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Research paper

The genetic profile of susceptibility to infectious diseases in Roman-Period populations from Central Poland

Magda Lewandowska *, Krystyna Jędrychowska-Dańska, Alicja Zamerska, Tomasz Płoszaj, Henryk W. Witas

Department of Molecular Biology, Faculty of Biomedical Sciences and Postgraduate Education, Medical University of Lodz, Lodz, 90-136, Poland

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ABSTRACT

For thousands of years human beings have resisted life-threatening pathogens. This ongoing battle is considered to be the major force shaping our gene pool as every micro-evolutionary process provokes specific shifts in the genome, both that of the host and the pathogen. Past populations were more susceptible to changes in allele frequencies not only due to selection pressure, but also as a result of genetic drift, migration and inbreeding. In the present study we have investigated the frequency of five polymorphisms within innate immune-response genes (*SLC11A1* D543N, *MBL2* G161A, *P2RX7* A1513C, *IL10 A*-1082G, *TLR2* –196 to –174 ins/del) related to susceptibility to infections in humans. The DNA of individuals from two early Roman-Period populations of Linowo and Rogowo was analysed. The distribution of three mutations varied significantly when compared to the modern Polish population. The TAFT analysis suggests that the decreased frequency of *SLC11A1* D543N in modern Poles as compared to 2nd century Linowo samples is the result of non-stochastic mechanisms, such as purifying or balancing selection. The disparity in frequency of other mutations is most likely the result of genetic drift, an evolutionary force which is remarkably amplified in low-size groups. Together with the F_{ST} analysis, mtDNA haplotypes' distribution and deviation from the Hardy-Weinberg equilibrium, we suggest that the two populations were not interbreeding (despite the close proximity between them), but rather inbreeding, the results of which are particularly pronounced among Rogowo habitants.

1. Introduction

Pathogens have constituted one of the strongest agents driving natural selection in humans for thousands of years (Fumagalli et al., 2011; Karlsson et al., 2014). The necessity to resist diseases was the major force shaping our past gene-pool. Alongside the development of humans' adaptability to hostile environments, pathogens were simultaneously undergoing a number of evolutionary changes - a process which is jointly referred to as host-pathogen coevolution. The process of this coevolution is still ongoing, however, in the past, the selection pressure of infectious diseases on the human genome was significantly higher. As all micro-evolutionary processes generate changes in allele frequencies (Sandoval-Castellanos, 2010), it may be assumed that gene variants which decrease fitness in specific environments should change their frequency upon events of selection.

The genomic signatures of natural selection have so far been studied mainly in modern populations using methods identifying a strong correlation between allele frequencies and a hypothesized selection driver, elevated linkage disequilibrium, reduced variation, unusually recent allele-age estimates or escalated rates of interpopulation and interspecies divergence (Jensen et al., 2016; Wilde et al., 2014). Of particular interest are the recent studies based on computer simulations modelling likely past events (Currat and Silva, 2013) or tracing selective sweeps (Racimo, 2016). However, the methods based on modern population genomic data have certain limitations, as it is not possible to distinguish actual selection signatures from patterns of variation provoked by other factors resembling natural selection, among which are past demographic processes, population size and structure changes or background selection (Jensen et al., 2016; Wilde et al., 2014). With the recent advance in ancient DNA (aDNA) studies resulting both from technological development and more comprehensive knowledge of the nature of ancient genetic material, it is now possible to seek signatures of local positive selection based on the simple assumption that a selected allele changes its frequency more rapidly than a neutral locus (Olalde and Lalueza-Fox, 2015). In 2014 Wilde et al. combined the testing of frequency changes of alleles involved in skin, hair and eye pigmentation with the forward simulation approach in Eneolithic and Bronze Age samples from Eastern Europe. Their findings suggest that depigmentation variants widespread in modern populations are the result of the combination of selective pressures, changes in diet and assertive mating, with selection coefficients ranging from 2 to 10%, which constitutes one of the strongest signals of recent selection in humans (Wilde et al., 2014). Using a similar molecular approach, researchers have recently reported other

* Corresponding author at: Department of Molecular Biology, Lodz 90-136, Poland.
E-mail address: magda.lewandowska@stud.umed.lodz.pl (M. Lewandowska).

