Genomic prediction with whole-genome sequence data in intensely selected pig lines

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Abstract

Background

Early simulations indicated that whole-genome sequence data (WGS) could improve prediction accuracy and its persistence across generations and breeds. However, results in real datasets have been ambiguous so far. Large data sets that capture most of the genome diversity in a population must be assembled so that allele substitution effects are estimated with higher accuracy. The objectives of this study were to use a large pig dataset to assess the benefits of using WGS for genomic prediction compared to using commercial marker arrays, to identify scenarios in which WGS provides the largest advantage, and to identify potential pitfalls for its effective implementation.

Methods

We sequenced 6,931 individuals from seven commercial pig lines with different numerical size. Genotypes of 32.8 million variants were imputed for 396,100 individuals (17,224 to 104,661 per line). We used BayesR to perform genomic prediction for 8 real traits and 9 simulated traits with different genetic architectures. Genomic predictions were performed using either data from a marker array or variants preselected from WGS based on linkage disequilibrium, functional annotation, or association tests. Both single and multi-line training sets were explored.

Results

Using WGS improved prediction accuracy relative to the marker array, provided that training sets were sufficiently large, especially for traits with high heritability and low number of quantitative trait nucleotides. The performance of each set of predictor variants was not robust across traits and lines. The most robust results were obtained

when preselected variants with statistically significant associations were added to the marker array. Under this method, average improvements of prediction accuracy of 2.5 and 4.2 percentage points were observed in within-line and multi-line scenarios, respectively, with training sets of around 80k individuals.

Conclusions

Our results evidenced the potential for WGS to improve genomic prediction accuracy in intensely selected pig lines. Although the prediction accuracy improvements achieved so far were modest at best, we would expect that more robust improvements could be attained with a combination of larger training sets and optimised pipelines.

Introduction

45 Whole-genome sequence data (WGS) has the potential to empower the
46 identification of causal variants that underlie quantitative traits or diseases [1–4],
47 increase the precision and scope of population genetic studies [5,6], and enhance
48 livestock breeding. Genomic prediction has been successfully implemented in the
49 main livestock species and it has increased the rate of genetic gain [7]. Genomic
50 prediction has provided many benefits such as greater accuracies of genetic
51 evaluations and the reduction of the generational interval in dairy cattle. However,
52 since its early implementations, genomic prediction is typically performed using
53 marker arrays that capture the effects of the (usually unknown) causal variants via
54 linkage disequilibrium. Alternatively, WGS are assumed to contain the causal variants
55 themselves. For this reason, it was hypothesized that such data could further improve
56 prediction accuracy and its persistence across generations and breeds. Early
57 simulations indicated that causal mutations from WGS could increase prediction
58 accuracy [8–13]. One simulation study indicated that the magnitude of prediction
59 accuracy improvement relative to dense marker arrays ranged from 2.5 to 3.7%, with
60 a persistence of over 10 generations [11]. Another one reached improvements of 30%
61 if causal variants with low minor allele frequency could be captured by the WGS [9].
62 However, benefits could be on the lower end of that range in standard livestock
63 populations due to small effective population sizes and long-term negative selection
64 [10].

65 During the last few years, there have been several attempts at improving the
66 accuracy of genomic prediction with the use of WGS in the main livestock species.
67 Results have been ambiguous so far. When predicting genomic breeding values within
68 breed or line, some studies found no relevant improvement of prediction accuracy for

69 WGS compared to marker arrays [14–18]. Other studies found small, and often
70 unstable, improvements (e.g., from 1 to 5% or no improvement depending on
71 prediction method [19–21], or trait-dependent results [21,22]). When predicting
72 genomic breeding values across populations, the identification of causal variants from
73 WGS can improve prediction accuracy [23–26], especially for small populations
74 where initial prediction accuracy was low or that were not included in the training
75 population [23,25–28].

76 One of the most successful strategies to exploit WGS consists in augmenting
77 available marker arrays with preselected variants from WGS based on their
78 association with the trait of interest [29–32]. In some cases, this strategy improved
79 prediction accuracy by up to 9% [31] and 11% [32]. However, it did not improve
80 prediction accuracies in other within-line scenarios [16]. Nevertheless, this shows
81 how identifying causal variants could enhance genomic prediction with WGS. Whole-
82 genome sequence data has already been applied in genome-wide association studies
83 (GWAS) to identify variants associated to a variety of traits in livestock [2,33–35],
84 including pigs [36,37]. However, the fine-mapping of causal variants remains
85 challenging due to the pervasive long-range linkage disequilibrium across extremely
86 dense variation.

87 High accuracy in estimating allele substitution effects and, ideally, the
88 identification of causal variants amongst millions of other variants are important for
89 the usefulness of WGS in research and breeding. This requires large data sets able to
90 capture most of the genome diversity in a population. Despite that low-cost
91 sequencing strategies have been developed, which typically involve sequencing a
92 subset of the individuals in a population at low coverage and then imputing WGS for
93 the remaining individuals [38–40], the cost of generating accurate WGS at this scale,

as well as the large computational requirements for the analyses of such datasets, have limited the population sizes or number of populations tested in some of the previous studies. This hinders the interpretation of results across studies, which are very diverse in population structures, sequencing strategies and prediction methodologies used. The largest studies on the use of WGS for genomic prediction to date have been performed in cattle, for which large multi-breed reference panels are available from the 1000 Bull Genomes Project [2,19,33]. This has enabled the imputation of WGS for cattle populations. The lack of such available reference panels has been cited as an important limiting factor for performing similar studies in other species, such as pigs [36].

We have previously described our approach to impute WGS in large pedigreed populations without the need for haplotype phased reference panels [41]. Following that strategy, we generated WGS for 396,100 pigs from seven intensely selected lines with diverse genetic backgrounds and numerical size. The objectives of this study were to use this large pig dataset to assess the benefits of using WGS for genomic prediction compared to using commercial marker arrays, to identify scenarios in which WGS provides the largest advantage, and to identify potential pitfalls for its effective implementation.

Materials and Methods

Populations and sequencing strategy

We performed whole-genome re-sequencing of 6,931 individuals from seven commercial pig lines (Genus PIC, Hendersonville, TN) with a total coverage of approximately 27,243x. Sequencing effort in each of the seven lines was proportional to population size. Approximately 1.5% (0.9 to 2.1% in each line) of the pigs in each

117 line were sequenced. Most pigs were sequenced at low coverage, with target coverage
118 of 1 or 2x, but a subset of pigs were sequenced at higher coverage of 5, 15, or 30x.
119 Thus, the average individual coverage was 3.9x, but the median coverage was 1.5x.
120 The number of pigs sequenced and at which coverage for each line is summarized in
121 Table 1.

122 The sequenced pigs and their coverage were selected following a three-part
123 sequencing strategy developed to represent the haplotype diversity in each line. First
124 (1), sires and dams with the highest number of genotyped progeny were sequenced at
125 2x and 1x, respectively. Sires were sequenced at a greater coverage because they
126 contributed with more progeny than dams. Then (2), the individuals with the greatest
127 genetic footprint on the population (i.e., those that carry more of the most common
128 haplotypes) and their immediate ancestors were sequenced at a coverage between 1x
129 and 30x (AlphaSeqOpt part 1; [42]). The sequencing coverage was allocated with an
130 algorithm that maximises the expected phasing accuracy of the common haplotypes
131 from the cumulated family information. Finally (3), pigs that carried haplotypes with
132 low cumulated coverage (below 10x) were sequenced at 1x (AlphaSeqOpt part 2;
133 [43]). Sets (2) and (3) were based on haplotypes inferred from marker array genotypes
134 (GGP-Porcine HD BeadChip; GeneSeek, Lincoln, NE), which were phased and
135 imputed using AlphaPhase [44] and AlphaImpute [45].

