Rheumatoid arthritis in Cardinal Carlo de' Medici (1595-1666): a confirmed macroscopic, radiologic and molecular diagnosis

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Abstract

Objectives

The paleopathological study of the skeletal remains belonging to Cardinal Carlo de' Medici (1595-1666), son of Ferdinando I (1549-1609) and Cristina of Lorena (1565-1637), has been presented previously. A diagnosis of Klippel-Feil syndrome, tuberculosis and a polyarthopathy, interpreted as rheumatoid arthritis, was suggested.
A revision of this case based on the analysis of the historical documents and of some radiological images of Carlo's bones has been proposed recently; according to the Authors, the Cardinal was affected by the "Medici syndrome", a combined Psoriatic-DISH arthropathy. This revision offers us the opportunity to discuss this complex case, comparing different points of view, and to present the results of the molecular analyses carried out on Carlo's bone samples.
We looked for the genetic risk factors of rheumatoid arthritis (RA) and psoriatic arthritis (PsA). We also searched for the primary candidate genes of RA and PsA, i.e. DR4 or DR1 and Cw6 or DR7 respectively, the latter predisposing also for psoriasis.

Methods

An original molecular protocol was applied to achieve an aDNA uncontaminated by exogenous sources and almost intact, starting from one of the Cardinal's rib pieces. The allele risk factors for both diseases were identified by PCR-SSP assay as HLA genotyping methodology.

Results

*Our data assigned Carlo the genotype DRB1*04/*11 for HLA-DRB locus and Cw*04/*12 for HLA-C locus.*

Conclusions

Since Carlo was infected by M. tuberculosis during infancy and was carrying the DR4 variant but not the Cw6, he surely had a predisposition to RA, not to PsA and/or psoriasis. The diagnosis of RA is thus confirmed.

Key words
rheumatoid arthritis, tuberculosis, Florence, renaissance, aDNA

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Introduction

In a previous work (1), the skeletal remains of Cardinal Carlo de' Medici (1595-1666), exhumed in the Basilica of San Lorenzo in Florence, were macroscopically and radiologically studied. This case was particularly interesting, as it revealed that Carlo was affected by different severe pathologies.

Ankylosis of the cervical column, associated with other facial and spine anomalies was attributed to a congenital disease, the Klippel-Feil syndrome. The lesions of C6 and C7 were diagnosed as the result of the tuberculosis (Pott's disease) the Cardinal suffered during his infancy. The post-cranial skeleton showed an ankylosing disease, mainly symmetrical and extremely severe, involving the large as well as the small articulations of the appendicular skeleton, characterised by massive joint fusion, that totally disabled the Cardinal in his last years of life. As a final diagnosis, an advanced, ankylosing stage of rheumatoid arthritis (RA) was suggested.

The second opinion about the paleopathological study of Cardinal Carlo de' Medici proposed by an Australian and Italian team (G.M. Weisz, M. Matucci-Cerinic, G.W. Albury, D. Lippi) offers us the opportunity to re-examine this complex case and to present the results of further analyses carried out on the skeletal remains of this member of the Medici family.

As stated in the introduction of the second opinion, the Authors based their observations on the archival data and literature on the Medici Project; in particular, some radiological images of Carlo's skeletal remains were examined. However, these researchers did not have access to the complete photographic documentation acquired during the study of the Cardinal, nor were they able to directly observe his skeletal remains and for this reason the diagnosis is only of a speculative type. Diagnosis of diseases in ancient human remains is a difficult task that requires long training and specialised competences, even when direct examination is possible, but it becomes a bold enterprise if the study is performed only on indirect documentation, as is the case of the second opinion about Cardinal Carlo; furthermore, no paleopathologist was involved in this historical and radiological study.

This paper intends to reply to the review of those Authors concerning the different illnesses of Carlo and to present the results of new molecular analyses carried out on his bone samples.

First of all, as concerns the clarification they have proposed about the family branch to which Carlo belonged, it seems necessary to clarify that Lorenzo was certainly an ancestor, but that the first important personage of this family line was Giovanni delle Bande Nere (1498–1526), considered even by the Grand Dukes as the real "father" of the branch from Cosimo I (1519–1574) to Gian Gastone (1671–1737); moreover, Anna Maria Ludovica (1667–1743) was not Grand Duchess, and therefore the junior branch of the family can be considered to have been extinguished with Gian Gastone (2).

Spinal anomalies

In our study of Carlo, a series of spinal anomalies was observed and the diagnosis of two different pathologies was formulated: the fusion of the cervical vertebrae was referred to a congenital disease, the Klippel-Feil syndrome (KFS), while the wedge-shaped deformity involving C6 and C7 was considered the classical manifestation of Pott's disease, *i.e.* the result of a tubercular infection. In the second opinion the diagnosis of KFS is basically accepted, but with some criticism, whereas the diagnosis of tuberculosis is rejected; our colleagues, however, do not propose an alternative diagnosis for the lesions affecting C6 and C7.

With regard to the criticisms about KFS, we confirm the presence of total atlanto-occipital fusion (AOF) (Fig. 1); the first cervical vertebra was separated from the occipital condyles caused by *post-mortal* damage and the mechanism of separation of the AOF was accidental, surely occurring before exhumation.

As for the tuberculosis, the column presents a clear picture of a non-severe form of Pott's disease, as demonstrated by the fusion of C6-7 and the wedge deformity of C6 and C7. The kyphotic

Fig. 1. Skull base of Carlo: the atlas is fused with the occipital condyles, but there is a post-mortal fracture, occurred before exhumation.

Fig. 2. Cervical and thoracic column of Carlo: the gibbus is present only in the lower cervical tract.

angulation of 20 degrees at C6 was not sufficient for externally detectable *gibbus* (humpback), as observed in the second opinion; the formation of a severe *gibbus* was prevented by a corset that was applied by the physician Girolamo Fabrici d'Acquapendente, called at court by the father of Carlo, Ferdinando I, to treat the neck deformity of his son (2). Pieraccini hypothesised an upper thoracic deformity on the basis of the archival document description, and not on the basis of a direct observation of Carlo's remains. The *gibbus* deformity is without doubt in the lower cervical tract, as clearly visible observing the cervical and thoracic column (Fig. 2).

A further point of discussion concerns the calcification of the anterior longitudinal ligament (ALL) observed in the first opinion at the level of T9 and T10 and another calcification between C2 and C3 observed by the Authors of the second opinion, who conclude that these fusions are manifestations of diffuse idiopathic skeletal hyperostosis (DISH). However, for diagnosis of DISH the standard criteria require involvement of at least 4 contiguous vertebrae of the thoracic spine (3, 4). Utsinger lowered the threshold for spinal involvement to 3 contiguous vertebral bodies (5); Julkunen *et al*. consider bridges connecting 2 vertebral bodies in at least 2 sites of the thoracic spine to be characteristic for DISH (6). In conclusion, also considering the opinion of different Authors, the Cardinal does not satisfy the criteria for diagnosis of this condition. Moreover, considering the age of Carlo, if he had been affected by DISH, the disease would have reached an advanced stage, with extensive ossification forming a continuous line of bumps, as observed for example in Ferdinando I (1549–1609) (7). The fusion of T9 and T10 is simply the result of degenerative arthritis of the column, with typical vertebral osteophytosis, leading to the formation of a bony bridge; the osteophytes are visible in other vertebrae also on the left side (*i.e*. between L2 and L3, and L3 and L4), but without the formation of a bony bridge (Fig. 3).

As for the neck fistula, the word "*fistula*" is simply referred to a skin opening with pus discharge. The term "*scrofula*" is generally used to indicate the enlarged nodes of the neck affected by tuberculosis, not necessarily ulcerated (8). The term "fistula" is not quoted, but the description given is that of a fistula: "*fontanella*" or "*rottorio*" that "*spurga gran quantità di materia*" (2, p. 422); there is no evidence of a possible *scrofula*.

