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Brief Communication: Effect of Nomadic Subsistence Practices on Lactase Persistence Associated Genetic Variation in Kuwait

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ABSTRACT Lactase persistence (LP)—the ability to digest lactose in adulthood—is paradigmatic of Holocenic dietary change affecting the evolutionary trajectory of specific populations. Kuwait represents one location of high LP where the variation in associated genomic regions has not been examined. Here, we present new sequence data from a 427 bp amplicon 14 kb upstream of the *LCT* (lactase) gene for two Bedouin tribal populations, the Ajman and Mutran. We estimate the frequency of known LP associated alleles and discuss the impact of nomadic-pastoralism on the associated genetic variation. We observe high frequency (56% on average) of the *−13,915*G* allele in both tribes, which is consistent with the high prevalence of LP in Kuwait. Whilst LP associated alleles occur in Kuwait at a similar frequency to other regional populations, we suggest that the *−13,915*G* allele frequency among the Kuwaiti Bedouin may be higher than among non-Bedouin Kuwaitis, possibly due to greater historical reliance on milk consumption or genetic drift. Am J Phys Anthropol 152:140-144, 2013.

Lactase persistence (LP) is a genetically controlled trait in which high lactase enzyme ("lactase-phlorizin hydrolase") activity is retained into adulthood (Sahi et al., 1973; Wang et al., 1998). In the ancestral human phenotype and other mammals, lactase activity declines post-weaning (Sebastio et al., 1989; Buller et al., 1990; Lacey et al., 1994). This results in lactase non-persistence ("adult-type hypolactasia;" "lactose intolerance") and inability to digest lactose in milk.

LP associates with several single nucleotide polymorphisms (SNPs) in intron 13 of *MCM6* (minichromosome maintenance complex component six) (OMIM 601806), upstream of *LCT* (lactase) (OMIM 603202) on chromosome two (Enattah et al., 2007). Confirmed phenotypically causative SNPs include; *−14,010*C* (Jensen et al., 2011), *−13,915*G* (Tishkoff et al., 2007), *−13,910*T* (Lewinsky et al., 2005), and *−13,907*G* (Tishkoff et al., 2007). The trait shows simple Mendelian inheritance, with LP associated alleles dominant (Enattah et al., 2002).

Levels of LP vary globally (Itan et al., 2010), being highest (>80%) in pastoralist populations (Holden and Mace, 1997) across Europe, the Middle East, and parts of Africa. Causative genetic variants are differently geographically distributed, occurring on different haplotype backgrounds (Hollox et al., 2001; Poulter et al., 2003; Ingram et al., 2007; Tishkoff et al., 2007). Mutations *−14,010*C* and *−13,907*G* are spread and probably evolved in sub-Saharan Africa, whereas *−13,915*G* likely originated in the Arabian Peninsula (Mulcare, 2006; Enattah et al., 2007; Imtiaz et al., 2007; Ingram et al., 2007; Enattah et al., 2008; Al-Abri et al., 2012). The *−13,910*T* allele is most common in Northern Europe, but also occurs in India (Gallego Romero et al., 2011), and sporadically among African and Asian populations (Itan et al., 2010).

In many global pastoralist populations, strong signals of positive selection have been identified on regions surrounding LP associated alleles (Bersaglieri et al., 2004; Coelho et al., 2005; Sabeti et al., 2007; Tishkoff et al., 2007). This suggests that LP has been under historical selection in populations where milk has been a substantial dietary component. It has been suggested that nomads may have historically encountered more intense selection for LP than sedentary pastoralists, due to their greater reliance on milk (Cook and Al-Torki, 1975; Ingram et al., 2007). Among African nomadic-pastoralists such as the Nuer, Dinka and Fulbe, the genetic basis of LP is largely unknown (Blench, 1999; Mulcare et al., 2004; Coelho et al., 2005; Itan et al., 2010). However, nomadic populations in the Arabian Peninsula typically show high frequencies of LP and associated alleles; particularly of *−13,915*G*, with allele frequencies ranging up to 0.489 in Saudi Arabian Bedouin (Ingram et al., 2007).

