Bad News:
They're all Carriers of Something
Broken Genes in the Beef Business

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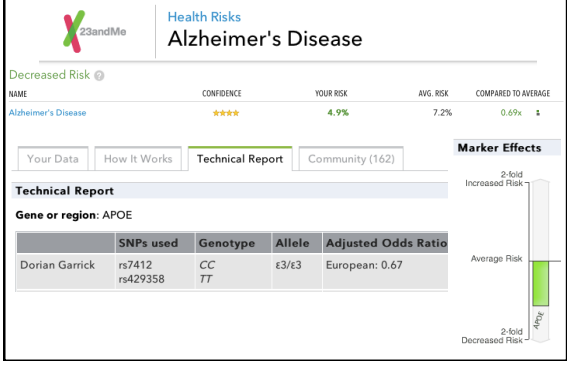

Good News:
They're all Carriers of Something
Broken Genes in the Beef Business

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Outline

- Inherited phenotypic disorders
  - e.g. birth defects
- Inherited disorders based on SNP genotypes
- Inherited disorders from sequence data
- Managing inherited disorders and other genetic variants

Phenotype-based Defects

Genetics defects monitored by US breed cattle breed associations

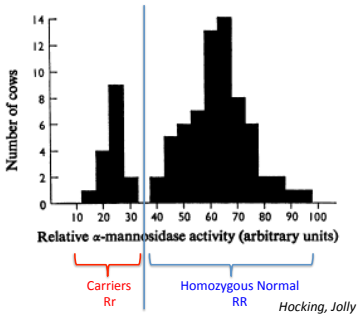
Genetic Abnormality	*Primary Breed(s) of Incidence*	*Lethal or Nonlethal*	*DNA Test Available*
Alpha (α)-Mannosidosis	Red Angus	Lethal	Yes
Arthrogryposis Multiplex (AM)	Angus	Lethal	Yes
Beta (β)-Mannosidosis	Salers	Lethal	Yes
Contractural Arachnodactyly (CA)	Angus	Nonlethal	Yes
Neuropathic Hydrocephalus (NH)	Angus	Lethal	Yes
Hypotrichosis (hairless calf)	Hereford	Nonlethal	No
Idiopathic Epilepsy	Hereford	Nonlethal	Yes
Osteopetrosis	Angus and Red Angus	Lethal	Yes (Red Angus)
Protoporphyria	Limousin	Nonlethal	Yes
Pulmonary Hypoplasia and Anasarca (PHA)	Maine-Anjou and Shorthorn	Lethal	Yes
Tibial Hemimelia (TH)	Shorthorn and Maine-Anjou	Lethal	Yes

Spangler http://ianrpubs.unl.edu/live/g2055/build/g2055.pdf

Mannosidosis

- Hereditary recessive disease first known in Angus, Murray Grey and Galloway cattle (Australia, 1957)
  - In 1970 about 10% Angus in NZ were carriers
- Homozygous recessive individuals:
  - develop signs of head tremors, have difficulty walking, demonstrate aggressive behavior and failure to thrive
  - Most die shortly after birth or within their first year
- Caused by deficiency of lysosomal α-*mannosidase*, an enzyme that degrades mannose-containing oligosaccharides – results from various SNP

Allele Dosage



Testing started in 1972
Carriers were identified by low enzyme levels and were culled

Hocking, Jolly and Batt (1972) Biochem J

Hairy Phenotype

Affected heifer

A "widely-used" young bull

- Yearling Holstein bull called Matrix
  - 12,000 AI inseminations
  - 6,000 offspring
  - 3,000 daughters
  - 1,500 "affected"
- Have to be caused by a dominant mutation

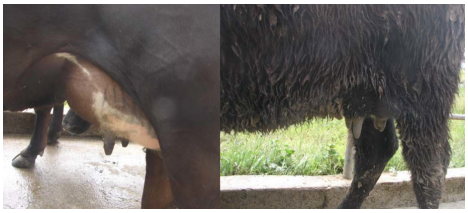
Unpublished: Dr. Richard Spelman, Livestock Improvement Corporation

Phenotype

Affected animals are hairy and seek water and mud

Caused by amino acid substitution

Cows exhibited poor, if any, lactation performance after calving



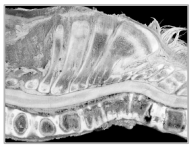
Caused by a *de novo* (new) mutation not present in ancestors

Whose liability?

