ABSTRACT

Objectives. The environmental and life-style conditions of the Kola Sami could have influenced the population-specific frequencies of the AGXT Pro11Leu allele, and certain alleles of APOE and LCT genes, involved respectively, in the metabolism of animal proteins, lipids and milk sugar.

Study Design. DNA samples were collected from the Sami population of Lovozero settlement (Murmansk Region) in 2005.

Methods. The analysis of the traditional diet of the Kola Sami was made using the data of ethno-graphic studies conducted in the nineteenth and beginning of the twentieth centuries. Frequencies of the AGXT Pro11Leu, APOE*e4 alleles and LCT gene CC-1910 genotype were defined by molecular-genetic analysis.

Results. The specificity of the Kola Sami gene pool is in the lower frequency of APOE*e4 allele compared with the Sami of Finland (0.205 and 0.310, respectively) and when compared with other groups (except the Skolt) in the higher frequency of hypolactasia conditioned by the CC-13910 genotype of the LCT gene (0.484).

Conclusions. The high prevalence of the AGXT allele T bearers among Kola Sami (0.273) does not contradict the hypothesis of the adaptive role this allele plays in populations with a traditionally high intake of meat. (Int J Circumpolar Health 2008; 67(1):56-66)

Keywords: Sami, Kola Peninsula, diet, genetics, AGXT Pro11Leu, APOE, LCT
INTRODUCTION

The Sami (population of 1,991 individuals in 2002) of the Russian Federation inhabit the Kola Peninsula. The population includes 3 dialect groups: Kildin, Akkala and Teri Sami (up to the 1940s, the Western part of the Kola Peninsula had also been inhabited by the Skolt Sami, who later moved to Finland). Differences in life-styles between the representatives of these groups in the middle of the twentieth century became negligible; therefore, in this work, the existing groups are pooled as “Kola Sami.”

The Sami are a circumpolar indigenous people, whose complex physiological and genetic adaptations to the environment attract researchers (1). Their long-term adaptation to a specific “arctic” diet could be one of the factors that have determined the Sami gene pool.

Main ethnographic data concerning the cuisine of Kola Sami were obtained at the end of the nineteenth and beginning of the twentieth centuries (2–4). Consequently, when discussing the “traditional diet,” we mean the type of diet that the Kola Sami have had since the end of the nineteenth century.

The diet of the Kola Sami can be regarded as a variant of the traditional cuisine common for the people of circumpolar Eurasia, in spite of some adaptations made in the early eighteenth century (the most important to be mentioned are active use of imported flour and the development of “dairy reindeer farming”). This type of diet is characterised by a low diversity of products (5). As the analysis of ethnographic data shows, the cuisine of the Kola Sami at the end of the nineteenth and beginning of the twentieth centuries was based on approximately 30 local and 20 imported products (2–4,6,7).

The data on the average annual consumption of the main foods by a family (mean size of a Sami family in the mid–1920s was 7 persons) were presented in the works of F. Ivanov-Djatlov (3) and T. Lukjanchenko (6). Using these data, we estimated the annual consumption per capita (Table I). It should be taken into account, however, that the food composition varied during the year and the list in the table is not complete but contains only the main products. Therefore, we propose that the derived materials are suitable for a qualitative comparison of the Sami diet to the diets of other high-latitude populations.

| Kind of food          | Per family (7 pers.) | Per capita |
|-----------------------|----------------------|------------|
| Venison               | 820                  | 117        |
| Fish                  | 490                  | 70         |
| Wildfowl (mainly ptarmigan) | 57          | 8          |
| Flour                 | 820                  | 115        |
| Sugar                 | 82                   | 12         |
The listed rates of consumption are close to those common for the Nenets reindeer herders of circumpolar Europe in the 1920s and 1930s. In Nenets families, the consumption of venison (per capita) varied from 90 to 200 kg; flour from 80 to 160 kg; and the consumption of sugar averaged about 5 kg per capita per year. Poor families had the lowest supply level (8).

