Gene-culture coevolution between cattle milk protein genes and human lactase genes

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Milk from domestic cows has been a valuable food source for over 8,000 years, especially in lactose-tolerant human societies that exploit dairy breeds. We studied geographic patterns of variation in genes encoding the six most important milk proteins in 70 native European cattle breeds. We found substantial geographic coincidence between high diversity in cattle milk genes, locations of the European Neolithic cattle farming sites (>5,000 years ago) and present-day lactose tolerance in Europeans. This suggests a gene-culture coevolution between cattle and humans.

Some, but not all, human populations have the genetically determined ability to digest milk lactose in adulthood, thereby benefiting from the rich food resources in cow's milk[1]. These societies (*e.g.*, Northern Europe) are lactose-tolerant and highly dependent on milk products. Lactose tolerance is an example of selection-based evolutionary change in humans from milk-drinking cultures[2]. Has there also been a detectable evolutionary change in the gene pool of domestic cattle from these cultures?

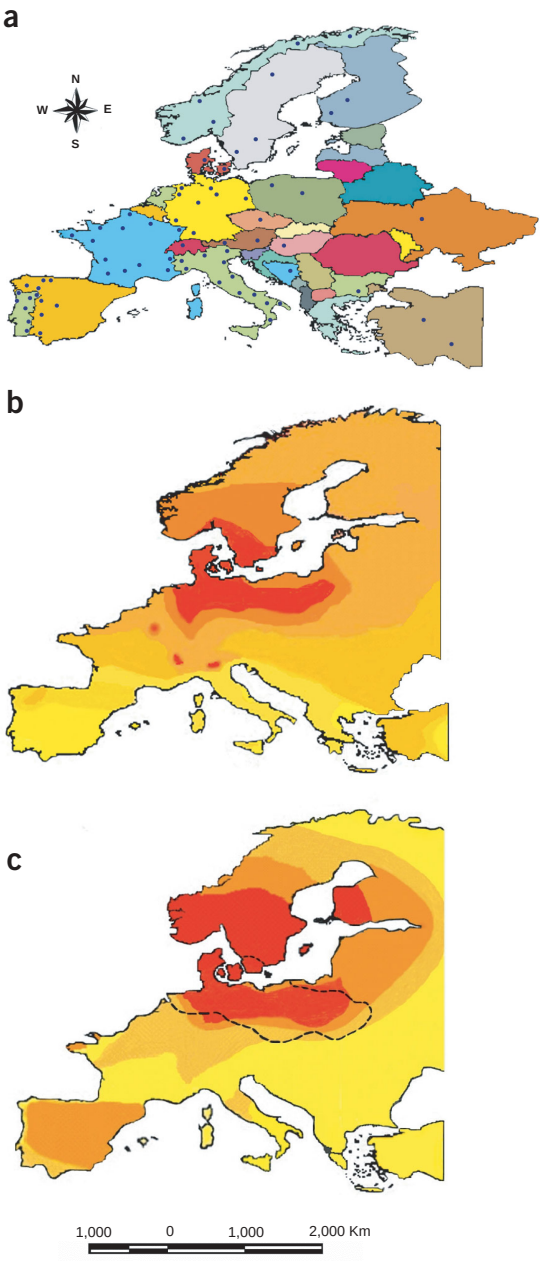

Figure 1 Geographic coincidence between milk gene diversity in cattle, lactose tolerance in humans and locations of Neolithic cattle farming sites in NCE. (**a**) Geographic distribution of the 70 cattle breeds (blue dots) sampled across Europe and Turkey. (**b**) Synthetic map showing the first principal component resulting from the allele frequencies at the cattle genes. The dark orange color shows that the greatest milk gene uniqueness and allelic diversity occurs in cattle from NCE. (**c**) Geographic distribution of the lactase persistence allele in contemporary Europeans. The darker the orange color, the higher is the frequency of the lactase persistence allele. The dashed black line indicates the limits of the geographic distribution of early Neolithic cattle pastoralist (Funnel Beaker Culture) inferred from archaeological data[15].

