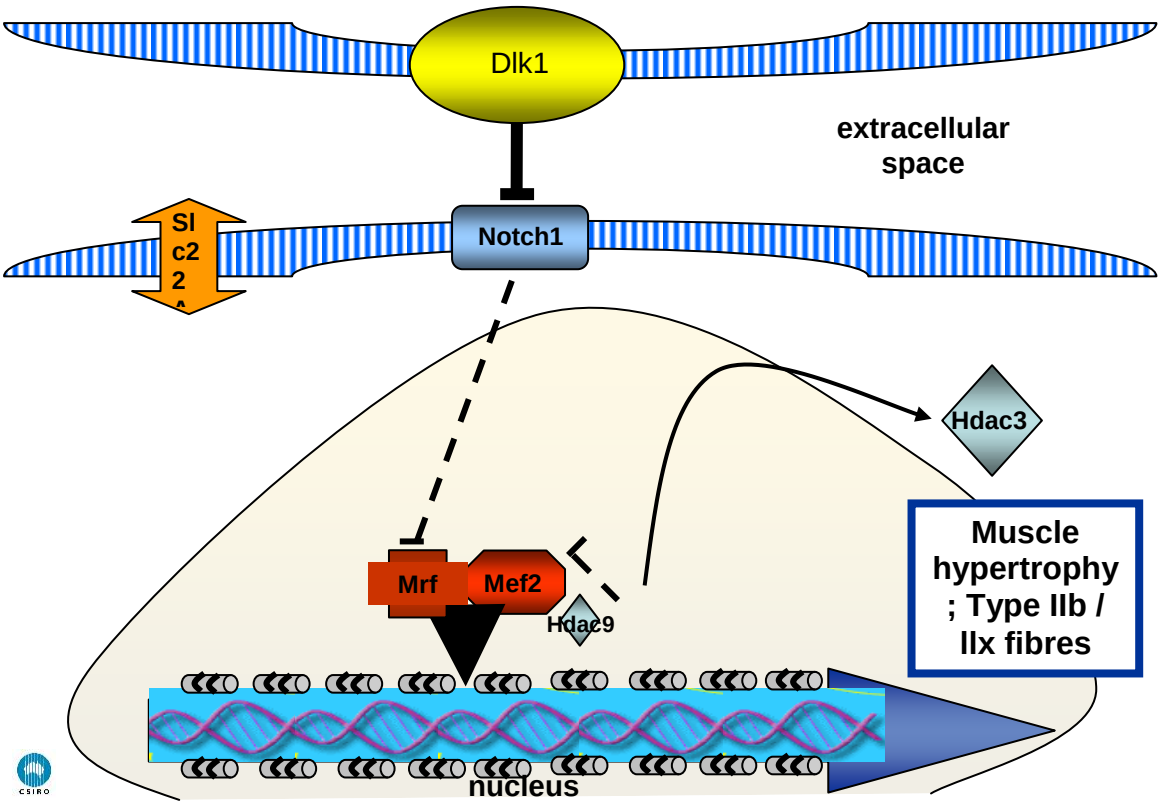

Figure 9. Model of muscle cell showing putative actions of DLK1, SLC22a3 and HDAC9 on regulation of muscle development in CLPG animals.

Class II HDAC modulation - inhibition

There is a number of therapeutics which inhibit the action of class II HDACs. Given that HDAC9 belongs to this class of proteins, studies were instigated in C2C12 cells. When C2C12 cells are pre-incubated in 5 or 10mM valproic acid (VPA) an increase in myotubes formation is observed after 4 days in culture. This is associated with a decreased level of cell proliferation under the same conditions. This seems to indicate that VPA functions to increase the number of cells available for myotubes formation leading to an increase4 in myotubes size (Figure 10, Chris Gorman, Pers Comm).

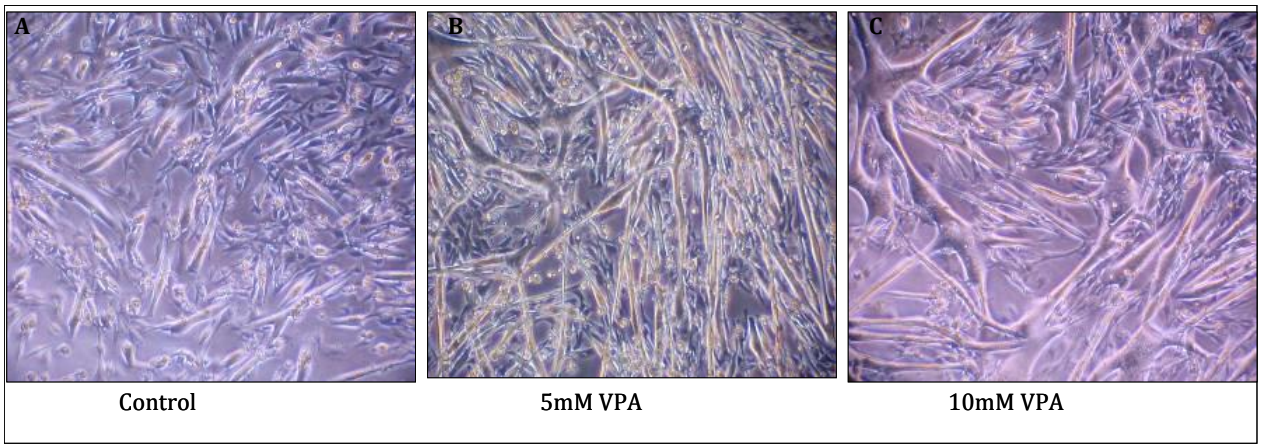

Figure 10. Effect of HDAC inhibitor Valproic acid (VPA) on differentiation of c2c12 cells.

Benfotiamine, a putative modulators of the Akt1 pathway, was tested in c2c12 cells. Results indicate minimal myotube formation (relative to controls) after 3-6 days post switch to differentiation conditions. This suggest that Benfotiamine at 50-100uM maintained c2c12 myoblasts in a proliferative state, and did not facilitate transition to differentiation and thus myoblast formation in a dose dependent manner.

Primary ovine myoblasts
Primary muscle cell lines derived from Callipyge lambs grew faster and had a lower rate of apoptosis than similarly prepared primary muscle cell lines from unaffected animals (Lavulo et al, 2008). Studies by Thu Nguyen (PhD student) demonstrated that there was a difference in sensitivity of primary muscle cells from Callipyge sheep cf normal (unaffected) sheep to IGF1.

Whole animal testing
Sheep

Proteomic studies demonstrated that Akt1 and associated pathways are altered in the Callipyge cf normal contrast. The Akt1 pathway is a druggable target as the vitamin B1 analogue, Benfotiamine, is a practical method to manipulate the Akt1 pathway, and in c2c12 cell lines Benfotiamine held muscle cells in a proliferative state in a dose dependent manner. It is thought that, Resveratrol (a naturally occurring polyphenolic compound) may decrease protein turnover in cells through a reduction in ubiquitin proteasomal degradation in the cell (NFkB pathway) and increases protein synthesis (through the AKT1 pathway, see Figure 11 below).

These compounds were added to lambs diets at 10 and 100mg/kg body weight (Benfotiamine) or 5 and 50 mg/kg body weight (Resveratrol) to determine their effect alone and in combination on growth, carcass composition, efficiency of feed use and characteristics that underpin eating quality in lamb.

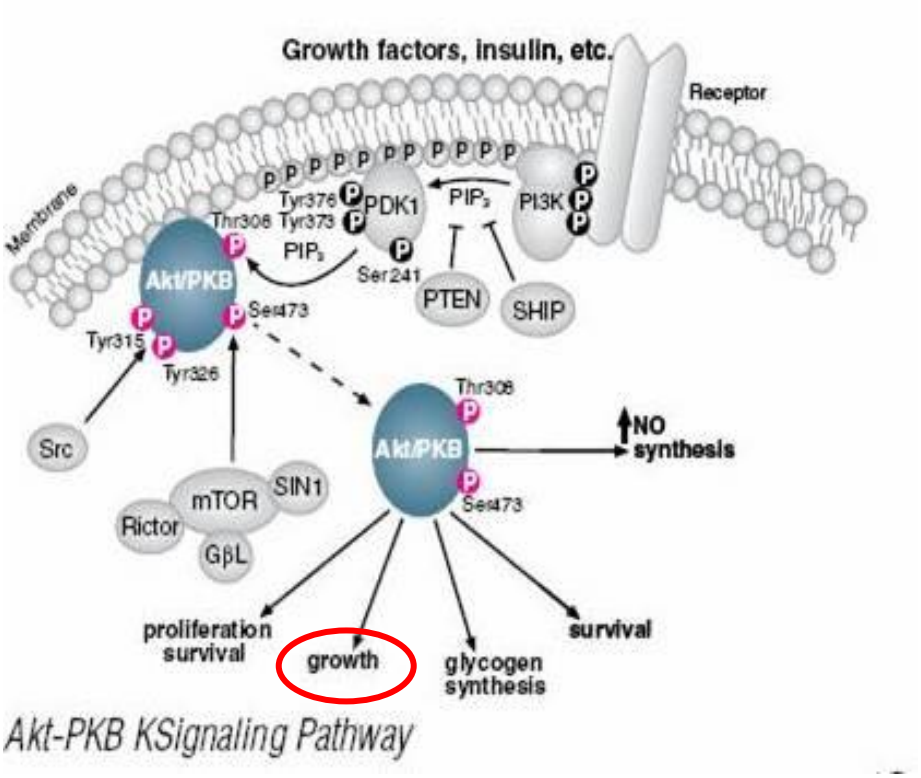

Figure 11. The AKT1 signalling pathway. Benfotiamine (and thiamine, Vitamin B1) increase phosphorylation of Thr308 and Ser473 of AKT1 and stimulate cell growth. It is noteworthy that in the lamb studies reported here, the effects of Benfotiamine, and Resveratrol, were greatest on gene expression in adipose tissue.

Benfotiamine alone at high dose (100mg/kg body weight) and Benfotiamine and Resveratrol together at low dose (10mg and 5 mg/kg body weight respectively) decreased the rate of fat gain and increased the rate of lean gain and over an 8 week period in 20 week old lambs (as assessed by Dual X-Ray Absorption Spectroscopy (DXA) analysis of the live animals). In the carcass there was a significant reduction in fatness due to the high dose of Benfotiamine and combined low dose Benfotiamine and Reseveratrol treatment. Expression of genes thought to be associated with the pathways influenced by Resveratrol and Benfotiamine were significantly affected in fat, but not in muscle. There were no significant effects of Benfotiamine and / or Resveratrol treatment on ultimate pH of the meat or meat colour, objective measures of tenderness (Warner Bratzler shear force) of the meat, cooking loss, average cross section of muscle fibres, size of fibre relative to number of nuclei or on oxidative profile of muscle as assessed using NADH staining. The magnitude of the reduction in fatness (from 400-600 g/animal) and increase in lean gain (480g in the live animal, less than 200g in the carcass) due to these treatments, over an 8 week period is very promising, and of the same order of magnitude as effects of a single copy of Carwell or GDF8 g+6723A in sheep. It is strongly recommended that this work be repeated, with a view to exploring commercialization if the same outcomes are found.

5.1.8 Carwell/EBV contrasts

Project :- SG109

Principal Investigators:- Ross Tellam, Tony Vuocolo, James Kijas, Hutton Oddy

Transcriptional analysis

Analysis of gene expression in progeny from sires of high and low muscle EBV was conducted. Progeny were approximately 18 months of age and were derived from a study conducted by Kirstie Martin and Kirstie Thompson of UNE / Sheep CRC.