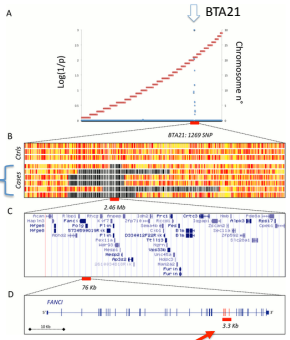
Brachyspina (Short spine)

- Lethal, gross lesions: growth retardation
- Vertebral malformation, brachyspina, long and slender limbs
- Inferior brachygnatism, malformation of the heart, kidneys and testicles
- < 1 in 100,000 births

6 samples



Brachyspina



Black shows SNP Homozygous segments

Shown from 6 affected cases to be caused by a deletion of 3,300 bases

Charlier et al PLoS ONE 7:e43085

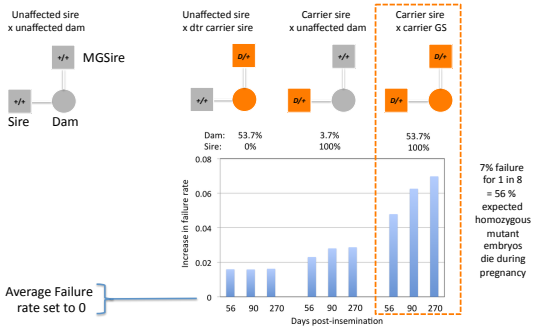
Phenotype first defects

- Rely on reports from producers
  - Also require collection of DNA samples (e.g. hair)
  - Many producers (especially seedstock) are reluctant to report such occurrences
    - Many well known defects have yet to be characterized because of lack of availability of DNA samples
- What about
  - embryonic lethals?
  - mutations that slightly reduce performance?

Brachyspina is an EL

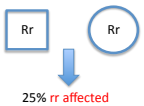
- Recessive defect was imputed onto 50k haplotypes
  - 7% Holstein sires were carriers of the defect
    - 1 in 200 matings would be between carriers
    - 1 in 4 such matings would produce homozygote
  - Expect more than 1 in 1,000 births to be affected
  - Embryonic lethality could cause low disease incidence but high carrier frequency

Embryonic lethality and fertility



Embryonic Lethals

- Expected impact on conception rate and calving date



25% rr affected
75% normal pregnancy

RR Normal
Rr Carrier
rr Recessive

Embryonic Lethals

- Expected impact on conception rate and calving date

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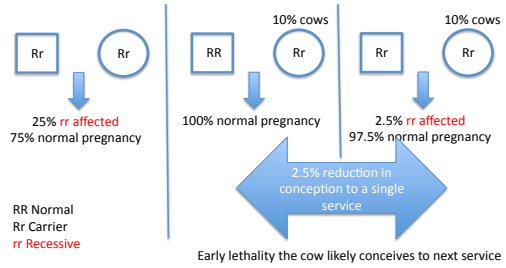


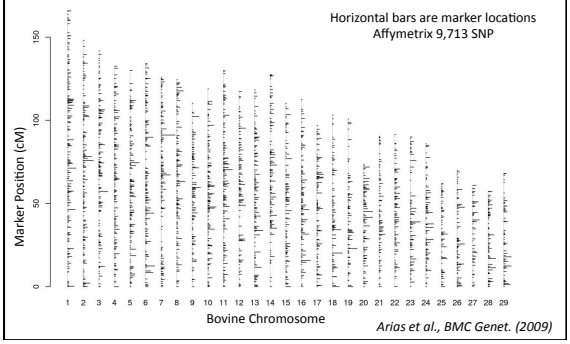
Embryonic Lethals (EL)

- Requires high proportion of carriers before it might be detected from reduced conception rate, increased failure rate or more days to calving
- Unlikely to be detected in small herds, or herds using many sires with few offspring per sire
- No chance of detection without total herd recording (THR)
- Could there be many such rare causes of EL?