136 Most sequenced pigs and their relatives were also genotyped either at low
137 density (15k markers) using the GGP-Porcine LD BeadChip (GeneSeek) or at high
138 density (80k markers) using the GGP-Porcine HD BeadChip (GeneSeek). Quality
139 control of the marker array data was based on the individuals genotyped at high
140 density. Markers with minor allele frequency below 0.01, call rate below 0.80, or that

141 failed the Hardy-Weinberg equilibrium test were removed. After quality control,
142 38,634 to 43,966 markers remained in each line.
143

Sequencing and data processing

144 Tissue samples were collected from ear punches or tail clippings. Genomic
145 DNA was extracted using Qiagen DNeasy 96 Blood & Tissue kits (Qiagen Ltd.,
146 Mississauga, ON, Canada). Paired-end library preparation was conducted using the
147 TruSeq DNA PCR-free protocol (Illumina, San Diego, CA). Libraries for
148 resequencing at low coverage (1 to 5x) were produced with an average insert size of
149 350 bp and sequenced on a HiSeq 4000 instrument (Illumina). Libraries for
150 resequencing at high coverage (15 or 30x) were produced with an average insert size
151 of 550 bp and sequenced on a HiSeq X instrument (Illumina). All libraries were
152 sequenced at Edinburgh Genomics (Edinburgh Genomics, University of Edinburgh,
153 Edinburgh, UK).
154 DNA sequence reads were pre-processed using Trimmomatic [46] to remove
155 adapter sequences from the reads. The reads were then aligned to the reference
156 genome *Sscrofa11.1* (GenBank accession: GCA_000003025.6) using the BWA-MEM
157 algorithm [47]. Duplicates were marked with Picard
158 (http://broadinstitute.github.io/picard). Single nucleotide polymorphisms (SNPs) and
159 short insertions and deletions (indels) were identified with the variant caller GATK
160 HaplotypeCaller (GATK 3.8.0) [48,49] using default settings. Variant discovery with
161 GATK HaplotypeCaller was performed separately for each individual and then a joint
162 variant set for all the individuals in each population was obtained by extracting the
163 variant positions from all the individuals.

164 We extracted the read counts supporting each allele directly from the aligned
165 reads stored in the BAM files using a pile-up function to avoid biases towards the
166 reference allele introduced by GATK when applied on low-coverage WGS [50]. That
167 pipeline uses the tool pysam (version 0.13.0; https://github.com/pysam-
168 developers/pysam), which is a wrapper around htslib and the samtools package [51].
169 We extracted the read counts for all biallelic variant positions, after filtering variants
170 in potential repetitive regions (defined as variants that had mean depth values 3 times
171 greater than the average realized coverage) with VCFtools [52]. This amounted to a
172 total of 55.6 million SNP (19.6 to 31.1 million within each line) and 10.2 million
173 indels (4.1 to 5.6 million within each line). A more complete description of the
174 variation across the lines is provided in [53].
175

Genotype imputation

176 Genotypes were jointly called, phased and imputed for a total of 483,353
177 pedigree-related individuals using the 'hybrid peeling' method implemented in
178 AlphaPeel [54,55]. This method used all the available marker array and WGS.
179 Imputation was performed separately for each line using complete multi-generational
180 pedigrees, which encompassed from 21,129 to 122,753 individuals each (Table 1).
181 We have previously published reports on the accuracy of imputation in the same
182 populations using this method [41]. The estimated average individual-wise dosage
183 correlation was 0.94 (median: 0.97). Individuals with low predicted imputation
184 accuracy were removed before further analyses. An individual was predicted to have
185 low imputation accuracy if itself or all of its grandparents were not genotyped with a
186 marker array or if it had a low degree of connectedness to the rest of the population.
187 These criteria were based on the analysis of simulated and real data on imputation

188 accuracy [41]. A total of 396,100 individuals remained, with each line comprising
189 between 17,224 and 104,661 individuals (Table 1). The expected average individual-
190 wise dosage correlation of the remaining individuals was 0.97 (median: 0.98)
191 according to our previous estimates. We also excluded from the analyses variants with
192 a minor allele frequency lower than 0.023, as their estimated variant-wise dosage
193 correlations was lower than 0.90 [41]. After imputation, 32.8 million variants (14.5 to
194 19.9 million within each line) remained for downstream analyses, out of which 9.9
195 million segregated across all seven lines.
196

Traits

197 We analysed data of 8 traits that are commonly included in selection
198 objectives of pig breeding programmes: average daily gain (ADG, g), backfat
199 thickness (BFT, mm), loin depth (LD, mm), average daily feed intake (ADFI, kg),
200 feed conversion ratio (FCR), total number of piglets born (TNB), litter weight at
201 weaning (LWW, kg), and return to oestrus 7 days after weaning (RET, binary trait).
202 Most pigs with records were born during the 2008–2020 period. Breeding values were
203 estimated by line with a linear mixed model that included polygenic and non-genetic
204 (as relevant for each trait) effects. Deregressed breeding values (dEBV) were obtained
205 following the method by VanRaden and Wiggans [56]. Only individuals in which the
206 trait was directly measured were retained for further analyses. The number of records
207 for each trait used in the analyses of each line is detailed in Table 2.
208

209 *Simulated traits*

210 To assist in the interpretation of results, we also created 9 simulated traits with
211 different numbers of quantitative trait nucleotides (QTN; 100, 1,000 or 10,000 QTN)

212 and heritability levels (h^2; 0.10, 0.25 or 0.50). Positions of the QTN were sampled
213 randomly amongst all variants called across all lines. Because QTN were sampled
214 from all variants, some QTN were fixed in some of the lines while segregating in
215 others. There were only negligible differences in the number of segregating QTN per
216 line (53 to 61, 531 to 583, or 5375 to 6058, respectively). Marker effects of the QTN
217 were sampled from a gamma distribution with shape=2 and scale=5. After a polygenic
218 term was calculated for each individual using these marker effects, residual terms
219 were sampled from a normal distribution with a variance parameter adjusted to
220 produce the desired heritability level. The number of records for the simulated traits is
221 detailed in Table 2. In these simulations, we used the imputed genotypes as real
222 genotypes and, therefore, implicitly cancelled any errors that might arise from the
223 processing of the sequencing reads and genotype imputation.
224

Training and testing sets

225 We split the individuals in each population into training and testing sets. The
226 testing sets were defined as those individuals from full-sib families from the last
227 generation of the pedigree (i.e., individuals that did not have any progeny of their
228 own). Only families with a minimum of 5 full-sibs were considered. The training set
229 was defined as all those individuals that had a pedigree coefficient of relationship
230 lower than 0.5 with any individual of the testing set. This design was chosen to mimic
231 a realistic situation in which breeding companies evaluate the selection candidates
232 available in the selection nucleus at any given time.
233

Genome-wide association study

To assess whether variants from the WGS could provide a finer mapping of causal variants than marker array data, and to provide an association-based criterion to preselect variants for the genomic prediction tests, we performed a GWAS for each trait and line. This step included only the individuals in the training set. We fitted a univariate linear mixed model that accounted for the genomic relationship matrix as:

$$\mathbf{y} = \mathbf{x}_i \beta_i + \mathbf{u} + \mathbf{e},$$

where $\mathbf{y}$ is the vector of dEBV, $\mathbf{x}_i$ is the vector of genotypes for the ith SNP coded as 0 and 2 if homozygous for either allele or 1 if heterozygous, β_i is the additive effect of the ith SNP on the trait, $\mathbf{u} \sim N(0, \sigma_u^2 \mathbf{K})$ is the vector of polygenic effects with the covariance matrix equal to the product of the polygenic additive variance σ_u^2 and a genomic relationship matrix $\mathbf{K}$, and $\mathbf{e}$ is a vector of uncorrelated residuals. Due to computational limitations, the genomic relationship matrix $\mathbf{K}$ was calculated using only imputed SNP genotypes in the marker array regardless of whether the association study involves the SNPs in the marker array or the variants in WGS. We used the FastLMM software [57,58] to fit the model.

We used the same p-value threshold ($p<10^{-6}$) for both marker array and for sequence associations, because while the WGS contains many more variants, they are also expected to be in higher linkage disequilibrium. This threshold was based on Bonferroni's multiple test correction assuming that the markers from the marker array were independent. For the simulated traits, we defined genomic regions that contained significant associations and assessed whether or not they contained a QTN. These regions were defined by overlapping 500-kb segments centered on the significant markers.

257

Genomic prediction in within-line scenarios

258 To test whether variants from the WGS could provide greater prediction
259 accuracy than the marker array, we tested genomic prediction using variants from the
260 marker array, from the WGS, or combining them. The marker array data (referred to
261 as 'Chip') was set as the benchmark for prediction accuracy. It contained all ~40k
262 variants in the marker array. For the sequence-based predictors, we preselected sets of
263 variants because currently available methods for genomic prediction are not yet
264 capable of handling datasets as large as the complete WGS. We tested different
265 alternative strategies for preselecting the predictor variants:

266 • *LDTags.* Tag variants retained after pruning based on linkage disequilibrium.
267 Variants were removed so that no pairs of SNPs with r^2>0.1 remained in any 10-
268 Mb window (windows slid by 2,000 variants) using Plink 1.9 [59]. The number
269 of predictor variants preselected by this method was on average of 30k variants
270 (range: 5k to 80k).

271 • *Top40k.* Variants preselected based on GWAS analyses. To mimic the number of
272 variants in Chip, we preselected the variants with the lowest p-value (not
273 necessarily below the significance threshold) in each of consecutive non-
274 overlapping 55-kb windows along the genome. In addition, to test the impact of
275 variant density on prediction accuracy, we preselected 10k, 25k, 75k, or 100k
276 predictor variants following the same criterion.

