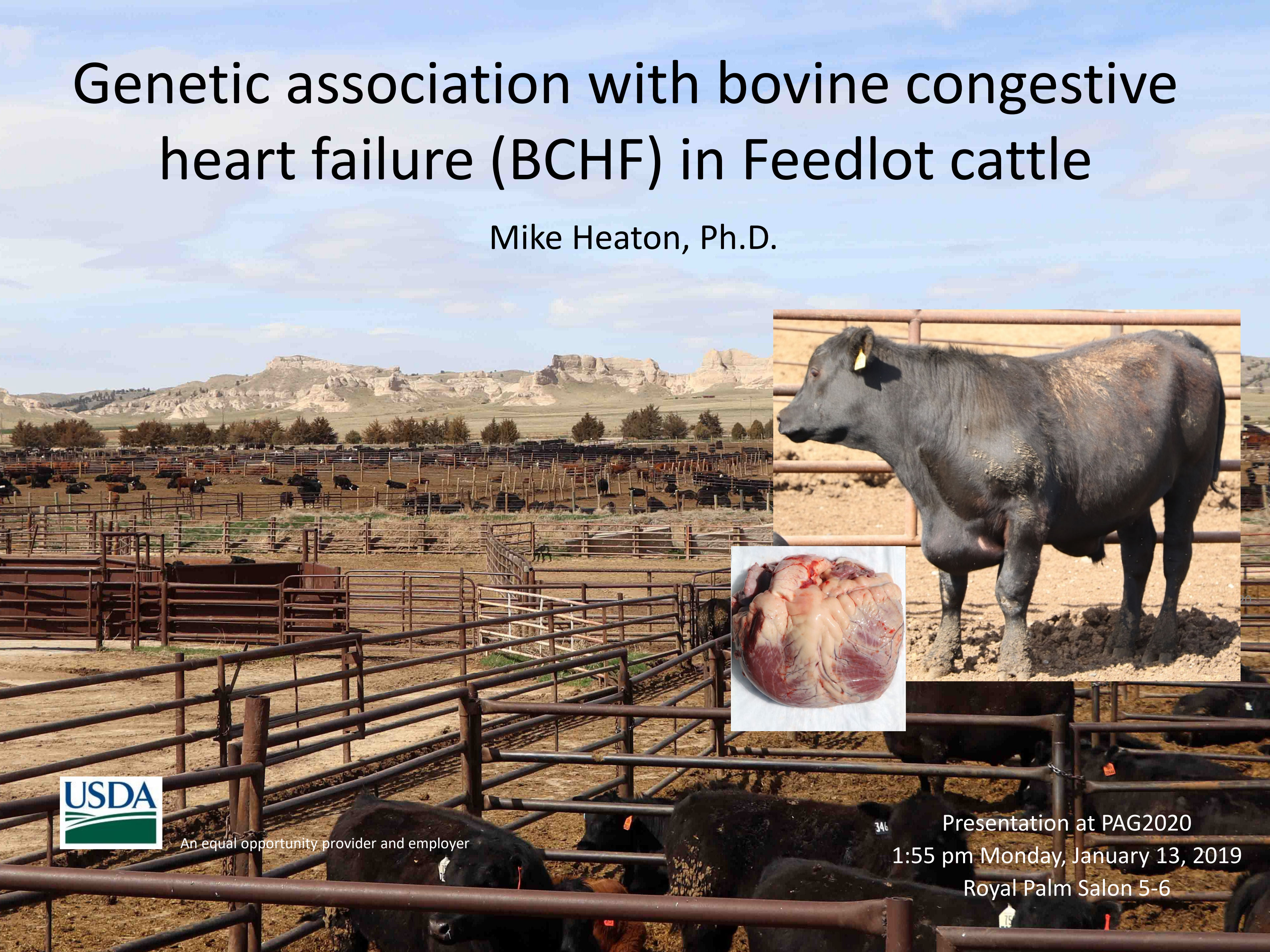
Genetic association with bovine congestive heart failure (BCHF) in Feedlot cattle

Mike Heaton, Ph.D.



USDA

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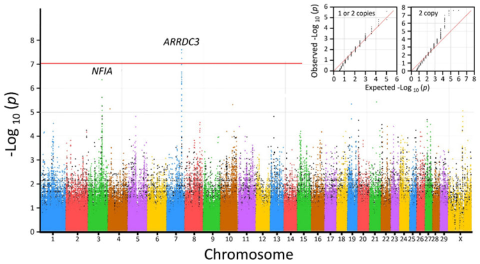
Presentation at PAG2020
1:55 pm Monday, January 13, 2019
Royal Palm Salon 5-6

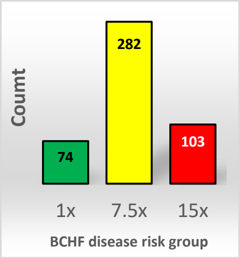
Topics

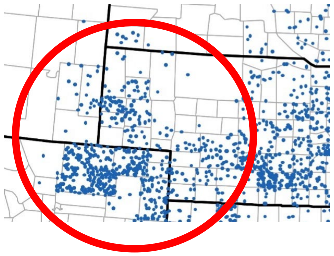
- The problem of congestive heart failure in beef cattle

- Discovery of genetic risk factors for BCHF



- Identifying animals at risk for disease





1 dot = 5k cattle on feed sold

Cattle with congestive heart failure are appearing with increased frequency in Western Plains feed yards

- These yards are experiencing significant losses to “brisket disease”
- These are well managed cattle with high genetic merit
- For some operations, this disease is their largest single economic loss
  - Nebraska Producer 1: > $250 k annually
  - Nebraska Producer 2: = $944 k from 2013 to 2018

