

PREVALENCE OF THE CCR5 DELTA32 MUTATION IN THE TUNDRA NENETS OF YAMAL

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Resume. CCR5 delta 32 is a genetic variant of CCR5 – deletion mutation of a gene that has a specific impact on the adhesive function of T cells. It is believed that this mutation arose approximately two and a half thousand years ago and eventually spread in Europe. It has been hypothesized that this allele was favored by natural selection during the Black Death. In the heterozygotes, this mutation strongly reduces the chance of HIV infection. In the homozygotes, it completely protects against HIV infection. CCR5 delta32 mutation in the heterozygous state occurs in Europe with a frequency of 5–14 %. The incidence of CCR5 delta32 outside of Europe is very low, and in many non-European ethnic groups it is completely absent. The prevalence of this mutation in the population of the Nenets has not previously been investigated. It was assumed to have a lower frequency than in Europeans. We carried out an investigation with 54 blood samples of Nenets living in Yamalo-Nenets Autonomous District. DNA was extracted with QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer protocol. To identify the CCR5 delta32 deletion polymerase chain reaction was conducted using primers: CCR5-D32-F: 5'CTTCATTACCTGCAGTC3', CCR5-D32-R: 5'TGAAGATAAGCCTCACAGCC3' under the following conditions: 95°-5'x1; 95°-15"→55°-15"→72°-60"x40; 72°-10"x1→4°-∞. Products of the reaction were separated in 2 % aga-rose gel for 1.5 hours. Gel documentation was performed using a Gel Doc XR Plus (Bio-Rad, USA). The prevalence of CCR5 delta32 mutation in Nenets was found to be 9.3 %. This was a higher than expected frequency, and it corresponds to the average prevalence of CCR5 delta32 mutation in Europe. Our finding suggests possible European origin of the Nenets.

Key words: CCR5 delta32; Nenets; ethnic groups.

INTRODUCTION

CCR5 delta 32 is a genetic variant of CCR5 receptor with 32 nucleotides deletion that affects its ligands binding. CCR5 is recognized as a number of cytokines including RANTES, MIP-1α and MIP-1β. These cytokines are expressed by immune cells, including NK-cells, T-lymphocytes, macrophages, and play a crucial role in the migration and activation of immune cells. Thus, any change in the sequence of the CCR5 gene or its expression can be associated with a dysfunction of the immune system and the development of autoimmune diseases [3]. CCR5 delta 32 mutation reduces number of CCR5 receptors on the surface of T lymphocytes. The CCR5 expression levels influence migration of inflammatory cells into the synovium and, as a result, the susceptibility to juvenile idiopathic arthritis [4]. In the heterozygotes this mutation significantly reduces HIV infection and in the homozygotes it fully protects against HIV infection [12]. Meta-analysis data indicated that CCR5 delta32 polymorphism might act as a protective factor in type 1 diabetes development, and as a risk factor for Kawasaki disease and Behcet's disease [5, 10].

Coalescence theory analysis of modern haplotype genealogy placed the origin of the CCR5 delta32-containing ancestral haplotype at about 700 years ago, with an estimated range of 275–1875 years [11] in one study and 700–3500 years ago in the other study [8], and eventually spread to Europe. Analysis of all available data

on CCR5 delta32 frequencies in the Old World allowed for construction of a geographical gene map to analyze possible correlations between allele frequencies and eight climatic-geographic parameters [6]. A strong positive correlation was found between the allele frequency and latitude, a strong negative correlation with annual radiation balance, and a weaker negative correlation with longitude. Exclusion of latitude reduced the negative correlation between the allele frequency and annual radiation balance, but it still remained large and significant. The existence of correlations between the cline of CCR5 delta32 frequencies and climatic-geographic parameters suggest for an effect of either natural environmental factors or large-scale population movements on the distribution of this allele [6].

Distribution of CCR5 gene mutations in populations has distinct ethnic and racial features. The geographic cline of CCR5 delta32 frequencies and its recent emergence are consistent with a historic strong selective event such as an epidemic of a pathogen similar to HIV-1 that utilizes CCR5 therefore driving its frequency upward in ancestral Caucasian populations (Table 1) [11].

Frequency of CCR5 delta 32 allele in 16 European populations was found to form a gradient from north to south with the highest frequency of the allele in Denmark and lowest in Corsica [7]. Prevalence of CCR5 delta 32 outside of Europe is extremely low and in many non-European ethnic groups it is completely absent [9].

MATERIALS AND METHODS

The materials for the study were 54 blood samples (dried on filter paper) of Tundra Nenets children living in boarding Seyaha village, Yamal-Nenets Autonomous District.