A key feature of this analysis was that when analysis of the Carwell effect on gene expression was carried out far fewer differentially expressed genes were found than when the analysis was carried out using the EBV contrast. This is not surprising, because Carwell is thought to be a single major gene affecting area of eye muscle, whereas EBVs are derived from quantitative analysis of phenotypes including scanned eye muscle area, scanned fat thickness and liveweight on progeny, their siblings, parents, grand parents and all available predecessors. Accordingly, it is expected that selection on EBVs would involve the concentration (selection) of many more genes, and as a consequence much greater diversity in gene expression, than contrasts involving animals thought to be heterozygous for putative major genes (or genes from one chromosomal region). In the case of Carwell, it also may be the case that the haplotype markers were not diagnostic, and hence the contrast between animals thought to carry the Carwell or not may not have been valid.

This represents a challenge in determining potential usefulness of the information. It is expected that a single mutation will leave a gene expression signature that includes cis-activated genes (those directly affected by the mutation) and trans-activated genes (those affected as a consequence of the mutation, but downstream to the mutation). Trans activated genes might have major effects on phenotype, but require changes in expression secondary, and distant, including on other chromosomes, to the causative mutation. Examples include those reported above for the effect on the Callipyge mutation on gene expression. In addition to those genes differentially expressed around the Callipyge locus (DLK-1[1], GTL-2, PEG-11) there are many more differentially expressed genes from elsewhere in the genome (including SLC22A3[1], HDAC9[1], Atf3, Fos, Rasd1, Lrrc2, a putative methyl transferase, Dnttip1, Frzb). The Callipyge mutation has taught us that derivation of function from gene expression is not easy. However, it has also shown that the candidate approach works, the differential gene expression profiles clearly point to a region near DLK-1 and PEG-11 on oar18. The CLPG mutation lies in a non coding region between these two differentially expressed genes. Similarly the observation that HDAC9 is differentially regulated in CLPG has spurred significant effort to modulate histone deacetylases generally (see below).

Consequently, it is not surprising that gene expression analysis of animals which differ in EBV (possibly involving many genes of small effect) finds many more differentially expressed genes, and from many more pathways, than those from a single “gene” or chromosomal region. This leads to discovery of many potential candidates, but refinement of the list of potential candidates to a more likely list is not simple. A suggested method is to combine EBV based gene expression analyses with whole genome SNP association studies. It would be anticipated that through this process, differentially regulated genes associated with QTL regions may be indentified and the potential list of candidates reduced

[1] See results of testing candidate genes and modulators with immortalized ovine and murine c2c12 cell lines elsewhere in this report

to those acting in cis with the genomic region. It is anticipated that this approach would reduce the search space for predictive gene markers.

Unfortunately we were unable to use the above procedures, so reducing the list of potential candidates to testable hypotheses required some knowledge of function of the particular gene, This remains an imprecise process (for example, it is likely that many more potential candidates are indicated than are actually present), but nonetheless shows some promise. Examples of such candidates are given below, with justification based on current knowledge of function. The information remains speculative until studies have been conducted to test each hypothesis.

In the case of HDAC9 such studies have been conducted both in sheepGENOMICS and elsewhere. These have shown that modulators of histone deacetylases affect the capacity of c2c12 muscle cells to differentiate into mature myofibres (Tellam and Vuocolo, Knight and McDonagh; Iezzi et al, 2005).

The mechanism of action by which HDAC inhibitors (i.e. TSA, sodium butyrate and VPA) increase the number of differentiated myotubes in culture and in animal models this occurs via increased follistatin expression. This increased production of follistatin is totally distinct from the conventional mechanisms for increasing myotube differentiation (i.e. muscle growth) which includes IGF-1 and IL-4 (Iezzi *et al.* 2004). Further evidence to support the theory of Iezzi and co-workers is seen in transgenic mice that over-express follistatin in the presence of a GDF8 knock-out. The animals show a dramatic increase in muscle mass when compared to the wild-type mouse (Lee and McPherron 2001). It is also well documented in the literature, that follistatin can block myostatin activity, a negative regulator of muscle growth *in vivo* (Lee 2007). It is less well recognized that follistatin can act to increase the number of muscle cells independent of GDF8 status.

Thus, myotube differentiation can be modulated by inhibiting the activity of HDAC enzymes. This suggests that HDAC inhibitors are an ideal target for modulating postnatal muscle growth in animals. This is because HDAC inhibitors have been shown to promote the fusion of undifferentiated myoblasts into multinucleated myotubes.

Identification of candidates

In those studies in which EBV contrasts were made, a significant number (~500) of differentially regulated genes were found. Of these three are standout candidates based on differential expression, potential function and indirect evidence from QTL studies either in sheep or closely related species.

Fstl1 (Follistatin like – 1) is related to follistatin (FST) which binds to GDF-8 and enhances muscle mass. While the functional relationship between Fstl1 and FST is not clear, it is noteworthy that both FST and follistatin like-3 (Fstl3) have large effects on muscle. FST overexpression coupled with myostatin knock-out results in a 4 fold (400%) increase in muscle mass of mice (Lee, 2006).

Fstl1 is located on oar1 @ 210.3Mbp (in cattle BTA1 @ 61.15Mbp, and BTAun). It may underly a QTL for meat (carcass) yield in beef cattle, and there is some evidence for a QTL near the FSTL1 locus in Suffolk sheep (Walling et al, 200?). Kijas (Milestone x, SG132) has located a number of SNP within the FSTL1 gene including one within a micro RNA recognition site.

Animals from shgen003 have been genotyped for a number of SNPs and microsatellite markers (the latter to ascertain phase) and analysis is currently underway to complete an association study.

Dicer1 High muscle EBV sheep had approx 1.5 fold increase in Dicer1 expression. Dicer1 processes all miRNA precursors and is therefore potentially a master regulator. Specific miRNAs are known to be involved in regulation of muscle development (see Clop et al, 2006). Dicer1 is located on oar18 @ 87.14Mbp, close to the region associated with Carwell, and within a region associated with muscle and fat weight in Texel sheep (Walling et al, 2001, 2005 & 200?) and near the rib eye muscling locus identified by Nicholl et al (1998). Dicer1 is less than 5Mbp from the CLPG mutation, and lies approx 5 Mbp from the Carwell diagnostic haplotype (although Dicer1 gene expression was not different in the Carwell cf non Carwell contrast). miRNA's are clearly involved in regulation of muscle growth (e.g. Clop et al, 2006; Wong & Tellam, 2008). It is unclear if there is a quantitative association between Dicer1 gene expression and production of miRNAs. If there is then Dicer1 would be a strong candidate.

Mustn1 Mustn1 is a transcriptional regulator highly expressed in skeletal muscle, differentially expressed in high cf low muscled EBV sheep. There is little known about its function. It is located on oar19 @ 52.24 – there is no meat related QTL nearby in bovine (Bta 22 @ 41.65Mbp), although there are several meat yield (Back Fat, Lean meat weight) and quality related (meat colour, pH) traits near the Mustn1 locus in the pig.

5.1.9 Summary of outcomes – gene function

- Extensive development of gene expression data in selected muscles of CLPG and normal lambs (both microarray and RT-PCR procedures). First use of MPSS to explore gene expression in muscle of CLPG and normal sheep. Development of a list of differentially expressed genes in muscle from CLPG, Carwell and animals from sires with different EBVs, and assessment of this list for potential candidates.

- Testing of potential candidates from the list of differentially expressed genes (FSTL1 as a candidate gene – mutation discovered and animals genotyped to determine association, Notch inhibitors (DAPT) and inhibitors of HDAC9 (specific siRNAs) tested in muscle cell lines, DLK1 and SLC22A3 transfection systems tested in immortalised sheep muscle cell lines).

- Proteomics assessment of differentially regulated proteins in organelles isolated from CLPG v normal muscle.

- Development of a list of potential modulatory pathways from differentially regulated proteins and testing of potential modulators in sheep (benfotiamine and resveratrol)

- Development of the only immortalized sheep muscle cell lines in the world, and use of these cells to evaluate the effect of candidate genes

5.2 Discovery of new major genes

The intent of this component of the Muscle subprogram was to discover and fine map major genes associated with meat production and quality traits. The expectation was that some causative mutations would be discovered and these could then provide phenotyped and genotyped animal resources to the discovery of gene function component of the Muscle subprogram.

It quickly became apparent that the FMFS resource would not be available in time for such a strategy to be implemented. Accordingly DNA and phenotypes from shgen.003 an MLA project that preceded sheepGENOMICS were used for initial gene discovery projects.

The first target was Carwell, a muscle phenotype associated with increased loin eye area, subsequent targets included mutations in regions around Myostatin, Calpastatin, Calpain 1 and Follistatin-like 1. The latter region was identified from work carried out within the discovery of gene function component of the Muscle subprogram.

At the time of writing, genotypes from the ovine SNP50 beadchip have become available and association studies using the FMFS population have commenced. It is anticipated that this will lead to an explosion of information about QTL positions and traits (for all subprograms – muscle, host resistance to parasites, wool and reproduction).

5.2.1 Gene discovery

Projects :- SG107, SG117

Principal investigators:- James Kijas, Julius van der Werf, Russell, McCulloch, Vicki Whan

a) Carwell. The “Carwell” muscling phenotype (increase in loin area, but not extending to other parts of the body) was described in the early 1990’s as a result of CT scanning in the MRC supported Meat Elite program (Banks, 1997). The chromosomal region responsible for the increased muscling was thought to be towards the distal end of oar18 (Barendse, 1995, Nichol et al, 1998) and at one time was thought to be allelic to Callipyge. Since that time, limited information has been published on the markers thought to be associated with Carwell, although oar18 has been implicated in increased muscle development in Suffolk and Texel breeds of sheep (Walling et al, 2002, 2004). A commercial test for Carwell “LoinMax”, consisting of 2 microsatellite markers and 1 SNP on oar18 (J.C. McEwan pers comm) is available. It is claimed that a paternally derived copy of the Carwell allele increases the weight of loin by 10% (http://www.catapultsystems.co.nz/products/30_loinmax.cfm).

In a separate study within sheepGENOMICS (Kijas et al, 2005) reported an association between a haplotype consisting of 151 allele of Mulge5 and 193 allele of Mulge6 (position on the sheep integrated map v4.2 114.94 and 114.14 cM respectively) and eye muscle area and eye muscle depth measured on the carcass. These are within the QTL reported by Walling et al (2004) for muscling in Texel sheep on oar18 (Figure 6). Kijas (2005) claimed based on commonality of haplotype and sire association with *Carwell* ancestry that the microsatellite markers above were potentially diagnostic of *Carwell*. One copy of the *Carwell* haplotype resulted in a 7.6% increase in carcass eye muscle area (in the three families in which *Carwell* was segregating in SHGEN.003). This is similar to the effect of one copy of the Carwell Haplotype on weight of the bone out saddle (9.6%) but larger than the 2.6% increase in lean in the loin measured using CT scanning (SG.116). SG.116 reports new results for Carwell. We were previously unaware of significant association between one copy of the Carwell haplotype and overall fat deposition in the carcass (overall reduction of 3.6%, 3% in shoulder fat and 4.5% in loin fat). These results and others are discussed in more detail below.

