

Effects of Tenderness Genotypes and of Experimental Site on the Expression of Fat and Ribosomal Module Genes

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Introduction

Skeletal muscle is plastic and able to respond to external stimuli through changes in gene expression profiles, as illustrated previously by a number of microarray studies (Lehnert, Wang, Tan et al. 2006; Li, Zhu, Li, X., et al. 2008; Wang, Bower, Reverter et al. 2009). As part of the process of turning data into biological information, analysis and interpretation of this type of data have focussed on using gene ontology and databases to identify key differentially expressed genes, pathways and processes. Whilst informative, this type of analysis often results in lists of genes that are difficult to interpret. In this paper we aim to illustrate a simplified systems biology approach for analysing microarray data. A previous microarray experiment used Longissimus dorsi muscle (LD) samples from 2 divergent cattle cross-breeds (Piedmontese x Hereford and Wagyu x Hereford) and 10 developmental time points from 60 days post-conception to 30 months of age (Hudson, Reverter and Dalrymple 2009). A series of co-expression networks of genes across all these conditions were identified (Hudson, Reverter, Wang et al. 2009). Subsets of these genes were grouped into modules, each having been annotated with a functional category (Hudson, Reverter and Dalrymple 2009; Dalrymple et al. unpublished). From these, the six modules used in this paper are: cell cycle (49 genes), fat (12), immune (22), mitochondria (33), muscle/glycolysis (44) and ribosomal (22). We hypothesise that concerted changes in gene expression within a module and disruptions in the cohesiveness of a module are indicative of major functional changes between the conditions/comparisons under investigation. In order to illustrate this concept, our paper aims to explore the genes from the modules as a perspective through which to view our new data, which was generated using the same species, tissue type and platform.

Materials and Methods

Cattle, environment and genotype. Sixteen Brahman steers were included in this experiment and form part of a larger study conducted at two experimental sites in New South Wales (NSW) and Western Australia (WA), Australia. Further details of the full experiment is provided by Cafe, McIntyre, Robinson et al. (unpublished). Briefly, prior to sample collection in the feedlot, cattle from NSW were fed a grain-based ration for 103 days, whilst

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in WA they were fed a grain-based ration for only 66 days. The ration composition in the two sites also differed. In NSW the ration had of 12.0 MJ ME and 160g CP per kg dry matter (DM). In WA the ration had of 10.8 MJ ME; 134g CP per kg DM (Cafe, McIntyre, Robinson et al. unpublished). In addition, the cattle had been selected based on their tenderness genotypes at the *CAST3-84*, *CAPN3JK* and *CAPN1-4751* loci. They were genetically classed as either “tough” (no copies of favorable alleles) or “tender” (2 copies of favorable *CAST3-84* and *CAPN3JK* and 1 copy of *CAPN1-4751* alleles).

Microarray Experiment. Needle biopsy samples were collected from the LD and total RNA was extracted using methods previously described (Lehnert Wang, Tan et al. 2006). Each of the 16 samples were labelled with Cy3 fluorescent label and hybridised to a 44k Agilent bovine array following a previously published protocol (Hudson, Reverter and Dalrymple 2009). As this was a single-colour array experiment, no dye swapping was done. Each RNA sample was spiked with 10 different viral polyadenylated RNAs to function as reference samples. Fluorescence intensity was measured using the GenePix 4000A scanner (Molecular Devices, Sunnyvale, CA) at a resolution of 10µm.

Bioinformatics. Modules in this paper refer to groups of genes in LD muscle that were previously shown to be co-expressed across development in 2 breeds (Hudson, Reverter, Wang et al. 2009). Each module has been annotated with a specific functional category. Module genes refer to the individual genes contained within each module. The differential expression (DE) of a gene is a measure of change in expression from one condition compared to another. The modules of genes have been overlaid onto the new data presented in this paper, where the effects of site and genotype are investigated.

Results and Discussion

We found that the majority of modules retained their consistency of co-expression, exhibiting neither a site nor a genotype effect (Figure 1). The genes in the mitochondrial module provide a good example of this with the genes tightly clustered around zero (Figure 1d). In contrast, the ribosomal (Figure 1f) and fat modules (Figure 1b) both exhibited patterns consistent with genotype and/or site effects. The genes in the ribosomal module show a small, but consistent site and genotype difference, the genes are slightly up regulated in the tough genotype compared to the tender genotype in NSW and vice versa in WA. The coherence of DE of the fat module is lower than for the other modules and is affected by genotype. Genes from the fat module are more highly expressed in the tough than in the tender genotype in both NSW and WA. Furthermore, in order to illustrate the effects on levels of expression, Figure 2a shows that the expression of fat genes is higher in WA than in NSW samples regardless of genotype. The ribosomal genes also had a slightly higher expression in the WA samples, but only for the tender genotype. Again, illustrating a coherent module, the levels of expression of the genes in the mitochondrial module show no effect from site or genotype (Figure 2c). Despite the large difference in the expression of fat module genes, no significant effects of genotype or site were found for IMF (Cafe et al. unpublished). A likely interpretation is that these changes are reflecting metabolic changes, such as turnover rates of lipids, especially considering the different nutritional regimens between the 2 sites, particularly the time on concentrate feed and the nutrient density of the feed.

