



NSW DEPARTMENT OF  
PRIMARY INDUSTRIES



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## Genetic testing at EMAI

The Elizabeth Macarthur Agricultural Institute (EMAI) molecular genetics laboratory has made significant contributions in the characterisation of deleterious inherited traits in animal species. The outcome of this sustained research contribution over more than ten years is the development of the diagnostic division of the laboratory and availability of 23 commercial diagnostic tests for genetic disease and production traits in cattle. The establishment of these tests in conjunction with education programs for breed societies and seedstock producers has paved the way for 'genetically smarter' and more consumer sensitive breeding programs.

The following genetic tests are available through the University of Queensland, Animal Genetics Laboratory or through EMAI directly:

Inherited trait	Classification	Breed
$\alpha$ -mannosidosis	disease	Angus/Murray Grey
$\alpha$ -mannosidosis	disease	Galloway
$\beta$ -mannosidosis	disease	Salers
Bovine leukocyte adhesion deficiency	disease	Holstein
Citrullinemia	disease	Holstein
Factor XI deficiency	disease	Holstein
Pompes Disease (E7)	disease	Brahman
Pompes Disease (E13)	disease	Brahman
Pompes Disease (E18)	disease	Shorthorn
Congenital myasthenic syndrome	disease	Brahman
Inherited congenital myoclonus	disease	Poll Hereford
Maple syrup urine disease	disease	Poll Hereford
Maple syrup urine disease	disease	Shorthorn
Protoporphyrria	disease	Limousin
Myophosphorylase deficiency	disease	Charolais
Freemartinism	disease	Multiple breeds
Red coat colour	boutique trait	Multiple breeds
Black coat colour	boutique trait	Multiple breeds
$\beta$ -lactoglobulin	production trait	Dairy breeds
$\kappa$ -casein	production trait	Dairy breeds
Muscular hypertrophy	production trait	Multiple breeds

## **BOVINE GENETIC DISEASES**

### **$\alpha$ -mannosidosis**

$\alpha$ -mannosidosis is an autosomal recessive lysosomal storage disorder found in Angus, Murray Grey and Galloway cattle. It is caused by deficiency in activity of lysosomal  $\alpha$ -mannosidase, resulting in an accumulation of mannose rich oligosaccharides in various tissues.

Affected Angus and Murray Grey calves either fail to survive the immediate postnatal period, or if they do, they show severe, progressive neurological disease characterised by tremors of the head, ataxia, and aggression. The phenotype in Galloways is more severe, with most affected cases aborted or stillborn.

#### *Angus /Murray Greys*

The mutation causing  $\alpha$ -mannosidosis in Angus and Murray Greys is identical, but differs from the mutation causing this disease in Galloways. The mutation in Angus and Murray Greys is a T→C transition at nucleotide 961 in the  *$\alpha$ -mannosidase* gene, resulting in a Phe321Leu substitution.

#### *Galloways*

The mutation causing  $\alpha$ -mannosidosis in Galloways is unique to this breed, and is a G→A transition in exon 5 at nucleotide 662 in the  *$\alpha$ -mannosidase* gene, resulting in an Arg221His substitution.

### **$\beta$ -mannosidosis**

$\beta$ -mannosidosis is an autosomal recessive lysosomal storage disorder found in Salers cattle. It is caused by deficiency in activity of lysosomal acidic  $\beta$ -mannosidase, resulting in an accumulation of mannose rich oligosaccharides in various tissues. Affected calves are usually born alive, but exhibit severe neurological abnormalities. These include weakness, incoordination, head-swaying, splaying of the front legs, and a poor sucking reflex.

The mutation responsible for bovine  $\beta$ -mannosidosis is a G→A transition at position 2574 in the  $\beta$ -mannosidase cDNA that creates a premature termination codon near the 3' end of the protein coding region.

### **Bovine leucocyte adhesion deficiency**

Bovine leucocyte adhesion deficiency (BLAD) is an autosomal recessive disease due to deficiency of CD18, a subunit of adhesion molecules on the surface of neutrophils. Adhesion of neutrophils to endothelial cells is a prerequisite to their egress from the circulation into tissue to combat infection. Consequently, homozygous mutant calves suffer recurrent infections that impair growth, and their survival is dependent on antibiotic therapy.