277 • *ChipPlusSign.* Variants preselected based on GWAS analyses as in Top40k, but
278 only significant variants ($p \leq 10^{-6}$) were preselected and merged with those in
279 Chip. When a 55-kb window contained more than one significant variant, only
280 that with the lowest p-value was selected as a proxy, in order to reduce the

281 preselection of multiple significant SNPs tagging the same causal variant. On
282 average, 309 significant variants were identified per trait and line (range: 23 to
283 1083; Table 3). These significant variants were merged with those in Chip.

284 • *Functional.* Variants that were annotated as loss-of-function or missense
285 according to Ensembl Variant Effect Predictor (Ensembl VEP; version 97, July
286 2019) [60]. The most severe predicted consequence type for each variant was
287 retrieved. The number of predictor variants preselected by this method was on
288 average of 35k variants (range: 27k to 40k).

289 • *Rand40k.* The same number of predictor variants as in Chip, chosen randomly.

290 Genomic prediction was performed by fitting a univariate model with BayesR
291 [61,62], with a mixture of normal distributions as the prior for variant effects,
292 including one distribution that sets the variant effects to zero. The model was:

$$\mathbf{y} = \mathbf{1}\mu + \mathbf{X}\boldsymbol{\beta} + \mathbf{e},$$

293 where $\mathbf{y}$ is the vector of dEBV, $\mathbf{1}$ is a vector of ones, μ is the general mean, $\mathbf{X}$ is a
294 matrix of genotypes, $\boldsymbol{\beta}$ is a vector of variant effects, and $\mathbf{e}$ is a vector of uncorrelated
295 residuals. The prior variance of the variant effects in $\boldsymbol{\beta}$ had four components with
296 variances $\sigma_1^2 = 0$, $\sigma_2^2 = 0.0001\sigma_g^2$, $\sigma_3^2 = 0.001\sigma_g^2$, or $\sigma_4^2 = 0.01\sigma_g^2$, where σ_g^2 is the
297 total genetic variance. We used a uniform and almost uninformative prior for the
298 mixture distribution. We used a publicly available implementation of BayesR
299 (https://github.com/syntheke/bayesR; accessed on 30 April 2021), with default
300 settings. Prediction accuracy was calculated in the testing set as the correlation
301 between the genomic estimated breeding value and the dEBV. Bias of the prediction
302 accuracy was calculated as the regression coefficient of the dEBV on the genomic
303 estimated breeding values.

304 It has been noted that using the same reference individuals for preselecting
305 variants through GWAS and for training the predictive equation can reduce prediction
306 accuracy and bias the predicted breeding values [16,63]. To account for that, we
307 reanalysed some of the scenarios after splitting the training set into two exclusive
308 subsets, one for GWAS to preselect the predictor variants and one for training the
309 predictive equation. The GWAS subset was defined by randomly selecting either 10%
310 or 50% of the individuals in the original training set. Those individuals were excluded
311 from the subset used for training the predictive equation afterwards.
312

Genomic prediction in multi-line scenarios

313 We considered multi-line scenarios in which the training set consisted of
314 merging the training sets that had been defined for each line. All analyses were
315 performed as for the within-line scenarios but with a line effect. In the multi-line
316 scenarios, all SNPs from the marker array that passed quality control and were
317 imputed for at least one line were included in the baseline (referred to as 'ML-Chip').
318 For ease of computation, the strategies for preselection of predictor variants from
319 WGS were applied only to the subset of 9.9 million variants that had been called and
320 imputed in all seven lines. Thus, we defined the predictor sets 'ML-Top40k' and
321 'ML-ChipPlusSign' by preselecting variants following the same criteria as in within-
322 line scenarios, but using a multi-line GWAS analyses with line effect instead. For
323 ML-ChipPlusSign, 60 to 7247 significant variants were identified per trait (Table 3)
324 and merged with those in ML-Chip. For comparison purposes, prediction accuracy
325 was calculated for the testing set of each individual line.
326

Results

Prediction accuracy within line

327 Whole-genome sequence data can improve prediction accuracy of marker
328 array data when there is a sufficiently large training set and if an appropriate set of
329 predictor variants is preselected. Figure 1 shows the prediction accuracy for the case
330 with the largest training set using different sets of predictor variants. In this case, all
331 tested sets of variants from the WGS, except for LDTags, yielded increases of
332 prediction accuracy that ranged from +2.0% to +9.2%. Using WGS also reduced bias
333 relative to Chip in some scenarios. However, the performance across predictor
334 variants set was not robust for the most part, and differed for each trait and line
335 (Additional File 1), often leading to no improvements of prediction accuracy or even
336 reduced prediction accuracy relative to Chip. One stable feature of the results was
337 LDTags showing a noticeable decrease in prediction accuracy in most traits and lines.
338 The size of the training set was one of the main factors that determined the
339 capacity of predictor variants from the WGS to improve the baseline prediction
340 accuracy of Chip. Figures 2 and 3 show the difference in prediction accuracy of
341 Top40k and ChipPlusSign with respect to the baseline of Chip against the number of
342 phenotypic records available in the training set. We observed large variability for the
343 difference in prediction accuracy, especially when the training set was small. This
344 variability was larger in Top40k than in ChipPlusSign, in a way that shrinkage of
345 variation as the training set was larger was more noticeable in ChipPlusSign. Gains in
346 prediction accuracy were low-to-moderate in the most favourable cases. In the most
347 unfavourable ones we observed large losses in prediction accuracy for Top40k but
348 more restrained losses for ChipPlusSign with moderate training set sizes. For both
349 sets of predictor variants, there was a positive trend that supported the need for large

350 training sets. This trend was clearer in ChipPlusSign than in Top40k, because of the
351 apparent lower robustness of the latter. Results for the other sets of predictor variants
352 are provided in Additional File 2.

353 The genetic architecture of the traits was also related to the success of WGS
354 for improving prediction accuracy. As the true genetic architecture of real complex
355 traits is mostly unknown, we used simulated traits to show that traits with high
356 heritability and low number of QTN were more likely to show larger improvements in
357 predictive performances. With Top40k (Figure 4), heritability seemed to be the main
358 factor that affected the expected improvement with large training sets (from null
359 improvements when h^2=0.1 to improvements of approximately 0.05 when h^2=0.5,
360 regardless of number of QTN, with a training set of 92k individuals). With
361 ChipPlusSign (Figure 5), the expected improvements with the same training set (92k
362 individuals) were not only greater in magnitude but depended on both heritability and
363 number of QTN (from null improvements when h^2=0.1 to improvements of
364 approximately 0.03 to 0.10 when h^2=0.5 with a number of 100 to 10k QTN,
365 respectively). Results confirmed the trends observed for the real traits (Figures 4 and
366 5); for instance, the higher robustness of ChipPlusSign compared to Top40k.

367 We observed diminishing returns when we increased the density of the
368 predictor variants. Increasing the number of predictor variants from the 40k in
369 Top40k to 75k selected in the same way yielded small improvements in prediction
370 accuracy compared to Top40k, but increases up to 100k variants provided smaller or
371 null additional gains (Additional File 3).

372 Splitting the original training set into two exclusive subsets, one for the
373 GWAS-based preselection of the variants and one for the training of the predictive
374 equation did not improve the prediction accuracy (Additional File 4). For

375 ChipPlusSign, this strategy reduced the bias but prediction accuracy decreased too,
376 probably because of the smaller subset available for training the predictive equation.
377

Prediction accuracy in multi-line scenarios

378 The performance of genomic predictors trained with multi-line datasets was
379 systematically lower than in the within-line scenarios (Additional File 5).
380 Nonetheless, the ML-ChipPlusSign predictor variants in general increased prediction
381 accuracy relative to ML-Chip (Figure 6). The increase in genomic prediction accuracy
382 for each line was largely dependent on the number of individuals of each line in the
383 training set. Therefore, the greatest improvements were achieved for the largest lines.
384 However, in the multi-line scenarios we observed increases of prediction accuracy for
385 some traits and lines for which no improvements were observed in the within-line
386 scenarios (Figure 7). In contrast, results for ML-Top40k were not robust (Additional
387 File 6).
388

Association tests

389 First, we assessed the performance of GWAS using the simulated traits. Table
390 4 shows the number of regions with significant associations that were detected using
391 either Chip or WGS, and whether they contained zero, one or multiple true QTN. The
392 WGS allowed the detection of a much larger proportion of true QTN than the Chip,
393 especially for the traits with high heritability and with large population sizes. The
394 most favourable scenarios for identifying regions that contained unequivocally a
395 single QTN with WGS were those in which the trait was controlled by a low number
396 of true QTN. However, even though the genetic architecture was very simple and
397 consisted of additive effects alone, the regions with significant associations only

398 captured a small fraction of the QTN that segregated within each line. Moreover,
399 using WGS also increased the number of regions with significant associations that
400 contained no QTN, which could therefore be considered as false positives. Some of
401 the selected regions contained multiple QTN, which could indicate either a 'hit by
402 chance' or an inability to disentangle multiple causal variants. While false positives
403 also occur with Chip, their incidence was more severe with the WGS, especially for
404 traits with a large number of QTN. Large population sizes further aggravated the
405 inflation of genome-wide p-values.

