Genome-wide runs of homozygosity revealed sources of inconsistencies in the AxiomTM Equine Genotyping array

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Runs of homozygosity (ROH), haplotypes identical-by-descent (IBD), are key tools for deriving genomic inbreeding[1]. The genomic inbreeding coefficient (F_{ROH}) for an animal is derived by dividing the sum of all homozygous segments (S_{ROH}) by the total length of the genome. In livestock, previously reported studies have shown high concordance rates between F_{ROH} and pedigree derived inbreeding (F_{PED}). In the framework of a global genetic diversity study of modern horse breeds, we recently observed notably low F_{ROH} values compared to the corresponding F_{PED}. In this study, a total of 4'520 horses from 21 different breeds were genotyped on the 670K Axiom™Equine Genotyping array (602,131 autosomal SNPs mapped to EquCab3.0)[2, 3]. Quality of genotyping was considered acceptable with a dish QC (DQC) [?] 0.82 and QC call rate (CR) [?] 97 according to Affymetrix Axiom best practices[4]. Runs of homozygosity segments (N_{ROH}) for each horse were determined with an overlapping window approach in PLINK v1.9[5] with the following parameters: a minimum SNP density of one SNP per 50 kb, a maximum gap length of 100 kb, a minimum length of homozygous segments of 500 kb (including more than 80 homozygous SNPs), and allowing for one heterozygous SNP per segment[6]. In total there were 18 horses with N_{ROH} equal to 0 and another 62 horses with N_{ROH} less than 30. Within the Franches-Montagnes (FM) breed it was particularly notable, that five purebred horses had fewer ROH segments ($N_{ROH} < 88$) compared to F1 outcrosses. Of particular concern were one horse with N_{ROH} equal to 0 and two others with N_{ROH}less than 60. We re-genotyped these horses, along with two additional control horses that had N_{ROH} between 123 and 180, using the same DNA sample and genotype platform. Based on this data, we computed genotype concordance rates between the two SNP batches. We found that FM horses with none or few N_{ROH} exhibited low genotype concordances rates (Figure 1a; green dots). The same result was observed for eight re-genotyped Lusitano (LUS) horses, with N_{ROH} ranging from 1 to 99 (Figure 1a; brown dots). A comparison of discordant SNPs between the three FM outlier horses suggested that individual genotype errors occurred randomly, as there was only an 8% overlap in erroneous markers among the horses (Figure 1b). Notably, we observed over 50,000 discordant SNPs for the FM horse showing none N_{ROH}. Based on the updated genotype information, all horses (FM and LUS) exhibited significantly higher N_{ROH}, ranging from 148 to 175. Our findings demonstrate that N_{ROH} is a reliable indicator for assessing the genotype quality of individual horses. Consequently, we recommend excluding horses exhibiting extremely low N_{ROH} (e.g., $N_{ROH} < 30$) from downstream analyses. However, further research is necessary to enhance the reproducibility of Axiom™ Equine Genotyping array.

Figure 1: Analysis of runs of homozygosity segments (N_{ROH}), genotype concordance rate, and discordant SNPs in re-genotyped horses. (a) Correlation between $N_{ROH}$and genotype concordance between two independent genotyping efforts using the same DNA extract and SNP platform: This panel illustrates the association between N_{ROH} and genotype concordance in Franches-Montagnes (FM) and Lusitano horses. (b) Overlap of discordant SNPs: The Venn diagram shows the common and unique discordant SNPs among the three re-genotyped FM horses.

Ethics approval

This study was conducted on data sampled under permits VD3527b, VD2976.1 and VD2227.2, each approved by the cantonal veterinary office of Vaud, Switzerland.

Availability of data and materials

Original and updated genotype information of all re-genotyped horses (FM and LUS) are deposited as described in the Supporting information.

Competing interests

The authors declare that they have no competing interests.

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