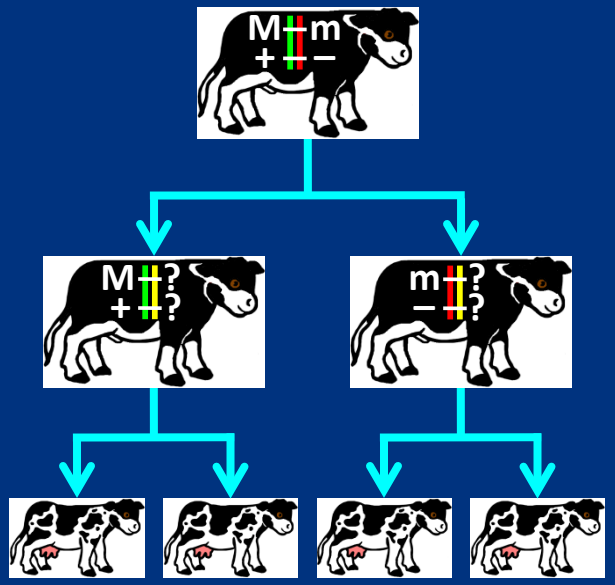
Revisiting the "a posteriori" granddaughter design



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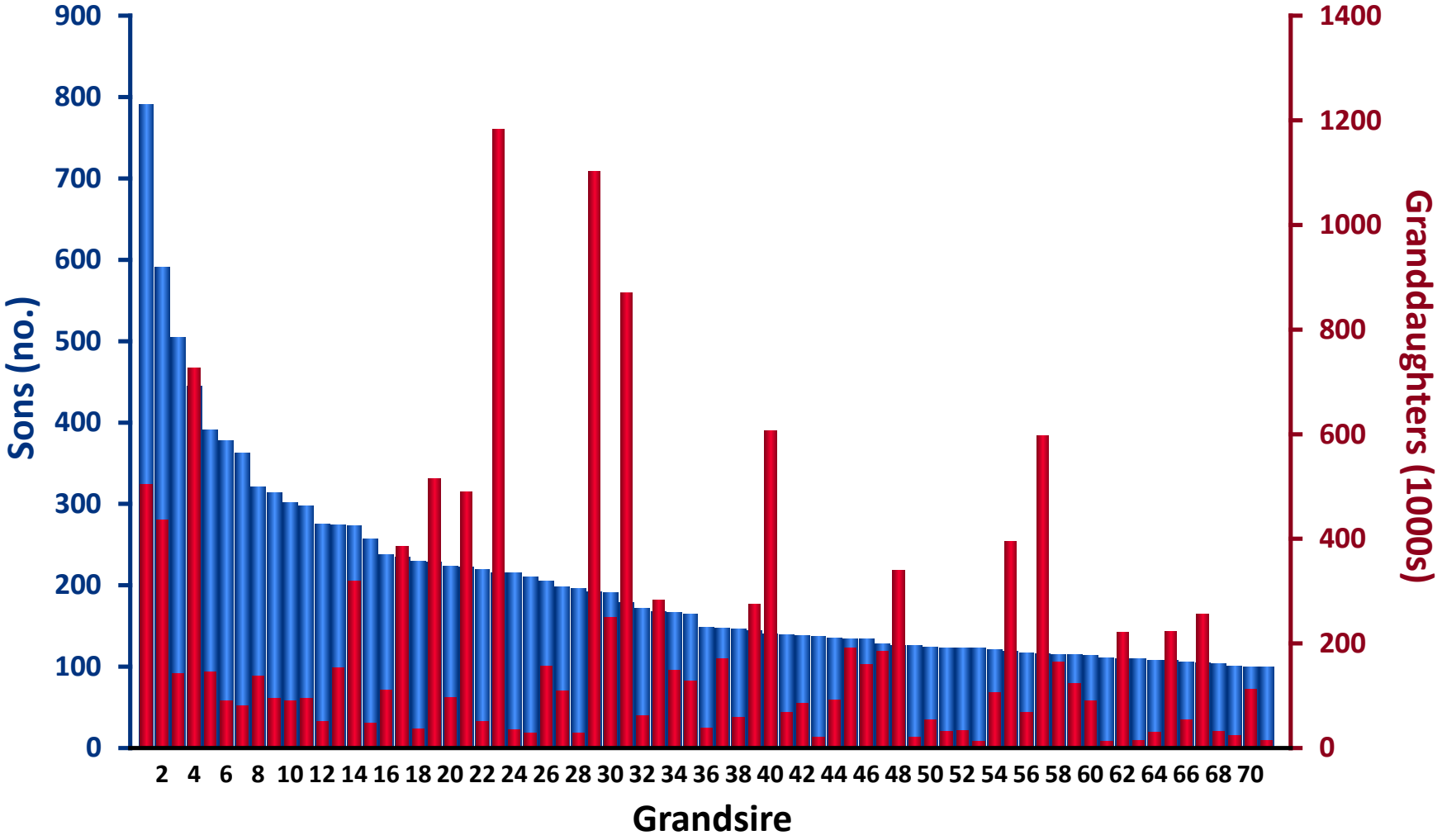
Granddaughter design