Taking into account all results from the muscle subprogram, it is clear that Carwell is not allelic to Callipyge. Although the putative Carwell haplotype is within 1Mb of the Callipyge mutation, comparison of gene expression changes indicate that the extreme elevation of

DLK1 observed in the muscle of paternal heterozygote Callipyge animals displaying the extreme muscle phenotype is not replicated in heterozygous Carwell animals (SG109).

b) Myostatin. Four new polymorphic SNPs were identified within 3,835 bases of the myostatin gene (including UTR's) by resequencing a wide range of lambs from different populations. The effects of one of these, the g+6723A allele at the Myostatin locus are broadly consistent with those published by ourselves and others (Johnson et al, 2005; Laville et al 2004; Clop et al, 2006; Kijas et al, 2007). The results show that a putative reduction in myostatin protein as a consequence of the g+6723A allele is associated with general reduction in fatness and increase in lean deposition in the carcass. The results here also suggest that the effect is greatest in the forequarter and loin and least in the leg / rump region of the carcass. The data presented here do not support an effect of one copy g+6723GA on ultrasound scanned traits on live animals. Hadjipavlou et al, 2008 were also unable to detect a significant difference in muscle or fat depth between g+6723GG and g+6723GA genotypes in Charollais sheep, but found that the g+6723AA genotype was associated with significantly greater muscle depth than the g+6723GG and g+6723GA genotypes. We were unable to show an effect on muscle depth of the g+6723AA genotype, principally because of the low number of lambs with g+6723AA alleles in the current study. Limited evidence from the CT scan data suggest that the presence of the g+6723AA genotype is associated with markedly increased lean deposition and reduced fat deposition in the carcass compared with the g+6723GG and GA genotypes. Moreover the limited evidence also suggests an increased birth weight of lambs with the g+6723AA genotype. Further data are required to confirm this observation.

c) CAST (Calpastatin). Eleven new polymorphic SNPs were identified within a(n)? 868 base region with the CAST gene. SNP and microsatellite markers at the Calpastain locus were identified and tested to determine if there were any associations between variation at the CAST locus and attributes of muscle and objective measures of tenderness and aging associated with eating quality.

A summary of the effects of one polymorphic SNP - CAST 270 a G/T allele substitution within CAST intron 14 - is shown below. There were significant main effects on the traits shown and there was a significant interaction between the g+6723A allele of myostatin and the difference in peak force of m. longissimus dorsi between 1 and 3 days aging and eye muscle depth and width measured on the carcass.

Trait	Intercept	Gene / Allele	Effect	Effect in SD units	Significance
Average peak force in LD muscle after 3 days aging	3.265 kg	CAST TT GT GG	 0.16 0.142	 0. 286 0. 254	P<0.05
Difference in peak force between 1 and 3 days aging	-5.71 kg	CAST TT GT GG	 0.082 1.44	 0.044 0.771	P<0.06
Eye muscle depth 1	26.37 mm	CAST TT GT GG	 -0.34 -0.46	 -0.21 -0.28	P<0.05
Muscle Glycogen	0.426 g/100g	CAST TT GT GG	 0.062 0.064	 0.577 0.59	P<0.05

d) CAPN1 (Calpain 1). Nine new polymorphic SNPs were identified within 1091 bases of the CAPN1 gene. SNP and microsatellite markers around the Calpain 1 & 3 loci were identified and tested to determine if there were any associations between variation at the CAST locus and attributes of muscle and eating quality. No traits were shown to be significant for the main effects of a CAPN1 SNP polymorphism located near the boundary of exon 15 and intron 16.

Work to complete the analysis of effects of many of the different SNP polymorphisms and microsatellite markers around CAST and CAPN1 is continuing.

e) Follistatin-Like 1 (FSTL1). Following identification of variation in gene expression of Follistatin Like 1 associated with variation in the EBV for eye muscle depth (SG014), a search around the FSTL1 locus in sheep identified a number of polymorphisms (including one in a non-coding miRNA binding site) that were subsequently investigated as potential targets in association analysis.

There were significant associations between haplotypes of markers at the FSTL1 locus and meat yield (lean and fatness) and quality attributes (objective and sensory measures of tenderness and meat colour). Work is currently underway to determine the location of the effect and to identify a potentially diagnostic haplotype. Unfortunately, there was no association between a FSTL1 3'utr SNP (the non coding miRNA binding site) and carcass or meat quality traits.

This is a promising result for several reasons.

1. The first is that the suggestion to look at the FSTL1 locus came from gene expression data obtained from muscle of animals with different EBVs for muscle depth.
2. The second is that variation around the FSTL1 locus is significantly associated with traits of industry relevance (and that the effects make biological sense).

Together these observations provide strong support for the suggestion that gene expression data can be used to inform a gene discovery program.

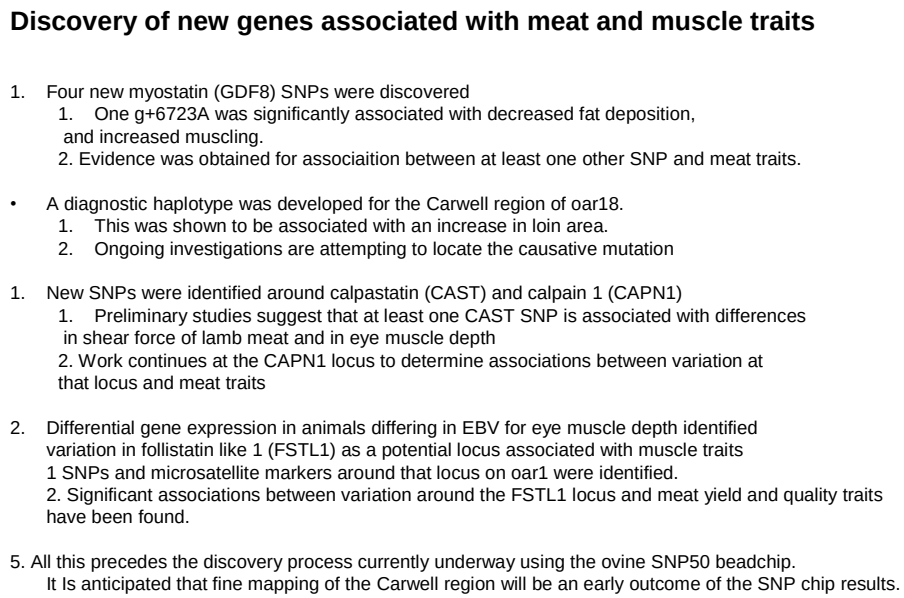

Figure 12. Discovery of new genes associated with meat and muscle traits

5.2.2 Phenotyped resources for further gene discovery and development of marker assisted breeding values

Projects :- SG118, SG120, SG122, SG123, SG124, SG125, SG127, SG128, SG129, SG130, SG134, SG135, SG138

7.2.2.1 FMFS ’05, ’06

Principal Investigators :- Jason White, Jason Sidddle, Peter Allingham, Robyn Warner, Matthew Kerr, Paul Greenwood, Hutton Oddy

The measurement of meat and muscle associated phenotypes on FMFS animals required standardization of protocols and in some cases development of entirely new procedures for high throughput screening of muscle samples. A major advantage was, with the exception of 1 kill, the remainder were in the same abattoir. All were carried out by the same team of researchers. All the phenotyping protocols used for live animal and carcass, meat and muscle traits are documented and available at:-

http://www.sheepgenomics.com/soptree.aspx

Most phenotype measurements were made using standard industry procedures. However a number of new traits were developed and used throughout the program. Methods developed specifically for use within the sheepGENOMICS muscle subprogram included:-

a) High throughput assessment of oxidative capacity (crude measure of fibre type), fibre cross sectional area and fibre number.

Oxidative Capacity

An indirect measure of oxidative capacity was developed by using a histochemical (NADH) stain specific for mitochondria. The basis for this technique was that the darker the staining across the entire section the more mitochondria and thus the greater the oxidative nature of the muscle sample. This was combined with the calculation of average fibre cross sectional area (CSA) on the same section which allowed for the calculation of fibre number in the loin. From the pixel intensity distribution data (derived from the pixel intensity in Figure 13 the mode (pixel intensity with highest frequency) was calculated. This trait was designated as Peak Pixel. Further work is underway to relate pixel intensity to muscle fibre type determined by more conventional staining procedures. An automated process was used to determine the average cross sectional area of muscle fibres and the number of muscle fibres in the cross section of the loin muscle was estimated by division of eye muscle area by fibre cross sectional area after adjustment for the proportion of connective tissue in the field.

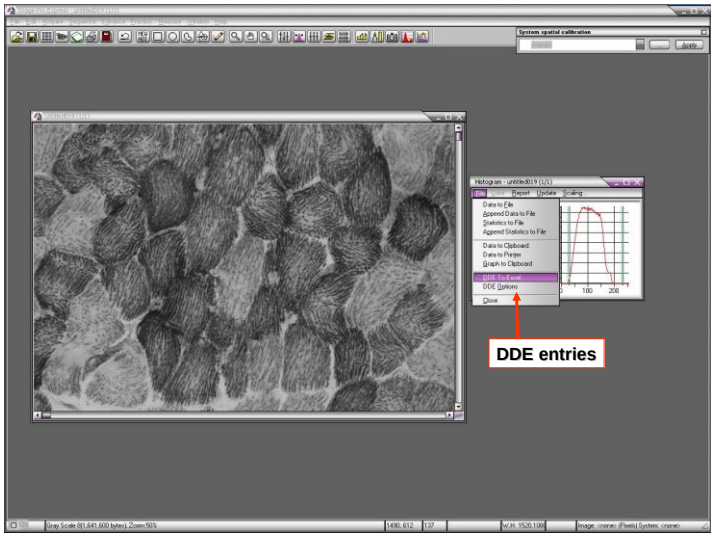

Figure 13 Screen shot showing histogram output from pixel intensity scan. Individual frequency for each pixel value (0-256) are exported to Excel for calculation of mode and average.

Loin muscle fibre number and cross sectional area

Fibre number in the loin was calculated as described and is summarised below in Figure 14., and average LD muscle fibre cross sectional area is shown in Figure 15.

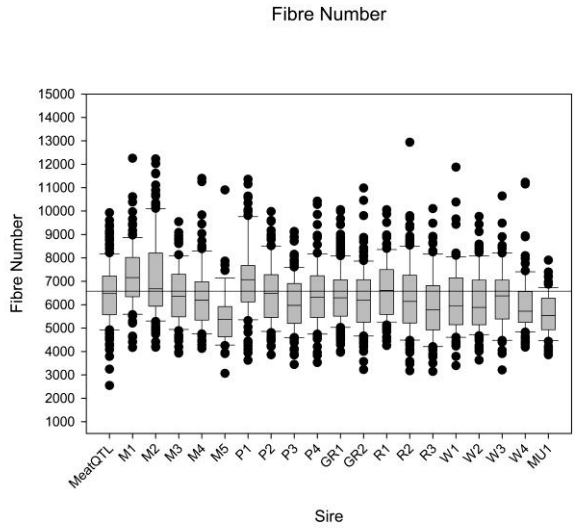

Figure 14. Range in muscle fibre number observed in progeny of FMFS sires. Note data are for entire cross section of the LD.

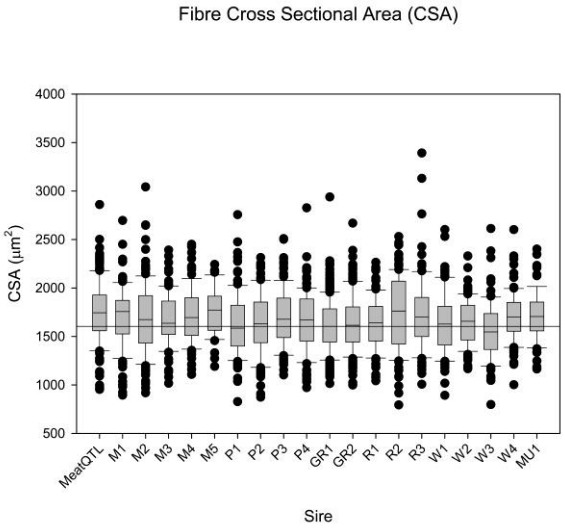

Figure 15. Range in average cross sectional area of LD muscle fibres in progeny of FMFS sires.