Haplotype First - Disequilibrium

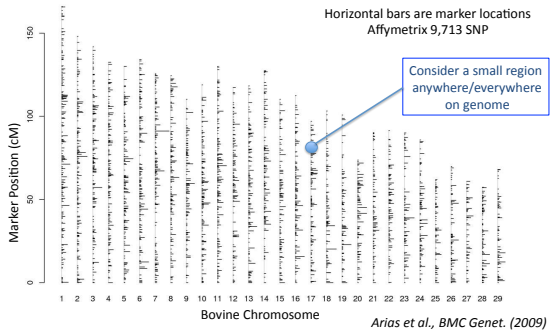
- An alternative approach to find embryonic lethals and other unfavorable mutations is to inspect haplotype distributions and haplotype combinations

Genome Structure



Arias et al., BMC Genet. (2009)

Genome Structure



Arias et al., BMC Genet. (2009)

Typical Region of 1% Chromosome

- Contains 20 SNP markers on the 50k panel
  - Each is labeled one of AA, AB, or BB corresponding to 0, 1 or 2 copies of the B allele
- Genotype of an individual would be expressed
  - Like 01012021120220012120 at such a region

Genotype vs Haplotype

- Chromosomes are paired so the genotype represents the allele inherited from the sire and the allele inherited from the dam
  - Consider 01012021120220012120 (diploid)
  - Paternal 0_0_101__101100_1_10 (haploid)
  - Maternal 0_0_101__101100_1_10 (haploid)
  - Where _ represents either 0 or 1 copies of B allele
- If the alleles can be filled in (or phased) we have the haplotypes of the individual

Many Potential Haplotypes

- At 2 loci there are 4 possible haplotypes
  - "00", "01", "10", and "11"
- At 3 loci there are 8 possible haplotypes
  - "000", "001", "010", "011", "100", "101", "110", "111"
- At *k* loci there are 2^*k* possible haplotypes
- At 20 loci (e.g. 1% or 1 Mb chromosome on 50k) there are >1 million possible haplotypes

Few Haplotypes are Present

- In 1,000 Herefords we only see up to 40 common haplotypes in any 1% chromosome region
  - On average there are 20 such common haplotypes

Common haplotypes in 1 Mb regions

20 SNP can have >1m haplotypes

Haplotypes from Beagle

7_93 (11 SNP)		6_38 (23 SNP)		20_4 (28 SNP)	
1075	01101110111	216	10010000000111000010111	761	01001100110111000011101011111
253	00101110111	132	10111000010101011000110	136	01101001101100100110011101111
181	11101110111	121	10011101011111010010101	124	01100101010001110110111110011
71	11101110101	118	10110100010111011010111	107	11101100110000000100101000011
58	01001110101	113	00110101001101100010111	88	11000110110010000101011111111
33	00100110101	95	10111000011101011000100	52	01101111110101110000111110001
29	01000110101	80	00011101111011010010101	50	01011101011110010100111111010
27	11001110101	74	11111101010110011101100	49	11110100110011100110110000001
24	11111110111	68	10111010101010111000110	49	01001101101011100000101000010
22	11001110111	67	10010001000111000010111	31	01001101110010100011010111111
20	01100110111	63	11111100010110011101100	26	01100001010000000110111110011
19	00101110100	53	10111101011010111000100	22	01001001110010100010110000010
10	01101110100	52	1 11111011100100011111100	20	11000110110110000101011111111

Note there are no tag SNPs!

