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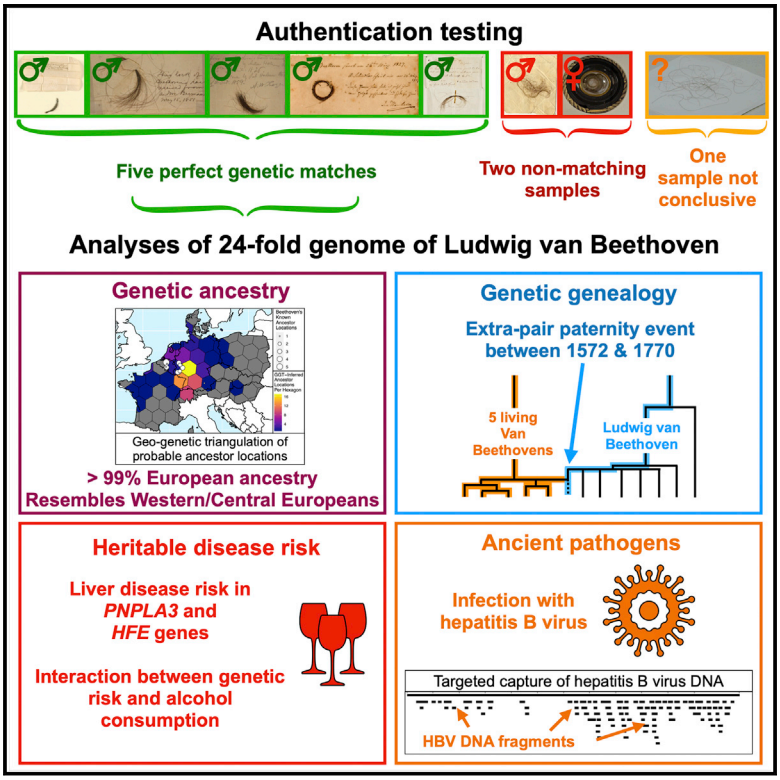
Genomic analyses of hair from Ludwig van Beethoven

Graphical abstract



Highlights

- Eight locks of hair attributed to Ludwig van Beethoven underwent genomic analyses
- We deemed five of these authentic and sequenced Beethoven's genome to high coverage
- Beethoven had a predisposition for liver disease and became infected with hepatitis B
- We also discovered an extra-pair-paternity event in Beethoven's paternal line

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In brief

Begg et al. perform genomic analyses of eight locks of hair attributed to composer Ludwig van Beethoven. They sequence a high-coverage genome, finding strong genetic risk for liver disease that may have been compounded by alcohol and an infection with hepatitis B. An extra-pair paternity event is discovered in Beethoven's direct patrilineage.

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Genomic analyses of hair from Ludwig van Beethoven

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SUMMARY

Ludwig van Beethoven (1770–1827) remains among the most influential and popular classical music composers. Health problems significantly impacted his career as a composer and pianist, including progressive hearing loss, recurring gastrointestinal complaints, and liver disease. In 1802, Beethoven requested that following his death, his disease be described and made public. Medical biographers have since proposed numerous hypotheses, including many substantially heritable conditions. Here we attempt a genomic analysis of Beethoven in order to elucidate potential underlying genetic and infectious causes of his illnesses. We incorporated improvements in ancient DNA methods into existing protocols for ancient hair samples, enabling the sequencing of high-coverage genomes from small quantities of historical hair. We analyzed eight independently sourced locks of hair attributed to Beethoven, five of which originated from a single European male. We deemed these matching samples to be almost certainly authentic and sequenced Beethoven's genome to 24-fold genomic coverage. Although we could not identify a genetic explanation for Beethoven's hearing disorder or gastrointestinal problems, we found that Beethoven had a genetic predisposition for liver disease. Metagenomic analyses revealed furthermore that Beethoven had a hepatitis B infection during at least the months prior to his death. Together with the genetic predisposition and his broadly accepted alcohol consumption, these present plausible explanations for Beethoven's severe liver disease, which culminated in his death. Unexpectedly, an analysis of Y chromosomes sequenced from five living members of the Van Beethoven patrilineage revealed the occurrence of an extra-pair paternity event in Ludwig van Beethoven's patrilineal ancestry.

INTRODUCTION

On March 27, 1827, the day after Beethoven's death, two of his associates discovered several documents stored within a hidden compartment in his writing desk, including an unusual document written in 1802 and addressed to his brothers, which is now known as the Heiligenstadt Testament. In it, Beethoven confessed that he had been "hopelessly afflicted" with a progressive

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hearing loss. Stating that only virtue and his art held him back from committing suicide, he explained that he could not leave the world "before I had produced all the works that I felt the urge to compose." Beethoven then requested that following his death, his disease be described by his favorite physician, Dr. Johann Adam Schmidt (1759–1809), and made public.[1]

Although Beethoven outlived Dr. Schmidt by 18 years, medical biographers have since attempted to determine the most likely causes of Beethoven's various health complaints. Such research has relied principally on documentary sources, including Beethoven's letters, diaries, and conversation books, and accounts from Beethoven's contemporaries including physicians' notes, an autopsy report, and descriptions of skeletal material following exhumations in 1863 and 1888. In addition, analyses of tissue sources claimed to originate from Beethoven have been performed, including toxicological analyses of hairs of unknown authenticity[2–4] and paleopathological and toxicological examinations of skull fragments,[5] at least two of which are inauthentic.[6]

These sources attest to a number of health complaints varying in severity and impact on Beethoven's life and career. Foremost among these were a bilateral, late-onset, progressive, and predominantly sensorineural form of hearing loss, as well as chronic gastrointestinal problems and, toward the end of Beethoven's life, liver disease. Beethoven's hearing loss began in his mid- to late 20s, characterized initially by tinnitus, loudness-recruitment, and the loss of high-tone frequencies, and would end his career as a performing artist by his mid-40s.[7,8] From at least the age of 22, Beethoven suffered from debilitating abdominal complaints that continued throughout his adult life, characterized primarily by abdominal pains ("Kolik") and attacks and remissions of often prolonged bouts of diarrhea. In the summer of 1821, Beethoven began to exhibit symptoms of liver disease when the first of at least two attacks of jaundice occurred, culminating in his death, considered most likely due to cirrhosis,[7,8] on March 26, 1827. Several lines of evidence indicative of the regular consumption of moderate to large quantities of alcohol[9] have led some medical biographers to conclude that Beethoven was alcohol dependent,[8,10] which is a known risk factor for liver cirrhosis.[11] While several of Beethoven's contemporaries insisted that Beethoven usually consumed alcohol in moderation,[12–14] one close friend is alleged to have stated that in ca. 1825–1826, Beethoven had been consuming at least a liter ("Mass") of wine with lunch every day.[9] Although little is known with certainty about the medical history of Beethoven's immediate family, a family history of alcohol dependence and liver disease has been noted.[7,8]

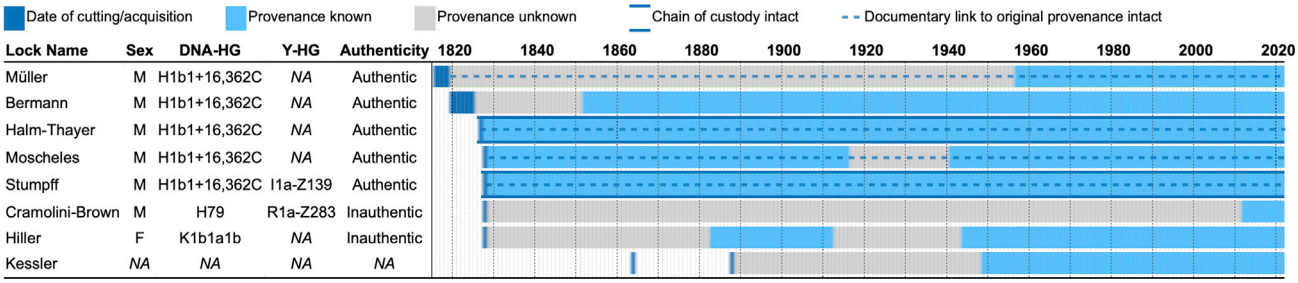
In addition to the three areas of illness mentioned above, Beethoven also showed other symptoms during his life, somatic and possibly also psychological.[7–9] In clarifying possible genetic causes of Beethoven's illnesses, we limit our investigation to the three somatic disease areas that dominate the medical biographical literature because they represent Beethoven's main health restrictions and are widely documented by Beethoven's own reports, as well as reports from Beethoven's contemporaries and physicians.

We sought to sequence Beethoven's whole genome to high coverage from authenticated strands of hair in order to improve our understanding of his health. On the basis of genetic data and supporting provenance information, we assessed the authenticity of eight locks of hair claimed to originate from Beethoven. We required authentic samples to derive from a single male individual and to exhibit DNA damage patterns consistent with the reported antiquity of the samples. We sequenced a 24-fold coverage genome from the best-preserved sample among five matching samples using a highly sensitive protocol for historical hair. We then performed ancestry analyses on the expectation that this individual's ancestry would be consistent with Beethoven's documented genealogy. As part of our ancestry analyses, we introduce a novel geo-genetic triangulation (GGT) technique using long identity-by-descent (IBD) segments shared with individuals in FamilyTreeDNA's genealogically explicit consumer database to determine the likely locations of Beethoven's ancestors. In addition, we compared this genome to two groups of genealogically documented living relatives. We extensively analyzed Beethoven's genome for genetic causes of and risk for somatic disease, in addition to metagenomic screening for evidence of infections, followed by targeted DNA capture.

RESULTS

Authentication of hair samples

Strands of hair from eight locks attributed to Ludwig van Beethoven were acquired from public and private collections for analysis, which were determined to have had independent provenances (Figure 1; STAR Methods). We refer to these as the Müller, Bermann, Halm-Thayer, Moscheles, Stumpff, Cramolini-Brown, Hiller, and Kessler Locks (Methods S1A–S1H). These locks can conservatively be estimated to fall within



Lock Name	Sex	DNA-HG	Y-HG	Authenticity
Müller	M	H1b1+16,362C	*NA*	Authentic
Bermann	M	H1b1+16,362C	*NA*	Authentic
Halm-Thayer	M	H1b1+16,362C	*NA*	Authentic
Moscheles	M	H1b1+16,362C	*NA*	Authentic
Stumpff	M	H1b1+16,362C	I1a-Z139	Authentic
Cramolini-Brown	M	H79	R1a-Z283	Inauthentic
Hiller	F	K1b1a1b	*NA*	Inauthentic
Kessler	*NA*	*NA*	*NA*	*NA*

Figure 1. Provenance summaries and authenticities of hair locks

Timelines, sex determination, mitochondrial and Y chromosome haplogroup determinations, and authenticity assessments for eight tested locks of hair claimed to have originated from Beethoven. The Kessler lock has a date of acquisition 36 to 61 years after Beethoven's death, during one of his exhumations in 1863 or 1888. See also Tables S1 and S2 and Figure 5.

a transect of Beethoven's life between ca. November of 1821 and his death in March of 1827, with the provenance histories for two of these locks, the Stumpff and Halm-Thayer Locks, bearing intact chains of custody (Figure 1). We expected authentic and independently sourced locks of Beethoven's hair to derive from a single male with predominantly Central European ancestry. We further expected the presence of terminal C-T deamination caused by DNA degradation over time, consistent with their provenances in the early 19th Century.

We performed shallow shotgun sequencing to permit assessments of DNA preservation and authenticity (Figure 1; Data S1A). Five samples, the Müller, Bermann, Halm-Thayer, Moscheles, and Stumpff Locks, shared identical mitochondrial genomes of haplogroup H1b1+16,362C with a private mutation at C16,176T, and had male XY karyotypes (Figure 1; Table S2; STAR Methods). Relatedness testing of autosomal and X chromosome DNA demonstrated that these five matching samples originated either from a single individual or monozygotic twins (Figure 2; Table S5; STAR Methods). All matching samples had DNA damage patterns consistent with their provenances in the early 19th Century (Data S1A).

In light of their provenance histories, we considered these genetic findings to be compelling evidence for the identity of these five independent locks of hair and proceeded under the working hypothesis that they are authentic. We determined that the Stumpff Lock (Figure 3) was marginally the best preserved of the five matching samples (Methods S1I) and sequenced a nuclear genome to an average of 24-fold coverage, incorporating laboratory and bioinformatics protocols optimized for the ultra-short DNA fragments characteristic of historical hair samples (mean fragment length 29.62 bp)[15–21] (STAR Methods). We restricted further analyses to the 1.64 Gb of the genome to which short reads could be confidently mapped ("accessible genome"; STAR Methods).

Principal component analyses (PCAs) performed on the high-coverage Beethoven genome placed it among Europeans, clustering with modern Germans (Figures S1–S3). Testing for admixture among five global populations using ADMIXTURE[22] revealed that Beethoven's ancestry was >99% European (Figures S4 and S5). We assessed geographic clustering of ancestors of 665 FamilyTreeDNA customers who share long ($\geq$6 cM) autosomal IBD segments with Beethoven and used a novel GGT method (STAR Methods) to analyze the place names documented in their genealogical records. We found strong geographic clustering of matches along the Rhine River and within present-day North Rhine-Westphalia in Germany (Figure 4; Methods S1L–S1O), largely consistent with the geographic distribution of the birthplaces of Beethoven's German ancestors.[23] We were unable to identify any shared ancestors with Beethoven among genealogical records for 30 customers with the longest shared IBD segments. Beethoven's I1a-Z139 Y chromosome haplogroup is common and widespread in Europe (Data S1D).

Of the non-matching hair samples tested, our sequence data show that the highly publicized Hiller Lock originated from a woman with close autosomal affinity in PCA space to present day North African, Middle Eastern, and Jewish populations[24] (Methods S1K). Its mitochondrial haplogroup, K1a1b1a (Table S2), is highly prevalent among Ashkenazi Jews.[25] Toxicological analyses of hairs extracted from this lock have been used to argue that Beethoven's health problems were caused or compounded by plumbism, and to refute suggestions that he was administered opiates during the course of his final illness and mercury for a hypothesized infection with syphilis.[2–4,26,27] We now conclude that these findings do not apply to Beethoven. We additionally demonstrate that patterns of longitudinally distributed lead isotope concentrations believed to have been shared between hair strands from the Hiller, Halm-Epstein, and Erdödy Locks[3] do not constitute proof of their authenticity, as the Hiller Lock is inauthentic.

We found that the Cramolini-Brown Lock originated from a male of European autosomal ancestry (Figure 1; Methods S1J), belonging to the mitochondrial haplogroup H79 and the Y chromosome haplogroup R1a-Z283 (Figures 1 and S4). As this sample differs from our five matching samples and we could not confirm its provenance prior to 2012, we conclude that it is almost certainly inauthentic.

Both the Hiller and Cramolini-Brown Locks exhibited levels of DNA damage similar to the five matching Beethoven samples (Data S1A).[28,29]

The Kessler Lock lacked sufficient DNA preservation for sex chromosomal karyotyping or ancestry determination, mitochondrial contamination estimation, or mitochondrial haplogroup assignment (Data S1A). We were therefore unable to assess its authenticity.

On the basis of these genetic data, and in light of their known provenance histories, we conclude that the Müller, Bermann,

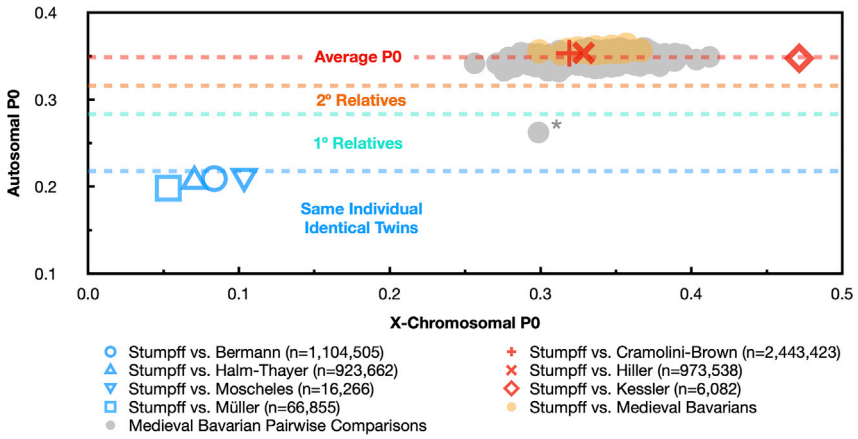


Figure 2. Relatedness testing of eight locks of hair attributed to Beethoven

Relatedness testing of eight locks of hair attributed to Beethoven, relative to an external reference panel of 41 medieval Bavarians. The proportions of non-matching alleles (P0) per pair and estimated degrees of relatedness are calculated from pseudo-haploid genotype calls using READ. The numbers of SNVs in each comparison are denoted by n. *Known medieval Bavarian sibling pair (STR355c/STR491). See also Table S5.

Halm-Thayer, Moscheles, and Stumpff Locks almost certainly authentically derive from Beethoven, the Cramolini-Brown Lock is almost certainly inauthentic, the Hiller Lock is definitely inauthentic, and the authenticity of the Kessler Lock could not be determined.

In order to support the authenticity of the matching samples further, we compared the Y chromosome from Beethoven's high-coverage genome against high-coverage Y chromosomes sequenced from five living men belonging to the Van Beethoven patrilineage. These individuals were identified through analyses of genealogical records, which document Aert van Beethoven (1535–1609) as a patrilineal ancestor shared by Ludwig van Beethoven and our research participants (Methods S1P).[23] Consistent with genealogical records, these five individuals share nearly identical Y chromosomes falling within the R-FT446200 haplogroup within R1b, with an average of 4.8 [3–7] private mutations having arisen along each lineage (Figure 5; STAR Methods). The pedigree reconstructed on the basis of these private mutations reproduced the documented pedigree among the participants (Figure 5). These Y chromosomes did not, however, match the Y chromosomes from either the five matching Beethoven hair samples within I1a-Z139 or the Cramolini-Brown Lock within R1a-Z283 (Figure 5).

Considering the strong historical and genetic evidence for the authenticity of the five matching hair samples, and our Y chromosome evidence for the lack of discontinuity in the paternal lineage between Aert van Beethoven and the five living descendants, we conclude that the most plausible explanation for our observations entails that at least one extra-pair paternity (EPP) event occurred on Beethoven's direct paternal line, between the conception of Aert van Beethoven's son Hendrik in Kampenhout, Belgium, in c.1572, and the conception of Ludwig van Beethoven seven generations later in 1770, in Bonn, Germany.

In order to further investigate the details of an EPP scenario and possibly ascertain Beethoven's genetic patrilineage, we queried the FamilyTreeDNA Y chromosome database, including >52,500 user records at high sequence resolution.[30] We identified five closely related profiles descending from the I-FT396000 lineage within I1a-Z139, with a mean time to most recent common ancestor (TMRCA) of 1,018 (95% CI 714–1,419) years before present (Figure 5; Methods S1Q; Table S6). However, all five of these participants carried dissimilar surnames, consistent with the fixation of surnames in many parts of Europe occurring several centuries after the most probable TMRCA for I-FT396000. We were therefore unable to establish Beethoven's direct genetic patrilineage and the surname of the individual involved in an EPP event.

In addition to testing the Y chromosomes of living Van Beethovens, we tested for IBD segment sharing among three living descendants of Beethoven's nephew, Karl van Beethoven, who are documented as 7th-degree genetic relatives to Beethoven.[23] Using IBIS,[31] which can accurately detect IBD segments $\geq$7 cM, we detected no IBD segments $\geq$7 cM shared between Beethoven and the three participants. The IBD-sharing and mitochondrial relatedness detected among the participants internally, however, was consistent with their documented genealogy.

In order to better interpret this result, we performed 100,000 simulations on a reconstructed pedigree using pedSIM,[32] including the three living descendants of Karl van Beethoven, and including Ludwig and Karl's father, Kaspar Anton Karl van Beethoven (1774–1815), as full siblings. These simulations estimated an average of 47.23 detectable cM among 2.46 IBD segments $\geq$7 cM shared with Beethoven per descendant. The simulated probability for detecting zero IBD segments $\geq$7 cM shared between Beethoven and each individual participant averaged 9.08% (95% CI 8.9%–9.26%), while the probability of detecting zero $\geq$7 cM IBD-segment sharing with Beethoven among all three participants combined was 0.851% (95% CI 0.79%–0.91%).

However, given the virtual certainty that an EPP event occurred in Beethoven's direct paternal ancestry, we could not assume with confidence that Ludwig and Kaspar Anton Karl were full siblings. In the event that Ludwig and Kaspar Anton Karl were half-siblings, the probability of detecting zero $\geq$7 cM IBD-segment sharing with Beethoven among all three participants combined was 8.34% (95% CI 7.81%–8.9%). As a result, we are unable to conclusively prove or disprove relatedness between Beethoven and the descendants of Karl van Beethoven, and are unable to provide further verification of sample authenticity on this basis.

Genetic variants associated with somatic disease

Diseases differ in terms of the degree of genetic causation and the number of genes involved. In monogenic and some complex diseases, an accurate diagnosis can be made by identifying the responsible mutation(s) in the patient. In multifactorial diseases, many genes are involved in interaction with non-genetic factors,

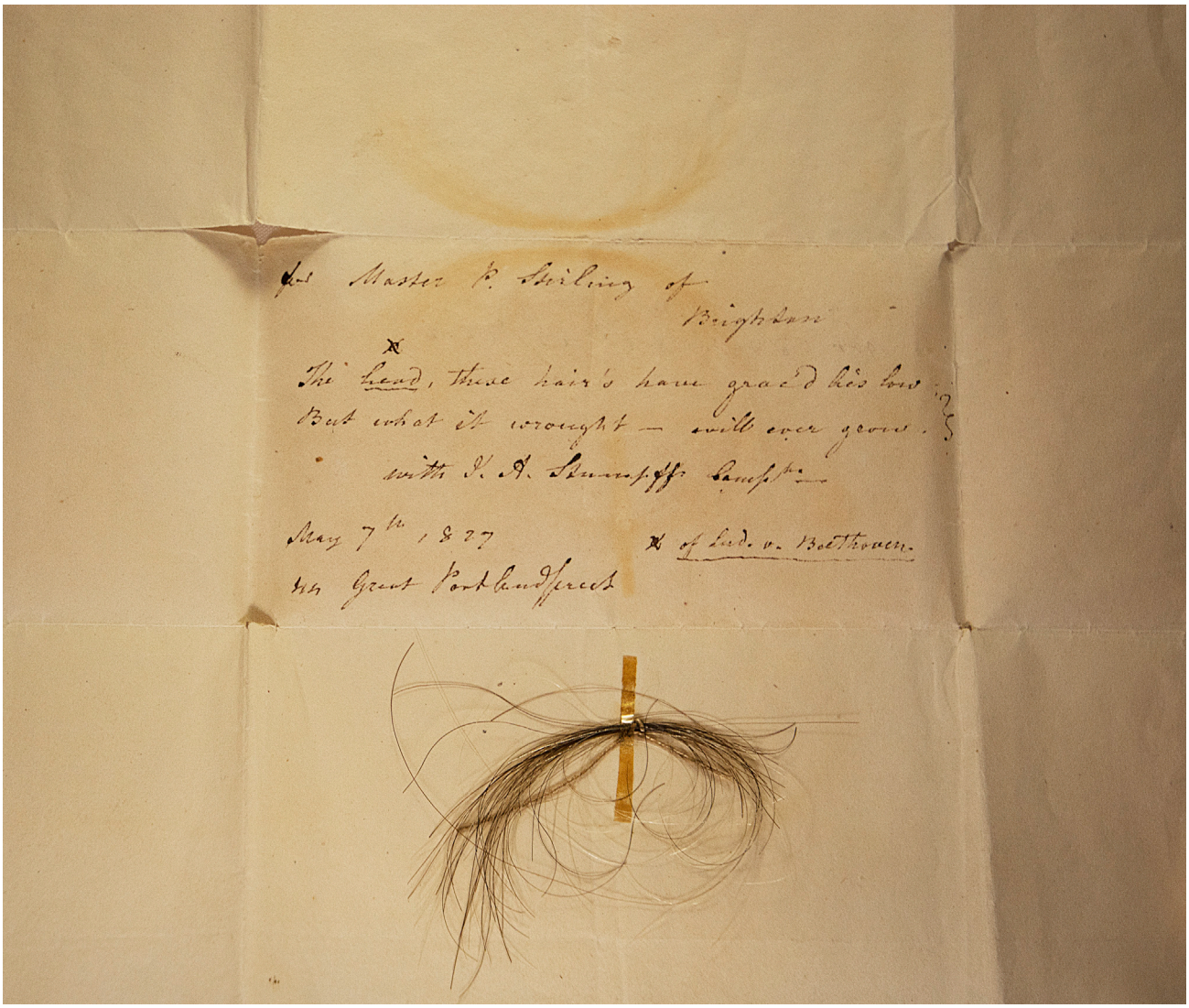


Figure 3. The Stumpff Lock

The Stumpff Lock from which Beethoven's high-coverage genome was sequenced. The lock is affixed to a letter from Johann Andreas Stumpff to Patrick Stirling, dated May 7, 1827. Stumpff's poem reads, "The head[⊠], these hair's have grac'd lies low; But what it wrought — will ever grow. [⊠]of Lud. v. Beethoven." Photographed in July of 2018 by American Beethoven Society member Kevin Brown.

and without exception, not all of the genes involved are yet known. For multifactorial diseases, the genetic contribution known so far can be summarized in a polygenic risk score (PRS). When attempting to determine an individual's polygenic predisposition for a multifactorial disease, the individual's PRS is usually compared against a distribution of PRSs generated from a suitable reference population. As a rule, an individual PRS does not provide sufficient predictive accuracy for a diagnosis. This must be kept in mind when interpreting the molecular genetic findings reported in the following.