These new phenotyping procedures enabled the sheepGENOMICS muscle subprogram to collect data on muscle oxidative capacity, myofibre cross sectional area and total number of muscle fibres in the loin on 2346 lambs. It is anticipated that this data will provide strong biological basis to help understand the components that influence potential yield of lean muscle in sheep.

b) Dual X-Ray Absorption Spectroscopy (DXA) calibration of sheep carcasses for chemical composition of primals and estimation of retail cuts – use on all carcasses

DXA calibration – 55 lambs were selected on the basis of expected wide range of fat, lean and bone component weights and retained after DXA scanning for bone-out to retail cuts and for analysis of fat, protein and ash in primal cuts (and half carcass) to develop equations that enabled the accurate estimation of retail cut weight, and half carcass and primal chemical composition.

The calibration equations (shown in the link http://www.sheepgenomics.com/soptree.aspx) were applied to all lamb carcasses (n = 2346).

This was the first time that the DXA instrument had been calibrated for chemical composition, and for retail cut weight of lamb carcasses. The development of these calibration equations allowed accurate measures of body composition and retail cut weight to be determined on all lamb carcasses generated through FMFS.

The total number of animals with full complement of meat traits measured on the '05 and '06 FMFS progeny is shown in the table below.

Sire	**'05 Progeny**	**'06 Progeny**	**Total**
Meat QTL	97 (2 females)	92	189
M1	61 (2 females)	58	119
M2	73	53 (1 female)	126
M3	53	43	96
M4	25	80	105
M5	0	56	56
GR1	94 (2 females)	139 (1 female)	233
GR2	69	96	165
P1	49	60	109
P2	67	51	118
P3	44 (1 female)	87	131
P4	48	59 (1 female)	107
R1	37	80	117
R2	77	47	124
R3	54 (1 female)	50	104
W1	50	35	85
W2	27	82	109
W3	44	72	116
W4	38	67 (1 female)	105
MU1	0	81 (1 female)	81
UNK*	2	9	11
Total	1009	1397	**2406**

* = sire unknown (since recovered by genotyping data)

The following table summarises the traits measured by the muscle subprogram on the progeny of the sheepGENOMICS FMFS flock. The table also provides a comparison of traits with those measured in MLA project shgen.003 which were used for gene discovery and evaluation of effects of putative markers by a number of projects within the muscle subprogram, while the FMFS flock was being developed and phenotyped. The table also

shows the traits currently being measured in the Information Nucleus of the Sheep CRC. It does not show the additional wool, parasite, behavioural and reproduction traits measured in FMFS and the Information Nucleus of Sheep CRC.

sheepGENOMICS (FMFS flock)	**Shgen.003**	**Sheep CRC (Information Nucleus)**
2004-2007	2001-2003	2007-
N= 4800 (both sexes)	n = 1280 (both sexes)	n = >5000 (both sexes)
Live and Scanned traits (wt, fat, emd)	"	"
Carcass traits (Hot Cwt, GR, EMD, EMW, EMA) (n = 2453, males only)	"	" + additional carcass fat measures
Yield traits (primal weights, fat, lean, bone of each primal, retail yield, chemical composition by DXA)	ViaScan yield, loin wt,	loin fat, loin wt, topside Wt, round wt, femur bone wt, some chemical composition by CT scan
Meat Quality Traits (shear force, d1 and d5, chewiness, drip loss, color (Minolta) pHu, IMF on 300)	" + sensory measures on > 400, muscle glycogen, IMF	" + pH decline, OH-Pro, IMF
Muscle characteristics (CSA, Fibre No, Aerobicity)		ICDH
Other (IGF1, Whole genome scan)	IGF1, Leptin, Urea	FE, Zn, Myoglobin, LCFA, MeFA (Whole genome scan)

This phenotyping effort (combined with data generated from other contracts…) has resulted in the creation of a phenotype database with more than 0.75 million data points that can be directly correlated with traits collected in other subprograms relating to parasite resistance, wool growth and fibre attributes, structure, behaviour and ewe reproduction traits. This is one of the most comprehensive phenotype datasets created in sheep. More than 4000 of the FMFS progeny will be genotyped on the ovine SNP50 beadchip (of which more than 2300 are males with comprehensive post slaughter data) this will enable high accuracy association studies to be carried out.

Development of a well phenotyped animal resource for gene discovery

1. More than 4800 progeny from 21 industry sires were generated at Falkiner Memorial Field Station (FMFS) in 2005 and 2006.
   1. All the FMFS lambs were measured with standard industry tests for meat traits (along with parasite, wool, behaviour, reproduction traits)
   2. 2346 male (wether) lambs were slaughtered and measured for a full range of traditional carcass and meat quality traits.

2. New traits were developed for increasing accuracy of body composition and retail meat yield traits
   1. DXA (Dual Emission Xray Absorption) was used to measure body composition on 2346 lambs
   2. New equations to calibrate DXA to chemical composition were derived
   3. New Equations to calibrate DXA to boned out retail cuts were derived
   4. These equations were used to derived new traits for chemical composition in primal cuts which provided novel high accuracy measures of distribution of components of yield throughout the carcass

3. New high throughput methods were developed to assess number, size and oxidative potentiall of muscle fibres
   1. Unique data on muscle fibre size, number of fibres and pixel intensity were obtained on muscle from 2346 lambs
   2. These have already been used to confirm that reduction in GDF8 (myostatin) protein is associated with an increase in muscle fibre number.

5. This work has provided a unique resource for gene discovery. There are more than ¾ million data points in the phenotype database, for more than 150 clearly unique traits.

Figure 16. Highlights of the FMFS phenotyping component of the Muscle subprogram

5.2.3. Evaluation of effects of DNA markers (for major genes) on phenotypes

Projects :- SG116, SG132, SG133
Evalaution of DNA markers for variation in MSTN and Carwell

Principal investigators :- Hutton Oddy, Julius van der Werf, James Kijas, Jason Siddell, Peter Allingham, Cedric Gondro

The following tables summarise all the studies conducted to estimate the size of effect of the myostatin marker g+6723G>A and the putative Carwell diagnostic haplotype.

In general it can be seen that for a copy of MSTN g+6723A the effects are approx 0.2 – 0.3 phenotypic standard deviations for lean traits and approx 0.3-0.5 phenotypic standard deviations for fat related traits. The effects of g+6723A on objective measures of tenderness are approx 0, but there are significant effects on sensory measures of tenderness (approx 0.3 phenotypic standard deviations). These are most likely driven by the significant reduction in intramuscular fat (-0.5 phenotypic SD units). The size and direction of effects of myostatin g+6723A on fatness and meat yield reported in studies from the UK, Belgium and NZ (Table n) are consistent with those obtained within sheepGENOMICS.

Compared to a copy of the MSTN g+6723A allele, one copy of the putative Carwell haplotype has approximately half the effects on lean and fatness related traits (lean, approx 0.1-0.2; fat, approx -0.15 phenotypic SDs). The effects of both MSTN and Carwell markers seem to be greater in the shoulder and loin than the hind leg.
The table (n) below summarizes the effect of 1 copy of a mutation in the myostatin locus on fatness and meat yield and quality traits. These results are similar to those reported by Walling et al (2004) and in more detail by Macfarlane et al (2009) for a QTL on oar18 in British Texel lambs that was associated with an increase in loin muscle weight (although all the animals in that study also had one copy of the myostatin g+6723A allele). It is not clear if the Carwell and Texel Muscling QTL on oar18 are allelic.

Table n. Summary of previously effects of 1 copy of myostatin g+6723A mutation or a myostatin associated microsatellite haplotype on carcass and meat traits reported previously (effects shown as proportion of phenotypic SD, "-" signifies reduction)

Trait Group	Laville *et al* (2004), Clop *et al* (2006)	Walling *et al* (2004)	Johnson *et al* (2005)	Hadjipavlou et al (2008)
Fatness	- 0.4*	- 0.4-0.5	-0.4-05	Not reported
Muscle weight / Yield	+ 0.2-0.3*	Not significant	+0.2-0.3	0.3-0.4
Tenderness	Not Determined	Not Determined	Not Determined	Not Determined

*Half effect of 2 copies of myostatin 3'UTR mutation, assuming no significant dominance effects

Table n. Summary of effects of 1 copy of myostatin g+6723A in experiments conducted within sheepGENOMICS (values are proportion of phenotypic SD accounted for, ns = not significant, - = not determined) * results from shgen.003 published in Kijas et al (2007). Remaining results as yet unpublished

Trait Group	Shgen.003*	FMFS Core	FMFS ’07	KEV
Live				
Birth wt	-	-0.4	0.17	-
Scanned emd	Ns	ns	Ns	Ns
Scanned fat	Ns	ns	Ns	Ns
Carcass				
CCWT		0.16	Ns	
Dressing %	-	0.28	Ns	-
GR	-0.44	-0.21	Ns	
Cfat	-0.45	-0.22	-	
Ema	0.33	ns	-	
Lean	-	0.16	0.2	
Fat	-	-0.25	-0.29	
Meat Traits				
IMF%	-0.5	ns	-	
Color a (redness)	Ns	-0.39	-	
WBS d1	Ns	ns		
WBS d3-5	Ns	0.2		
Overall satisfaction	-0.32	-	-	
Muscle fibre no	-	0.31	-	
CSA	-	ns	-	
Peak Pixel	-	ns	-	

Table n. Summary of effects of 1 copy of putative Carwell haplotye on a range of traits (values are proportion of phenotypic SD accounted for, ns = not significant, - = not determined)

Trait Group	Shgen.003	FMFS Core	FMFS ’07	KEV
Live				
Birth wt		ns	0.17	
Scanned emd	0.1 (ns)	ns	Ns	
Scanned fat	Ns	ns	-0.11	
Carcass				
Dressing %		ns		
GR		ns	-0.14	
Cfat		-0.19		
Ema	0.23	0.19		
Lean		ns	0.09	
Fat		ns	-0.16	
Loin Wt		-0.18	-	
Eye of Loin		-	0.46	
Meat Traits				
IMF%	-0.16	ns		
Color a		ns		
WBS d1	0.17	ns		
WBS d3-5	0.23	0.31		
Overall satisfaction	-	-	-	
Muscle fibre no	-	ns		
CSA	-	ns		
Peak Pixel	-	ns		

Issues around evaluation of gene (DNA) marker effects in industry populations.

Evaluation of effects of gene markers, particularly using industry flocks, is not a straight forward process. A major limitation to obtaining high quality data was the inability to accurately assign progeny to sire in syndicate mated flocks (FMFS ’07 and KEV). Use of only a limited set of a microsatellite markers for parentage assignment resulted in either a high rate of mis-assignment, and hence data wastage (FMFS ’07 progeny) or inability to analyse the data at all (KEV). This is a critical issue in design of such studies. Attention must be give to development of a set of markers for high accuracy parentage identification if such work is to be repeated. There is hope that such markers will become available. Within the

ISGC Mike Heaton (USDA) is working up a set of SNP markers with no interfering SNPs within range of the oligo's used to detect SNPs on different assay platforms. That SNPs can be used to correctly assign parents has been demonstrated within the FMFS Core flock where Jill Maddox and Mohammad Shariflou have assigned progeny of unkown sires using both a large (~8000) and small (~100) set of SNPs from the ovine SNP50 beadchip (personal communication).

Other constraints are statistically based. Where two (or more) genes likely to affect the same trait are present, the models currently used to determine effects require many more numbers than are currently feasible to collect. Accordingly the simple additive model is used, but with almost no way of testing that it is correct. By combining datasets across studies, it may eventually become possible to obtain sufficient data to determine if the effects of multiple markers are additive or have epistatic effects. This has become a problem with testing of the effect of Carwell in the presence of myostatin g+6723A. In progeny of commercial composites born to composite / maternal breed ewes, it is highly likely that a proportion of those progeny carry copies of each of myostatin g+6723A and Carwell, yet it is extremely difficult to obtain data to determine the nature of the interaction (if any) between the effects of the markers when 2 or more are present. This observation has significant implications for gene discovery processes using many (tens of) thousands of SNPs and comparatively few (10's of) phenotypes.

