

HUMAN BIOLOGICAL SURVEY

An association between lactose intolerance and anthropometric variables in the Sudanese *Shagia* tribe (East Africa)

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Abstract

Background: The culture of contemporary Sudanese tribes is not homogeneous. One of the three main tribes in northern Sudan is the *Shagia* tribe. This study is part of the large-scale research project to anthropologically and genetically describe the *Shagia* population, who inhabited three villages in an isolated region of the Fourth Nile Cataract. This population is extremely homogeneous as a result of geographical, genetic and cultural isolation.

Aim: The aim of the study was to analyse the frequency of two single nucleotide polymorphisms (SNPs), C/T-13910 and G/C-14010, within the isolated population. These SNPs are closely associated with lactase persistence. In addition, this study has correlated the SNPs with anthropometric measurements.

Subjects and methods: Buccal swabs were collected from 126 subjects. The DNA was extracted and the occurrence of the two alleles at each SNP was analysed using real-time PCR. An anthropometric examination of 64 adult individuals was used for an analysis of body measurements and proportions.

Results: At the C/T-13910 SNP, the CT genotype frequency was 3.2%, whilst 96.8% of individuals were homozygous for the C allele. The presence of the T allele showed a strong association with body mass index (BMI) and waist circumference. At the G/C-14010 locus, all the examined subjects were homozygous for the G allele.

Conclusions: The C/T-13910 polymorphism correlated with anthropometric measurements. Identification of the T allele of C/T-13910, in this isolated tribe, may be linked to their previously nomadic lifestyle and could provide important information on the ancestry of the tribe and the admixture of European genes.

Keywords

4th Nile Cataract region, lactase intolerance, RT-PCR, Sudan

History

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Background

Africa is characterized by a wide variety of genetically distinct populations, particularly in the eastern part of the continent. Intercontinental variations in morphological features are a consequence of the natural selection that is associated with adaptation to changing environmental conditions. This remains an ongoing subject for genetic research (Coop et al., 2009; Myles et al., 2007). Sudan was formerly known as Nigritia, Balad as Saudawan (in Arabic 'land of the Blacks'). The name 'Sudaan' means 'two dams' and denotes the first two cataracts of the Nile, which separate this country from Egypt.

The *Shagia* tribe is one of the three main tribes in northern Sudan. There is a small isolated population of this tribe who were found in the Fourth Nile Cataract region precisely at the

junction of two deserts (N 18° 56.413' and E 032° 07.547'). The geographical location of the tribe has led to the population remaining genetically and culturally isolated, with no indication of the villages on world maps. The difficult natural conditions have made these people barely accessible. The *Shagia* people are farmers and their homesteads are located along the Nile, from Kurtia to the third Cataract. The language they speak and write is Arabic, but there are few literate people in these sparsely populated villages. The houses are made from bricks, which have been created from mud and animal faeces, and the roofs are covered with straw to reduce the inside temperature. Each village consists of a few farmsteads and is inhabited by 40–60 people. Families consist of generations living together and the elderly only occasionally go outside. The men are the leaders, but often they are not the eldest members of the family. Most work, including the field work, is performed by women.

The *Shagia* people tend to have a vegetarian diet, with a prevalence of leguminous plants (beans, broad beans and crown vetch). Although the country is rich in citrus fruits, such as grapefruits, oranges and lemons, they are rarely eaten because of their high price. Vegetables are the main source

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of vitamins and are mostly tomatoes, onions and chives. On special occasions, such as weddings or the Ram Holiday, the *Shagia* people eat mutton or goat meat, which is bought at the town market. The daily food is based on bread made from flour called 'gurassa' and an overcooked broad bean dish called 'fud'. After every meal, either black tea with sugar or an infusion of mint or chokeberry is drunk. The *Shagia* do not breed dairy animals and do not consume animal milk or dairy products.