Several diseases have been proposed to account for Beethoven's hearing loss, including otosclerosis,[8] Paget's disease of bone (PDB),[33] complications from Crohn disease (CD) or ulcerative colitis (UC),[34] sarcoidosis,[35] and systemic lupus erythematosus (SLE).[36] Genome-wide association study (GWAS) summary statistics with sufficient power for meaningful disease risk stratification via PRS could not be obtained for otosclerosis, PDB, or sarcoidosis, limiting our assessments of Beethoven's polygenic risk to CD, UC,[37] and SLE[38] (Data S1H; Methods S1R–S1X; STAR Methods). Only Beethoven's PRS for SLE was found to confer notably elevated polygenic risk, placing him within the 93rd polygenic risk percentile, and conferring an odds ratio (OR) relative to the mean polygenic score among controls of approximately 2.96 (1.54–5.67) (Methods S1U; Data S1H).

In addition to evaluating polygenic risk for multifactorial diseases underlying Beethoven's hearing loss, we evaluated the hypothesis of a monogenic etiology. We prioritized 55 genes[39,40] in which variants could cause monogenic post-lingual hearing loss and further analyzed an extended set of an additional 209 genes predominantly related to pre-lingual hearing loss (Data S1F). In addition, we analyzed 137 genes causing rare monogenic

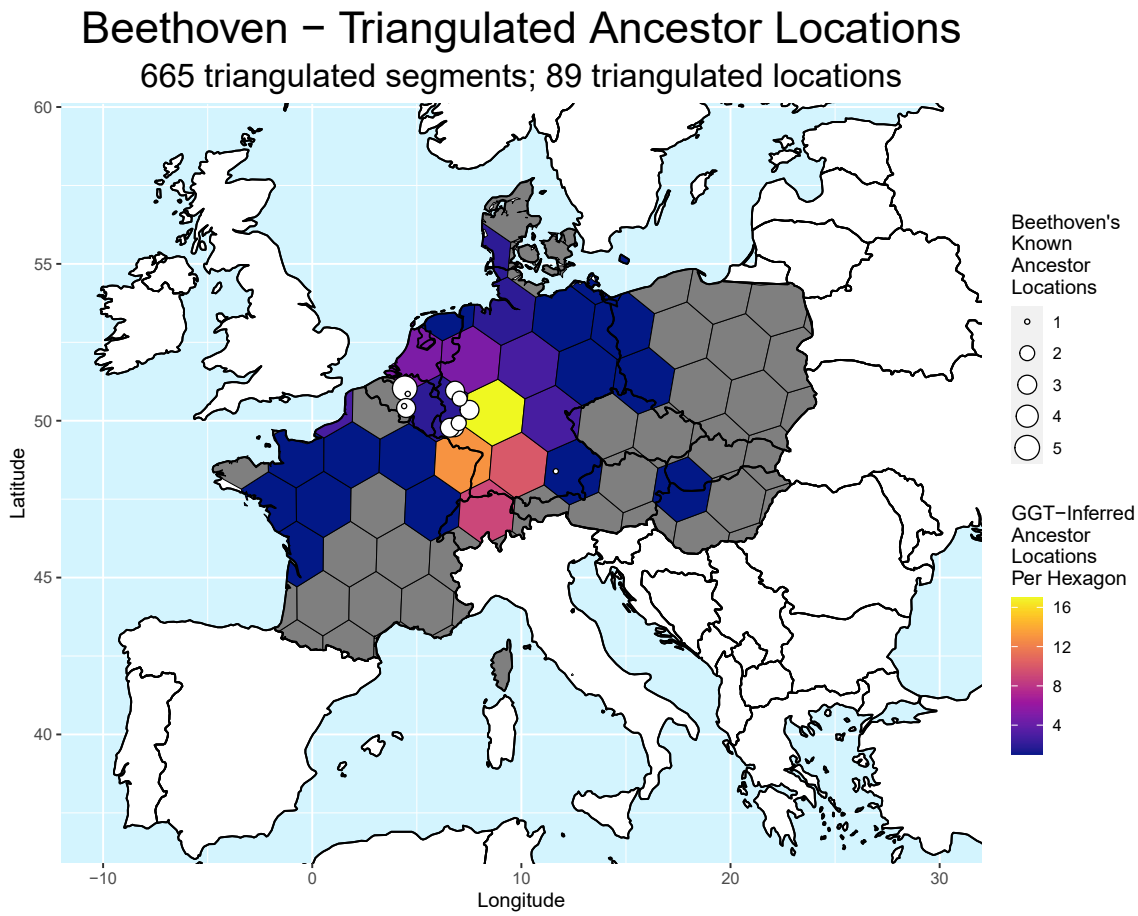


Figure 4. Geo-genetic triangulation of Beethoven's probable ancestor locations

Geo-genetic triangulation, showing probable locations of Beethoven's autosomal ancestors. Regional intensity per hexagon represents number of shared ancestors between Beethoven and modern FTDNA customers, with the following criteria: (1) IBD segments (n = 665) must be shared (triangulated) between at least three individuals to be genetically validated and (2) ancestor locations (n = 89) of matching individuals must occur within the same hexagon to be geographically validated. Areas of Western and Central Europe that were considered but lack any probable ancestors are shown in gray. See also Methods S1L–S1O.

subforms of SLE, inflammatory bowel disease-like syndromes, PDB, sarcoidosis, and otosclerosis (Data S1F; STAR Methods). To estimate the sensitivity of our analysis, we analyzed the coverage of these prioritized genes. On average, 83% (SD ±19%) of the protein or tRNA/rRNA coding sequences of these genes (n = 390) were within the accessible genome, and 64% (SD ±24%) were within the accessible genome and covered by at least 20 reads (Figure S6). While acknowledging the limited sensitivity of our analyses, we were unable to identify any unambiguous disease-causing variants. Several variants of uncertain significance and with weak evidence for pathogenicity were, however, identified (Data S1G). In summary, we could not reliably evaluate most hypothesized multifactorial causes of Beethoven's hearing loss, nor did we identify a monogenic origin.

In the overwhelming majority of cases worldwide, cirrhosis of the liver can be attributed to the effects of alcohol[41] or infections by the hepatitis B virus (HBV) or the hepatitis C virus (HCV),[42] acting on a background of individual genetic predisposition. Both singly and acting in combination, alcoholic liver disease (ALD) and viral hepatitis are the most frequently proposed hypotheses for Beethoven's liver disease.[7] However, liver cirrhosis may also occur in the context of specific underlying diseases, which are typically multifactorial in origin. Primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), SLE, sarcoidosis, and complications from CD or UC have previously been hypothesized for Beethoven.[7] Several monogenic etiologies have also been considered as underlying conditions, including hereditary hemochromatosis (HH), alpha-1 antitrypsin deficiency (AATD), Wilson's disease, and cystic fibrosis (CF).[7]

We analyzed Beethoven's polygenic risk for liver cirrhosis,[43] which placed Beethoven within the 96th risk percentile (Methods S1X; Data S1H; STAR Methods). Consistent with his PRS polygenic risk for liver cirrhosis, Beethoven was found to be homozygous for the variant consistently implicated as the most strongly associated locus for liver cirrhosis in GWASs, at rs738409 in *PNPLA3*.[44] A significant modulating effect on rs738409 has been observed at rs2294918, also in *PNPLA3*, which attenuates risk among rs738409 carriers.[45] Beethoven lacked the risk attenuating allele and was homozygous for the highest known risk diplotype in *PNPLA3*, I148M-K434E. Beethoven's polygenic scores for PBC[46] and PSC[47] did not confer disease risk, placing him

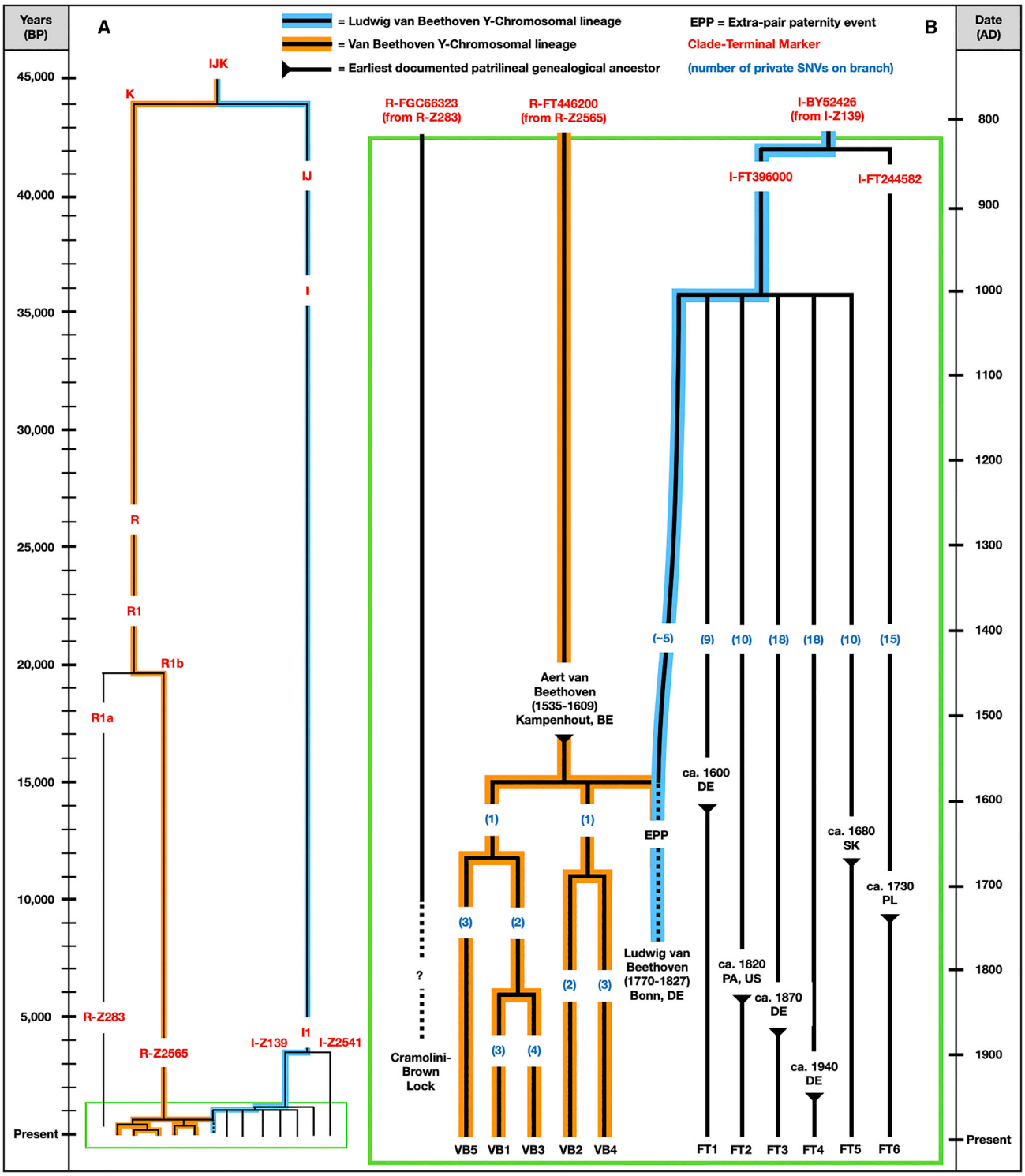


Figure 5. Y chromosome phylogeny of all male samples tested

Y chromosome phylogeny containing all male samples tested, including Ludwig van Beethoven, the six closest known living Y chromosome relatives of Ludwig van Beethoven within the FamilyTreeDNA database (FT1–FT6), five living descendants of Aert van Beethoven (VB1–VB5), and the Cramolini-Brown Lock. (A) Deep phylogenetic overview within the last 50,000 years of three major clades harboring Ludwig van Beethoven and his closest living Y chromosome relatives (I-Z139), the living Aert van Beethoven descendants (R-Z2565), and the Cramolini-Brown Lock (R-Z283).

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within the 22nd and 59th polygenic risk percentiles, respectively (Methods S1V and S1W; Data S1H).

Additionally, in order to investigate a possible monogenic condition underlying Beethoven's liver cirrhosis, we analyzed the genes responsible for hypothesized monogenic diseases including AATD, HH, Wilson's disease, and CF, as well as 47 genes that may cause rare monogenic forms of hypothesized multifactorial diseases already analyzed via PRS (Figure S6; Data S1F; STAR Methods). While no mutations causal to AATD, Wilson's disease, or CF were identified, Beethoven was found to be a compound heterozygote for two variants causal to HH, at rs1799945 (p.His63Asp) and rs1800562 (p.Cys282Tyr) in the *HFE* gene.

In order to assess the combined effect of Beethoven's heritable risk factors for liver disease with heavy alcohol consumption, retrospective cohort analyses were performed on cohorts of UK BioBank males matching Beethoven's genotypes at rs738409 and rs2294918 in *PNPLA3*, and rs1799945 and rs1800562 in *HFE*, including males meeting ICD-10 criteria for "Harmful use" and "Alcohol dependence" (ICD-10 codes F10.1 and F10.2), whom we term heavy drinkers. The disease prevalence of all-cause liver disease (ICD-10 code K7*) and liver cirrhosis[43] (Data S1H) among heavy drinking males homozygous for Beethoven's *PNPLA3* diplotype was found to be 30.95% (95% CI 24.77%–37.68%) and 21.43% (95% CI 16.08%–27.6%) compared to a prevalence among all male heavy drinkers of 20.64% (95% CI 19.43%–21.89%) and 10.34% (95% CI 9.44%–11.3%), respectively (Figure 6; Data S1K and S1L). Prevalence for all-cause liver disease (24% [95% CI 16.02%–33.57%]) and cirrhosis (14% [95% CI 7.87%–22.37%]) was also significantly elevated among male heavy drinkers with compound heterozygosity in *HFE*, as compared to baseline rates among heavy drinkers (Figure 6; Data S1M). The combined effect of Beethoven's *PNPLA3* and *HFE* genotypes among heavy drinking males could not, however, be accurately determined owing to a low number of matching individuals (n = 4).

Although many medical biographers favor irritable bowel syndrome (IBS) as the cause of Beethoven's gastrointestinal symptoms, several have proposed one of the two inflammatory bowel diseases, i.e., CD or UC.[7,8] In the vast majority of cases, all three of these diseases are multifactorial in origin. We additionally queried for genetic origins for other causes of gastrointestinal distress, including celiac disease, and monogenic diseases and conditions such as lactose intolerance, CF, and monogenic forms of inflammatory bowel disease or inflammatory bowel disease-like syndromes.[48]

Beethoven's polygenic scores for CD and UC[37] placed him in the 36th and 61st percentiles, respectively (Methods S1R and S1S; Data S1H). Beethoven's polygenic score for IBS[49] placed him within the 9th polygenic risk percentile, conferring a protective status against IBS with an OR of 0.39 (0.15–1) (Methods S1T; Data S1H). However, due to our use of UK BioBank IBS cases during parameter optimization, which were also included during the generation of the GWAS summary statistics we used,[49] this result may be confounded by overfitting.

Beethoven additionally lacked the HLA-DQ2 and DQ8 alleles, which are a prerequisite for celiac disease (STAR Methods). Beethoven was most likely lactose tolerant, carrying heterozygous genotypes for both lactase persistence alleles at rs4988235 and rs4988235 near the *LCT* gene.[50,51] Furthermore, we were unable to identify any disease-causing variants for CF (Data S1F).

Hepatitis B virus DNA recovered from Beethoven's hair

An infection with viral hepatitis has been considered by several of Beethoven's medical biographers[7,52–55] as a plausible cause for his liver disease. Using the metagenomic screening pipeline MALT,[56,57] we screened all sequence data from the Müller, Bermann, Halm-Thayer, and Stumpff Locks for HBV DNA. Three DNA libraries prepared from the Stumpff Lock yielded putative traces of HBV DNA (HEB001.B0102, HEB001.F0101, and HEB001.M0103; Data S1I). Although only four HBV-mapping reads were identified, these appeared to represent specific matches and were well distributed along the genome. We therefore performed hybridization capture to enrich libraries for HBV DNA. After sequencing, deduplication, and filtering of low-copy-number reads of HBV-DNA-enriched libraries, 92 unique reads from 20 libraries remained (Data S1I), resulting in a mean HBV genome coverage of 1.26-fold (Figure S7A), with all positive libraries deriving from the Stumpff Lock (STAR Methods). No clear damage pattern was observed, which likely resulted from the relatively low number of recovered reads (Figure S7B). Our phylogenetic analysis placed the reconstructed sequence within subgenotype D2 with 100% support (Figure 7), irrespective of the HBV reference genome used for read mapping (STAR Methods). Following HBV-DNA enrichment of the 25 extraction and library preparation blanks, three unique reads were found from three libraries (LIB002.A0116, LIB002.A0139, and LIB002.A0141; Data S1J), all mapping to an ~200-bp section of the S gene in the HBV genome (pos. 296–487).

DISCUSSION

Authenticity of Beethoven's genome

Of the eight locks of hair analyzed here, seven yielded sufficient DNA for interpretation, and we found five of those to derive from a single male individual with ancestry and DNA damage patterns consistent with originating from Ludwig van Beethoven. Four main considerations support our conclusion that the individual in question is indeed Ludwig van Beethoven.

First, the documentary evidence supporting the authenticity of these five locks (Figure 1; STAR Methods) is very strong. In particular, the Halm-Thayer Lock is recorded to have been presented in 1826 by Beethoven himself to fellow musician Anton Halm and his wife; Halm presented it in 1859 to the Beethoven scholar, Alexander Thayer; and it remained with the Thayer family in the United States until American Beethoven Society member Kevin Brown

(B) Detailed phylogeny within the last thousand years outlining Ludwig van Beethoven's Y chromosome lineage (blue outline; haplogroup I-FT396000), the living Van Beethoven Y chromosome lineage (orange outline; haplogroup R-FT446200), and the approximate hypothesized location of an extra-pair paternity (EPP) event within the Van Beethoven Y chromosome tree (dotted line). In blue parentheses are the numbers of confidently ascertained private variants (for Ludwig van Beethoven, see those with a 3-star rating in Data S1E).
See also Methods S1P and S1Q.

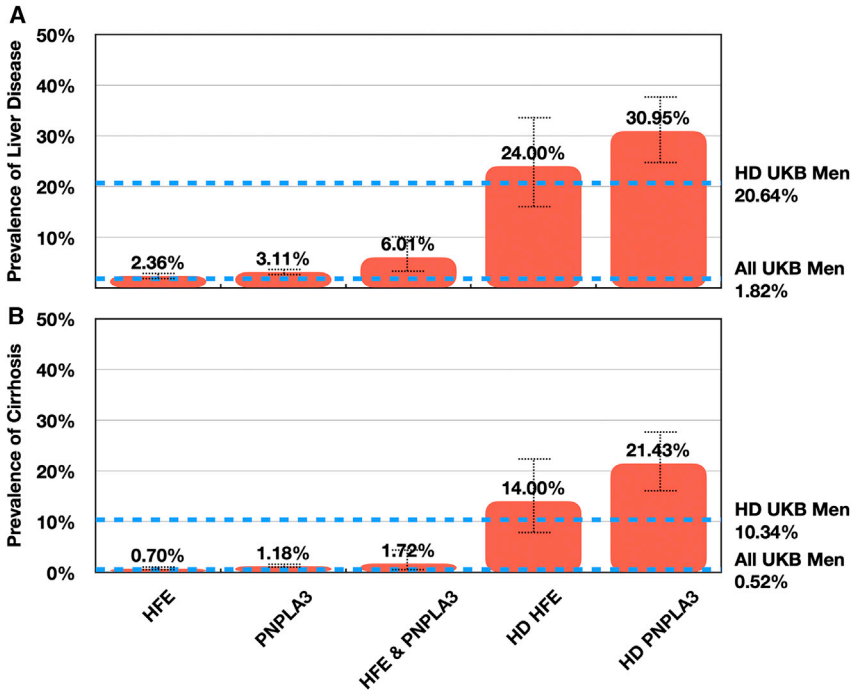

Figure 6. Liver disease prevalence in cohorts sharing Beethoven's risk factors

Prevalence and 95% confidence intervals of (A) all-cause liver disease (ICD-10 code K7*) and (B) liver cirrhosis following Emdin et al.[43] among cohorts of UK BioBank (UKB) men (n = 227,602) with compound heterozygosity at rs1799945 and rs1800562 in *HFE* (HFE; n = 5,004), homozygosity at rs738409 and rs2294918 in *PNPLA3* (PNPLA3; n = 10,485), and heavy drinkers (HD; ICD-10 codes F10.1 and F10.2; n = 4,235) by individual risk factors and in combinations of risk factors (HFE & PNPLA3, n = 233; HD HFE, n = 100; HD PNPLA3, n = 210; HD HFE & PNPLA3, n = 4). See also Data S1K–S1M.

purchased it in 2017 for the Society, which made a sample from it available for the present study. Similarly, following Beethoven's death in 1827, the Stumpff Lock was sent by family friend Johann Streicher, acting for Johann Schickh, who was organizing Beethoven's funeral, to harp-maker Johann Stumpff in London, who, within a month, sent it to Patrick Stirling, member of a prominent Scots family of musical patrons; when put up for auction in 2016, still attached to a document bearing Stumpff's signature, Sotheby's described the lock as having "come down to the present owners by direct descent," and at that auction it was purchased by Kevin Brown for the American Beethoven Society, which made a sample available. By contrast, there are gaps in the known ownership and whereabouts of the Müller, Bermann, and Moscheles Locks: before 1851 when Thayer acquired the Bermann Lock; between 1917 and 1940 in the case of the Moscheles Lock; and between 1820 and 1956 when the Beethoven-Haus Bonn acquired the Beethoveniana collection of Hans Conrad Bodmer, including the Müller Lock, on his death. Nonetheless, these three locks all have documented 19th Century origins. In the cases of the Müller and Moscheles Locks, like the Halm-Thayer and Stumpff ones, the associated documentation is original. In all recorded cases, the historical custodians of the locks have been known Beethoven acquaintances, musicians, scholars, collectors, enthusiasts, and institutions.