The Sami usually had small reindeer herds, comparable to those of the poor Nenets. In the 1860s, the Kola husbandries had mainly from 20 to 30 animals (9). In 1926–1927, 42% of herds comprised less than 25 reindeer, while 43% of owners had from 26 to 100 animals (10). At this level of herding, the necessity of having animals for transportation purposes and for reproduction of the herd meant it was a minor possibility to use reindeer as food.

It can be concluded that the reindeer-herding production was, among the Kola Sami, at a level common for the poor Eurasia herders. Moreover, venison was included in the Sami’s diet only during the winter season, from November to April (2). The estimated 117 kg per year (Table I) would provide during these 6 months 600–650 g of meat, fat and edible entrails per day.

In summer, the reindeer meat was almost totally substituted by fish. Here is a typical description provided by N. Kharuzin (2, p. 90): “In winter the Lapps almost always have venison, eaten fresh, salty, or dried. In summer, venison is out of the question, since reindeer are slaughtered only in winter, and the main food is fresh fish; which, in turn, is very seldom eaten in winter and is replaced by either salty or dried fish.”

In fact, fish were the second important source of animal protein for the Sami. This high level of fish was found in no other group of reindeer herders, except for some local groups of Nenets, especially poor ones (8). River and lake fish were the main source for the Sami, but they also caught some anadromous species, mainly herring and salmon. Marine species (cod) were fished in small amounts and only by a few coastal groups.

Gathering added diversity to the Kola Sami diet, but did not alter the ratio of the main nutrients considerably. Mushrooms were not considered an edible product and were eaten only in exceptional cases. At the end of the summer, the Sami collected and preserved berries (3), but the consumption is difficult to evaluate.

The Kola Sami consumed flour in amounts close to those reported for Nenets in the 1920s. Consumption of sugar among the Sami averaged 12 kg per year – similar to that of the Chukchi and Siberian Eskimo (Yupik) in the middle of the 1930s (8,11). Therefore, the amount and composition of carbohydrates in the Sami diet did not differ greatly from those of other reindeer herders in the first third of the twentieth century.

The question of whether the Sami used reindeer milk as an additional source of proteins, lipids and sugar (lactose) is of a special interest. As it is seen in the ethnographic literature, the reindeer dairy farming of the Scandinavian and Finnish Sami developed rather late (possibly in the eighteenth century) and did not spread across the Kola Peninsula (12,13). In particular, the 2 most numerous groups of Kola Sami, Skolt and Kildin, did not use reindeer milk as a food (14,15). Therefore, reindeer milk made no significant contribution to the nutrient intake. Similarly, as it was in other circumpolar groups, milk sugar (lactose) was available only to breastfed infants.
A quantitative evaluation of the traditional diet of the Kola Sami was made in the study conducted in 1926–1927 (3). It was a statistical survey of 80 families of Kola reindeer herders and fishers. We calculated the contribution of the main nutrients in the ration (see Table II), taking the physiological energy values of protein, fat and carbohydrate, respectively, as equal to 4.0, 9.0 и 4.0 kcal/g (16). The data allowed us to compare the diets of the Kola Sami and other circumpolar groups.

Intake of proteins (almost exclusively of animal origin) was as high in the Kola Sami as in other indigenous Arctic populations (Table III). Lipids contributed to the Sami diet considerably less than in the diet of other reindeer herders (Chukchi) and marine hunters (Greenland Inuit), but practically as much as in the diet of the Evenks. The subsistence of the latter – similar with that of the Sami – is based on a combination of reindeer herding with another food-producing activity (hunting in Evenks, fishery in Sami). The share of carbohydrates in the diet of the Kola Sami was rather high by the beginning of the twentieth century, close to the “European” level. Flour products supplied the main part of the carbohydrate intake, while consumption of sugars (mainly sucrose) was low.

