

DNA analysis of dust particles sampled from the Turin Shroud

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Abstract. The Turin Shroud is traditionally considered the burial cloth in which the body of Jesus Christ was enveloped after his death about 2000 years ago. Here we report the main findings from the analysis of genomic DNA extracted from dust particles, which were vacuumed from the backside of Turin Shroud corresponding to internal parts of the body image and the lateral edge used for its radiocarbon dating. Specific plant chloroplast DNA (cpDNA) and human mitochondrial DNA (mtDNA) target regions were analyzed to identify plant taxonomic entities and human genetic lineages. Plant species native to the Mediterranean countries and widespread in the Middle East (Vavilov's centers of origin V and IV, respectively) were identified, in addition to others living in temperate and boreal regions of the northern hemisphere or having their primary center of origin and distribution in central and eastern Asia (mainly China, I) or native only to the Americas. Since many of these species were introduced into Europe after the Marco Polo travels and Christopher Columbus voyages, our findings suggest a geographic scenario for which only some of the detected plant cpDNAs are compatible with the supposed origin and trail of the relic, whereas others are likely from a historical interval later than the Medieval period. As for human mtDNAs, our analyses allowed the detection of sequences from multiple subjects, which clustered into a number of western Eurasian haplogroups, including some known to be typical of western Europe (H1 and H3), the Near East (H13 and H33), the Arabian Peninsula (R0a) and the Indian sub-continent (M56 and R8). Such mitogenome diversity could be due to contacts with subjects of different ethnic origins in recent centuries, but it is also compatible with the historic path followed by the Turin Shroud during its supposed 2000-year journey from the Near East. Furthermore it raises the possibility of an Indian manufacture of the linen cloth.

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1 Introduction

According to many scholars, the long journey of the Turin Shroud began in Jerusalem in the year 30 or 33 AD. After concealment for years, the shroud was moved to Edessa (now Şanlıurfa in Turkey) and then to Constantinople (now Istanbul in Turkey) in 944 AD. After the Sack of Constantinople in 1204, the shroud was most likely taken by the crusaders and transferred to Athens (Greece), where it remained until 1225. It reappeared in France at Lirey around the years 1353 to 1357 and was kept at Chambéry from 1502 to 15784. Apart from some brief displacements in an effort to hide it during war periods, the shroud has been conserved in the Cathedral of Turin (Italy) from 1578 to the present (for comprehensive reviews see [1-6] and references therein).

In this study, we performed DNA analyses to determine the biological sources of the dust particles (*i.e.*, pollen grains, cell debris and other minuscule organic specimens, such as plant-derived fibers and blood-like clots) vacuum-collected on the occasion of official samplings from the Turin Shroud. In particular, all these specimens were derived from different filters corresponding to various areas of the Turin Shroud and some of the dust particles have been removed from the filters and supplied to scientists for investigative studies. Genetic analyses of DNA isolated from plant fibers and cell debris or human blood crusts deriving from samples of the Turin Shroud have been postulated several times. In the past decades, hundreds of pollen grains have been identified and classified in terms of genus and species, using both optical and scanning electron microscopes, in order to be compared with geographic areas in which the corresponding plants did originate and now live. Independent cytological observations have clearly demonstrated that the identified geographic areas are fully compatible with the supposed historic paths travelled by the Turin Shroud [6-10]. In more recent years, molecular results have apparently supported the existence of human DNA. Nevertheless, the DNA as a whole of the Turin Shroud remains still uncovered.