Clinical features

Pair #26: WY01_20170927_5263_6447

Unaffected Clinical disease

Other clinical signs

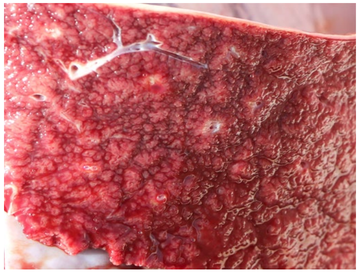
Pair #22 NE04_20170822_854_25

Jugular distension

Pair #89 NE04_20180409_916_262

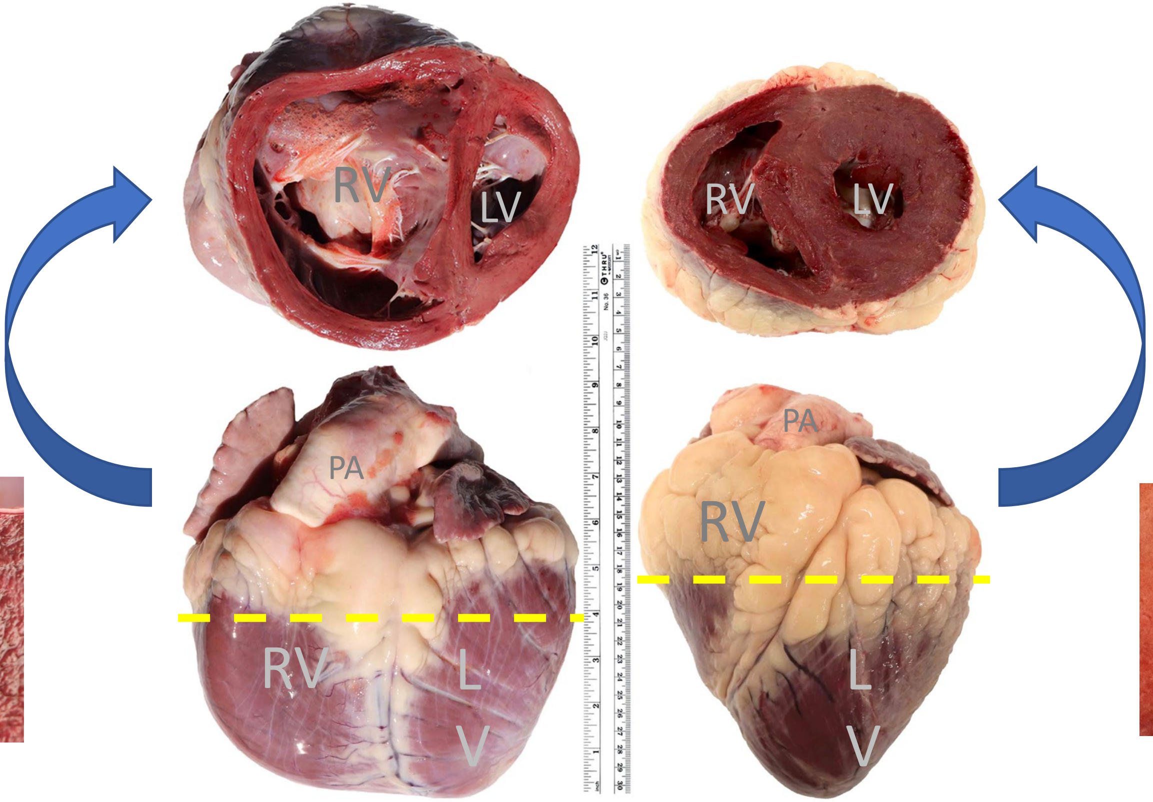
Intermandibular edema

Postmortem differences



NE01_20180314_750_70414

Clinical liver



Clinical heart

Unaffected heart

USMARC_20180512_20170387

Unaffected liver

Outbreaks clustered by source

Up to 7% loss observed in single-source groups (lot)

- 40 of 600 (May, 2017)
- 39 of 500 (January, 2018)

Disease clustering by source suggests underlying genetic causes

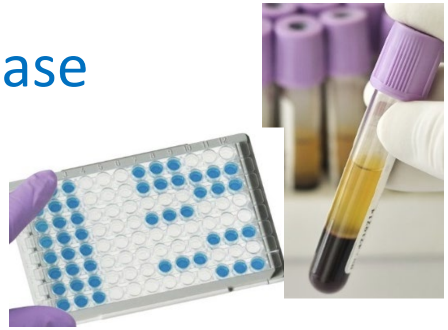
Goals of our research

1. Understand how the disease works

- Essential for prevention and treatment

2. Develop a DNA test for animals at risk

- Facilitate selective breeding for reduced risk
- Reduce the number of diseased cattle
- Identify and manage cattle at risk



3. Develop a blood test for diagnosing disease

- Manage cattle with signs of heart failure

Project collaborators

University of Nebraska Lincoln

Dr. Dale Grotelueschen

Dr. Brian Vander Ley

Dr. Kathy Whitman

Dr. Halden Clark

Dr. Adam Bassett **Sang In Lee**

Jaden Carlson

Madeline Pelster

Helen Smith

N *School of Veterinary Medicine and Biomedical Sciences*
GREAT PLAINS VETERINARY EDUCATIONAL CENTER

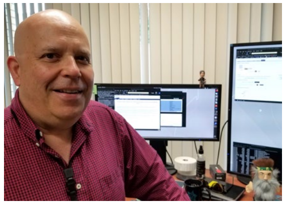
University of Colorado Anschutz Medical Campus

Colorado State University

Dr. Greta Krafsur

USDA Agricultural Research Service

USMARC



Dr. Greg Harhay

Dr. Aspen Workman

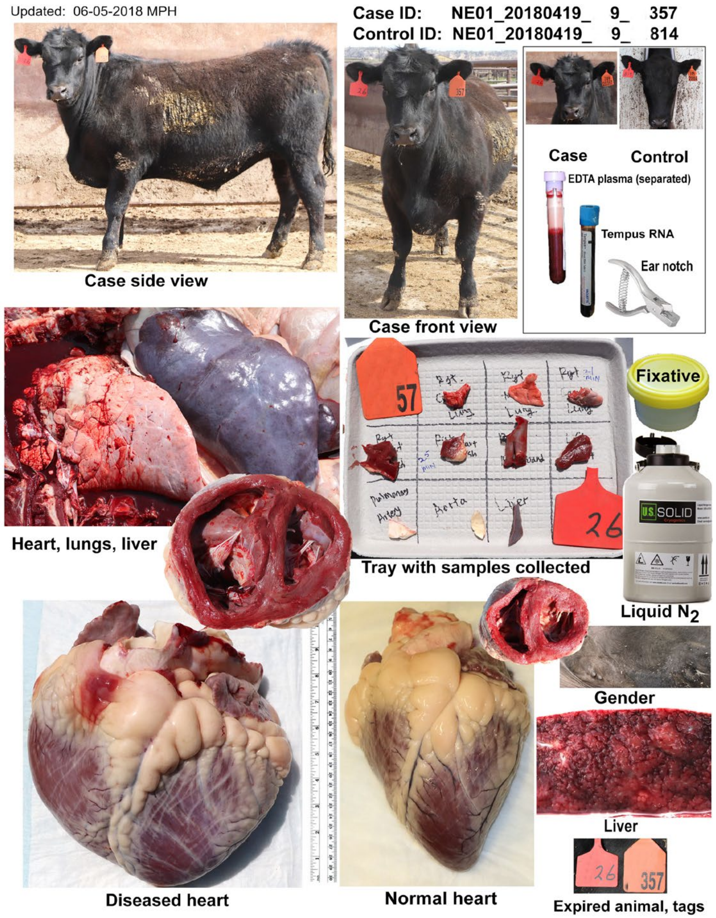
Dr. Tim Smith

Dr. Larry Kuehn

Study design (2016)

- 100 matched case-control pairs
  - Four feedyards ~4000 ft
  - Pen riders identify clinical cases

- Presumptive diagnosis
  - Clinical presentation, necropsy, histopathology
- Matched controls
  - Source, arrival date, gender, and breed type
  - None developed clinical BCHF before harvest
- Preserve tissues
  - DNA, RNA, protein



Heaton et al., F1000Research 2019, 8:118

21 trips over 15 months and 15,000 miles

Sample Summary

Site	Altitude (ft)		Pairs	Sources
NE01	4,075		76	20
NE02	3,816		17	9
WY01	4,143		6	6
WY02	4,198		3	2
Average:	4,058	Totals:	102	37

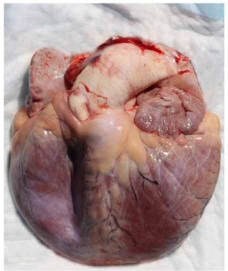
- 95 black, 5 red, 2 red whiteface
- 71 males, 31 females
- 2017 - 2018

What types of clinical cases did we see?