The environmental conditions have contributed to the morphological structure of the *Shagia*. Deep-rooted tribal affiliation has also sustained the typical features of this ethnic group. The role of body proportions in the racial diversity of modern humanity is not clear. Some researchers believe that the taxonomic categories should be based on comparisons between the length and width of the body, whilst others have reservations about using this technique. The use of genetic analysis may aid in the prediction of geographic origin of a population.

The lactase gene, LCT, is emerging as a new candidate gene for association with anthropometric measurements (Corella et al., 2011). In a recent genome-wide association study (GWAS), carried out by Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE), strong correlations were found between various single nucleotide polymorphisms (SNPs) in the lactase gene (LCT) and both waist circumference and body mass index (BMI) (Corella et al., 2011; Heard-Costa et al., 2009). Lactose digestion depends on the activity of the enterocyte brush-border enzyme, lactase. The majority of people lose their ability to digest milk before they reach maturity because the lactase enzyme is deactivated between the third and fifth year of life (Aurisicchio & Pitchumoni, 1994). Inability to digest lactose manifests as digestive complaints that vary from mild flatulence to severe abdominal pain and diarrhoea (Aurisicchio & Pitchumoni, 1994). Lactase persistence (LP), which is the ability to digest lactose throughout adulthood, occurs in most northern Europeans (in 90% of Scandinavians), but is less common in southern Europe and in the Middle East. Lactase persistence is also present in some African shepherd tribes (e.g. the Tutsi and the Fulani) (Durham, 1992; Ingram et al., 2007; Swallow, 2003).

It has been shown that LP is associated with the T allele of a C to T base transition in intron 13 of the MCM6 gene (Enattah et al., 2002; Tishkoff et al., 2007). This is found upstream from the LCT locus, LCT-13910. In addition, Mulcare et al. (2004) showed that other polymorphisms, which include G/C-14010, T/G-13915 and C/G-13907, may be implicated in lactose tolerance amongst the residents of East Africa.

In this study, analysis of the allele frequencies of LCT polymorphisms, along with measurements of body proportions of the *Shagia* people, were performed to verify the hypothesis of an association between the SNPs and anthropometric variables. Two polymorphisms were assessed. The first was the LCT-13910C>T base change, which is generally found at high frequencies in populations of European descent. However, it is very rare in Africans. The second was the G/C-14010 base change, which is primarily observed in African populations.

Methods

Subjects

The inhabitants of the Fourth Nile Cataract region were at risk of displacement due to the erection of the Merowe High Dam (Merowe Dam, Merowe Multi-Purpose Hydro Project). In February 2005, a scientific expedition was led by the director of the Archaeological Museum in Gdansk (Poland), Henryk Paner. A total of 126 individuals from the region were subjected to genetic analysis. The subjects occupied three villages (Abu Haraz, Shibabit, El Higiena) and identified themselves as members of the *Shagia* tribe (75 women and 51 men, mean age = 23.9 ± 17.3 years, median age = 20 years). Anthropometric measurements were taken from 64 adults (35 women and 29 men). The remaining individuals were children.

The research was conducted in accordance with the principles of the Declaration of Helsinki and local law. The participants were informed about the aim of the study and consented to take part. Oral consent was obtained in the presence of the local government representative since most of the subjects were illiterate. The research project was approved by the Bioethical Commission of the Pomeranian Medical University in Szczecin, Poland (KB-0012/129/10).

Genotyping

Buccal swabs (saliva and epithelium) were collected from all individuals. Swabs were dried in separate areas in order to avoid cross-contamination. The DNA was extracted using a BuccalAmp DNA Extraction kit (Epicentre, Madison, WI). Oligonucleotide primers and TaqMan probes were designed for the LCT polymorphisms, i.e. LCT-13910C>T and G/C-14010 (rs4988235 and rs4988233, respectively). Oligonucleotide and probes were designed and synthesized by Applied Biosystems (ID Assay: C_15769614_10 and C_42578736_10, respectively). PCR amplification and an end-point read was performed on Fast 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA).

