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 ($2.4 \times 10^{-42}$) 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 $-\log P$*

*Effects with bimodal CI deleted
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**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Chromosome</th>
<th>Location (bp)**</th>
<th>Probability Australia</th>
<th>Probability U.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein %</td>
<td>3</td>
<td>15,632,410</td>
<td>3.2×10⁻³⁰</td>
<td>2.4×10⁻⁴²</td>
</tr>
<tr>
<td>Fat yield</td>
<td>5</td>
<td>93,945,655</td>
<td>7.9×10⁻¹⁵</td>
<td>1.1×10⁻³⁷</td>
</tr>
<tr>
<td>Fat %</td>
<td>5</td>
<td>93,945,655</td>
<td>2.0×10⁻³⁸</td>
<td>9.8×10⁻⁴⁰</td>
</tr>
<tr>
<td>Protein %</td>
<td>20</td>
<td>31,228,912</td>
<td>1.3×10⁻³⁴</td>
<td>2.4×10⁻³³</td>
</tr>
<tr>
<td>Protein %</td>
<td>29</td>
<td>41,989,397</td>
<td>7.9×10⁻⁴¹</td>
<td>5.6×10⁻⁰⁷</td>
</tr>
</tbody>
</table>

*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

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

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