Genetic identification of putative remains of the famous astronomer Nicolaus Copernicus

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We report the results of mitochondrial and nuclear DNA analyses of skeletal remains exhumed in 2005 at Frombork Cathedral in Poland, that are thought to be those of Nicolaus Copernicus (1473–1543). The analyzed bone remains were found close to the altar Nicolaus Copernicus was responsible for during his tenure as priest. The mitochondrial DNA (mtDNA) profiles from 3 upper molars and the femurs were identical, suggesting that the remains originate from the same individual. Identical mtDNA profiles were also determined in 2 hairs discovered in a calendar now exhibited at Museum Gustavianum in Uppsala, Sweden. This calendar was the property of Nicolaus Copernicus for much of his life. These findings, together with anthropological data, support the identification of the human remains found in Frombork Cathedral as those of Nicolaus Copernicus. Up-to-now the particular mtDNA haplotype has been observed only 3 times in Germany and once in Denmark. Moreover, Y-chromosomal and autosomal short tandem repeat markers were analyzed in one of the tooth samples, that was much better preserved than other parts of the skeleton. Molecular sex determination revealed that the skeleton is from a male individual, and this result is consistent with morphological investigations. The minimal Y-chromosomal haplotype determined in the putative remains of Nicolaus Copernicus has been observed previously in many countries, including Austria, Germany, Poland, and the Czech Republic. Finally, an analysis of the SNP located in the *HERC2* gene revealed the C/C genotype that is predominant in blue-eyed humans, suggesting that Copernicus may have had a light iris color.

eye-color marker | hairs | human remains | identification | mitochondrial and nuclear DNA

The world's most famous astronomer, Nicolaus Copernicus (in Polish, Mikołaj Kopernik), author of *De Revolutionibus Orbium Coelestium*, was born in 1473 in Thorun (in Polish, Toruń), Poland. Copernicus' father died when he was only 10 years old. Nevertheless, thanks to his eminent uncle, bishop of Warmia, Lucas Watzenrode, Nicolaus Copernicus obtained a very thorough education including studies at the Jagiellonian University in Cracow (in Polish, Kraków) and at the Italian universities in Bologna, Padova, and Ferrara. Most of the astronomical observations that formed the basis for his extraordinary discoveries were made in the Polish city of Frombork, where he served as a canon in the Cathedral. Nicolaus Copernicus died at age 70 in the year 1543, and was buried in the Frombork Cathedral. Unfortunately, Frombork Cathedral has >100 tombs, and the majority are unnamed. Nevertheless, for over 200 years, attempts have been made to find Copernicus' grave. Even Napoleon played a part in these efforts when he ordered one of his officers to perform such a search in 1807 (1). In 2004 a group of Polish scientists launched a new search for Copernicus' grave. The exact location was uncertain, but it has been thought that the grave could be located near the St. Cross Altar because Copernicus was in charge of this altar during his tenure as priest at the Cathedral (2). Several skeletons were discovered near the St. Cross Altar in 2005, including one incomplete skeleton that appeared to be that of Copernicus based on a facial reconstruction (3).

In addition to morphological studies, DNA analysis is commonly used for individual identification of historical or even ancient remains. In the case of the putative Copernicus remains, a genetic identification was possible because the remains, especially the teeth, were found to be well preserved. A challenge here, however, was to find a possible source of reference material. The uncle, Lucas Watzenrode, would provide a common maternal lineage if his biological material had been available. However, a thorough search for his remains, and those of other relatives to Copernicus, has failed so far. In lack of DNA from maternal or paternal relatives, the hope to find a reference DNA that could be linked to Copernicus was focused on an astronomical reference book, *Calendarium Romanum Magnum* by Johannes Stoeffler that was used by him for many years. This book had been taken to Sweden as "war booty" after the Swedish invasion of Poland (the so-called "Deluge") in the mid 17th century, and currently is the property of Museum Gustavianum at Uppsala University. A careful examination of the book revealed several hairs, and a likely source of the hairs is the book's owner and principal user, namely Copernicus himself. Therefore, these hairs were evaluated as a possible reference material for a genetic comparison with the teeth and bone material recovered from the St. Cross Altar tomb.

