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Bovine mammary gland X chromosome inactivation

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ABSTRACT

X chromosome inactivation (XCI) is a process by which 1 of the 2 copies of the X chromosomes present in female mammals is inactivated. The transcriptional silencing of one X chromosome achieves dosage compensation between XX females and XY males and ensures equal expression of X-linked genes in both sexes. Although all mammals use this form of dosage compensation, the complex mechanisms that regulate XCI vary between species, tissues, and development. These mechanisms include not only varying levels of inactivation, but also the nature of inactivation, which can range from being random in nature to driven by parent of origin. To date, no data describing XCI in calves or adult cattle have been reported and we are reliant on data from mice to infer potential mechanisms and timings for this process. In the context of dairy cattle breeding and genomic prediction, the implications of X chromosome inheritance and XCI in the mammary gland are particularly important where a relatively small number of bulls pass their single X chromosome on to all of their daughters. We describe here the use of RNA-seq, whole genome sequencing and Illumina BovineHD BeadChip (Illumina, San Diego, CA) genotypes to assess XCI in lactating mammary glands of dairy cattle. At a population level, maternally and paternally inherited copies of the X chromosome are expressed equally in the lactating mammary gland consistent with random inactivation of the X chromosome. However, average expression of the paternal chromosome ranged from 10 to 90% depending on the individual animal. These results suggest that either the mammary gland arises from 1 or 2 stem cells, or a nongenetic mechanism that skews XCI exists. Although a considerable amount of future work is required to fully understand XCI in cattle, the data reported here represent an initial step in ensuring that X chromosome variation is captured and used in an appropriate manner for future genomic selection.

Key words: RNA sequencing, X chromosome inactivation, genomic selection, cattle

INTRODUCTION

The mammalian X chromosome is a large, gene-rich chromosome. Females have 2 X chromosomes, whereas males have a single X chromosome and a small, gene-poor Y chromosome. If not for X chromosome inactivation (**XCI**) taking place during early embryonic development, the difference in X chromosome gene dosage between the sexes would lead to X-linked gene expression differences in males and females.

Although dosage compensation through XCI has been the subject of intense study for more than 50 yr, the overwhelming majority of research has focused on understanding its regulation in model organisms, particularly mice and *Caenorhabditis elegans*. To date, few reports have described XCI in cattle beyond investigation of gene expression from in vitro generated preimplantation embryos (De La Fuente et al., 1999; Peippo et al., 2002; Xue et al., 2002). X chromosome gene expression studies from these in vitro bovine embryos are consistent with well-studied model organisms, in that XCI occurs by the blastocyst stage of development. However, substantial developmental differences between embryos produced in vivo and those produced in vitro are well documented (Niemann et al., 2008) and care is still required when data are extrapolated to in vivo embryo development. The only in vivo data of XCI in cattle have been generated from *Bos taurus*/*Bos indicus* crosses (Dindot et al., 2004; Chen et al., 2016). Analysis of expression patterns from a single X-linked gene (*XIST*) indicated that XCI was occurring in a parent of origin manner in extraembryonic tissues and in a random manner in the embryo proper (Dindot et al., 2004). However, this conflicts with extensive analysis of X-linked gene expression in extraembryonic membranes where XCI appeared random in nature (Chen et al., 2016). Convincing evidence is lacking on XCI timing and mechanisms in the developing bovine embryo, fetus, or adult tissues. These conflicting results contribute to the challenge of understanding the contribution of genetic variation from the X chromosome to phenotypes

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of interest. These challenges have largely resulted in the exclusion of this large gene-rich chromosome from genomic selection strategies.

In the absence of cattle-specific data, we must rely on XCI data from mice to infer potential mechanisms and timings for this process. In mice, 2 forms of XCI have been widely reported during embryogenesis (Deng et al., 2014). Beginning at the 4-cell stage, imprinted XCI exclusively silences the paternal X chromosome. Later, around the time of embryonic implantation (developmental d 3.5), epiblast cells of the inner cell mass, which give rise to the embryo, reactivate the paternal X chromosome and undergo a random form of XCI (Deng et al., 2014). Random XCI in the inner cell mass is permanent and therefore gives rise to patches of clonally derived cells having the same X chromosome inactivated in the adult.