Within sheepGENOMICS there have been 4 separate studies carried out within the muscle SP to obtain data for evaluation of the effects of the myostatin g+6723A allele and the putative Carwell haplotype DNA markers. It is essential that all this data be pooled and a single analysis be carried out to arrive at an estimate of marker effects that include as many animals as possible. To conduct this work, it will be necessary for different phenotypes measured in each study and within industry to be compiled into a single database and the best predictor of phenotypes common to all studies be estimated for further analysis. Alternatively it may be possible to prepare tables such as those above that show the magnitude and commonality of effects of DNA markers across traits classes, so that statements about effects of a DNA marker(s) on for example fat, lean, yield and eating quality traits can be made. In summarizing the data above, it seems that an approach such as this is feasible, even if it does not provide unequivocal quantitative estimates of the size of effect of a DNA marker on a trait.

Evaluation of effects of DNA markers in Australian Sheep

1. The myostatin mutation g+6723A was found to increase lean mass by 0.2-0.3 phenotypic standard deviation (pSD)units, to decrease fat mass by 0.3-0.5 pSD units. These results are similar to those reported from overseas studies. Although there was no effect of g+6723A on objective measures of meat quality, it was associated with a reduction consumer satisfaction by 0.3 pSD units in one study. The g+6723A allele was also associated with more muscle fibres (0.3 pSD) and less intramuscular fat (0.5 pSD). There was no apparent effect on scanned eye muscle or fat depth in live lambs.

2. The Carwell haplotype was associated with a reduction in carcass fat (, -0.14, -0.16 pSD for GR, and by CT scan respectively) and an increase in carcass lean (0.09 pSD, CT scan). Although there was a negative effect on IMF (-0.16 pSD), limited evidence suggested no negative effect on objective measures of tenderness. There was a small effect of 1 copy of the Carwell haplotype on scanned fat depth in one study.

3. Work is underway to complete evaluation of CAST, CAPN1 and FSTL1 in lambs.

4. Use of industry flocks for genotype evaluation requires high accuracy pedigree tools. A major limitation in industry studies was correctly assigning progeny to sire in syndicate mated flocks. This will need to be overcome in development of new resources for evaluating effects of gene markers on industry or novel traits. There is a particular problem with obtaining numbers of progeny with multiple copies of different genes for evaluation of epistatic effects.

5. This work has provided a first estimates of effect of gene discovered or further developed within the muscle subprogram of sheepGENOMICS.

Figure 17. Summary of the outcomes from evaluation of DNA markers (for major genes) in Australian sheep.

5.2.4 Summary of outcomes - Gene discovery

- Development and application of new phenotyping procedures
    - Muscle fibre number and cross sectional area with NADH staining intensity in the same sample which allows higher throughput and is a lower cost method for obtaining such data on large numbers of lambs
    - Use of DXA on primals and half carcasses to measure chemical composition and estimate retail yield on large numbers of lambs
    - Measurements of industry standard traits for growth, fat and muscle phenotypes on more than 4800 lambs from the sires used at FMFS
    - Measurement of carcass, meat yield, meat quality and muscle characteristics on more than 2350 lambs (from the sires used at FMFS)

- Discovery and evaluation of the effects of the myostatin (GDF8) polymorphism g+6723G->A and other polymorphisms in the myostatin region on production, carcass and meat eating quality traits in sheep.

- Discovery and evaluation of the effects of a putative Carwell haplotype marker on production, carcass and meat eating quality traits in sheep.

- Discovery and evaluation of the effects of polymorphisms in the calpastatin and calpain 1 and Follistatin Like 1 gene regions on production, carcass and meat eating quality attributes in sheep

- Development of an industry based resource of DNA and phenotypes that can be used to evaluate the effects of other markers and SNP panels on meat related traits in sheep.

6. Capability and capacity building aspects of the Muscle subprogram

Development of the field of genomics applied to animal production was not an initiative of the RDCs (MLA and AWI) acting alone, but came about in part as a response to the Australian Commonwealth Governments "National Research Priorities" suggesting the Rural Development Corporations (RDCs) invest in (amongst other things) "Frontier Technologies for Building and Transforming Australian Industries". See - http://www.dest.gov.au/sectors/research_sector/policies_issues_reviews/key_issues/national_research_priorities/priority_goals/transforming_australian_industries.htm

The sub-programs of sheepGENOMICS, were directed by both MLA and AWI to develop and execute a "World Class" R & D program. The science and program management structures put in place were designed to facilitate excellence in science and its management.

The Scientists responsible for the leadership and development of the sub-programs were chosen specifically to ensure the highest quality science was brought to bear on the opportunities presented by genomic technologies to the sheep industry.

Dr Greg Harper (CSIRO, Livestock Industries) was appointed as inaugural sub-program leader. Dr John Bass (formerly AgResearch and then Liggins Institute, University of Auckland, New Zealand) and Prof Miranda Grounds (University of Western Australia) were appointed as external members of the Scientific Advisory Committee. Initial projects implemented within the Muscle sub-program were selected by application and interview. It is worthy of note that only one third of those submitted were supported. The highest standards of scientific review were adhered to in initiating and reviewing progress of projects within the muscle sub-program.

The muscle sub-program engaged at least eight scientists who never before worked for the Australian livestock and red meat industry. These included :- six new post-doctoral scientists (White, Knight, Vuocolo, Lavulo, Pagel, Kijas), two Professors (Mackie, Grounds) with expertise in human muscle, bone and connective tissue organization, development and function, three postgraduate students (Gorman, Meadows, Thu). During the course of the sub-program it has seen promotion of sub-program participants to Professorial and Deputy Chief of Division level within the University sector and within CSIRO.

The muscle sub-program pioneered the web based information system that became the sheepGENOMICS website. It used a web based system to enable sharing of resources across sites in Perth, WA (University of Western Australia), Melbourne (University of Melbourne and DPI Vic), Brisbane (CSIRO, Livestock Industries), Armidale, NSW (University of New England). The initial design of the "intelligence system" included the capacity to exchange documents and track editing of them in real time. Unfortunately this facility was unable to be included in the sheepGENOMICS website.

The muscle sub-program contributed actively to the development of the ARC Network for Genes and Environment in Development (NGED), and sent students and contributing papers on sub-program work to the NGED once it was established. In this way the sub-program extended its reach into complementary fields in other species.

By engaging with scientists who had no previous industry experience into the field, the sheepGENOMICS muscle sub-program increased exposure to the red meat industry of a pool of new people and organizations with complementary skills. At the time the sheepGENOMICS muscle sub-program was developed, existing meat science researchers were actively involved with the MLA supported Sheep Meat Eating Quality (SMEQ) program

and with the Sheep CRC. A deliberate policy of expanding the range of people and skills (rather than further over-committing those currently engaged with MLA) was employed to provide the red meat industry with options for future development.

This strategy worked, although at a cost. There are now more scientists aware of the opportunities for R & D within the meat industry. New skill sets are available to the industry, new genomic and phenotyping resources (and tools and data) are available to industry.

Unfortunately, there has been some resistance to this change. In particular, relationships between sheepGENOMICS and the Sheep CRC in the field of muscle growth and meat science have been strained despite the engagement of a significant number of the same scientists by both the Sheep CRC and sheepGENOMICS. Failure to generate a seamless expansion of the pool of skills in the field available to the meat industry is possibly due as much to separation of priorities of the differing “organizations” – sheepGENOMICS and SheepCRC – as well as the inevitable competition for resources and “air time” between the ventures. A lack of appreciation of the wider range of complementary skills available to the meat industry as a consequence of decisions to engage new scientists (see above) rather than continue just with those already engaged by MLA, needs to be addressed. It is important that different managers within MLA responsible for strategic and near to market investments recognize that such tensions between “organizations” and between “established” and “up and coming” researchers will potentially arise and act to minimize them.

In the event that strategic investments such as sheepGENOMICS were to continue, or equivalent ventures in other fields be supported by MLA, it seems to me to be essential that MLA Managers recognize the inherent requirements for operating in complementary spaces and ensure (and nurture) due recognition of the differences between strategic investments designed to grow capacity (people, skills, resources, data), and those from more short term (lower risk) investments designed to capitalize on better known scientific principles. Both investments are important for industry development, albeit the timelines are different and some of the same people may be operating in both spaces.

MLA and AWI do not operate alone, but in concert with the Commonwealth Government, and the various Research Organisations in the field. Investors are totally reliant upon the capability and capacity of the Research Organizations, as well as their willingness to work with them. Accordingly, it would be helpful to the Research Organizations in particular if a broader picture of policies designed to grow particular expertise and resources (most of which will reside within their own organizations long after any RDC, and Commonwealth Government, funding initiative is spent) were articulated and adhered to.

Summary of outcomes

- Development of capability to conduct genomics research on meat sheep
    - eight new scientists, post-doctoral fellows and PhD students exposed to the Australian meat and livestock Industry
    - Development of cross disciplinary (and organization) teams to carry out fundamental muscle R & D for the meat & livestock industry
    - Development of sheep specific genomic resources (linkage and physical maps, virtual genome, many SNPs and location on genome, use of Affymetrix Bovine array for sheep gene expression studies, unique immortalized ovine muscle cell lines)

7. Impact of this work on the meat and livestock industry

A principal outcome of Sheep Genomics Muscle subprogram has been to develop world class research infrastructure (genomic tools, muscle specific techniques for integrating muscle cell biology, gene expression, proteomics, methods for measuring muscle related phenotypes in lambs) to conduct genomic research on meat industry opportunities. The Muscle subprogram has further developed skills within key researchers throughout a number of research agencies. This provides the Australian sheep (and livestock) industry with highly skilled people able to conduct world class genomics research and the resources to conduct and / or implement outcomes of genomic research on meat production in the future. It is heartening that the strategy used within the muscle subprogram of sheepGENOMICS has been replicated elsewhere, most notably within the pharmaceutical giants Pfizer and Merck. Through their new companies, Pfizer Genetics and Merial Pty Ltd, they have recently entered the production animal gene testing space with the intent of discovering genes that regulate phenotype in farm production animals. They are entering the marker with the expectation that with this new information, they will discover new therapeutic compounds to manipulate animal health and production, or sell more product targeted to specific genotyped populations.

The important role played by sheepGENOMICS is perhaps best illustrated below (Figure 18). This illustrates the development of genomic resources generally. The structures of research and development activities which clearly show sheepGENOMICS positioned within the medium to longer term investment horizon applies equally well to the outcomes and positioning of the Muscle sub-program. This was recognized in the reviews carried out by Philip Pogson (Leading Partnerships) and Prof John Gibson (University of New England), both of which strongly advocated the development of a Sheep Industry Genetics Plan that incorporated a pipeline from strategic investments to industry delivery. I am not aware that this is being addressed.