Results

The cranium (Fig. 1 *A* and *B*) and postcranial material (Fig. 1*C*) exhumed from the St. Cross Altar tomb appear to be from a person who died at 60–70 years of age. Sequence analysis of the hypervariable region I (HVI) and hypervariable region II (HVII) was possible for the tooth samples. These 2 highly polymorphic regions located within the control region of the mitochondrial genome are commonly used in forensic identification cases when analysis of nuclear markers fails. An identical HVI and HVII mtDNA profile [with the polymorphisms: 16129A; 16316G; 263G; 315.1C according to revised Cambridge reference sequence (rCRS)] was confirmed in the teeth by 3 independent laboratories using slightly different procedures. Analysis of the HVI region was also successful from the more degraded femur samples, providing additional support of the findings from the tooth analysis. Taken together, these data

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See Commentary on page 12215.

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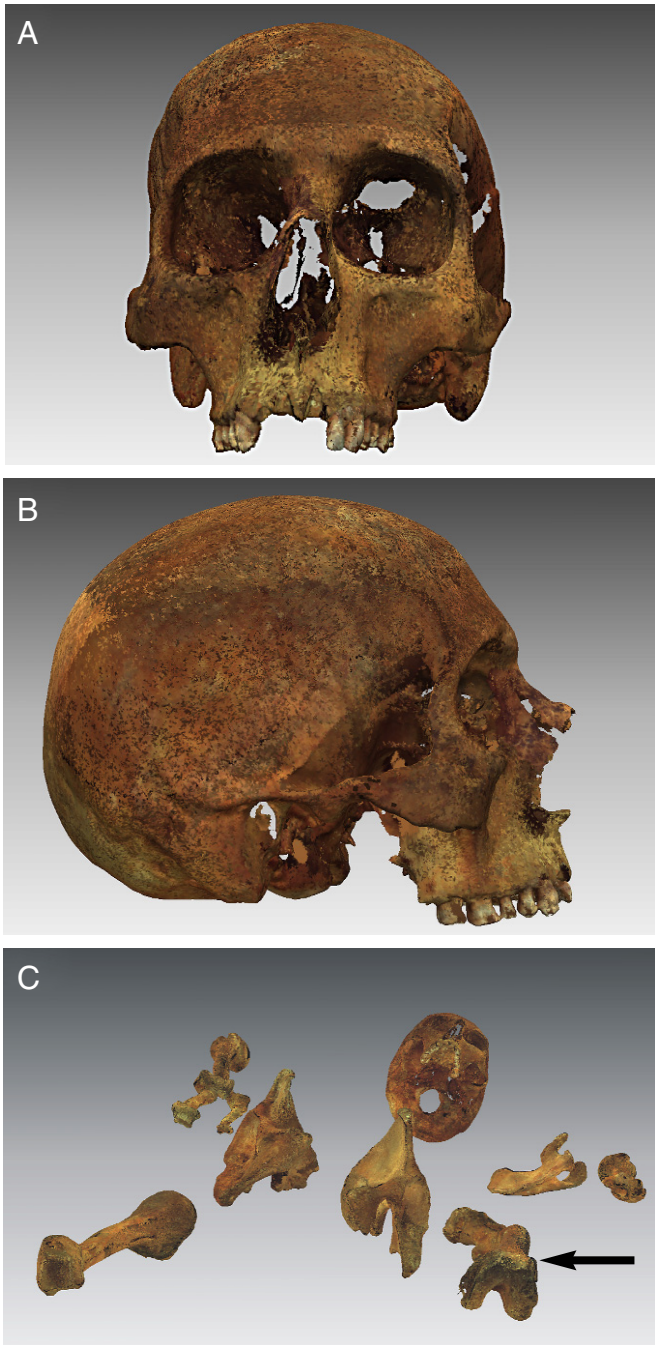

Fig. 1. Frontal (*A*) and lateral (*B*) views of the cranium (no mandible found), and 3-dimensional view of some bones from the putative skeleton of Nicolaus Copernicus (*C*). The femur is indicated by the arrow. Scanning was done using a Konica-Minolta Vivid 9i.

demonstrate that the skull and the remainder of the skeleton are likely to be from one single individual.