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signals of indigenous positive selection such as: polymorphism adapting to high altitudes in Andean Peruvians (Fehren-Schmitz and Georges, 2016), lactase persistence (Sverrisdottir et al., 2014; Witas et al., 2015) or mutations in the *DRD4* connected to novelty-seeking in pre-Columbian South America (de Rubira et al., 2016). Furthermore, several aDNA studies have so far been focused on polymorphisms in the immune system such as assessing the evolutionary history of TLR4 variants in Europe (Plantinga et al., 2012) or genotyping the la Braña 1 individual (Olalde et al., 2014).

In the present study we focus on the assumption that frequency changes of an allele under environment-specific selection are faster than those of alleles under neutrality (Olalde and Lalueza-Fox, 2015). From an evolutionary perspective, the genes of innate immune response are worthy subjects for such studies, as this immunity is fully hereditary, which means than any mutations will remain in the population gene pool unless they are removed by stochastic or directional evolutionary force. In contrast, adaptive immunity provides a broader range of protection, being both heritable and adaptable during the lifespan however, the acquired benefits are not passed down to future generations (Meyer et al., 2016). We chose four single nucleotide polymorphisms (SNPs) and one deletion in genes involved in innate immune response that have been proven to influence the susceptibility to mycobacterial diseases in Caucasians (Hart and Tapping, 2012; Li et al., 2011; Mokrousov et al., 2008; Velez et al., 2010; Zhang et al., 2011). Mycobacteria, such as *M. tuberculosis* or *M. leprae* are pathogens with a long-standing relation to *Homo sapiens,* which makes them likely to drive the natural selection of their hosts' resistance. However, as the innate immune response is not only the first line of defence in fighting against a wide range of pathogens but also play a crucial role in autoimmune disorders (Wen and Wong, 2005) or cancer combating (Liu and Zeng, 2012) it remains evident that possible changes in allele frequencies over time cannot be indisputably assigned to any single selection event. Moreover, it bears stressing that human susceptibility to infectious diseases is affected by other, non-genetic factors, such as climate, dietary deficiencies, stress, trauma and population density (Spigelman et al., 2015). Nevertheless, here we decided to focus on genetic polymorphisms that influence the response to intracellular bacteria as infectious diseases have accompanied humans since the dawn of their history with possible multiple selection events, while the immune response to mycobacteria is proven to be highly dependent on an individual's genotype.

