Short communication: Imputation of markers on the bovine X chromosome

Xiaowei Mao,*†¹ Anna Maria Johansson,† Goutam Sahana,* Bernt Guldbrandtsen,* and Dirk-Jan De Koning†

*Department of Molecular Biology and Genetics, Center for Quantitative Genetics and Genomics, Aarhus University, PO Box 50, DK-8830, Tjele, Denmark

†Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Box 7023, 75007 Uppsala, Sweden

ABSTRACT

Imputation is a cost-effective approach to augment marker data for genomic selection and genome-wide association studies. However, most imputation studies have focused on autosomes. Here, we assessed the imputation of markers on the X chromosome in Holstein cattle for nongenotyped animals and animals genotyped with low-density (Illumina BovineLD, Illumina Inc., San Diego, CA) chips, using animals genotyped with medium-density (Illumina BovineSNP50) chips. A total of 26,884 genotyped Holstein individuals genotyped with medium-density chips were used in this study. Imputation was carried out using FImpute V2.2. The following parameters were examined: treating the pseudoautosomal region as autosomal or as X specific, different sizes of reference groups, different male/female proportions in the reference group, and cumulated degree of relationship between the reference group and target group. The imputation accuracy of markers on the X chromosome was improved if the pseudoautosomal region was treated as autosomal. Increasing the proportion of females in the reference group improved the imputation accuracy for the X chromosome. Imputation for nongenotyped animals in general had lower accuracy compared with animals genotyped with the low-density single nucleotide polymorphism array. In addition, higher cumulative pedigree relationships between the reference group and the target animal led to higher imputation accuracy. In the future, better marker coverage of the X chromosome should be developed to facilitate genomic studies involving the X chromosome.

Key words: imputation, pseudoautosomal region, X chromosome, nongenotyped

Short Communication

Both genomic selection (Meuwissen et al., 2001) and genome-wide association studies (McCarthy et al., 2008) require genotyping of large numbers of individuals, which can be costly and time consuming. To cope with these problems, young cattle are frequently genotyped with a relatively cheap, lower density panel or not genotyped at all. Genotypes may be imputed to a higher density panel using information from a reference population (Marchini and Howie, 2010). The genotypes of nongenotyped animals may to some extent be inferred from their relatives' genotypes. The majority of imputation studies in cattle have examined the accuracy of imputation from low-density to high-density SNP arrays (Ma et al., 2013; Brøndum et al., 2014; Pryce et al., 2014), but only a few studies have imputed nongenotyped animals to a higher density (Boison et al., 2014; Bouwman et al., 2014; Sargolzaei et al., 2014).

However, most of these studies focused on imputation of autosomes (Brøndum et al., 2014; van Binsbergen et al., 2014). Hickey and Kranis (2013), using 1,255 individuals from a pedigree of 4 generations, showed that the Z chromosome in chickens with 25 SNP could be imputed to a higher density (1,137 SNP) with relatively high accuracy (approximately 0.9). However, this study did not consider the pseudoautosomal region (**PAR**) that recombines with the W chromosome. In dairy cattle, ignoring the X chromosomes could miss important biological functions and affect genomic evaluation (Sandor et al., 2006; Lyons et al., 2014; Su et al., 2014; de Camargo et al., 2015). Regarding biological functions, nonsynonymous mutations on the X chromosome were shown to be associated with andrological and growth traits in beef cattle (Lyons et al., 2014). The X chromosome is important due to its relatively long length (148,823,899 bp), accounting for approximately 6% of the total physical genome (Zimin et al., 2009). ENSEMBL (release 82) lists 19,981 protein coding genes in the cattle genome out of which 833 (4.2%) are found on the X chromosome. Regarding the genomic evaluation, Su et al. (2014) reported that incorporating markers on the X chromosome to genomic predictions

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¹Corresponding author: xiaowei.mao@mbg.au.dk

contributes 0.3 to 0.5% to the reliability of genomic predictions. They also reported that the X chromosome accounted for on average 1.7% of the total additive genetic variance for 15 indices included in the Nordic Total Merit index (<http://www.nordicebv.info>). However, in their study only genotypes from males were used. Moreover, imputation of the X chromosome for nongenotyped animals has not been investigated in these studies. Imputing genotypes for nongenotyped animals with phenotypic records, especially for records that are difficult or expensive to measure, could potentially provide new insights.

The objective of our study was to assess the imputation accuracy of markers on the X chromosome in dairy cattle for nongenotyped animals and animals genotyped with low-density chips.

All data were recorded for routine dairy cattle management and genetic evaluations, and no additional tests or perturbations were performed for the purpose of this study. Our data set contained 26,884 animals. By breed, these 26,884 animals included 26,383 Holstein and 501 Red Holstein; by sex, they included 17,996 males and 8,888 females. These animals were genotyped by Illumina BovineSNP50 BeadChip version 1 and 2 (**50K**; Illumina Inc., San Diego, CA; Matukumalli et al., 2009). The minimal accepted GenCall score for individual genotype was 0.60. There were a total of 55,298 SNP in the 50K chips including SNP on 29 BTA as well as the X chromosome. The number of SNP included for analysis varied between 2,502 on the BTA1 and 894 on the X chromosome. The UMD v3.1 assembly (Zimin et al., 2009) was used as the reference for genomic position.

