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Extremely high frequency of autoimmune-predisposing alleles in medieval specimens*

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Abstract: The precise etiology and reasons for the increase in incidence of autoimmune disorders still remain unclear, and although both genetic and environmental factors have been proven to shape individual predisposition, it is not known which of the factors, if not both, is responsible for the boom observed during the last decades. In order to establish whether a higher frequency of autoimmune-predisposing alleles may explain this increase we took advantage of ancient DNA methodology to establish the genetic predisposition, conferred by cytotoxic T lymphocyte associated antigen-4 (*CTLA4*) +49A/G and human leukocyte antigens (*HLA*) *DQB1*^{57}, in population inhabiting Poland in the Middle Ages. After successful typing of 42 individuals from a 12th~14th's century archeological burial site, we found that frequencies of the predisposing alleles in the medieval population were higher than they are at present, suggesting thus that the recently observed incidence increase results most probably from factors of other than genetic nature.

Key words: Ancient DNA (aDNA), Autoimmunity, Cytotoxic T lymphocyte associated antigen-4 (*CTLA4*) gene, *HLA DQB1*, Type 1 diabetes (T1D)

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INTRODUCTION

It is estimated that up to 3%~5% of the world's population is affected by at least one disorder that results from autoimmunity (Marrack *et al*., 2001), and the incidence of at least some of the autoimmune diseases has been growing steadily for the last decades (Onkamo *et al*., 1999; Ivarsson *et al*., 2000; Haynes *et al*., 2004). It is estimated that autoimmune-related disorders are the third (after cancer and atherosclerosis) cause of mortality and morbidity in developed countries (Chatenoud, 2006). A number of loci have been thus far proven to exert an influence on predisposition towards autoaggressive responses (Aune *et al*., 2004), among which the most influential are *HLA* (human leukocyte antigens) genes, *CTLA4* (cytotoxic T lymphocyte associated antigen-4) and *PTPN22* (protein tyrosine phosphatase non-receptor type 22) (Pearce and Merriman, 2006). However, studies on monozygotic twins and affected families indicate that autoimmunity is triggered by other than genetic factors. There have been many possible environmental factors found to influence autoimmune response, such as certain drugs, pathogens, pollutants, diet, newly introduced chemicals or stress (Gerstein, 1994; Longnecker and Daniels, 2001; Roivainen, 2006; Dahlquist, 2006). Nevertheless, none of the genetic factors, nor an environmental one, was proven to be responsible for the observed boom of the diseases incidence.

It is feasible, that since the living conditions improved and more affected individuals reach procreation age due to advances in medical treatment, the 'autoimmune' alleles are preserved in the gene pool, making populations more prone to this kind of disorders. To find out whether this might be at least partly the case, we decided to study the balance in the case of two predisposing alleles' frequencies in past populations, by means of ancient DNA (aDNA) analysis, in order to compare with their contemporary presence. We chose *CTLA4* +49A/G and *HLA DQB*^{57}

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polymorphisms—the only confirmed for the Polish population sites involved in the onset of certain autoimmune mediated diseases and found to influence predisposition towards type 1 diabetes (T1D) and coeliac disease among others. We decided to analyse a population inhabiting Poland in the Middle Ages. Taking advantage of such a relatively old group ensures that the frequency of genotypes was not influenced by any major human movements or chemicals introduced to the environment during the later periods of industrial changes.

MATERIALS AND METHODS

Materials

Analyzed medieval DNA was isolated from teeth of over 100 non-related individuals, collected on an archeological burial site in Stary Brzesc Kujawski (SBK-4) central Poland, dated back to 12th~14th century.

The reference groups (Witas *et al*., 1998; Witas and Młynarski, 1999) comprised individuals inhabiting contemporarily the same region of Poland.