During random XCI, the 2 X chromosomes theoretically have equal chances of being inactivated. Hence, the percentage of cells with the paternal X chromosome inactivated is expected to be approximately 50%. However, in practice, XCI can be skewed in one or more tissues of an individual. This skewing of XCI can occur by random chance, be mediated by genetic mechanisms (Thorvaldsen et al., 2012; Calaway et al., 2013), or be a result of an X-linked mutation that affects cell proliferation or survival (Belmont, 1996).

Although XCI silences the majority of the X chromosome, several genes are known to escape this inactivation to some extent (Valencia and Wutz, 2015). Whereas many genes that escape XCI are located within the pseudo-autosomal regions (**PAR**) of the X chromosome and have a homolog on the Y chromosome, others have been located outside the pseudo-autosomal regions. Although the mechanisms of escape from XCI of non-PAR genes are not fully understood, both the number and distribution of these genes across the X chromosome differ significantly between species (Valencia and Wutz, 2015).

One gene that escapes random XCI (but retains monoallelic expression) in mice is the RING finger ubiquitin ligase *Rnf12/RLIM* gene (Bach et al., 1999; Ostendorff et al., 2002). Genetic analyses in mice have demonstrated that *Rnf12/RLIM* expressed from the paternal X chromosome serves as a survival factor for milk-producing alveolar cells in mammary glands of pregnant and lactating females (Jiao et al., 2012, 2013). These studies also reported that only the paternal *Rnf12/RLIM* allele was expressed in the majority of mammary epithelial cells, leading Jiao et al. (2013) to suggest that this gene displayed an imprinted XCI pattern of expression in this cell type. However, to date no further evidence supports this suggestion in other mammals, and although many aspects of XCI appear well conserved throughout mammals, extrapolation of *Rnf12/RILM* regulation to other mammals should be undertaken with caution.

To date, genome-wide association studies and genomic selection strategies undertaken in cattle have for the most part not included genetic variation on the X chromosome due to challenges around Mendelian transmission and lack of data on XCI in cattle regarding whether this mechanism is random in nature or determined by parent of origin. The implications of X chromosome inheritance patterns and random/imprinted XCI in the mammary gland are considerable in terms of undertaking genomic selection for improved production performance. For example, if parent of origin XCI resulted in expression of only the paternally inherited X chromosome, selection of bulls with desirable X chromosome variation would lead to rapid improvement in production performance for all their daughters. Conversely, if XCI resulted in expression only from the maternally inherited X chromosome, improvement in production would take many generations and rely entirely on increasing the frequency of desirable alleles in the population. These implications are particularly important in the context of dairy cattle breeding where a relatively small number of bulls pass their single X chromosome on to all of their daughters. This project has undertaken analyses to understand XCI in lactating mammary glands of New Zealand dairy cattle as a first step in including X chromosome variation in genomic selection strategies.

MATERIALS AND METHODS

Ethics Statement

Animal ethics approval was granted for all animal work by the Ruakura Animal Ethics Committee, Hamilton, New Zealand. No animals were killed for this study. Mammary tissue samples were obtained by needle biopsy in accordance with protocols approved by the ethics committee (approval AEC 12845).

DNA Genotypes

To determine levels of heterozygosity in a representative sample of the New Zealand dairy population, whole genome sequencing, read mapping, and variant calling was undertaken on 556 individuals as previously described (Littlejohn et al., 2016). Variants were called across the entire genome including the X chromosome. BCFtools stats (Li et al., 2009) was used to obtain the per sample counts for heterozygous SNP at each position from the sequence reference set of variants. Proportion heterozygosity was calculated in each in-