It can be concluded that the Sami’s adjustment to local food resources included adapting to a high intake of animal protein and a moderate intake of fat (almost exclusively animal). It was not necessary to adapt to digesting various sugars, since natural sugars were consumed in negligible quantities and the variety of sugars consumed was poor (lactose-containing milk was available in noticeable amounts only during infancy). The low intensity reindeer herding could not provide a sufficient food supply, increasing the risk of hypocaloric stress.

These environmental and lifestyle conditions could influence the population-specific frequencies of the AGXT Pro11Leu allele and certain alleles of APOE and LCT genes; these genes are involved, respectively, in the metabolism of animal proteins, lipids and milk sugar.

The alanine-glyoxylate transferase gene (AGXT, OMIM 604285) controls the activity of the enzyme, catalysing the transformation of glyoxylate into glycine in the liver. In

| Table II. Average daily consumption of the main nutrients by the Kola Sami at the beginning of the twentieth century. |
|---------------------|-------|-------|-------|
| Daily consumption   | Proteins | Fats | Carbohydrates |
| In grams            | 207.95 | 54.25 | 448.85 |
| In calories         | 831.8  | 488.25 | 1795.40 |
| Per cent of daily energy intake | 26.7   | 15.7  | 57.6  |

| Table III. Caloric values of the main nutrients (per cent of daily energy intake) in the diet of circumpolar populations. |
|----------------------------------------------------------|-------|-------|-------|
| Population     | Subsistence     | Proteins | Fats | Carb. | Reference |
| Kola Sami      | Herders / fishers | 27     | 16   | 57    | 3        |
| Evenki         | Hunters / herders | 33     | 19   | 48    | 17       |
| Chukchi        | Reindeer herders  | 30     | 32   | 38    | 18       |
| Greenland Inuit| Marine hunters   | 26     | 37   | 37    | 19       |
humans, the enzyme tends to be localized in peroxysomes.

However, some nucleotide substitutions in the AGXT gene (resulting in the substitution of amino acid in the protein) alter subcellular localization and transfer up to 90% of enzymes to mitochondria. This induces the formation of oxalate in peroxysomes from the accumulated glyoxylate. Oxalate deposits in the form of calcium oxalate can lead to the development of kidney stone disease (20). In mammals, the mitochondrial enzyme localization is common in carnivores, while the peroxisomal localization is common in herbivores (21). It was suggested that the transfer of some amount of enzymes to mitochondria, induced by the substitution of Proline-11 with Leucine in the signal peptide of the protein (Pro11Leu polymorphism), may be adapted by humans whose diet is high in meat (22).

Apolipoprotein E gene (APOE, OMIM 603202) encodes the protein involved in the metabolism and transport of lipids. There are 3 common APOE gene alleles e2, e3 and e4 (for a review, see 23). The APOE*e4 allele in modern populations of Europeans is associated with high plasma cholesterol and LDL cholesterol levels, and is considered a risk factor for Alzheimer’s disease and coronary heart disease (23,24). However, in the groups with a traditional life-style and subsistence diet, the e4 allele is not associated with higher cholesterol level (25).

The lactase gene (LCT, OMIM 107741) determines the age-persistent or restricted lactase activity. Lactase persistence is associated with C->T transition in position -1910 upstream LCT gene (26). The individuals with the CC-1910 genotype have gradual decline of enzyme production with age – restricted lactase synthesis or hypolactasia. Following up-to-date data, the frequency of hypolactasia in European populations (Finnish, Swedish, Russian) is agrees with the frequency of CC_{1910} genotype (26–28). Lactase persistence is prevalent in groups living in the temperate zone and practising dairy farming (29). Restricted lactase activity is the evolutionary initial variant, common for all mammals and found in most non-Europeans and in almost all circumpolar human populations.

The aim of our research is to analyse the allele frequencies of the metabolism-related genes AGXT, APOE and LCT in the Kola Sami in comparison with other populations.

