Mitochondrial DNA single nucleotide polymorphism associated with weight estimated breeding values in Nelore cattle (Bos indicus)

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Abstract

We sampled 119 Nelore cattle (Bos indicus), 69 harboring B. indicus mtDNA plus 50 carrying Bos taurus mtDNA, to estimate the frequencies of putative mtDNA single nucleotide polymorphisms (SNPs) and investigate their association with Nelore weight and scrotal circumference estimated breeding values (EBVs). The PCR restriction fragment length polymorphism (PCR-RFLP) method was used to detect polymorphisms in the mitochondrial asparagine, cysteine, glycine, leucine and proline transporter RNA (tRNA) genes (tRNAasn, tRNAcys, tRNAgly, tRNAleu and tRNApro).

The 50 cattle carrying B. taurus mtDNA were monomorphic for all the tRNA gene SNPs analyzed, suggesting that they are specific to mtDNA from B. indicus cattle. No tRNAcys or tRNAgly polymorphisms were detected in any of the cattle but we did detect polymorphic SNPs in the tRNAasn, tRNAleu and tRNApro genes in the cattle harboring B. indicus mtDNA, with the same allele observed in the B. taurus sequence being present in the following percentage of cattle harboring B. indicus mtDNA: 72.46% for tRNAasn, 95.23% for tRNAleu and 90.62% for tRNApro.

Analyses of variance using the tRNAasn SNP as the independent variable and EBVs as the dependent variable showed that the G → T SNP was significantly associated (p < 0.05) with maternal EBVs for weight at 120 and 210 days (p < 0.05) and animal’s EBVs for weight at 210, 365 and 455 days. There was no association of the tRNAasn SNP with the scrotal circumference EBVs. These results confirm that mtDNA can affect weight and that mtDNA polymorphisms can be a source of genetic variation for quantitative traits.

Key words: bovine, mitochondria, mtDNA, SNP, weight.

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Introduction

Mitochondria are eukaryotic cell organelles involved in various cellular functions, including cell proliferation, apoptosis and, mostly important, energy production (Birch-Machin, 2006) by oxidative phosphorylation (Taanman, 1999). These organelles are responsible for approximately 90% of the energy produced by the mammal cell (Boettcher et al., 1996b). Mitochondria have their own genome, which is maternally inherited in mammals and is an important source of cytoplasmic genetic variation (Gibson et al., 1997; Birky, 2001). In cattle, direct maternal effects have been discussed since Wagner (1972) but the contribution of cytoplasmic effects estimated for growth and milk production are not consistent between populations (Gibson et al., 1997).

Boettcher et al. (1996b) demonstrated bias in the heritability, permanent environmental variance, and accuracy estimation in the animal model when the cytoplasmic effects were ignored. Moreover, the effect of cytoplasmic inheritance has been studied in respect to milk production (Bell et al., 1985; Tess et al., 1987; Schutz et al., 1992; Boettcher et al., 1996a) and beef cattle growth traits (Tess and Robison, 1990; Northcutt et al., 1991; Tess and MacNeil, 1994; Quintanilla et al., 1999).
Recently, molecular biology has furthered our understanding of the function and inheritance of the mitochondrial genome and its importance on livestock development and production (Smith and Alcivar, 1993; Smith et al., 2000). Since Anderson et al. (1982) published the complete mitochondrial DNA (mtDNA) sequence, nucleotide variants in coding and non-coding regions have been studied to associate molecular markers with the production of Bos taurus breeds (Ron et al., 1993; Mannen et al., 1998; Mannen et al., 2003). Pegoraro et al. (1996) identified sequence alterations in mitochondrial transfer RNA (tRNA) genes and in the origin of light strand (Ori L) replication of transfer RNA (tRNA) genes and in the origin of light strand (1996) identified sequence alterations in mitochondrial DNA (mtDNA) sequence, nucleotide variations differ in estimated breeding values (EBVs) for growth and reproductive traits. The aims of this study were to estimate the frequency of single nucleotide polymorphisms on weight and scrotal circumference estimated breeding values at different ages.