The investigation of the astronomical calendar at Museum Gustavianum in Uppsala revealed 9 hair samples that were collected. The analysis provided mtDNA sequence data for 4 of the hairs. Of the obtained profiles, 2 were identical to each other and to the profile from the skeletal remains recovered from the St. Cross Altar tomb. The 2 other hairs contained profiles that differed from each other and from the profile in the remains, and can thus be excluded as being from the same source.

In addition to the hypervariable region analysis, 16 haplogroup informative SNP positions were examined (709G, 1719G, 1811A, 3010G, 6365T, 6776T, 7028C, 8251G, 8697G, 9055G, 11251A, 12372G, 13708G, 14766C, 14798T, and 15904C). Analysis of these haplogroup informative mtDNA polymorphisms indicates that the examined individual belongs to haplogroup H, which is the most frequent of the 6 European-specific haplogroups. Approximately 40% of the population in Europe can be classified into this haplogroup, which is uniformly distributed throughout the continent (4–7).

Table. 1. Genotype frequencies (%) at rs12913832 of *HERC2* vs. iris color

	rs12913832 genotype		
Eye color	C/C	C/T	T/T
Blue/gray	83.5	15.8	10.5
Green	13.5	10.1	5.3
Hazel	3.0	46.8	47.4
Brown/black	0	27.3	36.8

N = 388; data from ref. 8

Only 6 markers (representing the shortest amplicons) out of 15 short tandem repeat (STR) loci included in the Identifiler kit (Applied Biosystems) gave positive genotyping results: D8S1179– 11, 14; D3S1358– 16, 18; TH01– 9.3; D19S433– 13; VWA– 14, 15; D5S818– 12. PCR products were also obtained for the amelogenin sex marker, and this result is in concordance with the previous anthropological finding that the examined skeleton was a male (XY). Male sex was further confirmed by the analysis of 16 STR loci located on Y chromosome included in the Yfiler amplification kit (Applied Biosystems): DYS456– 16; DYS389I– 13; DYS390– 23; DYS389II– 29; DYS458– 19; DYS19– 14; DYS385– 11, 13; DYS393– 13; DYS391– 11; DYS439– 12; DYS635– 23; DYS392– 13; Y GATA H4– 12; DYS437– 15; DYS438– 12; DYS448– 19.

Additionally, we analyzed a single SNP position, rs12913832 located in an evolutionarily conserved region within intron 86 of the *HERC2* gene (8). This analysis revealed a homozygous C/C genotype prevalent among individuals with light eye coloration (Table 1).

Discussion

Previously successful analyses of old human material have been performed in several cases including: 24 Neolithic skeletons of the first European farmers (9); a 5,000-year-old mummified human body found in the Tyrolean Alps (10); the evangelist Luke (11); the Italian poet and scholar Francesco Petrarca (12); a putative son of Louis XVI, king of France and Marie-Antoinette (13); the legendary outlaw Jesse James (14); and the Tsar family Romanov (15).

Here, we present an additional case of successful DNA analysis for human individual identification. The mtDNA profile in the putative remains from the St. Cross Altar tomb at the Frombork Cathedral and 2 of the hair samples from Copernicus' calendar match completely. We can only speculate in that the 2 hair samples with different profiles originate from other readers of the book. The poor amplification efficiency indicates that they do not originate from a contemporary source. A search in the EMPOP mtDNA population database revealed a maximum match probability for this particular mtDNA profile equal to 0.2067% (16). This means that ≈1 in 483 randomly chosen individuals would have this haplotype.

The search in the EMPOP mtDNA database (17) showed that the mtDNA profile found in St. Cross Altar skeletal remains occurred in 4 of 3,830 West Eurasian haplotypes present in the database. The matching profiles were previously seen in individuals derived from Germany (1 from Rostock and 2 from Ulm) and Denmark (Copenhagen). No identical haplotype was found

in other population groups (of a total of 4,527 haplotypes in the database).