With regard to vertebral tubercular osteomyelitis, according to the second opinion there is no definite radiological sign of such a picture in Carlo's column. However, there is evident bone sclerosis in the body of C6 at x-rays; this sclerosis, with C6-C7 total body fusion, and the clear round osteolytic defect of C6, visible at CT, are pathognomonic of recovered tuberculosis (9, 10). This picture is similar to Figure 31-c, regarding a healed case of C5-C6 tubercular spondylitis, and many other recovered cases (*i.e*. see Figs. 9d-e, 24c) reported in the fundamental book by Thijn and Steensma (11). Remission from vertebral osteomyelitis is a common finding in paleopathology, and in literature many cases of Pott's disease are referred to healed individuals (12); long-term survival is also possible, considering the good alimentation and lifestyle of the members of an elite class (13).

Ossification of the anterior ligament is possible in tuberculosis, while ossifica-

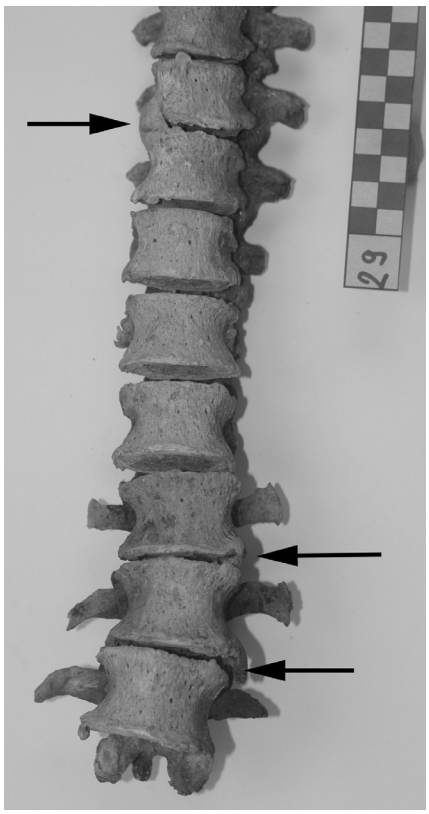

Fig. 3. Fusion of T9 and T10 on the right, referable to degenerative arthritis of the column, with typical vertebral osteophytosis; osteophytes are visible between L2 and L3, and L3 and L4, but only on the left-hand side and without the formation of a bony bridge.

tion of the lateral ligaments, not visible in Carlo's radiological image and mentioned by colleagues, is not described in Pott's disease (11).

Finally, Carlo was affected by tubercular infection at the age of 9, and therefore the dental abscesses causing submentorial discharge proposed by our colleagues, must be ruled out.

In conclusion, there seems to be no doubt about the diagnosis of tuberculosis. Both historical sources and paleopathological evidence agree with this diagnosis.

Rheumatoid or psoriatic arthritis?

In the second opinion the diagnosis of rheumatoid arthritis (RA) is rejected in favour of psoriatic arthritis (PsA). The supposed references to dermatological signs of the disease are reported: our colleagues mention a generic Miscellaneous Medici Archive among the sources, but the specific documents containing these references are not indicated.

In any case, a mention of "*rogna*" is actually reported by archival documents on April 25th, 1620.

The other references to skin disorders are disputable: in 1601 there is the news of a skin outbreak ("*rottura di pelle*", *i.e.* skin ulcer), but in 1601 Carlo was only 5 years old, and it is impossible for the onset of psoriatic arthritis to have been so precocious; in 1644 and 1654-55 there is the news of "*grattatura*" (skin erosion) and erysipelas ("*resipola*", *i.e.* acute skin inflammation), not "*rogna*".

As correctly pointed out by our colleagues, ancient "*rogna*" is an erythematous condition often associated with pruritus (14) that could fit with psoriasis. However, the report of a single episode of a very generic slight dermatitis named "*finissima* (very slight) *rogna*" is insufficient because the picture of skin psoriasis is very severe and evident, with recurrent and dramatic attacks. In such an advanced stage of the disease the manifestation of psoriasis would have been massive, and the archival documents would have mentioned this problem. Finally, in the pre-antibiotic era and in the poor hygienic conditions of 16th century, even if in the higher social classes, infectious dermatologic diseases were certainly very common, and therefore the mention of "*rogna*" is unlikely to be referred to psoriasis, but could be the manifestation of a large number of dermatological problems, mainly infectious.

As for the conditions of the appendicular articulations, the Authors of the second opinion affirm that they could not detect definite subchondral erosions in the examined images of Carlo and that they found joint surfaces intact rather than destroyed. However, subchondral erosions are present only in the first stages of RA and not in the very advanced stages (as is the case of the Cardinal), where only the final condition of RA, ankylosis, is present; furthermore, there is clear bone destruction with total symmetrical ankylosis not only in the knees but, very evident, in the feet, ankles, hands, carpal bones, wrists and elbows. The colleagues refer to Larsen's grading (15), according to which ankylosis is the last out of the five grades of RA, and only present after destruction of the joint surfaces: this is exactly the RA phase of Cardinal Carlo! The osteoporosis is not only subchondral because Carlo was not in the acute phase of the disease; the immobility to which he was condemned in the last years of his life by the generalised articular ankyloses well explains the severe and diffuse osteoporosis. In a letter to a nephew he writes: "*Y*(our) *H*(ighness) *please excuse me for not being able to sign in my own hand (writing), because my hand does not function*" (4th December 1658) (2).

Finally, according to the second opinion, the ankylosis between the third proximal and middle phalanxes of both hands is a Boutonnière deformity, and not a swan neck deformity.

Indeed, as clearly appears from the superior and lateral view of the affected fingers, there is a proximal interphalangeal (PIP) joint hyperextension, with probable distal interphalangeal (DIP) joint hyperflexion (the third phalanx is missing): this is the typical aspect of swan neck deformity, while Boutonnière's deformity is characterised by PIP flexion with DIP hyperextension.

Diagnosis

The alternative diagnosis of combined psoriatic-DISH arthropathy proposed in the second opinion is not convincing.

Firstly, as mentioned above, Carlo presents a fusion with a bony bridge on the right side only between T9 and T10; as widely reported in literature, the involvement of only two vertebrae is not sufficient for a diagnosis of DISH.

Secondly, as already discussed, the presence of psoriasis is not clear: there is only one episode of "*rogna*", but this is a generic definition that could reflect a wide range of dermatologic diseases, not necessarily psoriasis. Furthermore, as specified earlier, subchondral erosions are present only in the first stages of RA, not in the very advanced stage of Cardinal Carlo. The colleagues of the second opinion followed the CASPAR classification for diagnosis of psoriatic arthritis, suggesting that Carlo's condition scored point 4 according to these classification criteria (16): 2 points for the presence of psoriasis, 1 point for the family his-