The causative mutation is an A→G transition at nucleotide 383 in the *CD18* gene, resulting in an Asp128Gly substitution.

### **Citrullinaemia**

Citrullinaemia is an inborn error of metabolism due to deficiency in activity of the urea cycle enzyme, argininosuccinate synthetase (ASS). It is characterised by a pronounced accumulation of citrulline in tissues and body fluids due to the failure of normal synthesis of argininosuccinic acid. Interruption of the urea cycle results in an inability to dispose of excess ammonia (by conversion to urea) derived from the metabolism of protein. It is this increased concentration of ammonia in the brain that accounts for the severe, progressive neurological disease that is characteristic of this disease.

Affected Friesian calves are born relatively normally, but within 12-24 hours they become depressed, then show tremors, head-pressing and apparent blindness. By 48 hours, affected calves develop seizures, opisthotonus, coma and death.

The mutation causing citrullinaemia in Holstein-Friesians is a C→T transition at nucleotide 258 in the *ASS* gene, converting Arg86 into a termination codon.

### **Factor XI deficiency**

Factor XI deficiency is an autosomal recessive defect reported in humans, dogs and Holstein cattle. Factor XI is one of a number of proteins involved in the process of clot formation. In humans and cattle, Factor XI deficiency presents as a bleeding disorder that may be life threatening. The disease was first diagnosed in Holstein cattle in Ohio USA in 1969. The characteristic haemophilic-like symptoms were later observed in Holstein-Friesians in Canada, England and Australia.

Bovine Factor XI (FXI) deficiency is the result of a 76 bp insertion in exon 12 of the gene encoding FXI. Most of the insertion consists of long adenine repeats, but one part codes for a premature stop signal. As a result, the mutation prevents synthesis of the full length, active protein, thereby disrupting the clotting cascade in homozygous mutant subjects.

## Pompes Disease

Generalised glycogenosis (Pompe's Disease) is an autosomal recessive, lysosomal storage disease, caused by deficiency in activity of acidic  $\alpha$ -glucosidase (AAG). Clinical signs are progressive muscular weakness, incoordinated gait and ill-thrift, with a life expectancy of less than 12 months.

### E7

The E7 mutation is the most common of two mutations (E7 & E13) identified in the *acidic  $\alpha$ -glucosidase* gene in Brahmans that cause generalised glycogenosis, and is a dinucleotide deletion in exon 7 (1057 $\Delta$ TA). The resultant frame-shift introduces a premature termination codon in exon 8.

### E13

The E13 mutation is the least common of two mutations identified in this gene in Brahmans, and is a nonsense mutation in exon 13 (1783C $\rightarrow$ T, Arg595Ter).

### E18

The E18 mutation is the only known mutation causing generalised glycogenosis in Shorthorns. It is the result of a dinucleotide deletion (2454 $\Delta$ CA) in exon 18, which introduces a premature termination codon in exon 19.

## Congenital myasthenic syndrome

Congenital myasthenic gravis (congenital myasthenic syndrome, CMS) is an autosomal recessive disease that has been reported in Red Brahman calves in South Africa. The condition is the result of a deletion of 20 nucleotides at position 470 in exon 5 of the gene encoding the  $\epsilon$  subunit (bov*CHRNE*) of the nicotinic acetylcholine receptor (AChR). The deletion causes a frame shift, followed by a premature stop codon in the predicted bov*CHRNE* protein, resulting in loss of function and subsequent impairment of neuromuscular transmission.

Clinical signs are similar to those seen in Pompe's Disease, with the exceptions that the onset of the disease is earlier and progression much more rapid. Muscular weakness develops at 3 to 4 weeks of age, and progresses within weeks to the point where the calf can no longer rise unassisted. Life expectancy is less than 6 months.