The result of the EMPOP database search is interesting from the perspective of Copernicus' maternal lineage. His maternal ancestors may have originated from Silesia, and can thus be of German descent. Copernicus' grandmother, Catherina, was first married to Heinrich Peckau, who was a member of the council of Thorun. After Heinrich's death, Catherina was married to a trader and famous enemy of the Teutonic Knights—Lucas Watzenrode. Together they had 3 children, Christina, Lucas, and Nicolaus Copernicus' mother, Barbara (18).

In the case of the paternal lineage, the search of the YHRD Y chromosome population database (19) did not reveal the haplotype found in the examined human remains among the 2,595 complete haplotypes comprising the Eurasian metapopulation and among all of the 10,243 complete haplotypes included in the database originating from all over the world. The YHRD database size varies significantly based on the number and character of loci that are included in the search profile. By limiting their number to the core set called the minimal haplotype (most often analyzed Y-STR loci) the searchable data in the YHRD database were significantly extended, giving the total number of 63,369 haplotypes. In this larger dataset, a minimal Y-chromosomal haplotype, derived from the putative Copernicus remains, was present 47 times, 44 times in a European metapopulation consisting of 31,762 minimal Y-chromosome haplotypes. The same haplotype has been found in individuals from many countries, including Austria, Germany, Poland, and the Czech Republic. It is interesting to note that Copernicus' paternal ancestors may also have originated from Silesia. Copernicus' father, also named Nicolaus, was a known trader in Cracow. He moved to Thorun ≈1458 where he married Barbara Watzenrode. Nicolaus Copernicus was their youngest son. The Y-chromosome data that we obtained will be useful if reference samples from some of Copernicus' relatives along the paternal lineage are ever collected.

Analysis of the SNP position located in the *HERC2* revealed the homozygous C/C genotype, which is the predominant genotype among blue or gray-eyed humans (≈80%). This genotype is rare among people with dark iris coloration (8, 20, 21). The result indicates that Copernicus might have had light iris color, a finding that is rather unexpected given that he is usually shown in portraits with dark eyes. Nevertheless, it is difficult to unambiguously interpret this finding because, although it is significantly less probable, the genotype C/C in rs12913832 can be associated with dark (but not brown/black) irises. One possible explanation for the discrepancy is that early portraits of Copernicus, i.e., those made during his lifetime, were often made using a chalcography technique, which does not reflect actual colors. Thus, it is possible that the initial impression of dark eye color created by a faulty technique color could have been replicated by other artists.

Taking all data into consideration, i.e., the identical genetical profiles in the skeletal remains and reference hairs along with the other anthropological and archeological information, we conclude that the skeletal remains derived from the St. Cross Altar tomb at Frombork Cathedral are those of the great Polish astronomer, Nicolaus Copernicus. This is the end of a search that has lasted for at least 2 centuries, and a clear demonstration of the value of using both molecular and morphological approaches in the investigation of historical remains.