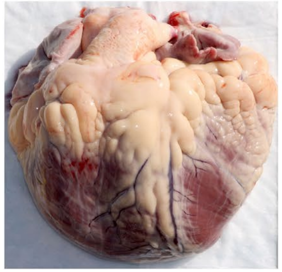
Day 1 (bull)

Day 30 (heifer)

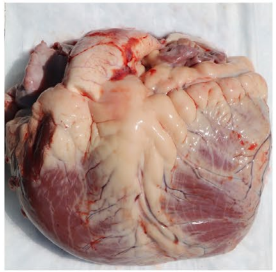
Day 238 (steer)



Affected, pair 24

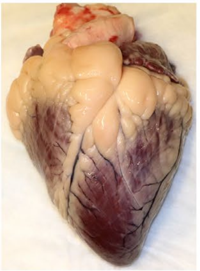


Affected, pair 32



Affected, pair 15

Day 240 (control)



USMARC20060372

Clinical cases at every stage of the feeding cycle

First result: *EPAS1* was not associated with BCHF

F1000Research
Open for Science

RESEARCH ARTICLE

Evaluation of *EPAS1* variants for association with bovine congestive heart failure

Michael P. Heaton[1], Adam S. Bassett[2], Katherine J. Whitman[2], Greta M. Krafsur[3], Sang In Lee[2], Jaden M. Carlson[2], Halden J. Clark[2], Helen R. Smith[1], Madeline C. Pelster[2], Veronica Basnayake[4], Dale M. Grotelueschen[2], Brian L. Vander Ley[2]

The search was expanded to the whole genome

- 102 matched case-control pairs

- Illumina BovineHD BeadChip (777 k markers)

  2 months, ~$20k

Dr. Mark Boggess

- Whole genome sequencing (30 M variants)

  2-3 years, $300k, 78/204 (38%) sequenced Dec. 2019

Dr. Tim Smith

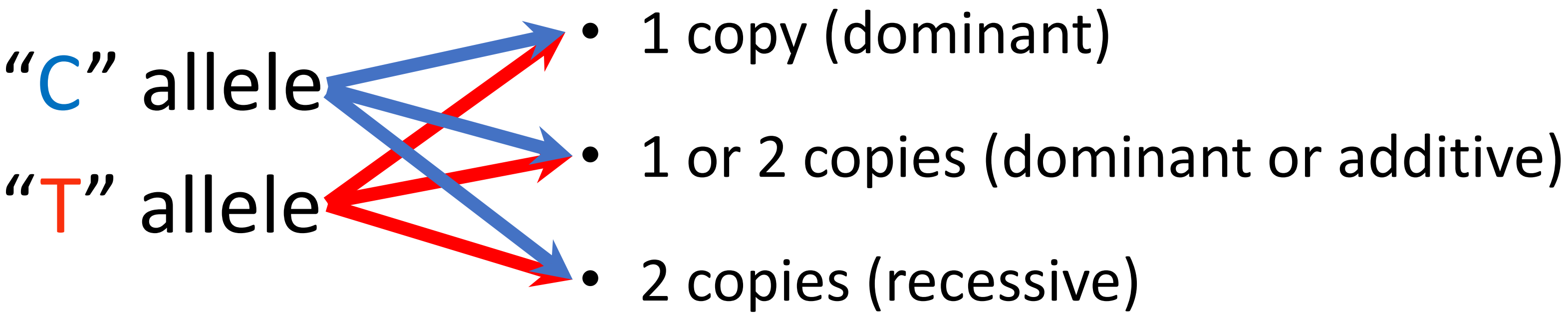
McNemar’s test for association

- PLINK does not have a suitable McNemar’s analysis package
  - Fisher's exact test, Cochran-Mantel-Haenszel test
- Custom software written in MATLAB programming language

Dr. Greg Harhay

- Each of 562k SNPs were analyzed 12 ways: “risk factor” vs “protective factor”