Among 941 Herefords

Haplotypes Identical by Descent

- Common autosomal haplotypes must be in many sires and many dams
- Imagine a common haplotype contains an old mutation (r)
  - Not necessarily known

Sire / Dam	010r11 (20%)	Others (80%)
010r11 (20%)	rr (4%)	common
Others (80%)	common	common

- Absence of the homozygous form suggests lethality
- Direct testing of mating pairs adds power (need THR)

Findings

- US research in dairy cattle found 5 regions that included a common haplotype that was never observed as homozygous
- French researchers found
  - In Holstein: CVM, Brachyspina, HH1 and HH3
  - In Montbeliarde: a new one at 9% another at 7%
  - 9 new mutations contained in the causal haplotypes and likely to cause the lethality were discovered

US: VanRaden et al., 2011 J Dairy Sci
French: Fritz et al., 2013 PAG

New mutation in common haplotype

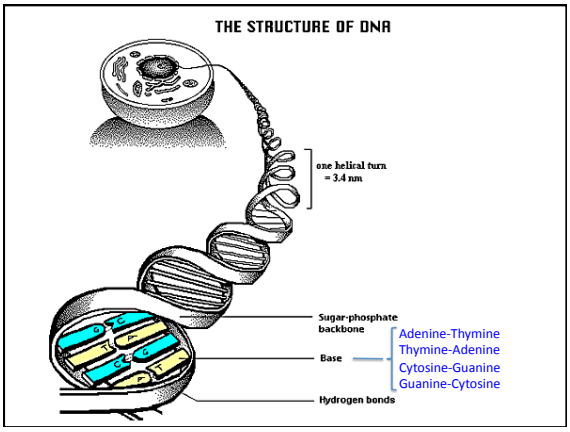
- Mutations are most likely to occur in common haplotypes
- Haplotypes could look the same using 50k markers but differ at sequence level
- Deleterious mutations in just some copies of a common haplotype may result in the homozygous haplotype being underrepresented rather than absent

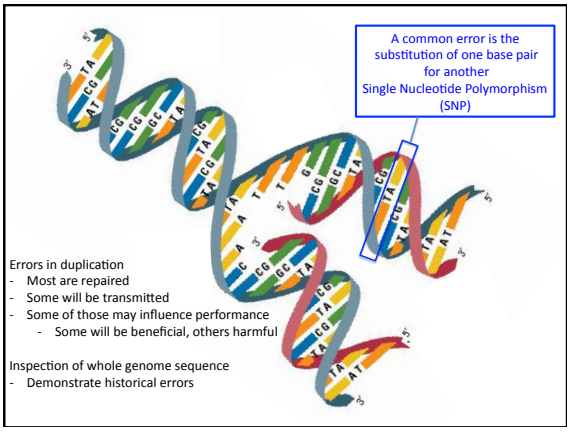
Application to Beef Cattle

- But haplotypes that contain any unfavorable (but not lethal) allele would be underrepresented in selected sires and dams
- Ideally, haplotyping and research into homozygote deficiency needs to be done in unselected animals
  - Most beef cattle 50k training populations for genomic prediction have focused on widely-used sire with reliable EPDs

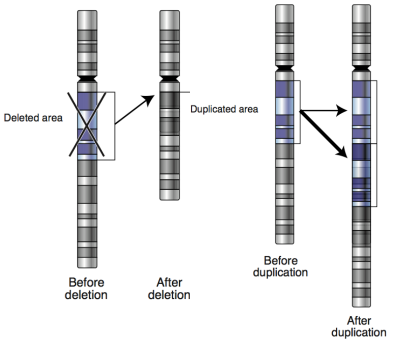
Gene Sequence First - LOF

- Identifying loss of function mutations from genome sequence
  - Gene product has less or no function





Replication Errors



These include
- Deletions
- Duplications
- Inversions
- Translocations
- Substitutions