Second, the histories of these locks are, with one small exception, independent of each other. The exception is that the Bermann and Halm-Thayer Locks were held together in a picture frame while in Thayer's ownership. That apart, there are no historical opportunities for the locks to have been confused, amalgamated, contaminated, or replaced by one another. The Bermann and Moscheles Locks could not share a source later than Beethoven himself, as the chronological gaps in their documentation do not overlap. Yet all these five almost entirely independent locks, two of them with impeccable provenance, two with good provenance, and a fifth with moderate provenance, are genetically identical.

Third, the two locks that do not genetically match the five matching locks not only fail to match each other, too, but also have weaker supporting documentation than the matching locks. Although a genuine Cramolini Lock may exist, the lock known as Cramolini-Brown has no secure documentation before 2012. Similarly, while there is earlier documentation of a Hiller Lock, there is no surety that the lock that came to light in the difficult circumstances of 1943 is that same lock.

Fourth, plausible hypotheses whereby five separate locks attributed to Beethoven could share the same genetic source individual other than him are extremely hard to construct. Any fraudulent manipulation of the locks and documentation would have had to pre-date by many decades any concept, verified in 1985, that genetic data could be derived from hair.[59] It would require a coordinated effort to disperse at least five locks of hair derived from a single individual among a diverse group of Beethoven's close affiliates and/or subsequent collectors, most likely within Beethoven's lifetime or immediately after his death, in tandem with forgery of supporting provenance documents. Any later effort, undertaken during gaps in their known custodianship, would have to have been made before 1850 for the Bermann Lock but after 1915 for the Moscheles Lock. The feasibility of such complex operations seems impossible to credit, as does any apparent motivation for it, whether financial or otherwise.

Comparison with living individuals' genomes

We were not able to confirm Beethoven's genetic relationship with either a set of five Belgian male research participants sharing his surname or a set of three Austrian research participants genealogically documented as his collateral descendants. These two analyses are very different.

The five living men belonging to the Van Beethoven patrilineage are only distantly interrelated genealogically but have matching Y chromosome haplogroups, consistent with descent from the common patrilineal 16th century ancestor Aert van Beethoven, also identifiable as such from genealogical data. Genealogical data also identify Aert as ancestral to Ludwig van Beethoven,

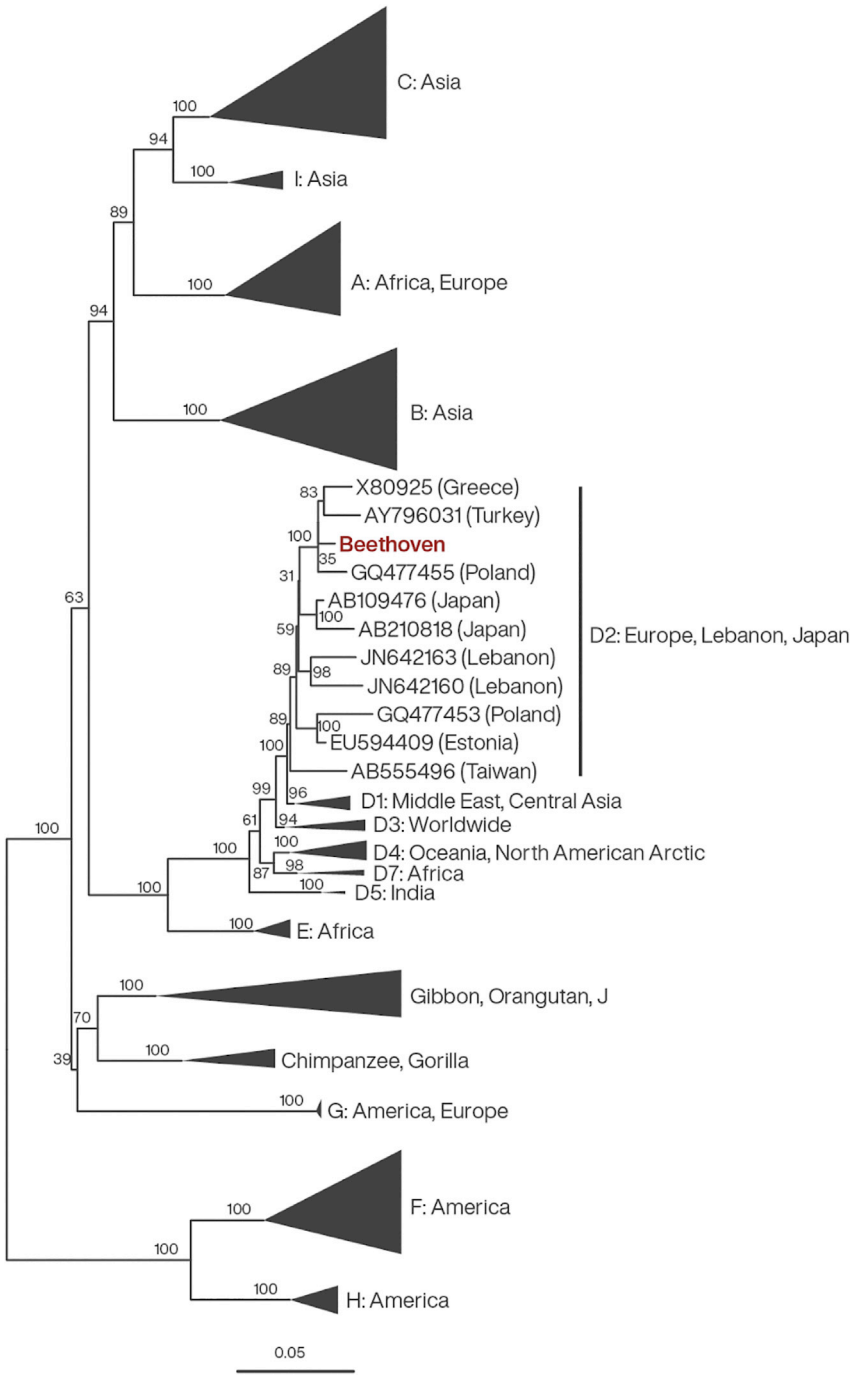

Figure 7. Phylogenetic tree of HBV

Phylogenetic tree of HBV with branches in substitutions per site estimated using RAxML.[58] Clades corresponding to the main genotypes were collapsed and annotated with their typical geographic location, except for subgenotype D2, in which the HBV genome recovered from Beethoven was placed. Bootstrap supports are reported on the nodes. See also Figure S6 and Data S1I and S1J.

but the Y chromosome data do not match in this case. What scenarios could explain this? We have concluded above against the possibility that an individual other than Ludwig van Beethoven could be the source of the five genetically matching hair samples. Could there have been an error in Beethoven's legal genealogy? We conclude against this possibility also, given the well-established paternal genealogy for Ludwig van Beethoven.[23,60] With the exception of his father, Johann van Beethoven (ca. 1739–1792), each step in the patrilineage is documented in at least two different archival records, which were reviewed thoroughly on observing the mismatch. Moreover, the strong concordance between surname and Y chromosome haplogroup observed in historical Belgium,[61] supporting the accuracy of local record-keeping, renders the mismatch very difficult to account for by genealogical error. The possibility that remains, therefore, is that an EPP event took place in one of the generations between Aert and Ludwig van Beethoven. In Western Europe over the last 400 years, such events were rare but did take place, at an average frequency of 1%–2% per generation.[62] One Beethoven biographer[63] has previously suggested, on circumstantial grounds, that Ludwig senior may not have been Johann van Beethoven's biological father. Our genetic findings, however, do not allow us to favor any particular generation for the occurrence of an EPP event.

A second analysis focused on three closely related living descendants of Ludwig van Beethoven's nephew Karl, documented as 7th-degree genetic relatives of Ludwig. Because Karl's patriline is now extinct, Y chromosome analysis was impossible, so a more probabilistic method involving analysis of autosomal IBD segments was adopted. We were unable to detect any IBD-segment sharing ≥ 7 cM between Beethoven and these living individuals and could not determine conclusively whether this finding reflects an unusually low level of IBD sharing for this level of relatedness or an EPP event.

Origins of Beethoven's diseases

The genomic sequence data are a novel and unbiased primary source that offer the potential to improve our understanding of Beethoven's health problems. It must be emphasized, however, that our approach has important limitations. First, the ultra-short read data characteristic of historical hair samples significantly impact the data quality and coverage of analyzed genes. For example, deletions and duplications, which have been shown to cause a relevant proportion of cases of monogenic non-syndromic hearing loss,[64–66] were not considered in our analyses due to these limitations. Second, despite great advances in medical genetics, the genetic causes of

many diseases are not yet fully understood, especially in the case of multifactorial diseases, which are further complicated by the fact that non-genetic causes may also substantially contribute to the development of disease.

Taking these limitations into account, we did not find a molecular genetic cause for Beethoven's hearing loss. However, important differential diagnoses, such as otosclerosis, which is frequently suggested in the literature, could not be evaluated due to a lack of reference data. Substantiation of the previously hypothesized role of plumbism as a causative or contributory factor to Beethoven's hearing loss[3,4,26,27] must await analyses of samples authenticated via genetic testing.

Similar to the results for hearing loss, we did not find a molecular genetic explanation for Beethoven's gastrointestinal complaints. However, we were able to render some important diagnoses less likely. For example, celiac disease and lactose intolerance can almost be ruled out as causes. IBS, often suspected as a cause, is less likely on the basis of the PRS findings but, given the limited diagnostic power of the IBS PRS, is still possible.

Our most significant results concern Beethoven's liver disease. The elevated PRS for liver cirrhosis, which includes homozygosity for a risk variant in *PNPLA3*, the strongest known genetic risk factor, suggests that Beethoven inherited a considerable genetic predisposition. Compound heterozygosity in the *HFE* gene may have made an additional contribution. Due to the low penetrance of the *HFE* diplotype,[67–69] it cannot be assumed that Beethoven suffered from clinically relevant hemochromatosis. Nonetheless, the role of iron overload caused by mutations in the *HFE* gene, for which excessive alcohol consumption is a co-morbid risk factor[69] (Data S1M), may have had an additional, unfavorable effect on liver health[70] (Figure 6; Data S1K and S1L). Our retrospective cohort analyses demonstrated that Beethoven's risk for liver disease would have been heavily contingent on the extent of his alcohol consumption (Figure 6; Data S1K–S1M). If Beethoven was regularly consuming sufficiently large quantities of alcohol, the combined risk conferred by alcohol consumption and his substantial genetic predisposition may constitute a plausible causative explanation for his liver disease. In addition, we demonstrated that Beethoven had an HBV infection at least during the months leading to his death. Our analyses presently lack the sensitivity to determine the nature and timing of this infection, which would have strongly influenced the extent of its causal involvement with Beethoven's liver disease. A chronic perinatal or childhood HBV infection would have been a strong driver of liver disease, no doubt exacerbated by his genetic risk and alcohol consumption, whereas an HBV infection closer to the end of Beethoven's life would have been of lesser relevance (Hepatitis B virus DNA in Beethoven's hair; STAR Methods). Nonetheless, we conclude that Beethoven's substantial genetic predisposition, HBV infection, and alcohol consumption all present plausible causal factors in his liver disease, although the exact causal pattern cannot presently be determined.

Hepatitis B virus DNA in Beethoven's hair

HBV is an important current global public health problem as a major cause of liver cirrhosis and cancer.[71] This virus can be transmitted from mother to child during birth, via sexual contacts, or through surgery with contaminated instruments. It may cause chronic infections (especially when contracted during childhood), which result in liver complications after decades in a large proportion of cases. Acute HBV infections are usually asymptomatic or mild but can lead to lethal fulminant hepatitis in rare cases.

Hair has recently been revealed as a potential reservoir of HBV DNA in individuals suffering from both chronic and acute HBV infections,[72,73] supporting the plausibility of HBV DNA fragments surviving in ancient and historical hair samples from HBV-positive individuals. Screening of shotgun sequencing data and the hybridization capture experiment indicated the presence of HBV DNA in several libraries prepared from the Stumpff Lock (Data S1I). Ninety-two sequencing reads mapping on the HBV genomes were recovered, after filtering of low-copy-number reads and deduplication. These were well distributed along the HBV genome sequence, as expected from fragments genuinely originating from a target organism. This allowed the reconstruction of a significant proportion of it (63%; Figure S7A). In contrast, only three reads mapping on the HBV genome were recovered from the 25 negative controls after HBV-DNA enrichment, and these were all found within a small (~200-bp-long) section of the HBV genome, more indicative of cross-mapping due to the presence of another DNA molecule sharing a local similarity with the HBV genome (Data S1J). Of note, extraction and library blanks corresponding to the hair samples yielding the strongest signal (i.e., HEB001.A and HEB001.B) did not contain any HBV-mapping reads. Therefore, it appears unlikely that the signal recovered from the Stumpff Lock arose from laboratory contamination, which is, in general, not expected for HBV. Furthermore, our phylogenetic analysis indicated that the HBV genome recovered from the Stumpff Lock belonged to a single subgenotype D2 (Figure 7). Subgenotype D2 is one of the most prevalent HBV variants in Europe today[74,75] and has been shown to be present in the region since at least the Middle Ages.[76] These results are consistent with an authentic HBV infection.

Only hairs from the Stumpff Lock were proven positive for HBV, plausibly spanning a period of growth no later than the summer to winter of 1826, and likely earlier (STAR Methods). However, owing to differential sequencing efforts between the four samples tested and random fluctuations in HBV viremia,[77] the absence of detection in older samples does not necessarily imply that the infection was acquired toward the end of Beethoven's life. Owing to these limitations, we are unable to determine how or when Beethoven's infection with HBV occurred.

Future directions

Genomic sequence data from authenticated locks of Beethoven's hair provide Beethoven studies with a novel primary source, already revealing several significant findings relating to Beethoven's health and genealogy, including substantial heritable risk for liver disease, infection with HBV, and EPP. This dataset additionally permits numerous future lines of scientific inquiry. This initial series of five hair samples, spanning approximately the last 7 years of Beethoven's life, is hoped to be expanded through the authentication testing of additional independent locks of hair, and enables future testing for infections, informative biomarkers, and exposures to environmental causes of or contributors to disease. The further development of bioinformatics methods for risk stratification and continued progress in medical genetic research

will allow more precise assessments both for Beethoven's disease risk and for the genetic inference of additional phenotypes of interest. Increases in the size of consumer genetics databases, as well as the testing of additional hypothesized relatives both living and deceased, will lend further clarity to our understanding of Beethoven's genetic genealogy. This study illustrates the contribution and further potential of genomic data as a novel primary source in historical biography.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

T.J.A.B., W.R.M., and J.K. conceptualized the research. T.J.A.B., J.K., W.R.M., T.K., M.H.D.L., M.M.N., A. Schmidt, and D.K. designed the research. T.J.A.B., P.A.M., A.K., and A. Schmidt visualized the research. W.R.M., T.J.A.B., M.H.D.L., M. Boecker, and J.D.W. acquired samples. T.J.A.B., W.R.M., and T.K. acquired funding. T.J.A.B., E.R., G.B., A.K., C.F., A.T., M. Burri, J.O., R.B., and F.A. performed the laboratory work. T.J.A.B., A. Schmidt, A.K., G.R., P.A.M., M.S., K.P., T.K., A. Szolek, A.P., R.B., C.M., and S.C. performed bioinformatics analyses. W.R.M., J.D.W., M.H.D.L., J.R., and T.J.A.B. performed historical research. T.K., R.D.A., M.M.N., D.K., J.K., and W.R.M. supervised the research. T.J.A.B., T.K., and R.D.A. wrote the original draft. T.J.A.B., R.D.A., T.K., A. Schmidt, C.M., A.K., G.R., P.A.M., M.H.D.L., J.D.W., R.B., A. Szolek, M.S., C.S., K.P., D.K., W.R.M., M.M.N., and J.K. reviewed and edited the final draft with contributions from all authors.

DECLARATION OF INTERESTS

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
0.5 M EDTA, pH 8.0	Thermo Fisher Scientific (Life Technologies)	Cat#EP0052
10% Criterion TBE-Urea Polyacrylamide Gel, 18 well, 30 μl	BioRad	Cat#3450089
10x GeneAmp PCR Gold Buffer and MgCl2	Thermo Fisher Scientific (Life Technologies)	Cat#4379874
2-Propanol	Merck	Cat#1070222511
20% SDS Solution	Serva	Cat#39575.01
20x SCC Buffer	Thermo Fisher Scientific (Life Technologies)	Cat#AM9770
2x TBE-Urea Sample Buffer	BioRad	Cat#1610768
3M Sodium Acetate buffer pH 5.2	Sigma-Aldrich	Cat#S7899-500ML
5 M Sodium chloride (NaCl)	Sigma-Aldrich	Cat#S5150-1L
5 M Sodium chloride (NaCl)	Sigma-Aldrich	Cat#S5150-1L
ATP	New England Biolabs	Cat#P0756S
BSA 20 mg/ml	New England Biolabs	Cat#B9000S
Bst 2.0 DNA Polymerase	New England Biolabs	Cat#M0537S
Buffer Tango 10x	Thermo Scientific	Cat#BY5
D1000 Reagents	Agilent Technologies	Cat#5067-5583
D1000 ScreenTapes	Agilent Technologies	Cat#5067-5582
Denhardt's solution	Sigma-Aldrich	Cat#D9905-5Ml
dNTP Mix 25 mM each	Thermo Scientific	Cat#R1121
DTT 5g	Thermo Fisher Scientific (Life Technologies)	Cat#R0861
Dynabeads MyOne Streptavidin C1	Thermo Fisher Scientific (Life Technologies)	Cat#65002
Dynabeads MyOne Streptavidin T1	Thermo Fisher Scientific (Life Technologies)	Cat#65601
Ethanol	Merck	Cat#1009832511
FastAP Thermosensitive Alkaline Phosphatase	Thermo Scientific	Cat#EF0652
GeneRuler Ultra Low Range DNA Ladder	Thermo Fisher Scientific (Life Technologies)	Cat#SM1211
Guanidine hydrochloride	Sigma-Aldrich	Cat#G3272-500g
Herculase II Fusion DNA Polymerase	Agilent Technologies	Cat#600679
Human Cot-1 DNA	Thermo Fisher Scientific (Life Technologies)	Cat#15279011
Klenow fragment	Thermo Scientific	Cat#EP0052
Oligo Length Standards 20/100 Ladder	IDT	Cat#51-05-15-02
PEG 8000 Powder, Molecular Biology Grade	Promega	Cat#V3011
Pfu Turbo Cx Hotstart DNA Polymerase	Agilent Technoloogies	Cat#600412
Polyethyleneglycol 8000 50%	Jena Bioscience	Cat#CSS-256
Proteinase K	Sigma-Aldrich	Cat#P2308-100MG
Sera-Mag Speed CM	GE Healthcare Lifescience	Cat#GE65152105050250
Silica Magnetic Beads	G-Bioscience	Cat#GENO786-915
Sodiumhydroxide Pellets	Fisher Scientific	Cat#10306200

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
SYBR Gold Nucleic Acid Gel Stain (10,000X Concentrate in DMSO)	Thermo Fisher Scientific (Life Technologies)	Cat#S33102
T4 DNA Polymerase	New England Biolabs	Cat#M0203L
T4 DNA-Ligase	Thermo Scientific	Cat#EL0013
T4 Polynucleotide Kinase	New England Biolabs	Cat#M0201L
T4 RNA ligation Buffer	New England Biolabs	Cat#B0216L
TE buffer pH 8.0 low EDTA	Panreac AppliChem	Cat#A8569,0500
Tris-HCl, pH 8.0	Thermo Fisher Scientific (Life Technologies)	Cat#15568025
Tween 20	Sigma-Aldrich	Cat#P9416-50ML
UltraPure Salmon Sperm DNA Solution	Thermo Fisher Scientific (Life Technologies)	Cat#15632011
UltraPure TBE Buffer, 10X	Thermo Fisher Scientific (Life Technologies)	Cat#15581044
USER Enzyme	New England Biolabs	Cat#M5505L
Water HPLC Plus	Sigma-Aldrich	Cat#34877-2.5L-M
Critical commercial assays		
Oragene OG-500 Saliva collection kit	Oragene DNA	N/A
Infinium GSA-24 v2.0 Kit	Illumina	Cat#20024446
High Pure Viral Nucleic Acid Large Volume Kit	Roche	Cat#5114403001
DyNAmo Flash SYBR Green qPCR Kit	Thermo Fisher Scientific	Cat#F415L
MinElute PCR Purification Kit	QIAGEN	Cat#28006
Quick Ligation Kit	New England Biolabs	Cat#M2200L
Oligo aCGH/Chip-on-Chip Hybridization Kit	Agilent Technologies	Cat#5188-5220
HiSeq 4000 SBS Kit (50/75 cycles)	Illumina	Cat#FC-410-1001/2
HiSeq 3000/4000 PE Cluster Kit	Illumina	Cat#PE-410-1001
QIAquick Nucleotide Removal Kit	Quiagen	Cat#28304
Oligo aCGH/Chip-on-Chip Hybridization Kit	Agilent Technologies	Cat#5188-5220
NextSeq 500 High Output Flow Cell Cartridge v2	Illumina	Cat#15065973
DNBseq (100 cycles) PE Kit	BGI	N/A
Deposited data		
Human reference genome NCBI build 37, GRCh37	Genome Reference Consortium	http://www.ncbi.nlm.nih.gov/projects/genome/ assembly/grc/human/
Downloadable genotypes of present-day and ancient DNA data	Allen Ancient DNA Resource	https://reich.hms.harvard.edu/allen-ancient-dna-resource-aadr-downloadable-genotypes-present-day-and-ancient-dna-data
Thousand genomes project phase 3 release	The International Genome Sample Resource	http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/phase3/
FamilyTreeDNA consumer database	goranr@genebygene.com	N/A
GWAS summary statistics: Cirrhosis	Emdin et al. 2021[43]	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8035329/bin/NIHMS1680867-supplement-1.pdf
GWAS summary statistics: Systemic lupus erythematosus	Julià et al. 2018[38]	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST005001-GCST006000/GCST005831/
GWAS summary statistics: Primary biliary cirrhosis	Cordell et al. 2015[46]	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST003001-GCST004000/GCST003129/
GWAS summary statistics: Primary sclerosing cholangitis	Ji et al. 2016[47]	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST004001-GCST005000/GCST004030/