MATERIAL AND METHODS

The physical-anthropological study of the indigenous population of the Kola Peninsula was performed in 2005. The study agreed with the Russian Association of Indigenous Peoples of the North (RAIPON). Biological samples were collected in the group of Kola Sami living in Lovozero village (Murmansk Region). The biological samples collected were buccal swabs in children and venous and capillary blood samples in adults. In schoolchildren, the samples were collected in collaboration with the Lovozero District Department of Education. The blood samples were collected by the personnel of the Lovozero District Hospital during medical and/or diagnostic procedures for adult patients without chronic diseases. In all cases, the samples were collected with the patients’ informed consent.

DNA was prepared from buccal swabs using commercial DNA Isolation Kit (Isogene Moscow, Russia), and from peripheral blood
samples using a standard phenol/chloroform extraction and the isopropanol precipitation method.

The *LCT* C/T<sup>1910</sup> genetic polymorphism was analysed using a real-time PCR assay based on fluorescence resonance energy (27,30).

The *APOE* e2, e3 and e4 alleles genotyping was carried out using PCR amplification and digestion of PCR products with the Hha I endonuclease restriction according to the method of Hixon and Vernier (31).

The genotyping of the Pro11Leu polymorphism of *AGXT* gene (previously described as C154T) was carried out according to the method of Caldwell et al. (22).

The contemporaneous non-indigenous residents of Murmansk Region have their origins in the various territories and ethnic groups of the Russian Federation. An intensive population inflow to the Kola Peninsula began in 1915. During the second half of the twentieth century, the region went through several waves of migration, which resulted in a significant genetic transfusion. The gene pool of the contemporaneous non-indigenous population does not reflect neither regional nor ethnic specificity. Therefore, we have chosen the Kostroma Russians as a reference population. The northern Kostroma Region in European Russia was less affected by migration than the Kola Peninsula. The Kostroma Russians are a group of “Northern Russians” that began to settle along the White Sea Coast in the fourteenth and fifteenth centuries.

We collected samples from 64 individuals, both of whose parents self-identified as Kola Sami, and from 108 Kostroma Russians. Since not every sample allows extracting all the target-genes, we indicate the number of samples for every gene in our results.

### RESULTS

The frequencies of the studied genotypes and alleles in Kola Sami are shown in Table IV.

For all the tree genes studied – *LCT*, *AGXT* and *APOE* – the observed genotype frequencies in the Sami sample are in accordance with the Hardy-Weinberg equilibrium.

The allele and genotype frequencies of the *LCT* and *APOE* genes in the Kostroma Russians have been defined previously, but they were unknown for the *AGTX* gene. That is why in this study we accomplished the genotyping of the Pro11Leu (C154T) polymorphism of the *AGXT* gene in the sample of 108 Kostroma Russians. It was found that the CC, CT and TT genotypes have 7, 8 and 5 individuals, respectively. The T allele frequency is 0.176±0.026, and the C allele

| Genotypes: | LCT (n=64) | Genotypes: | AGXT (n=54) |
|-----------|-----------|-------------|-------------|
| CC (n=31) | 0.484     | CC (n=32)   | 0.593       |
| CT (n=27) | 0.422     | CT (n=20)   | 0.370       |
| TT (n=6)  | 0.094     | TT (n=2)    | 0.037       |
| Alleles:  |           | Alleles:    |             |
| C         | 0.70±0.04 | C           | 0.778±0.040 |
| T         | 0.30±0.04 | T           | 0.222±0.040 |

| Genotypes: | APOE (n=56) |
|-----------|-------------|
| e2/e2 (n=0) | 0.000       |
| e2/e3 (n=6)  | 0.107       |
| e2/e4 (n=3)  | 0.054       |
| e3/e3 (n=29) | 0.518       |
| e3/e4 (n=16) | 0.286       |
| e4/e4 (n=2)  | 0.036       |
| Alleles:    |             |
| e2         | 0.080±0.026 |
| e3         | 0.714±0.042 |
| e4         | 0.205±0.038 |
frequency is 0.824±0.026. The distribution of genotypes is in accordance with the Hardy-Weinberg equilibrium.