406 Despite this, with the real traits we found that GWAS using WGS can
407 contribute to a better understanding of the genetic mechanisms that underlie the traits
408 of interest. To illustrate this, we examined the GWAS results for BFT in line A, for
409 which a large number of phenotypic records were available. Figure 8 shows the
410 results for chromosome 1 as an example, while Additional File 7 shows the results for
411 six genomic regions of interest. The main genomic regions and candidate genes
412 associated to BFT detected with Chip in the same genetic lines studied here were
413 reported elsewhere [64]. We will use the candidate genes reported there to refer to the
414 genomic regions with significant associations. Using Chip, we identified 6 genomic
415 regions ($p<10^{-6}$). Using WGS (with a more stringent significance threshold of $p<10^{-9}$
416 to focus on the most significant associations), we confirmed 3 of these genomic
417 regions that co-located to candidate genes *MC4R*, *DOLK*, and *DGKI* or *PTN*.
418 However, the most associated variants in each of these genomic regions located
419 outside the coding region of these putative causal genes. These signals sometimes had
420 very strong evidence of association for some variants that were relatively distant from
421 our candidate functional gene, which could cast doubts about the fine-mapping of the
422 causal mutation. The region at SSC18, 9–13 Mb, contained two candidate genes

423 *DGKI* and *PTN*, but the WGS revealed significantly associated variants within *DGKI*
424 and none within *PTN*, despite that the strongest associations were away from both
425 genes at 10.5-11 Mb. Using the WGS we also detected 24 additional genomic regions
426 that contained candidate genes such as *CYB5R4*, *IGF2*, and *LEPR*. These genes were
427 previously detected in other lines using the Chip but not in this one [64], sometimes
428 because there were no markers for the associated region in Chip (SSC2, 0–4 Mb). The
429 region at SSC1, 52.5–53.5 Mb, showed many significant variants that encompassed
430 not only the previously identified candidate gene *CYB5R4*, but also *MRAP2*
431 (annotated with functions on feeding behavior and energy homeostasis). In contrast,
432 candidate gene *LEPR* was located within the region at SSC6, 146.5–147.0 Mb, where
433 many significant variants were located, although the most significant variants were
434 not in the coding regions of the gene. Using the WGS we also identified additional
435 candidate genes that had not been previously detected in any of the lines, such as
436 *CYP24A1* (annotated with functions on fatty acid omega-oxidation and vitamin D
437 metabolism; not shown). For many of the other genomic regions, it was difficult to
438 pinpoint a candidate gene with the available information or there were no annotated
439 genes.
440

Discussion

441 Our results evidenced the potential for WGS to improve genomic prediction
442 accuracy in intensely selected pig lines, provided that the training sets are large
443 enough. Improvements achieved so far were modest at best. On one hand, these
444 modest improvements indicated that the strategies that we tested were likely
445 suboptimal. On the other hand, the positive trend for the largest training sets indicated
446 that we might have not reached the critical mass of data that is needed to leverage the

447 potential of WGS, especially in scenarios where genomic prediction with marker
448 arrays is already yielding high accuracy. The results from several traits and lines with
449 different training set sizes and the use of simulated phenotypes allowed us to identify
450 the most favourable scenarios for genomic prediction with WGS. We will discuss (1)
451 the prediction accuracy that we achieved with WGS compared to commercial marker
452 array data and the scenarios in which WGS may become beneficial, and (2) the
453 potential pitfalls for its effective implementation and the need for an optimised
454 strategy.
455

Prediction accuracy with whole-genome sequence data

456 We compared the genomic prediction accuracy of the current marker array
457 (Chip) with sets of preselected sequence variants in a way that the number of variants
458 remained similar across sets. Improvements of prediction accuracy can be limited if
459 current marker arrays are already sufficiently dense to capture a large proportion of
460 the genetic variance in intensely selected livestock populations. These populations
461 typically have small effective population size [10,19]. Nevertheless, modest
462 improvements have been achieved under certain scenarios. In our study, the most
463 robust results were obtained for the ChipPlusSign set, where variants that showed
464 statistically significant associations to the trait were preselected and added to the
465 information from the marker array. This is consistent with previous reports that
466 showed an improvement of prediction accuracy under similar approaches [29–32].
467 We added 23 to 1083 significant variants to those in Chip in different scenarios. In the
468 most successful ones, at least around 200 significant variants were added and average
469 improvements of prediction accuracy of 2.5 percentage points were observed with
470 training sets of around 80k individuals. In other instances, however, additions of a

471 larger number of variants have been proposed. The addition of 1623 variants
472 (preselected as the combination of 3-5 variants for each of the top QTL per trait and
473 breed) to a 50k array increased prediction reliability (accuracy squared) by up to 5
474 percentage points in Nordic cattle [29]. Adding the 16k SNPs with largest estimated
475 effects to a 60k array increased prediction reliability on average by 2.7 (up to 4.8)
476 percentage points in Holstein cattle [30]. For the custom 50k array for Hanwoo cattle,
477 it has been reported that adding at least around 12k SNPs (3k for each of four traits)
478 improved prediction accuracy by up to ~6 percentage points [32]. The addition of
479 ~400 variants preselected by GWAS with regional heritability mapping to a 50k array
480 increased prediction accuracy by 9 percentage points in sheep [31]. In other cases in
481 Nordic cattle, however, the addition of ~1500 variants preselected by GWAS to a 54k
482 panel produced negligible improvements in the prediction of traits with low
483 heritability [65].

484 Preselecting an entirely new set of predictor variants from WGS, as in
485 Top40k, proved more challenging than ChipPlusSign. In Top40k, we preselected the
486 variants with the lowest p-value in each of consecutive non-overlapping 55-kb
487 windows along the genome. This strategy did not perform much differently from just
488 taking random variants from these windows, as in Rand40k. One possible reason for
489 these results is that at this variant density, random variants effectively tag the same
490 associations as Top40k thanks to linkage disequilibrium. Denser sets of predictor
491 variants provided only small further improvements of prediction accuracy with
492 diminishing returns.

493 The modest performance of ChipPlusSign and Top40k could also be a
494 consequence of the difficulty for fine-mapping causal variants through GWAS with
495 WGS. Theoretically, the identification of all causal variants associated with a trait

496 should enable the improvement of prediction accuracy [12]. Even though WGS allows
497 the detection of a very large number of associations, problems such as false positives
498 or p-value inflation also become more severe in a way that added noise might offset
499 the detected signal. For instance, results in cattle showed that GWAS with WGS did
500 not detect clearer associated regions relative to marker arrays and failed to capture
501 QTL for genomic prediction [14], as the effect of potential QTL were spread across
502 multiple variants. Therefore, WGS performed better with simple genetic architectures
503 (i.e., traits with low number of QTN). This is consistent with expectations and
504 simulation results [8] that indicated that the benefit of using WGS for genomic
505 prediction would be limited by the number and size of QTN. When there are many
506 QTN with small effects it becomes much more difficult to properly estimate their
507 effects accurately. Therefore, for largely polygenic traits (as most traits of interest in
508 livestock production), training sets need to be very large before WGS can increase
509 prediction accuracy [8].

510 The advantage of using WGS might be limited by the current training set
511 sizes, especially in scenarios where marker arrays are already yielding high prediction
512 accuracy [14,20]. Multi-line training sets could be particularly beneficial with the use
513 of WGS because they allow a larger training set with low pairwise relationship degree
514 among individuals. Previous simulations suggested that WGS might be the most
515 beneficial with multi-breed reference panels [66], especially for numerically small
516 populations. Our results with a multi-line training set indicated that WGS can improve
517 prediction accuracy in scenarios that are less optimised than within-line genomic
518 prediction. The average improvements of prediction accuracy of 4.2 percentage points
519 were observed for the populations that contributed around 80k individuals to the
520 training set. However, in general those predictions were still less accurate than using

521 variants preselected under within-line training sets. In our multi-line scenarios we
522 only used variation that segregated across all seven lines. We observed that
523 population-specific variation accounted only for small fractions of genetic variance
524 [53] and it seems unlikely that they would contribute much to prediction accuracy
525 across breeds. Another possible obstacle is the differences in the allele substitution
526 effects of the causal mutations across breeds. This can be caused by differences in
527 allele frequency, contributions of non-additive effects and different genetic
528 backgrounds, or even gene-by-environment interactions among others [24,67].