Here we document novel and robust genetic data used for taxonomic identification of plant species on the basis of chloroplast DNA (cpDNA) barcodes and molecular reconstruction of human mitochondrial DNA (mtDNA) haplotypes by exploiting short DNA sequences recovered from pollen grains, cell debris and other minuscule organic specimens, such as plant-derived fibers and blood-like clots found into the dusts sampled in the Turin Shroud by STURP Members. In particular, the dust particles analyzed in this study belong to different filters of the back of the Turin Shroud, corresponding to face, hands, glutei and feet of the body image, and the lateral edge, which was used for radiocarbon dating. Our main task dealing with the attribution of DNA residuals to living organisms or fossils of known genetic identity was engaged from a molecular point of view by isolating, amplifying, subcloning and sequencing specific regions of the genome. In particular, we focused our investigations on nuclear markers (rDNA intergenic transcribed spacers, ITS) and extra-nuclear markers, of both chloroplast (cpDNA barcodes RuBisCO or *rbcL*, *trnH-psbA*, and *trnL-intron*) and mitochondrial (mtDNA targets CYB, COI and NADH dehydrogenase-subunit 5 along with the hypervariable regions HVRI and HVRII, the so-called D-loop) origin for the analysis of plant and human microscopic residuals, respectively. Organic remnants restrained by the dusts of the Turin Shroud allowed us to extract DNA molecules that were then amplified by Polymerase Chain Reaction (PCR) assay and sequenced by Next Generation Sequencing (NGS) technology in order to recover genic and intergenic markers suitable to identify plant species as well as human haplotypes.

2 Results

According to chloroplast DNA and ITS sequences, several plant taxa native to the Mediterranean area were identified (distinct Clovers, Ryegrass, Chicory, Horsetail and Plantains) as well as species with a primary center of origin in the Asian continent (rare species of Plum and Pear, Cucumber, Common Hop, Grape, Hawthorn, Walnut and Hornbeam) or the Americas (Black locust and Nightshades) but introduced in a historical interval later than the Medieval period and currently widely spread throughout Europe (**Table 1**). The most abundant species belongs to the genus *Picea*, which includes

a few closely related Spruces native to Europe and several other forms widespread in temperate and boreal forest regions of the Northern Hemisphere (for additional information see [11]). Clearly some of the species identified on Turin Shroud were not introduced to Europe before the XVI century, after the discovery of America by Christopher Columbus, while others originating from China were likely introduced to Mediterranean territories from the XIII century and thereafter, following the travels of Marco Polo.

Among the mtDNA amplicons generated and sequenced, different human sequences corresponding to distinct mtDNA loci were identified. This finding not only indicates that human DNA was indeed unequivocally present in the dust particles sampled from the Turin Shroud, but also that the sources of human DNA could be ascribed to numerous individuals. In fact, the mtDNA haplotypes were found to belong to different branches of the human mtDNA tree, even after having excluded all the mtDNA sequences that could be theoretically attributed to operator contaminations. In particular, our analyses allowed the detection of sequences from multiple subjects, which clustered into a number of Western Eurasian haplogroups, including some known to be typical of Western Europe (H1 and H3), the Near East (H2a, H13 and H33), the Arabian Peninsula (R0a) and the Indian sub-continent (M56, R7 and R8). Such mitogenome diversity could be due to contact with individuals of different ethnic origins in recent centuries, though it is also compatible with the proposed historic path (*i.e.*, Jerusalem in Israel until approximately 500 A.D., Sanliurfa in Turkey until 944, Constantinople in Turkey until 1204, Lirey and Chambery in France from 1353 until 1578, Turin in Italy to date). A schematic overview of the current geographical distribution of the human mtDNA haplogroups and sub-haplogroups identified in the Turin Shroud is reported in **Figure 1** (for additional information see [11]).

Table 1. Plant cpDNA and ITS sequences identified in the dust particles of the Turin Shroud along with their putative species or genus source. All plant cpDNA and ITS sequences deriving from PCR amplicons were verified through replicated sequencing of both strands and deposited in GenBank with accession numbers JQ007354-JQ007431 and JQ082521-JQ082524.