**DISCUSSION**

**Alanine-glyoxylate transferase gene AGXT**

The frequency of the *AGXT* allele T (which corresponds to the *AGXT*<sup>Pro11Leu</sup> minor allele, encoding Leu in position 11) in the Kola Sami is very close to the frequency of this allele in the Swedish Sami – 0.279 (22). The other groups in the neighbouring regions have considerably lower *AGXT*<sup>Pro11Leu</sup> frequencies: 0.197 in Norwegians (22) and 0.176 in Russians (Table V).

**Apolipoprotein E gene APOE**

In Europe, the frequencies of the allele *APOE*e4 increase northward: from 0.05–0.08 in Mediterranean populations to 0.20 in Scandinavian populations (34,35,36). The frequency of *APOE*e4 allele in the Kola Sami is 1.5 times lower than in the Finnish Sami, having the highest proportion of the allele in Europe: 0.31 (37). However, according to the “European scale,” the allele e4 prevalence in the Kola Sami is rather high and conforms to the data for other indigenous circumpolar populations of Eurasia: 0.12–0.22 (Table VI).

### Table V. Frequency of AGXT allele T (*AGXT*<sup>Pro11Leu</sup>) in Sami and other populations.

| Population              | n   | Allele T frequency | Reference |
|-------------------------|-----|--------------------|-----------|
| Kola Sami               | 54  | 0.222              | This work |
| Sami (Sweden)           | 34  | 0.279              | 22        |
| Norwegians              | 76  | 0.197              | 22        |
| Russians (Kostroma)     | 108 | 0.176              | This work |

### Table VI. APOE allele frequencies in Sami and other populations.

| Population                      | N   | APOE alleles | Reference |
|---------------------------------|-----|--------------|-----------|
| Kola Sami                       | 56  | 0.080        | 0.714     | 0.205  | this work |
| Sami of Finland                 | 70  | 0.050        | 0.640     | 0.310  | 37      |
| Khanty, Mansi (WSiberia)        | 36  | 0.030        | 0.780     | 0.190  | 38      |
| Komi-Izhems (North Komi)        | 66  | 0.045        | 0.735     | 0.220  | 33      |
| Evenki, Siberia                 | 124 | 0.004        | 0.843     | 0.153  | 39      |
| Evenki, Vanavara (Siberia)      | 41  | 0.037        | 0.841     | 0.122  | 33      |
| Chukchi (Chukotka)              | 89  | 0.034        | 0.764     | 0.202  | 33      |
| Finns (Helsinki)                | 2087| 0.062        | 0.760     | 0.178  | 40      |
| Finns (Finland, 5 areas)        | 1577| 0.390        | 0.767     | 0.194  | 41      |
| Norwegians (Norway)             | 798 | 0.058        | 0.744     | 0.198  | 42      |
| Swedes (Huddinge)               | 407 | 0.078        | 0.719     | 0.203  | 43      |
| Russians (Kostroma)             | 79  | 0.133        | 0.728     | 0.139  | 33      |
| Russians (Moscow)               | 205 | 0.080        | 0.800     | 0.120  | 44      |
| Russians (St. Petersburg)       | 403 | 0.108        | 0.773     | 0.119  | 45      |
The lower APOE*e4 frequency in the Kola Sami compared with the Sami of Finland may result from the Kola Sami’s recent genetic mixing through intermarriages with the Russians, in which populations the prevalence varies from 0.12–0.15 (33,44,45).

**Lactase gene (LCT)**

The frequency of the LCT gene CC\textsubscript{13910} genotype in the Kola Sami is very close to the frequency of hypolactasia (lactase restriction) – 48%, estimated earlier by the lactose-tolerance test (12). The similarity of genotype and phenotype frequencies confirms the hypothesis that genotype CC\textsubscript{13910} determines hypolactasia in Sami, as in other European populations (Table VII).