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
GWAS summary statistics: Crohn disease	de Lange et al. 2017[37]	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST004001-GCST005000/GCST004131/
GWAS summary statistics: Ulcerative colitis	de Lange et al. 2017[37]	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST004001-GCST005000/GCST004131/
GWAS summary statistics: Irritable bowel syndrome	Eijsbouts et al. 2021[49]	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90016001-GCST90017000/GCST90016564/
HLA reference panel	Szolek et al. 2014[78]	https://github.com/FRED-2/OptiType
HBV reference genome; genotype D	GenBank	GenBank: NC003977
HBV reference genome; genotype F	GenBank	GenBank: FJ657525
Phylotree Build 17	van Oven 2015[79]	https://www.phylotree.org/builds/mtDNA_tree_Build_17.zip
Mediavel Bavarian reference panel	Veeramah et al. 2018[80]	https://www.ebi.ac.uk/ena/browser/view/PRJEB23079?show=reads
Hg19	UCSC Genome Browser	http://hgdownload.cse.ucsc.edu/goldenpath/hg19/bigZips/
hg38	UCSC Genome Browser	ftp://hgdownload.soe.ucsc.edu/goldenPath/hg38/
UK BioBank	UK BioBank	Approved Research ID: 54169 ‘ The Beethoven Genome Project’
ClinVar build 2021-03-15	Clinical Genome Resource	https://ftp.ncbi.nlm.nih.gov/pub/clinvar/
OMIM	Online Mendelian Inheritance in Man	https://omim.org/downloads
Deafness Variation Database version 9, build 2021-01-04	Deafness Variation Database	https://deafnessvariationdatabase.org/public/releases/v9/
Oligonucleotides		
IS5 (AATGATACGGCGACCACCGA)	Sigma-Aldrich	N/A
IS6 (CAAGCAGAAGACGGCATACGA)	Sigma-Aldrich	N/A
IS7 (ACACTCTTTCCCTACACGACGC)	Sigma-Aldrich	N/A
IS8 (GTGACTGGAGTTCAGACGTGTGC)	Sigma-Aldrich	N/A
BO4.P7.part1.R (GTGACTGGAGTTC AGACGTGTGCTCTTCCGATCT[Phos])	Sigma-Aldrich	N/A
BO6.P7.part2.R (CAAGCAGAAGACGG CATACGAGAT[Phos])	Sigma-Aldrich	N/A
BO8.P5.part1.R (GTGTAGATCTCGGT GGTCGCCGTATCATT[Phos])	Sigma-Aldrich	N/A
BO10.P5.part2.R (AGATCGGAAGAGCG TCGTGTAGGGAAAGAGTGT[Phos])	Sigma-Aldrich	N/A
Software and algorithms		
Bcftools version 1.10.2	Li, 2011[81]	https://samtools.github.io/bcftools/
Burrows-Wheeler Aligner (BWA) version 0.7.12	Li and Durbin, 2009[82]	http://bio-bwa.sourceforge.net/
Samtools version 1.9	Li et al. 2009[83]	http://samtools.sourceforge.net/
GATK version 3.5	McKenna et al. 2010[84]	https://software.broadinstitute.org/gatk/
mapDamage2.0	Jónsson et al. 2013[28]	https://ginolhac.github.io/mapDamage/
Haplogrep2.0	Weissensteiner et al. 2016[85]	http://haplogrep.uibk.ac.at/
PLINK version 1.9	Purcell et al. 2007[86]	http://pngu.mgh.harvard.edu/purcell/plink/
Schmutzi	Renaud et al. 2015[87]	https://github.com/grenaud/schmutzi
IGV version 2.14	Thorvaldsdóttir et al. 2013[88]	https://software.broadinstitute.org/software/igv/
PRSice2.0 version 2.3.3	Choi et al. 2019[89]	https://www.prsice.info
Variant Effect Predictor	McLaren et al. 2016[90]	http://grch37.ensembl.org/Homo_sapiens/Tools/VEP/Edit

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
pedSIM	Caballero et al. 2019[32]	https://github.com/williamslab/ped-sim
snpAD version 0.3.4	Prüfer, 2019[20]	https://bioinf.eva.mpg.de/snpAD/
FlashPCA2.0	Abraham et al., 2017[91]	https://academic.oup.com/bioinformatics/article/33/17/2776/3798630
R	R Core Team, 2021[92]	https://www.r-project.org
AdaptorRemoval	https://github.com/MikkelSchubert	https://github.com/MikkelSchubert/adapterremoval
ANGSD	Korneliussen et al. 2014[93]	http://www.popgen.dk/angsd/index.php/ANGSD
MALT version 0.3.8	Herbig et al. 2016[56]	https://software-ab.informatik.uni-tuebingen.de/download/malt/welcome.html
READ	Kuhn et al., 2018[94]	https://bitbucket.org/tguenther/read
BEAGLE version 5.1	Browning et al. 2011[95]	https://faculty.washington.edu/browning/beagle/b5_1.html
ADMIXTURE	Alexander et al. 2009[22]	https://www.genetics.ucla.edu/software/admixture/
Preseq	Daley and Smith, 2014[96]	https://github.com/smithlabcode/preseq
EAGER version 1.92.38	Peltzer et al. 2016[97]	https://eager.readthedocs.io/en/latest/
EIGENSOFT	Patterson et al. 2006[98]	https://github.com/DReichLab/EIG
IBIS	Seidman et al. 2020[31]	https://github.com/williamslab/ibis
Rx_identifier	Mittnik et al. 2016[99]	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5051676/bin/pone.0163019.s003.r
leehom version 1.1.5	Renaud et al. 2014[100]	https://github.com/grenaud/leeHom
PATHd8	Britton et al. 2007[101]	https://rdrr.io/github/fkeck/diatobc/man/pathd8.html
BEAST version 2.5.2	Bouckaert et al. 2014[102]	https://www.beast2.org
MEGAN version 6.13.1	Huson et al. 2016[103]	https://software-ab.informatik.uni-tuebingen.de/download/megan6/welcome.html
Picard tools	http://broadinstitute.github.io/picard/	http://broadinstitute.github.io/picard/
DamageProfiler	Neukamm et al. 2021[104]	https://github.com/Integrative-Transcriptomics/DamageProfiler
MAFFT version 7.475	Katoh and Standley, 2013[105]	https://mafft.cbrc.jp/alignment/software/
Gblocks	Castresana, 2000[106]	https://home.cc.umanitoba.ca/~psgendb/doc/Castresana/Gblocks_documentation.html
RAxML version 8.2.12	Stamatakis, 2014[58]	https://github.com/stamatak/standard-RAxML
OptiType	Szolek et al. 2014[78]	https://github.com/FRED-2/OptiType

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Johannes Krause (krause@eva.mpg.de).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Sequence data generated from the five matching locks have been deposited at the European Nucleotide Archive under the accession number ENA: PRJEB56343 and are publicly available as of the date of publication. The raw data from non-matching locks of hair reported in this study cannot be deposited in a public repository because the identity of the donors could not be confirmed and consent could not be provided.

- Original code for the Geo-genetic triangulation method has been deposited at Dryad: https://datadryad.org/stash and is publicly available as of the date of publication. https://doi.org/10.5061/dryad.k0p2ngfc4.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Beethoven hair samples

We identified 34 locks of hair attributable to Ludwig van Beethoven that have been described in primary sources and/or reside in public and private collections around the world, of which 25 are believed to have independent provenances (Table S1; Methods S1A–S1H). We tested eight locks of hair of independent provenance, namely the Müller (ca. 1815-1820; Methods S1A), Bermann (ca. 1821; Methods S1B), Halm-Thayer (April 25th, 1826; Methods S1C), Moscheles (March 24th, 1827; Methods S1D), Cramolini-Brown (March 27th, 1827; Methods S1E), Stumpff (March 26th-28th, 1827; Figure 3; Methods S1F), Hiller (March 27th, 1827; Methods S1G) and Kessler Locks (October 13th-23rd, 1863 or June 22nd, 1888; Methods S1H). Of the five genetically matching locks of hair, the Halm-Thayer and Stumpff Locks bear perfect chains of custody, the Müller and Moscheles Locks have incomplete chains of custody, but intact documentary links to their original provenances, while the Bermann Lock has no documentation attesting to its initial acquisition (Figure 1). The Müller, Moscheles and Stumpff Locks remain affixed to or associated with documents bearing the signatures of their original name-sake recipients (Methods S1A, S1D, and S1F). Of the non-matching locks of hair, the Hiller Lock lacks a contemporary account of its acquisition, while Ludwig Cramolini's (1805-1884) account of the Cramolini Lock's acquisition was first published after his death in 1907. The provenance of the Hiller Lock is unknown prior to the 1880s, and that of the Cramolini-Brown Lock unknown prior to 2012. The Kessler Lock was reputedly recovered during one of Beethoven's two exhumations in 1863 or 1888, with the earliest known reference to its acquisition dating to 1948.

Hair locks were named according to their first intended recipient or long-term owner, rather than the initial acquirer, who in a number of cases acted only as a brief intermediary (eg. Anton Schindler for the Moscheles Lock; Johann Valentin Schick and Johann Baptist Streicher for the Stumpff Lock). In cases where either a lock of hair is documented to have been split into two or more locks, or additional locks of hair bearing the same name are known to exist elsewhere, the last name of a subsequent confirmed owner is appended to the name of the first owner to specify the lock of hair in question (eg. Halm-Thayer Lock; Cramolini-Brown Lock). Below we describe the provenance histories of each tested lock.

Müller Lock

The Müller Lock has a moderately documented historical provenance, lacking a clear first-hand account of its original acquisition from Beethoven, in addition to having an incomplete chain of custody. An accompanying provenance (Methods S1A) note nonetheless associates the lock with two individuals, Nannette Streicher and Elise Müller, whose interactions with Beethoven are well documented within the time-frame of the lock's documented acquisition, on November 4th, 1820, by Elise Müller. The Müller Lock would eventually be acquired by the renowned collector of Beethoven memorabilia, Hans Conrad Bodmer, after which it would be bequeathed to the Beethoven-Haus Bonn, where it permanently resides. The Müller Lock is one of three locks of hair tested which remains associated with a provenance note bearing the signature of its namesake owner. Genetic testing has demonstrated that the Müller Lock is almost certainly authentic.

The Müller Lock is currently part of the collections of the Beethoven-Haus, Bonn, where it is cataloged and briefly described under the identification HCB V 12 (Hans Conrad Bodmer collection):

> Lock of Beethoven's hair; brown strands with some white hairs; enclosed in [folded] paper and wrapped in two paper covers. On the inner paper cover [is found] the handwritten remark: "Beethoven's hair / obtained by [Mrs.] Streicher / on Nov. 4. 1820'; on the outside paper cover [exists] the handwritten remark "Beethoven's hair. / Elise Müller'; the second signature of this piece: R 1 b.
>
> 1815–1820
>
> Concerning the dating: Nannette Streicher was particularly close to Beethoven after 1815. It remains an open question, whether she obtained the strands of hair from Beethoven at the request of Elise Müller, or whether she had already possessed it earlier.
>
> Provenance: Nannette Streicher in Vienna; Elise Müller in Bremen; unknown owner(s); Hans Conrad Bodmer in Zürich.
>
> Date of accession: 1956, Hans Conrad Bodmer Bequest.
>
> [translation William Meredith]

The first name in the set of paper covers, Nannette Streicher (1769-1833; née Stein), was an esteemed fortepiano manufacturer and important figure in Beethoven's life. She was born in Augsburg in 1769 to the famous organ and fortepiano manufacturer, Johann

Andreas Stein (1728-1792). After her father's death, she took over the firm. In 1794 she married Johann Andreas Streicher (1761-1833) and they moved, with her brother Matthäus, to Vienna. In 1802 Nannette and her brother separated as business partners and the firm was renamed "Nannette Streicher, née Stein." Nannette probably first met Beethoven in Augsburg in 1787 when he visited the city; her husband, who frequently assisted Beethoven in his business dealings, stated in letters that he had known the composer since 1788. Unusually for Beethoven, his relationship with the couple has been described as "uniformly serene", but Nannette was especially helpful to him in the years 1817-18, as documented in numerous letters and notes. At this time he was living near them in the Landstraße, and Nannette greatly assisted Beethoven in advising him on how to hire a housekeeper and a kitchen maid. Professionally, he greatly admired the fortepianos her firm produced, writing in 1817 that they had been his preferred instruments since 1809, an extraordinary statement.[1] That same year, he asked them to rent him one of their instruments that had been specially altered to accommodate his hearing loss.

The second name in the set of paper covers, Elise Müller (1782-1849), was a pianist, piano teacher, and song composer. She was born to Dr. Wilhelm Christian Müller (1752-1831), an important teacher and writer on music, and his wife Maria Amalia, in Bremen. Elise gave her first public concert at the age of ten; in 1807 the famous Leipzig music periodical, the *Allgemeine musikalische Zeitung*, said that her playing was distinguished by "fluency, assuredness, and expression."[107] She revered the works of Beethoven, which were one of her specialties, and her father characterized her as "Pianoforte-Spielerin Beethoven'scher Werke" (piano player of Beethoven's works). After Dr. Müller retired in 1817, he traveled extensively. In 1820 he and his daughter traveled to Vienna on their way to Italy, arriving in the city in October.

During their time in Vienna, the Müllers visited Beethoven twice. The first visit was on October 26th, documented in a book about Dr. Müller's travels.[108,109] The second is an account of Dr. Müller's observations of Beethoven eating in a restaurant, published in an obituary for Beethoven in the *Allgemeine musikalische Zeitung* No. 21 on May 23rd, 1827, entitled "Etwas über Ludw. van Beethoven" ("Some Things About Ludwig van Beethoven").[14,110] In his book, Müller stated that he and his daughter had been in correspondence with Beethoven for several years before meeting him. It is likely that a statement in the diary of Fanny Giannatasio on January 31st, 1817, that Beethoven had received letters and a gift from a lady in Bremen, refers to Elise.[1,111] Little of this correspondence survives, except a collection of poems commemorating Beethoven's 49th birthday authored by Elise Müller and Dr. Carl Iken, editor of the *Bremer Zeitung*, in December of 1819,[112] and a brief note Beethoven wrote during the Müllers' stay in Vienna.[1] In 1822 Beethoven asked the Berlin publisher of his Sonata in E Major, Op. 109, to send Elise a copy of the first edition.[112] The date of Elise Müller's acquisition of the lock of hair from Nannette Streicher on November 4th, 1821, was the Müllers' last day in Vienna during their trip.[108]

The Beethoven-Haus' provenance description is careful in its dating of when Nannette cut the lock of hair, only giving a probable five-year timespan when she and the composer were closely connected. The handwritten note thus only gives a *terminus ante quem* for the date of the cutting. The date does, however, indicate that Elise received the locket from Nannette that day, and it would have been natural for a dedicated pianist and piano teacher to visit the famous manufacturer at her shop, which also served as a famous music salon.

Nothing is known about the provenance of the lock between its ownership by Elise in 1820 (though one might presume it was in her collection until her death in 1849) and the unknown date upon which it was acquired by the great Swiss doctor and Beethoven collector Hans Conrad Bodmer (1891-1956). Bodmer also owned a lock of hair given to Robert Schumann in 1845 (HCB Br 115), one that belonged originally to Beethoven's friend Karl Holz (HCB V 11), one that belonged to Peter Simrock (HCB BBi 11/28), a lock from 1825 given to Schlesinger (HCB V 6), a lock given by "Cramolini in Darmstadt" to someone (HCB V 10), and a lock with a miniature portrait of the composer (HCB Mh 49).

Two DNA extractions, each consisting of 25cm of hair, were performed on the Müller Lock, for a total of 50cm of hairs sampled.

Bermann Lock

The original provenance of the Bermann Lock is almost entirely unknown. It was most likely acquired by Jeremias Bermann, a publisher of one of Beethoven's compositions in 1821, whose direct associations with Beethoven are likewise largely unknown. The American-born Beethoven biographer, Alexander Wheelock Thayer (1817-1897), records receiving the lock from a "Mr. Bermann" in 1851 during Thayer's biographical research on Beethoven. The Bermann Lock would remain within Thayer's family until its acquisition by the American Beethoven Society in 2017. Genetic testing has demonstrated that the Bermann Lock is almost certainly authentic.

The Bermann Lock's first documented owner, a "Mr. Bermann," is believed to have either been Jeremias Bermann (1770-1855), or Jeremias' son, Joseph Bermann (1810-1886). Jeremiah had married Anna Eder (1790-1859), the daughter of Joseph Eder (1760-1835), a publisher of several of Beethoven's early compositions in the late 1790s. The two most significant of the father's editions are the first edition of the three Fortepiano Sonatas, Op. 10 and the first Titelauflage of the *Pathétique* Sonata, Op. 13. The firm's specialty was the printing of visiting cards and New Year's cards. The Edersche Kunsthandlung was in a building called Zum schwarzen Elephanten (Black Elephant) on the famous Graben street.

In 1811, Bermann joined Eder's publishing firm, taking over operations in 1815 and publishing further Beethoven compositions. In June of 1821 Jeremias Bermann published the first edition of Beethoven's Bagatelles, Op. 119, Nos. 7-11 (published as Nos. 28-32), as part of the third volume of the Wiener Piano-Forte-Schule von Friedrich Starke, Kapellmeister. The manuscript of No. 7 is dated "am 1 = ten Jenner 1821." This date helps us locate Bermann's direct connection to Beethoven to between late 1820 and the early summer of 1821. Unfortunately, there is a gap in the conversation books and correspondence of Beethoven from 1820-22, and

Bermann is not mentioned in any of the surviving conversation books, nor is he mentioned in any Beethoven letter. Thus, while we know Bermann had a direct connection to the composer in 1820-21, there is no surviving document connecting them or detailing how or when Bermann acquired the lock. A New York Tribune Illustrated Supplement article from February 13th, 1898 states describing the recent auctioning of Thayer's library, and details that on May 15th, 1851, Alexander Wheelock Thayer visited the Edersche Kunsthandlung, purchasing a number of Beethoven autographs and acquiring the Bermann Lock. By 1851, the ownership of the firm, then named Jeremias Bermann & Sohn, had been passed on to Jeremias' son, Joseph.

Although we can deduce that Bermann most likely directly interacted with Beethoven ca. 1821 concerning the publishing of Op. 119, Nos. 7-11, the lock itself may have been acquired at any time between 1815 and 1821, and possibly later than 1821. Thayer records in an accompanying provenance note, "This lock of Beethoven's hair received from a Mr. Bermann. May 15, 1851," (Methods S1B).

The picture frame, containing both the Bermann Lock and the Halm-Thayer Lock described below, as well as several other items belonging to Alexander Wheelock Thayer including a military uniform and hat, dress sword, a shirt believed to have belonged to Beethoven, and numerous documents, were acquired as a single collection from living descendants of Thayer in the summer of 2017 by American Beethoven Society members Kevin Brown, William Meredith, and other members of the American Beethoven Society. Two DNA extractions, each consisting of 25cm of hair, were performed on the Bermann Lock, for a total of 50cm of hairs sampled. The Bermann Lock is currently in the collection of American Beethoven Society member Kevin Brown.

Halm-Thayer Lock

Among the eight locks of hair tested, the Halm-Thayer Lock is peerless in the completeness of his provenance history, having a perfectly documented chain of custody, as well as a detailed first-hand account of its acquisition (Figure 1). Evidence of the interactions and events leading up to its original acquisition are furthermore corroborated in numerous letters and conversation book entries. The Halm-Thayer Lock is the only lock of hair tested which is documented to have been received personally from Beethoven. Genetic testing has demonstrated that the Halm-Thayer Lock is almost certainly authentic.

The lock originated between March and April of 1826 as a practical joke perpetrated by Beethoven's friend and then-secretary, Karl Holz (1798-1858), on the wife of one of Beethoven's acquaintances, Anton Halm (1789-1872). Karl Holz was a close friend of Beethoven's and a skilled violinist, from 1823 playing second violin in the famous Schuppanzigh Quartet, which premiered many of Beethoven's string quartets. During the years 1825 to 1826, Holz acted as an unpaid secretary to Beethoven, replacing Beethoven's former secretary, Anton Schindler (1795-1864). Anton Halm was formerly a Lieutenant in the Austrian military who, in 1811, resigned his commission in order to pursue a career as a pianist and composer.[113] At the time of the lock's acquisition, Halm was completing a four-hand piano transcription of Beethoven's *Große Fuge*, Op. 133, having been commissioned to do so by Mathias Artaria (1793–1835), cousin to the founder of the eminent publishing house, Artaria & Co.[112] Halm's wife wanted a lock of Beethoven's hair, and Halm asked Holz to convey the wish to Beethoven in a conversation book. Several days later, Frau Halm received a lock of hair, which Holz had removed from a goat. On April 24th, Halm wrote to Beethoven that he would deliver the manuscript of the piano transcription to him, and when he visited the next day, Beethoven told Halm they had been deceived and gave Halm a white sheet of paper with "a significant quantity of his hair," saying "Das sind meine Haare!" ("That is my hair!").[114] Halm regarded this as a triumph but his wife was indignant with Holz about the dirty trick.

Several accounts of Halm's acquisition of the lock exist. The most authoritative account was told to Alexander Wheelock Thayer by Anton Halm, presumably during the same interview in 1859 when Thayer records receiving the lock from Halm. This account was published posthumously in 1908 from Thayer's notes in the fifth volume of his biography of Beethoven:

> Around the time of the preparations for and performance of the B-flat major quartet, there took place a mischievous joke, whereby a liberty was taken with a lock of Beethoven's hair.
>
> We have the first-hand account of the immediate recipient, as told by the piano player and composer Anton Halm to Thayer, who recorded it in his notes. Schindler's narrative is somewhat corrected and supplemented by this account. In the rehearsal for Schuppanzigh's concert, Frau Halm, "née Sebastiani from Trier, whom Beethoven always referred to as his fellow countrywoman", was also present. She had wished to own a lock of Beethoven's hair, a favor of which few could boast; Beethoven usually replied: "Leave me alone!"
>
> "My wife asked me to ask Beethoven for a lock of hair during this favorable occasion. But since Beethoven could not hear, and several people were present, I declined out of politeness to negotiate personally with Beethoven through his notebook. I therefore asked Karl Holz to present my wife's request to Beethoven. After a few days, my wife received a lock of hair from a third party, which was supposed to be Beethoven's hair."
>
> In the interim, Beethoven asked Halm to arrange the quartet fugue for the pianoforte four-hands.
>
> "Meanwhile, Karl Groß, an accomplished amateur cellist, had asked me, shrugging his shoulders: 'Who knows whether the hair is genuine?,' And yet I had no suspicions. After the piano arrangement was finished, I brought it to [Beethoven].-" [...]

> "When I was about to leave, he met me with a fearfully serious expression on his face, saying: 'You have been cheated with the lock of hair! You see, I am surrounded by such terrible creatures that they put aside all the respect they owe respectable people. You have the hair of a nanny-goat.' And speaking like this, he gave me a significant quantity of his hair in a sheet of white paper, which he had cut entirely from the back of his head, with the words: 'That is my hair!' - He had probably cut off the hairs from behind because they were still black there, while in front everything was already snow-white. - So I went home with this seldom-received gift, in triumph. - Not so my wife. She was indignant about Karl Holz's wickedness and immediately wrote a letter appropriate to the circumstances."