of 1 or more bases

Do Errors Matter ? Gene Structure

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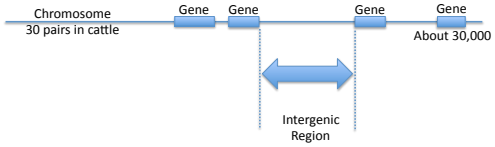
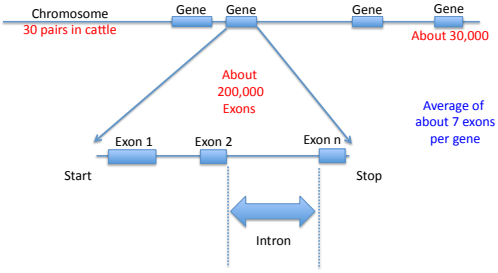
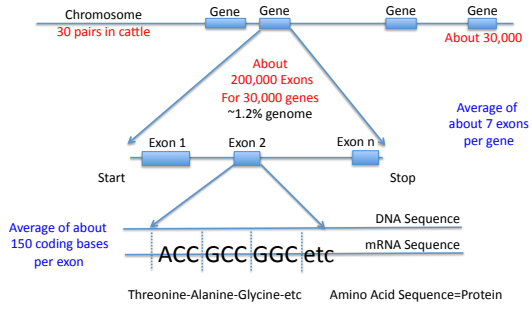


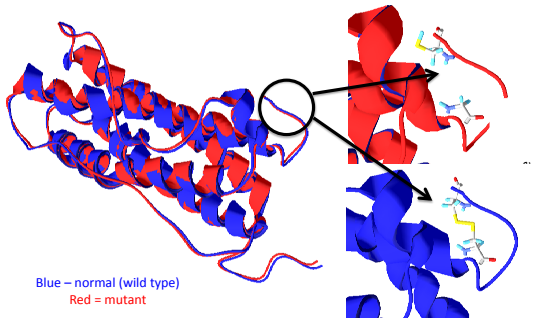
Do Errors Matter ? Gene Structure



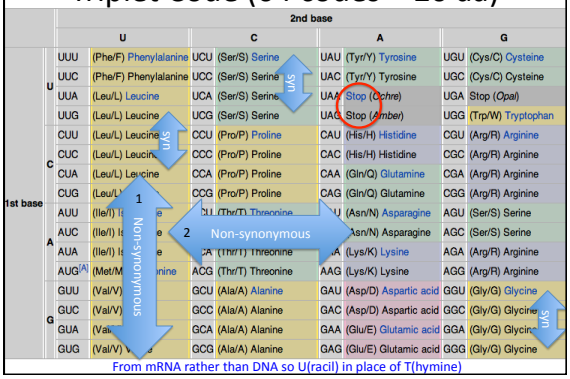
Do Errors Matter ? Gene Structure



Amino acid sequence & protein shape



Triplet Code (64 codes – 20 aa)



1st base	2nd base U	2nd base C	2nd base A	2nd base G
U	UUU (Phe/F) Phenylalanine	UCU (Ser/S) Serine	UAU (Tyr/Y) Tyrosine	UGU (Cys/C) Cysteine
	UUC (Phe/F) Phenylalanine	UCC (Ser/S) Serine	UAC (Tyr/Y) Tyrosine	UGC (Cys/C) Cysteine
	UUA (Leu/L) Leucine	UCA (Ser/S) Serine	UAA Stop (*Ochre*)	UGA Stop (*Opal*)
	UUG (Leu/L) Leucine	UCG (Ser/S) Serine	UAG Stop (*Amber*)	UGG (Trp/W) Tryptophan
C	CUU (Leu/L) Leucine	CCU (Pro/P) Proline	CAU (His/H) Histidine	CGU (Arg/R) Arginine
	CUC (Leu/L) Leucine	CCC (Pro/P) Proline	CAC (His/H) Histidine	CGC (Arg/R) Arginine
	CUA (Leu/L) Leucine	CCA (Pro/P) Proline	CAA (Gln/Q) Glutamine	CGA (Arg/R) Arginine
	CUG (Leu/L)	CCG (Pro/P) Proline	CAG (Gln/Q) Glutamine	CGG (Arg/R) Arginine
A	AUU (Ile/I)	ACU (Thr/T) Threonine	AAU (Asn/N) Asparagine	AGU (Ser/S) Serine
	AUC (Ile/I)	ACC (Thr/T) Threonine	AAC (Asn/N) Asparagine	AGC (Ser/S) Serine
	AUA (Ile/I)	ACA (Thr/T) Threonine	AAA (Lys/K) Lysine	AGA (Arg/R) Arginine
	AUG (Met/M)	ACG (Thr/T) Threonine	AAG (Lys/K) Lysine	AGG (Arg/R) Arginine
G	GUU (Val/V)	GCU (Ala/A) Alanine	GAU (Asp/D) Aspartic acid	GGU (Gly/G) Glycine
	GUC (Val/V)	GCC (Ala/A) Alanine	GAC (Asp/D) Aspartic acid	GGC (Gly/G) Glycine
	GUA (Val/V)	GCA (Ala/A) Alanine	GAA (Glu/E) Glutamic acid	GGA (Gly/G) Glycine
	GUG (Val/V)	GCG (Ala/A) Alanine	GAG (Glu/E) Glutamic acid	GGG (Gly/G) Glycine