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Table 1 Spearman correlation coefficient values between the first principal component from the milk protein gene frequencies, the lactase persistence allele frequency and the presence or absence of archeological evidence for Neolithic cattle pastoralists

	Spearman correlation	Degrees of freedom	*P*
First principal component versus neolithic cattle pastoralists	–0.750	21.2–27.3	<0.0005
First principal component versus lactase persistence allele frequency	–0.593	17.7–24.6	<0.01
Neolithic cattle pastoralists versus lactase persistence allele frequency	0.730	19.3–24.8	<0.0005

Our study of nonsynonymous mutations in six milk protein genes in ~20,000 cattle from 70 breeds across Europe (**Fig. 1a**) found high allelic richness and genetic distinctiveness in the native cattle from North Central Europe (NCE), as illustrated by the synthetic map of cattle milk protein genes (**Fig. 1b** and **Supplementary Tables 1** and **2**, **Supplementary Fig. 1** and **Supplementary Methods** online). Notably, this synthetic map (inner contour) closely matches the European distribution of the allele for human lactase persistence that is most frequent in NCE ($P < 0.0005$; **Table 1**). This is in stark contrast to the lower levels of lactose tolerance found in people of Southern Europe and the Near East. There was also strong concordance ($P < 0.001$) of the geographic distribution of cattle milk gene diversity with the early Neolithic distribution of a European cattle pastoralist society[3] (**Fig. 1c**).

How can we explain the strong geographic concordance between cattle milk gene diversity, human lactose tolerance and the distribution of the earliest European cattle pastoralists? We propose that since Neolithic times, there has been gene-culture coevolution between the domestic cattle and human culture driven by the advantages conferred by milk consumption. This led to the maintenance of larger herds and selection for increased milk yield and altered milk protein composition. This coevolution seemingly influenced the frequencies of the important milk protein genes in cattle and the gene encoding lactase in humans. In fact, a recent study suggested that the relatively old variant for lactose tolerance was only recently driven to high frequencies in North Central Europeans after the introduction of dairy culture in this region[4].

This scenario is also supported by evidence for selection at milk protein loci in bovids[5]. For example, directional selection can explain high intraspecific divergence and low intraspecific polymorphism in *k*-casein sequences across bovids[5]. Our data also show patterns consistent with selection: 19 NCE breeds deviated significantly from neutrality (Ewens-Watterson test, 32% of 114 tests with $P < 0.01$ versus 2% of 306 tests with $P < 0.01$ in the 51 non-NCE breeds; Fu test and Tajima test, all NCE breeds showed $P < 0.05$ versus 4 of 51 non-NCE cattle).

Our genetic data corroborate recent archaeological evidence suggesting that the early European cattle pastoralists in NCE were dependent on milk[6,7], as early Neolithic sites in NCE are rich in cattle remains[4]. Based on the analysis of intratooth change in nitrogen isotope ratios from archaeological cattle teeth, it seems that cattle herds were managed for early weaning of calves, making cow's milk more available for human consumption. Meat production, practiced outside NCE, necessitates later weaning to optimize weight gain[7].

Among several phenomena that might have shaped our data, selection seems the most probable explanation. Recent studies have shown that high diversity in human genes can evolve rapidly due to selection[8]. In addition, analysis of bovine myostatin alleles showed signals of balancing selection in a number of independently occurring mutations that cause double-muscling in beef breeds[9].

Given that population surveys of mtDNA sequence, microsatellite markers and protein polymorphisms in European cattle breeds show no evidence of elevated diversity in NCE[10-12], it is likely that selection pressure imposed by early pastoralists and their successors in different regions of NCE has left the legacy of high allelic diversity at these specific milk genes. It is also possible that some of the diversity represents relatively recent mutation (<10,000 years), although, under a neutral model, mutation rates are too low (10^{-6}–10^{-9}; ref. 5) for this to be a primary factor. Selection may have maintained many favorable new mutations by protecting them from the normal process of attrition due to drift.

Another possible source of the unique diversity found in cattle in NCE is historical gene flow from an as yet unidentified origin. Two candidates for this source are local wild aurochs (*Bos primigenius*), which persisted in NCE until the sixteenth century, and domestic cattle other than those that gave rise to present day European cattle (outside NCE). Extensive wild auroch introgression seems unlikely, and no mtDNA sequences have been detected in European cattle which match aurochs sequences identified using ancient DNA sequencing[13].

Notably, our findings contradict the results of previous surveys of genetic variation in European cattle[10–12], which suggested that diversity declines with distance from the Fertile Crescent region. This discrepancy could be explained by selection on the milk genes, and it may also reflect different sampling strategies. Our analysis is based on a sample set that is unprecedented in size, geographic coverage and breed diversity. Furthermore, unlike previous studies, we analyzed only nonsynonymous polymorphisms in strong candidate genes most likely to yield unusual geographic patterns in milk gene diversity.