Material and Methods

Cattle sample

The sample (n = 119) consisted of 69 purebred adult Nelore cattle registered as pure origin imported (POI) in the Brazilian Association of Zebu Breeder’s Herdbook and identified as carriers of B. indicus mtDNA plus a further 50 purebred B. indicus Nelore cattle which were carriers of B. taurus mtDNA and not registered as POI. A blood sample was collected from each animal and total DNA extracted according to Sambrook et al. (2001). The presence of B. indicus and B. taurus mtDNA was confirmed by the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method by the absence (B. indicus) or the presence (B. taurus) of HindIII restriction site at nucleotide 12178 on the NADH dehydrogenase subunit 5 gene (Meirelles et al., 1999).

Detection of polymorphisms

The single nucleotide substitutions (SNP) in five transfer RNA (tRNA) mitochondrial genes were detected by PCR-RFLP using endonuclease enzymatic digestion of the amplicons. Primers were designed using the B. indicus mtDNA sequence as a reference (GenBank AY126697). The PCR was performed using 150 ng of total DNA, 1x PCR buffer (20 mM Tris-HCl, 50 mM KCl, Invitrogen, Brazil), 3 mM MgCl2, 0.2 mM of each dNTP, 0.2 μM of each primer (Table 1) and 1.5 units of Taq DNA polymerase (Invitrogen, Brazil) in a final volume of 50 μL. Cycling was set for 5 min at 95 ºC followed by 35 cycles of 40 s at 95 ºC, 30 s at the specific temperature of each primer and 40 s at 72 ºC, with a final 72 ºC extension for 5 min. The PCR products (15 μL) were digested with 1 unit of Bsr I, Mnl I, Spe I, Dra I or Bsm Al restriction endonuclease specific for each tRNA gene sequence for one hour at the temperature recommended by the supplier (New England Biolabs, USA). The mitochondrial tRNA PCR amplicons, specific primer sequences, annealing temperatures, SNP positions and restriction enzymes used in this study are pre-

<table>
<thead>
<tr>
<th>tRNA gene</th>
<th>Primer sequence (5’-3’)</th>
<th>AT (ºC)</th>
<th>AP (bp)</th>
<th>SNP position</th>
<th>RE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparagine (tRNAAsn)</td>
<td>TCCTCACAGACTGTGGG GGGTGGAGAATGCTCAGGG</td>
<td>52</td>
<td>277</td>
<td>G5501 → T</td>
<td>Bsr I</td>
</tr>
<tr>
<td>Cysteine (tRNACys)</td>
<td>AGCTACTGCGTCTTCCAAT ACCAAAATAGTGAATAAAGG</td>
<td>50</td>
<td>257</td>
<td>C5612 → T</td>
<td>Mnl I</td>
</tr>
<tr>
<td>Glycine (tRNA Gly)</td>
<td>AGCGGTAGTGGTCTCAC GATTGCGATTGAGCAGTAG</td>
<td>55</td>
<td>300</td>
<td>T9768 → C</td>
<td>Spe I</td>
</tr>
<tr>
<td>Leucine (tRNA Leu)</td>
<td>AGAACCGGTTAATGTGTTT AACCTAGAATTTGACC</td>
<td>52</td>
<td>189</td>
<td>A3051 → G</td>
<td>Dra I</td>
</tr>
<tr>
<td>Proline (tRNAPro)</td>
<td>GACAGGTTTCTTGTAGTACTC GTAGGTAATTCTACTGG</td>
<td>55</td>
<td>302</td>
<td>T15751 → C</td>
<td>Bsm Al</td>
</tr>
</tbody>
</table>
sent in Table 1. Electrophoresis was carried out on 2% (w/v) agarose gel and DNA was stained with ethidium bromide and the fragments visualized under ultraviolet light. Mitochondrial transfer RNA SNP frequencies were obtained by direct counting of the PCR-RFLP results.