Kuwaiti Bedouin have traditionally lived as nomadic-pastoralists in small patrilocal, tribal groups, relying on meat and milk products from camels, sheep, and goats (Dickson, 1959; Cole, 2003; Mohammad et al., 2009). Phenotypic measurements suggest LP is common within Kuwait (Al-Sanae et al., 2003), yet the genetic variants conferring LP remain unidentified. Itan et al. (2010) pinpointed Kuwait as one of two locations on the Arabian Peninsula which exhibit significant differences between

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known LP associated genotype and phenotype frequencies ($P < 0.01$). Previous study has only attempted to identify LP associated variants at the *−13,910* locus (Mulcare, 2006), finding no evidence of the LP associated allele. Our study aims to improve the understanding about the genetic basis of high LP in Kuwait, determining the frequency of LP associated variants which have remained unstudied in Kuwait. Identifying the LP associated allele in the Kuwaiti population studied here represents a further step toward understanding adaptations for dairying in nomadic populations, especially within the Arabian Peninsula.

Through sequencing a 427 bp region upstream of *LCT* in 66 Kuwaiti Bedouin samples, this study attempts to identify genomic variants previously associated with LP. We consider whether the nomadic history of the Bedouin, including practices of living in small endogamous groups and heavy consumption of milk products, has affected LP associated allele frequency.

MATERIALS AND METHODS

Samples

DNA samples represent a subset of those studied for short tandem repeat (STR) and SNP loci by Mohammad et al. (2009), and were collected from ancestrally distinct (for at least three generations) male Kuwaiti Bedouin. Samples originated from the Mutran (Adnani lineage) ($n = 30$), and the Ajman tribes (Qahtani lineage) ($n = 39$). Samples were collected from the Al Farwaniyah Governorate and the Al Ahmadi Governorate, respectively (for details and ethical permissions, see Mohammad et al., 2009).

Analyses

Samples were polymerase chain reaction (PCR) amplified for a 427 bp amplicon 14 kb upstream of the *LCT* gene using primers MCM6i13 and LAC-CL2, reported in Ingram et al. (2007). This amplicon was designed to include SNPs previously functionally associated with LP; *−14,010G>C*, *−13,915T>G*, *−13,910C>T* and *−13,907C>G* (Lewinsky et al., 2005; Tishkoff et al., 2007; Enattah et al., 2008). Diluted samples were sequenced by Macrogen using the forward primer.

Polymorphic SNPs were identified and genotypes called by visual assessment of chromatograms using Sequencher® version 5.0 (2011). Genotypes were confirmed by repeated PCR and sequencing the reverse complement strand. Two samples (AJM1 and MUT5) had indeterminable genotypes and were excluded from analyses. Consequently, 29 Mutran and 37 Ajman samples were genotyped.

The Ajman and Mutran populations were tested for allele frequency differences to each other at the *−13,915* locus using a Pearson's chi-square test in IBM SPSS version 19 (IBM Corp, 2010). LP associated loci in both tribes were tested for genotypic deviation from Hardy Weinberg equilibrium (HWE) using an exact-test in Arlequin version 3.5.1.3 (Excoffier and Lischer, 2010).

Assuming dominance of derived SNPs, we tested whether the frequency of LP associated genotypes among the Bedouin was sufficient to explain the phenotypic frequency of LP in Kuwait, as measured in a different cohort by Al-Sanae et al. (2003). The hydrogen breath testing technique used by Al-Sanae et al. (2003) to determine LP phenotypes frequently produces false negatives (a lactose intolerant person appears lactose tolerant) and false positives (a lactose tolerant person appears lactose intolerant), averaged across an aggregation of studies to be 9/132 and 5/120, respectively (Mulcare et al., 2004). To account for these potential errors and sampling effects, we adopted the Monte Carlo-based "GenoPheno" method of Mulcare et al. (2004). To include the effect of alleles at different loci, we summed all alleles associated with LP, using this as a combined "derived allele frequency." This approach is justifiable under the assumptions that derived alleles are dominant and that they occur mutually exclusively on a chromosome because of their genomic proximity [evidenced by the different haplotype backgrounds associated with different alleles (Hollox et al., 2001; Poulter et al., 2003; Ingram et al., 2007; Tishkoff et al., 2007)]. "True" population frequency of LP was then estimated from sample allele frequencies, assuming adherence to HWE (confirmed through the HWE test described above), and calculated as $p^2 + 2p(1 - p)$, where p is the LP associated allele frequency. This frequency was compared to the frequency of the phenotype measured by Al-Sanae et al. (2003), accounting for errors in the hydrogen breath testing technique (Mulcare et al., 2004). The Monte Carlo simulations were run 100,000 times.