Anthropological examination

The participants assumed an anthropometric position (a position at attention, barefooted, with the upper limbs hanging freely along the trunk, hands close to thighs and the head in the Frankfurt plane) for the examination. Length (basis–vertex, basis–suprasternale, basis–symphision, basis–acromion, basis–dactylion III) and width (acromion–acromion, thoracolaterale–thoracolaterale, illicristale–illicristale, xiphoidale–thoracospinale, epicondylion medialis–epicondylion lateralis) measurements were taken. The arms, stomach and shoulder blade skin-folds were measured, as was the circumference of the arms, chest, waist, hips and shins. The measurements were used to calculate selected anthropological indicators.

Anthropometric measurements, taken with a medical scale, anthropometer and anthropological centimetre (body mass, height, hip, chest, shin and forearm measurements), were used to calculate the BMI, body density (D), total fat as a percentage of body weight (F%) and in kilograms (Fkg) and active tissue as a percentage of body weight (TA%) and

in kilograms (TA kg). The BMI values were compared with the range of values given by the World Health Organization.

Active tissue (TA) was determined by the Piechaczek's formula (Malinowski et al., 2000; Piechaczek, 1975). For men:

$$\text{TA kg} = -103.85484 + 0.446921x_1 + 0.13343x_2 + 0.458056x_3 + 0.838393x_4.$$

For women:

$$\text{TA kg} = -61.719697 + 0.339491x_1 + 0.540846x_4 + 0.26024x_3 + 0.407343x_5,$$

where x_1 = body height (cm), x_2 = hip size (cm), x_3 = chest size (cm), x_4 = shin size (cm), x_5 = forearm size (cm).

Adipose tissue, in kilograms (F kg), was calculated following the formula:

$$\text{body mass kg} - \text{TA kg}.$$

Active tissue, as a percentage of body weight (TA%), was calculated as:

$$\text{TA kg} / \text{body mass kg} \times 100\%.$$

Total fat, as a percentage of body weight (F%), was calculated as:

$$100\% - \text{TA}\%.$$

Adipose tissue distribution was assessed using the waist-to-hip ratio (WHR). In accordance with the anatomical classification of obesity, two types of obesity were assessed: android and gynoid.

Statistical analysis

Statistical calculations were performed using STATISTICA PL 9.0. All data were expressed as arithmetic means and standard deviations (SD). The Shapiro–Wilk test was used to assess normality of the distribution. The significance of differences between the mean values was assessed with a *t*-test. Pearson's linear correlation coefficient was used for the analysis of the correlations between F%, F kg, TA%, TA kg, D and BMI index. Differences were considered to be significant at the level of $p < 0.05$.

Results

The anthropometric measurements are presented in Table 1. The *Shagia* men were significantly taller than the women (by ~13 cm, on average). The tallest man (188.4 cm) lived in the village of Shibabit and the shortest (158.0 cm) came from Abu Haraz. Both the tallest and the shortest women (171.4 cm and 146.1 cm, respectively) came from Shibabit.

The *Shagia* men have slender bodies (62.1% of the leptosomic type), whilst the women are stout (57.2% of the pyknic type). The largest amount of subcutaneous fat in the bodies of men was found on the arms (31.1 ± 8.9 mm), under the scapular bone (24.9 ± 9.8 mm) and on the stomach (24.1 ± 9.3 mm). This was also the case in women, with measurements of 29.52 ± 6.7 mm on the arms, 8.7 ± 7.4 mm under the scapula bone and 16.4 ± 8.4 mm on the stomach.

Table 1. Anthropometric data of the studied *Shagia* tribe.

