

Marker discovery and associations with beta-carotene content in Indian dairy cattle and buffalo breeds.

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Summary

Vitamin A is essential for human health and current intake levels in many developing countries such as India are too low due to malnutrition. Milk can be an important source of beta-carotene and three key genes (BCMO1, BCO2, and SCARB1) that are involved in the modification and transports of beta-carotene and vitamin A have been identified to have variants that may be associated with varying levels of beta-carotene. The aim of this work was to identify Single Nucleotide Polymorphisms (SNPs) in these three genes associated with beta-carotene levels in Indian cattle and buffalo breeds. Data already available from whole genome sequencing were collected from several breeds. Polymorphic SNPs within coding regions for buffalo and cattle were used to build a custom Sequenom panel and used to genotype 2,144 animals belonging to Indian cattle and buffalo breeds and crosses for which beta-carotene level in milk was measured. A general linear model was used to determine association of these SNPs with beta-carotene levels. Several SNPs were found for cattle breed crosses, but none overlapped across the crosses. For buffalo breeds only 2 out of the 4 breeds considered had associated SNPs with one overlapping SNP for the BCMO1 gene. These SNPs could be used to develop selection strategies for improved beta-carotene in milk of Indian cattle and buffalo breeds.

Keywords: cattle, buffalo, beta-carotene, BCMO1, SCARB1, BCO2, Indian.

Introduction

Vitamin A is essential for human health and current intake levels in many developing countries such as India are too low due to malnutrition. According to the World Health Organization (WHO), an estimated 250 million preschool children are vitamin A deficient globally. This number excludes pregnant women, and nursing mothers who are particularly vulnerable. Vitamin A deficiency can be addressed through provision of foods that are naturally rich (in the form of beta-carotene, which is then metabolized to vitamin A) or artificially fortified foods. One cup of regular milk supplies 16 percent and 12 percent of WHO recommended daily intakes for women and men, respectively. The metabolism of beta-carotene to form vitamin A in milk is a nutritionally important process in mammals. Recently,

genomic technologies have facilitated the identification of three key genes: BCMO1 and BCO2 (e.g. Berry *et al.*, 2009) and SCARB1 (Valacchi *et al.*, 2011) that are associated with the amount of beta-carotene in milk. Some cattle, with favourable naturally occurring genotypes, have been shown to have as much as 80% more beta-carotene in their milk.

The aim of this work is to identify Single Nucleotide Polymorphisms (SNPs) in the BCMO1, SCARB1 and BCO2 genes associated with beta-carotene levels in Indian cattle and buffalo breeds.

Material and methods

SNP discovery

Reads from whole genome sequencing of 202 Cattle (141 *Bos taurus*, 61 *B. indicus*) and 35 Buffalo (*Bubalus bubalis*) non-indian animals from several breeds were collected. Cattle reads were aligned against the UMD3.1 reference, due to the high genomic similarity and due to the lack of a *B. indicus* reference genome. *B. bubalis* reads were aligned to the buffalo reference genome MD_CASPUR_WB_2.0. A preliminary filter step was applied to discard all reads with mapping quality <10. Then, the standard pipeline of the Samtools or GATK software was applied to call the variants in all the samples for BCMO1, BCO2 and SCARB1 genes.

Building of the Sequenome custom panel and SNP association analyses with beta carotene

SNPs in the coding regions were used to build a Sequenome panel gathering together SNPs from all the two species. A total of 1,011 cattle and 1133 buffalo samples were collected (Table 1). Beta-carotene was measured from milk, DNA was extracted and genotyped with the Sequenome panel. For each breed, only high-quality SNPs (call rate >90) and belonging to the same genus (cattle and buffalo) were considered. With the high-quality SNPs, a general linear model (GLM) analysis for association with beta-carotene levels was first performed considering all the retained SNPs as fixed effects. Then, a second GLM was performed with a similar model, considering as fixed effect only SNPs with $P \leq 0.25$ in the first GLM analysis. After that, only SNPs significantly associated ($P < 0.05$) or suggestively associated ($P < 0.1$) with beta-carotene levels were considered and Least Squares means (LSM) and allele frequencies were calculated for the genotypes of each SNP if more than a few animals were represented.

Results and discussion

Approximately 1,400 SNPs were detected in the 3 genes for *B. taurus*, 1,600 for *B. indicus* and 2,300 in *B. bubalis*. The number of SNPs detected in the coding regions varied from 16 to 26 in the three species, with 5 overlapping SNPs between *B. taurus* and *B. indicus*. All these SNPs were used to build a custom Sequenom array. The beta-carotene analyses demonstrated a significant difference in beta-carotene level in milk as expected among cattle and buffalo with buffalo showing a lower beta-carotene content than cattle. This may be because buffalo can convert it directly in Vitamin A (Khan, 2002). Moreover, breed differences in beta-carotene content were detected within cattle and buffalo breeds (Table 1).

The GLM analyses on the high-quality SNPs detected several SNPs suggestively or

significantly associated with beta carotene content for cattle breeds, in the three genes for Holstein Cross and in 2 genes for Jersey cross. No overlapping SNPs were detected (Table 2).

