

1 **Genome-Wide Association Testing for Haemorrhagic Bowel**

2 **Syndrome in a Swiss Large White Pig Population**

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ABSTRACT

24 **Background**

25 The porcine haemorrhagic bowel syndrome (HBS) is a multifactorial disease causing fatal
26 gastrointestinal disturbances and sudden death in fattening pigs. HBS is the leading cause of
27 deaths during fattening in Swiss pigs, with unclear etiology. Environmental and
28 management factors are associated with HBS incidence, but recent findings also suggest a
29 potential genetic predisposition. Pigs sired by a Swiss Large White (SLW) line appear more
30 prone to HBS. Here we conduct genome-wide association studies (GWAS) for HBS between
31 cases and controls to investigate potential genetic factors for the disease in Swiss fattening
32 pigs.

33 **Results**

34 Our study included 1,036 HBS cases and 4,080 controls with available microarray genotypes
35 or whole-genome sequencing data. Variant positions were determined according to the
36 current porcine reference assembly (Sscrofa11.1) or a HiFi-based SLW haplotype assembly
37 which we constructed using trio-binning. GWAS for HBS were conducted using 12.49 to
38 15.46 million biallelic variants in three mapping cohorts consisting of purebred animals from
39 SLW sire and dam lines, or crosses between these two parental lines. The statistical model
40 applied for the GWAS accounted for animal relatedness, population structure, and an
41 imbalanced case/control ratio. No sequence variants significantly associated with HBS were
42 identified, regardless of the cohort analysed and the reference sequence considered.

43 **Conclusions**

44 The lack of genetic associations despite a relatively large sample size suggests that
45 susceptibility to HBS in the studied SLW population is not due to large effect variants but
46 may be influenced by numerous small effect genetic variants, in addition to environmental
47 and management factors.

48 MAIN TEXT

49

50 **Background**

51 Haemorrhagic Bowel Syndrome (HBS) in pigs is a multifactorial disease that has become a
52 substantial threat to swine production. The disease manifests in fatal gastrointestinal
53 disturbances and sudden death in finisher pigs [1]. Key characteristics of HBS include pallor
54 and abdominal distention in carcasses, with intestinal torsion frequently observed during
55 necropsy [2,3]. Despite its substantial impact on animal welfare and the economics of pig
56 production, the etiology of HBS is not fully understood. Several factors contributing to the
57 incidence of the disease have been identified, including feeding systems [4], the origins of
58 the fattening pigs, cleaning frequency of feed distribution systems, and the width of feeding
59 places [2,5]. Seasonal variations [1,3,6], antimicrobial usage in feed [2,7], gut microflora
60 composition [3], and infectious agents such as *Clostridium perfringens* and
61 *Enterobacteriaceae* [2,4] have also been implicated.

62 A genetic component to HBS susceptibility has been proposed by previous studies [1,4]. A
63 recent study by Holenweger et al. [5] also revealed that fattening pigs descending from a
64 Swiss Large White (SLW) sire line were overrepresented among affected animals. These
65 recent findings suggest that genetic variants may contribute to the susceptibility to HBS and
66 may explain at least part of the observed across-breed variability to develop the disease.

67 This study aimed at identifying genetic variants associated with HBS in Swiss pigs through
68 genome-wide association testing, thereby contributing to a better understanding of the
69 etiology of the syndrome and facilitating the development of breeding strategies to prevent
70 disease incidence.