Parameter	Male (n = 29)	Female (n = 35)	p
	Mean ± SD	Mean ± SD	
Age (years)	35.76 ± 12.17	36.97 ± 14.57	0.72
Height (cm)	170.00 ± 8.00	157.39 ± 6.65	<0.0001
Weight (kg)	64.06 ± 10.14	56.69 ± 11.21	0.03
BMI (kg/m ²)	22.24 ± 3.07	19.00 ± 4.00	0.98
Waist (cm)	82.99 ± 10.24	85.69 ± 12.77	0.37
Hip (cm)	92.69 ± 7.06	96.74 ± 10.02	0.19
Chest (cm)	88.56 ± 6.20	89.83 ± 10.82	0.48
Forearm (cm)	26.93 ± 2.27	26.15 ± 2.60	0.21
Shin (cm)	31.19 ± 3.01	31.01 ± 3.50	0.21
Fat (%)	19.45 ± 0.06	24.15 ± 0.06	0.002
Fat (kg)	12.67 ± 5.01	14.18 ± 5.89	0.28
Active tissue (%)	80.55 ± 0.06	75.84 ± 0.06	0.002
Active tissue (kg)	51.39 ± 7.75	42.51 ± 5.99	<0.0001

Table 2. Association of the -13910 C>T polymorphism with anthropometric variables.

Parameter	CC (n = 61)	CT (n = 3)	p
	Mean ± SD	Mean ± SD	
Age	36.7 ± 13.6	31 ± 8.5	0.48
Height (m)	1.6 ± 0.1	1.6 ± 0.1	0.53
Weight (kg)	59.5 ± 11.2	70.5 ± 8.5	0.09
BMI (kg/m ²)	22.4 ± 3.5	27.7 ± 0.2	0.012
Waist (cm)	83.5 ± 11.1	104.2 ± 2.0	0.002
Hip (cm)	95.5 ± 8.9	93.3 ± 9.4	0.68
Chest (cm)	89.4 ± 9.1	87.4 ± 7.2	0.7
Forearm (cm)	26.5 ± 2.5	26.7 ± 3.1	0.91
Shin (cm)	31.1 ± 3.3	30.7 ± 4.2	0.82
Fat (kg)	13.5 ± 5.6	12.7 ± 5.4	0.81
Fat (%)	22.1 ± 6.3	19.7 ± 3.9	0.52
Active tissue (kg)	46.4 ± 8.1	49.9 ± 8.5	0.45
Active tissue (%)	77.9 ± 6.3	80.3 ± 3.9	0.52

The anthropometric analyses showed that 82.3% of women were at high risk of android obesity or were already obese. All men were at a risk of, or had, gynoid obesity.

On average, the men weighed 12 kg more than the women ($p = 0.03$) (Table 1). No significant differences were observed between the range of BMI values for women and the range for men. The BMI values indicated that 17.24% of men and 28.57% of women were underweight. Of the men, 24.14% were overweight, compared with 14.29% of women. Obesity was observed in 6.90% of men and 11.43% of women. The majority of the population was well nourished (51.72% men and 45.71% women). The Pearson's correlation coefficient values suggested a significant dependence between BMI and body density, total fat active tissue or waist circumference ($p < 0.0001$ for each correlation). Moreover, significant correlations were also observed between body mass and waist circumference ($r = 0.71$; 95% CI = 0.56–0.81; $p < 0.0001$) and between height and waist circumference ($r = 0.36$; 95% CI = 0.63–0.85; $p < 0.0001$).

Analysis of the LCT-13910C>T polymorphism (rs4988235) identified three subjects with the CT genotype (3.2%), whilst all others were homozygous for the C allele (96.8% of lactase non-persistent individuals) (Table 2). The CT genotype was present only in men, who showed a significantly higher weight, body mass index and waist circumference ($p = 0.009$, $p = 0.012$ and $p = 0.002$,

respectively), but did not differ in height, compared with the CC individuals (Table 2). In regards to the second polymorphism, i.e. C-14010 (rs4988233), all subjects had the GG genotype.