DNA was isolated using QIAamp Mini Kit (QIAGEN) according to the attached protocol. To identify deletions CCR5 delta32 used polymerase chain reaction with the flanking primers: CCR5-D32-F: 5'CTTCATTACACCTGCAGTC3', CCR5-D32-R: 5'TGAAGATAAGCCTCACAGCC3' under the following conditions 95°-5'x1; [95°-15»→55°-15»→72°-60»]x40; 72°-10'x1→4°-∞; products of the reaction were separated on 2% agarose gels for 1.5 hours; gel documentation was carried out using a Gel Doc XR Plus (Bio-Rad). As a result of PCR amplification are produced two fragments 196 bp — wild type and 164 bp — delta32 mutation (Figure 2).

RESULTS

CCR5 delta 32 mutation in heterozygous form was detected in 5 children, and homozygous mutations were not found at all. Therefore we found the prevalence of heterozygous form of CCR5 delta 32 mutation in Nenets population to be 9.3%.

DISCUSSION/CONCLUSION

Prevalence of CCR5 delta32 mutation was expected to be significantly lower than among Europeans. However, the prevalence of the studied allele among Nenets living in the Yamal-Nenets Autonomous District, was close to the average prevalence among Europeans.

Nenets belong to the so-called “Samoyed” people (corrupted self-reference *Saamoa*) which is the same as *Saami* (formerly Lapps or Lapons) in Finland (*Suomi* — the Finnish name for Finland), or transliterated into English *Samodi*.

Saamoa speak the Samoyedic language which is a branch of the Uralic language family and it is known they moved from farther south in Siberia to the northernmost part of what later became Russia before the 12th century [12].

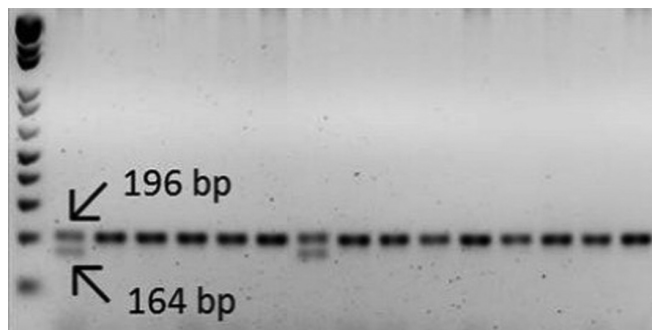


Fig. 2. Results of gel documentation of PCR

It is known that the allele CCR5 delta32 is rare in African, Asian, Middle Eastern, and American Indian populations, suggesting its recent origin [11]. It is assumed that high frequency of CCR5 delta32 mutation in Europe is a results of strong selection from bubonic plague (11). This hypothesis is popular in population genetic and medical literature, though quantitative assessment is absent. Three lines of evidence (predictions from a population genetic model, the geographical distribution of the allele, and the clinical effect of the deletion) indicate that the smallpox *Variola major* virus is a more likely candidate [13].

It is known that the population of people living in the northernmost part of Siberia declined tremendously after several outbreaks of smallpox [14], though little is known about the outbreaks of plague [14].

Our results support the hypothesis that the high frequency of the variant of this allele in European and in Yamal populations arose through strong selection from rather smallpox than from plague.

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- ♦ **Resume.** CCR5 delta32 — генетический вариант CCR5, представляющий собой делецию 32 пар оснований, который приводит к нарушению адгезивных свойств кодируемого ею белка CCR5 Т-клеток. Предполагается, что эта мутация возникла примерно две с половиной тысячи лет назад и со временем распространилась в Европе. В гетерозиготном состоянии эта мутация сильно уменьшает шанс инфицирования клетки ВИЧ, в гомозиготном, по-видимому, приводит к полной невозможности инфицирования ВИЧ. Мутация CCR5 delta32 в гетерозиготном состоянии встречается в Европе с частотой 5–14 %. Встречаемость CCR5 delta32 за пределами Европы крайне мала, во многих неевропейских этнических группах она полностью отсутствует. Распространенность данной мутации в популяции ненцев ранее не исследовалась, предполагалось, что она должна быть ниже, чем у европейцев. Нами было проведено исследование 54 образцов крови ненцев, проживающих на территории Ямало-Ненецкого автономного округа. Для выделения ДНК использовался QIAamp DNA Mini Kit (QIAGEN) в соответствии с прилагаемым протоколом. С целью идентификации делеции CCR5 delta32 применялась полимеразная цепная реакция с применением праймеров: CCR5-D32-F: 5'CTTCATTACACCTGCAGTC3', CCR5-D32-R: 5'TGAAGATAAGCCTCACAGCC3' при следующих условиях: 95°-5'x1; 95°-15»→55°-15»→72°-60»x40; 72°-10'x1→4°-∞; продукты реакции были разделены в 2%-м агарозном геле в течение 1,5 часов; гель-документирование проводилось с помощью Gel Doc XR Plus (Bio-Rad, США). По результатам проведенного исследования распространенность исследуемой мутации среди ненцев составила 9,3 %. Таким образом, распространенность мутации CCR5 delta32 среди ненцев, проживающих в Ямало-Ненецком округе, соответствует ее распространенности у европейцев. Это может свидетельствовать о европейском происхождении ненцев.
- ♦ **Key words:** CCR5 delta32; ненцы; этнические группы.

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