> "One or two years later, my wife was standing by Beethoven's open grave, on the 29th of March, 1827, and she saw Holz, standing on the other side weeping, too ashamed to look at her directly. Touched by that, she held out her hand to him across the grave as reconciliation."[114] [translation Tristan Begg & Robert Attenborough]

Halm's account begins during the first rehearsals of Beethoven's String Quartet No. 13 in B♭ major, which would first be premiered by the Schuppanzigh Quartet on March 21st, 1826.[115] This account is corroborated by numerous lines of evidence, including letters and conversation book entries. The involvement of Karl Holz is recorded in an entry in Beethoven's conversation books around March 31st, 1826, confirming that Holz had indeed given a lock of hair to Halm's wife:

> I have already given the hair to Halm.[116] [translation Tristan Begg]

In a conversation book entry on April 16th, Halm confirms his receipt of the initial, inauthentic lock of hair, and, on behalf of his wife, thanks Beethoven for the lock:

> My wife respectfully thanks you as a fellow countrywoman for the exceedingly dear memento (the hairs), and if we are not too irritating, we will call on you for a visit.[116] [translation Tristan Begg]

Interestingly, the words "die Haare" (the hairs) were added to this entry after it was initially written; Halm may have had to clarify what the memento was.

On the 24th of April, 1826, after the completion of his arrangement for the *Große Fuge*, Op. 133 for piano four-hands, Anton Halm wrote to Beethoven, confirming their intention to meet the following day:

> I have finished your Fugue, which I have the honor of sending along, with the greatest possible diligence and care! At every bar, I was amazed at your power of harmony and its flow, as well as the musical motives that you used and their development to the point of exhaustion!
>
> Concerning my arrangement, it was unfortunately not possible always to keep the subjects in their original shape; rather more frequently they had to be broken. Otherwise it is so brilliant, so advantageously playable, and, as I hope, still intelligible enough, that your most elevated masterwork will be acknowledged as that which it is. I shall take the liberty of delivering your manuscript at a quarter past three tomorrow afternoon, at the latest, to get your kind opinion of my arrangement.[112] [translation Theodore Albrecht]

In addition to Halm's first hand account as recorded by Thayer, as well as supporting lines of evidence from the letters and conversation books, two accounts of Halm's acquisition of the lock were related by Anton Schindler in different editions of his biography of Beethoven. These accounts contain several inconsistencies, both internally, and when compared to Halm's account. The accuracy of Schindler's discrepancies cannot be confirmed. The first account, from 1840 reads:

> Though Beethoven was throughout his whole life a prey to misfortune and disappointment, yet there were moments in which he did not scruple to inflict pain and disappointment on others. Nevertheless, it must be observed that in most cases of this kind he acted under some other influence than that of his own feelings. The following circumstance occurred in the latter years of his life.
>
> The wife of M. Halm, an esteemed piano-forte player and composer, residing in Vienna, was a great admirer of Beethoven, and she earnestly wished to possess a lock of his hair. Her husband, anxious to gratify her, applied to a gentleman who was very intimate with Beethoven, and who had rendered him some service. At the instigation of this person, Beethoven was induced to send the lady a lock of hair cut from a goat's beard; and Beethoven's own hair being very gray and harsh, there was no reason to fear that the hoax would be very readily detected. The lady was overjoyed at possessing this supposed memorial of her saint, proudly showing it to all her acquaintance; but when her happiness was at its height, some one, who happened to know the secret, made her acquainted with the deception that had been practised on her. In a letter addressed to Beethoven, her husband warmly expressed his feelings on the subject of the discovery that had been made. Convinced of the mortification which the trick must have inflicted on the lady, Beethoven determined to make atonement for it. He immediately cut off a lock of his hair, and enclosed it in a note, in which he requested the lady's forgiveness of what had occurred. The respect which

> Beethoven previously entertained for the instigator of this unfeeling trick was now converted into hatred, and he would never afterwards receive a visit from him.
>
> This is not the only instance that could be mentioned, in which our great master was influenced by vulgar-minded persons to do things unworthy of himself.[117] [translation Ignaz Moscheles]

Anton Schindler's first account contains several inconsistencies with Halm's account. Schindler is initially uncertain of the date of the episode. Although it is unclear whether Karl Holz delivered the goat hairs directly to Frau Halm, as suggested by the conversation book entry, or through a "third party" as indicated in Halm's account, Halm's account nonetheless contests Schindler's assertion that his wife received the goat hairs directly from Beethoven. Schindler's assertion that Beethoven "never afterwards received a visit" from the "instigator", undoubtedly Karl Holz, is incorrect; numerous letters documenting their continued friendship and correspondence following this incident refute this, in particular a letter from Beethoven to Holz dating from April 26th, 1826, the day after Beethoven's meeting with Halm, which reads:

> Beloved Friend!
>
> You may rest assured that I have completely forgotten the recent incident and that it will never alter my feelings of gratitude to you. Please, therefore, do not show anything of this in your behaviour. You will always be welcome to me. I hope that next Sunday you will not despise my dinner table. [...] If you have time to visit me this week I shall be delighted if you do so. You will find me quite unchanged, the same as usual. I shall expect you on Sunday for certain.
>
> Ever your friend
>
> Beethoven
>
> [1] [translation Emily Anderson]

Schindler's re-telling of the story, from 1860, omits several earlier inconsistencies. As well as providing a more specific date, Schindler clarifies that Karl Holz was the "instigator" alluded to in his first account. Consistent with his first account, Schindler again implicates Beethoven personally in the prank, while still laying the blame on Holz:

> A Juvenile Trick
>
> We promised our readers an example of our master's disposition, despite his misfortunes and frequent ill-humor, towards buffoonery and practical joking. The wife of Anton Halm, the pianist and composer, wanted a lock of Beethoven's hair. The request was made through Karl Holz, who persuaded the master to send his ardent admirer some hairs from the beard of a goat, actually not too different from Beethoven's own coarse gray hair. The lady, delighted with the memento of her musical idol, boasted far and wide of the gift, but before long she learned how she had been duped. Her husband was still deeply sensitive of his honour as a military officer, and in an aggrieved letter to our master related what he had heard. When Beethoven realized that his prank had been taken as an insult, he atoned for it by cutting off a lock of his own hair and sending it to the lady with a note begging for forgiveness. This incident occurred in 1826.[14] [translation Constance S. Jolly]

Halm's account states that it was Beethoven who informed Halm personally of the deception, who atoned for it with a lock of his own hair. As Schindler was not acting as Beethoven's secretary at the time of this incident, his accounts must be interpreted with caution. In addition, considerable animosity existed between Schindler and Holz, and Schindler's three biographies are replete with slanders against his personal enemies, including Holz, which likely colored Schindler's accounts. It remains unclear from all of the surviving accounts and the surviving documentary evidence whether Beethoven was personally involved in or aware of the prank, a detail to which Anton Halm may not have been privy, or which he may not have wanted to disclose.

Alexander Wheelock Thayer received the Halm-Thayer Lock from Anton Halm on October 12th, 1859, while interviewing Halm during his biographical research on Beethoven. Thayer's pencil inscription accompanying the hairs reads, "Hair from Beethoven's head received from himself by Anton Halm, on 25th April, 1826. Given me by Ant. Halm this 12th Oct. 1859. A. W. Thayer," (Methods S1C).

In addition to the hairs Thayer received from Halm, a second lock of hair drawn from the original Halm Lock, the Halm-Epstein Lock, is also known to exist. The Halm-Epstein Lock was given to Halm's pupil, the pianist Julius Epstein (1832-1926). The Halm-Epstein Lock, along with the Erdödy, Hiller and Bernard Locks, is one of only four locks of Beethoven's hair for which results from scientific analyses have yet been published.[3,4] It was last sold at the Sotheby's auction house on June 11th, 2019. A third lock of hair possibly originating from the Halm Lock, belonging to a "Herr Oberstleutnant August Halm," is described as part of an exhibit of Beethoven memorabilia displayed in Vienna during the 1927 centenary of Beethoven's death. The exhibition's description of the lock states: "Beethoven's hair, enclosed with a letter proving its authenticity." The whereabouts of this lock are currently unknown.

Two DNA extractions, each consisting of 25cm of hair, were performed on the Halm-Thayer Lock, for a total of 50cm of hairs sampled. The Halm-Thayer Lock, purchased together with the Bermann Lock in the summer of 2017 from Thayer's living

descendants for analysis in The Beethoven Genome Project, is currently in the collection of American Beethoven Society member Kevin Brown.

Moscheles Lock

The Moscheles Lock has a well-documented provenance, with a first-hand account of its initial acquisition and only a single break in its chain of custody between ca. 1917 and ca. 1940. Although it was separated from its original provenance letter in 1911, it nonetheless remains affixed to a provenance note bearing the signature of its namesake recipient, Ignaz Moscheles. The Moscheles Lock was cut from Beethoven's head by his friend and secretary, Anton Schindler, on March 24th, 1827, two days before Beethoven's death, but while Beethoven was "still fully conscious",[112] and mailed to Ignaz Moscheles. The Moscheles Lock was retained in the Moscheles family until ca. 1917 when Moscheles' son, Felix, died childless. The Moscheles Lock resurfaced in 1940 in the possession of the Ukrainian-American violin virtuoso, Louis Krasner. The lock then remained in the Krasner family until 2010, when it was acquired by the American Beethoven Society. Genetic testing has demonstrated that the Moscheles Lock is almost certainly authentic.

On March 24th, 1827, Beethoven's amanuensis, Anton Schindler, mailed the Moscheles Lock, along with a detailed letter and newspaper clipping, to a prominent musician and friend of Beethoven in London, Ignaz Moscheles (1794-1870). The letter, which arrived on April 5th, is now on permanent loan to the Beethoven-Haus; a portion of it was published in Volume 5 of the *Harmonicon* magazine in an article announcing Beethoven's terminal illness and death in May 1827:

> I have just come from Beethoven. He is already dying, and before this letter is beyond the walls of the capital, the great light will have been extinguished forever. He is still fully conscious, however. I hasten to dispatch this letter, in order to run to him. I have just cut these hairs from his head and am sending them to you. God be with you![112] [translation Theodore Albrecht]

Anton Schindler's reputation suffered a serious blow in the 1970s, when it was discovered that he had inserted more than 150 entries in his own hand (writing as himself) into Beethoven's conversation books many years after the composer's death.[118] Though this was apparently done primarily to enhance his own reputation,[119] each of these entries must be confirmed by another source before it can be trusted as accurate. While any claims Schindler made must be examined with great care, existing customs stamps on the provenance letter, as well as subsequent accounts by the Moscheles family, support the veracity of Schindler's account.

Following Ignaz Moscheles' death in 1870, his widow, Charlotte (1805-1889), stated in her two-volume biography of Ignaz Moscheles that all of the Beethoven memorabilia were given to their son, Felix Moscheles (1833-1917):

> The lock of Beethoven's hair, the sketches in his own hand, the metronome tempi of the 9th Symphony, and the sketch-book which Schindler sent him, were always kept and regarded as the most sacred relics, and are now in the possession of his son Felix.[120] [translation A. D. Coleridge]

Most of these items were sold as the "Moscheles Collection" before Felix's death by the famous Berlin antiquariat, Leo Liepmannssohn, on November 17th-18th, 1911. The collection consisted mainly of music manuscripts, but listed on p. 3 are items concerning "Beethoven's last illness and death, contained in letters of Beethoven and Schindler to Moscheles." A detailed inventory appears on pp. 7-12; Schindler's letter is auction number 5/section II/letter 6. However, neither the lock of hair nor the periodical clipping were sold along with the letter (https://digi.ub.uni-heidelberg.de/diglit/liepmannssohn1911_11_17/0031).

Schindler's letter came to the Beethoven-Haus on permanent loan in 1998 as part of the Wegeler Collection (Karl Wegeler had purchased the Beethoveniana of the Moscheles collection at the Liepmannssohn auction), but it did not come with the accompanying lock of hair or newspaper clipping. The intact chain of custody of the Moscheles Lock thus ends ca. 1917.

In 1940, a lock of hair described as the Moscheles Lock, separated from the original provenance letter, was exhibited in Boston. The lock was affixed to a piece of paper bearing the signature of Ignaz Moscheles and quoting from the original Schindler letter, and was described in some detail in the Boston Symphony Orchestra's Concert Bulletin of their 60th season (1940-41) by the Beethoven biographer John N. Burk. By this time, this lock was part of the collection of the Ukrainian-American violinist Louis Krasner (1905-1993). Burk described the lock in a segment titled "Rare Beethoven Relics":

> In a case in the First Balcony Gallery are several items of Beethoven memorabilia, lent by Mr. Louis Krasner, which are of unusual interest because of their initial showing in Symphony Hall. […] There is also a copy made by Moscheles for Robert Schumann of a sketch of Beethoven drawn from life by Hornemann.
>
> In the same frame with the sketch are a flower picked on Beethoven's grave in 1852 by the English musician, George Doane, and a lock of Beethoven's hair, sent to Moscheles by Schindler in 1827. Moscheles has written an accompanying note stating: "L. v. Beethoven died on 26th March, 1827. A. Schindler wrote me on the 24th of March: 'This hair I have today cut from his [Beethoven's] head and I am sending this to you.'"[121]

Over his lifetime, Krasner amassed a large collection of musical manuscripts and memorabilia, most of which were donated to the Houghton Library at Harvard University by members of the Krasner family between 1986-2001. The circumstances by which Louis Krasner acquired the lock are not currently known. The lock of hair, exactly as arranged in the 1940 description, was acquired by the

American Beethoven Society for genetic testing from the New York based antiquarian dealers J & J Lubrano in the fall of 2010 (Methods S1D). The Society gifted the lock to the Ira F. Brilliant Center for Beethoven Studies, San Jose State University, San Jose, California that same year.

On July 15th, 2016, then director of the Ira F. Brilliant Center for Beethoven Studies, William Meredith, engaged two conservators to open the frame at the conservation laboratory of the Legion of Honor Museum, San Francisco, extracting 24 hairs, amounting to approximately 130 cm of hair. During this process, conservators noted that more exposed portions of the hairs appeared to be discolored, consistent with a process known as 'photo-aging' during which melanin breaks down when exposed to light.[122] Two DNA extractions, each consisting of 40cm of hair, were performed on the Moscheles Lock, for a total of 80cm of hairs sampled. The remaining hairs that were not sampled remain in the collections of the Ira F. Brilliant Center for Beethoven Studies.

Cramolini-Brown Lock

The Cramolini-Brown Lock has a relatively poor historical provenance, with no documentation tracing it to the original Cramolini Lock, for which a detailed first-hand account of acquisition does exist. There currently are two locks of hair purported to be from the original Cramolini Lock, and a third which may yet exist and whose provenance may be traceable to the original. The Cramolini-Brown Lock which we analyzed was acquired from RR Auction. Another lock of hair is in the collections of the Beethoven-Haus in Bonn, as item V 10 of the Hans Conrad Bodmer Collection, with a note of provenance tracing it to Darmstadt, Germany, the town in which Ludwig Cramolini eventually settled to pursue his career as an operatic tenor singer and theater director, and start a family. The third lock, whose existence and possible whereabouts are unknown, is recorded as being gifted in 1827 to Ludwig Cramolini's then fiancée, Nanette Schechner (1804-1860), who had performed with high praise in a rendition of Beethoven's opera, *Fidelio*, in 1826.[115] Genetic testing has demonstrated that the Cramolini-Brown Lock is almost certainly inauthentic.

The original Cramolini Lock is documented as being removed from Beethoven's corpse on March 27th, 1827, on the day following his death, by the renowned operatic tenor, Ludwig Cramolini (1805-1884). Cramolini had removed the lock without formal permission, resulting in a confrontation between himself and Beethoven's friend and secretary, Anton Schindler. Ludwig Cramolini recorded his account of the acquisition of the hairs in a diary, excerpts of which would eventually be published a 1907 *Frankfurter Zeitung* article entitled "An Beethovens Leiche" (On Beethoven's Corpse) and, in an abridged English translation, in a 1907 *New York Times* article entitled "Beethoven as Seen Through Diary of Man Who Knew Him." Cramolini's full account of his visits to Beethoven during the final months of his life, as well as his participation in commemorations during Beethoven's funeral, would be published in the second volume of Friedrich Kerst's 1913 collection of Beethoven reminiscences.[123] Cramolini's account of the acquisition of the hairs reads:

> On the 27th, after the rehearsal for A. Müller's operetta 'Die erste Zusammenkunft', I drove to Beethoven's apartment, a small pair of scissors in my pocket. There I found Schindler, who had already fended off a great number of people curious to see Beethoven, but he let me pass. And so I stood before the covered corpse, which rested on long wooden boards upon chairs, as was customary in those days. In the presence of an old woman (Beethoven's housekeeper, I believe), I lifted the shroud, quickly clipped off a ringlet of hair and wanted to depart immediately, when Schindler entered. I embraced him, wept, and admitted that I had cut some hair from Beethoven's head as an eternal memento for myself and Nanette Schechner (singer at the Vienna Opera). Schindler behaved like a lunatic, demanded that I return the hair, said it was an insult, and all this before the body of the great Beethoven, which angered me so that I asked him to follow me into the antechamber, so that I might answer him outside the presence of the divine master; for here, I thought, it was a crime. I waited for Schindler quite a while - in vain. He failed to come, and thus I returned home and later gave Nanette Schechner some of the hair, for which she was exceedingly grateful. I still have my share of the booty, as Schindler called it.[124] [translation William Meredith]

As a result of splitting the hair between himself and Nanette Schechner, the original Cramolini Lock is documented as two locks of hair. It is currently unknown what has become of the Cramolini-Schechner Lock. There is no doubt that Ludwig Cramolini was in Vienna at the time of Beethoven's death as Cramolini's own account,[123] as well as records in Beethoven's conversation books,[125] document him and Nanette Schechner visiting Beethoven and singing to him during the course of Beethoven's final illness. Ludwig Cramolini was also among the singers at Beethoven's funeral.

The Cramolini-Brown Lock is folded in a small slip of paper with what may be preliminary notes for a funeral procession: "1. leader with staff. 2. 8 children 2 girls with candles 2 girls w/ flower baskets wherein flws. & fruit / 2 boys w/ candles 2 children with pitchforks, scythes, flowers…Soprano, Alto & Basso"[124] (Methods S1E). As Beethoven's funeral was being planned on March 27th, this detail may be consistent with the circumstances of the hair's removal and concealment in the immediate aftermath of Beethoven's death. This document has been interpreted as preliminary arrangements for Beethoven's funeral.[124] On the reverse is twice penciled in the name "Beethoven" (Methods S1E).

The Cramolini-Brown Lock was first offered for sale in October 2012 by the respected antiquarian dealer Thomas Kotte in Roßhaupten, Germany, for €35,000 (approximately $45,600 at the time).[124] Either because of the high price, the lack of authentication, or both factors, the lock did not sell. It reappeared at an auction in Amherst, Massachusetts, from the auction house RR Auction. At the Kottke auction in 2012, a large number of items related to the Cramolini family were additionally advertised. These include what was claimed to be the dried placenta of Ludwig Cramolini's grandson (also named Ludwig Cramolini), numerous letters, and a family tree tracing the ancestry of the Cramolini family to Ludwig Cramolini's grandson.

The Cramolini-Brown Lock was acquired at auction on March 11, 2015 from RR Auction, by three members of the American Beethoven Society for analysis in The Beethoven Genome Project.[126] The entire Cramolini-Brown Lock consists of approximately 250 hairs, an average of 3" in length, of blond, brown, gray and black hairs (Methods S1E). In total, 109 hairs of varying lengths with at least 15 bulbs adhering were sent to the University of Tübingen's Paleogenetics Department. The majority of hairs with bulbs were long, black curly hairs. Four DNA extractions of 25cm each, and three DNA extractions of 2 bulbs with approximately 1.5cm adhering to them, were destructively sampled in seven separate extractions, for approximately 109 cm of hair ultimately sampled.

Stumpff Lock

The Stumpff Lock (Figure 3) is the second of the locks of hair tested which boasts an intact chain of custody (Figure 1). While a first-hand account of its cutting does not survive, its provenance in the immediate aftermath of its cutting, between March 28th and May 7th, 1827 is well documented. The Stumpff Lock furthermore remains affixed to a document bearing the signature of its original, namesake owner, Johann Andreas Stumpff (Methods S1F). The Stumpff Lock is stated by Sotheby's to have remained within the family of its eventual owner, Patrick Stirling, until its acquisition at Sotheby's in London in November of 2016 by a member of the American Beethoven Society. Genetic testing has demonstrated that the Stumpff Lock is almost certainly authentic.

The lock was originally sent to London-based Thuringian-born harp maker Johann Andreas Stumpff (1769-1846) on March 28th, 1827, in a letter written by a mutual friend of Stumpff and Beethoven, Johann Baptist Streicher (1796-1871), the son of Beethoven's close friend Nannette Streicher (see Müller Lock). Also included in this letter was a small sheet of music manuscript. Both items were sent to Stumpff by Streicher on behalf of another mutual friend of Beethoven, the prominent art and culture journalist Johann Valentin Schickh (1770-1835), who was taking responsibility for Beethoven's funeral at that time and was too busy to write. The hairs were therefore first acquired at some point between Beethoven's death on March 26th and Streicher's sending of the letter on March 28th, 1827. Below is the text of Streicher's letter to Stumpff of March 28th, 1827, relating to the hair and music manuscript:

> Since Herr Schickh has eagerly taken responsibility for Beethoven's funeral, he is prevented at the moment from writing to you and Herr Schultz himself. Meanwhile, he sends you the enclosed lock of Beethoven's hair, cut after his death, as well as a little piece of manuscript; a larger will follow.[112] [translation Theodore Albrecht]

This correspondence was, along with the letter of Schindler to Moscheles from four days before, reproduced in the same volume of *Harmonicon* that announced Beethoven's death (see Moscheles Lock). Stumpff then acknowledges receipt of the hair and music shortly after receiving them in a letter sent to Streicher on April 16th, 1827:

> The passing of that irreplaceable great German man, our friend Beethoven, pierced me deeply. Here I sit, bent over your dear letter that confirmed the news of it for me, and stare at the lock that adorned the head from which flowed the immortal works, which are and shall remain the admiration of all cultivated nations. […] Now, my dear friend, I thank you most sincerely for the lock of hair and music of our departed friend that you sent, with the request that you give Herr von Schickh my many regards, and extend my thanks for the tender proof of his friendly sentiments toward me.[112] [translation Theodore Albrecht]

The lock next appears affixed to a letter from Stumpff to the thirteen-year-old orphan and inheritor of the Keir branch of the Scottish Stirling clan estates, Patrick Stirling (1813-1839) (Figure 3; Methods S1F).[127] The letter contains two notable features idiosyncratic to Stumpff's correspondence, one of which is a short poem, and the other Stumpff's exceedingly fine handwriting. The text of this letter is reproduced below:

For Master P. Stirling of

Brighton

The head⊠, these hair's have grac'd lies low

But what it wrought - will ever grow. }

with J. A. Stumpff Compts -

May 7th, 1827 ⊠of Lud. v. Beethoven.

44 Great Portland Street

While it is not yet clear what prompted Stumpff to send these hairs to the orphan Patrick Stirling, it is notable that the Stirling family patronized several prominent Romantic era musicians. Patrick's sister, Mary Wedderburn, was godmother to Edvard Grieg (1843-1907),[128] while Patrick's aunt, Jane Wilhelmina Stirling, famously patronized Frédéric Chopin (1810-1849). An as yet unexplained detail worthy of mention, and consistent with an origin for the hairs in early nineteenth-century Vienna, can be found on the accompanying note in which the Stumpff Lock was originally folded (Methods S1F). On the reverse of this note is penned in dark ink

'Beethovens hair,' as well as the lightly penciled name 'Schuppanzigh,' in a separate hand (Methods S1F). Ignaz Schuppanzigh (1776-1830) was a close associate of Beethoven's, having premiered many of his string quartets as first violinist of the Schuppanzigh Quartet, as well as the Ninth Symphony, Op. 125.