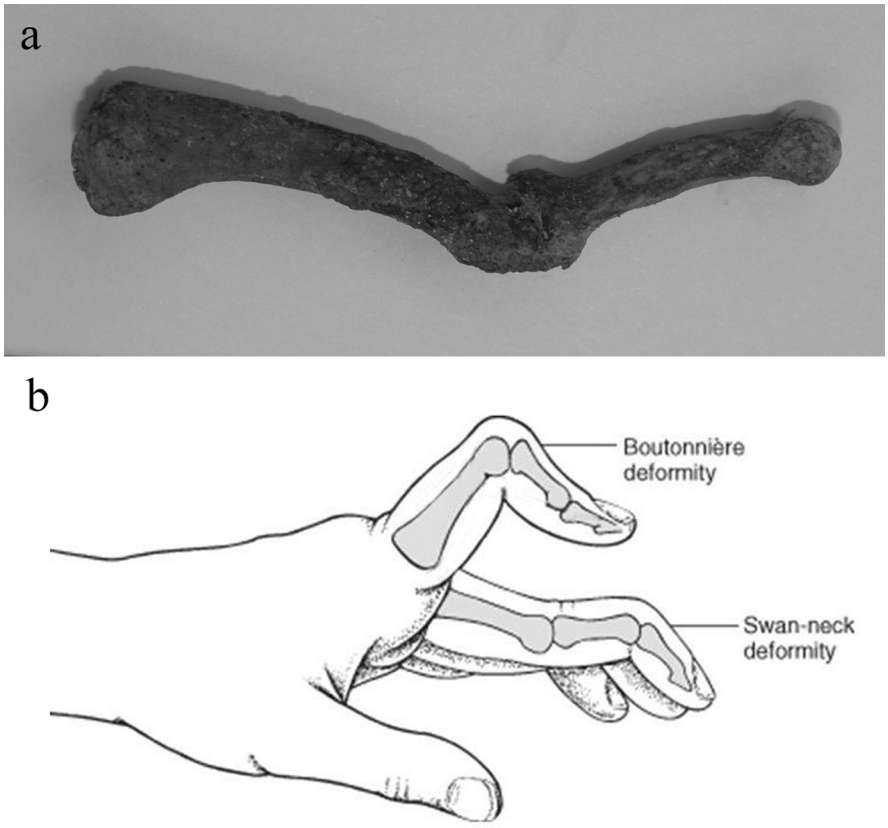

Fig. 4. A scheme of the Boutonnière and swan-neck deformities, compared to Carlo's right finger : hyperflexion of the DIP joint (the third phalanx is missing) clearly fit with a swan-neck deformity.

tory of psoriasis, 1 point for dactylitis, 1 point for juxta-articular bone formation. However, it should be pointed out that the CASPAR criteria were established according to the clinical features of living patients, not applicable to osteoarchaeological material. In fact, evidence of psoriasis in Carlo and of family psoriasis in his ancestors is not demonstrated but only supposed, and there is no radiographic evidence of juxta-articular new bone formation in the hands or the feet; only dactylitis can be supposed on the basis of bony fusion.

As correctly reported by the Authors of the second opinion, the condition observed in Carlo is very similar to that of Piero il Gottoso (1416-1469). Evidence of this rheumatologic disease in the family supports the diagnosis of RA, whose aetiology is linked to genetic factors (17).

On the basis of previous considerations, also the existence of the so-called "Medici syndrome", a combined pathology of psoriasis and DISH affecting the male members of the primary branch of the family, recently proposed (18), is not supported by convincing and scientific proofs. We propose a less problematic explanation for the presence of DISH in the males of the Medici family: more simply, recent studies have highlighted a link between the incidence of DISH and high social status, with particular regard to lifestyle and nutritional patterns, diabetes and obesity (19), confirming the association between DISH in mature age and elite status (20-23), as is the case of the Medici family.

New molecular results

Introduction

Macroscopic and radiological examinations of Carlo's skeleton exhibited a number of pathologies, including Klippel-Feil's Syndrome, a congenital disease marked by ankylosis of the cervical column, Pott's disease, as a consequence of juvenile tuberculosis infection and, finally, many signs of an advanced stage of rheumatoid arthritis (RA), although psoriatic arthritis (PsA) has also been suggested (1).

The association of specific HLA alleles and certain autoimmune diseases is known to be well established. The HLA system, a highly polymorphic genomic region playing a central role in the immune response regulation, encodes for glycoproteins expressed on cell membranes and classified as HLA-class I, -class II and -class III molecules (24). A variety of HLA loci act as genetic risk factors which, after contact with particular environmental components (in particular, viruses and bacteria determinants), can trigger a strong autoimmune reaction through a number of molecular mechanisms able to break down the tolerance of the self-reactive T cell towards self-epitopes (25). RA is a multifactorial autoimmune disease (AID), the development of which is closely related to HLA-DRB1 loci belonging to HLA-class II, which includes the allelic variants DRB1*01 (serotype DR1) and DRB1*04 (phenotypically DR4) (26).

PsA is another AID affecting the joints, a subtype of spondyloarthropathy developing in approximately 10–30% of patients showing psoriasis (27). The appearance of psoriasis pre-dates arthritis: skin lesions generally precede arthritic symptoms in 75% of cases and develop about 10 years prior to the onset of the joint symptoms (28). It is extensively documented that polygenic factors are involved in the aetiology of PsA. Although some non-HLA candidate genes (for instance TNF-β, TNF-α, and other inflammatory cytokines) have been identified recently, their role in PsA is still to be defined, whereas the strongest primary allele predisposing to PsA maps on the HLA class I genomic subregion and is related to the Cw*06 genetic variant (29, 30). It should be emphasised that the absence of the specific predisposing alleles cited above excludes the appearance of both RA and PsA. By contrast, in genetically susceptible individuals, these diseases could be elicited by environmental factors (for instance *Proteus mirabilis*, *Mycobacterium tuberculosis* for RA, but also stress and obesity for PsA).

Firstly, our work was designed to define the HLA genotype of Cardinal Carlo

de' Medici by PCR-SSP typing, a useful molecular methodology to evaluate his predisposition to RA. Secondly, we focused on what we could acquire about genetic susceptibility to PsA.

We upheld this research by developing a protocol to purify ancient DNA (aDNA), which was well preserved in terms of concentration and authenticity, so to be successfully amplified. Heavy aDNA damages (loss of nucleotides, hydrolysis and fragmentation) and contamination by microorganism and contemporary DNA, often made its recovery from ancient specimens extremely difficult (31-32). These destructive phenomena produce DNA fragments in the percentage between 100 and 300 bp, depending on many different factors (such as conditions of preservation and antiquity of the specimens) (31, 33-35), with more than 100-fold decrease of the DNA yield, resulting in the failure and/or decreased fidelity of PCR amplification (36). Fortunately, the bone is a hard material that preserves nucleic acids better than other tissues because aDNA is contained within cells which usually retain structural integrity.

In short, after DNA extraction and purification, we performed HLA genotyping from a fragment of Cardinal Carlo's bone.