Common Name	Genus/Species	TS filters	Gene/Barcode	No. sequences
Horsetail	<i>Equisetum</i> spp.	I	rbcl	1
Spruce	<i>Picea</i> spp.	E, F, G, H, I	trnL	17
Common hop	<i>Humulus lupulus</i> L.	I	trnH-psbA	2
Cucumber	<i>Cucumis</i> spp.	I	trnH-psbA	2
Grape	<i>Vitis</i> or <i>Parthenocissus</i> spp.	G	trnL	1
Knotted clover	<i>Trifolium striatum</i> L.	I	ITS	3
White clover	<i>Trifolium repens</i> L.	I	trnL	2
Tobacco	<i>Nicotiana</i> spp.	I	rbcl	1
Nightshades	<i>Solanaceae</i> fam.	I	rbcl	1
Annual ryegrass	<i>Lolium multiflorum</i> Lam.	E	ITS	4
Hornbeams	<i>Carpinus</i> spp.	I	rbcl/trnL	2
Chicory	<i>Cichorium intybus</i> L.	F	trnL	2
Persian walnut	<i>Juglans regia</i> L.	I	trnL	2
Hawthorn	<i>Crataegus</i> spp.	I	rbcl	1
Black locust	<i>Robinia pseudoacacia</i> L.	E, F	ITS	4
Strawberry clover	<i>Trifolium fragiferum</i> L.	I	trnL	2
Plantain	<i>Plantago argentea</i> Chaix	I	trnL	1
Chinese plum	<i>Prunus kansuensis</i> Rehder	I	trnH-psbA	2
Almond-leaved pear	<i>Pyrus spinosa</i> Forssk	I	trnH-psbA	1
Shrub willow	<i>Salix suchowensis</i> W.C.	I	trnH-psbA	3
Soybean	<i>Glycine max</i> (L.) Merr.	I	rbcl	1

On the basis of our findings, two scenarios are possible for the Turin Shroud: i) it is authentic – 2000 years old: the burial cloth in which the body of the “Man of the Shroud” was wrapped after his death came in contact with different folks/devotes across different lands during its long journey; ii) it is a medieval fake (XIII–XIV century): most DNA contaminants derive from various ethnic groups of the Middle East, Caucasus, North-East Africa, Central Asia and India who traveled towards Europe (France and Turin).

3 Discussion

DNA extracted from dust particles that were vacuumed from the Turin Shroud showed sequence profiles that identify numerous plant cpDNA specific types and correspond to several distinct human mtDNA haplogroups. Combination of sampling and sequencing methods allowed us to study a broad range of particles, leading to the isolation and identification of several plant and human DNA molecules.

These results not only confirm that plant fibers and pollen grains are present on the shroud, as previously reported by optical microscopy, but also reveal that multiple human individuals touched or otherwise left traces of their DNA on this relic. The detection of such a variety of DNA sources is extremely valuable in assessing whether there are possible parallelisms between the areas of origin and distribution of identified land plant species and human mitochondrial haplogroups and the documented temporal and spatial paths of the Turin Shroud.

Most of the plant DNA sequences recovered from the total genomic DNA isolated from the Turin Shroud belong to taxonomic species native to and spread in the Old World. In fact, based on cpDNA and ITS sequences, we identified plant species that have an origin and are now widespread in regions around the Mediterranean basin, from the Iberian Peninsula to Palestine (*e.g.*, Clovers, Ryegrasses, Plantains and Chicories). Despite the nature of the cloth, the lack of recovery of DNA sequences ascribable to *Linum usitatissimum* is not surprising because flax fiber cells at maturity are typically dead [12] and the hydrolytic damage of the DNA, which is expected for old specimens [13], is also favored by the procedures commonly adopted for the removal of fibers from the plant stem [14].