The Stumpff Lock was sold at auction to American Beethoven Society member Kevin Brown at auction in late November 2016 for analysis in The Beethoven Genome Project.[129] Sotheby's description of the Stumpff Lock stated that "This lock of hair has come down to the present owners by direct descent," (https://www.sothebys.com/en/auctions/ecatalogue/2016/music-continental-books-manuscripts-l16406/lot.5.html). Two DNA extractions, each consisting of 25cm, or four hairs, were initially performed for authentication purposes, amounting to a total of 50cm, or 8 hairs, removed from the lock. Subsequently, the Stumpff Lock was chosen, owing to marginally superior DNA preservation (Methods S1I) for further extractions to generate libraries, for production sequencing of a high-coverage genome. An additional 56 hairs, amounting to 275cm, were removed and destructively sampled in 11 additional DNA extractions, for a total of 325cm of hair removed altogether. The Stumpff Lock at the time of writing resides in the collection of American Beethoven Society member Kevin Brown.

Hiller Lock

The Hiller Lock, elsewhere referred to as the Guevara Lock,[2] has a comparatively poor provenance history, lacking a first-hand account of its acquisition, as well as having an incomplete chain of custody (Figure 1). It is first mentioned as being inherited as a birthday present in 1883 by Paul Hiller. Paul Hiller claims that the lock was initially acquired by his father, Ferdinand Hiller, on March 27th, 1827. However, Ferdinand Hiller recorded no account of the lock's acquisition, either in his diary at the time, or in his subsequent memoirs. The lock remained in Paul Hiller's possession until at least 1911. The lock's whereabouts between 1911 and 1943 are not known with certainty. In 1943, the lock was acquired by a Danish doctor aiding in the escape of Danish Jews to neutral Sweden. In 1994, the Hiller Lock was acquired by members of the American Beethoven Society. Genetic testing has demonstrated that the Hiller Lock is definitely inauthentic.

Ferdinand Hiller (1811-1885) was a composition pupil of Johann Nepomuk Hummel (1778-1837), and later became a prominent Romantic era composer who succeeded Felix Mendelssohn (1809-1847) as director of the Leipzig Gewandhaus Orchestra. In his 1871 reminiscences, Hiller records four visits to the dying Beethoven with Hummel, between March 8th and March 23rd, 1827.[130] During one of these visits, Hummel's wife, Elisabeth ("Betty"), would acquire a lock of hair, which was acquired as part of the Yvonne Hummel Collection by the American Beethoven Society from the Hummels' descendants.[131] An additional lock of Beethoven's hair attributed to Elisabeth Hummel resides in the collections of the Beethoven-Haus Bonn as item R 1 e. Despite this, Hiller makes no mention of either viewing Beethoven's corpse, or removing a lock of hair following Beethoven's death.[2,130]

The earliest known mention of the Hiller Lock, believed to have been penned by Ferdinand Hiller's son, Paul Hiller, consists of a fragmentary inscription that was discovered when the locket containing the hairs was opened following its acquisition by the American Beethoven Society. Upon this damaged inscription can be discerned the words "Beethoven", "abgeschnitten", and "Ferdinand Hiller" (Methods S1G). On the reverse of the inscription is a portion of a page from a 44-volume periodical, *Exposé de la Situation Générale de L'Algérie*, published in 1881 (Methods S1G).

The inscription found on the locket today is a replacement of an unknown date, though likely added around the time of the locket's refurbishment by Cologne-based art dealer, Hermann Großhennig, on December 18th, 1911. The inscription reads, "This hair was cut off of Beethoven's corpse by my father, Ferdinand v. Hiller, on the day after Ludwig van Beethoven's death, that is 27 March 1827, and was given over to me as a birthday present in Cologne on 1 May 1883. Paul Hiller" (Methods S1G). On the reverse of the current inscription is a note documenting the locket's refurbishment in 1911: "Newly pasted to make it dust-free. Original condition improved"[2] [translation Russel Martin].

Between 1911 and 1943, nothing is known with certainty about the lock's whereabouts. As attested by Thomas Wassard Larsen, one of the owners of the lock prior to its acquisition by the American Beethoven Society, the lock was received by a Danish doctor, Kay Alexander Fremming, in the port town of Gilleleje, Denmark, from an unknown Jewish refugee seeking the safety of neutral Sweden in October of 1943:

> My name is Thomas Wassard Larsen, and i am writing to you about a lock of Beethovens hair, sold by Sotheby's auctions i London. I hope you understand the meaning with this letter, because i'm not very good at writing in english.
>
> The lock was owned by my mother, who had to sell it due to her economical situation. My mother Michele was born in France a cupple of years before 2.nd world war. During w.w.2 my grandmother had 8 kids including my mother, and she could not feed them all so therefore my mother was adopted by a nice family in Denmark. She was no in the age of 8 years.
>
> My mothers new parents were a Doctor and a nurse who lived in a little town in North Sealand called Gilleleje. This little town was one of the closest to Sweden, to witch many judes fled during 2.nd w.w. Many of these judes were wery poor and some of them had som awful deceases.
>
> My mothers new father who was a doctor helped many of these judes, in the start only with medicin, but later he worked together with the local fishermen, in the night to smuggel judes to Sweden. It was one of these judes who gave the lock of Beethovens hair to him for his help. My grandfather kept this medallion until his dead in 1969, the same year that i was born.[2]

A historically well known community of approximately 7,800 Ashkenazi Jews in Copenhagen had survived in relative safety in Nazi-occupied Denmark until the local Gestapo were ordered to detain them on October 1st, 1943. This prompted a mass exodus of Copenhagen's Jews to neutral Sweden, many of whom used the port town of Gilleleje, 40 miles north of Copenhagen and allowing a short sea voyage to Sweden. Ultimately 7,220 Jews and 686 non-Jewish spouses escaped with the help of the Danish Resistance movement.[132]

The Hiller Lock of hair would remain within the Fremming family between 1943 and 1994, until being acquired by members of the American Beethoven Society at auction at Sotheby's in London. At auction, the lock consisted of 582 hairs, approximately 4.5'' in length, after which 160 hairs were given to the principal investor, Dr. Alfredo Guevara, and 422 were kept by the Ira F. Brilliant Center for Beethoven Studies (Methods S1G). The hairs are blond, brown, gray and black.

Accepted by many as authentic, the hairs have been subjected to a number of scientific tests, which have led some of Beethoven's medical biographers to conclude that Beethoven's health problems, hearing loss, and death may have been caused or compounded by plumbism.[3,4,26,27,133] It has additionally been concluded from these analyses that Beethoven did not receive mercury treatment for a hypothesized infection with syphilis, and that he did not receive opiates during the treatment of his final illness. The Hiller Lock is the only lock of hair putatively originating from Beethoven for which data from a DNA analysis exists, consisting of a partial PCR and Sanger Sequencing based analysis of several variants within hypervariable regions I and II carried out by LabCorp in 1999.

Five hairs, totaling approximately 25cm, were sent to the University of Tübingen's Paleogenetics Department by Dr. Alfredo Guevara, and subsequently sampled in a single DNA extraction.

Kessler Lock

The Kessler Lock is of enigmatic provenance, with only a single known statement from 1948 attesting to its acquisition. The Kessler Lock nonetheless appears to have a complete chain of custody (Figure 1). The Kessler Lock was reputedly recovered during one of Beethoven's two exhumations, in 1863 or 1888, in Vienna's Währinger Ostfriedhof, by the father of musicologist Hubert Kessler (1898-1985). In 1948, Hubert Kessler received the lock in a letter from his uncle, approximating 50 strands of hair, and accompanied by several fragments of textile (Methods S1H). The relics were contained within an envelope with the inscription, ''Aus Beethoven's Sarg *[From Beethoven's coffin]*'' (Methods S1H). Owing to poor DNA preservation, the authenticity of the Kessler Lock could not be determined.

> ''Dearest Hubert!
>
> Having received your pleasant letter from the third of this month, let me inform you that, according to what I remember, my honored father (that is, your grandfather) was present in person at the exhumation. Beethoven's remains were examined by P. Also as the representative of his superior and friend Domböck and brought home the relic (Beethoven's hair), which was always greatly esteemed by us.'' [translation Birgit Lodes]

Neither of Beethoven's exhumation reports from 1863 or 1888 describe hairs as being preserved in Beethoven's grave.[7,134] However, Schubert's entire head of hair was recovered during the 1863 exhumation, supporting the notion that the conditions in the Währinger Ostfriedhof may have been conducive to the survival of hair. Schubert, who died 20 months after Beethoven, was separated from Beethoven by two grave plots, and exhumed simultaneously. The exhumation report notes, however, that the soil in Schubert's grave was 'damp' and the coffin much better preserved, whereas the soil in Beethoven's grave was 'dry' and 'loamy,' presumably responsible for the poorer preservation of organic materials.[134] The 1863 exhumation report goes on to state:

> […] the members of the administration took individual parts of the remnants of clothing and the wood of the coffin of Beethoven as well as of Schubert; parts were given over to the few persons present at this serious act who were visibly moved by strong emotions. Most of these remnants, however, were put aside and for the time being looked over by Dr. v. Breuning[134] [translation William Meredith]

However, the report later states that the remnants of clothing kept by Dr. v. Breuning were placed in a large tin box, soldered shut, and reinterred with Beethoven's remains.[134] It is unclear from the 1863 exhumation report whether all of these recovered materials were reinterred, or if some were still retained by those present.

The individual referred to as 'P' in the letter to Dr. Kessler is likely Dr. Carl von Patruban, who, along with Dr. Standthartner, examined Beethoven's remains as they were being exhumed on October 13th, 1863. Of the 32 people counted as present during Beethoven's first exhumation, only 13 are named in the exhumation report. None by the name of Domböck can be identified, although a 'Dobyhak' is mentioned as being present on October 22nd.[134]

A contemporary newspaper account published on September 1st, 1888 in the *Evening Post* states that, apart from the three anthropologists present during Beethoven's second and final exhumation in 1888, "very few persons witnessed the exhumation, and most of these were officials,'' rendering the recovery of the hairs during the 1888 exhumation less likely.

A single DNA extraction on what appeared to be a bulb was carried out, followed by a second extraction on four hairs, amounting to approximately 17cm. The Kessler Lock and associated textile fragments are currently owned by the University of Illinois Music Department.

Identification of living patrilineal descendants of Aert van Beethoven

The paternal lineage of Ludwig van Beethoven has been the subject of much research and has been reconstructed with reasonable certainty at least as far back as 1535[23] (Methods S1P). In almost all respects, this reconstruction is widely accepted, but two areas worthy of discussion remain. First, the correct identification of Beethoven's putative great-great-grandfather, Kornelius, has been met with some reservations. Of the two men bearing this name and living within the vicinity of Beethoven's known ancestors, genealogists overwhelmingly favor Kornelius van Beethoven, born on October 20th, 1641, at Bertem near Louvain, in present-day Belgium.[115] However, another Kornelius van Beethoven, born in 1630, has been considered, with reservations, as a candidate.[135] Nonetheless, both of these candidates are believed to be closely related, and are not expected to disrupt the Y chromosome pedigree. Second, anomalously, no baptismal record has yet been found for Beethoven's father, Johann van Beethoven.[23,115]

Extra-pair paternity (EPP) is a known danger to the reconstruction of genetic patrilineages, necessitating the adoption of candidate selection strategies designed to minimize the probability of EPP. EPP rates are known to have ranged from 1-2% in Western Europe across the last 400 years, and, historically, have varied from as low as 0.31% to as high as 6% in regions comprising present-day Belgium, depending on rural versus urban setting, as well as socio-economic status.[136] In order to best mitigate the possibility of EPP, these five research participants were selected among numerous candidates to maximize the number of independent lineages, additionally taking into account genealogical documentation of urban vs. rural settlement and the socioeconomic status of each patrilineage. These potential donors were always separated by at least twelve meioses from each other in the direct paternal line.

Five individuals were selected, who met all of the above criteria and represented independent lineages sharing a common ancestor with Aert van Beethoven (1535-1609), Beethoven's great-great-great-great-great grandfather. A genealogy of these five individuals is presented in Methods S1P; living individuals are not named for privacy reasons. All five consented to provide saliva samples for Y chromosome testing. Saliva samples were collected using the Oragene OG-500 kit. Lysing of cells took place immediately after the collection of the saliva. DNA was extracted and purified in accordance with the manufacturer's recommended instructions (prepIT L2P PD-PR-006 https://www.dnagenotek.com/us/pdf/PD-PR-006.pdf). One participant requested that any remaining DNA extract be incinerated following sequencing, which was carried out.

Identification of living descendants of Karl van Beethoven

We identified, from a published genealogy of Ludwig van Beethoven,[23] three living individuals descending from Karl van Beethoven (1806-1858), the son of Beethoven's younger brother Kaspar Anton Karl van Beethoven (1774-1815) and his wife, Johanna van Beethoven (née Reiß; 1786-1869). We received ethical approval to test for IBD-segment sharing between them and the Beethoven genome (Human Biology Research Ethics Committee, University of Cambridge, application HBREC.2020.48, December 18th, 2020; confirmed by the Director of the Research Ethics Commission, Austrian Academy of Sciences, as raising no concerns, September 16th, 2020). All three of these individuals are documented as 7th-degree genetic relatives of Beethoven.

Permits

Ethical approval was granted (Medical Ethical Committee UZ Leuven/KU Leuven, procedure number S61715; Department of Archaeology, University of Cambridge, approval date May 1st, 2019), allowing that up to five relatives from different branches of the Van Beethoven patriline may be approached.

Ethical approval was granted to test for IBD-segment sharing between three genealogically documented descendants of Karl van Beethoven and the Beethoven genome (Human Biology Research Ethics Committee, University of Cambridge, application HBREC.2020.48, December 18th, 2020; confirmed by the Director of the Research Ethics Commission, Austrian Academy of Sciences, as raising no concerns, September 16th, 2020).

METHOD DETAILS

Hair sample decontamination, DNA extractions, initial double-stranded library preparation and indexing amplifications were performed in the dedicated ancient DNA cleanroom facilities in the University of Tübingen's Paleogenetics Department in Tübingen, Germany (sample ID prefixes JK & TU) and the Max Planck Institute for the Science of Human History's Department of Archaeogenetics in Jena, Germany (sample ID prefix HEB) (Data S1A).

Decontamination, extraction and purification

For each hair sample, hair shafts were decontaminated either in four immersions in sterile, UV-irradiated water prior to extraction, or a single 10 s immersion in 0.5% final concentration bleach followed by four immersions in sterile, UV-irradiated water (Data S1A). The hairs were then placed in 1000μl of active extraction buffer comprising an inactive extraction buffer containing 10mM final concentration Tris buffer (pH 8.0), 10mM NaCl, 5mM CaCl, 2.5mM EDTA (pH 8.0), and 2% SDS. To create the active extraction buffer, 40 μl of 1M DTT and 100μl of Proteinase K were mixed with 860 μl of inactive extraction buffer, per extraction.[16] The DNA extract was subsequently incubated overnight at 37°C while rotating at 15 rpm.[15]

After centrifuging the digested hairs into a pellet, the resulting DNA extract was purified into 100μl of TET buffer. Samples processed in Tübingen were purified using a silica-based Qiagen MinElute column affixed to a Hi-Pure Extender Assembly, whereas samples purified in Jena utilized a Hi-Pure Viral DNA silica-column with included extender assembly.[137] The volume and composition of the binding buffer was optimized for the retention of ultra-short DNA fragments and low copy number DNA templates such as those

expected in historical hair samples.[18] Standard Qiagen and Hi-Pure washing buffers[137] were used following manufacturer recommended protocols.

Library preparation, indexing and sequencing

Following purification, either 10μl or 20μl of purified extract from the Cramolini-Brown, Hiller, Kessler, Moscheles, Stumpff, Halm-Thayer and Bermann Lock samples underwent initial double-stranded library preparation and double-indexing protocols compatible with Illumina sequencing technologies, incorporating amplification strategies and polymerases intended to reduce PCR biases or artifacts during indexing amplification of ancient DNA libraries.[17,138,139] Negative controls were incorporated during extraction and library preparation stages in order to gauge the presence of background levels of DNA during lab work prior to double-indexing.[140] Quality control checks included library-based real-time qPCRs with the Roche LightCycler96 following both initial library-preparation and subsequent indexing amplification, as well as the generation of two fragment size profiles to assess molarity prior to sequencing, using an Agilent Bioanalyzer 2100.[141] A mitochondrial DNA capture was additionally performed on a library prepared from the Kessler Lock.[142] Single-stranded libraries using 30μl of purified extract from the Stumpff, Bermann, Halm-Thayer and Müller Locks were additionally prepared at the Max Planck Institute for Evolutionary Anthropology's Department of Paleogenetics.[19] Sequencing was performed on Illumina HiSeq2500, HiSeq4000 and NextSeq500 platforms (Illumina, San Diego, CA), using a variety of sequencing chemistries (Data S1A). Three libraries (TU50.BH6.1U, TU50.BH6.2U, TU50.BH6.3U) from the Cramolini-Brown Lock were prepared using UDG-treatment.[143]

QUANTIFICATION AND STATISTICAL ANALYSIS

Bioinformatics processing of initial low-coverage data

Initial processing of reads for assessments of DNA preservation and authentication were performed using EAGER version 1.92.38,[97] including quality checking of FASTQ files with FastQC, and clipping and merging of paired-end reads with Clip&Merge. Autosomal and sex chromosomal alignments were performed against hg19 using BWA version 0.7.12, with a lower read-length cut-off of 30 base pairs with MAPQ $\geq$ 30 and disabling of seeding with -l 1000 being specified.[82] The mapping algorithm CircularMapper was used to perform mitochondrial alignments, employing BWA while extending both ends of chrMT of hg19 by 500 bases to avoid spurious low coverage calls at either end of the chrMT reference. Duplicate removal, sorting and indexing of BAM files were performed using samtools version 1.9.[83] Library complexity extrapolations were performed using default settings with the preseq tool lc_extrap[96] in order to assess relative sample preservation (Methods S1I) and to estimate the number of additional extractions and libraries required to attain a high coverage genome. Genotyping was performed using GATK version 3.5.[84]

Mitochondrial contamination estimation, haplogroup assignment, and regional haplogroup frequency analysis

Wherever possible, consensus mitochondrial genomes were called from non-UDG treated, double-stranded libraries, and paired-end data preferred over single-end data. Only the Müller Lock consensus genome was called from shallow shotgun data sequenced from single-stranded libraries.

Final endogenous mitochondrial contamination rates were assessed with Schmutzi, using the share/schmutzi/alleleFreqMT/197/freqs/ panel of putative contaminants included in the program.[87] With the exception of the Kessler Lock, which lacked sufficient preservation for contamination estimation to be performed, all samples were found to contain between 0-3% contamination, with an average of 1% contamination (Data S1A).

Endogenous consensus mitochondrial genomes were called using the Schmutzi tool endocaller using default parameters.[87] Endogenous consensus FASTA files were uploaded into Haplogrep2.0 version 2.2,[85] querying Phylotree build 17,[79] in order to assign mitochondrial haplogroups and to detect local private mutations (Table S2). The Kessler Lock was found to lack sufficient coverage for a full endogenous mitochondrial consensus genome to be called, despite undergoing two separate extractions and an mtDNA capture (Data S1A).

Regional frequencies of the H1b1+16,362C mtDNA haplogroup, shared among the Müller, Bermann, Halm-Thayer, Moscheles and Stumpff Locks, were assessed by comparison against FamilyTreeDNA's mtFull database, comprising 203,514 full mitochondrial genomes at the time of analysis. No individuals completely matching this mitochondrial genome were identified. However, 219 individuals within the H1b1+16,362C haplogroup, differing only by the lack of the private mutation, were found. The geographic distribution of these individuals was found to be broadly in Western and Central Europe, as well as countries harboring recent European diasporas (Data S1D). No specific clustering within or among countries was observed.

High-coverage Beethoven autosomal genome sequencing and genotype calling

Nuclear DNA in hair has an extremely low average fragment length owing to the activity of endonucleases expressed during hair formation.[144,145] To make use of the overwhelming fraction of ultra-short reads recovered during single-stranded library preparation (Data S1A and S1B), a lower read-length cut-off of 20bp was introduced after adapter trimming (leehom 1.1.5),[100] mapping (bwa 0.7.12, parameters: -n 0.01 -o 2 -l 16500)[82] and indel realignment (GATK 3.5).[84]

An accessibility mask was created to account for the greater chance of misalignments due to the short read length. For this, reads were first filtered for a mapping quality of at least 25. Reads shorter than 35bp were required to contain a genome-wide unique k-mer while permitting for up to one mismatch MapL procedure in de Filippo et al.[21] Sites were only included which overlapped with the

map35_100 mappability regions developed for the Altai Neanderthal genome, and which did not overlap with tandem repeat regions or indels; see supplementary section 5b in Prüfer et al.[146] Any regions falling within the upper- and lowermost 2.5% of the coverage distribution were excluded, after correcting for local GC content. This accessibility mask reduced the length of the autosomal genome to approximately 1.64Gb, while raising average coverage from 19.68-fold coverage across the full autosomal reference to 24.06-fold within accessible regions (Table S4).

Genotypes were called using snpAD (version 0.3.4), which employs an error model that takes the type of substitution and the location within a given read into account.[20] SnpAD was run with 10 position dependent error matrices on either end of reads. Approximately 7.5 sites per 10,000 were called as heterozygous on the autosomes within accessible regions. The reference allele was supported on average by 57% of reads at heterozygous sites with one reference and one non-reference allele. Autosomal contamination was estimated by testing of the X-chromosome with ANGSD (version 0.910) to be approximately 2.9%.[93]

In order to recover any common variants removed during filtering steps, and to provide genotype phasing information, imputation and phasing were performed using Beagle version 5.1[95] using the full 1000 Genomes Project Phase 3 release as a reference panel,[147] retaining positions with a Genotype Probability (GP) $\geq$ 0.99.

HLA genotyping

We applied a development version of OptiType[78] to sequence data from FASTQ files merged from all available libraries, mapped against OptiType's custom Human leukocyte antigen (HLA) reference panel containing 1025 alleles with "common" or "intermediate" CIWD 3.0 designation (https://github.com/FRED-2/OptiType, tag DER). We obtained the top 3 HLA class I and class II genotypes for the loci HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DPA1 and HLA-DPB1. Haplotypes were assigned based on previously reported frequencies and properties such as linkage disequilibrium.[148,149] Alleles were genotyped and both haplotypes could be unambiguously called. The resulting haplotypes are:

HLA-A*01:01∼B*08:01∼C*07:01∼DQA1*03:03∼DQB1*03:01∼DRB1*04:01∼DRB4*01:03∼DPA1*01:03∼DPB1*04:01

HLA-A*11:01∼B*56:01∼C*01:02∼DQA1*01:01∼DQB1*05:01∼DRB1*01:01∼NULL∼DPA1*01:03∼DPB1*57:01

These haplotypes are consistent (according to frequencies observed in modern European populations) with those haplotypes commonly found in central/western Europe. The second HLA-B allele besides HLA-B*08:01 could not be resolved between HLA-B*54:01, B*55:01 and B*56:01 due to a lack of unique read support in the regions distinguishing them. All reads covering the SNPs distinguishing these three alleles are either co-mapping to HLA-B*08:01 or the subject's HLA-C alleles. In any case, since all three belong to the HLA-B22 serological family, the allele's exact identity bears no additional clinical significance. Considering the frequency of the three alleles and their haplotypes in modern populations, the most plausible allele is HLA-B*56:01.