Discussion

The data were obtained from an interdisciplinary study of the *Shagia* tribe. Genetic and anthropological analyses of the *Shagia* people, from three isolated villages, were used to contribute to the conservation of the cultural heritage of the Nile Basin. This is an area of historical significance for the evolution of modern civilization. Our research is unique and cannot be repeated because all data was collected before the *Shagia* people from this region were expatriated due to the erection of the Merowe Dam. As a consequence of the new dam, their homeland was flooded by a newly created lake; therefore the *Shagia* from these villages moved in the different grounds occupied by the other tribes.

Our study showed that the *Shagia* men have slender bodies, whilst the women are stout. Analysis of the WHR index revealed that the *Shagia* women have android obesity, whilst gynoid obesity was found in all examined men. The study demonstrated that a strong build predominated within the *Shagia*, irrespective of gender. Both women and men had more subcutaneous fat on their arms than on their stomach or the areas around the shoulder blades. Despite uncomfortable living conditions, such as high temperatures of up to 50 °C, desert areas and a shortage of drinking water, the *Shagia* people of this region make excellent use of natural resources.

Our genetic analysis showed the presence of the CT genotype (3.2%) at the LCT-13910 locus, which is in contrast to the report by Bayoumi et al. (1981) who did not observe the T allele in the *Shagia* tribe. The discordant result may be due to the small number of participants in the former study ($n = 9$ vs $n = 126$ in the present study). Although the presence of the CT genotype was found in only three individuals in our study, we observed that these individuals had higher values for some basic anthropometric measurements, such as body mass and waist circumference. Our observations are in agreement with the results described by Corella et al. (2011).

Identification of the LCT-13910C>T polymorphism may indicate an association with the previously nomadic lifestyle of the *Shagia*. Enattah et al. (2007) showed that a high frequency of the LP phenotype is typical of shepherds that use the milk of farm animals. In Africa, the CT and TT genotypes of the LCT-13910C>T polymorphism can be found in tribes living a nomadic lifestyle, in shepherds, in farmers who breed cattle (e.g. the *Fulani* and the *Dogon*) and in populations residing near Cameroon (Enattah et al., 2007; Lokki et al., 2011). However, this polymorphism is absent in several groups from sub-Saharan Africa and does not occur in Nigeria, Malawi, Ethiopia and the larger part of Senegal (Mulcare et al., 2004). Thus, the difference in the frequency of occurrence is most likely to result from the history of animal domestication and dairy culture (Enattah et al., 2008).

All individuals had the GG genotype at the G/C-14010 locus, which is consistent with previously published reports (Tishkoff et al., 2007). In Afro-Asiatic populations, the C allele appeared ~2000–16 000 years ago. It is common in

Nilo-Saharan populations from Tanzania (39%) and Kenya (32%), but is absent in the *Hadza* populations (Tishkoff et al., 2007). Cavalli-Sforza et al. (1994) stated that the C allele appeared in Sudanese Nilo-Saharan populations after migration from southern Sudan. The C allele is ‘younger’ than the T allele, which is why it has not been found among the *Shagia* people. This is not surprising considering the lack of environmental pressures.

This study examined the genetic variability of a Sudanese tribe and provided a description of an ethnic group which had not previously been investigated. It is difficult to clearly determine the ethnic affiliation of the *Shagia* people, but the present study complements our previous data that described the admixture of European genes in the *Shagia* (Kempinska-Podhorodecka et al., 2012a,b, 2013). To further investigate the admixture level in this tribe, the use of more powerful biomarkers, such as the Y chromosome or mitochondrial DNA, will be necessary. Unfortunately, the nature of the research, the small amount of material and its unique character will make further investigations impossible. After this study the *Shagia* tribe was displaced from their territory due to the erection of the Merowe Dam. As a consequence, the area of the Fourth Nile Cataract was flooded by a newly created lake, which prevents any further work on the isolated *Shagia* people.

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Declaration of interest

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