Estimated breeding values

The cattle selected for mtDNA genotyping were part of the Nelore cattle breeding program (NCBP) at the University of São Paulo (ANCP, http://www.ancp.org.br/sumarios) which estimated breeding values for approximately 900,000 cattle. The estimated breeding values used in this study for association analysis were kindly provided by the NCBP.

The traits analyzed were maternal effects for weight (MW) at 120 and 210 days (MW120 and MW210), animal weight (AW) at 120, 210, 365, and 450 days (AW120, AW210, AW365, and AW450) and scrotal circumference (SC) at 365, 450, and 550 days (SC365, SC450, and SC550).

Briefly, breeding values for each animal were estimated using an animal model applying the best linear unbiased predictor (Boldman et al., 1995). The model used for genetic parameter estimations was

\[ y_{ij} = \mu + X\beta + Z_1a + Z_2m + Z_3p + e, \]

where \( y_{ij} \) is the vector of records, \( \beta \) the vector of fixed effects (herd-year-season, sex, dam age class), \( X \) is the matrix associating \( \beta \) with \( y \), \( a \) is the vector of estimated breeding values for direct genetic effects, \( Z_1 \) is the matrix associating \( a \) with \( y \), \( m \) is the vector of estimated breeding values for maternal genetic effects, \( Z_2 \) is the matrix associating \( m \) with \( y \), \( p \) is the vector of permanent environmental non-additive genetic effects contributed by dams to the records of their progeny, \( Z_3 \) is the matrix associating \( p \) with \( y \), and \( e \) is the vector of residual effects (Gunski et al., 2001).

Association analysis

Maternal and animal estimated breeding values were independently compared between animals with and without the Bsr I restriction site (G or T nucleotide) on the tRNA\textsuperscript{Cys} gene by analysis of variance (ANOVA). The linear model used was:

\[ y_{ij} = \mu + N_i + e_{ij}, \]

where \( y_{ij} \) is the breeding value for each trait of the \( i \)-th animal, \( N \) is the fixed effect of the \( i \)-th tRNA\textsuperscript{Cys} nucleotide (G or T) and \( e_{ij} \) is the random error effect associated with the \( i \)-th observation. Means were compared using the Tukey Studentized Range Test in the SAS program (SAS Institute Inc., 2001). Differences with \( \alpha < 0.05 \) were considered statistically significant.

Nelore Heard-book genealogy data incorporated in the breeding value estimations allowed us to obtain the estimated breeding values for all parents and relatives from each genotyped animal. However, only those ascending the animals harboring \( B. indicus \) mtDNA were considered for further analysis. Because mitochondrial DNA is inherited maternally, females and their offspring from the same matrilineal lineage were assumed to have the same mitochondrial tRNA\textsuperscript{Cys} nucleotide at position 5501. This allowed us to group females from the same lineage with previously genotyped animals. The new data set was based on 345 observations for each characteristic analyzed and was used for ANOVA and the Tukey test.

Results and Discussion

All the cattle genotyped showed no sign of heteroplasy for the five single nucleotide polymorphisms (SNPs) analyzed, suggesting maternal inheritance and homoplasmic distribution within tissues (Attardi, 1985; Smith et al., 2000; Birky, 2001). The 50 cattle genotyped as carrying \( B. taurus \) mtDNA were monomorphic for the five mitochondrial tRNA gene SNPs analyzed in this study, suggesting that the SNPs evaluated are specific to mtDNA from \( B. indicus \) cattle.