Broader geographic patterns

To illustrate the results from Kuwait in a global context, maps of interpolated allele frequencies were created using published data from 244 populations (Supporting Information Table 1). Typically, the exact geographical provenance was not known, so co-ordinates were generated for the center of the geographical range of the population or country using a web-based program (http://www.itouchmap.com/latlong.html, 2012). Frequencies of *−13,915*G*, *−13,910*T*, and *−13,907*G* were plotted, and interpolated maps produced using ArcGIS (ESRI, 2011). Interpolations were cropped using image editing software (Brewster et al., 2011).

RESULTS

Considering all 66 samples, the amplicon was variable at three sites (Table 1), all previously identified as functionally associated with LP; *−13,915T>G*, *−13,910C>T*, and *−13,907C>G*. The *−13,915*G* allele appeared at high frequency in the Ajman (0.57) and Mutran (0.55). The *−13,910*T* allele occurred in heterozygous state in one Ajman sample and *−13,907*G* at heterozygous state in two Mutran samples.

TABLE 1. Genotype and allele frequencies of variant LP associated alleles

		Locus and genotype								
		−13,915T>G			*−13,910C>T*			*−13,907C>G*		
Pop.[a]		TT	TG	GG	CC	CT	TT	CC	CG	GG
Ajman	GF[b]	0.16	0.54	0.30	0.97	0.03	0.00	1.00	0.00	0.00
(37)	AF[c]	0.57 (0.058)			0.01 (0.013)			0.00 (0.000)		
Mutran	GF	0.17	0.59	0.28	1.00	0.00	0.00	0.93	0.07	0.00
(29)	AF	0.55 (0.065)			0.00 (0.000)			0.03 (0.024)		
Total	GF	0.17	0.55	0.29	0.98	0.02	0.00	0.97	0.03	0.00
(66)	AF	0.56 (0.043)			0.01 (0.008)			0.02 (0.011)		

[a] Population studied and number of individuals sampled.
[b] Genotype frequency.
[c] Derived allele frequency at each locus (standard errors within parentheses).

Neither the Ajman, Mutran, nor the combined Bedouin population deviate from the expectations of HWE at *−13,915* ($P = 0.740$, $P = 0.713$, and $P = 0.459$, respectively). This justifies the assumption of HWE used in the GenoPheno method.

Assuming dominance of derived SNPs and acknowledging that LP associated SNPs appear on different haplotype backgrounds so are unlikely to occur together on the same chromosome (Hollox et al., 2001; Poulter et al., 2003; Ingram et al., 2007; Tishkoff et al., 2007), determined genotypes indicate overall LP prevalence of 0.84 within the Ajman sample, and 0.90 within the Mutran sample.

Predicted phenotypic frequency in the Ajman and Mutran populations (accounting for sampling to produce an estimate of true population phenotypic frequency, rather than simply the frequency we observed within our samples) was approximated using the HWE formula, $p^2 + 2p(1 - p)$, where p is the frequency of the LP associated allele, giving LP prevalence of 0.82, 0.83, and 0.83 among the Ajman, Mutran, and overall Kuwaiti populations, respectively. The predicted true frequency of LP based on Kuwaiti genotype data was compared to measurements of phenotypic LP frequency of 0.53 (37/70 individuals) in a population of "Kuwaiti Arabs" (Al-Sanae et al., 2003). Under these assumptions, Kuwaiti Bedouins would have a significantly higher prevalence of LP than indicated phenotypically in generalized Kuwaiti Arabs ($P = 0.00016$).

Interpolated geographic distributions of LP associated alleles (Supporting Information Figs. 1–3) show that Kuwaiti Bedouin populations are characteristic of other nearby populations.