A shorter version of the first haplotype, HLA-A*01:01∼B*08:01∼C*07:01∼DRB1*04:01 has been previously reported in populations from central and western Europe such as the Netherlands, England and Austria,[150,151] among other populations. A shorter version of the second haplotype, HLA-A*11:01∼B*56:01∼C*01:02∼DRB1*01:01, has been reported for the Canary Islands, Croatia, Poland, France[151–153] and Russia (Khamaganova, unpublished, reported in Allele Frequency DataNet), among other populations.

Sex chromosomal karyotyping

Sex chromosomal karyotyping was performed on shallow sequence data for all eight purported Beethoven hair samples using an R script developed for low coverage shotgun sequence data, which compares the X chromosome read count (Rx) against the average read count across the autosomes.[99]

Relatedness testing of autosomal and X chromosome DNA among locks of hair

In order to explicitly test if the five samples with matching mitochondrial genomes derived from a single individual, we assessed levels of autosomal relatedness among all eight samples using READ[94] (Figure 2; Table S5). We maximized the number of genotype positions included in each pairwise comparison by comparing the high-coverage Stumpff Lock genome against the low coverage Müller, Bermann, Halm-Thayer, Moscheles, Cramolini-Brown, Hiller and Kessler Lock genomes. We selected a publicly available and ancestry-matched dataset of 41 low- to medium-coverage medieval Bavarian genomes as an external reference panel.[80] We used this reference panel to calculate an appropriate average proportion of non-matching alleles (P0) among all independent pairwise comparisons to assess degrees of relatedness.

READ is able to identify up to second-degree relatives with a false-positive rate of 3% using as few as 1,000 shared single nucleotide variants (SNVs) among pairwise comparisons between samples, after filtering for minor allele frequency (MAF) $\geq$ 0.10.[94] We generated pseudo-haploid autosomal genotype calls from the BAM files of all samples, including the medieval Bavarian reference panel, using ANGSD version 0.921.[93] We then filtered genotypes according to an intersection of the accessible genome, and a list of sites with MAF $\geq$ 0.10 among the EUR superpopulation of the 1000 Genomes Project Phase 3 release,[147] retaining 3,060,820 positions. In order to provide an additional axis to graphically represent autosomal relatedness, we repeated the READ analysis exclusively using pseudo-haploid calls from the X-chromosome, again using the intersection of our accessibility mask and sites on the X-chromosome with MAF $\geq$ 0.10.

Damage rate assessment

DNA accumulates characteristic damage patterns over time, whereby cytosines deaminate into uracils, in increasing frequencies towards the 5′ end, which are subsequently misread by polymerases as thymines. This is termed 5′ C-T deamination.[154] We determined

damage rates for all samples using mapDamage2.0, reporting nuclear and mitochondrial damage rates separately (Data S1A). All samples, including non-matching samples, displayed damage patterns characteristic of ancient DNA, and consistent with their documented or presumed antiquity.

Principal components analyses

PCA's were initially performed for the high-coverage Stumpff Lock genome and low-coverage Cramolini-Brown and Hiller Lock genomes using global reference panels from the Phase 3 release of the 1000 Genomes Project[147] for analyses of ancestry on a global scale (Figure S1). A second set of PCA's were performed on the Cramolini-Brown and Hiller Lock low-coverage genomes using using reference panels of 21 and 50 modern and historical West Eurasian populations, respectively, drawn from the Allen dataset (https://reich.hms.harvard.edu/allen-ancient-dna-resource-aadr-downloadable-genotypes-present-day-and-ancient-dna-data) (Methods S1J and S1L). Each genome was merged with its respective reference panel using the mergeit tool from the EIGENSOFT package.[98,155] PCA's were performed using smartpca with default settings.

Higher resolution PCA's for the high-coverage Beethoven genome were performed using individuals from the FamilyTreeDNA customer database. We used the PCA function in PLINK,[86] which uses the implementation from the program GCTA[156] (Figures S2 and S3). Individuals were queried from each European country in the FamilyTreeDNA database, and closely related individuals were removed using the software KING.[157] Up to 150 individuals per country were randomly selected for analysis to keep sample sizes balanced. For the marker set, we took the intersection of the 574,384 SNVs genotyped in Beethoven's genome, and the 132,268 SNVs found on all of the Illumina genotyping arrays used. SNVs were pruned for linkage disequilibrium using the flag –indep-pairphase 100kb 1 0.9, and only SNVs with a genotyping rate of 95% were kept, resulting in a final count of 45,329 SNVs.

ADMIXTURE analyses

We used the full 1000 Genomes Project phase 3 reference panel[147] to assess Beethoven's ancestry composition among a diverse panel of five global populations, containing 26 subpopulations, with ADMIXTURE.[22] Beethoven's genome was filtered to include only accessible regions (see section High-coverage Beethoven autosomal genome sequencing and genotype calling), followed by filtering for DP $\geq$ 14 and QC $\geq$ 30. The reference panel was first filtered by MAF $\geq$ 0.05, and to only include filtered SNV sites from Beethoven's genome. The two datasets were merged and converted into PLINK using –geno 0.01, –mind 0.01 and –hwe 1e-50, resulting in 157,634 converging SNV positions among 2,505 individuals. ADMIXTURE was then run using K = 1 to K = 12 using five-fold cross-validation. We found that the cross-validation errors for K = 5 to K = 12 were roughly comparable (CV error = 0.51997–0.52114). Results are plotted for K = 5 (Figure S5), broadly representing European (EUR), East Asian (EAS), South Asian (SAS), African (ASW & AFR) and Native American (AMR) ancestry, and K = 12 (Figure S4) for a more detailed breakdown of ancestries within these global populations. All filtering and merging steps were performed using bcftools version 1.10.2[81] and PLINK version 1.90.[86]

Geo-genetic triangulation with IBD-segments

We estimated the spatial distribution of Beethoven's likely ancestors using a novel method we call Geo-Genetic Triangulation (GGT) (Figure 4; Methods S1L–S1O). GGT ensures that only locations with a high likelihood of being ancestral to Beethoven are selected. Broadly, there are three steps, which we detail below: (1) segment matches between the subject and other genetic testers are identified; (2) genetic triangulation between three or more individuals identifies segments inherited identical-by-descent (IBD) from a recent common ancestor; (3) each triangulated segment is screened for coinciding ancestor locations which increases the confidence of recent shared ancestry. When an IBD-segment is inherited by two individuals, only one of many ancestral lines is typically shared between the two. The GGT method substantially reduces the noise of irrelevant ancestor locations.

First, we generated a match list between the subject and FamilyTreeDNA database. We considered matches to be those with the longest segment $\geq$ 6cM, and we removed any "micro-segments" < 2cM. We repeated this procedure pairwise between each match and the other matches.

Next, segments shared between the subject and the subject's matches were triangulated by looking for groups of matches where two or more members shared a segment with the subject and also an overlapping segment between each other. If numerous matches had segments overlapping the same genomic range, we applied a threshold for the maximum number of checked permutations of triangulation group member candidates to reduce the execution time. We found 720 permutations to be a good compromise between execution time and the number of identified triangulating segments. If segments were clustered into separate match groups but had nearly identical start and end points (within 0.5 cM) and shared members, we de-duplicated them into the same match group. Any segments with a total overlap between group members of < 2cM were removed.

Lastly, we queried available genealogies for all matches sharing triangulated segments with the subject and removed any dubious relationships (older than 10 generations). We used the Google Geocoding API (https://developers.google.com/maps/documentation/geocoding) to convert free text locations into coordinates. Locations that were simply country names were removed, and for any provincial locations we randomly chose a point within that province. We derived administrative boundaries using the Level 1 polygons from the Database of Global Administrative Areas (GADM; https://gadm.org/), and sampled points using the spsample function of the sp package in R.[92] For each triangulated segment group in the previous step, we identified ancestor locations shared by more than one group member. These coincident locations are the most likely ancestral origins of the IBD segment shared by the subject and his matches. In order to determine whether ancestor coordinates were proximate enough to be considered coincident, we grouped points into equally sized spatial units. We created a hexagonal sampling grid using the spsample function with cell-size of

200 km, and the HexPoints2SpatialPolygons function. We limited the scope of our study area to Western and Central Europe (Austria, Belgium, Czech Republic, Denmark, France, Germany, Hungary, Luxembourg, Netherlands, Poland, Switzerland, and Slovakia) based upon the broad ancestry results from PCA and ADMIXTURE.[22] Results of the GGT analysis were plotted using the ggplot2 package.

Six control cases were run through the same GGT pipeline to validate the method (Methods S1M–S1O). Each control case was chosen based upon four or five generations (16 or 32 ancestors) of known ancestry from within 80 km of the same location. Given the expectation of Beethoven's origins in western Germany, we drew two control cases from each of three locations surrounding western Germany: northern Netherlands, southwestern Germany, and eastern Germany into the Czech Republic. We used the same number of matches (ca. 10,000) as for the main subject to maintain comparable sample sizes between each analysis.

Y chromosome analyses

Thirty-fold coverage whole-genome sequencing of living descendents of Aert van Beethoven via 100-cycle paired-end sequencing was performed by BGI in Hong Kong using the DNBseq sequencing platform. An average of 1.11 billion reads were generated per sample following BGI's data filtering steps, with over 97% passing BGI's in-house Q20 filtering (Data S1C). GC content was estimated by BGI at an average of 41% for each sample. De-multiplexing was performed by BGI, and raw FASTQ files were delivered via physical hard-drive, whereupon they were uploaded onto the secure MPI-SHH-DAG computing cluster. FASTQ files were renamed for compatibility with the EAGER (version 1.92.56)[97] pipeline for downstream processing, which was used for adapter removal, mapping, and duplicate removal. Mapping was performed using BWA version 0.7.12[82] only against the hg19 Y-chromosome, with MAPQ $\geq$ 30 and seeding of l = 32 enabled. Files were subsequently converted from SAM to BAM, sorted and indexed using samtools.[83] Duplicate reads were removed using DeDup version 0.12.1. Following duplicate removal, reads were again sorted and indexed using samtools.

For Y chromosome comparisons of both historical hair samples and living relatives against the FamilyTreeDNA database, we realigned reads to hg38 using BWA-ALN, and used mapDamage2.0 to downscale the base quality of C>T and G>A transitions for historical samples.[28,82] We required a base quality and read mapping quality (MAPQ) $\geq$ 30 during variant calling. Variants that were shared with modern NGS results on the same branch were not taken into consideration as private variants, as their placement was already known. Variants that were found to be highly recurrent in the FamilyTreeDNA customer database were given less weight. We checked for each remaining private variant in a custom-built database compiled from thousands of published ancient DNA results. Variants observed within this ancient genome dataset from different haplogroups and not seen in any modern results were given less weight. We further gave less weight to variants in regions known to be problematic in ancient DNA, including the centromere, Yq12 heterochromatic region and the DYZ19 repeat.

Time to most recent common ancestor estimates

FamilyTreeDNA built the Y chromosome phylogeny based on all available Big Y results from customers using a combination of automated shared variant detection and manual curation. All available SNVs from the non-recombining Y (NRY) that passed FamilyTreeDNA's variant filters were considered.

Analyses were restricted to SNV mutations within FTBED which covers approximately 11.25 Mbp of NRY. Private and shared SNVs within these regions were automatically determined and validated from the Big Y test results from present-day individuals (private variants in the LvB result were not considered). Reoccurring SNVs that were found to have occurred more than five times across the entire haplotype tree were automatically excluded from the analysis. Adjacent SNVs with the same phylogenetic placement located within 150 bp of each other were classified as multi-nucleotide polymorphisms (MNPs) or incorrect alignments and were also excluded from the analysis. FTBED SNV coverage was recursively calculated for each branch using the intersection of coverage for any two immediate child branches, which themselves were calculated as the union of all their downstream child branches.[158] The resulting intersect coverage was used to adjust the number of SNVs associated with each branch, to account for varying coverage in the NGS data.

We used a modified version of the PATHd8 algorithm[101] to convert the mean path length of each clade into a divergence time. For calibration points of the major backbone clades in the Y-DNA haplotype tree, we first used BEAST 2.5.2.[102,159] We created an alignment with 91 Big Y sequences spanning the major clades of the tree, using the GTR+Γ model, strict clock, and non-parametric Coalescent Bayesian Skyline model for the tree prior. We ran the model in two MCMC chains of 5×10^7 steps sampled every 10^3 steps, checked for convergence, and discarded 20% as burn-in. These calibration points were used to adjust downstream age estimates based on SNV counts in the PATHd8 algorithm. We converted SNV counts into time using the equation $T = S / (\mu \times C)$, where S is the SNV count, μ is the Y-DNA mutation rate estimated by Poznik et al.,[160] and C is the intersect coverage of the downstream samples. For all branches less than 2,000 years old, we also averaged the SNV-based time estimates with Short Tandem Repeat (STR) based estimates to reduce stochastic variation caused by either marker set. STR pairwise genetic distances were ordinated against SNV-based time estimates, and modeled as a general additive model (GAM) with log link function in the mgcv package[161] of R 3.5.1.[92] The mean path lengths were modeled as gamma distributions to incorporate uncertainty both in interval between mutations, and mutation rate.

Y-STR imputation

The Y chromosomal SNVs of Beethoven were analyzed to determine his placement on the Y-DNA tree. His two closest Big Y-tested paternal relatives, FT5 and FT6, in haplogroup I-FT244582 were identified. Their 111 Y-STR marker results were compared to the

modal values of the outgroup (I-FT126507) in order to reconstruct the most-likely STR haplotype of Beethoven. Markers were classified in different categories.

A) Match between FT5 and FT6 but different from the modal value of the outgroup
B) Match between FT5, FT6 and the outgroup modal value
C) Difference between FT5 and FT6, and FT5 matched the outgroup modal value
D) Difference between FT5 and FT6, and FT6 matched the outgroup modal value
E) Other (no clear pattern)

For category A the FT5/FT6 allele was used and given extra weight. For category B markers the shared allele was used. For category C and D the common allele was used and the uncommon allele was assumed to be the result of a private mutation in FT5 or FT6. Category E markers were not used.

100 Y-STR marker alleles were estimated in this way and compared to FamilyTreeDNA's STR matching database with over 500,000 Y-DNA haplotypes ranging from 12 to 111 marker resolution. The closest matches to the reconstructed haplotype were in turn correlated with their closest Y-STR matches in the general database and their Y-SNV test results were used to narrow down the list to the best candidates for belonging to the I-FT244582 haplogroup.

The final candidates were contacted and invited to participate in the study and their consent was sought to upgrade their Y-DNA results to Big Y-700. Eight customers, FT1-FT4 and FT7-FT9 were upgraded and four of them, FT1-FT4, were found to belong to I-FT244582 and formed a new subclade together with FT5; I-FT396000. FT7-FT9 were found to belong to outgroups of the target clade.

Analyses of living descendants of Karl van Beethoven

Beethoven's genome was initially filtered according to the accessibility mask, after which it was filtered for depth $\geq$ 10-fold and genotype quality $\geq$ 30 and converted to PLINK.[86] Genotype data for the living descendants generated using the HumanOmniExpress-24 chip were downloaded from FamilyTreeDNA, converted into PLINK, and merged with Beethoven. We filtered the merged PLINK file by –geno 0.01 resulting in 373,023 shared SNVs with zero missingness, after which the plink file was annotated with a recombination map.

We ran the phase-unaware IBD-detection algorithm IBIS[31] on the merged PLINK file using the default detection thresholds of $\geq$ 7cM, $\geq$ 436 SNVs, and tolerating a homozygous SNV mismatch rate of 0.004.

In order to simulate the expected IBD-sharing and the probabilities of detecting zero IBD-sharing with Beethoven both per individual and in combination, we used pedSIM[32] to generate 100,000 pedigree structure simulations on a reconstructed pedigree, including individual sex information and using a sex-specific recombination map.[162] We converted pedSIM .seg output files to .coef files with the seg2coef tool from IBIS[31] using a genome length of 3400 cM. We then calculated total expected IBD-sharing and probabilities for zero IBD-segment sharing $\geq$ 7 cM per individual and among all three individuals collectively.

Polygenic risk scoring

We generated polygenic scores for several complex diseases hypothesized by Beethoven's medical biographers (Data S1H; Methods S1R–S1X). We used publicly available GWAS summary statistics to assess polygenic risk for Crohn's disease (CD; Methods S1R), ulcerative colitis (UC; Methods S1S),[37] irritable bowel syndrome (IBS; Methods S1T),[49] systemic lupus erythematosus (SLE; Methods S1U),[38] primary biliary cirrhosis (PBC; Methods S1V),[46] primary sclerosing cholangitis (PSC; Methods S1W),[47] and cirrhosis (Methods S1X).[43] For parameter optimization during polygenic risk scoring for complex diseases, we considered case-control data from the UK Biobank using respective ICD-10 codes for each condition.[163]

Beethoven's genome was filtered within accessible regions, with only genotype calls with a genotype quality $\geq$ 30 being included, after which it was merged with the full UK Biobank genotyped dataset. Individuals and positions within the merged UK Biobank-Beethoven dataset then underwent quality filtering recommended for polygenic risk scoring.[164] Genotype calls were filtered by minor allele frequency ($\geq$ 0.05), genotyping rate ($\geq$ 99%), and Hardy-Weinberg Equilibrium ($p \geq$ 1E-06). Individuals were filtered for heterozygosity and relatedness using quality control criteria provided by the UK Biobank, as well as ancestry, with non-Europeans being excluded based on a list provided by the UK BioBank. Individuals were further filtered by imposing an individual missingness rate $\leq$ 0.01. The tool FlashPCA2[91] was used to calculate principle components from an LD-pruned version (window-size 200bp, step-size 50 variants, LD $R^2 \leq 0.25$) of the merged and quality filtered UK Biobank-Beethoven file, with the first 50 principal components being incorporated as ancestry covariates during polygenic scoring, in addition to sex. In total, the merged and quality filtered UK Biobank-Beethoven dataset converged on 195,504 positions in 385,136 individuals. LD pruning was subsequently performed for each polygenic score generated using default settings in PRSice 2.0.[89] All filtering steps were performed in PLINK version 1.9.[86]

GWAS summary statistics files underwent similar stringent quality filtering, including the removal of duplicate and ambiguous (C-G, G-C, A-T, T-A) variants, where strand-flipping may not be detectable. Where such information was reported, sites were filtered by MAF $\geq$ 0.05 and imputation score INFO $\geq$ 0.9.

15,000 population matched controls were drawn from the above mentioned quality filtered UK BioBank dataset for polygenic scoring for all diseases, from which cases were subsequently excluded on a per-disease basis. Individuals who reported their country of birth as "Germany" (Data Field 20115) and an ethnic background of "white" or "any other white background" (Data Field 21000)

were selected as an independent German cohort (n = 1,153), not included in regression analyses, to assess potential bias in polygenic scoring arising from population stratification. Polygenic risk scoring was performed using the clumping-and-thresholding tool PRSice-2, version 2.3.3.[89] Results were reported for the p-value threshold with the highest Nagelkerke R^2 value. For each PRS, we report the partial R^2 attributable to PRS with respect to the full model including sex and the first 50 principal component values, and after adjustment for disease prevalence.

For cirrhosis, we were able to retrieve only 12 genome-wide significant SNVs from publicly available association summary statistics.[43] We therefore performed polygenic risk scoring using imputed genotypes from the UK BioBank, and imputed genotypes from the Beethoven genome, after disabling both clumping-and-thresholding and the removal of ambiguous variants. Imputed genotype data from the UK BioBank was filtered for INFO $\geq$ 0.9 and converted to PLINK using QCTOOL v2.

Variant Effect Predictor

To potentially identify rare and high-effect variants we considered variants with a read depth above two reads and with more than one sequence read of the alternative allele. Additionally, insertions or deletions (indels) were called in genes that could cause phenotypes relevant to Beethoven (Data S1F) by the UnifiedGenotyper in GATK, version 3.5.0.[84] We then annotated and filtered the variants with VEP version 96 in combination with external databases (gnomAD version 2.1.1, dbNSFP version 4.1, spliceAI and ClinVar 2021-10-02).[90] After annotation we excluded common variants (allele frequency of > 2% in subcohorts of the population-based databases gnomAD, Exome Variant Server (EVS), and 1000Genomes; or > 4 reported homozygous occurrences in gnomAD). Variants that were present in ClinVar but were not rated as being benign or likely benign were kept, even if the frequency in the general population exceeded the thresholds noted above. We then conducted two analyses. First, we analyzed variants in genes that could cause relevant phenotypes (for a list of the phenotypes/genes, see Data S1F). Second, we extended the analysis to variants in genes that are linked to phenotypes according to OMIM (2021-07-11). For the first analysis we filtered for variants with 1) a high impact according to VEP, with 2) a moderate impact, or with 3) a SpliceAI-score above 0.2 in any category. For the second analysis we used stricter filter criteria: We required variants with 2) a moderate effect to have a Combined Annotation-Dependent Depletion (CADD) score above 25 or 3) to have a spliceAI score above 0.5 in any category. Additionally, we required the variants of the second analysis to have a QUAL score above 30 and to have at least 3 sequence reads of the alternative allele. The sequencing reads in the region containing the potentially relevant variants were first manually inspected with the Integrative Genomics Viewer (IGV).[88] Variants without convincing evidence for validity, such as probable false calls likely to have arisen due to DNA damage, mapping and deduplication failures, were discarded. Remaining variants were further assessed according to the variant interpretation criteria of the American College of Medical Genetics and Genomics (ACMG) guidelines.

Analysis of coverage

The 1.64Gb of Beethoven's genome retained within our accessibility regions contained 78.2% of the coding-sequence of protein coding genes, in which we identified a total of 16,692 variants after quality filtering of QUAL $\geq$ 30.

Coverage in regions that might contain causative variants for monogenic diseases was analyzed. Using Ensembl Biomart (GRCh37, version 104), transcripts with the longest protein-coding sequence in protein-coding genes were first selected. The coding sequences of these genes were then used for analysis. For non-protein coding genes, the range from gene start to gene end was chosen according to Ensembl Biomart. First, the overlap of the genomic regions obtained with the accessibility filters was determined. Then, for the overlapping regions, coverage in the aligned reads was determined using the Mosdepth tool.[165]

For groups of genes that were analyzed in a prioritized manner, results of the analysis can be found in Table S7 and in Figure S6. The values of individual genes that were prioritized can be found in Data S1F.

Retrospective cohort studies

We extracted encoded anonymized participant ID's (EIDs) for UK Biobank[163] males matching Beethoven's genotypes at rs1799945 and rs1800562 in HFE, and rs738409 and rs2294918 in PNPLA3 from genotyped and imputed UK Biobank participant genetic data using qctool v2 and PLINK version 1.9.[86] We then queried the UK Biobank ICD-10 Main and Secondary databases for ICD-10 codes matching Beethoven's genetic, infectious and lifestyle risk factors both singly and, where sufficient sample sizes permitted, in combinations of risk factors. Odds-Ratios, 95% confidence intervals and p-values were calculated for each retrospective cohort study using Fisher's Exact Test for Count Data in R[92] (Data S1K–S1M). Heavy drinking (HD) males were included using ICD-10 Main and Secondary codes of F10.1 'Harmful use' and F10.2 'Alcohol dependence.' We must caution, however, that in a recent analysis, Beethoven was not found to meet the DSM-IV criteria for 'Alcohol abuse,' analogous to ICD-10 F10.1 'Harmful use', and only tentatively was argued to meet the minimum necessary criteria for 'Alcohol dependence'.[8] Thus our heavy drinking cohort is chosen to illustrate disease prevalences in a hypothetical scenario in which Beethoven may have met criteria for one or both of these diagnoses.