Materials and methods

The sample consisted of a rib fragment, which was put into a 15 mL tube and kept at 4°C until analysis. All precautions were taken for pre-treatment and handling of the sample. Since contemporary contaminations with foreign genetic material can easily become a source of false-positive results in ancient DNA (aDNA) research, laboratory personnel wore protective clothes (body suit, hairnets, hoods, gloves and safety-masks). All reagents, containers and tools, as well as the same bone sample, were left under UV light for at least half an hour to cross-link any contaminating DNA on working surfaces. Prior to UV exposure, the bone was washed with TE Buffer (10 mM Tris-HCl pH 8.0, 1mM EDTA) for 30 min with continuous vertical rotation and dried. The rib was pulverised with a Dremel tool. In order to achieve the best yield of DNA we applied three different approaches. The first two samples, named CC01.04EE and CC04.04EE, differed only in the time of bone digestion but not in the remaining DNA extraction procedure. 200 mg of both CC01.04EE and CC04.04EE bone powder were digested in 4.2 mL of a lysis buffer consisting of 0.5 M Na_2EDTA, pH 8.0, 10 mM Tris-HCL, pH 7.8, NaCl 100 mM, SDS 0.1%, and Proteinase K added to a final concentration of 250 μg/mL. The first sample was left in a lysis buffer for a shorter time than the second one (12 hours instead of 2 days). DNA extraction and purification steps were associated with the "open tube" control test, a strategy aimed at assessing the foreign nucleic acid introduction during molecular analysis: three 1.5 mL tubes containing sterile water were left open in all working areas during the entire processing of the specimens, from bone powdering to PCR reaction mix preparation. Some aliquots of tube water were then amplified along with the samples to verify the presence of contaminating DNA. 600 uL of the digestion solution were placed into 15 ml of a conic polypropylene tube with a sterile disposable pipette and centrifuged at 7000*g* for 20 min. 350 μL of the clear supernatant containing DNA were transferred to a 1.5 mL Eppendorf tube and purified by means of the Masterpure™ DNA Purification Kit (EPICENTRE, Madison, WI, USA), a method based on the Salting-Out procedure conceived by Miller (37) and further modified by us to obtain higher nucleic acid yield and purity. The lysis suspensions were mixed and vortexed with a protein precipitation reagent (180 μL of saturated NaCl solution containing CH_3COONa: sodium acetate) to precipitate the debris by centrifugation at 10000*g* for 10 min. The supernatant was recovered and put into a clean microcentrifuge tube. This step was repeated twice. aDNA was precipitated with the addition of isopropanol 100% followed by high speed centrifugation, after freezing at -70°C for 20 min. The DNA pellet was dried, washed twice with 75% of ethanol and dissolved in 50 μL of Tris 10 mM, pH 7.8. The CC04.04EP sample was first treated by the PCIA method (Phenol/Chloroform/Isoamyl Alchohol, 25:24:1, v/v/v, Tris-EDTA buffered saturated, pH8) (38) starting from an 600 μL aliquot of the bone digested solution, followed by purification with the Masterpure™ DNA Purification kit of EPICENTRE, modified by us as described above.

Genotyping of Cardinal Carlo's body was performed by means of PCR-SSP (Polymerase Chain Reaction with Sequence-Specific Primers). This technique is a system to screen HLA human characteristics at the genomic level and is based on the amplification refractory mutation system (ARMS) described by Middleton *et al*. (39). To execute HLA-DRB and HLA-C analysis on Cardinal Carlo's DNA we used the low resolution Biotest DRB SSP kit for HLA-DRB and the low resolution Biotest HLA-C SSP kit for HLA-C, both tests supplied by Biotest (Dreiech, Germany). The PCR trays consist of a block of 200 μL tubes, each containing dried allele-specific or group allele-specific primers, together with an internal control (IC) represented by dried primers which amplify a 1069 bp fragment of the ubiquitous "housekeeping" gene for the human growth hormone (HGH).

Despite the commercial kit protocol, the PCR mix composition was modified by enrichment with Taq and $MgCl_2$ and the DNA was slightly diluted (1:1) with sterile double distilled water as precautions to avoid amplification failure for the presence of inhibiting impurities (*e.g*. Maillard products) (36). For HLA-DRB genotyping, 10 μL of PCR mix (146.2 μL dH_2O, 120 μL PCR cocktail, 3.6 μL Taq DNA polymerase, 30 μL DNA, 2 μL $MgCl_2$, glycerol and cresol red) were dispensed into each of the 23 tubes of a 24-tube PCR tray. The first tube of each PCR tray acts as negative control and contains the complete PCR reaction mix without DNA, which was replaced with an equal aliquot of sterile water. For the PCR analysis of HLA-C locus, equal aliquots of the amplification mix were pipetted into each of the 17 tubes of the 18-tube PCR tray, the first tube being the negative control. DNA samples were amplified in a GeneAmp PCR system 9600 (Perkin-

Elmer, Foster city, CA, USA). Cycle parameters were set according to the instructions provided to the users. The PCR products were loaded into each well of a 2% agarose gel stained with 4 µL of ethidium bromide (EtBr) solution (EtBr final concentration: 10 mg/mL). Electophoresis was realised in 1 X TBE Buffer (90 mM Tris-Borate and 2.5 mM EDTA prepared in demineralised sterile water) and the amplicons were visualised as bands with a UV transilluminator (BIO-RAD, Hercules, CA, USA). The allelic assignment was based on the presence or absence of amplicons and was carried out by reading the pattern of the specific bands on a reaction sheet supplied by the manufacturer.

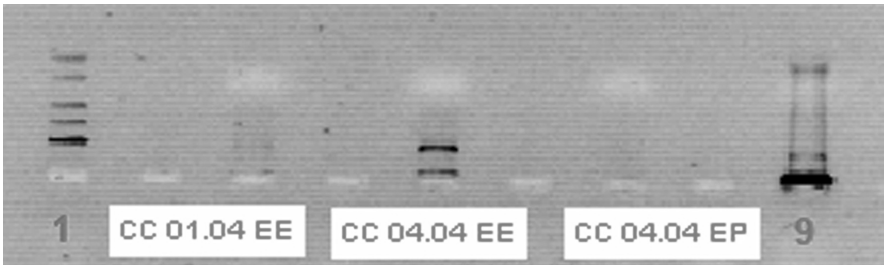

Fig. 5. Bands of genomic DNA purified from a rib fragment of Cardinal Carlo. 5 µL of DNA mixed with 1 µL of cresol red 1 mg/mL are loaded on agarose gel. Wells from left to right: *line 1*, DNA Size Markers consisting of dsDNA fragments with sizes of 50, 150, 400, 750 and 2564 bp; *line 3* (CC01.04EE); *line 5*, (CC04.04EE); *line 7* (CC04.04EP); *line 9*, modern sample of DNA. *Lines 2, 4, 6* and *8* are empty. CC: Cardinal Carlo. The four numbers following the acronym CC indicate the DNA extraction date from the bone: the first two numbers refer to the day, the second two to the month. EE: DNA purification by the NaCl-CH_3COONa/isopropanol method using Masterpure™ DNA Purification Kit EP: DNA extraction with PCIA followed by purification with NaCl-CH_3COONa/isopropanol (see text for more details).

Results

Spectrophotometric evaluations documented a good double-strand DNA (dsDNA) concentration (112 µg/mL). The A260/A280 nm ratio, measured to evaluate DNA purity, was 1.4. In order to further check DNA quality, gel electrophoresis was performed by loading the DNA isolated from all the samples differently processed (Fig. 5). The molecular amplicon weights were established by DNA-size Markers (One Lamba, Inc, Canoga Park, CA, USA). The difference in the digestion time results in the absence of DNA for CC01.04EE. Conversely, the CC04.04EE sample showed only two sharp bands of the DNA without severe fragmentation, an aspect indicative of its good preservation during extraction and purification procedures. No DNA trace was detected for the CC04.04EP sample, perhaps as a consequence of DNA loss during the PCIA step. All DNA samples were compared to the DNA purified from human fresh bone sampled at necropsy, used as modern control and treated in accordance with the protocol adopted for the CC04.04EE sample.

Figure 6 displays the result of the HLA-DRB PCR-SSP test showing the DRB1*04 allele amplification. Positivity for DRB1*04 (phenotipically DR4) is confirmed by the presence of the DRB4 gene (serotype DR53), notoriously associated with DR4. The assay also revealed positivity for DRB1*11 (phenotype DR11), which is linked with the DRB3 locus on the HLA-DRB sub-region. Only a few internal controls (IC) can be seen on the gel owing to the length of the HGH amplicon, a typical behaviour when one is working with aDNA.