529 We observed low robustness of genomic prediction with WGS across traits
530 and lines, and drops in prediction accuracy in those scenarios where genomic
531 prediction with WGS failed. Regarding bias, we did not observe a systematic increase
532 for ChipPlusSign despite using the same individuals for variant preselection and for
533 training the predictors [16,63]. When we split the training set into two subsets, one for
534 GWAS-based variant preselection and the other for training of the predictive
535 equations, we did not observe any improvement in accuracy or bias. One hypothesis is
536 that both subsets belong to the same population and therefore retained similar inter-
537 relationship degrees (i.e., they are not strictly independent sets of individuals).
538 Moreover, the reduction in individuals available for training the predictors negatively
539 affected prediction accuracy.

540 We did not directly test persistence of prediction accuracy, but previous
541 studies with real data found no higher persistence of prediction accuracy for WGS,
542 not even with low degree of relationship between training and testing sets [14]. We
543 would expect such obstacles to persistence of accuracy until causal variants can be
544 successfully identified.

545

Suboptimal strategy and pitfalls

546 The use of WGS for genomic prediction can only be reached after many other
547 steps are completed to produce the genotypes at whole-genome level. Each of these
548 steps has its pitfalls. It is unavoidable that the success of using of WGS is sensitive
549 not only to the prediction methodology itself but also to the strategy followed until
550 genotyping. This strategy includes the choice of which individuals to sequence, the
551 bioinformatics pipeline to call variants, the imputation of the WGS and choice of
552 variant filters. When combined with the multiplicity of prediction methods and the
553 preselection of predictor variants (which is unavoidable with current datasets,
554 predictive methodologies and computational capacities), there are many options and
555 variables in the whole process that can affect the final result and that are not yet well
556 understood. Therefore, a much greater effort for optimising such strategies is required.
557 Here we tested relatively simple approaches to see how they performed with large
558 WGS datasets. We have discussed what in our opinion are the main pitfalls of our
559 approach for selection of the individuals to sequence [55] and the biases that may
560 appear during processing of sequencing reads [50] elsewhere, and therefore here we
561 will focus on imputation of WGS and its use for genomic prediction.
562

563 ***Imputation accuracy***

564 It is widely recognized that imputation from marker arrays to WGS from very
565 few sequenced individuals can introduce genotyping errors and that genotype
566 uncertainty can be high [17,21,68,69]. The accuracy of the imputed WGS is one of the
567 main factors that may limit its performance for genomic prediction. In a simulation
568 study, van den Berg et al. [17] quantified the impact of imputation errors on

569 prediction accuracy and showed that prediction accuracy decreases as errors
570 accumulate, especially in the testing set.

571 Imputation of WGS is particularly challenging because typically we have to
572 impute a very large number of variants for a very large number of individuals from
573 very few sequenced individuals. We assessed the imputation accuracy of our approach
574 elsewhere [41,55] and recommended that ~2% of the population should be sequenced.
575 In our study, line D was the line where prediction accuracy with Top40k performed
576 the worst, mostly performing below Chip predictors. In this line, only 0.9% of the
577 individuals in the population had been sequenced and therefore lower imputation
578 accuracy could be expected. Although there was not enough evidence for establishing
579 a link between these two features (sequencing effort and prediction accuracy), we
580 recommend cautious design of a sequencing strategy that is suited to the intended
581 imputation method [55].

582 Prediction accuracy could be improved by accounting for genotype uncertainty
583 of the imputed WGS. For that, it could be advantageous to use allele dosages rather
584 than best-guess genotypes [69], although most current implementations cannot handle
585 such information.

586

587 ***Preselection of predictor variants***

588 Simply using WGS to increase the number of markers does not improve
589 prediction accuracy [18,21,24]. Due to the large dimensionality of WGS, there is a
590 need to remove uninformative variants [24,31,66,68,70]. Predictor variants must be
591 causal or at least informative of the causal variants, which depends on the distance
592 between the markers and the causal variants [13]. For this reason, variants that are in
593 weak linkage disequilibrium with causal mutations have a ‘dilution’ effect, i.e., they

594 add noise and limit prediction accuracy [24,31,70]. However, if too stringent filters
595 are applied during preselection of predictor variants, there is a risk of removing true
596 causal variants, and that would debilitate persistence of accuracy across generations
597 and across populations [66,71]. For instance, the impact of removing predictor
598 variants with low minor allele frequency can vary depending on the minor allele
599 frequency of the causal variants as well as the distance between predictor and causal
600 variants [13]. Losing causal or informative variants would negatively affect multi-line
601 or multi-breed prediction.

602 A popular strategy to preselect the predictor variants is based on association
603 tests. Genome-wide association studies on WGS are expected to confirm associations
604 that were already detected with marker arrays and identify novel associations (e.g.,
605 [36,72]). However, preliminary inspection of our GWAS results for the real traits
606 showed that the added noise could easily offset the added information and fine-
607 mapping remains challenging. Multi-breed GWAS [4] and meta-analyses [73] are
608 suitable alternatives for GWAS to accommodate much larger population sizes and for
609 combining results of populations with diverse genetic backgrounds. Multi-breed
610 GWAS can be more efficient to identify informative variants than single-breed
611 GWAS, which may benefit even prediction within lines [74]. Because the signal of
612 some variants may go undetected for some traits but not for other correlated traits,
613 combining GWAS information of several traits can also help identifying weak or
614 moderate associations [25]. We did not test whether combining the significant
615 markers from the different single-trait GWAS yielded greater improvements in
616 prediction accuracy [29,32]. Multi-trait GWAS models could be more suited for that
617 purpose [72,75]. To improve fine-mapping, other GWAS models that incorporate

618 biological information have been proposed (e.g., functional annotation [76] or
619 metabolomics [77]).

620 There have been other suggested methods that may improve variant
621 preselection for genomic prediction. VanRaden et al. [30] suggested that preselecting
622 variants based on the genetic variance that they contribute rather than the significance
623 of the association could be more advantageous, as the former would indirectly
624 preselect variance with higher minor allele frequency. Other authors proposed
625 preselection of variants using statistics that do not depend on GWAS, such as the
626 fixation index (F_{ST}) score between groups of individuals with high and low phenotype
627 values [70], as an alternative to avoid the negative impact of spurious associations.

628 Preselecting predictor variants based on functional annotation was not useful,
629 as it reduced prediction accuracy in several traits and lines. Previous studies showed
630 that subsets of variants based on functionality either did not improve or reduced
631 prediction accuracy [20] and that adding preselected variants from coding regions to
632 marker arrays produced lower prediction accuracy than just adding the same number
633 of variants without considering functional classification [32]. A plausible explanation
634 is that functional variants are enriched for lower minor allele frequency, which can be
635 less informative for prediction [13]. Furthermore, functional annotation does not
636 necessarily capture true effects, and the method we used is biased towards protein-
637 coding variants, which may lead to an underrepresentation of functional non-coding
638 variants that may explain a large fraction of quantitative trait variance. Xiang et al.
639 [78] found that expression QTL and non-coding variants explained more variation in
640 quantitative traits in cattle than protein-coding functional variants. When functional
641 annotation is not considered, intergenic variants are more likely to be preselected by
642 chance. Such variants tend to be more common and widespread across populations,

643 and therefore can act as tag variants and capture much larger fractions of trait variance
644 [53].
645 Another popular strategy to reduce the number of variants is to prune variants
646 based on linkage disequilibrium (LDTags). This strategy performed very poorly in our
647 populations. Other studies reported different outcomes, where pruning for r^2>0.9
648 provided positive results [18,21]. It is possible that this was in part due to the stringent
649 threshold (r^2>0.1) that we used in order to retain only a small number of variants.
650
651 ***New models and methods***
652 It is also likely that models, methods, and their implementations need to be
653 improved to handle the complexity of WGS and to efficiently estimate marker effects
654 of so many variants with high accuracy, among other features. This is a very active
655 area of research and multiple novel methodologies have been proposed over the last
656 years. Some examples are a combination of subsampling and Gibbs sampling [79],
657 and a model that simultaneously fits a GBLUP term for a polygenic effect and a
658 BayesC term for variants with large effects selected by the model (BayesGC) [26].
659 Testing alternative models and methods for genomic prediction was out of the scope
660 of this report. However, together with refinements in the preselection of predictor
661 variants, it remains an interesting avenue for further optimisation of the analysis
662 pipeline.
663 Some of the most promising methods are designed to incorporate prior
664 biological information into the models. One of such methods is BayesRC [23], which
665 extends BayesR by assigning flatter prior distributions to classes of variants that are
666 more likely to be causal [19,22]. Similarly, GFBLUP [80] could be used to
667 incorporate prior biological information from either QTL databases or GWAS as

genomic features [21,35,68]. The model MBMG [27], which fits two genomic relationship matrices according to prior biological information, has also been proposed for multi-breed scenarios to improve genomic prediction in small populations. Haplotype-based prediction methods could provide greater prediction accuracy with WGS than SNP-based methods in pigs [81] and cattle [82]. These methods reduce the number of model dimensions. However, the uptake of such methods has been limited so far due to their greater complexity, for example, to define haplotype blocks.