From mRNA rather than DNA so U(racil) in place of T(hymine)

Stop gain mutations in 9111

- Potentially lethal
  - CUBN- assoc with MEGALOBLASTIC ANEMIA 1 (malabsorption of vitamin B12)- neural tube defects
  - FBN1- causes marfans syndrome- lethal when homozygous
  - SOX6- Homozygous mice showed delayed growth and died within 2 weeks of birth
  - ERCC6- causes a number of growth disorders, although there is a lot of allelic heterogeneity
  - RYR2- paralogue of RYR1 causes stress syndrome in pigs
  - ABCA12- causes severe Ichthyosis-lethal before advanced medical treatment in humans
  - XBP1- causes major effective disorder 7 (X-linked gene)

Stop gain mutations in 9111

- Potentially damaging:
  - reproduction: DNAAF1 - abnormal sperm motility, neonatal stress
  - heart function: TTN, ERG, MYH7, RYR2, SOX6
  - epilepsy: RILP, GRIK2
  - diabetes: OAS1, Vasohibin1
  - autism: TEP1
  - oestrogen response: BRCA2, Vasohibin1

Managing Variation

- Commercial Producer
  - Crossbreed
  - Outbreed
  - Put up with it
    - Provide hair samples (for DNA) on suspicious animals

Managing Variation

- Seedstock Producer
  - Change "shoot, shovel and shut up" mentality
    - Provide DNA (hair) samples on suspicious animals
  - Use SNP genotyping (at least on all prospective herd sires)
  - Use DNA tests for known defects
    - Cull carrier parents
      - not recommended, especially for outstanding animals
    - Select clean offspring for subsequent use

Managing Variation

- Breed Associations
  - Collect as much phenotypic data as possible
  - Especially reproductive traits on THR herds
  - Encourage wider use of genomic panels
    - Particularly beneficial on entire (unselected) cohorts
  - Explore opportunities to deliver decision support tools
  - Expect to record more single gene information

Managing Variation

- AI Companies
  - Use SNP HD panels on all AI sires
  - Individually sequence all future bulls that are to be widely used
  - Liability?
    - "Fit for purpose"
    - Caveat emptor

Managing Variation

- Industry & Science Community
  - Sequence widely used historical bulls
    - Annotate variants that might be damaging
    - Populate SNP chips with interesting variants
    - Validate the effect of interesting variants
  - Communicate test results of validated effects
  - Further develop and implement decision support tools to manage selection and mating of carrier animals

Summary

- Finding unfavorable mutations
  - Phenotype – first: need producer sampling
    - Saying nothing slows progress
  - Haplotype – first : rely on (random) genotyping
    - Improved reproductive recording would help
  - Sequence – first : rely on bioinformatics
    - Promising for lethal recessives, harder for production
- All 3 methods produce information that will increase our ability to predict performance

This is good news

Summary

- Selection (and culling) should be aimed at:
  - Increasing the frequency of favorable alleles and reducing the frequency of unfavorable alleles
    - Avoiding matings that can produce homozygous recessives
  - Not eliminating unfavorable alleles and fixing favorable alleles
    - Both these latter behaviors are too expensive in terms of selection intensity
- Decision Support Tools can help this process

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