C/T SNP example

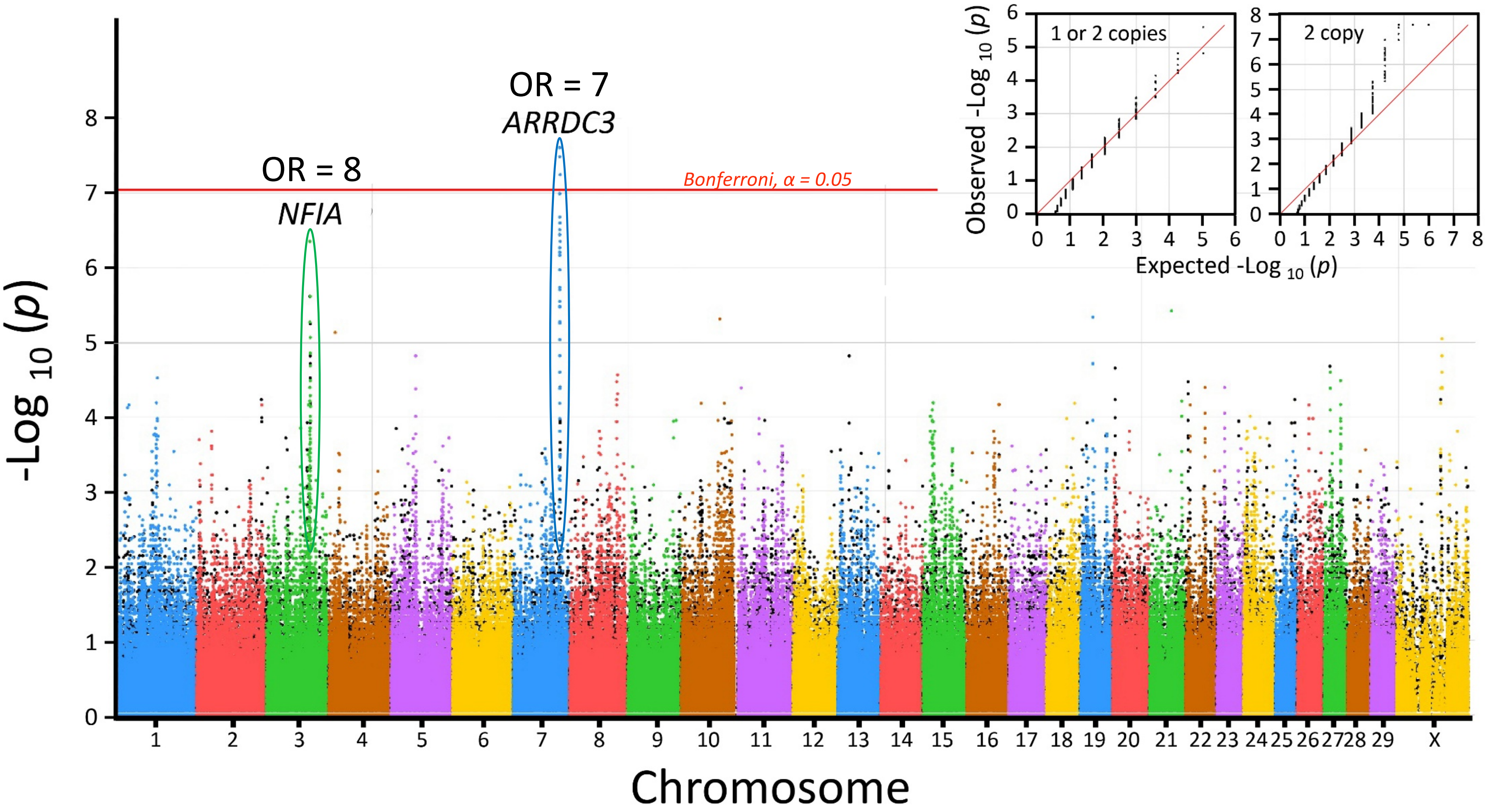


“C” allele

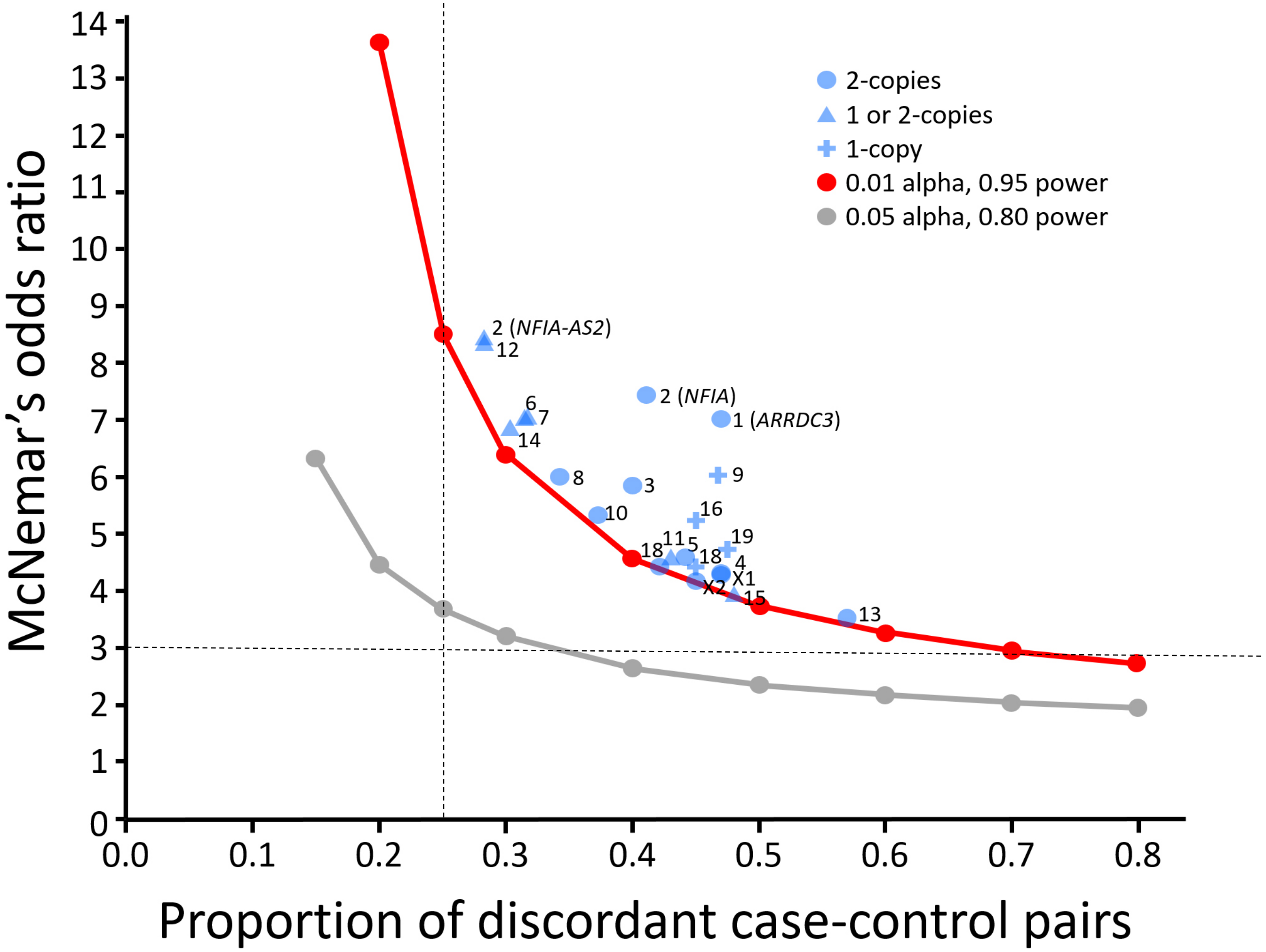
“T” allele

- 1 copy (dominant)
- 1 or 2 copies (dominant or additive)
- 2 copies (recessive)

Manhattan plot of McNemar’s test for association



21 SNPs significantly associated with BCHF



Top 21 SNP associations with BCHF

Table 2. SNPs associated with the highest risk of BCHF in feedlot cattle

									A1 Frequency					McNemar pairs								OR CI$_{95}$						
														1,1	1,0	0,1	0,0											
Rank[a]	Chr	SNP ID	UMD3.1 Chr. pos. (bp)	ARS1.2 Chr. pos. (bp)	Nearest genes	SNP location	A1[b]	A2	Case	Control	Ref.	Risk model[c]	Risk allele	*a*	*b*	*c*	*d*	*b + c*	*n*	*(b + c)/n*	OR	Lower	Upper	χ2[d]	*p* -value (exact)	-log(*p*)	SNPs in LD (χ2 >15)	Block size[e] (kb)
1	7	BovineHD0700027239★	93244933	90845941	ARRDC3 ★	Exon 4 C182Y tRt	A	G	0.794	0.583	0.219	2 copies	A	25	42	6	29	48	102	0.471	7.0	3.0	16.5	25.5	1.01E-07	7.0	56	956
2	3	BovineHD0300024307	85123495	84578325	NFIA	Intron 2	G	A	0.647	0.427	0.448	2 copies	G	7	37	5	53	42	102	0.412	7.4	2.9	18.8	22.9	4.43E-07	6.4	21	596
2	3	ARS-BFGL-NGS-103524★★	85253155	84706206	NFIA-AS2★★	Intron3	C	A	0.25	0.525	0.443	1 or 2 copies	A	72	26	3	1	29	102	0.284	8.7	2.6	28.6	16.7	1.52E-05	4.8	21	596
3	10	BovineHD1000021490	75580294	75267920	KCNH5	Intron 4	A	G	0.672	0.476	0.542	2 copies	A	14	35	6	47	41	102	0.402	5.8	2.5	13.9	19.1	4.87E-06	5.3	0	5
4	5	BovineHD4100003664	48418959	48188142	HMGA2_MSRB3	Intergenic	A	G	0.706	0.525	0.203	2 copies	A	15	39	9	39	48	102	0.471	4.3	2.1	8.9	17.5	1.52E-05	4.8	2	5
5	26	BovineHD2600006169	23929257	23685234	CNNM2	Intron 1	A	C	0.884	0.716	0.729	2 copies	A	41	37	8	16	45	102	0.441	4.6	2.2	9.9	17.4	1.54E-05	4.8	0	4
6	19	BovineHD1900007657	25991182	25381310	PITPNM3	Intron 4	A	C	0.799	0.941	0.760	1 or 2 copies	C	7	28	4	63	32	102	0.314	7.0	2.5	20.0	16.5	1.93E-05	4.7	9	30
7	22	BovineHD4100015417	5652914	5605128	GADL1_STT3B	Intergenic	A	G	0.576	0.430	0.552	1 or 2 copies	A	60	28	4	10	32	102	0.314	7.0	2.5	20.0	16.5	1.93E-05	4.7	0	5
8	20	BovineHD2000001515	4762743	4854589	BNIP1	Exon 6 3'UTR	G	A	0.451	0.652	0.859	2 copies	A	3	30	5	64	35	102	0.343	6.0	2.3	15.5	16.5	2.24E-05	4.7	0	8
9	9	BovineHD0900027458	96528831	95086861	SYTL3	Intron 3	A	C	0.490	0.368	0.391	1 copy	A	29	30	5	11	35	75	0.467	6.0	2.3	15.5	16.5	2.24E-05	4.7	0	2
10	4	BovineHD0400003776	12664064	12793811	ASB4	Intron 3	G	A	0.960	0.824	0.688	2 copies	G	61	32	6	3	38	102	0.373	5.3	2.2	12.8	16.4	2.43E-05	4.6	0	22
11	27	BovineHD2700003477	11978259	12942894	AGA_TENM3	Intergenic	A	G	0.451	0.657	0.698	1 or 2 copies	G	48	36	8	10	44	102	0.431	4.5	2.1	9.7	16.6	2.54E-05	4.6	4	48
12	8	BTB-01266056	95400554	93743405	SMC2	Intron 20	C	A	0.855	0.970	0.814	1 or 2 copies	A	3	25	3	71	28	102	0.283	8.3	2.5	27.6	15.8	2.74E-05	4.6	1	21
13	1	BovineHD0100023638	82311685	81722301	MAP3K13	Intron 10	G	A	0.882	0.721	0.792	2 copies	G	36	45	13	8	58	102	0.569	3.5	1.9	6.4	16.6	3.01E-05	4.5	0	4
14	8	Hapmap27238-BTA-163742	93655394	92016089	GRIN3A_CYLC2	Intergenic	A	G	0.825	0.955	0.823	1 or 2 copies	G	5	27	4	66	31	102	0.304	6.8	2.4	19.3	15.6	3.40E-05	4.5	2	6
15	22	BovineHD2200011041	38708159	38567450	CADPS	Intron 3	A	G	0.530	0.690	0.750	1 or 2 copies	G	41	39	10	12	49	102	0.480	4	1.9	7.8	16.0	3.85E-05	4.4	0	2
16	3	ARS-BFGL-NGS-110776	41119556	40971803	COL11A1_OLFM3	Intergenic	C	A	0.667	0.745	0.750	1 copy	A	17	31	6	28	37	82	0.451	5.2	2.2	12.4	15.6	4.13E-05	4.4	0	3
17	22	BovineHD2200002812	9438878	9404132	PDCD6IP_ARPP21	Intergenic	A	G	0.940	0.801	0.662	2 copies	A	53	35	8	6	43	102	0.422	4.4	2.0	9.4	15.7	4.19E-05	4.4	0	8
18	26	BovineHD2600008347	31264198	31000995	SMNDC1_DUSP5	Intergenic	A	G	0.767	0.891	0.651	1 copy	G	6	35	8	46	43	95	0.453	4.4	2.0	9.4	15.7	4.19E-05	4.4	0	12
19	24	BovineHD2400006360	23311439	23021459	NOL4	Intron 4	A	G	0.662	0.799	0.635	1 copy	G	12	33	7	32	40	84	0.476	4.7	2.1	10.7	15.6	4.23E-05	4.4	0	7
1	X	BovineHD3000025651	93114732	87952697	CCNB3	Intron 1	A	C	0.72	0.469	na	Homozygous[f]	A	28	39	9	26	48	102	0.471	4.3	2.1	8.9	17.52	1.52E-05	4.8	10	161
2	X	BovineHD3000025051	91049685	85788201	ZNF41	Intron 2	C	A	0.87	0.662	na	Homozygous	C	48	37	9	8	46	102	0.451	4.1	2.0	8.5	15.85	4.06E-05	4.4	1	1