Methods

Each tooth was mechanically and chemically cleaned, powdered and after pre-treatment with EDTA (ethylene diamine tetraacetic acid), proteinase K and PTB (N-phenacylthiazolium bromide), subjected to DNA isolation in MagNA Pure® Compact nucleic acid extraction system (Roche). Sequences of interest were subsequently amplified from isolated DNA fragments and identified using RFLP (restriction fragment length polimorphism) analysis (*CTLA4* +49A/G) or ASA-PCR (allele-specific amplification, polymerase chain reaction) (*HLA DQB1*^{57}). Alternatively, amplification products were sequenced. Primers used in PCR were designed to amplify as short sequences as possible (116 and 100 bp respectively), which are more likely to be extracted from aDNA.

As necessary with all aDNA isolation and analytical procedures, special attention was given not to contaminate the samples while collecting, and to provide controlled purity of environment for analysis. All work was done in a laminar air flow workstation, in rooms specially dedicated to aDNA work, where no contemporary DNA had ever been analyzed. All personnel involved wore disposable clothing and all disposables and reagents used throughout isolation and amplification procedures had been carefully selected from those found to be most reliable (Schmidt *et al*., 1995). Only those results were considered that could be confirmed in at least two independent DNA isolation procedures from different samples of the same specimen.

RESULTS AND DISCUSSION

Our preliminary results for *CTLA4* +49A/G polymorphism (Table 1) indicate that the frequency of the predisposing G allele was higher in medieval Poland than it is contemporarily observed. Similarly, the frequency value of the protecting Asp codon at *HLA DQB1*^{57} (Table 2) is closer to contemporary diabetic patients than to contemporary control group. Testing for trend in frequency distribution of predisposing alleles clearly confirmed the direction of changes from contemporary control through medieval close in value to contemporary diabetics: $P<0.001$ for linear frequency trend of GG vs remaining genotypes, allele G vs allele A frequency; XX vs Asp and Asp vs non-Asp alleles. This strongly suggests that the increase in autoimmune diseases incidence observed within the last decades, especially T1D, does not result from changes in predisposing alleles' frequencies of the studied loci, at least in Polish population. Moreover, the studied *HLA DQB1*^{57} and *CTLA4* +49A/G alleles' frequencies seem to confer even higher risk for the medieval population. The results remain in accordance with other authors' findings. Hermann *et al*.(2003) found the frequency of *HLA DRB1-DQA1-DQB1* T1D protecting haplotypes higher in contemporary Finland when compared to mid-20th century population, despite 2.5-fold increase in the disease incidence, which may, similarly to our results, point at environment changes as a reason for the recent boom of autoimmune diseases increase. Further work is being currently done to increase studied group quantity, to raise the number of studied sites, and to determine not only the aforementioned but other autoimmune-predisposing loci frequencies in past populations in order to add to the discussion on potential factors involved in the increase of autoimmune disorders incidence.

Table 1 Distribution of genotypes at position +49 of exon 1 of the *CTLA4* gene and alleles frequencies in contemporary controls and patients with T1D, as well as in the studied medieval population

Group	AA (%)	AG (%)	GG (%)	A (%)	G (%)
Contemporary control* (*n*=223)	40.8	51.6	7.6	66.6	33.4
Medieval population (*n*=42)	23.8	52.4	23.8	50.0	50.0
Contemporary diabetics* (*n*=207)	26.5	49.8	23.7	51.4	48.6

*According to Witas *et al.*(1998)

Table 2 Distribution of genotypes at the *HLA DQB1*^{57} and frequency analysis of Asp codon in contemporary controls and diabetics as well as in the studied medieval population

Group	Asp/Asp (%)	Asp/X (%)	X/X (%)	Asp (%)
Contemporary control* (*n*=78)	27.0	47.0	26.0	50.64
Medieval population (*n*=64)	14.1	31.2	54.7	29.69
Contemporary diabetics* (*n*=75)	0.0	25.0	75.0	12.67

*According to Witas and Młynarski (1999); X: Amino acid other than Asp coded at HLA DQB1^{57}

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