71

72 **Methods**

73 Tissue samples of 1036 pigs that died from HBS were collected on farm and at animal
74 carcass collection points in Switzerland over a period of six months, through SUISAG, the
75 service partner for Swiss pig producers. HBS was confirmed post-mortem based on the
76 inspection of the intestinal tract by stock veterinarians and veterinarians from SUISAG-SGD.
77 We prepared DNA from ear tissue samples of 1036 HBS-affected pigs using the Promega
78 Maxwell RSC DNA System (Promega, Dübendorf, Switzerland) and sent it to Gencove for
79 low-pass sequencing (<https://gencove.com/>). An average number of 945K (between 150K
80 and 5.15M) read pairs (2 x 150bp) corresponding to an average genome coverage of
81 approximately 1-fold were collected for the HBS cases. Genotypes for 45,100,556 biallelic
82 sequence variants (SNP) corresponding to the Sscrofa11.1 (GCA_000003025.6) reference
83 sequence [8] were provided by Gencove. To infer the population structure, we extracted a
84 subset of 48,919 SNPs from the genotypes that are also present on the SNP arrays routinely
85 used for genomic prediction in Switzerland. These SNP genotypes were combined with
86 array-derived genotype data of 17,006 pigs that were genotyped for routine genomic
87 prediction, resulting in a combined dataset of 18,042 animals. After removing 4,554 markers
88 with minor allele frequency (MAF) less than 5% using PLINK (v1.90) [9], a genomic
89 relationship matrix (GRM) was constructed based on 44k SNPs using GCTA (v1.94.1) [10] and
90 the top principal components were extracted and visualized to assess the structure of the

91 genotyped populations and select three ancestry-matched control cohorts. The cohorts
92 consisted of 1) purebred animals from a SLW sire line, (70 cases, 280 controls), 2) purebred
93 animals from a SLW dam line (61 cases, 244 controls), and 3) a mixed population of animals
94 from the sire and dam lines, their crosses, as well as crosses between either the sire or the
95 dam line and Landrace animals (1,020 cases, 4,080 controls), maintaining a 1:4 case-to-
96 control ratio in all cohorts. Boars and sows genotyped for routine genomic prediction whose
97 progeny were registered in the national herdbook and therefore not affected by HBS before
98 reaching reproductive age were considered as control animals.

99

100 Genotypes for the control animals were imputed to the whole-genome sequence level with
101 Beagle v5.4 [11] using a sequenced reference panel of 421 pigs that included mostly SLW
102 animals. The reference animals had genotypes at 22,018,148 autosomal and 350,478 X-
103 chromosomal sequence variants [12]. We retained 15.43 million autosomal 192,930 X-
104 chromosomal biallelic SNPs that had a model-based accuracy of imputation greater than
105 0.8. Sex of HBS cases was inferred based on allele frequency estimates of X-chromosomal
106 markers (--impute-sex flag in PLINK).

107

108 We considered SNPs with a minor allele frequency greater than 5% and less than 10%
109 missingness for the subsequent association tests. For the purebred cohorts we excluded
110 SNPs from genome-wide association testing that deviated significantly ($p < 1 \times 10^{-6}$) from
111 Hardy-Weinberg proportions. After the quality control, we retained between 12.49 and
112 13.46 million SNP for association testing in the three cohorts.

113

114 The genome-wide association studies (GWAS) in the three cohorts were performed using a
115 generalized linear mixed model implemented in SAIGE (v1.0.9) [13] which accounts for
116 sample relatedness and an imbalanced ratio of cases and controls. The top 10 principal
117 components derived from a GRM, and sex were included as fixed factors, and a GRM was
118 fitted as random effect. A Bonferroni-corrected significance threshold ($p=3.1 \times 10^{-9}$) was
119 applied to account for multiple testing.

120

121 A haplotype of the SLW breed was assembled through trio-binning [14], utilizing PacBio
122 high-fidelity (HiFi) reads from a male offspring originating from a crossing between a
123 purebred Swiss Large White boar and an Alpenschwein sow. High Molecular Weight (HMW)
124 DNA was extracted from a liver tissue sample of the F1 using the Monarch® HMW DNA
125 Extraction kit and sequenced on the PacBio Sequel IIe platform, employing three SMRT cells
126 to generate 4.93 million HiFi reads with an average length of 17.73 kb. Additionally, DNA
127 from maternal blood and paternal liver tissue samples was sequenced on an Illumina
128 Novaseq6000 to generate short reads with an average coverage of 28.7x. Haplotype-
129 resolved assemblies were then generated with hifiasm v0.16.1 [15] as outlined in [16]. The
130 paternal haplotype (SLW) assembly size was 2.406 Gb across 998 contigs, with an average
131 Quality Value (QV) of 49.96 and a contig N50 of 30.62 Mb. The assembly achieved a BUSCO
132 single-copy score of 87.9%.