[a] In the three models tested, the genetic risk factor were defined as having exactly 1, 1 or 2, or exactly 2 copies of the risk allele, respectively.

[b] A1 was defined in the McNemar's test analyses as the most frequent allele in the combined group of 204 cases and controls.

[c] In the three models tested, the genetic risk factor were defined as having exactly 1, 1 or 2, or exactly 2 copies of the risk allele, respectively.

[d] McNemar's chi-squared with continuity correction: (|b - c| - 1)2/(b + c)

[e] Distance between distal SNPs within block of LD where χ2 > 15. If no additional linked SNPs present, distance between non-linked adjacent SNPs.

[f] In the X-chromosome analysis, pairs of males and pairs of females were analyzed together. Since male X-chromosome genotypes are always homozygous, the risk factor was defined as being homozygous at the position. Both alleles were evaluated for being the risk factor.

★ Marker used in 2-SNP test: BovineHD0700027239 (BCHF5, *ARRDC3,* 2 copies, risk allele ="A")

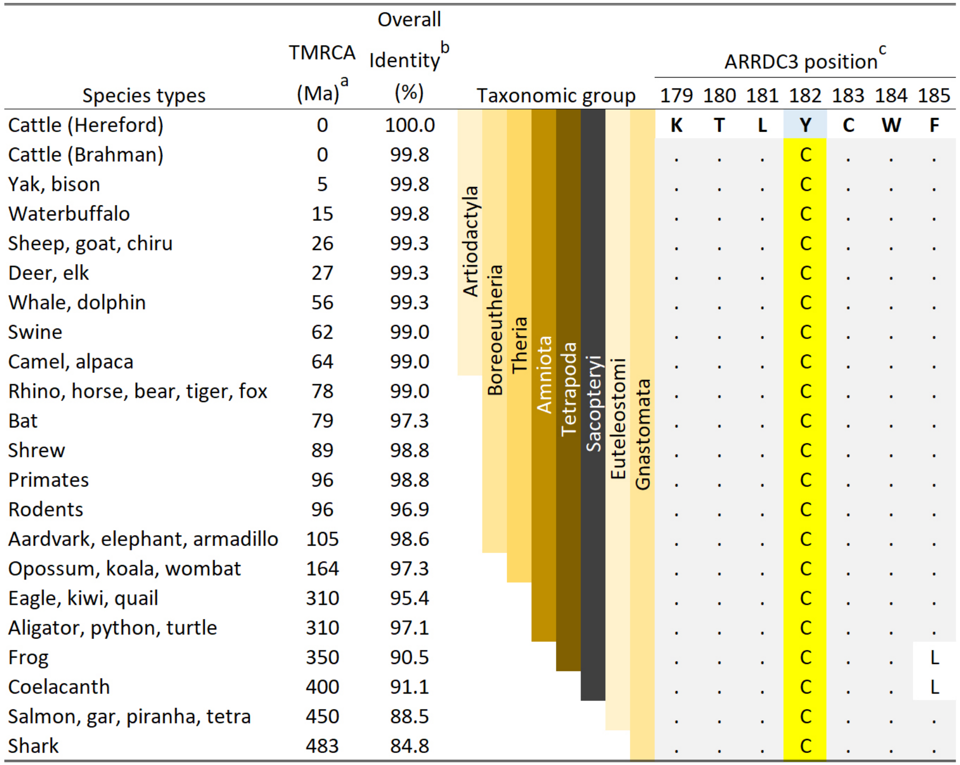
★★ Linked to marker used in 2-SNP test: BovineHD0300024366 (BCHF2, *NFIA-AS2,* 1 or 2 copies, risk allele ="A")

ARRDC3 missense variant C182Y associated with BCHF

Possible function: In mouse and human, the arrestin domain-containing 3 protein gene (*ARRDC3)* is widely expressed and regulates body mass and energy expenditure. Patwari et al., Cell Metab 2011;14: 671–683

It also interacts with β2-adrenergic receptor in the early endosome and prevents receptor recycling. Tian et al., J Biol Chem 2016;291:14510–14525

Table 3. Evolutionary comparison of ARRDC3 residues near the C182Y position in the jawed vertebrates.