In this Nelore sample (n = 119) no mitochondrial tRNA\textsuperscript{Cys} or tRNA\textsuperscript{Gly} mutations were found using PCR-RFLP. However, we did detect polymorphic SNPs in the tRNA\textsuperscript{Leu}, tRNA\textsuperscript{Cys} and tRNA\textsuperscript{Pro} mitochondrial genes of the cattle harboring \( B. indicus \) mtDNA (n = 69) where the B. taurus sequence (Anderson et al., 1982) was present in 72.46% of animals for tRNA\textsuperscript{Leu}, 95.23% for tRNA\textsuperscript{Cys} and 90.62% for tRNA\textsuperscript{Pro}, the remaining animals having a variant tRNA pattern similar to the B. indicus mtDNA GenBank sequence AY126997. Tracing the matrilineal genealogy, we found that each of the 19 cattle genotyped as having \( B. indicus \) tRNA\textsuperscript{Cys} were descendants of different females imported from India, suggesting that the nucleotide mutations in the tRNA\textsuperscript{Cys}, tRNA\textsuperscript{Leu} and tRNA\textsuperscript{Pro} mitochondrial genes, characteristic of \( B. indicus \) cattle, occurred in India and were brought to Brazil with imported Indian cows.

Because the mitochondrial tRNA\textsuperscript{Cys} and tRNA\textsuperscript{Gly} genes were not polymorphic for the SNPs tested in our sample and the mitochondrial tRNA\textsuperscript{Cys}, and tRNA\textsuperscript{Pro} genes lacked sufficient variability, we used the SNP on the tRNA\textsuperscript{Cys} gene to compare the estimated breeding values of the cattle harboring \( B. indicus \) mtDNA in our sample.

As stated above, maternal and animal estimated breeding values were independently compared between the cattle in our sample which had, or lacked, the Bsr I restriction site (G or T nucleotide). The guanidine to thymine substitution was significantly associated (p < 0.05) with changes in maternal breeding value for weight at 120 and 210 days and the animal breeding value for weight at 210, 365 and 455 days (Table 2). Cattle with the mitochondrial tRNA\textsuperscript{Cys} guanidine SNP had higher estimated breeding values compared to those with the thymine SNP (Table 3), although there were no differences for the scrotal circumference estimated breeding values between cattle with different nucleotides at this position (Tables 4 and 5).

Gunski et al. (2001) studied mitochondrial genome effects to compare pre-weaning growth traits between were in Nelore cattle harboring \( B. taurus \) mtDNA and \( B. indicus \) mtDNA, but found no significant (p > 0.05) differences for maternal and animal estimated breeding values.
Our results suggest an association between polymorphism in the mitochondrial tRNA_{asn} gene with growth traits in B. indicus Nelore cattle. The differences for maternal and direct weight estimated breeding values were not caused by a B. taurus nuclear genome effect since this sample were known to harbor the B. indicus mtDNA and were progeny of registered POI sires and cows.

There are reports associating cytoplasmic lineage and growth and milk traits effects in various cattle breeds. Tess et al. (1987) reported that cytoplasmic effects influenced pre-weaning and milk traits in Hereford cattle but later results did not confirm this effect (Tess and Robison, 1990), while Schutz et al., (1993) found that maternal lineage was responsible for 4% to 15% of the phenotypic variation in milk traits of Holstein-Friesian cattle. Boettcher et al., (1996b) reported that the inclusion of the maternal lineage effect in a simulated animal model gave better estimations of heritability and permanent environment variance and also accurate estimated breeding values. However, due to small number of different lineages in our data set we could not test if cytoplasmic lineage was associated with breeding value variation.

Table 3 - Weight estimated breeding values (EBV) for the G5501 → T (G, n = 247; T, n = 71) mitochondrial tRNA_{asn} gene single nucleotide polymorphism (SNP). The table shows the means and standard errors of the mean (SE) for maternal EBV for weight (MW) at 120 and 240 days and animal EBV for weight (AW) at 120, 240, 365 and 450 days.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>MW_{120}</th>
<th>MW_{240}</th>
<th>AW_{120}</th>
<th>AW_{240}</th>
<th>AW_{365}</th>
<th>AW_{450}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>1</td>
<td>1.59 ± 0.15</td>
<td>2.40 ± 0.19</td>
<td>-0.06 ± 0.25</td>
<td>-0.87 ± 0.38</td>
<td>2.86 ± 0.62</td>
<td>1.12 ± 0.68</td>
</tr>
<tr>
<td>Error</td>
<td>343</td>
<td>0.58 ± 0.28</td>
<td>1.30 ± 0.37</td>
<td>-0.57 ± 0.38</td>
<td>-2.48 ± 0.65</td>
<td>-0.80 ± 1.07</td>
<td>-2.83 ± 1.42</td>
</tr>
</tbody>
</table>

Values in columns with different superscript letters are significantly different by the Tukey test (p < 0.05).