133

134 Paired-end short reads from our imputation reference cohort consisting of 421 samples
135 were aligned to the herein generated SLW assembly using bwa-mem2 [17]. Variant calling
136 was then performed with DeepVariant v1.4 and GLnexus v1.4.1 [18] resulting in 29.73

137 million autosomal variants. From these, an imputation reference panel was constructed
138 using 24.75 million biallelic SNPs using the bcftools v1.6 [19] view command with the ‘-m2 -
139 M2 -v snps’ flags. We imputed the low-pass sequencing data of HBS cases to the sequence
140 level with Glimpse v1.1 [20]. Coordinates of SNP array genotypes of controls were lifted
141 over to the SLW assembly using nf-LO [21] and subsequently imputed up to the sequence
142 level with Beagle v5.4 [11]. The imputed case and control datasets were merged, retaining
143 19.78 million SNPs with a missingness of less than 10% and a MAF greater than 0.05 for
144 GRM preparation and principal component analysis. Following quality control performed
145 separately for each cohort, as previously detailed (--maf 0.05, --geno 0.1; --hwe 1×10^{-6} for
146 purebred cohorts), we retained between 13.71 and 15.46 million SNPs for GWAS with
147 SAIGE, as described earlier.

148

149 **Results and discussion**

150 Genome-wide association studies with partially imputed sequence variant genotypes were
151 performed to identify genomic loci associated with susceptibility to HBS in Swiss fattening
152 pigs. A principal components analysis identified three distinct cohorts for association
153 testing: purebred animals from the SLW sire line, purebred animals from the SLW dam line,
154 and crosses (Figure 1A, 1D, 1G). The majority of HBS cases fell into the latter cohort, as it
155 included the fattening pigs which were produced by the crossing of purebred parental lines.

156

157 Our initial case/control association analyses relied on between 12.49 and 13.46 million
158 variants for which genotypes were called from alignments against the current Sscrofa 11.1
159 genome assembly which was assembled from a Duroc boar [8]. Neither the within-breed

160 nor the multi-breed GWAS revealed markers significantly associated with HBS (Figure 1B,
161 1E, 1H). The choice of the reference assembly can impact sequence read alignment, variant
162 calling, and downstream genetic analyses [22], particularly if the target breed diverged from
163 the reference sequence. We repeated the GWAS with between 13.71 and 15.46 million
164 SNPs for which genotypes were called from a SLW assembly (Fig 1C, 1F, 1I), but again, found
165 no significant genetic variants associated with HBS in either of the cohorts tested. The lack
166 of significant associations is somewhat surprising as previous studies identified the SLW sire
167 line as the main risk factor for developing HBS [5]. Given a relatively low effective
168 population size of 72 and 44 for the sire and dam lines [23], respectively, and the fact that
169 both populations diverged less than 20 years ago, we are confident that our cohort was
170 large enough to identify large effect genetic variants for developing HBS. Therefore, our
171 findings suggest that the previously reported breed-specific predisposition to HBS is mainly
172 driven by small effect genetic variants, which are possibly modulated by environmental and
173 management factors.

174 Incorporating environmental variables into the case/control GWAS was not possible as the
175 case and control groups were reared under disparate conditions. Future research efforts to
176 better understand the genetic architecture for the development of HBS in fattening pigs and
177 to identify associated genetic variants should ideally select control pigs from the same
178 fattening farms as the cases, ensuring that these controls are closely matched to the cases
179 in terms of age and weight profile and have successfully reached slaughter. Unfortunately,
180 implementing such an effort is not straightforward as fattening pigs are not routinely
181 genotyped. Farms with high and low incidences of HBS identified before [5] could serve as
182 candidates for undertaking such genotyping efforts. However, considering that an average

183 Swiss fattening farm produces approximately 500 fattening pigs per year, and assuming an
184 HBS incidence of 1% [5], the sampling of a case/control cohort with sufficient statistical
185 power to identify trait-associated small effect variants appears to be a major undertaking.
186 Nevertheless, such a cohort may also serve as a reference for the implementation of
187 genomic prediction.