Species types	TMRCA (Ma)[a]	Overall Identity[b] (%)	Taxonomic group	ARRDC3 position[c] 179	180	181	182	183	184	185
Cattle (Hereford)	0	100.0	Artiodactyla	**K**	**T**	**L**	**Y**	**C**	**W**	**F**
Cattle (Brahman)	0	99.8		.	.	.	C	.	.	.
Yak, bison	5	99.8		.	.	.	C	.	.	.
Waterbuffalo	15	99.8		.	.	.	C	.	.	.
Sheep, goat, chiru	26	99.3		.	.	.	C	.	.	.
Deer, elk	27	99.3		.	.	.	C	.	.	.
Whale, dolphin	56	99.3		.	.	.	C	.	.	.
Swine	62	99.0		.	.	.	C	.	.	.
Camel, alpaca	64	99.0		.	.	.	C	.	.	.
Rhino, horse, bear, tiger, fox	78	99.0	Boreoeutheria	.	.	.	C	.	.	.
Bat	79	97.3		.	.	.	C	.	.	.
Shrew	89	98.8		.	.	.	C	.	.	.
Primates	96	98.8		.	.	.	C	.	.	.
Rodents	96	96.9		.	.	.	C	.	.	.
Aardvark, elephant, armadillo	105	98.6		.	.	.	C	.	.	.
Opossum, koala, wombat	164	97.3	Theria	.	.	.	C	.	.	.
Eagle, kiwi, quail	310	95.4	Amniota	.	.	.	C	.	.	.
Aligator, python, turtle	310	97.1		.	.	.	C	.	.	.
Frog	350	90.5	Tetrapoda	.	.	.	C	.	.	L
Coelacanth	400	91.1	Sacopteryi	.	.	.	C	.	.	L
Salmon, gar, piranha, tetra	450	88.5	Euteleostomi	.	.	.	C	.	.	.
Shark	483	84.8	Gnastomata	.	.	.	C	.	.	.

[a] TMRCA is the estimated time to most recent common ancestor in millions of years Hedges SB et. al., Mol Biol Evol. 2015; 32(4): 835–45.

[b] The full length ARRDC3 protein is 414 in cattle and most of the Amniota species

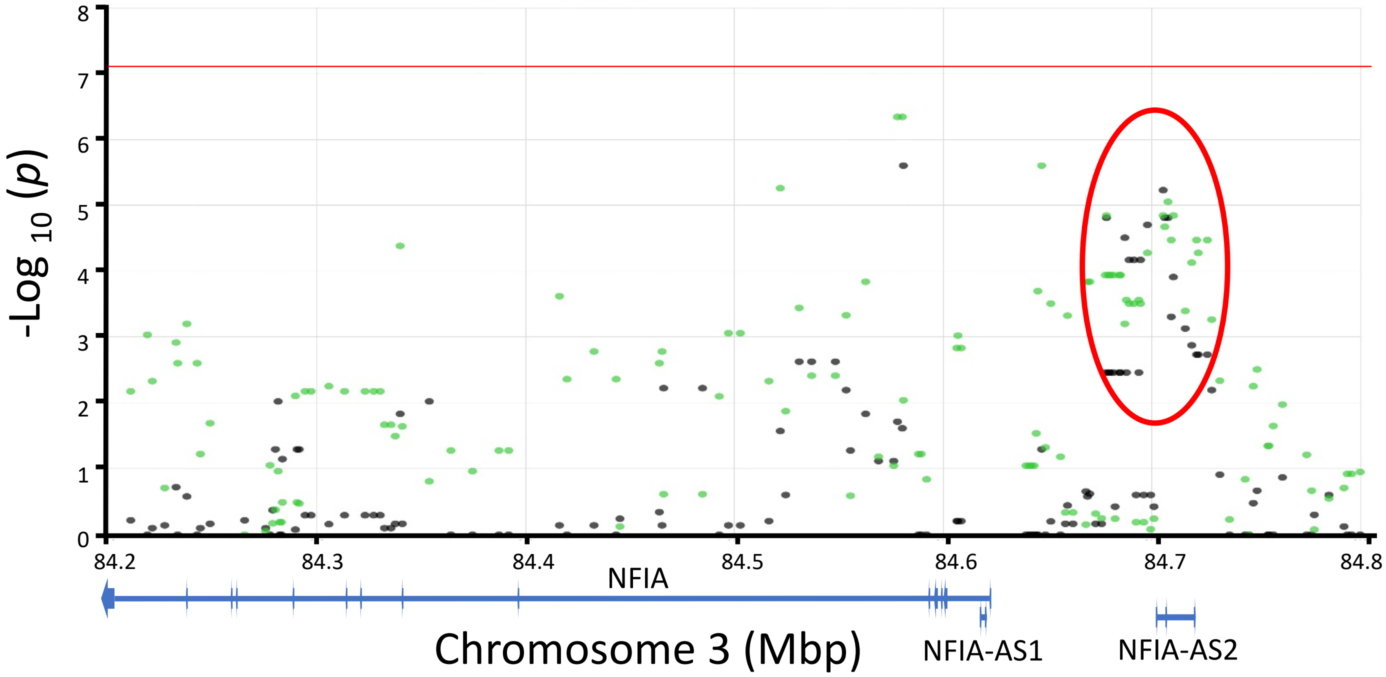
[c] The letters are IUPAC/IUBMB codes for amino acids. The dots are amino acid residues identical to those in cattle.

2-copies Y182: OR = 7.0
CI_{95} = 3.0 to 16.5
p-value = 1×10^{-7}

NFIA gene region associated with BCHF

Possible function: “..the transcription factor nuclear factor I-A (NFIA) is now shown to drive the brown fat genetic program through binding to lineage-specific cis-regulatory elements.” Shapira and Seale, Nat Cell Biol. 2017;19:1006–1007

NFIA-AS2: OR = 8.7
CI_{95} = 2.6 to 28.6
p-value = 2×10^{-5}



ARRDC3 + *NFIA*:
OR =15.3
CI_{95} = 4.8 to 49.3
p-value = 7×10^{-11}

What can we do with 2 SNPs today?

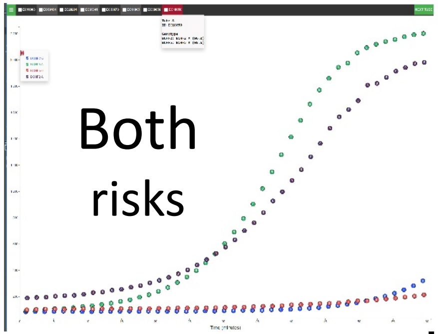
- It will take years to sort out all the effects of the top 21 genomic regions associated with BCHF.
- *ARRDC3* and *NFIA-AS2* markers are associated with up to 15-fold risk
- Can we make use of these markers now to help affected beef producers?

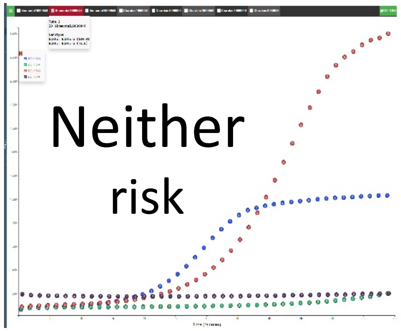
Can we test for disease risk with 2 SNPs?