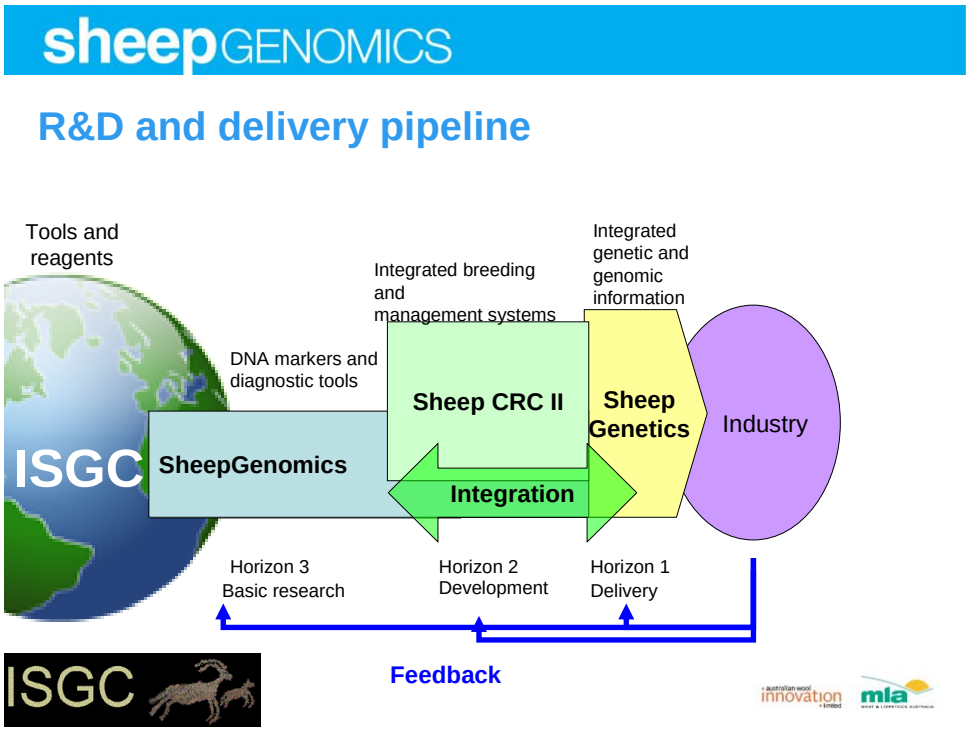

Figure 18. Diagram of the Research and Development and industry delivery landscape for sheep genetics and genomics technologies. This highlights the role played by each of the industry

investments and places them within a delivery horizon context. It can be seen that without ongoing investments in the sheepGENOMICS space that new information to assist development of new genetic improvement tools for the industry may well be delayed.

Specifically the muscle subprogram developed and applied new methods for measuring phenotypes on FMFS progeny. These have contributed new information on a sheep population ideally structured for discovery of QTL affecting muscle (and other) traits. Information on the FMFS ’06 progeny are immediately useful and have been transferred to Sheep Genetics, information on the FMFS’05 progeny will become useful to Sheep Genetics upon parentage SNP testing of their dams.

Additionally, information on specific gene mutations (or loci) affecting meat yield and objective meat quality attributes has been obtained. Information about Myostatin at least, has been published and made available to industry. Still to be delivered is information on the effects of the Carwell haplotype, and SNPs in association with Calpastatin, Calpain 1 and Follistatin Like 1. When this work is complete it may be utilized together with DNA SNP based tests for parentage and ASBVs to enhance rate of genetic gain in the lamb industry.

Beyond these specific loci, outcomes yet to come from the whole genome association study - anticipated to include Genomic EBVs for both existing and new meat production, yield and quality related traits – will be eventually made available through Sheep Genetics.

It is more difficult to predict the eventual utility of outcomes from discovery of gene function. The muscle subprogram has generated lists of differentially regulated genes and proteins in animals with genetic differences contributing to major changes in muscle phenotypes (Callipyge, Carwell, Various EMD ASBVs). Some of the genes and pathways identified through this work have been subject to evaluation using cell lines derived from sheep and mice, and in one case within lambs. These pilot studies have provided unique information on the function and utility of the target genes and pathways. In the case of HDAC9 use of knock down using siRNA has provided proof that the pathway is involved in regulation of muscle cell number and size. Use of Resveratrol and Benfotiamine as feed additives to modulate growth and fat / lean yield remains equivocal but worthy of a repeat study. Knowledge of the effect of reduction in Myostatin (GDF8) protein (due to the mutation g+6723 G->A) on lean and fat deposition has lead to further studies designed to test alternative methods for down regulation of myostatin protein in sheep. In summary, however, these studies are indicative and are likely to require substantial further investment before they provide practical tools for industry to use.

The full value of the lists of differentially regulated genes will only become apparent when they are combined with the locations of SNPs associated with muscle phenotypes – available July 2009. This will provide information to assist deduce functionality of specific SNPs and provide guidance as to likely candidate genes within specific genomic regions. In the longer run, this will contribute to discovery of the mutations responsible for moderate to large genetic effects on meat related traits.

A unique outcome of the muscle subprogram has been the development of a conditionally immortalized ovine muscle cell line. This has value as a tool for further research into the mechanisms of action of genes and gene pathways and their association with muscle phenotypes.

8. Conclusions and recommendations

For a strategic investment such as the Muscle sub-program of sheepGENOMICs to have long term impact it is critical that promising leads be followed up as the resources allow. It would be anticipated that by July 2009, knowledge of many genomic regions that are associated with meat related traits will be have been identified - a result of whole genome screens using the ovine SNP50 beadchip that are currently underway. This information will identify many promising genomic regions and highlight opportunities not currently imagined.

The muscle subprogram of sheepGENOMICS has contributed the following new outcomes:-

- Development and application of new phenotyping procedures
    - Muscle fibre number and cross sectional area with NADH staining intensity in the same sample which allows higher throughput and is a lower cost method for obtaining such data on large numbers of lambs
    - Use of DXA on primals and half carcasses to measure chemical composition and estimate retail yield on large numbers of lambs
    - Measurements of industry growth, fat and muscle phenotypes on more than 4800 lambs from the sires used at FMFS
    - Measurement of carcass, meat yield, meat quality and muscle characteristics on more than 2350 lambs (from the sires used at FMFS)

- Extensive development of gene expression data in selected muscles of CLPG and normal lambs (both microarray and RT-PCR procedures). First use of MPSS to explore gene expression in muscle of CLPG and normal sheep. Development of a list of differentially expressed genes in muscle from CLPG, Carwell and animals from sires with different EBVs, and assessment of this list for potential candidates.

- Testing of potential candidates from the list of differentially expressed genes (FSTL1 as a candidate gene – mutation discovered and animals genotyped to determine association, Notch inhibitors (DAPT) and inhibitors of HDAC9 (specific siRNAs) tested in muscle cell lines, DLK1 and SLC22A3 transfection systems tested in immortalised sheep muscle cell lines).

- Proteomics assessment of differentially regulated proteins in organelles isolated from CLPG v normal muscle.

- Development of a list of potential modulatory pathways from differentially regulated proteins and testing of potential modulators in sheep (benfotiamine and resveratrol)

- Development of the only immortalized sheep muscle cell lines in the world, and use of these cells to evaluate the effect of candidate genes

- Evaluation of the effects of g+6723G->A and other polymorphisms in the myostatin region on production, carcass and meat eating quality traits in sheep.

- Evaluation of the effects of polymorphisms in the calpastatin and calpain 1 and Follistatin Like 1 gene regions on production, carcass and meat eating quality attributes in sheep

- Development of capability to conduct genomics research on meat sheep

  - eight new scientists, post-doctoral fellows and PhD students exposed to the Australian meat and livestock Industry
  - Development of cross disciplinary (and organization) teams to carry out fundamental muscle R & D for the meat & livestock industry
  - Development of sheep specific genomic resources (linkage and physical maps, virtual genome, many SNPs and location on genome, use of Affymetrix Bovine array for sheep gene expression studies, unique immortalized ovine muscle cell lines).

- More than 10 full papers in refereed journals and over 39 papers / posters / communications have been presented at conferences from participants in the Muscle sub-program to date.

Recommendations

There are a number of technical tasks that remain to be completed. These should be given high priority for support.

- Skin samples from Callipyge (CN) and normal (NN) lambs have been collected. It would be useful to know if they differed in density and distribution of wool follicles (P+s/P, and secondary branching). The information from this small study would assist understand the putative role of DLK1 as a notch inhibitor in a tissue other than muscle.
- The Benfotiamine and Resveratrol trial should be repeated with more and younger lambs. Body composition changes should be measured more thoroughly than in the preliminary study. It is likely that the combination of the two natural compounds may reduce fatness. This may be valuable information for use of these compounds in applications other than manipulation of body composition of sheep (e.g. as additives for human diets to reduce obesity).
- The observation that methylation (of chromatin and DNA) patterns around different loci is affected by tissue location and by development has implications for wider studies of epigenetic regulation of tissue development. This work should be promoted to NHMRC and ARC for more fundamental studies on regulation of development.
- Lists of gene pathways and differentially expressed genes that may be candidates for differential muscle development should be compared with outcomes from the ovine SNP50 beadchip with a view to rapidly locating high priority candidates for gene discovery.
- All the data from evaluation of myostatin, Carwell and other DNA / gene markers should be combined into a single analysis to determine the “best bet” estimate of effects. This information needs to be passed to industry as quickly as possible.

In addition to the technical tasks above, activities to secure access to resources should be implemented:-

- The shgen.003 DNA should be secured in a safe and readily accessible place and together with phenotype data a process to ensure unencumbered access for the future is required. It is anticipated that this resource will become more valuable as the need to confirm associations between markers and traits in independent populations is recognized.
- An inventory of samples (DNA / blood / tissue) and all phenotype data from all studies conducted within the Muscle subprogram should be established. This is especially important because to date most of these studies have been used for validation of several markers. They provide an opportunity for evaluation of new markers and new panels of SNPs when they become available.

Given that results from the ovine SNP50 beadchip will become available before July 2009, the following should also be given high priority.

- Key scientists involved with development of FMFS phenotyped resources should remain engaged with the analysis of associations between ovine SNP50 beadchip and meat traits. The purpose of this recommendation is to ensure ongoing engagement of those doing the analysis of genotypic effects with those who have been instrumental in generating the phenotype data and developing hypotheses about the basis of different phenotypes. It is anticipated this will lead to more rapid discovery of underlying mutations. This work will not be undertaken within the Sheep CRC.

- Close collaboration between the ISGC Haplotype Mapping project and sheepGENOMICS muscle subprogram scientists should continue to ensure lessons learned about selection sweeps in the "global" sheep population inform future work on understanding genetic mechanisms that impact on growth and development of muscle. It is not planned that this work will be undertaken by the Sheep CRC.
- Close collaboration between key scientists in sheepGENOMICS and others in the field (SheepCRC and independently) be encouraged to develop DNA / gene markers that work across sheep breeds (i.e. almost certainly causal mutations). It is anticipated that knowledge of gene function and effect on phenotype will be required to do this, rather than simply associations between markers and traits. It is anticipated that this information will ultimately provide more robust solutions to industry than reliance on associations alone.

At the highest level it is strongly suggested that ongoing collaboration between the ISGC, sheepGENOMICS, Sheep CRC and Sheep Genetics be fostered. It is anticipated this would enable an integrated pipeline that co-ordinates strategic investment in development of resources in the field, exploration of the possibilities that can come from the application of the technologies that depend of those resources, and delivery of technical solutions for industry.