The presence of some alien tree species introduced from Eastern USA (*Robinia pseudoacacia* L.), and Northern or Southern China (*Salix suchowensis* W.C. Cheng, *Pyrus spinosa* Forssk and *Prunus kansuensis* Rehder) is not negligible, with the former species that currently has a distribution area centered in Europe and the Mediterranean basin, and the latter that are more widespread in temperate Asia, Southern Europe and Northern Africa. Overall, the various plant species and numerous taxonomic families identified on the Turin Shroud suggest that contamination may have occurred during the past centuries and support the scenario that the linen cloth was likely exposed to different locations across the Mediterranean area. We can assume that the Turin Shroud was likely displayed in, or in very close contact with, different types of natural and anthropological environments. The large variety of plant forms (*i.e.*, herbaceous plants and woody tress, with agricultural and/or forest interest, used as food source and ornamental purposes, etc.) is compatible with highly diversified geographic ranges, whose areas of radiation may vary from the farms of the cultivated plains to the forests of the uncontaminated mountains. Similarly, on the basis of the known ages of introduction of certain plant species from Northern and Southern America, and Eastern Asia, we can assert that the Turin Shroud may have followed a highly variable temporal path. In fact, some species have their center of origin and diversification in areas around the Mediterranean basin, including North Africa, and most of these species were widely distributed throughout Europe before Christ age. Other species among those identified in the Turin Shroud, however, were introduced to Europe not before the XVI century, after the discovery of America by Christopher Columbus (for example, the species of the genus *Robinia* and some others of the *Solanaceae* family). Finally, fruit plants of the genera *Prunus* and *Pyrus*, belonging to the Rosaceae family, are necessarily deriving from Central Asia and Middle East regions, and they were likely introduced in the countries bordering the Mediterranean sea from the XIII century onwards, after the travels of Marco Polo.

On the basis of the analyses performed so far, we can state to have ascertained the presence of human mtDNA in the Turin Shroud characterized by distinct haplogroups, including R0a, R7 and R8, several H haplotypes (*e.g.*, H1, H2a, H3, H13 and H33), and few L3c and M haplotypes.

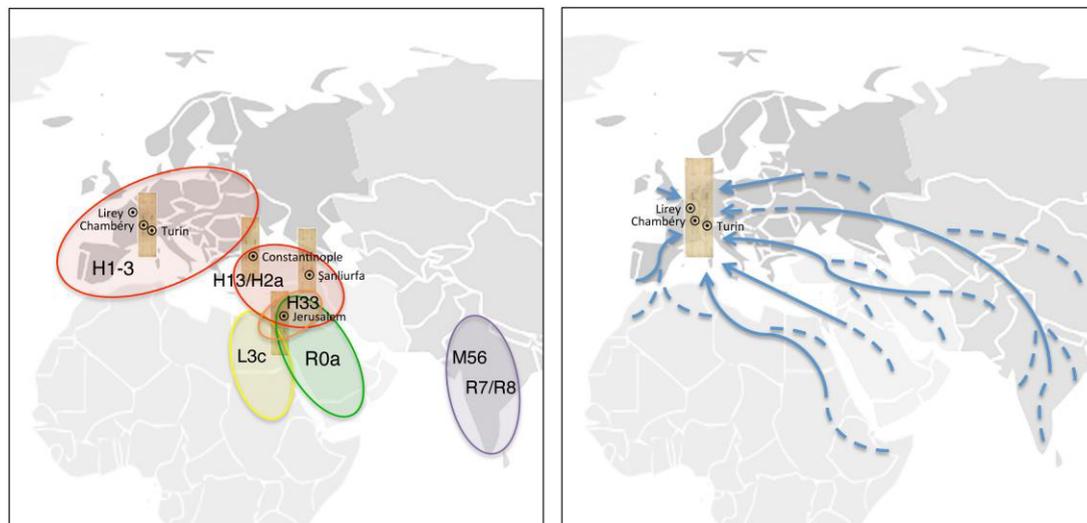


Figure 1. Schematic overview of the current geographical distribution of human mtDNA haplogroups and sub-haplogroups identified in the analyzed dust particles of the Turin Shroud. The haplotypes deciphered in this study are mostly related to haplogroups and subhaplogroups that belong to ethnic groups of Europe, Middle East and India. The human mtDNA sequences, as clusters deriving from the assembly of reads generated by 454 sequencing, were deposited in GenBank with accession numbers KP126143-KP126230.