Conclusion

Our results evidenced the potential for WGS to improve genomic prediction accuracy in intensely selected pig lines. The performance of each set of predictor variants was not robust across traits and lines and the improvements that we achieved so far were modest at best. The most robust results were obtained when variants that showed statistically significant associations to the trait were preselected and added to the marker array. With this method, average improvements of prediction accuracy of 2.5 and 4.2 percentage points were observed in within-line and multi-line scenarios, respectively, with training sets of around 80k individuals. We would expect that a combination of larger training sets and improved pipelines could help achieve greater improvements of prediction accuracy. The robustness of the whole strategy for generating WGS at the population level must be carefully stress-tested and further optimised.

Ethics approval and consent to participate

The samples used in this study were derived from the routine breeding activities of PIC.

Consent for publication

Not applicable.

Availability of data and material

The software packages AlphaSeqOpt, AlphaPhase, AlphaImpute and AlphaPeel are available from the AlphaGenes website (http://www.alphagenes.roslin.ed.ac.uk). The datasets generated and analysed in this study are derived from the PIC breeding programme and not publicly available.

Competing interests

The authors declare that they have no competing interests. BDV, CYC, and WOH are employees of Genus PIC.

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Authors' contributions

RRF, GG and JMH designed the study; CYC assisted in preparing the datasets; RRF, AW and MJ performed the analyses; RRF wrote the first draft; AW, CYC, BDV,

706 WHO, GG and JMH assisted in the interpretation of the results and provided
707 comments on the manuscript. All authors read and approved the final manuscript.

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Figures

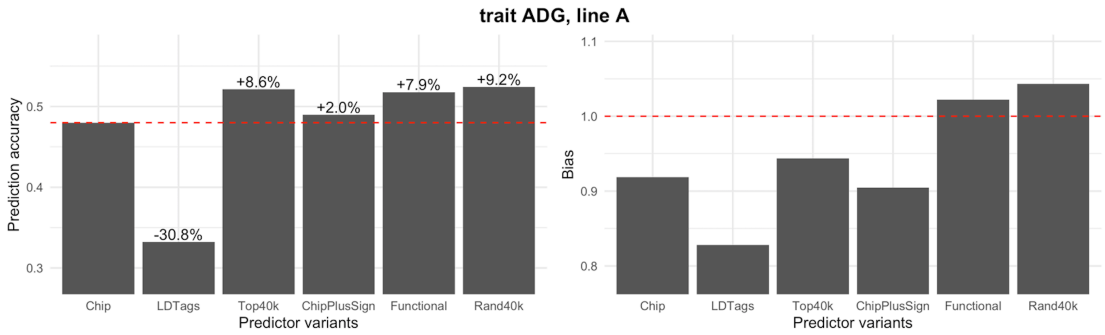


Figure 1. Prediction accuracy for each set of predictor variants for trait ADG in line A. Left: Correlation (left). Dashed line at value of Chip as a reference. Values indicate relative difference to Chip. Right: Bias. Dashed line at the ideal value.

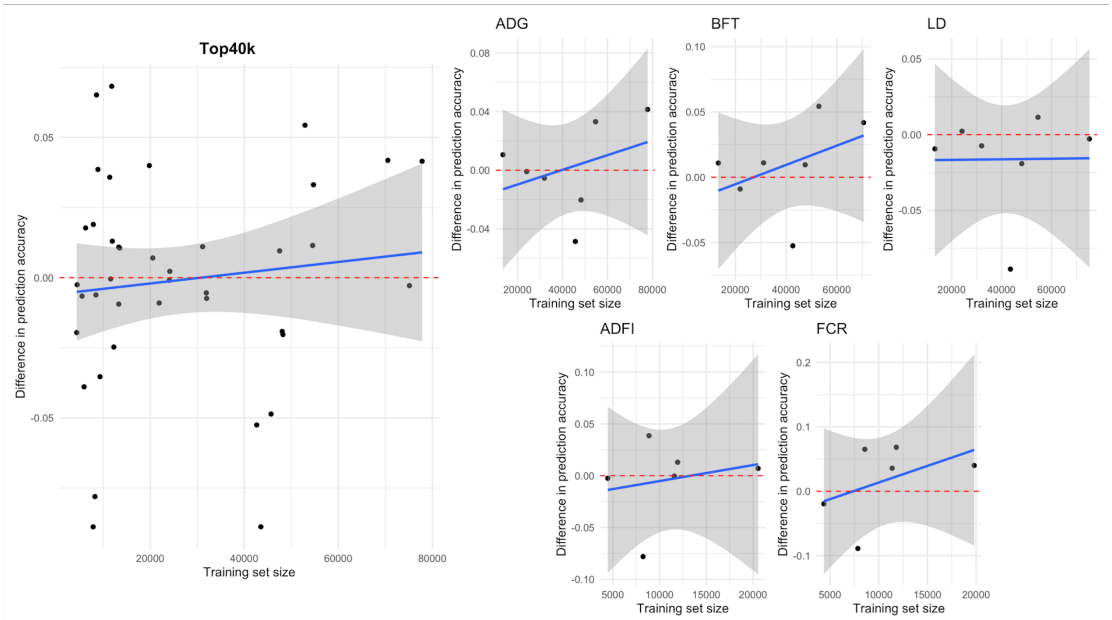


Figure 2. Genomic prediction accuracy with the Top40k predictor variants for the real traits. The difference of prediction accuracy between Top40k and Chip is shown, for all traits and lines (left) or by trait (right). Red dashed line at 'no difference'.

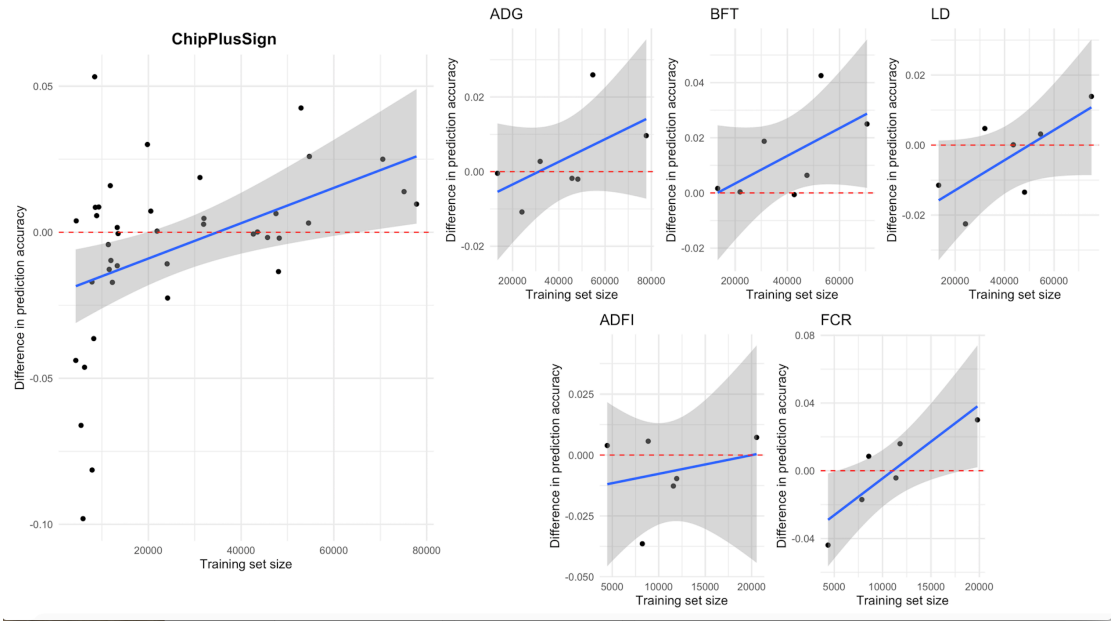


Figure 3. Genomic prediction accuracy with the ChipPlusSign predictor variants for the real traits. The difference of prediction accuracy between ChipPlusSign and Chip is shown, for all traits and lines (left) or by trait (right). Red dashed line at 'no difference'.

