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Molecular evidence of HLA-B27 in a historical case of ankylosing spondylitis

The association between HLA-B27 and ankylosing spondylitis (AS) is the strongest known association of any HLA antigen with human disease (1). AS belongs to the family of spondylarthropathies (SpA), a group of related chronic inflammatory rheumatic diseases sharing several clinical features (2). The most common one is their association with HLA-B27 (3).

Despite the strong linkage between HLA-B27 and AS, no clear evidence of B27 in ancient and historical samples has been reported to date. The present study was conducted to confirm a morphologic diagnosis and to describe the application of a method that may be used to expand the capacity of paleopathologic research.

The remains of a male individual (age 62 years [\pm 5 years] at the time of death) who had been buried at Blanche Eglise Church in La Neuveville, Switzerland has been subjected to macroscopic, radiologic, and genetic analysis. The individual died between the 14th and 18th centuries AD. We sought evidence of an HLA-B27 allele in this individual, who was assumed to have had AS based on findings described below.

We extracted DNA from 2 distal parts of the femur and carried out HLA-B27 sequence-specific polymerase chain reaction (PCR) studies. The extraction and testing of ancient DNA was performed under recommended conditions (4). The B27 sequence-specific primers have been described previously (5). Negative control procedures were conducted for all extractions and all PCRs, and, as expected, showed no positive results. Two of 3 PCR products from each extraction from the “La Neuveville” individual were sequenced directly, and 1 product from each extraction was cloned (10 clones and 12 clones, respectively, were sequenced) (results not shown). In addition, genetic typing of the historical individual and of all researchers involved in this study was performed, using the AmpFLSTR Profiler Plus PCR Amplification Kit according to the instructions of the manufacturer (Applied Biosystems, Foster City, CA).

Based on the spinal remains, the “La Neuveville” individual was presumed to have AS rather than a degenerative disease or another type of SpA. Macroscopic and radiologic pathodiagnostic findings included extensive syndesmophytes, ossified interspinous ligaments, ankylosed facet joints, and mild left convex scoliosis (Figure 1). B27 sequence-specific

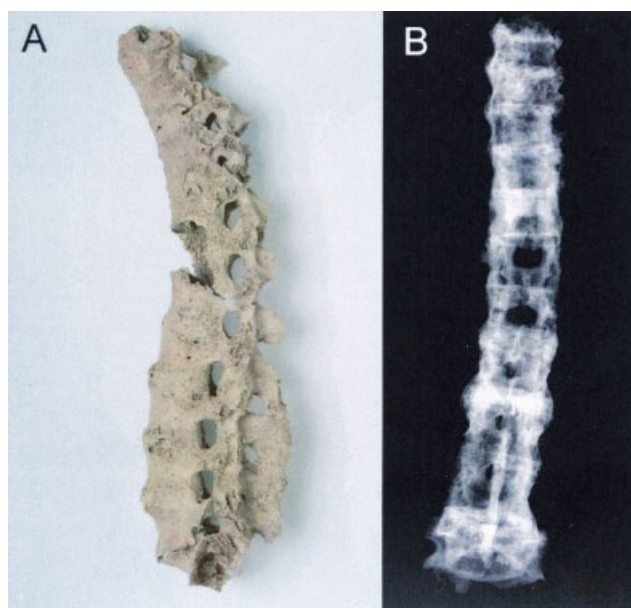


Figure 1. Lateral view of a gross specimen (A) and anteroposterior radiograph (B) of the osseous lower thoracic/lumbar spine, suggesting ankylosing spondylitis, in a man who died between the 14th and 18th centuries AD, at the age of \sim 62 years.

PCR of DNA extracted from 2 parts of the femur showed positive results matching those in recently obtained positive clinical control samples from a rheumatology outpatient unit (Johannes Gutenberg University Mainz). The retrieved sequences (GenBank accession nos. AY829003–AY829007) represent one of several known HLA-B27 alleles. Additional genetic typing of all researchers involved in this study supported the authenticity of the ancient DNA cloning and sequencing results (Table 1); no evidence of contamination was found, despite the application of rigorous authentication criteria (4).

In addition to the fact that the authentication criteria for analysis of ancient DNA were fulfilled, there was further evidence that the material tested was endogenous DNA from the “La Neuveville” individual in that 1) none of the study researchers was typed as B27 positive and 2) B27-positive and B27-negative controls were not brought into the building until after the extractions and PCR studies of the “La Neuveville” individual had been performed.

Although it is clear that HLA-B27 is the predominant predisposing genetic factor in AS, other genetic factors (within and outside the major histocompatibility complex) and environmental factors are likely involved as well. It should be noted that AS can also occur in the absence of this allele. In addition, the contribution of B27 to the genetic susceptibility to AS has been estimated to be \sim 20–30%. Thus, B27 testing alone is not clinically helpful. However, in combination with positive results from the case history, the physical examination, and the radiologic diagnosis, a positive result on B27 testing increases the probability that the patient has AS (2).