9. Bibliography (Publications arising)

1. Lavulo, L.T., Uaesootrachoon, K., Mirams, M., White, J.D., Cockett, N.E., Mackie, E.J. and Pagel, C.N. (2008) Myoblasts isolated from hypertrophy-responsive callipyge muscles show altered growth rates and increased resistance to serum deprivation-induced apoptosis. Cells Tissues Organs 187: 141-151
2. White, J.D., Vuocolo, T., McDonagh, M., Grounds, M.D., Harper, G., Cockett, N.E. and Tellam, R.L. (2008) Cellular analysis of the Callipyge phenotype through skeletal muscle development; association of Dlk1 with developing satellite cells. *Differentiation* 76, 283-298.
3. Kijas, J.W., McCulloch, R., Hocking-Edwards, J.E., Oddy, V.H., Lee, S.H. and van der Werf, J. (2007) BMC Genetics 8:80
4. Leeton, L. and Tellam, R.L. (2007) Adipogenesis in Mouse 3T3L1 Cells: the effects of Rtl1 over-expression. *Functional and Integrative Genomics* 7, 257-261
5. Fleming-Waddell, J.N., Wilson L.M., Olbricht G.R., Vuocolo, T., Byrne, K., Craig B.A., Tellam R.L., Cockett N.E. and Bidwell C.A. (2007) Analysis of gene expression during the onset of muscle hypertrophy in callipyge sheep. *Animal Genetics* 38, 28-36.
6. Vuocolo T., Byrne K., White J., McWilliam S., Reverter A., Cockett N.E. and Tellam R.L. (2007) Identification of a gene network contributing to muscle hypertrophy in callipyge skeletal muscle. *Physiological Genomics* 28, 253-272.
7. Tellam, R.L. (2007) The Impact of Genomics on Livestock Production. In *Redesigning Animal Agriculture – the challenge for the 21st century*. (Ed. Swain DL, Charmley E, Steel JW and Coffey SG) (CABI Publishing) pp 46-64.
8. Tellam, R.L. (2006) Capturing benefits from the bovine genome sequence. *Australian Journal of Experimental Agriculture* 47, 1039-1050.
9. Vuocolo, T., Cockett, N., Tellam, R.L. (2005) Expression of imprinted genes surrounding the callipyge mutation in ovine skeletal muscle. *Australian Journal of Experimental Agriculture* 45, 879-892.

10. Meadows, J.R.S., Chan, E. and Kijas, J.W. (2008) Linkage disequilibrium compared between five populations of domestic sheep. *BMC Genetics* **9:** 61-71.

Presentations at symposia, workshops and conferences

1. *Tellam, R. Rapid evolution of genomic imprinting* (2007) Joint Conference on Vertebrate Comparative Genomics. National Academy of Science USA and Australian Academy of Science (Los Angeles).
2. *Tellam, R. Rapid evolution of genomic imprinting in mammals* (2007) CSIRO Transformational Biology Workshop (Canberra).
3. *Tellam, R. Genomic imprinting at the callipyge locus in sheep – a taste for epigenetic control* (2007) CSIRO Nutrigenomics Workshop (Brisbane).
4. *Tellam, R. Determining Gene Function For Agriculture And As A Model Organism* (2005) The Bovine Genome Project: The Next Phase Workshop (Houston, USA).
5. *Tellam, R. Epigenetic changes in gene expression in Callipyge lambs* (2005) The Association for the Advancement of Animal Breeding and Genetics Conference (QLD, Australia).
6. *Tellam, R. Changes in skeletal muscle gene expression caused by the callipyge mutation in sheep*. (2005) The Sir Mark Oliphant Conference - International Frontiers

of Science and Technology. Epigenetic Regulation of Disease and Development (Canberra Australia).

7. Vuocolo T., Byrne K., White J., Cockett N.E. and Tellam R.L. (2006) Differential DNA Methylation is Associated with the Callipyge Mutation in Sheep. NGED Conference, Cairns, QLD.
8. Vuocolo, T., Byrne, K., McWilliam, S., Reverter, A., Cockett, N. and Tellam, R.L. (2006) *Transcriptional profiling of callipyge skeletal muscle*. Plant and Animal Genome XIV Conference, San Diego, California.
9. Vuocolo, T., Bryne, K., Leeton, L., Wong, F., Barris, W., Cockett, N., White J. and Tellam, R.L. (2007) *Differential methylation is associated with the callipyge mutation in sheep*. 28th Lorne Genome Conference, Victoria, Australia.
10. Byrne, K., Vuocolo, T., Leeton, l., Reverter, T., Cockett, N., White, J. and Tellam, R.L. (2006) *Changes in skeletal muscle gene expression caused by the callipyge mutation in sheep.* COMBIO2006 Combined Conference, Brisbane, QLD.
11. Fleming-Waddell, J.N., L.M. Wilson, G.R. Olbricht, T. Vuocolo, K. Byrne, B.A. Craig, R.L. Tellam and C.A. Bidwell. 2006. *Gene expression profiling of two hypertrophied muscles in callipyge lambs.* International Society of Animal Genetics. http://www.isag.org.uk/pdf/2006ISAG_Proceedings.pdf C514. (abstract, poster)
12. Wilson, L.M., J.N. Fleming-Waddell, M. Neary, N.E. Cockett, and C.A. Bidwell. *The callipyge mutation does not alter DLK1 expression in visceral or subcutaneous adipose tissue in sheep.* International Society of Animal Genetics. http://www.isag.org.uk/pdf/2006ISAG_Proceedings.pdf C529. (abstract, poster)
13. Fleming-Waddell, J.N., L.M. Wilson, G.R. Olbricht, T. Vuocolo, K. Byrne, B.A. Craig, R.L. Tellam and C.A. Bidwell. 2006. *Analysis of gene expression during the onset of muscle hypertrophy in callipyge lambs.* International Plant and Animal Genome Conference. http://www.intl-pag.org/14/abstracts /PAG14_P724.html. (abstract, poster)
14. Tellam R.L., Vuocolo, T., Byrne, K., White, J., McWilliam, S., Reverter A., and Cockett, N. (2006) *Transcriptional Profiling of Callipyge Skeletal Muscle during Development.* International Society for Animal Genetics, Porto Seguro, Brazil
15. Leeton, L., Vuocolo, T., Dalrymple B., and Tellam, R.L. (2005) *Analysis and modulation of murine PEG11, a paternally imprinted genes with a potential role in adipogenesis.* 26th Lorne Genome Conference, Victoria, Australia.
16. Leeton, L., Vuocolo, and Tellam, R.L. (2005) *Effect of modulation in 3T3L1 cells of murine RTL1, a paternally imprinted gene with a potential role in adipogenesis*. The Sir Mark Oliphant Conference - International Frontiers of Science and Technology. Epigenetic Regulation of Disease and Development (Canberra Australia)
17. Dalrymple, B., Barris, W., Reverter, T., McWilliam, S. and Tellam, R.L. (2005) *Comparative genomic analysis of tandem arrays of snoRNAs and miRNA precursors adjacent to the Callipyge mutation.* 26th Lorne Genome Conference, Victoria, Australia.
18. Dalrymple, B., McWilliam, S., Barris, W., Reverter-Gomez, T., Tellam, R.L. and Leeton, L. (2005) Evolution of SNORNAS and miRNas in the BEGAIN – DIO iii interval of Eutherian mammals. The Sir Mark Oliphant Conference - International Frontiers Of Science And Technology. Epigenetic Regulation of Disease and Development (Canberra Australia).
19. Dalrymple, B., McWilliam, S., Barris, W. and Tellam, R. (2004) *Identification of small non-coding RNAs in eukaryotes: the role of comparative genomics.* Lorne Genome

Conference. Lorne, Victoria, Australia.

20. Vuocolo, T., Leeton, L. and Tellam, R.L. (2004) *Expression of alternatively spliced mRNA variants arising from genes located at the imprinted Dlk1 locus*. Horizons in Livestock Sciences Conference – Gene Silencing and Therapeutic Innovations. Gold Coast, QLD.
21. Leeton, L., Vuocolo, T., Dalrymple, B. and Tellam, R.L. (2004) Analysis of the sequence of murine PEG11, a paternally imprinted gene that encodes a GAG-RVT retrotransposon-like polyprotein. Horizons in Livestock Sciences Conference – Gene Silencing and Therapeutic Innovations. Gold Coast, QLD.
22. Dalrymple, B., McWilliam, S., Barris, W., Leeton, L. and Tellam, R.L. (2004) Comparative genomic analysis of tandem arrays of snoRNAs and miRNA precursors adjacent to the callipyge mutation. Horizons in Livestock Sciences Conference – Gene Silencing And Therapeutic Innovations. Gold Coast, QLD.
23. NGUYEN, T., GROUNDS, M. HARPER, G.S. and WHITE, J.D., (2006) Links between the IGF1 signaling pathways and skeletal muscle hypertrophy in sheep. In: Wool meets Meat – Tools for a modern sheep enterprise. *Proceedings of the 2006 Australian Sheep Industry CRC Conference, Orange.* (eds. Cronje, P.B. and Maxwell, D.K.) The Australian Sheep Industry CRC, Armidale, NSW, Australia, 239-240.
24. NGUYEN, T., WHITE, J.D., GROUNDS, M. and HARPER, G.S. (2004) Links between the IGF1 signaling pathways and skeletal muscle hypertrophy in sheep. *Proceedings of the Matrix Biology Society of Australia and New Zealand* in September 2004, Rottnest Is. Western Australia.
25. NGUYEN, T., GROUNDS, M., HARPER, G.S. and WHITE, J.D. (2004) Links between the IGF1 signaling pathways and skeletal muscle hypertrophy in sheep. *Proceedings of the Australian Society for Biochemistry and Molecular Biology* 36, Pos-Mon-051.
26. NGUYEN, T., WHITE, J.D., GROUNDS, M. and HARPER, G.S. (2004) Links between the IGF1 signaling pathways and skeletal muscle hypertrophy in sheep. *Combined CRCs Postgraduate Conference,* Coffs Harbour, Sept 2004, (Gardner, G. ed.)

27. Meadows J, Chan E and Kijas J. Characterisation of Linkage Disequilibrium in Australian Sheep. Poster. Plant and Animal Genome XVI, San Diego, USA, January 12 – 16 2008.

28. Kijas JW, Meadows J, Pardeshi V, Kadoo, N, Gupta V, Hummel J, Drogemuller C, Moran C, O'Rourke B and Arthur P. Genetic Basis of the Australian Merino. Poster. The 30th International Society of Animal Genetics, Porto Seguro, Brazil, August 20 - 25, 2006.

29. Meadows JRS, Hanotte O, Drogemuller C, Calvo J, Godfrey R, Coltman D, Maddox JF, MarzanoN, Kantanen J, Kijas JW. 2006. Novel Y Chromosomal Haplotypes Reveal Wild and Domestic Sheep Diversity. The 30th International Conference Animal Genetics, Porto Seguro, Brazil, August 20 - 25, 2006.

30. Gorman CM. Mackie EJ. White JD. The Myogenic effect of compounds of the skeletal muscle extracellular matrix on cultured myoblasts. ComBio 2008 Combined Conference, Canberra, ACT, Australia. September 2008. ISSN 1328 4924

31. Gorman CM. Mackie EJ. White JD. The myogenic effects of components fo skeletal muscle extracellular matrix on cultured myoblast. Matrix Biology Society of Australia

and New Zealand Annual Meeting, Ettalong Beach, NSW, Australia. October 2008. ISBN 0 95875892 8 3

32. Gorman CM. Mackie EJ. White JD. The myogenic effects of components fo skeletal muscle extracellular matrix on cultured myoblast. The Australian Health and Medical Research Congress, Brisbane, Qld, Australia. November 2008. Issn 1447-6010.

33. White JD. Gorman CM. Cockett N. Oddy H. Greenwood P. Pagel C. Lavulo L. Mackie EJ. Cellular dissection of the callipyge skeletal muscle phenotype. ComBio 2008 Combined Conference, Canberra, ACT, Australia. September 2008. ISSN 1328 4924

34. White JD, Abdul-Raes R, Bachor K, Myers D, O'Brien T, Wark J, Mackie EJ, Tudor E. Anti-epileptic drug therapy and its influence on osteoblasts and myoblasts. Matrix Biology Society of Australia and New Zealand Annual Meeting, Ettalong Beach, NSW, Australia. October 2008. ISBN 0 95875892 8 3

35. White JD, Abdul-Raes R, Bachor K, Myers D, O'Brien T, Wark J, Mackie EJ, Tudor E. Are the changes in bone mineral density with anti-epileptic drug therapy due to altered osteoblast and myoblast activity? The Australian Health and Medical Research Congress, Brisbane, Qld, Australia. November 2008. ISSN 1447-6010.