Materials and Methods

Samples. Teeth (upper molars) and femur samples were chosen for DNA extraction and genotyping of the putative remains of Nicolaus Copernicus. The DNA extraction from bone material was performed in 3 laboratories i.e., (*i*) Institute of Forensic Research in Kraków, Poland (tooth T1, femur F1); (*ii*) Museum and Institute of Zoology of the Polish Academy of Sciences in Warsaw, Poland (tooth T2, femur F2) and (*iii*) Rudbeck Laboratory at Uppsala University, Sweden (tooth T3 and femur F3). The following precautions were undertaken in the laboratory to make every possible effort to prevent contamination: Full protective clothing and separate working localities for extraction, amplification, and sequencing setup were used. Extraction and PCR were performed in separate clean room facilities with HEPA-filtered air, positive pressure and LAF-benches. Furthermore, all working areas, including all equipment, were regularly UV-irradiated and cleaned with bleach. At least 2 different analysts performed all steps in the analysis, and 2 negative controls were included for each extraction and amplification performed. The extraction procedures were as follows: (*i*) Bone samples were treated with 15% bleach (a tooth or ≈1-cm^3 pieces of femur were submerged in bleach for 1 min), then repeatedly shaken with 70% ethanol and distilled water (dH_2O), and finally subjected to UV irradiation. Bone and tooth samples were subsequently pulverized using FreezerMill 6750 apparatus (Spex CertiPrep) and subjected to an organic extraction procedure. Briefly, ≈3 g of bone powder were incubated overnight at 56 °C with 3 mL of buffer (0.5 M EDTA, 10% SDS), 225 μL of proteinase K (10 mg/mL) and 120 μL of 1 M DTT. After incubation, all samples were subjected to double extraction with a buffered mixture of phenol-chloroform-isoamyl alcohol (Sigma). DNA extracts were then concentrated and purified with Centricon 100 columns (Millipore). (*ii*) Samples were treated with 15% bleach, then repeatedly shaken with 70% ethanol and dH_2O and UV irradiated. After decontamination, samples were individually crushed and the powder was transferred to a sterile tube. Samples were digested overnight at 55 °C in the lysis buffer containing Proteinase K (DNeasy Tissue Extraction Kit; Qiagen) and DNA was extracted following the protocol for isolation of total DNA from solid tissues using the DNeasy Tissue Extraction Kit (Qiagen). (*iii*) Bone and tooth extractions were performed individually using the same protocol. Before the extraction, a tooth or a bone piece (≈1 cm^3) was submerged in 6% sodium hypochlorite (bleach) for 15 min for decontamination of exogenous DNA. This process was followed by demineralization in 2 mL of 0.5 M EDTA (pH 8.0). Digestion of bone was achieved by addition of 3 mg proteinase K and incubation for ≈17 h at 65 °C. The protocols are from refs. 22 and 23 with minor modifications. A salting out procedure was performed using the Wizard Genomic DNA Purification Kit (Promega). The tooth extraction was performed as described for bone with pulverization of the tooth using liquid nitrogen. The powder was soaked at 37 °C in 0.5 M EDTA, 5% SDS, and 3 mg proteinase K, and thereafter extracted using the Wizard Genomic DNA Purification Kit (Promega).

A total of 9 hair samples were collected from the standard astronomical reference *Calendarium Romanum Magnum* by Johannes Stoeffler. This book, which belonged to Copernicus, is now in the possession of the Museum Gustavianum in Uppsala, Sweden. The hair specimens, serving as possible reference material, were analyzed in the Rudbeck Laboratory at Uppsala University. The samples were extracted and amplified separately. Each hair was cleaned in 0.4% SDS followed by 1 wash in 100% ethanol and 3 washes in dH_2O. Hairs were extracted in a total volume of 212 μL containing a final concentration of 1 × PCR buffer II (Applied Biosystems), 33 mM DTT, and 0.24 μg/μL Proteinase K (Sigma). A spin column, Microcon Y-30 (Millipore) was used to purify the samples.

Analysis of mtDNA. Procedures for sequencing of the hypervariable segments in mtDNA varied slightly among the 3 laboratories involved in the project. (*i*) PCR amplification was performed using previously described primer pairs (L15997-H16236 and L16159-H16401 (HVI); L48-H285 and L172-H408 (HVII) (24). Amplification was performed in GenAmp 9700 thermocycler (Applied Biosystems) in a total volume of 10 μL. The reaction mixture contained 5 μL of Qiagen multiplex PCR kit (Qiagen), 1 μL of PCR primers, 1 μL of Q solution, and 3 μL of template DNA. The temperature profile was as recommended by the kit manufacturer with an annealing temperature of 58 °C (HVI) or 60 °C (HVII). PCR products were checked on 2.5% agarose gel and the remaining volume was purified with Exo-SAP IT kit (Amersham Pharmacia). Sequencing reactions were performed using BigDye Terminator Cycle Sequencing Ready Reaction kit, v.1.1 (Applied Biosystems) with the primers used for amplification reactions. The products of sequencing reactions were resolved with an ABI PRISM 3100 genetic analyzer (Applied Biosystems), and analyzed using SeqScape computer software (Applied Biosystems).