## Inherited congenital myoclonus

Inherited congenital myoclonus (ICM) is an autosomal recessive disease, characterised by hyperesthesia and myoclonic jerks of skeletal musculature, which start *in utero* (hence the term ICM), and may occur spontaneously, or in response to tactile, visual and/or auditory stimuli. There are no pathological lesions in the central nervous system, and symptoms are unaltered by antiepileptic and anticonvulsive drugs. The severe spasms *in utero* almost invariably result in hip lesions, ranging from cracks in the acetabulum to fractures of the neck of the femur. Clinically, affected calves are bright and alert, but are unable to rise to suckle, so under field conditions, they rarely survive beyond 2-3 days.

Their suckling reflex is normal, but attempts to stand the calves result in a "saw-horse" type posture. Breathing ceases (apnoea) during these myoclonic spasms. Upon release to the ground, the calves relax within a minute, and normal breathing resumes.

Bovine ICM is the result of a severe disturbance of glycine-mediated neurotransmission in the spinal cord, due to a single base transversion (156C $\rightarrow$ A) in exon 2 of the  $\alpha$ 1 subunit of the glycine receptor (GlyR). The mutation deletes a tyrosine codon and creates a premature stop codon, leading to the synthesis of a truncated polypeptide that lacks function.

## Maple Syrup Urine Disease

Maple Syrup Urine Disease (MSUD) is an autosomal recessive disease found in both Poll Hereford and Poll Shorthorn cattle. It is caused by deficiency in activity of the E1 $\alpha$  subunit of the *branched chain keto acid dehydrogenase* gene (BCKADH). Calves are born relatively normal, but exhibit neurological disease within 24 hours. Their condition rapidly deteriorates with their inability to metabolise the branched chain keto acids, with opisthotonus, limb-paddling and death occurring within 72-96 hours of birth. There is molecular heterogeneity between the two breeds for this disease.

### Poll Herefords

The mutation in Poll Herefords is a C $\rightarrow$ T transition in the leader sequence at nucleotide 248, corresponding to codon -6, where glutamine is changed to a stop codon (Q-6St).

### Shorthorns

The mutation in Poll Shorthorns is a C $\rightarrow$ T transition at nucleotide 1380 in exon 9, predicting the substitution of leucine in place of a highly conserved proline at codon 372.

## Protoporphyrin

Erythropoietic protoporphyria is an autosomal recessive defect found in Limousin cattle, characterised by severe photosensitivity. Protoporphyrin is caused by a deficiency in activity of ferrochelatase, the enzyme responsible for inserting iron into protoporphyrin, which is the final step of the haem biosynthetic pathway.

Affected Limousins show lesions of the nares, ears, midline and perineal regions, and display an apparent aversion to sunlight. Lactating adults may have severe lesions of the udder and teats, which impacts upon their ability to successfully nurse their young. Affected animals have a markedly elevated concentration of protoporphyrin in blood, and a deficiency of ferrochelatase in tissues.

The mutation causative of protoporphyria in Limousin cattle is a G→T transversion at nucleotide 1250 in exon 11 of the *ferrochelatase* gene, converting the stop codon to a leucine codon. Translation is terminated when the next in-frame stop codon is reached, resulting in an additional 27 amino acids at the carboxy terminus of the protein.

## Myophosphorylase deficiency

Myophosphorylase deficiency is a glycogen storage disease (GSD Type V) that has been recorded in Charolais cattle in North America and New Zealand. The human condition is commonly referred to as McArdles Disease. GSD Type V is caused by a deficiency in activity of myophosphorylase, a muscle-specific enzyme that metabolises stored glycogen, releasing glucose as glucose 1-phosphate. As with Pompe's Disease (GSD Type II), deficiency in activity of the enzyme results in the accumulation of glycogen, leading to clinical signs that predominantly involve muscle.

Clinical signs in Charolais usually become apparent in calves at several weeks or months of age, and manifest as an intolerance to exercise. Affected calves lag behind the moving herd, and may collapse and become recumbent for several minutes following prolonged exercise. In the absence of such stress however, not all homozygous mutant calves are clinically obvious. In addition, some homozygous mutants have been known to breed despite muscle weakness.