This represents the first successful attempt to link allele HLA-B27 and its pathogenic effects in historical human remains. Future studies may focus on the episodic historical

Table 1. Genotyping results in the historical individual “La Neuveville” and all researchers involved in the study*

| | Amelogenin | D3S1358 | D8S1179 | D5S818 | vWA | D21S11 | D13S317 | FGA | D7S820 | D18S51 |
|-----------------|------------|---------|---------|--------|-------|---------|---------|---------|--------|--------|
| “La Neuveville” | X/Y | 14/17 | 11/13 | 12/13 | 17 | 30/32.2 | 8/9 | 19/21 | ld | ld |
| Researchers | | | | | | | | | | |
| 1 | X/Y | 14/18 | 13/14 | 11/13 | 16/17 | 29/30 | 12 | 22/26 | 8/11 | 12/14 |
| 2 | X/Y | 15 | 10/14 | 10/13 | 14/16 | 28/30 | 12 | 19/21.2 | 9/10 | 13/14 |
| 3 | X/Y | 14/15 | 13/14 | 11/13 | 16/17 | 30/30.2 | 9/12 | 20/25 | 9/10 | 15/19 |
| 4 | X/Y | 14 | 14 | 12 | 16/17 | 28/29 | 9/12 | 21/22 | 11/12 | 12/17 |
| 5 | X/Y | 14/15 | 13/14 | 12/13 | 16/19 | 29/31.2 | 8 | 21/23 | 7/8 | 15/16 |
| 6 | X | 15/16 | 12 | 12 | 16/18 | 29 | 8/9 | 21/25 | 11 | 12/13 |

* Results shown are the typed alleles from 9 autosomal short tandem repeat loci and the sex-determining locus amelogenin. Individual “La Neuveville” was typed 6 times (3 times per extraction), and the researchers were typed at least twice. ld = locus dropout (no amplification of long polymerase chain reaction products due to DNA degradation).

prevalence of the B27 allele, to provide further insight into its enigmatic evolutionary history and its connection to cases of suspected AS.

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Distribution of HLA–B27 subtypes in Sardinia and continental Italy and their association with spondylarthropathies

The association of spondylarthropathies with HLA–B27 is well documented; however, the presence of HLA–B27 is not uniformly distributed among the different forms of spondylarthropathy. Ankylosing spondylitis (AS) exhibits the strongest degree of association with HLA–B27 and psoriatic arthritis (PsA) exhibits the lowest degree of association. Furthermore, there is no definitive evidence of a role of

HLA–B27 molecules in the pathogenesis of any form of disease. It is also not clear if its role is the same in all spondylarthropathies, as the most conservative hypothesis would suggest. In this regard, association data may provide some insights. It has been reported that some HLA–B27 subtypes do not associate with AS: i.e., B*2709 and B*2706 are absent in patients with AS in Sardinia and Southeast Asia, respectively (1). Much less is known about other forms of spondylarthropathy, especially because their lower degree of association with HLA–B27 makes studying this association more demanding in terms of the number of patients to be analyzed.

We analyzed a large cohort of patients from Sardinia and continental Italy with 2 forms of spondylarthropathy: AS (271 patients), and PsA (263 patients). Patients with AS were selected based on fulfillment of the modified New York criteria (2), and PsA was diagnosed according to the Moll and Wright criteria (3). Patients and controls were recruited, via a program in conjunction with the Italian Ministry of Education, from several regions of Italy; all gave informed consent. Some of the patients and controls from Sardinia have been described in another report (4). B27 subtyping was performed using the HLA–B27 high resolution kit (Dynal, New Hyde Park, NY).

Table 1 shows the distribution of B27 and its subtypes in healthy controls and patients with each of the spondylarthropathies studied. The proportion of PsA patients who have been found to be positive for B27 in other studies ranges from 10% to 20%: these data were confirmed in our cohort. Twelve percent and 13% of patients with PsA in continental Italy and Sardinia, respectively, were B27 positive. PsA is a heterogeneous disease, and its association with B27 is usually higher in forms with axial involvement. Accordingly, our B27-positive

Table 1. Frequency of HLA–B27 among psoriatic arthritis (PsA) and ankylosing spondylitis (AS) patients in Sardinia and continental Italy*

| | Sardinia | Continental Italy |
|------------------|----------|-------------------|
| PsA (n = 54/209) | | |
| B27+ | 7 (13) | 26 (12) |
| B27– | 47 (87) | 183 (88) |
| AS (n = 128/143) | | |
| B27+ | 89 (70) | 108 (76) |
| B27– | 39 (30) | 35 (24) |

* Values are the number (%); n values are for Sardinia/continental Italy.