(*ii*) Amplification of the HVI and HVII was carried out with a thermocycler T1 (Biometra) using REDTaq Genomic Polymerase (Sigma) and the following thermal profile: 95 °C for 2 min followed by 38 cycles of 94 °C for 15 s, 58 °C for 20 s, 72 °C for 1 min, and a final elongation step of 72 °C for 3 min. We used primer pairs described in refs. 11 and 25: pairs L16055-H16139, L16122-H16379, and L16209-H16401 (HVI) and ref. 26: L00052-H00201, L00123-H00270, and L00260-H00397 (HVII). PCR products were visualized on 2.5% agarose gel and amplicons were subsequently cleaned using the QIAquick PCR Purification Kit (Qiagen). DNA sequencing was carried out using a DTCS quick start master mix (Beckman-

Coulter) and a CEQ8000 DNA Sequencer (Beckman-Coulter). The sequencing data were analyzed using CEQ8000 Genetic Analysis System (Beckman-Coulter).

(*iii*) The hypervariable regions (HVI and HVII) of the mtDNA were amplified using combinations of different primer pairs generating short amplification products (27–30). The PCR amplification reactions contained 1 × PCR Gold Buffer (Applied Biosystems), 2.4 mM $MgCl_2$, 0.2 μM of each primer, 5 U AmpliTaq Gold DNA Polymerase, 0.2 mM of each dNTP, 0.16 mg/mL BSA, and 10% glycerol in a total volume of 30 μL. To each reaction, 10 μL of DNA extract from hair, tooth, or bone was added. Amplification was performed in a GeneAmp 9700 PCR System (Applied Biosystems) by a 10 min incubation at 95 °C, followed by 40 cycles of 30 s at 95 °C, 45 s at 60 °C, and 60 s at 72 °C. The program was completed by an extension step at 72 °C for 7 min and a final hold at 4 °C. Amplicons were visualized on a 2% agarose gel. Purification of PCR products was performed using the QIAquick PCR Purification Kit (Qiagen). Each product was eluted in 40 μL of dH_2O. Forward and reverse sequencing was performed using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit, v.3.1 (Applied Biosystems) and the amplification primers as sequencing primers. Sequence analysis was performed on an ABI 3730 XL Analyzer (Applied Biosystems). The data were analyzed and compared to rCRS using Sequencher 4.5 software (Gene Codes). Additional mtDNA analysis was performed for 16 haplogroup informative SNP positions from the coding region of mtDNA using the procedure described in ref. 4.

Examination of Nuclear Markers. Tooth samples were in much better condition than other parts of the skeleton, and analysis of nuclear markers was only possible on tooth material. Sample T1 was subjected to examination of nuclear identification markers, i.e., Y-STR marker set included in AmpFlSTR Yfiler kit (Applied Biosystems) and autosomal STR loci included in AmpFlSTR Identifiler kit (Applied Biosystems). The Y-chromosome markers are particularly valuable in kinship studies (in the male inheritance line). The amplification procedures used were according to the manufacturer's recommendations with one modification relying on increased cycle number (34 instead of recommended 30 cycles) for amplification of the loci included in AmpFlSTR Yfiler kit (Applied Biosystems). PCR products were analyzed using ABI PRISM 3100 Avant capillary electrophoresis platform following the original protocols (Applied Biosystems). Sample T1 was also subjected to analysis of the rs12913832 SNP position recently implicated in eye color inheritance in humans (20–21). The C allele at rs12913832 leads to decreased expression of the *OCA2* gene, particularly within iris melanocytes, which is postulated to be the ultimate cause of blue eye color. Genotyping was performed using sequencing and SNaPshot protocols described previously (8) and additionally an alternative extension primer was applied: 5′-GGCCAGTTTCATTTGAGCATTAA-3 at a concentration of 0.2 μM.

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