DISCUSSION

This study provides the first evidence of high frequency of *−13,915*G* in Kuwaiti Bedouin; 0.57 in the Ajman and 0.55 in the Mutran tribe. The *−13,910*T* and *−13,907*G* alleles occur only at very low frequency. This is similar to previous measurements of LP associated genetic variation in other populations on the Arabian Peninsula (Supporting Information Figs. 1–3; Supporting Information Table 1). The high prevalence of *−13,915*G* characterized in this study likely explains a significant proportion of LP in the Kuwaiti population. However, the exact amount of genetic variation in Kuwait which this study explains is unpredictable without accurate knowledge of the proportion of Bedouin in Kuwait.[1]

Given previous findings based on extended haplotype homozygosity tests that the *−13,915*G* allele has been under selection in neighboring populations (Saudi Arabian, Moroccan, Arab, and Saharawi) (Enattah et al., 2008), it seems reasonable to hypothesize that the allele may have risen to high frequency in Kuwait as a result of similar selection pressures.

There is no evidence of significant differences in allele frequencies at the *−13,915* marker between the Ajman (0.57) and the Mutran tribes (0.55) ($\chi^2_{(1)} = 0.033$, $P = 0.856$, $n = 132$). Previous analyses of Y chromosome haplotypes within haplogroup J1, which has high frequency in the Middle East (encompassing 100% of Ajman and 93% of Mutran samples), have shown that Ajman and Mutran male lineages are distinct and lie on different branches of the median-joining network (Mohammad et al., 2009). The relatively large genetic differentiation based on neutral Y-chromosomal markers consequently contrasts with the near identical allele frequencies found in each tribe based on the LP associated alleles. Specific demographic and evolutionary hypotheses for the maintenance of these levels of diversity could be investigated in the future via coalescent models.

Based on the allele frequencies we found, dominance of LP associated alleles and accordance with HWE, 83% of Kuwaiti Bedouin would be expected to be LP. Comparing this frequency with that of the previously measured LP phenotype in the Kuwaiti Arab individuals (0.53) measured by Al-Sanae et al. (2003) indicates that the Bedouin populations might include significantly a higher frequency of LP individuals than among the overall Kuwaiti Arab population. Because the GenoPheno test we used (Mulcare et al., 2004) accounts for the error rate of phenotypic determination and sampling of the Bedouin population, and LP associated genotypes of the Kuwaiti Bedouin were checked through reverse sequencing, this difference is unlikely to reflect sampling or measurement errors. Holden and Mace (1997) and Ingram et al. (2007) have suggested that LP frequency correlates with population's subsistence habits, with milk-drinking groups exhibiting higher allele frequencies. Ingram et al. (2007) noted that Bedouin populations on the Arabian Peninsula—consuming a significant quantity of dairy products—have higher LP associated allele frequencies than neighboring non-Bedouin Arabs, who may drink less milk. Consequently, our result may suggest that Kuwaiti Bedouins have had historically greater selection pressure for LP than non-Bedouin Kuwaiti Arabs: a result of greater reliance on milk-based products.

Nevertheless, we cannot rule out alternative scenarios that LP associated allele frequencies have instead been rendered higher in the Bedouin population than in the non-Bedouin Arab populations because they have been more affected by drift in the Bedouin population, possibly as a consequence of the relatively low Bedouin N_e (Ajman male $N_e = 560$, 95% CI 250–1,500; Mutran male $N_e = 870$, 95% CI 400–2,200), (Mohammad et al., 2009) or allele surfing. Allele surfing occurs where a subset of individuals at the frontier of an expanding group settle in sparsely occupied regions, resulting in a high frequency of the subset's carried alleles in the new offshoot population (Hofer et al., 2009; Itan et al., 2009). Given claims that nomadic subsistence practices of the Bedouin may allow groups to expand to live in regions where sedentary pastoralism is impossible (Bloom and Sherman, 2005), it is plausible that allele surfing effects could have been common in the recent demographic expansion of the Ajman and Mutran Bedouin populations, estimated at 5.3–3.8 kya (Mohammad et al., 2009).

Finally, it is possible that secondary lactose intolerance (e.g., a consequence of environmental damage to the ileum) could cause a disparity between genotype and phenotype frequencies. Further tests involving direct genotype-phenotype assessment will be required to quantify the role of this confounding factor.

[1]According to Ghabra (1997), Bedouins may account for 65% of the Kuwaiti population, yet census data is inaccurate as a proportion of Bedouin remain officially stateless since the introduction of new citizenship laws.

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