A total of four SNPs (two for SCARB1 and two for BCMO1 genes) were detected for the Pandharpuri breed, while three SNPs (one for BCO2 and two BCMO1 gene) were detected for Murrah. A SNP on the BCMO1 gene overlapped between the two breeds, with the genotype AB for the Pandharpuri and the AA for Murrah that has the highest level of beta-carotene. No SNPs for Surti and Mehsana breeds were detected (Table 3).

Table 1. Beta-carotene (BC) means and standard error of the mean (SE) across species/breeds. Significant differences (P<0.05) within the buffalo or cattle breeds are indicated by the letters a, b and c.

Species/breed	N.	BC Mean (mcg/100ml)	(±SE)
Cattle	1011	4.99	0.20
- Jersey Cross ^(a)	517	3.88	0.16
- Holstein Cross ^(b)	494	6.15	0.38
Buffalo	1133	2.02	0.07
- Mehsana ^(c)	291	1.19	0.12
- Murrah ^(a)	283	2.66	0.16
- Pandharpuri ^(a,b)	279	2.20	0.15
- Surti ^(b)	280	2.06	0.14

Table 2. Association of SNPs with beta-carotene BC level for the significant or suggestive associations SNPs for cattle. Suggestive association is reported with the “” symbol in the SNP column. Gene, SNP number in the Sequenom panel with predicted effect (mis=missense, syn= synonym), % of genotypes when >1% (% freq), beta-carotene least squares means (BC lsm) and standard error (SE) are reported.*

Breed	Gene	SNP	Genotype	% freq	BC lsm (±SE)
Holstein cross	BCO2	7* (miss)	AA	93.46	5.88 (0.39)
			AB	6.54	8.82 (1.48)
	SCARB1	39 (syn)	AA	80.16	5.99 (0.41)
			AB	19.22	6.18 (0.85)
	BCMO1	43 (syn)	AA	93.89	6.06 (0.38)
			AB	5.91	6.12 (1.53)
Jersey cross	SCARB1	13 (syn)	AA	57.65	4.03 (0.21)
			BB	41.96	3.66 (0.25)
	BCMO1	3* (miss)	AA	96.88	3.84 (0.16)
			AB	3.12	5.15 (0.90)

Table 3. Association with beta-carotene level for the significant or suggested associated SNPs for the buffalo breeds. Suggestive association is reported with the “” symbol in the SNP column. Here gene, SNP number in the Sequenom panel with predicted effect (mis=missense, syn= synonym, -= not detected) % of genotypes % of genotypes when >1% (% freq), beta-carotene least squares means (BC lsm) and standard error (SE) are reported.*

Breed	Gene	SNP	Genotype	% freq	lsm (\pm SE)
Pandharpuri	SCARB1	52* (syn)	AA	29.12	1.86 (0.28)
			AB	48.28	2.42 (0.22)
			BB	22.61	2.20 (0.32)
	SCARB1	55 (syn)	AA	32.31	1.98 (0.26)
			AB	44.23	2.22 (0.22)
			BB	23.46	2.45 (0.31)
	BCMO1	62 (syn)	AA	92.06	2.15 (0.16)
			AB	7.58	3.15 (0.54)
	BCMO1	49* (-)	AA	89.71	2.10 (0.16)
AB			9.93	3.10 (0.47)	
Murrah	BCO2	65 (-)	AA	87.23	2.49 (0.17)
			AB	12.41	3.92 (0.45)
	BCMO1	66 (syn)	AA	64.26	2.54 (0.20)
			AB	31.41	3.09 (0.28)
			BB	4.33	1.07 (0.75)
	BCMO1	49 (-)	AA	69.18	2.98 (0.19)
			AB	27.24	2.03 (0.30)
			BB	3.58	1.02 (0.83)

Conclusions

The custom panel designed for genes related to beta-carotene production shows applicability in genotyping of cattle and buffalo in India and may be used for other developing countries. Several SNPs were significantly associated in cattle and in some buffalo breeds, providing markers that may be useful to develop genetic selection strategies that can increase beta-carotene content in milk of those populations.

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List of References

- Berry, S.D., S.R. Davis, E.M. Beattie, N.L. Thomas, A.K. Burrett, H.E. Ward, A.M. Stanfield, M. Biswas, A.E. Ankersmit-Udy, P.E. Oxley, J.L. Barnett, J.F. Pearson, Y. van der Does, A.H. Macgibbon, R. J. Spelman, K. Lehnert & R.G Snell, 2009. Mutation in bovine beta-carotene oxygenase 2 affects milk color. *Genetics* 182:923-926.
- Khan, M.S. 2002. Animals that Produce Dairy Foods | Water Buffalo. In: *Encyclopedia of*

Dairy Sciences (Second Edition), edited by J.W. Fuquay, Academic Press, San Diego, p 340-342,
Valacchi G, C. Sticozzi, Y. Lim, A. Pecorelli, 2011. Scavenger receptor class B type I: a multifunctional receptor. *Ann. N. Y. Acad. Sci.* 1229: E1-7.