36. Nguyen, T. Wong CF, White JD, Harper G, Grounds M. Links between the IGF-1 signalling pathways and skeletal muscle hypertrophy in sheep. 7th Pan Pacific Connective Tissue Societies Symposium, Cairns, QLD, Australia. October 2007. ISBN 0 9585892.

37. Gorman CM, White JD. The role of extracellular matrix proteins in myogenesis in vitro. 7th Pan Pacific Connective Tissue Societies Symposium, Cairns, QLD, Australia. October 2007. ISBN 0 9585892.

38. Lavulo LT, Uaesoontrachoon K, Mirans, M, White JD, Cockett NE, Mackie EJ, Pagel CN. An in-vitro approach to understanding the molecular mechanism of callipyge muscle hypertrophy. Exercise, Muscle & Metabolism 2007, Melbourne, Vic, Australia. November 2007.

39. Gorman C, Mackie EJ, White JD. Production animals models of muscle hypertrophy. Exercise, Muscle & Metabolism 2007, Melbourne, Vic, Australia. November 2007.

10. References cited

1. Freking, B.A. Murphy, S.K., Wylie, A.A., Rhodes, S.J., Keele, J.W., Leymaster, K.A., Jirtle, R.L. and Smith, T.P.L. (2002) Identification of the single base change causing the callipyge muscle hypertrophy phenotype, the only known example of polar overdominance in mammals. Genome Res 12: 1496-1506

2. Koohmaraie, M., Shackleford, S.D., Wheeler, T.L., Lonergan, S.M. and Doumit, M.E. (1995) A muscle hypertrophy condition in lamb (callipyge): characterization of effects on muscxle growth and meat quality traits. J. Anim. Sci. 73: 3596-3607

3. Carpenter, C.E., Rice, O.D., Cockett, N.E. and Snowder, G.D. (1996) Histology and composition of muscles from normal and callipyge lambs. J. Anim. Sci. 74: 388-393
4. Cockett, N.E., Jackson, S.P., Shay, T.L., Farnir, F., Berghmans, S., Snowder, G.D., Nielsen, D.M. and Georges, M. (1996) Polar overdominance at the ovine callipyge locus. Science 273: 236-238

5. Murphy, S.K. Nolan, C.M., Huang, Z., Kucera,K.S. Freking, B.A., Smith, T.P.L, Leymaster, K.A. Weidman, J.R., Jirtle, R.L. (2006) Callipyge mutation affects gene expression in cis: A potential role for chromatin structure**.** Genome Res. 2006 Jan 13; : 16415109

6. Macfarlane, J.M., Lambe, N.R., Bishop, S.C., Matika, O., Rius-Vilarrasa, E., McLean, K.A., Haresign, W., Wolf, B.T., McLaren, R.J. and Bunger, L. (2009) Effects of the Texel muscling quantitative trait locus on carcass traits in crossbred lambs Animal 3: 189-199

7. Hadjipavlou, G., Matika, O., Clop, A., Bishop, S.C. (2008) Two songle nucleotide polymorphisms in the myostatin (GDF8) gene have significant association with muscel depth in commercial Charollais sheep. Animal Genetics 39: 346-353

11. Appendix 1

Summary of Intellectual Property developed within the Muscle subprogram. Note Type s = support, m = modulators, g = gene markers, p = phenotypes.

MEUSP Project	Outputs	Specific IP	TYPE	Encumberances	Contributing IP
101 Cell resource development	Cell lines	Primary muscle cell lines from different muscle (LD and ST) and from Callipyge (CN) and Normal (NN) sheep. Conditionally immortalised sheep muscle cell lines from CN and NN sheep.	S M		
102 MEUSP intelligence unit 112 MEUSP intelligence unit	Bioinformatics platform	Web site for communications within scientific sub-programs. Contributed to development fo sheepGENOMICS web site.	S		
103 Phenotype characterisation 104 Phenotype characterisation 113 Phenotype charateristation	Various Various Candidates	Calipyge phenotypes- muscle attributes. Potential candiade molecules and associated genes derived from literature.	G S M		
105 Candiadte regulatory genes	Various	Information on transcripts around Callipyge locus and some associations between Callipyge transcripts and myogenic regulatory factors	M		
107 Genes affecting muscle phenotype 117 Kijas Molecular dissection 132 Validation of DNA tests for sheep industry 133 Validation of DNA tests for industry 138 Consultancy for Peter Allingham	Muscle DNA markers Muscle DNA markers Muscle DNA markers Muscle DNA markers FMFS database	Carwell Associated Haplotypes (microsatellites and SNP) and effects in shgen.003. MSTN 3'UTR and flanking microsatellite Haplotype information about associated effects in shgen.003. New SNPs and microsatellites flanking CAST, Calpain1 and Calpain 3 (117 +FLSTN 1)	G S M	ShGen.003 and associated contracts CSIRO patent re CAST (Calpastatin) and association with tenderness in beef cattle. Note that MLA "own" 100% of the data and DNA in ShGen.003	SFG.014 - suggested FSTL1 as candidate gene from analysis of Hi / Lo muscle EBVs Samples came from Sheep CRC 1 (although their contribution to IP would be minor if at all)
109 Muscle	Candidates	Information on differential gene expression in CLPG			

Hypertrophy		(CN,NN,NC,CC), Carwell (+/-) and hi / Lo muscle EBV lambs. Lists of differentially regulated transcripts and potential candidate genes and pathways. Specific knowledge of candidates such as FSTL1, HDAC9, SLC22A3, DLK1, Notch (list is available)	M
110 Callipyge animals Utah	Candidates	Samples of muscles from animals of known CLPG status	S M
116 Charateristation of novel muscle phenotypes and discovery of associated genotypes	Muscle DNA markers	Carwell haplotype and MSTN 3'UTR associations with meat yield and quality traits in industry and FMFS derived animals	G P
118 Extended phenotyping	FMFS phenotype database	FMFS muscle fibre number, size and NADH staining intensity information. Also includes some work on Fibre types of P1, P2 progeny	G P S
120 FMFS phenotyping -carcase 129 FMFS phenotyping -carcase 135 FMFS phenotyping -carcase	FMFS phenotype database FMFS phenotype database FMFS phenotype database	FMFS slaughter information and meat quality (shear force, compression, color, pHu measurements)	G P S
122 FMFS phenotyping sample collection	FMFS phenotype database	FMFS slaughter information	G P S
123 FMFS phenotyping DXA	FMFS phenotype	FMFS wethers - primal weights, estimated retail and	G

scanning 127 FMFS phentyping DXA scanning 134 FMFS phenotyping DXA scanning	database FMFS phenotype database FMFS phenotype database	chemical components derived by DXA on shoulder, loin, belly, leg and half carcase	 P S
124 FMFS visual assessments	FMFS phenotype database	Information on all FMFS progeny on visual assessment	G P S
128 FMFS DXA calibration	FMFS phenotype database	Calibration equations to enable conversion of raw DXA information into corrected chemical and retail components for half carcase and shoulder, loin, belly and leg	G P S
136 MEUSP Proteomics II	Candidates	Knowledge of effects of benfotiamine on c2c12 muscle cell lines, Knowledge of effects of HDAC and Notch inhibitors on c2c12 muscle cell lines. Knowledge of effects of benfotiamine and resveratrol (as feed additives) on lamb growth, body composition and specific gene expression.	M
137 Modulation of genes to enhance muscling	Candidates	Production of expression cassettes for 3 DLK1 variants, production of expression cassette for SLC22A3, Production of 4 siRNA variants targetterd to HDAC9 knock down and knowledge of the effect of HDAC9 knockdown on C2C12 muscle cell phenotypes	M
139 Utilisation of cell lines for functional	Cell lines	Insertion of 3 DLK1 expression cassettes into conditionally immortalised ovine muscle cell lines. Knowledge of effects of these on cellular phenotype. Insertion of SLC22A3 expression cassette into conditionally immortalised ovine muscle cell lines. Knowledge of effects of over-expression of SLC22A3 on ovine cell lines. Knowledge of effects of DAPT (Notch inhibitor) on immortalised ovine muscle cell lines.	S M

	Conditionally immortalised ovine muscle cell lines transfected with DLK1 constructs and SLC22A3.		

12. Appendix 2

Continued access to Muscle subprogram resources

The resources developed within (and used by) the Muscle subprogram are available, and can be obtained from the sites or persons listed below.

1. Gene Function component of the muscle sub-program

Primary ovine muscle cell lines ejmackie@unimelb.edu.au

Data on gene expression from primary muscle cell lines (qRT-PCR tony.vuocolo@csiro.au, microarray jasondw@unimelb.edu.au)

Immortalised ovine cell lines ejmackie@unimelb.edu.au jasondw@unimelb.edu.au

Immortalised ovine cell lines transfected with overexpression constructs of dlk-1 and scl22a3 ejmackie@unimelb.ed.au jasondw@unimelb.edu.au

Data from gene expression (affymetrix bovine microarray and qRT-PCR of muscle samples taken from 80, 100, 120 pc and within 2 days of birth and at 11-12 weeks from up to 4 muscles ss, ld, st, sm from up to 4 Callipyge genotypes CC, NC, CN, NN ross.tellam@csiro.au tony.vuocolo@csiro.au

Data from MPSS runs from selected CN and NN muscle (LD samples taken at 12 weeks of age were analysed) ross.tellam@csiro.au

Data from Proteomics studies accessible through matthew.mcdonagh@dpi.vic.gov.au

A list of more than 50 candidate genes generated from differential gene expression in Callipyge, Carwell and high low muscle EBV contrasts are available at

http://www.sheepgenomics.com/documents.aspx?DocumentID=J8Sg2SFhPgk%3d

Of the candidates listed those discussed above (section XXX) are high priority. Some have been explored further (section XXX,YYY).

In addition to candidate genes, pathways and potential protein modulation targets identified using differential proteomics in Callipyge contrasts are available at

http://www.sheepgenomics.com/documents.aspx?DocumentID=TfPtRojsI%2bk%3d

All gene expression data were deposited with the sheepGENOMICS Core Technologies databases.

2. Gene Discovery component of the Muscle subprogram

FMFS Core Blood and DNA – Currently stored at AGRF – contact ?
FMFS Core IGF1 blood cards – Where are these Jason?
FMFS Core Phenotypes - http://pheno.sg.angis.org.au/

Methods for measurement of new muscle phenotypes -
http://www.sheepgenomics.com/soptree.aspx

Shgen.003 – Blood stored at Pfizer Animal Genetics – Brisbane facility
http://www.geneticsolutions.com.au/content/v4_standard.asp?name=ContactUs
This needs to be secured. The original agreements with Genetic Solutions clearly indicate that the blood and DNA are owned by MLA, but and agreement over IP generated from the resource has not been achieved. Legal advice suggests that MLA may offer Genetic Solutions (and subsequent owners of Genetic Solutions) a non –exclusive license to the use of IP arising. Given the potential value of this resource for evaluation of marker effects it is imperative that the license arrangements for IP arising be resolved. Owners are MLA, UNE, Genetic Solutions – Head Agreement is shgen.102.

Shgen.003 – Phenotypes stored on SQL data file at UNE julius.vanderwerf@une.edu.au

FMFS ’07 progeny blood and DNA – Blood at AGRF DNA of subset with James Kijas – james.kijas@csiro.au
FMFS ’07 progeny meat phenotypes (on subset)- http://pheno.sg.angis.org.au/

FMFS ’08 progeny blood and DNA – Blood at AGRF DNA of subset with James Kijas
FMFS ’08 progeny meat phenotypes (on subset) – currently being measured (on 80 animals) - hutton.oddy@une.edu.au

Keiller Evaluation Study (KEV) Blood and DNA – james.kijas@csiro.au
KEV Phenotypes – Peter Allingham / Hutton Oddy – peter.allingham@csiro.au; hutton.oddy@une.edu.au