The PAR is homologous between the mammalian X and Y chromosomes. It is required for sex chromosome segregation during male meiosis (Das et al., 2009). The boundary between the PAR and X-specific region was assigned to the 5' end of the *GPR143* gene (chromosome X: 143,861,798–143,891,357) in previous studies using sequence-based analysis and fluorescence in situ hybridization (Van Laere et al., 2008; Das et al., 2009). Thus the PAR in our study was defined from 143,861,798 to 148,823,899 bp on the X chromosome. Heterozygosity of the X chromosome was also calculated using genotypes of 17,996 males to confirm our definition of the PAR, because males should be completely homozygous for the non-PAR region (barring unusual conditions such as XXY genotypes). The rest of the SNP on the X chromosome were defined as X-linked SNP.

Imputation was carried out using FImpute V2.2 (Sargolzaei et al., 2014), which combines both family and population-based information. Different scenarios of imputation were set up to evaluate the effect of the

following factors on imputation accuracy: treating SNP in the PAR either as autosomal or as X-linked, different sizes of reference groups, different proportions of males and females in the reference group, and the effect of the additive genetic relationship between reference group and target group. Target groups were generated by randomly masking a subset of 2,000 animals from the 26,884 50K animals to either nongenotyped (**0K**) animals in the scenario of imputing 0K animals to 50K, or to Illumina BovineLD BeadChip (Boichard et al., 2012) typed (**7K**) animals in the scenario of imputing 7K animals to 50K. In consequence, 24,884 animals remained as the reference group. Not all 0K animals were imputed because FImpute requires that animals in the target group have progenies in the reference group. Smaller reference populations were generated by taking random subsets of sizes 20,000, 15,000, and 5,000 from the complete reference group. To impute the X chromosome, genotype imputation rules were modified in FImpute to account for the sex chromosome inheritance. For the X chromosome, the sires of male individuals are set to unknown (Sargolzaei et al., 2014) because males inherit alleles only from the females. To include PAR into imputation, we treated them as autosomal alleles as suggested by Hickey and Kranis (2013). Chromosome 2 (BTA2) was also imputed to compare imputation accuracy with the X chromosome, because BTA2 is similar in size to the X chromosome.

Imputation accuracies were calculated for SNP on BTA2, X-linked SNP, and SNP in the PAR. Imputation accuracy was measured using 2 statistics: SNP-wise accuracy and animal-wise accuracy. The SNP-wise accuracy was the correlation between the imputed genotype with true genotype both expressed as gene content (0, 1, or 2). Animal-wise accuracy was calculated for each individual by correlating the true genotypes (0, 1, or 2) minus the mean gene content per SNP with the imputed genotype (0, 1, or 2) minus the mean gene content per SNP (Mulder et al., 2012). Mean gene content was calculated as $2p$, with p representing the frequency of the allele for which the homozygote is coded as 2 (Bouwman et al., 2014). For each individual, a cumulative pedigree relationship (**CPR**) was calculated as the sum of all additive genetic relationships greater than 0.25 with all animals in the reference group. For example, the CPR for animal A with reference group (animal B and C) is the sum of the additive genetic relationship between animal A and B, and animal A and C. This was done to measure the degree of relationship of this specific animal to the reference group.

The PAR was approximately 5 Mbp long and included 76 SNP, which accounted for 8.5% of the X chromosome (894) in terms of number of SNP. We compared

the imputation accuracy when splitting the PAR from the X chromosome as autosome or not. The results showed a considerable decrease of SNP-wise accuracy of the X chromosome when the PAR was treated as X linked (Table 1). For example, for a reference group of 24,884, the SNP-wise accuracy for the X chromosome decreased from 0.94 to 0.92 in the scenario of 0K to 50K and from 0.93 to 0.90 in the scenario of 7K to 50K. This suggests that the PAR should be imputed separately and treated as autosomal during imputation, which was expected because the PAR is inherited differently from the X-linked region. Software optimized to model the X chromosome and the PAR simultaneously should be developed. However, SNP in the PAR had much lower imputation accuracy compared with X-linked SNP. This is probably because the PAR region is much smaller than the X chromosome and other autosomes. As more phasing errors are expected at the beginning and end of segments, the overall imputation accuracy for the PAR is lower (Sargolzaei et al., 2014). Poor imputation accuracy of SNP in the PAR might also occur if PAR has a higher male recombination rate than X-linked SNP. Human studies have shown that the per-nucleotide recombination rate in the PAR for males is 17-fold higher than the genome-wide average, due to one obligatory crossing-over per male meiosis in the PAR (Rouyer et al., 1986; Hinch et al., 2014).

The effects of different male/female ratios in the reference group on the imputation accuracy of the X chromosome and BTA2 are shown in Table 1. In general, more females in the reference group increased the imputation accuracy, especially for the X chromosome.