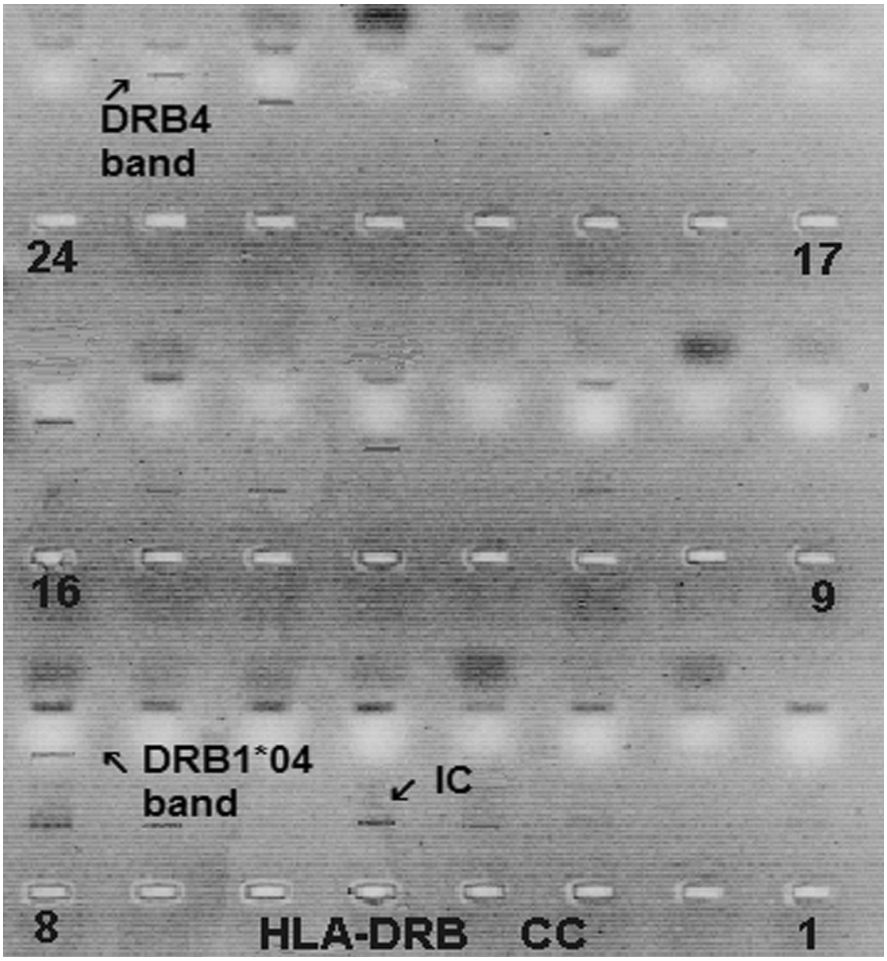

Fig. 6. Results of the PCR-SSP genotyping test for the HLA-DRB locus of Cardinal Carlo. The PCR products are loaded into each well of a 2% agarose gel stained with EtBr, electrophoresed and visualised under UV light. The arrows indicate the band positions of PCR products at *lines 8* and *23* corresponding to DRB1*04 and DRB4 alleles, respectively, and the band at *line 5* as an example of internal control (IC). *Line 1* represents the negative control. The remaining positive bands define the second alleles of Cardinal Carlo, exactly DRB1*11 (phenotypically DR11, *lines 13* and *16*) always co-expressed in conjunction with DRB3 (serotype DR52, *line 22*).

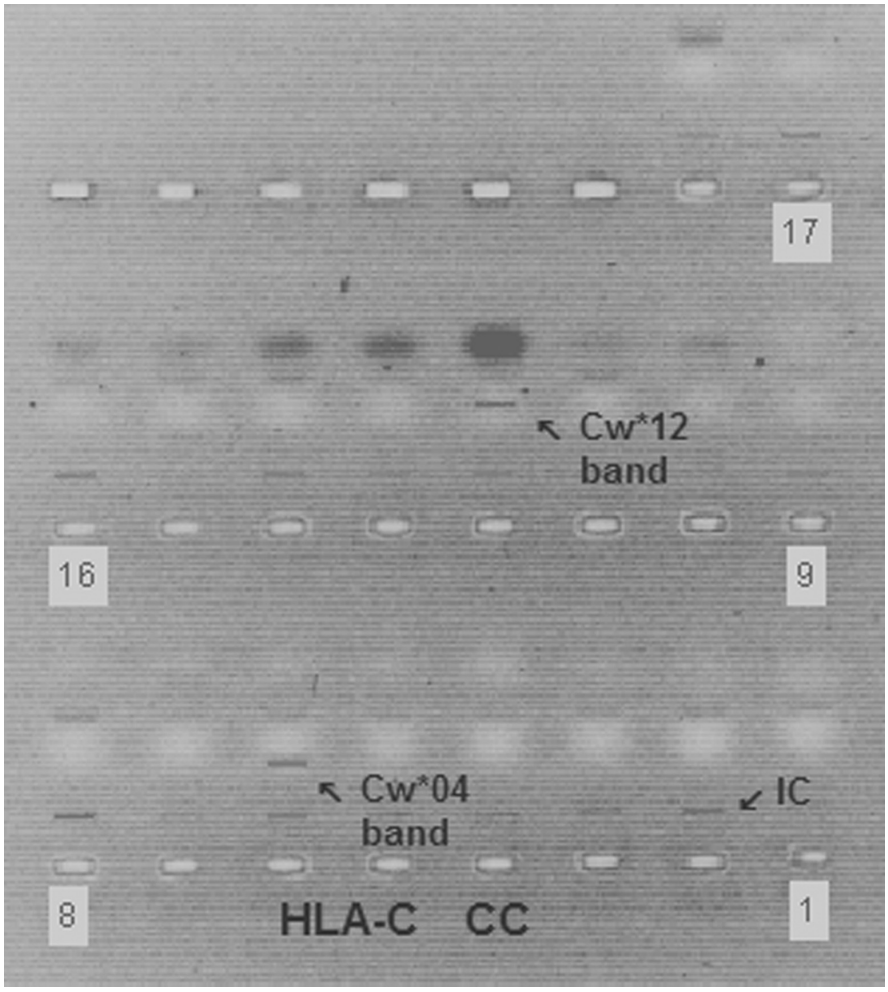

Fig. 7. Cardinal Carlo's genotype for HLA-C locus after PCR-SSP assay and visualisation on agarose gel with EtBr staining. The arrows indicate the presence of amplicons at *lines 6* and *12*, corresponding to Cw*04 and Cw*12 allele variants, respectively. In *line 2* the arrow shows an example of interna control (IC). *Line 1* contains the negative control.

With respect to HLA-C locus, the typing test allows the assignment of the Cw*04/Cw*12 genotype to Carlo. There is no evidence of the PCR product at the position corresponding to Cw*06 on the gel (Fig. 7).

All experiments (DNA isolation and molecular typing) were performed in triplicate and repeated at different dates, with the same results.

Discussion

An optimised protocol for aDNA isolation from the bone allowed us to obtain good amplifiable starting material to accomplish HLA typing at the genomic level of Cardinal Carlo de' Medici, whose genotype for DRB1 and C loci was DRB1*04/*11 and Cw*04/*12. Therefore, he was bearing the specificity HLA-DR4 predisposing to RA, but not HLA-Cw6 considered to be the strongest risk factor for PsA and psoriasis. DRB1*11, Cw*04 (Cw4 in serology) and Cw*12 alleles are not involved in the onset of RA, PsA or of other rheumatic diseases.

Our molecular results confirm that acetate-isopropanol purification is a suitable method for isolating aDNA, a feature strengthened by some literature data (40, 41). Furthermore, the very low degree of DNA fragmentation in the analysed sample confirms the good conditions of Carlo's skeleton, as referred by experts at the last body examination (1). Typical HLA genotyping analysis exhibits two advantageous characteristics, crucial when one is working with aDNA. Firstly, the PCR-SSP test specifically used in our work amplifies the nucleotide sequences with a length varying from 110 bp to 370 bp. This is confirmed by the more difficult PCR amplification observed for IC (1069 bp) that was not always visible on the gel. Secondly, all HLA genotyping kits have been designed to amplify only human DNA templates, thus excluding any interference deriving from the exogenous genomes of other species.