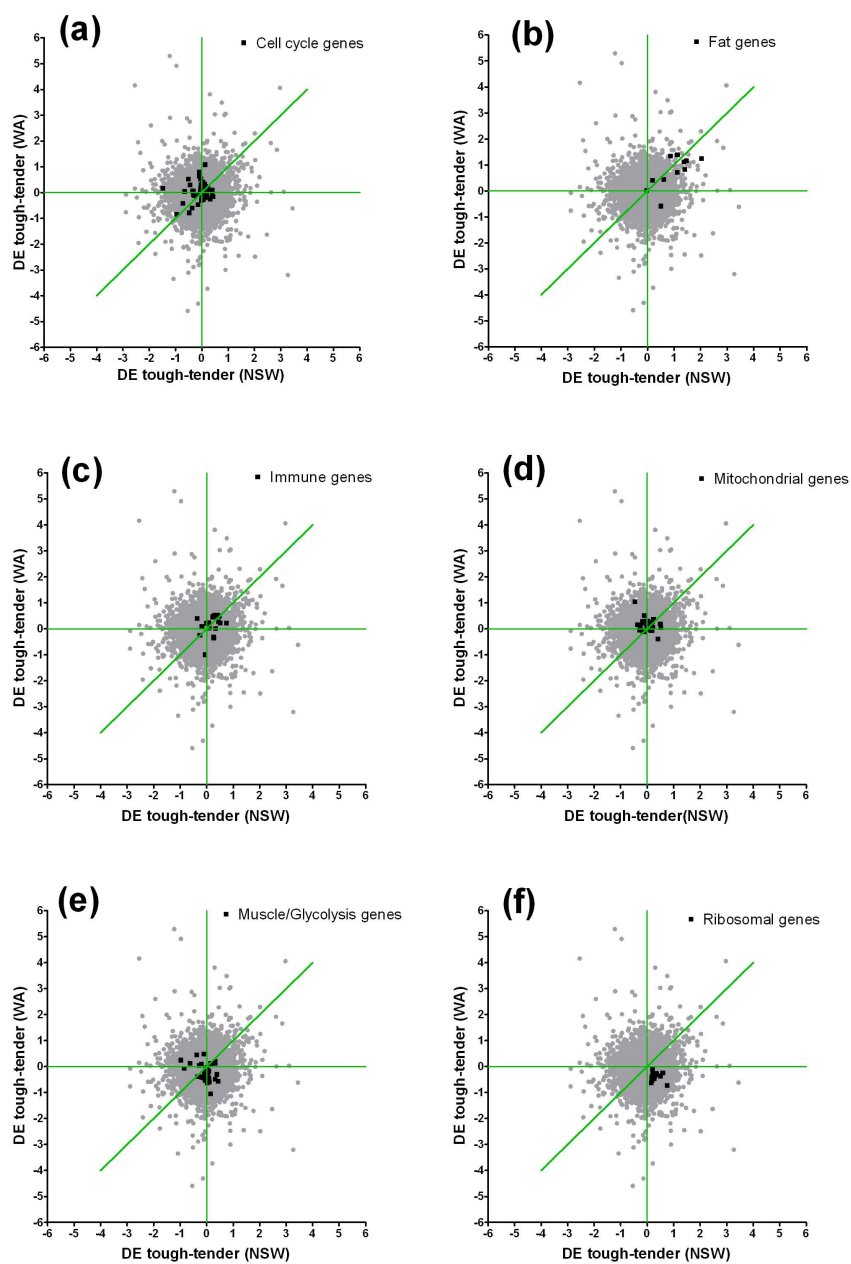


Figure 1. Differential Expression patterns of genes from 6 different modules (a-f) as affected by genotype (tough and tender) and site (NSW and WA). Grey points represent probes/genes and the black squares are the respective module genes. DE is measured as the change in intensity signal between tough and tender genotypes.

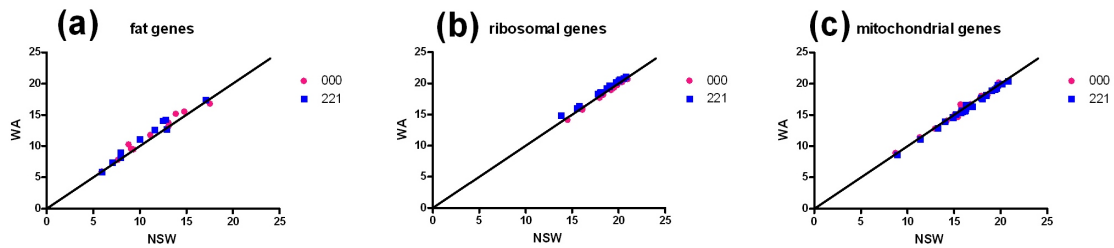


Figure 2. Relationship between the levels of gene expression for NSW and WA for both genotypes (tough and tender), in 3 modules of genes. The level of gene expression is measured in intensity of signal.

Conclusion

We have shown that previously defined modules of genes can be useful in terms of focussing the interpretation of high-throughput data from an independent microarray experiment conducted using the same sample type and array platform. We found unexpected effects of tenderness genotypes on the expression of genes in the fat module as well as the effect of both genotype and site on the expression of ribosomal genes. Further investigations into elucidating the molecular basis for these phenomena are currently underway.

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