With regard to the sources of human DNA, the detected haplotypes do not cluster randomly on the entire human mtDNA tree, but only on a specific subset of its branches, corresponding to haplogroups from Western Eurasia and surrounding areas. All these sequences are from geographic regions that were the homelands of most of the perhaps thousands of people who came into contact with the relic throughout the centuries. Taking into account only dust particles sampled from filters corresponding to the internal parts of the linen cloth, which enveloped the body of the man of the shroud, four partially overlapping geographic regions are generally outlined by the human haplogroups: i) the Middle East with haplogroups H13, H33 and R0a; ii) Southeast Europe and Turkey with haplogroups H1a, H2a and H13; iii) Western Europe, including France and Italy, with haplogroups H1j and H3; and iv) India with haplogroups M56 and R8.

The haplogroup R0a occurs frequently in the Arabian Plateau, especially in local populations of Saudi Arabia, with its highest frequency in Yemen (38%) and smaller frequency in North-East Africa, including Horn of Africa, Anatolia, Iranian Plateau and Dalmatia, whereas the highest frequency of haplogroup R8 occurs mainly towards the East India. The haplogroup H, for which several subgroups were identified in the Turin Shroud, is by far the most frequent in Europe. H1 is very common in the Western regions of Europe, but also among the populations of North Africa including the Berbers. The haplogroup H13 is known to be rare in Europe and West Asia, being also present in the Caucasus, whereas H33 is a very rare haplogroup nowadays mainly found among the Druze, a minority population of Israel, Jordan, Lebanon and Syria. Finally the M haplogroups are typical of Asia, while L3c is very common in North-Eastern Africa (*e.g.*, Egypt, including Ethiopian and Yemeni Jews) and it is also found in Southern Europe.

The lineage of a haplotype, in this case understood as the combination of allelic variants – based on single and informative nucleotide polymorphisms – along a segment of mitochondrial DNA (*i.e.* D-loop), reconstructed by using our data does not reflect neither the total genetic variation of the ancient populations, as with the genetic drift some variants of the mitochondrial DNA may have been lost, nor

the current geographical locations, as with the gene flow as a consequence of migration of individuals among populations of different countries some variants of the mitochondrial DNA may have been mixed in modern populations. The fact remains that the haplotypes deciphered up to now from the human DNA found in the Turin Shroud are all related to haplogroups that belong to ethnic groups of Europe, North Africa and Middle East, hence especially populations who live in countries that overlook the Mediterranean basin and in regions of the very large Arabian plateau, spanning from the Iberian Peninsula to Anatolia and Caucasus to the Horn of Africa.

In conclusion, results from this study are consistent with the presence of several plant species according to cpDNA barcodes and distinct human mtDNA haplogroups. Overall DNA data were compared with historical information to verify whether the geographic areas of origin and distribution of land plant species (embryophytes) and human mitochondrial haplogroups are coherent with the proposed temporal and spatial paths of the Turin Shroud. However, the detection of mtDNA haplogroups that are typically from India is somehow unexpected and raises the possibility that the linen cloth was weaved in India, as supported perhaps by the original name of the Turin Shroud – *Sindon* – which appears to derive from *Sindia* or *Sindien*, a fabric coming from India [11].

Acknowledgments

An extended and detailed version of this paper has been recently published by the same authors on *Scientific Reports* (<http://www.nature.com/articles/srep14484/>). Raw sequence reads and datasets are available in the BioSample database of NCBI (<http://www.ncbi.nlm.nih.gov/bioproject/283206>). In particular, all the sequences related to the MT- DNA loci (MT-DLOOP, MT-CO1, and MT-ND5) and obtained from the TS filters (samples EFGH, I and IR) have been deposited with the following accession numbers: SAMN03610574–SAMN03610579 and SAMN03610596–SAMN03610598 (Registration date: 6-May-2015).

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