The first studied allele is *SLC11A1* G1730A (rs17235409) in the gene coding for solute carrier family 11 number 1, formerly known as *NRAMP1*, located on 2q35, which is one of the genes most extensively studied for susceptibility to infectious diseases. It encodes a divalent cation transporter recruited to the phagolysosomal membrane during phagocytosis in macrophages and neutrophils (Gruenheid et al., 1997). Its main role is transporting cations out of the phagosome, which limits its accessibility to pathogens (Jabado et al., 2000), NO production, inducing apoptosis and decreasing *IL10* expression (Rojas et al., 1997). A missense mutation in the 543 codon (D to N) results in the impaired functionality of the transporter and limited bactericidal capability. Studies on Chinese Han populations reported D543N heterozygosity as a possible susceptibility factor for tuberculosis (TB) development (Tang et al., 2012) which is consistent with the finding of the D543N's influence on visceral leishmaniasis susceptibility in the Moroccan population (Ejghal et al., 2014). The mutated allele is rare in European populations ranging between 0.5 and 2%, which - along with its conservative character and role in the immune response - could point to recent or long-lasting purifying selection. The next analysed polymorphism is within the *MBL2* gene coding for mannose-biding lectin, located on 10q11. This calcium-dependent C-type lectin binds to the repetitive carbohydrate structures of pathogens and subsequently opsonizes them, promotes phagocytosis and activates the complement system (Bellamy, 2005; Hoal, 2002). A non-conservative polymorphism at codon 54 (G161A, rs1800450) leads to the production of non-functional and unstable protein inhibiting formation of the MBL tetramer, which is necessary for non-specific immune response (Turner, 2003). *MBL2* polymorphisms have been associated with several bacterial infections rather than with specific pathogens (Summerfield et al., 1995), most likely due to highly conserved character of *MBL2* gene among species (Verga Falzacappa et al., 2004). Furthermore, we focused on *P2RX7*, a gene located on the 12q24 which codes the P2X7 receptor. The protein product is expressed in human macrophages and has multiple antibacterial functions. When the P2X7 receptors are activated by the ATP, a cation-selective channel opens and Ca^{2+} enter the macrophage, which activates caspase cascade and results in apoptosis (Ferrari et al., 1999). Moreover, it activates phospholipase D which promotes phagosome-lysosome fusion and elimination of mycobacteria (Fairbairn et al., 2001). Change in exon 13 in the 1513 position (A1513C, rs3751143) leads to a change of the glutamic acid at position 496 to an alanine in the C-terminus of the receptor, which results in the complete loss of protein function in homozygotes and a partial loss in heterozygotes (Fernando et al., 2007). The mutation has been positively correlated with a number of conditions, such as osteoporosis (Husted et al., 2013) or chronic lymphocytic leukaemia (Wiley et al., 2002); however, it has so far been most strongly correlated with susceptibility to TB (Ben-Selma et al., 2011; Fernando et al., 2007; Mokrousov et al., 2008; Nino-Moreno et al., 2007). For the present study, we selected also SNP in the *IL10* gene located on the long arm of chromosome 1, encoding interleukin 10, a major anti-inflammatory cytokine produced predominantly by monocytes upon the pathogenic flora (Said et al., 2010). Its main functions include T-cell suppression, macrophage deactivation and interference with antigen-presenting cells. Several polymorphisms within the promoter region have been proven to alter the level of cytokine expression. A-1082G (rs1800896) has been associated with a wide range of conditions varying in different populations, however, in Europeans it is mainly associated with decreased susceptibility to tuberculosis in a dominant way (Zhang et al., 2011). The last studied polymorphism is 23-bp insertion/deletion in *TLR2* gene coding Toll-like receptor 2. Members of the TLR family belong to a wide group of pattern recognition receptors (PRR) and occur both intra- and extracellularly. In the presence of agonist TLR1 forms a heterodimer with TLR2 and this complex recognizes components of mycobacteria, such as ML.1966 or LpqH which initiates transmission of the signal to a macrophage (Krutzik et al., 2003) and activates NF-κB resulting in the expression of various pro-inflammatory cytokines. 23 bp insertion/deletion (−196 to −174 ins/del, rs111200466) occurs in the gene promoter upstream to the start codon (Hart and Tapping, 2012). The deletion allele lowers transcriptional activity and inferior IL-8 production. Velez et al. revealed that in Caucasians 23 bp deletion results in a reduced expression of the gene and increased sensitivity to mycobacterial diseases while homozygotes for insertion are fully functional and protect against bacterial infections (Velez et al., 2010).

The aim of this study was to test the null hypothesis that the frequency of polymorphisms in innate immune genes associated with susceptibility to infectious disease differs between past populations inhabiting Central Poland from the Roman Period and the present-day Polish population. Moreover, we aim to investigate the mechanism of alleles' profile changes, if any, considering recent local selection events, the increased power of genetic drift in low-size groups or interbreeding in subdivided populations as possible phenomena involved in the remodelling of the gene pool.

2. Materials and methods

The samples included in the study were unearthed at two archaeological sites: Rogowo ($n = 29$) and Linowo ($n = 16$), both of which were settlements from the early Roman Period according to tomb equipment dating to 2nd-3rd century and located as shown on Fig. 1. The selection of the samples for the presented study resulted from the excellent condition of preservation of bone material and the abundance