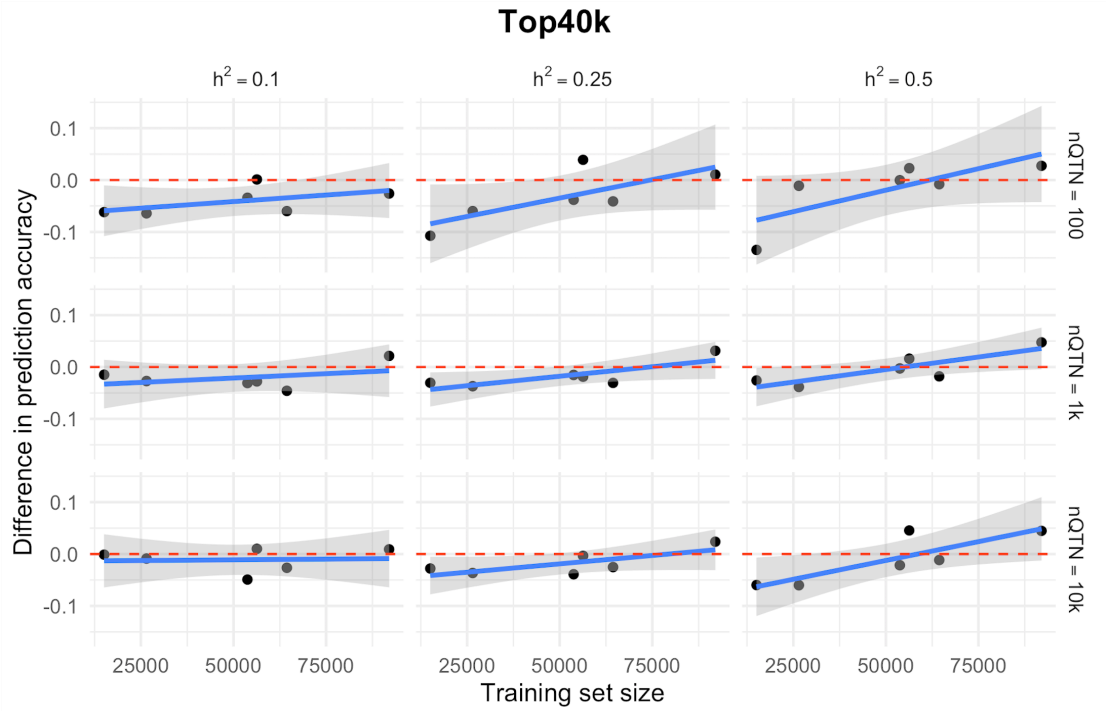


Figure 4. Genomic prediction accuracy with the Top40k predictor variants for the simulated traits. The difference of prediction accuracy between Top40k and Chip is shown by heritability (h^2) and number of quantitative trait nucleotides (nQTN) of the simulated traits. Red dashed line at 'no difference'.

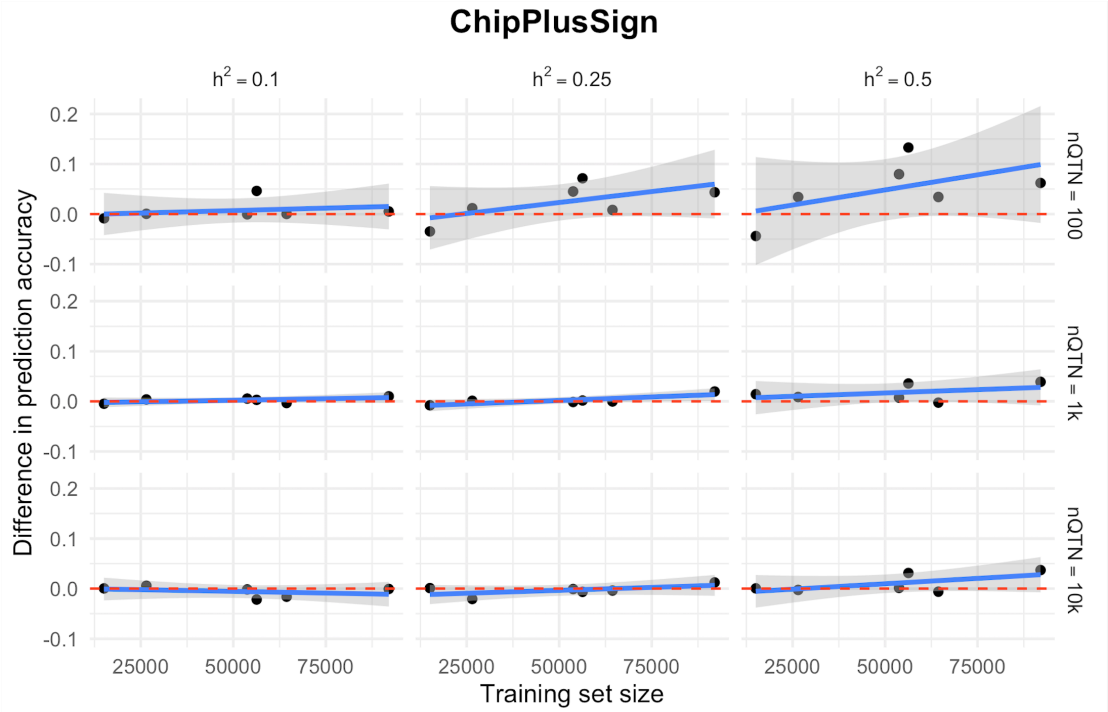


Figure 5. Genomic prediction accuracy with the ChipPlusSign predictor variants for the simulated traits. The difference of prediction accuracy between ChipPlusSign and Chip is shown by heritability (h^2) and number of quantitative trait nucleotides (nQTN) of the simulated traits. Red dashed line at 'no difference'.

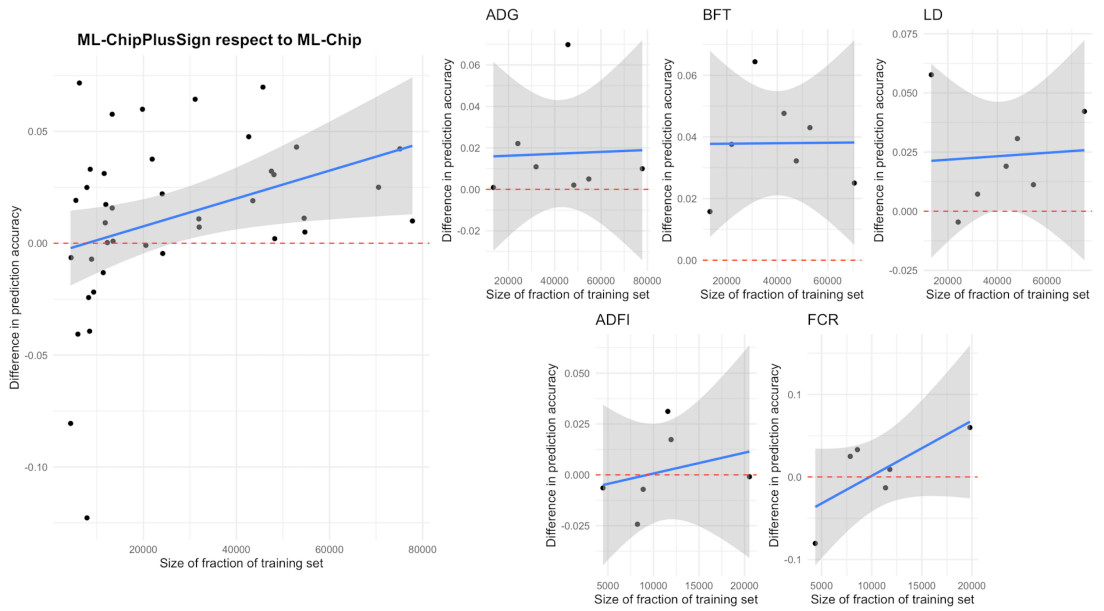


Figure 6. Genomic prediction accuracy with the ML-ChipPlusSign predictor variants for the real traits. The difference of prediction accuracy between ML-ChipPlusSign and ML-Chip is shown, for all traits and lines (left) or by trait (right). Red dashed line at 'no difference'.