The mutation is a C→T transition at position 1468 in the bovine *myophosphorylase* gene, changing tryptophan to arginine at amino acid 490 (W490R). The condition is inherited in an autosomal recessive manner.

## Freemartinism

Unlike most other species, more than 90% of twin calves are haemopoietic chimeras. This happens because the chorioallantoic blood vessels of the foetuses fuse during the second month of gestation, allowing the interchange of haemopoietic stem cells before the immune system is able to recognise and react to foreign material. This exchange of stem cells produces a state of immunological tolerance between the twins. The major implication with freemartinism in heifer twins is when the cotwin is male. Freemartinism is hormonal suppression of the developing female reproductive tract in utero, leading to infertile heifers. An indication of freemartinism is the presence of sequence specific to a gene on the Y chromosome in DNA isolated from the blood of the heifer.

## BOVINE BOUTIQUE TRAITS

### Coat Colour

Two coat colour PCR tests are available, courtesy of ABS Global, Wisconsin. They are based on the melanocyte-stimulating hormone (MSH) receptor having a major function in the regulation of black versus red pigment synthesis within melanocytes. Tyrosinase is the limiting enzyme involved in the synthesis of both pigments - a low level of tyrosinase leads to production of the red pigment, whereas high levels lead to the black pigment. Three alleles attributable to coat colour have been identified in the bovine *MSH-receptor* gene (Table 2). A test specific for the wildtype allele (which produces a variety of colours, with black animals being red/black, i.e. black with red tinge) is not available but can be deduced from results of both the recessive red and dominant black tests.

**Table 2:** Genotypes and corresponding phenotypes for coat colour alleles

Genotype	Phenotype
E <sup>D</sup> E <sup>D</sup>	Black, or black and white
E <sup>D</sup> E <sup>+</sup>	Black, or black and white
E <sup>+</sup> E <sup>+</sup>	Various – red/black
E <sup>D</sup> e	Black, or black and white
E <sup>+</sup> e	Red, or red/black
e e	Red, or red and white

#### *Dominant black*

The dominant black allele ( $E^D$ ) is caused by a point mutation (296T→C), resulting in black coat colour. The mutation (Leu→Pro) makes the receptor work more efficiently, replacing the red/black wild type colour with black.

#### *Recessive Red*

The recessive red allele (e) is caused by a frameshift mutation at nucleotide 311 ( $\Delta G$ ), producing a prematurely terminated receptor and no melanin synthesis. Homozygous e/e animals have a red coat colour.

### **BOVINE PRODUCTION TRAITS**

#### **$\kappa$ -casein**

Genetic variants of the bovine  $\kappa$ -casein gene are associated with protein content of milk, and have significant influence on rennet clotting time, firmness and cheese yield of milk. Milk of the BB genotype is considered superior to the AA genotype, leading to a preference for sires with  $\kappa$ -casein genotypes of BB or AB.

#### **$\beta$ -lactoglobulin**

Bovine  $\beta$ -lactoglobulin is the major component of milk whey protein. Genetic variants of the bovine  $\beta$ -lactoglobulin gene are associated with significant differences in milk composition, and its cheese-processing properties, including rennet clotting time, rate of firmness, curd firmness and cheese yield. Genotyping is considered useful for early identification of the  $\beta$ -lactoglobulin variants in dairy cattle selection programs.

#### **Muscular hypertrophy**

Muscular hypertrophy, is due to an increase in the number of muscle fibres (hyperplasia). The phenotype in cattle is the result of polymorphisms in exons 2 and 3 of the *myostatin* gene (MSTN) (also known as GDF-8 (Growth Development Factor 8)). The carcasses of animals with muscular hypertrophy have significantly increased muscle mass as expressed by retail product yield but fertility and calving ease may be compromised in the extreme phenotype (double muscling).

The laboratory has the capability to genotype for each of the six known mutations in the *myostatin* gene attributable to muscular hypertrophy. This includes the frameshift mutations at nucleotide (nt) 419 (del7:ins10) and nt821 (del11) in exons 2 and 3 respectively; the nonsense mutations at nt610 (C→T) and nt676 (G→T) in exon 2, and a missense mutation at nt938 (G→A) in exon 3.

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