The HLA genetic system, mapping on the major polymorphic region within the human genome, contains a large number of genes associated with numerous AIDs, among which RA, PsA and psoriasis (42). The molecular mechanisms underlying the manifestation of HLA-restricted AIDs are still poorly understood for a variety of non-mutually exclusive reasons including survival of autoreactive T cell clones, unknown target autoantigens, epistatic effects, dosage and penetrance of the expressing genes and existence of a strong linkage disequilibrium (LD) within the same HLA region and between the HLA and non-HLA genes (43, 44). The link between RA and the risk alleles DR1 and DR4, both widespread in the Caucasoid population (45, 46), can be well explained by "molecular mimicry", a reliable theory consisting of a strong autoimmune response involving the synthesis of autoantibodies cross-reacting with DR1/DR4 variants, some pathogen determinants and/or *self*-epitopes, all of which bear common or similar amino-acid sequences (47, 48). All DR4 allelic variants predisposing to RA carry a EQK/RRAA peptide motif, known as "shared epitopes" located in the HLA molecule groove (49, 50, 51, 52), the site responsible for binding of antigen-derived peptides produced inside the antigen presenting the cells (APCs: macrophages, dendritic cells, B-cells and Type A synoviocytes). The HLA-peptide complex interacts with $TCD4^+$ cells to initiate the immune reaction (26). After a microbial infection, autoantibodies recognise as *non-self* the DR4 molecules and/or host determinants that share similarity sequence with the microbe itself (47). Numerous examples of sequence homology between DR4 variants and many microbial peptides have been described,

like the *Epstein-Barr virus* glycoprotein gp100 (bacterial homologous sequence: QK/RRAA) (53), *Escherichia coli* heat shock proteins DnaK and DnaJ (QKRAA) (54, 55), *Proteus mirabilis* haemolysin (ESSRAL) (56, 57) and *Cytomegalovirus* (LGRPN) (58). Recently, other structural homologies have been identified in the DR4 molecule and cartilage components like proteoglycans (59) and Type II collagen (CII) (60-62), considered as arthritogenic peptides. It follows that tissue-specific damages in RA occur for the presence, in the joint fluid and synovium, of dendritic cells (DCs) and Type A (macrophage-like) synoviocytes and cartilage components (63, 64), all of these expressing DR4 molecules which become targets of autoantibody attack (65). Circulating antibodies towards CII (66, 67, 68) and the complex HLA-DR4/HCgp-39$^{263\text{-}275}$ (a peptide of human cartilage) (59) were found in the rheumatic synovial membrane when combined with inheritance of HLA-DR4 or -DR1.

Interestingly, the *Mycobacterium tuberculosis* 65-kDa heat shock protein (hsp) possesses a peptide (KDLL) common to three subsets of the DRB1 loci: DR1, DR3 and DR4. This sequence homology can trigger an immune reaction with massive release of antibodies against *self*-determinants, like the DR4 itself (69). A work reports on the existence of a nine-aminoacid *M. tuberculosis* motif shared with that of proteoglycans (70), and aggressive T-cells towards mycobacterial 65-kDa hsp from rheumatoid synovial fluid have been isolated (71). All these findings might explain how molecular mimicry mechanism induced by the structural similarity of *M. tuberculosis* antigens and synovial *self*-peptides evokes DR4-restricted RA and causes local inflammation with progressive joint injury (59, 70).

The immunogenetic basis of PsA remains unclear due to its polygenic nature and the strong LD between HLA-C and other supposed causative loci. A number of genes, *e.g.* some HLA-B alleles (HLA-B13, B57, B39) and cytokines, have been suggested as further possible candidates (72, 73), and HLA-Cw6 specificity is thought to be the main determinant of susceptibility to psoriasis and PsA (74). This assertion, confirmed by several studies (75, 76, 77), is above all supported by the observation that HLA-C locus lies in a genomic region characterised by very strong LD (many hundreds of bp), also encompassing the other supposed predisposing genes (30, 73). In any case, HLA-DRB1*04 alleles are not associated with PsA (72).

Our results along with rich literature data enable us to state that Carlo was bearing a genetic risk marker, the DR4, certainly declaring his predisposition to develop RA after a microbial infection. Even though the microbial agents triggering RA are quite widespread, the *M. tuberculosis* can certainly be taken into consideration since Carlo suffered from Pott's disease during his infancy. On the contrary, the Cardinal is rather unlikely to have been affected by PsA as he did not carry the genes considered to be the first important genetic risk factors for this disease, namely HLA-Cw6 and HLA-DR7 (77, 78, 79).

Therefore, the presence of DR4, absence of Cw6 and DR7 and previous infection with *M. tuberculosis* are all elements addressing to RA rather than to PsA. *Vice versa*, evidence in favour of PsA (and opposing to RA) should have implied the lack of HLA-DR4 and presence of HLA-Cw6 or HLA-DR7. Moreover, negativity for Cw6 suggests that Carlo could not even suffer from psoriasis.

Conclusions

The paleopathological study of the skeletal remains of Carlo revealed that:

- the Cardinal was affected by a tubercular infection in infantile age, as confirmed by the signs of a healed Pott's disease in the lower cervical column;
- the macroscopic picture of arthropathy is more similar to that of rheumatoid arthritis than psoriasic arthritis;
- Carlo had a clear predisposition to rheumatoid arthritis, and not to psoriatic arthritis, as demonstrated by the new molecular data.

In conclusion, the diagnosis of rheumatoid arthritis in an advanced stage of evolution is confirmed.

References

1. GIUFFRA V, VITIELLO A, GIUSIANI S *et al*.: Rheumatoid arthritis, Klippel-Feil syndrome and Pott's disease in Cardinal Carlo de' Medici (1595-1666). *Clin Exp Rheumatol* 2009; 27: 594-602.
2. PIERACCINI G: La stirpe dei Medici di Cafaggiolo. vol. 2, Firenze, Nardini Editore, 1986.
3. RESNICK D, NIWAYAMA G: Radiographic and pathologic features of spinal involvement in diffuse idiopathic skeletal hyperostosis (DISH). *Radiology* 1976; 119: 559-68.
4. VERLAAN JJ, ONER FC, MAAT GJR: Diffuse Idiopathic Skeletal Hyperostosis in Ancient Clergymen. *Eur Spine J* 2007; 16: 1129-35. DOI 10.1007/s00586-007-0342-x
5. UTSINGER PD: Diffuse idiopathic skeletal hyperostosis. *Clin Rheum Dis* 1985; 11: 325-51.
6. JULKUNEN H, HEINONEN OP, KNEKT P, MAATELA J: The epidemiology of hyperostosis of the spine together with its symptoms and related mortality in a general population. *Scand J Rheumatol* 1975; 4: 23-7.
7. GIUFFRA V, GIUSIANI S, FORNACIARI A, VILLARI N, VITIELLO A, FORNACIARI G: Diffuse Idiopathic Skeletal Hyperostosis in the Medici, Grand Dukes of Florence (XVI century). *Eur Spine J* 2010; 19/2: 103-7.
8. BLOCH M: I re taumaturghi, studi sul carattere sovrannaturale attribuito alla potenza dei re particolarmente in Francia e in Inghilterra. Torino, Einaudi, 1989.
9. AUFDERHEIDE C, RODRIGUEZ-MARTIN C: The Cambridge Encyclopedia of Human Paleopathology. Cambridge, Cambridge University Press, 1998.
10. ORTNER DJ: Identification of Pathological Conditions in Human Skeletal Remains. New York, Smithsonian Institution Press, 2003.
11. THIJN CJP, STEENSMA JT: Tuberculosis of the Skeleton. Berlin, Springer-Verlag, 1990.
12. PÁLFI GY, DUTOUR O, DEÁK J, HUTÁS I (eds): Tuberculosis Past and Present. Budapest, Golden Book Publisher, 1999.
13. ROBERTS CA, BUIKSTRA JE: The bioarchaeology of tuberculosis: a global view on a reemerging disease. Gainesville, University Press of Florida, 2003.
14. MARCOVECCHIO E: Dizionario Etimologico Storico dei Termini Medici. Firenze, Festina Lente, 1993.
15. LARSEN A, DALE K, EEK M: Radiographic evaluation of rheumatoid arthritis and related conditions by standard reference films. *Acta Radiol Diagnosis* 1997; 18: 481-91.
16. TAYLOR W, GLADMAN D, HELLIWELL P *et al*.: Classification Criteria for Psoriatic Arthritis. Development of new criteria from a large international study. *Arthritis Rheum* 2006; 54: 2665-73.
17. Criswell LA, Gregersen PK: Current understanding of the genetic aetiology of rheumatoid arthritis and likely future developments. *Rheumatology* 2005; 44/4: iv9-iv13.
18. WEISZ GM, MATUCCI-CERINIC M, ALBURY WR, LIPPI D: The Medici syndrome, a medico-historical puzzle. *Intern J Rheumatic Dis* 2010; 13: 125-31.
19. SMYTHE H, LITTLEJOHN G: Diffuse idiopathic skeletal hyperostosis. *In* KLIPPEL JH,