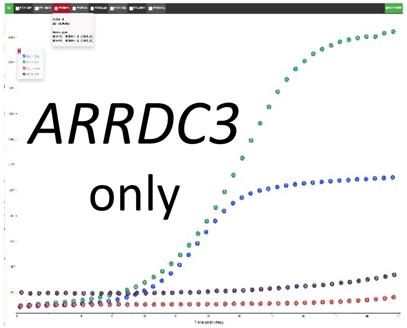
MatMaCorp

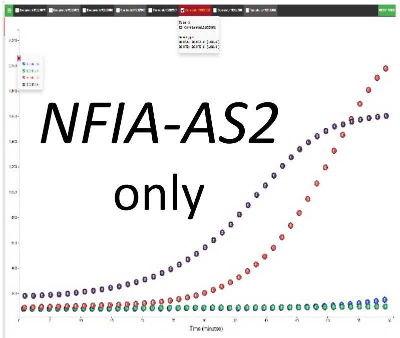
Dr. Abe Oommen

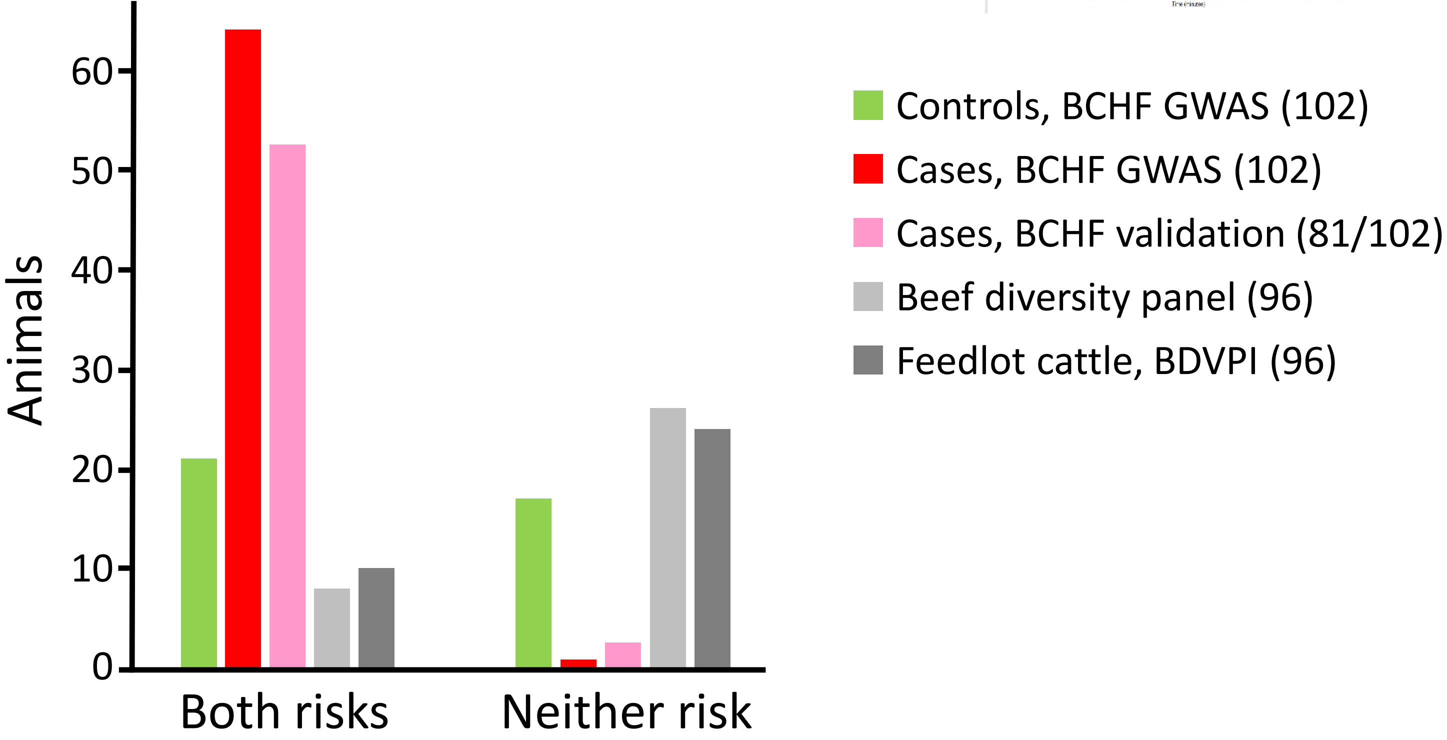
BovineHD0300024366 (BCHF2, *NFIA-AS2,* 1 or 2 copies, risk allele =“A”)
BovineHD0700027239 (BCHF5, *ARRDC3,* 2 copies, risk allele =“A”)









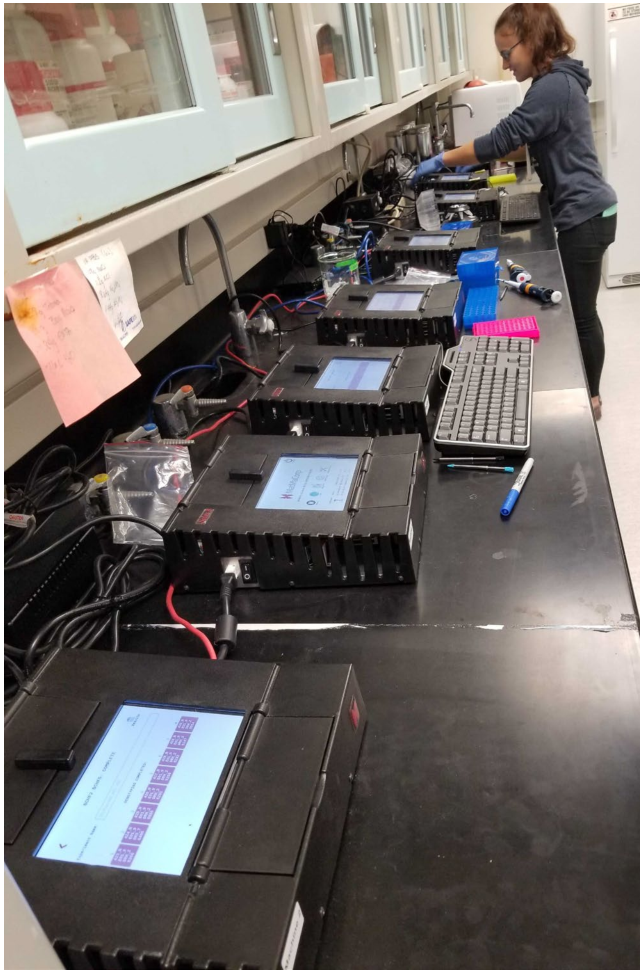


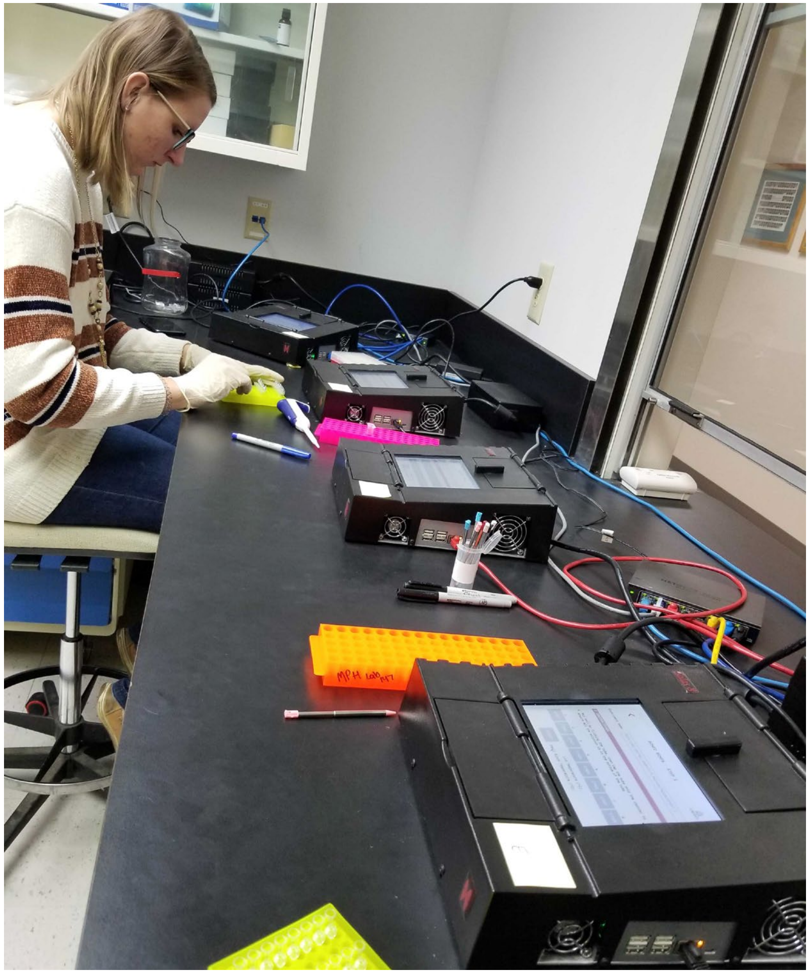
Collecting 1077 ear notches from an affected herd