188 The lack of pedigree information for the mostly cross-bred HBS cases, together with the
189 structured and related case/control cohorts, makes it difficult to estimate heritability of
190 HBS. Although the statistical methods implemented in SAIGE and fastGWA-GLMM [24]
191 minimize type I errors attributable to relatedness and imbalances between cases and
192 controls in GWAS, the variance components they estimate through penalized quasi-
193 likelihood are not precise enough for calculating heritability [13, 24]. Documenting pedigree
194 information for fattening pigs could contribute to a better understanding of the genetic
195 architecture underlying the disease.

196 Finally, the potential impact of structural variations (SVs), which may not be adequately
197 tagged by the SNPs used in our study, should also be considered. Although the newly built,
198 breed-specific SLW assembly makes variants overlapping insertions with respect to the
199 Duroc-based reference sequence amenable to association mapping, the short-read
200 sequencing-based approach doesn't allow to comprehensively study SVs [25]. The
201 establishment of a porcine SV imputation reference panel for pigs could enable future HBS
202 research by integrating SV data for a more comprehensive association analysis.

203

204 **Conclusions**

205 We report the first GWAS for HBS in Swiss pigs reported to be at risk for HBS, a sporadically
206 occurring disorder characterized by sudden death in fattening pigs. Our comprehensive
207 genetic analysis, spanning several breeds and two different reference genomes, did not
208 reveal any significant genetic markers associated with HBS. This finding suggests that the
209 genetic susceptibility to HBS is likely to involve small effect genetic variants and/or more
210 complex SVs that may interact with environmental and management factors, rather than
211 large effect genetic variants. Future research should prioritize the selection of cases and
212 controls from the same fattening farms, allowing a clearer distinction between genetic
213 predisposition and environmental influences.

214 **List of abbreviations**

215 BUSCO: Benchmarking Universal Single-Copy Orthologs

216 GRM: Genomic Relationship Matrix

217 GWAS: Genome-Wide Association Studies

218 HBS: Haemorrhagic Bowel Syndrome

219 HiFi: High Fidelity

220 HMW: High Molecular Weight

221 MAF: Minor Allele Frequency

222 QV: Quality Value

223 SLW: Swiss Large White

224 SNP: Single Nucleotide Polymorphism

225 SV: Structural Variation

226

227 **Declarations**

228 **Ethics approval and consent to participate**

229 Tissue samples for the HIS cohort were collected from dead pigs. The sampling of blood
230 from the F1 and its parents used for constructing the de novo assembly was approved by
231 the veterinary office of the Canton of Zurich (animal experimentation permit ZH077/2022).

232

233 **Consent for publication**

234 Not applicable

235

236 **Availability of data and materials**

237 Low-pass sequencing data of pigs affected by HBS have been deposited at the European
238 Nucleotide Archive (ENA) of the EMBL at BioProject PRJEB62539. Raw sequence read data of
239 pigs used to prepare the imputation reference panel are available at the European
240 Nucleotide Archive (ENA) of the EMBL at BioProjects PRJEB38156 and PRJEB39374. Long and
241 short sequencing reads from the trio used to generate the SLW assembly are at PRJEB74562.

242

243 **Competing interests**

244 The authors declare that they do not have any competing interests.

245

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250 and interpretation of data and in writing the manuscript.

251

252 **Authors' contributions**

253 AM performed the analyses and wrote the first draft of the manuscript with input from ASL
254 and HP. ASL assembled the SLW haplotype. SN collected samples from the trio used for SLW
255 assembly. HP and AH conceptualized the study. CD, AG, AH, NK and HP conceptualized the
256 project. All authors read and approved the final manuscript.

257

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263

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335 **Figures:**

336 **Figure 1 title:** Principal component analysis (PCA) and genome-wide association study
337 (GWAS) results across three cohorts. PCA plots highlighting cases (red) and controls (green)
338 selected for the three cohorts: purebred Swiss Large White sire line (A), purebred Swiss
339 Large White dam line (D), and a mixed population including these two lines their crosses,
340 and crosses between either of those lines and Landrace (G). The corresponding Manhattan
341 plots (B, E, H) illustrate the GWAS results using the Sscrofa11.1 reference genome. Plots (C,
342 F, I) depict GWAS results utilizing the herein established Swiss Large White reference

343 assembly. The red line across all Manhattan plots represents the Bonferroni-corrected
344 significance threshold.