- Sires with many progeny-tested sons genotyped for genetic markers
- Sons of heterozygous sire divided into 2 groups based on which paternal allele they received
- Significant difference in genetic evaluations for 2 son groups indicates sire is segregating for QTL linked to genetic marker for trait of interest
- *A posteriori* granddaughter design (APGD)

Application of APGD to U.S. Holsteins

- Original application
  - August 2012 evaluation
  - 9,180 bulls
  - Sons of 52 sires (≥100 genotyped, progeny-tested sons/sire)
- Update
  - April 2015 evaluation
  - 14,246 bulls
  - Sons of 71 sires (100–791 genotyped sons/sire)

Genotyped sons and their daughters



Traits analyzed

- Milk production (5 traits)
- Somatic cell score
- Productive life
- Calving (4 traits)
- Fertility (3 traits)
- Conformation (18 traits)
- Net merit

Genotype and haplotype determination

- Entire genome (including sex chromosomes) divided into 621 segments (~100 markers each)
- Specific number of markers adjusted to achieve near equality within chromosome
- Haplotypes determined using findhap program

Analysis

- Genomic EBV
- No SNPs on X chromosome (sons receive Y rather than X chromosome from sire)
- 19,932 tests (604 segments × 33 traits)
- Nominal significance levels of 0.05 or 0.01 meaningless

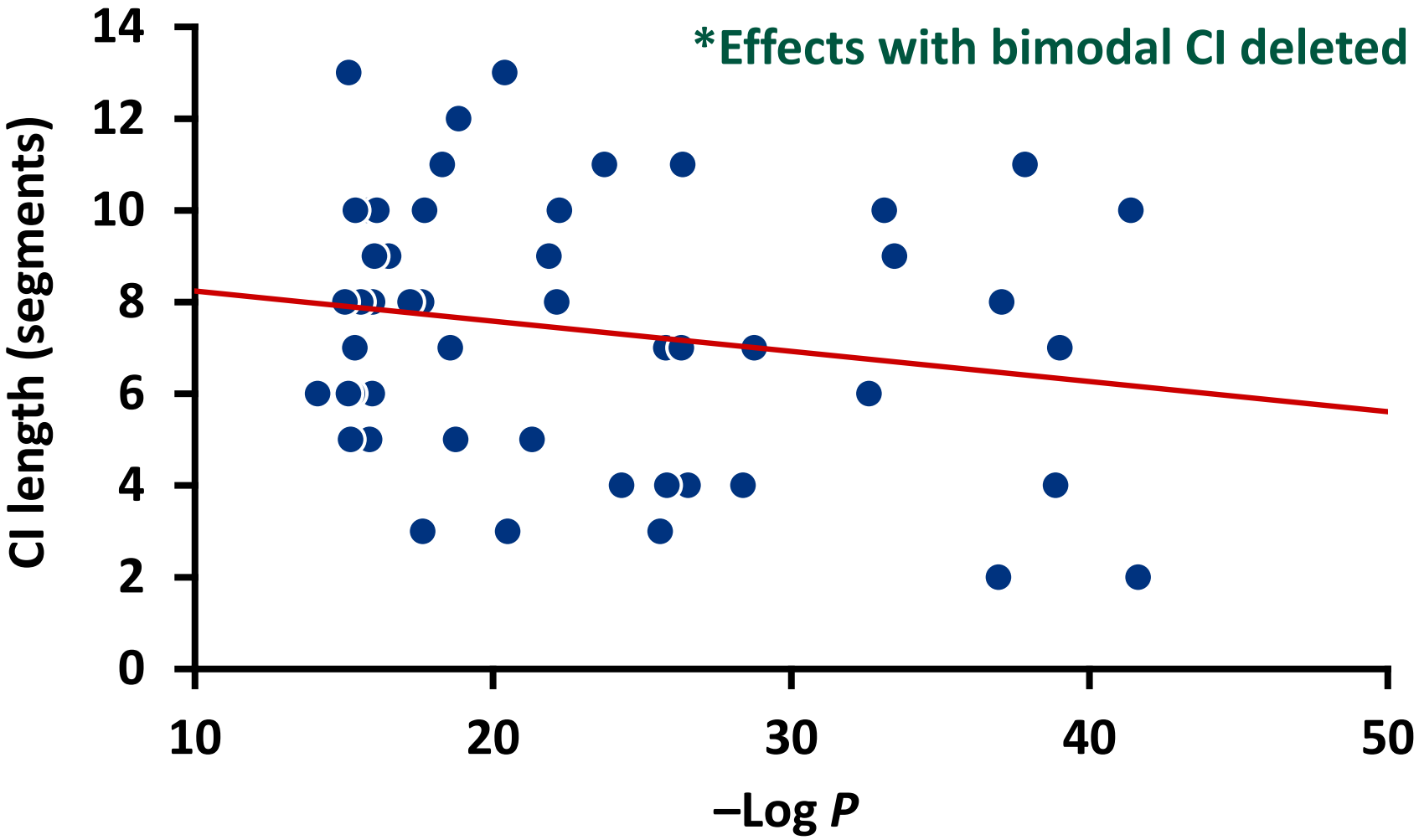
Effects by trait

- Only segments with nominal $P < 10^{-15}$ considered to be significant
- 55 chromosomal regions met criterion (30 regions in 2012)
- At least 1 significant effect for all traits (except protein yield, daughter stillbirth rate, and 4 conformation traits)
- Lowest probability (2.4×10^{-42}) for protein percentage on chromosome 3