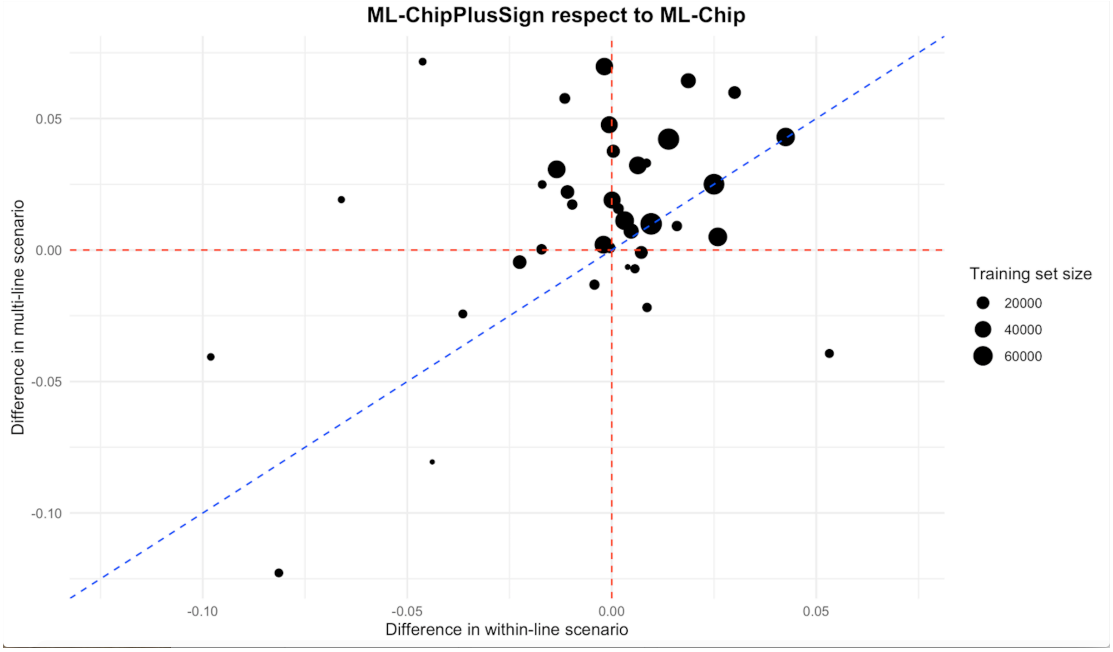
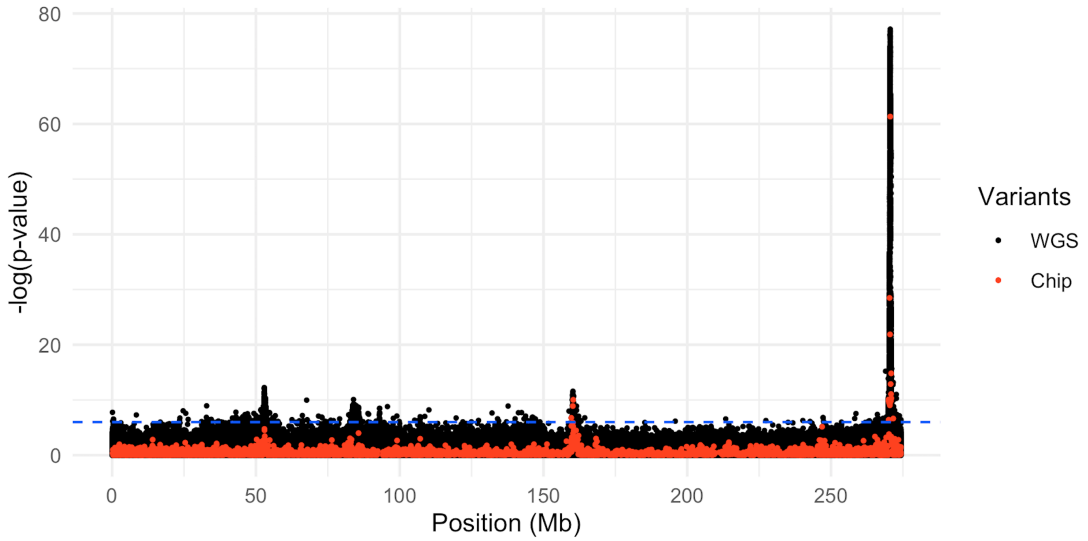


Figure 7. Comparison of the difference in genomic prediction accuracy in the multi-line scenarios (between ML-ChipPlusSign and ML-Chip) and in the within-line scenarios (between ChipPlusSign and Chip). Red dashed line at 'no difference'. Blue dashed line is the bisector.



1007

1008 **Figure 8.** Genome-wide association study results for trait BFT in line A. Only
1009 chromosome 1 is displayed as an example. In red, results for the variants in the
1010 marker array (Chip); in black, results for the whole-genome sequence data (WGS).
1011 The blue dashed line indicates significance threshold with Bonferroni's multiple test
1012 correction assuming that the markers from the marker arrays were independent (p-
1013 value $\leq 10^{-6}$).

1014

Tables

1015 **Table 1.** Number of sequenced pigs and pigs with imputed data.

Line	Individuals sequenced	Individuals sequenced by coverage				Individuals used in analyses	
		1x	2x	5x	15–30x	Pedigree	Imputed
A	1,856	1,044	649	73	90	122,753	104,661
B	1,366	685	545	44	92	88,964	76,230
C	1,491	628	728	54	81	84,420	66,608
D	731	362	311	16	42	79,981	60,474
E	760	394	274	27	65	50,797	41,573
F	381	193	137	16	35	35,309	29,330
G	445	217	176	15	37	21,129	17,224

1016

1017 **Table 2.** Number of phenotypic records per trait and line.

Trait	A	B	C	D	E	F	G
ADG	88,342	64,285	56,173	51,061	35,423	26,335	15,452
BFT	80,146	62,027	55,233	47,509	34,527	23,872	15,268
LD	85,233	64,141	56,026	48,509	35,495	26,453	15,274
ADFI	21,960	9,525	9,062	12,256	12,444	4,105*	4,851
FCR	21,200	9,217	8,654	12,044	12,316	4,016*	4,754
TNB	13,581	10,721	9,626	7,729*	6,506*	-	3,230*
LWW	-	9,112	7,251	-	-	-	2,813*
RET	-	6,978	6,327	-	-	-	1,669*
Simulated	104,661	76,230	66,608	60,474	41,573	29,330	17,224

1018 *ADG* average daily gain, *BFT* backfat thickness, *LD* loin depth, *ADFI* average daily

1019 feed intake, *FCR* feed conversion ratio, *TNB* total number of piglets born, *LWW* litter

1020 weight at weaning, *RET* return to oestrus 7 days after weaning.

1021 *Included in multi-line scenarios, but excluded in within-line scenarios because of the

1022 limited size of the testing set.

1023

1024 **Table 3.** Number of significant variants from the whole-genome sequence data that

1025 were added to the marker array in ChipPlusSign.

Trait	A	B	C	D	E	F	G	Multi-line
ADG	646	581	424	498	279	219	143	4731
BFT	1083	758	664	518	1030	218	237	6149
LD	633	579	458	518	222	215	43	7247
ADFI	145	224	169	23	183	-	119	767
FCR	198	224	162	95	56	-	134	1369
TNB	71	117	161	-	-	-	-	248
LWW	-	32	73	-	-	-	-	480
RET	-	184	31	-	-	-	-	60

1026 *ADG* average daily gain, *BFT* backfat thickness, *LD* loin depth, *ADFI* average daily

1027 feed intake, *FCR* feed conversion ratio, *TNB* total number of piglets born, *LWW* litter

1028 weight at weaning, *RET* return to oestrus 7 days after weaning.

1029

1030 **Table 4.** Number of significantly associated genomic regions in the genome-wide
1031 association study for the simulated phenotypes that contained 0, 1 or 2 or more
1032 quantitative trait nucleotides (QTN).

h^2	nQTN	Line size	Chip		Whole-genome sequence		
			0 QTN	1 QTN	0 QTN	1 QTN	≥2 QTN
0.10	100	27k	4	1	8	6	0
		56k	11	3	19	19	0
		92k	10	7	44	19	0
	1k	27k	1	0	4	0	1
		56k	1	0	16	3	1
		92k	1	0	283	9	0
	10k	27k	1	0	1	0	0
		56k	0	0	16	2	1
		92k	2	0	186	17	12
0.25	100	27k	11	6	26	15	1
		56k	22	8	44	28	3
		92k	20	7	90	34	1
	1k	27k	0	0	8	1	3
		56k	3	0	34	15	6
		92k	6	0	692	49	16
	10k	27k	0	0	2	0	0
		56k	0	0	90	9	22
		92k	4	0	564	56	164
0.50	100	27k	18	9	24	24	1
		56k	30	13	116	41	3
		92k	17	9	425	44	1
	1k	27k	6	0	22	9	6
		56k	5	1	238	59	32
		92k	11	1	903	169	120
	10k	27k	0	0	4	0	0
		56k	0	0	360	77	172
		92k	10	0	379	116	508

1033