The highest prevalence of the hypolactasia phenotype among the Sami groups was found in Skolt (60%), and the lowest (25%) in Inari “Fisher” Sami (46). The Kola Sami with 48% of hypolactasia are “in the upper part of Sami list.” However, when compared with other circumpolar peoples, Sami populations have rather low frequencies of lactase restriction. For example, the hypolactasia phenotype was found in more than 70% of Khanty and Mansi from Western Siberia (51), and the frequency of the LCT CC\textsubscript{13910} genotype in Chukchi is 0.98 (27).

Our analysis has shown that the level of consumption of reindeer milk by the Sami could not considerably alter the selective pressure to favour the bearers of the lactase persistence phenotype (13). We suggest that the main factor changing the initial frequency of the LCT gene CC\textsubscript{13910} variant in various groups of Sami is the genetic mixing with the neighbouring populations: Norwegians and Finns in Scandinavia and Finland, and Slavonic people (mainly Russians) on the Kola Peninsula.

**Conclusions**

The presented data show that the allele frequencies of the metabolic-related genes (APOE, AGXT, LCT) in the Kola Sami are close to those in other Sami groups. The specificity of the Kola Sami gene pool is in the lower frequency of APOE*e4 allele compared

| Ethnic group, country/region | Phenotyped Samples | Genotyped Samples |
|-----------------------------|-------------------|------------------|
|                             | Lactose intolerance | CC\textsubscript{13910} | genotype |
| Kola Sami                   | 50 0.48            | 12 0.484          |
| Skol Sami                   | 176 0.60           | 46 --             |
| Mountain Sami (Inari)       | 75 0.37            | 46 --             |
| Mountain Sami (Utsjoki)     | 158 0.34           | 46 --             |
| Inari “Fisher” Sami         | 110 0.25           | 46 --             |
| Finns                       | 315 0.17           | 46, 47 0.181     |
| Swedes (Finland)            | -- 0.077           | 46 0.076         |
| Swedes                      | -- 0.01-0.07       | 46 0.051         |
| Norwegians                  | -- No data         | -- 0.038         |
| Russians                    | 136 0.37\textsuperscript{1} | 48 0.36\textsuperscript{2} |

\textsuperscript{1}Moscow region.
\textsuperscript{2}Kostroma.
with the Sami of Finland (0.205 and 0.310, respectively) and, in comparison with other groups (except the Skolt), in the higher frequency of hypolactasia conditioned by the \( CC_{-13910} \) genotype of the \( LCT \) gene. We suppose that these peculiarities have resulted from the gene inflow from the Slavonic (mainly Russian) inhabitants of the Kola Peninsula. The \( APOE^{*}e4 \) allele frequency in Russians is lower than in Finns and Norwegians (Table VI), and the frequency of the \( CC_{-13910} \) genotype in Russians is higher (Table VII).

Consequently, as a condition of mixing with the neighbouring populations, in the Fennoscandian groups of Sami one could expect a smaller decline of the \( APOE^{*}e4 \) allele frequency as compared with the Kola Sami and a faster decline of the \( C_{-13910} \) allele frequency. The outcomes of our study are in the full agreement with this assumption.

The high prevalence of the \( AGXT \) allele \( T \) bearers among Sami does not contradict the hypothesis of the adaptive role this allele plays in populations with a traditionally high intake of meat (22). The allele \( T \) frequencies in Russian and Norwegian populations are close to each other. Therefore, it is impossible to assess the influence of the gene inflow to the Sami from these populations, if it existed, by the changes in the allele \( T \) prevalence.

As a whole, the study results are in strong agreement with the data of nutritional anthropology. The allele frequencies of the \( APOE, AGXT \) and \( LCT \) genes reflect the high adaptability of the Kola Sami to a diet based on circumpolar hunting, fishing and reindeer herding.

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