Confidence intervals (CIs)

- Nonparametric bootstrap analysis (Visscher *et al.*, 1996, *Genetics*) applied to chromosome with haplotype segments with $P < 10^{-15}$
- 100 samples generated for each trait × chromosome combination by sampling 14,246 sons with repeats
- For each sample, all haplotype segments along chromosome analyzed by APGD, and segment with lowest *P* selected
- 90% CI determined by distribution of segments with lowest *P*

CI as function of –log *P**



CI results

- In all cases, 90% CI that spanned only part of chromosome determined
- Included only 2 segments for fat yield (chromosome 5) and protein percentage (chromosome 3)
- Narrowed as $-\log_{10} P$ increased, but regression not significant
- At least 6 regions with bimodal effect distribution in bootstrap analysis, including net merit (chromosome 18)
- For net merit, >1 QTL segregating on chromosome and consistent with Cole and VanRaden (2011, *JABG*)

Looks convincing, but …

- Literature full of QTL reports, but vast majority not validated

- Discovery of QTLs for milk production traits in Australian dairy cattle (Kemper *et al.*, 2015, *JABG*)
  - Holstein analysis included 8,478 cows and 3,049 bulls
  - Only effects significant by 2 criteria considered for further analysis

QTLs in Australian population*

Trait	Chromo-some	Location (bp)**		Probability	
		Australia	U.S.	Australia	U.S.
Protein %	3	15,632,410	16,097,418	3.2×10^{-30}	2.4×10^{-42}
Fat yield	5	93,945,655	92,115,327	7.9×10^{-15}	1.1×10^{-37}
Fat %	5	93,945,655	92,115,327	2.0×10^{-38}	9.8×10^{-40}
Protein %	20	31,228,912	31,393,193	1.3×10^{-34}	2.4×10^{-33}
Protein %	29	41,989,397	42,770,336	7.9×10^{-41}	5.6×10^{-07}

*Significant effect at $P < 10^{-15}$; *ABCG2* and *DGAT1* excluded

**Australia location is SNP with greatest effect; U.S. location is relative to 1st SNP in segment with greatest effect

Confirmation of fertility effects

- Haplotypes with major negative effects in Holsteins: HH1, HH2, and HH3 on chromosomes 5, 1, and 8 (VanRaden *et al.*, 2011, *JDS*)
- Causative mutations identified for HH1 and HH3, but not HH2
- APGD significant effects for cow conception rate (CCR) and daughter pregnancy rate (DPR) on chromosomes 1 (HH1) and 5 (HH2), but not chromosome 8

Study comparison

Study	HH1 (chr. 5)	HH2 (chr. 1)
VanRaden *et al.* (2011, *JDS*)		
Location (bp)	63,150,400	94,860,836–96,533,339
Effect, conception rate (%)	–3.0 ± 0.8	–3.2 ± 0.4
Frequency (%)	1.92	1.66
APGD CCR (%)		
Greatest effect (bp)	92,115,327–96,166,308	64,592,861–68,997,018
APGD *P*	1.7×10^{-29}	6.9×10^{-14}
CI (bp)	65,922,088–96,166,308	…
APGD DPR (%)		
Greatest effect (bp)	88,359,142–92,115,327	88,167,139–92,958,471
APGD *P*	1.6×10^{-26}	7.7×10^{-17}
CI (bp)	65,922,088–96,166,308	64,592,861–111,573,593

Fertility effect conclusions

- HH1
  - Same CI for CCR and DPR
  - CI did not include position of causative mutation (Adams *et al.*, 2012, *PAG XX*)
- HH2
  - DPR CI included location of HH2 haplotype
  - CCR CI not computed because minimum $P > 10^{-15}$

The next step

- 42 grandsires sequenced and available through 1000 Bull Genomes Project

- Remaining 29 bulls to be sequenced as part of BARD project
  - Initially sequence to depth of 10–15×
  - Haplotype determination will enable accurate and nearly complete sequence for most bulls
  - Additional sequencing as necessary to determine complete sequence

Conclusions

- At least 1 significant effect found for all but 6 traits
- Results for yield traits correspond to those for Australian Holsteins
- Results will be used to identify promising regions of sequence data for discovery of causative mutations
- QTN determination
  - Increase rates of genetic gain
  - Aid in understanding mechanisms that affect traits

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