Quantitative traits have also been associated with mtDNA polymorphisms. D-loop variation has been associated to carcass traits such as longissimus muscle area and beef marbling score (Mannen et al., 1998), milk production as measured by yield, fat content and estimated milk energy (Schutz et al., 1994), and calving rates (Sutarno et al., 2002). Furthermore, polymorphic sites on rRNA genes have also been associated with milk traits (Boettcher et al., 1996a).

The effect of variation in the mitochondrial genome on milk and beef traits is thought to be a consequence of changes in metabolic rate or the energy available for milk production and muscle development, the differences reported by us in this study tend to support this hypothesis.

We investigated scrotal circumference (SC) because this has been correlated with reproductive traits in Nelore cattle (Martins-Filho and Lôbo, 1991) and the heritability of scrotal circumference is considered moderate to high at 0.36 to 0.47 (Garnero et al., 2001; Pereira et al., 2002; Silveira et al., 2004). However, no studies have suggested that the mitochondrial genome may influence fertility traits.

Table 4 - Analysis of variance between scrotal circumference (SC, in centimeters) estimated breeding values for the G5501 → T (G, n = 247; T, n = 71) mitochondrial tRNA_{asn} gene single nucleotide polymorphism (SNP). The table shows the means and standard errors of the mean (SE) for SC estimated breeding values at 365, 450 and 550 days.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SC_{365}</th>
<th>SC_{450}</th>
<th>SC_{550}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>1</td>
<td>0.00</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Error</td>
<td>343</td>
<td>0.02</td>
<td>0.56</td>
<td>0.51</td>
</tr>
<tr>
<td>P &gt; F</td>
<td></td>
<td>0.97</td>
<td>0.75</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Values in columns with the same superscript letter do not differ statistically by the Tukey test (p > 0.05).
and the polymorphism tested by us was not associated with scrotal circumference estimated breeding values.

In humans, there are approximately 108 disorders that are associated with SNPs of mitochondrial tRNA genes. Three of these disorders involve the \( tRNA^{\text{asn}} \) gene (Brandon et al., 2005), indicating that SNPs of mitochondrial tRNA genes have an important role in mitochondria. Mitochondrial \( tRNA^{\text{asn}} \) gene SNP (G5501 → T) could affect maternal and direct weight estimated breeding values due to a direct effect on the functional efficiency of the \( tRNA^{\text{asn}} \) gene. Otherwise, the \( tRNA^{\text{asn}} \) gene SNP genotyped in our study may be associated to other functional polymorphisms or a group of polymorphisms (haplotype). While our results are not conclusive concerning the effect of the \( tRNA^{\text{asn}} \) gene SNP on Nelore estimated breeding values, they provide important evidence that nucleotide variation within the mitochondrial genome should be considered as genetic markers for the assisted selection of quantitative traits.

The \( B. \text{taurus} \) and \( B. \text{indicus} \) mitochondrial genomes have been completely sequenced but the existing polymorphisms between these two breeds need to be studied to further characterize the existing genetic variability in these breeds and elucidate the effects of such polymorphisms on production traits. The association of weight estimated breeding values with a \( tRNA^{\text{asn}} \) gene SNP confirms that mitochondrial DNA is a source of genetic variation that influences growth during the bovine pre-weaning and post-weaning periods.

Acknowledgments

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References


Internet Resource

Nelore cattle breeding program (NCBP) at the University of São Paulo, under the auspices of the Brazilian National Association of Breeders and Researchers of Nelore Cattle Association Nacional de Criadores e Pesquisadores – ANCP) http://www.ancp.org.br/sumarios.

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