- A pig ear-notcher and pickling brine solution
- Processed cattle at full speed (120/hour)
- Stable at room temperature storage until use

Genotyping *ARRDC3* and *NFIA-AS2* for 1077 calves

1 day, 2 people, 128 ear notches, 16 machines (1 run)

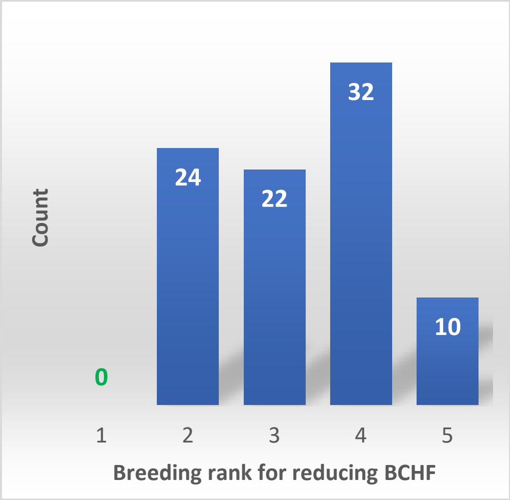




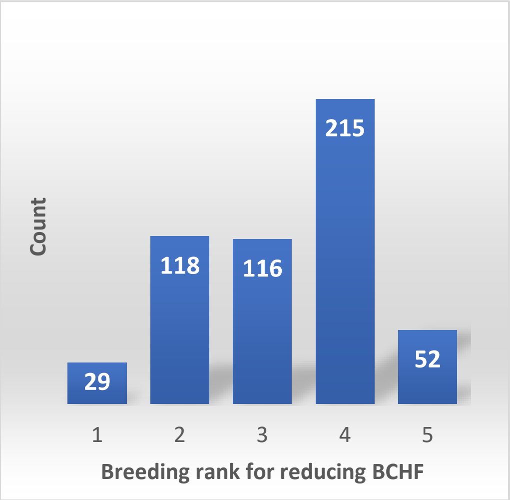
Sorting animals for breeding in an affected herd

Breeding rank	Potential risk transmission to calves
1	0%
2	25%
3	50%
4	75%
5	100%

Bulls (88)

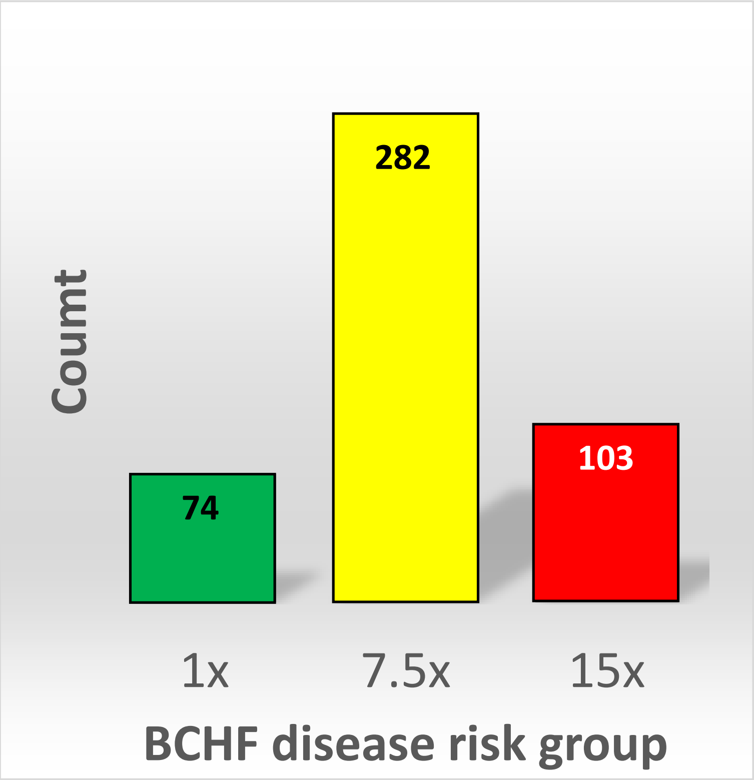


Heifers (530)

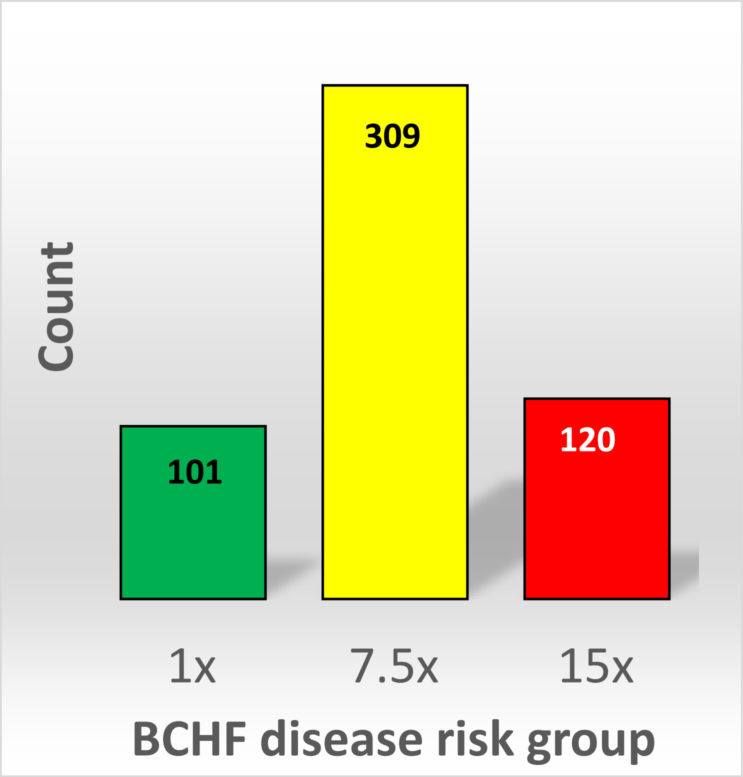


Sorting animals for disease risk in an affected herd

Steers (459)

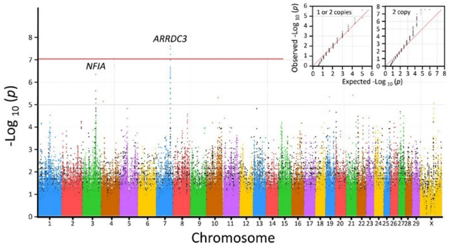


Heifers (530)

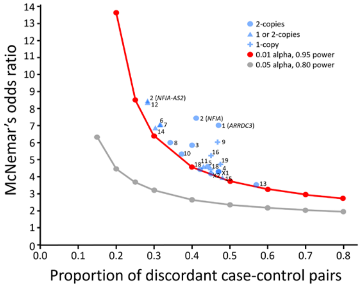


Conclusions

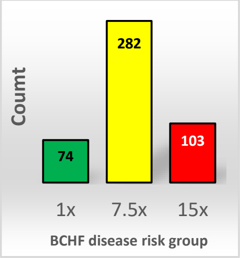
- *ARRDC3* and *NFIA* variants are major risk factors for heart failure in feedlot cattle.



- Other loci were also significantly associated



- A 2-SNP test sorts animals by risk group



Special credit and thanks go to the pen riders and feedyard operators that made this project possible