DIEPPE PA (Eds.): *Rheumatology*. 2nd edn, Vol. 2 London, Mosby 1998: 8.10.1-6.
20. JANKAUSKAS R: The Incidence of Diffuse Idiopathic Skeletal Hyperostosis and Social Status Correlations in Lithuanian Skeletal Materials. *International Journal of Osteoarchaeology* 2003; 13: 289-93. DOI: 10.1002/oa.697.
21. ROGERS J, WALDRON T: DISH and the monastic way of life. *International Journal of Osteoarchaeology* 2001; 11: 357–65.
22. JANSSEN HAM, MAAT GJR: Canons Buried in the "Stiftskapel" of the Saint Servaas Basilica at Maastricht AD 1070-1521. A Palaeopathological Study. Leiden, *Barge's Anthropologica*, 1999.
23. VERLAAN JJ, ONER FC, MAAT GJR: Diffuse Idiopathic Skeletal Hyperostosis in Ancient Clergymen. *Eur Spine J* 2007; 16: 1129-35. DOI 10.1007/s00586-007-0342-x
24. GOUGH SCL, SIMMONDS MJ: The HLA region and autoimmune disease: association and mechanisms of action. *Curr genomics* 2007; 8: 453-65.
25. FRIDKIS-HARELI M: Immunogenetic mechanisms for the coexistence of organ-specific and systemic autoimmune disease. *J Autoimmune Dis* 2008; 5: 1-7.
26. GONZALEZ-GAY MA, GARCIA-PORRUA C, HAAIER AH: Influence of human leukocyte antigen DRB1 on the susceptibility and severity of rheumatoid arthritis. *Semin Arthritis Rheum* 2002; 31: 355-63.
27. MEASE F: Psoriatic Arthritis Update. *Bulletin of the NYU Hospital for Joint Diseases* 2006; 64: 25-31.
28. GIROLOMONI G, GISONDI P: Psoriasis and systemic inflammation: underdiagnosed enthesopathy. *J Eur Acad Dermatol Venereol* 2009; 23 (Suppl. 1): 3-8.
29. GLADMAN DD, CHEUNG C, NG CM, WADE JA: HLA-C locus alleles in patients with psoriatic arthritis (PsA). *Hum Immunol* 1999; 60: 259-61.
30. NAIR STUART PE, NISTOR I, HIREMAGALORE R *et al*.: Sequence and Haplotype Analysis Supports *HLA-C* as the Psoriasis Susceptibility 1 Gene. *Am J Hum Genet* 2006; 78: 827-51.
31. WILLERSLEV E, COOPER A: Ancient DNA. *Proc R Soc B* 2005; 272: 3-16.
32. HOFREITER M, SERRE D, POINAR HN, KUCH M, PÄÄBO S: Ancient DNA. *Nat Rev Genet* 2001; 2: 353-9.
33. PÄÄBO S: Ancient DNA: extraction, characterization, molecular cloning and enzymatic amplification. *Proc Natl Acad Sci, USA* 1989; 86: 1939-43.
34. HANDT O, HÖSS M, KRINGS M, PÄÄBO S: Ancient DNA: methodological challenges. *Experientia* 1994; 50: 524-9.
35. STONE AC: DNA analysis of archaeological remains. *In* KATZENBERG M.A and SAUNDERS S.R. (Eds.): Biological Anthropology of the Human Skeleton. New Jersey, Wiley & Sons, Hoboken, 2008: 461-83.
36. ROGAN PK, SALVO JJ: Study of nucleic acids isolated from ancient remains. *Yearbook of Physical Anthropology* 1990; 33: 195-214.
37. MILLER SA, DIKES DD, POLESKI HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; 16: 1215.
38. CAPELLI C, TSCHENTSCHER F: Protocols for ancient DNA typing. *Nethods Mol Biol* 2005; 297: 265-78.
39. MIDDLETON D, WILLIAMS F: A history of DNA typing for HLA. *In* TERASAKI and GJERTSON (Eds.): HLA 1977. Los Angeles, California, UCLA Tissue Typing Laboratory 1977: 61-75.
40. HÄNNI C, BROUSSEAU T, LAUDET V, STEHELIN D: Isopropanol precipitation removes PCR inhibitors from ancient bone extracts. *Nucleic Acids Res* 1995; 23: 881-2.
41. KALMAR T, BACHRATI CZ, MARCSIK A, RASKO I: A simple and efficient method for PCR amplifiable DNA extraction from ancient bones. *Nucleic Acids Res* 2000; 28: E67.
42. SHIINA T, INOKO H, KULSKI JK: An update of the genomic region, locus information and disease association. *Tissue Antigens* 2004; 64: 631-49.
43. CAILLAT-ZUCMAN S: Molecular mechanisms of HLA association with autoimmune diseases. *Tissue Antigens* 2008; 73: 1-8.
44. FERRACCIOLI G, BRACCI-LAUDIERO L, ALIVERNINI S, GREMESE E, TOLUSSO B, DE BENEDETTI F: Interleukin-1β and inteleukin-6 in arthritis animal models: roles in the early phase of transition from acute to chronic inflammation and relevance for human rheumatoid arthritis. *Mol Med* 2010; 16: 552-7.
45. FUGGER L, SVEIGAARD A: Association of MHC and rheumatoid arthritis. HLA-DR4 and rheumatoid arthritis: studies in mice and in men. *Arthritis Res* 2000; 2: 208-11.
46. WORDSWORTH BP, LANCHBURY JSS, SAKKAS LI, WELSH KI, PANATI GS, BELL JI: HLA-DR4 subtype frequencies in rheumatoid arthritis indicate that DRB1 is the major susceptibility locus within the HLA class II region. *Proc Natl Acad Sci USA* 1989; 86: 10049-53.
47. WILSON C, TIWANA H, EBRINGER A: HLA-DR4 restriction, molecular mimicry and rheumatoid arthritis. *Immunol Today* 1997; 18: 96-7.
48. WATANABE T, TOKUNAGA K, MATUKI K *et al*.: Putative amino sequence of HLA-DRB chain contributing to rheumatoid arthritis susceptibility. *J Exp Med* 1989; 169: 2263-8.
49. BONGI SM, PORFIRIO B, ROMBOLÀ G, PALASCIANO A, BENEFORTI E, BIANUCCI G: Shered epitope HLA-DRB1 alleles and sex ratio in Italian patients with rheumatoid arthritis. *Joint Bone Spine* 2004; 71: 24-8.
50. TIAN X, JIANG M, QIU C: HLA-DRB1genes in 5 rheumatic disease multi-case families. *Clin Med J* 1998; 111: 208-10.
51. ATOUF O, BENBOUAZZA K, BRICK C *et al*.: HLA polymorphism and early rheumatoid arthritis in the Maroccan population. *Join Bone Spine* 2008; 75: 554-8.
52. DELGADO VEGA AM, ANAJA JM: Meta-analysis of HLA-DRB1 polymorphism in Latin American patients with rheumatoid arthritis. *Autoimmun Res* 2007; 6: 402-8.
53. ROUDJER J, PETERSEN J, RHODES G, LUKA J, CARSON DA: Susceptibility to rheumatoid arthritis maps to a T cell epitope shared by the HLA-Dw4 DR beta 1 chain and the Epstein Barr virus glycoprotein gp110. *Proc Natl Acad Sci USA* 1989; 86: 5104-8.
54. ALBANI S, TUCKWELL J, ESPAZA L, CARSON D, ROUDJER J: The susceptibility sequence to rheumatoid arthritis is a cross reactive B epitope shared by the E.coli DNAJ heat shock protein and the HLA-DRB1*0401 molecule. *J Clin Invest* 1992; 89: 327-31.
55. AUGER I, ROUDIER J: A function for the QKRAA amino acid motifs: mediating binding of DNAJ to DNAK. *J Clin Invest* 1997; 99: 1818-22.
56. WILSON C, TIWANA H, EBRINGER A: Molecular mimicry beteen HLA-DR alleles associated with rheumatoid arthritis and Proteus mirabilis as the aetiological basis for autoimmunity. *Microbes Infect* 2000; 2: 1489-96.
57. TIWANA H, WILSON C, ALVAREZ A, ABUKNESHA R, BANSAL S, EBRINGER A: Cross-reactivity between the rheumatoid arthritis-associated motif EQKRAA and structurally related sequence found in Proteus mirabilis. *Infection and Immunity* 1999; 67: 2769-75.
58. FUJIAMI RS, NELSON JA, WALKER L, OLDSONE MBA: sequence homology and immunologic cross reactivity of human Cytomegalovirus with HLA-DR-β chain: a means for graft rejection and immunosuppression. *Journal of virology* 1988; 62: 100-5.
59. BAETEN D, STEENBAKKERS PGA, RIJNDERS AMW, BOOTS AM, VEJS EM, DE KEJSER F: Detection of major histocompatibility complex/human cartilage gp-39 complexes in rheumatoid arthritis synovitis as a specific and independent histologic marker. *Athritis Rheum* 2004; 50: 444-51.
60. DE ROSA MC, GIARDINA B, BIANCHI C *et al*.: Modelling the ternary complex TCR-Vβ/collagen II(261-273)/HLA-DR4 associated with rheumatoid arthritis. PLoS ONE 2010; 5: 1-11.
61. ROSLONIEC EF, WHITTINGTON KB, HE X, STUART JM, KANG AH: Collagen induced arthritis mediated by HLA-DR1 (*0101) and HLA-DR4 (*0401). *Am J Med Sci* 2004; 327: 169-79.
62. COPE AP, PATEL SD, HALL F *et al*.: T cell responses to human cartilage autoantigen in the context of rheumatoid arthritis-associated and non associates HLA-DR4 alleles: *Arthritis Rheum* 1999; 42: 1497-507.
63. CREMER MA, YE XJ, MYERS LK, BRAND DD, ROSLONIEC EF, KANG AH: T cell immunity to type II collagen in the biobreeding rat: the identification and characterization of RT1^u-restricted cell epitopes on α1(II). *The Journal of Immunology* 2004; 173: 1795-801.
64. ZANELLI E, BREEDVELD FC, DE VRIES RRP: HLA association with autoimmune disease? A failure to protect? *Rheumatology* 2000; 39: 1060-6.
65. RANJENI T, MACDONALD KPA, PETTIT AR, CAVANAG LL, PADMANABHA J, ZEHENTER S: Dendritic cells and the pathogenesis of rheumatoid arthritis. *J Leukoc Biol* 1999; 66: 286-92.
66. CREMER MA, ROSLONIEC EF, KANG AH: The cartilage collagens: a review of their structure, organization and role in the pathogenesis of experimental arthritis in animals in human rheumatic disease. *J Mol Med* 1998; 76: 275-88.
67. TSARK EC, WANG W, TENG YC, ARKFELD