Our study provides evolutionary insights and identifies high diversity in cattle genes that are economically important, suggesting that cattle in NCE are a potentially precious genetic resource for future agricultural productivity. Farming practices since the Neolithic seem to have left reciprocal genetic signatures in cattle and human populations from NCE. This may represent a rare example of cultural-genetic coevolution between humans and another species. Other examples of coevolution have been documented for human genes and genes of parasites, such as *Plasmodium*[14]. But our study represents the first non-disease-related example of genetic coevolution between humans and domestic animals, reflecting the extent to which domestication has shaped human societies and the genomes of both humans and cattle.

Note: Supplementary information is available on the Nature Genetics website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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Mutations in the polyglutamine binding protein 1 gene cause X-linked mental retardation

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We found mutations in the gene *PQBP1* in 5 of 29 families with nonsyndromic (MRX) and syndromic (MRXS) forms of X-linked mental retardation (XLMR). Clinical features in affected males include mental retardation, microcephaly, short stature, spastic paraplegia and midline defects. PQBP1 has previously been implicated in the pathogenesis of polyglutamine expansion diseases. Our findings link this gene to XLMR and shed more light on the pathogenesis of this common disorder.

XLMR is a prominent unsolved problem in clinical genetics. Based on the distribution of linkage intervals in 125 unrelated families, we recently showed that roughly one-third of all mutations underlying MRX are clustered on proximal Xp[1]. This observation prompted us to search for mutations in families with linkage intervals overlapping this region.

In 5 of 29 families studied, we detected mutations in *PQBP1* that cause frameshifts in the fourth coding exon (**Supplementary Methods** online), which contains a stretch of six AG dinucleotides in the DR/ER repeat (**Fig. 1** and **Supplementary Fig. 1** online). In two families (family N9 (not previously reported) and family SHS with Sutherland-Haan syndrome (MRXS3; ref. 2)), all affected males carry an extra AG dinucleotide (3898_3899dupAG), whereas in two others (family N45 (not previously reported) and family MRX55; ref. 3), two AG dinucleotides are deleted (3896_3899delAGAG). A single AG unit (3898_3899delAG) is deleted in affected males of family N40 (ref. 4). In all families, these mutations segregated with the disease and were present in all obligate heterozygotes that we tested. Except for one, all obligate heterozygotes that we examined have random X-chromosome inactivation (data not shown) and have IQs in the normal range. Apart from a single-nucleotide polymorphism (IVS2–3C→T), we found no sequence variation in control X chromosomes.

The duplication observed in families N9 and SHS and the deletion found in families N45 and MRX55 give rise to almost the same frameshift (**Fig. 1b**). Still, there is considerable inter- and intrafamilial phenotypic variation (**Supplementary Table 1** online). For example, males with SHS show mental retardation, short stature, microcephaly, brachycephaly, spastic diplegia, small testes and anal stenosis or atresia, whereas there is no spastic diplegia or small testes in family N9, with an identical mutation. In both families the disease is not progressive. In family MRX55, in whom the predicted mutant protein differs by only two amino acids, affected individuals are moderately retarded but have no other clinical signs, except for a somewhat smaller body size in one individual (height was 159 cm, ≥2 s.d. below normal at the age of 20 years). In contrast, in addition to mental retardation, all affected individuals in family N45 have microcephaly, one has anal atresia and another has complete situs inversus. Some of this clinical variability may be due to differences in the genetic background. Family MRX55 is from Morocco, families N9 and N45 are from the Netherlands and family SHS has English ancestry.

In family N40, all affected males have congenital heart defects in addition to severe mental retardation, microcephaly, spasticity, short stature, cleft or highly arched palate and other craniofacial abnormalities[4]. The mother of two of the affected individuals has a corrected atrial septal defect. Facial features coarsened with age.

Several *PQBP1* splice variants have been described[5]. All but one very rare variant contain exon 4, which is mutated in the five families. The three different types of mutations cause frameshifts that lead to premature stop codons, resulting in truncated PQBP1 proteins that lack several important domains.

The particularly severe clinical phenotype seen in family N40 may be due to the fact that the C-terminal end of the predicted truncated protein is entirely different from that of the mutant proteins in the other families (**Fig. 1b**) and may give rise to aberrant protein-protein interactions.

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