For SNP-wise accuracy, a reference group of 8,000 males gave an accuracy of 0.76 for the X chromosome in the scenario of imputing from 0K to 50K, whereas 8,000 females resulted in an accuracy of 0.96. In addition, more females in the reference group raised the accuracy more when imputing from 0K to 50K than from 7K to 50K. The reason might be that females provide more information about the X chromosome than males, because females have 2 copies of the X chromosome whereas males have only one. This extra information for the X chromosome could be more beneficial when imputing the nongenotyped individuals. In livestock populations, some elite dams have already been genotyped with high density. As genotyping costs continue to decrease, more females could be genotyped. As a consequence, the X chromosome could be imputed more accurately in the future using more information from females.

In general, imputation from 0K to 50K was less accurate than from 7K to 50K. For example, SNP-wise accuracy of 0K to 50K for BTA2 was on average 10% lower than 7K to 50K (Table 1). This result agreed with previous studies (Sargolzaei et al., 2014; Bouwman et al., 2014). If the lower density panel was denser, shared haplotypes between distant relatives can be found more easily and precisely. The results also showed that SNP-wise accuracy of X chromosome is higher than that of BTA2 in the scenario of imputing from 0K to 50K. One explanation might be that males were completely homozygous for the X-linked region of the X chromosome, so phasing errors for the X chromosome were lower than for autosomes. Another reason could be due to that imputation of the X-linked and PAR SNP in the

Table 1. The SNP-wise imputation accuracy in different scenarios

Item	No. of animals ¹	Imputed ²	0K-50K ³			Imputed	7K-50K ⁴		
			BTA2	X ⁵	PAR ⁶		BTA2	X	PAR
No-PAR ⁷	24,884	899	0.89	0.92	NA	2,000	0.97	0.90	NA
Size of reference	24,884	899	0.89	0.94	0.79	2,000	0.97	0.93	0.81
	20,000	773	0.88	0.94	0.78	2,000	0.96	0.92	0.73
	15,000	642	0.87	0.94	0.77	2,000	0.96	0.90	0.63
	5,000	320	0.86	0.98	0.74	2,000	0.95	0.91	0.46
Male/female	8,000/0	450	0.85	0.76	0.66	2,000	0.95	0.89	0.62
	6,000/2,000	438	0.85	0.92	0.73	2,000	0.95	0.89	0.61
	4,000/4,000	441	0.85	0.95	0.73	2,000	0.95	0.91	0.62
	2,000/6,000	395	0.85	0.95	0.73	2,000	0.96	0.93	0.62
	0/8,000	318	0.87	0.96	0.75	2,000	0.96	0.95	0.55

¹No. of animals = number of animals in the reference group.

²Imputed = number of successfully imputed animals.

³0K-50K = scenario of imputing nongenotyped animals to 50K.

⁴7K-50K = scenario of imputing 7K animals to 50K.

⁵X = X chromosome.

⁶PAR = pseudoautosomal region.

⁷No-PAR = did not treat pseudoautosomal region in the X chromosome as autosome when imputing.

scenario of 0K to 50K had a higher missing rate compared with autosomes, especially when the reference size was small. This might be because X-linked and PAR SNP are quite sparsely genotyped, and shared haplotypes were hard to find with small-size reference groups. In the future, better coverage of the X chromosome should be developed to facilitate genomic studies involving the X chromosome. Regarding the size of the reference group, imputation accuracy decreased when the size of the reference group was reduced. This observation was in agreement with Sargolzaei et al. (2014).

This study demonstrated that imputation accuracy increased dramatically when the CPR increased, for both 0K to 50K (Figure 1) and 7K to 50K (results not shown). Most animals had a CPR less than 50. In

general, the smaller the CPR, the more variation of animal-wise accuracy. Similarly, the larger the CPR, the higher the animal-wise accuracy. This is probably due to better family information and better population information when CPR is larger. Better family information was reflected by SNP that were imputed more accurately through more progenies, parents, and grandparents. Better population information was reflected by more relatives resulting in more accurate haplotype blocks that the SNP can fit into. These results agree with previous studies (Boison et al., 2014; Bouwman et al., 2014). We also found that imputation from 0K to 50K was more affected by CPR. For example, lower CPR often resulted in low imputation accuracy (results were not shown). This is probably because imputation

Figure 1. Animal-wise accuracy in the scenario of imputing 0K to 50K, when the size of the reference group is 24,884. The x-axis is the cumulative pedigree relationship, and the y-axis is the animal-wise accuracy. Black represents the imputation accuracy for chromosome 2. Red represents the imputation accuracy for the X chromosome. Color version available online.

for nongenotyped animals is more dependent on the availability of close family information.

In conclusion, imputation of the X chromosome was improved if PAR was treated as autosomal because the PAR has inheritance rules that are different from those of the X-linked region. Including more females in the reference group also improved the imputation for the X chromosome. In general, imputation for nongenotyped animals had lower accuracy compared with animals genotyped with the low-density SNP array. In addition, the larger the CPR between the reference group and the target animal, the higher the imputation accuracy was for this animal. Our study was restricted by limited genotype coverage on the X chromosome, so better coverage of the X chromosome should be developed to facilitate genomic studies involving the X chromosome.

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