D, DODGE GR KOVATS S: Differential MHC Class II-mediated presentation of rheumatoid arthritis autoantigens by human dendritic cells and Macrophages. *J Immunol* 2002; 169: 6625-33.

68. COOK A, ROWLEY M, MACKAY I, GOUGH A, EMERY P: Antibodies to type II collagen in early rheumatoid arthritis. Correlation with disease progression. *Arthritis Rheum* 1999; 39: 1720-7.
69. ÁGUAS AP, ESAGUY N, SUNKEL CE, SILVA MT: Cross-reactivity and sequence homology between the 65-kilodalton mycobacterial heat shock protein and human lactoferrin, transferring and DRβ subset of major histocompatibility complex class II molecules. *Infect Immun* 1990; 58: 1461-70.
70. COHEN IR: The self, the world and autoimmunity. *Sci Am* 1988; 258: 34-42.
71. QUAYALE AJ, WILSON KB, LI SG *et al*.: Peptide recognition, T cell receptor usage and HLA-restriction elements of human heat-shock protein (hsp) 60 and mycobacterial 65-kD hsp-reactive T cell clones from rheumatoid synovial fluid: *Eur J Immunol* 1992; 22: 1315-22.
72. FITZGERALD O, WINCHESTER R: Psoriatic arthritis: from pathogenesis to therapy. *Arthritis Res Ther* 2009; 11: 214-22.
73. JENISCH S, HENSELER T, NAIR RP *et al*.: Linkage analysis of human leukocyte antigen (HLA) markers in familial psoriasis: strong disequilibrium effects provide evidence for a major determinant in the HLA-B/-C Region. *Am J Hum Genet* 1998; 63: 191-9.
74. MURRAY C, MANN DL, GERBER LN *et al*.: Histocompatibility alloantigens in psoriasis and psoriatic arthritis Evidence for the influence of multiple genes in the major histocompatibility complex. *J Clin Invest* 1980; 66: 670-5.
75. SCARPA R, COSENTINI E, MANQUSO F *et al*.: Clinical and genetic aspects of psoriatic arthritis "sine psoriasis" *J Rheumatol* 2003; 30: 2630-40.
76. OLIVIERI I, PADULA A, D'ANGELO S, CUTRO MS: Psoriatic arthritis "sine psoriasis". *J Rheumatol Suppl* 2009; 83: 28-9.
77. IKÄHEIMO I, TIILIKAINEN A, KARVONEN J, SILVENNOINEN-KASSINEN S: HLA risk haplotype Cw6, DR7, DQA1*0201 and HLA-Cw6 with reference to the clinical picture of psoriasis vulgaris. *Arch dermatol Res* 1996; 288: 363-5.
78. SAKKAS LI, LOQUEMAN N, BIRD H, VAUGHAN RW, WELSH KI, PANAYI GS: HLA class II and T cell receptor gene polymorphism in psoriatic arthritis and psoriasis. *J Rheumatol* 1990; 17: 1487-90.
79. SZCZERKOWSKA-DOBOSZ A: Human leukocyte antigens as psoriasis inheritance and susceptibility markers. *Arch Immunol Ther Exp* 2005; 53: 428-33.