Screening, capture, sequencing and analysis of hepatitis B virus DNA

Shotgun sequencing data generated from the Stumpff (HEB001), Bermann (HEB002), Halm-Thayer (HEB003) and Müller (HEB004) Locks were screened for traces of hepatitis B virus (HBV) DNA using MALT v. 0.3.8,[57] as previously described.[76,166] Reads assigned to HBV were inspected using MEGAN v. 6.13.1[103] and blasted against the NCBI-NT database to further assess the specificity of the match.

Some of the libraries showed putative traces of HBV DNA based on the screening of shotgun sequencing data (Data S1I). In order to confirm this initial result, we then performed HBV-DNA enrichment of all libraries using in-solution capture, as previously described.[76,166] Enriched libraries were pooled equimolarly (10 mM final concentration), and prepared for shotgun sequencing on an Illumina Miseq platform (Illumina, San Diego, CA) with 2x75 paired-end cycles. Additionally, all corresponding DNA extraction and library preparation blanks were sequenced on a separate Miseq run after HBV-DNA enrichment to provide negative controls.

Sequencing reads were demultiplexed based on sequenced P7 and P5 indices (allowing for one mismatch per index). Adapter trimming and paired-read merging were then performed using AdapterRemoval 2.3.0.[167] Only merged reads were retained, and fragments smaller than 30 bp were excluded. The following steps were then conducted for each library separately as well as for the whole data combined. Reads were mapped against the HBV reference genome (GenBank: NC_003977; genotype D) using the EAGER pipeline[97] with the CircularMapper option and a mismatch parameter (-n) of 0.01, a quality filtering (-q) of 30 and an elongation factor of 500. Mapped reads were deduplicated with Markduplicates (part of the Picard tools; http://broadinstitute.github.io/picard/) and realigned around indels using the GATK toolkit.[168] Damage assessment was performed using DamageProfiler.[104] We then filtered HBV-mapping reads based on their copy number in order to limit potential inter-sample contamination due to index-jumping.[169] As previously described,[76,170] reads were kept only if their copy number was significantly higher than those carrying non-expected index combinations (assumed to have arisen from index-jumping), as assessed by the modified Thompson-test for outlier detection. BAM files were then used to produce consensus HBV sequences using a 1-fold coverage threshold and a 50% majority rule.

Most of these reads were highly duplicated (cluster factor: 60), suggesting that sequenced libraries were largely exhausted. After deduplication and filtering of low-copy-number reads, 92 unique reads remained, resulting in a mean HBV genome coverage of 1.26-fold. All positive libraries were prepared from the Stumpff lock, with the exception of one library prepared from the Bermann lock (HEB002.B0102). However, no reads remained from this library after low-copy-number filtering, suggesting that they had arisen from inter-sample contamination due to index-jumping (Data S1I). Reads appeared well distributed along the HBV genome sequence, with higher coverage in the double-stranded region[171] of the genome (Figure 6A).

The reconstructed sequence was aligned with a set of modern HBV genomes representative of the currently described diversity of the virus using MAFFT v7.475[105] with the iterative refinement method for global alignments. The alignment was inspected and corrected around large indels when necessary. We then used Gblocks[106] to remove highly divergent and potentially misaligned regions, allowing for a maximum of 50% of gaps and using default parameters otherwise. The resulting alignment was used to construct a phylogenetic tree with RAxML v. 8.2.12,[58] using the GTRCAT substitution model and the rapid bootstrap algorithm with the autoMRE bootstopping criterion (Figure 7). In order to assess the robustness of the phylogenetic placement with respect to reference bias, we repeated the analysis after mapping the reads on a HBV reference genome belonging to genotype F (i.e. the most dissimilar to genotype D; GenBank: FJ657525).

If the levels of HBV viremia observed in the Stumpff Lock hairs are assumed to have been constant over the entire interval during which hairs from the Müller, Bermann, Halm-Thayer and Stumpff Locks were forming, we would have expected to have sequenced small numbers of HBV DNA from the Müller, Bermann and Halm-Thayer Locks, respectively. However, the assumption of stable viremia is violated by known random fluctuations in HBV viremia,[77] which can span between two to three orders of magnitude. Owing to the limited sensitivity of our analyses, differences in extraction and sequencing efforts and numbers of libraries prepared from our samples, differences in preservation between samples, differences in library preparation methods (a majority of libraries from the Stumpff Lock were singled-stranded, whereas a larger proportion of libraries from the earlier locks were double-stranded) as well as unpredictable HBV viremia, the absence of HBV reads recovered from the Müller, Bermann and Halm-Thayer Locks from earlier in Beethoven's life cannot be taken to conclude that Beethoven was definitely HBV-negative during the intervals in which hairs from those locks were forming. Although an acute infection occurring shortly before Beethoven's death cannot be ruled out, a chronic infection appears statistically much more likely (~3.5% of the world population lives with an HBV chronic infection today, and this proportion can reach up to ~20% in non-vaccinated areas).[71,172]

Although the provenance histories of several of the locks of hair document their acquisition on specific dates, the hair shafts tested correspond to periods of growth that would have preceded their acquisition by a period of months. Precise estimates of the length of Beethoven's hair at the time of cutting, and the corresponding interval of growth which tested hair shafts represent, cannot be determined. However, a tentative estimate of the latest probable period of Beethoven's life during which he was definitely HBV-positive can be deduced by comparing the minimum probable length of his hair as depicted in sketches contemporaneous to the cutting of the Stumpff Lock against the recorded average length of tested HBV-positive hairs and known scalp hair growth rates in European males.

The Stumpff Lock hairs lack any bulbs and represent the distal ends of Beethoven's hair. Judging from the length of Beethoven's hair as depicted in two of Josef Eduard Teltscher's three deathbed sketches of Beethoven on or before March 26th, 1827 (which, unlike the sketches by Josef Danhauser on March 28th, were made prior to Beethoven's autopsy and craniotomy), Beethoven's hair appears to have been at least 4" (101.6 mm) in length at the time of his death. Using an average European male scalp hair growth rate of 0.368 ± 0.058mm per day,[173] and judging from the average length of the sampled Stumpff Lock hairs of approximately 49mm, the latest plausible interval of growth that the sampled HBV-positive hairs represent would have been between approximately June to November of 1826 at the latest, though likely earlier. Taken in view of the incubation period for an acute infection that likely would have been necessary for HBV DNA levels to have risen to detectable levels, Beethoven's three paracenteses, performed on December 20th, 1826, January 8th, and February 3rd, 1827,[7] are thus unlikely routes for HBV infection.

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Supplemental Information

Genomic analyses of hair from Ludwig van Beethoven

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Figure S1. Scatter plot of first two principal components projecting Beethoven onto a global reference panel, related to Results: Authentication of hair samples and STAR Methods: Principal components analyses: Principal components analysis of Beethoven's high-coverage genome, projected onto principal components derived from a globally representative sample of 2,530 modern genomes. Broader geographic classifications (e.g. European, South Asian) are drawn from the 1000 Genomes Project[S1] nomenclature and are represented by large colored opaque circles, within which are ethnicity-specific symbols. See also Figures S2 and S3.

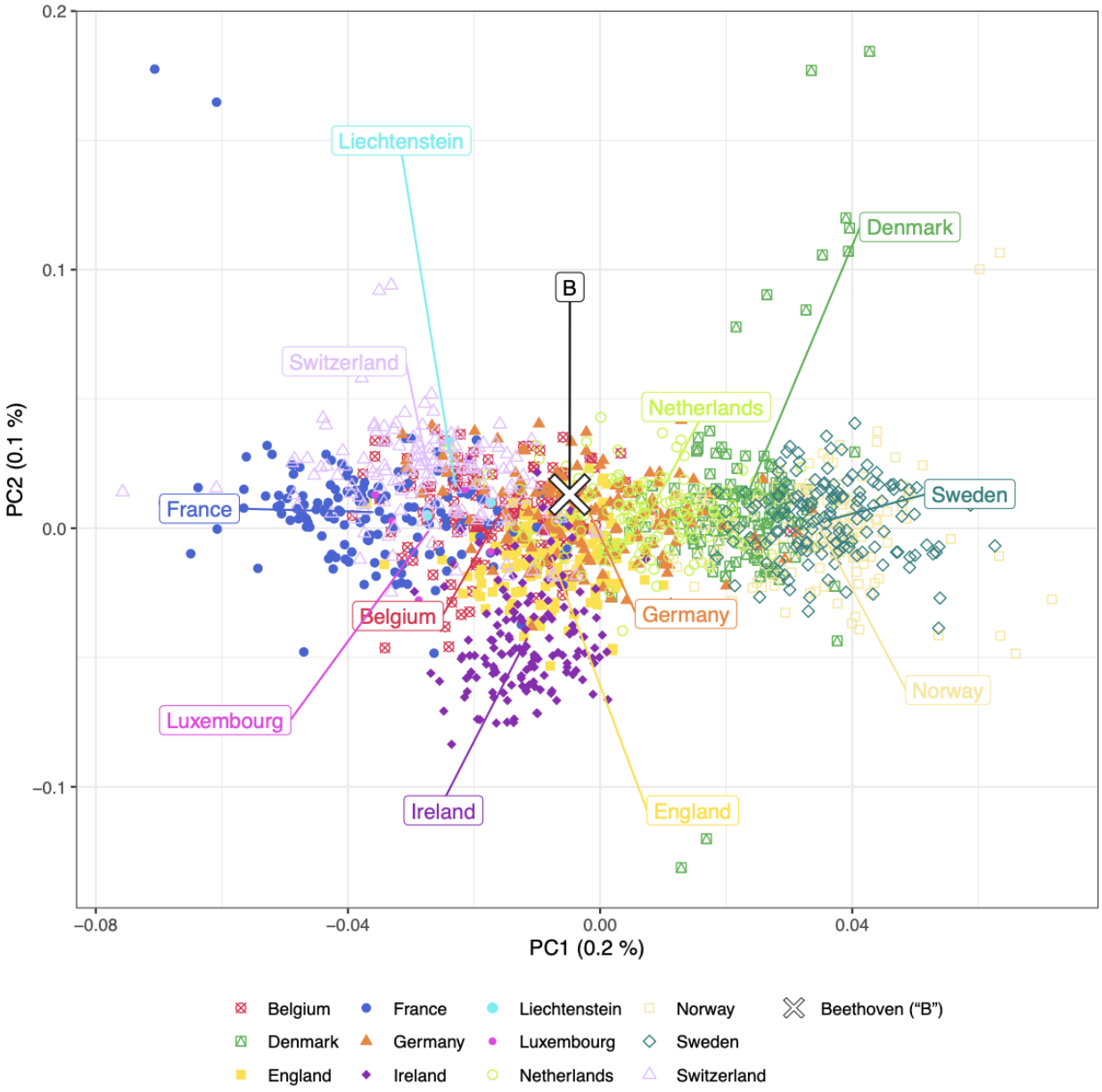

Figure S2. Scatter plot of first two principal components projecting Beethoven onto a Western, Central and Northern European reference panel, related to Results: Authentication of hair samples and STAR Methods: Principal components analyses: Principal components analysis of Beethoven's high-coverage genome (B), projected onto principal components derived from cohorts of up to 150 FamilyTreeDNA customers each representing a country within Western, Central and Northern Europe. See also Figure S1 and S3.

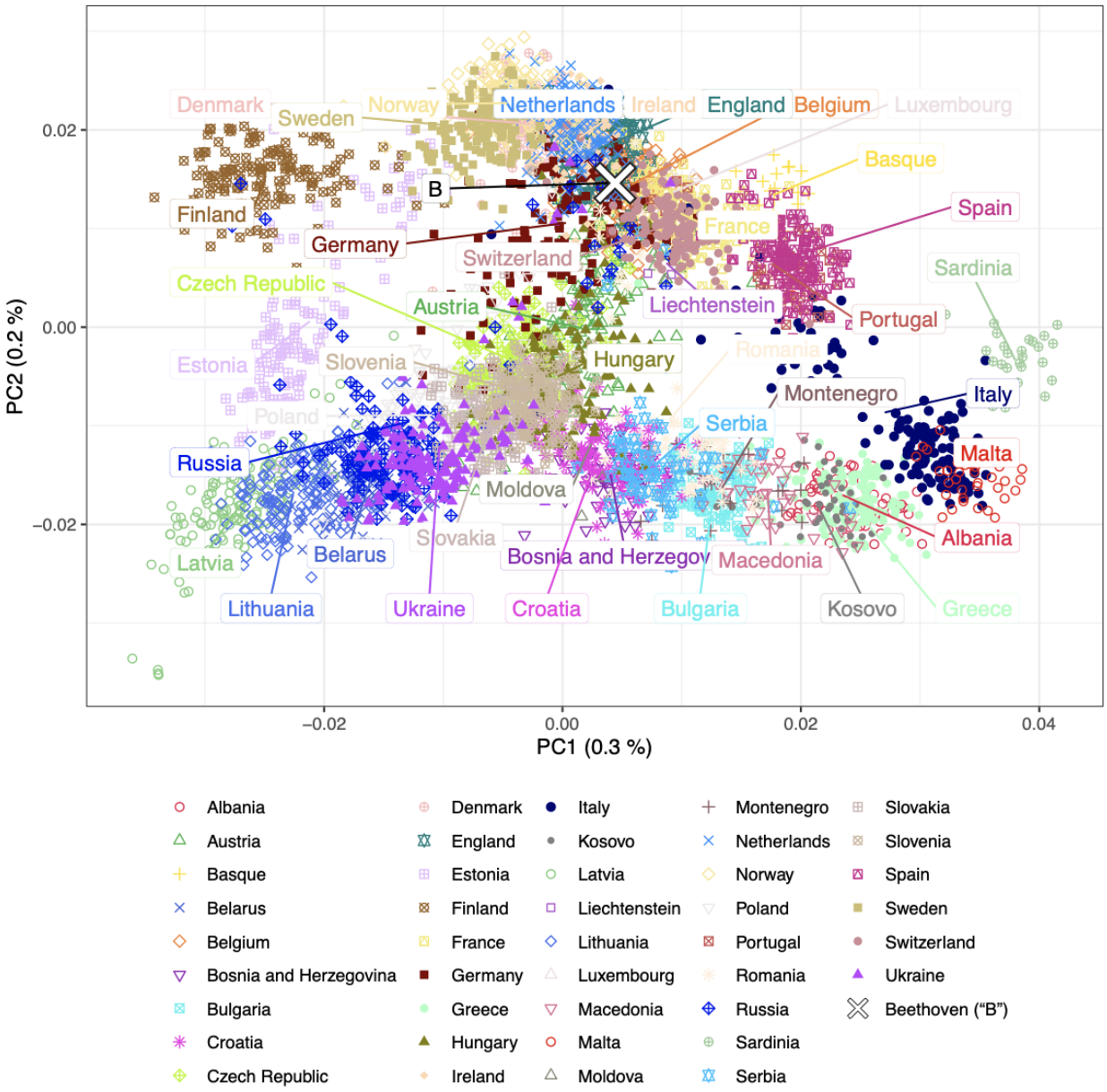

Figure S3. Scatter plot of first two principal components projecting Beethoven onto a European reference panel, related to Results: Authentication of hair samples and STAR Methods: Principal components analyses: Principal components analysis of Beethoven’s high-coverage genome (B), projected onto principal components derived from cohorts of up to 150 FamilyTreeDNA customers each representing a country within Europe. See also Figures S1 and S2.

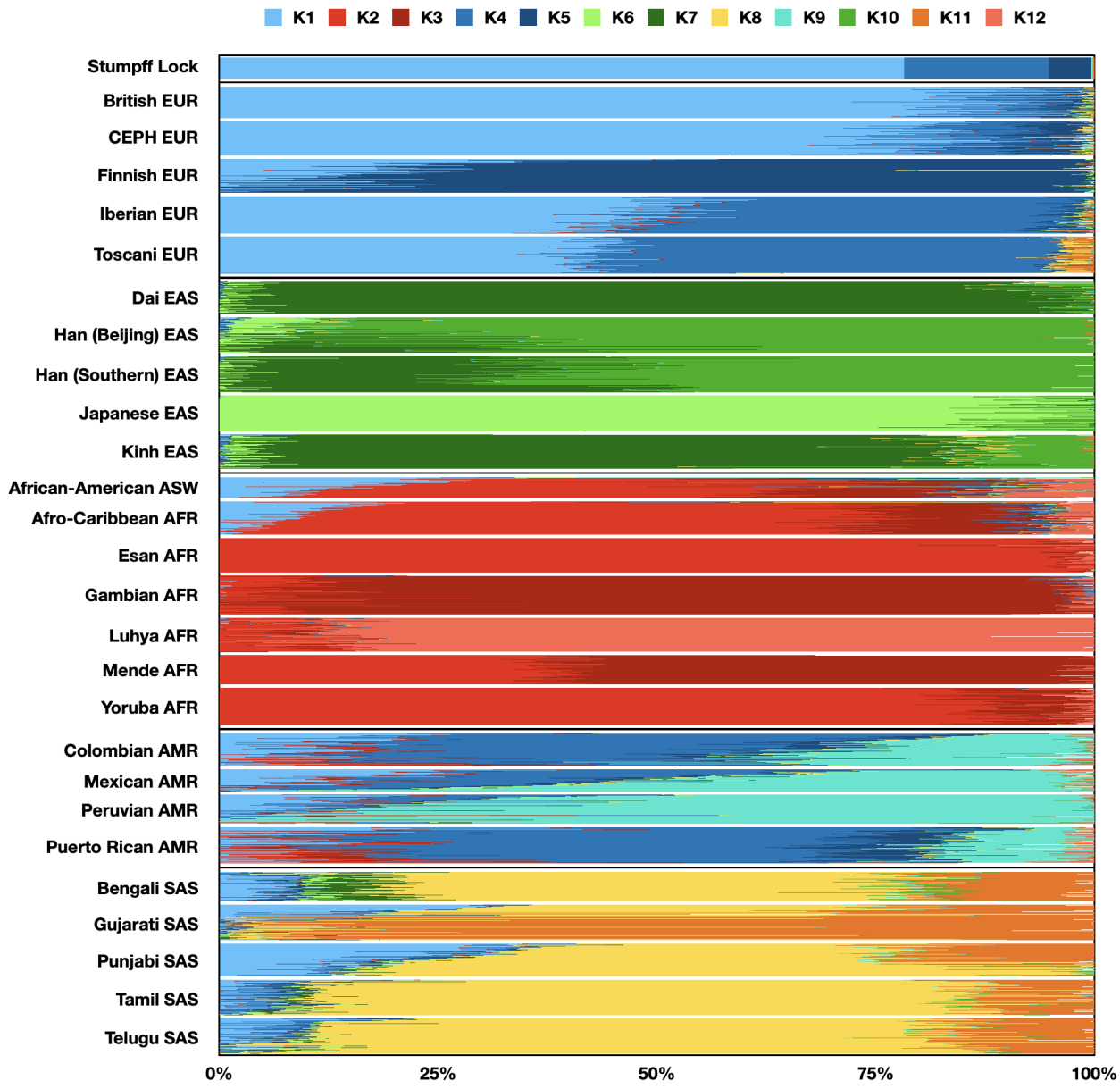

Figure S4. ADMIXTURE analysis of Beethoven using a global reference panel at K=12, related to Results: Authentication of hair samples and STAR Methods: ADMIXTURE analyses: ADMIXTURE[S2] results for a global panel of 2,505 individuals[S1] with K=12. EUR = European; EAS = East Asian; ASW = African Southwest; AFR = African; AMR = American; SAS = South Asian. See also Figure S5.

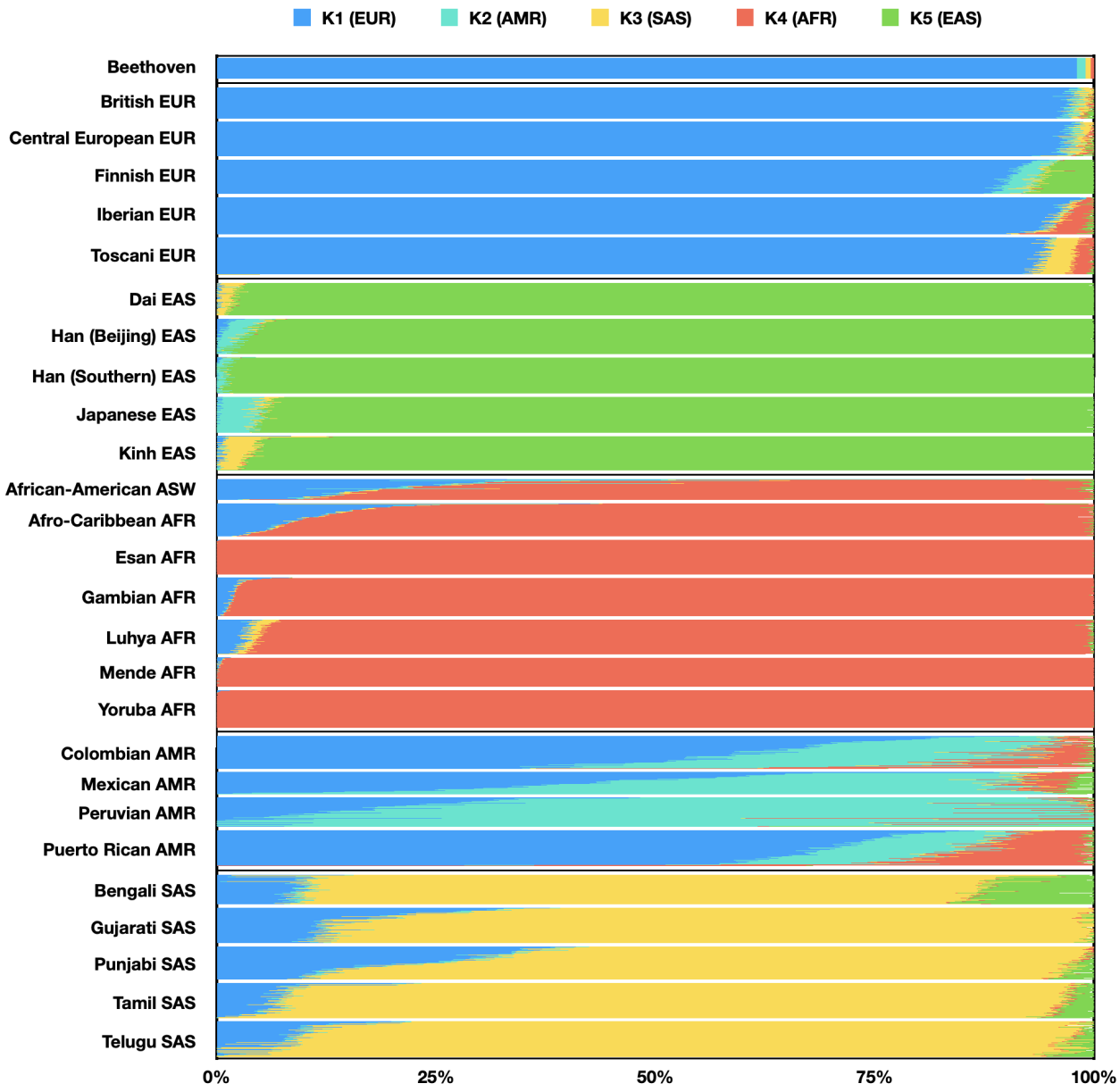

Figure S5. ADMIXTURE analysis of Beethoven using a global reference panel at K=5. Related to Results: Authentication of hair samples and STAR Methods: ADMIXTURE analyses: ADMIXTURE results for a global panel of 2,505 individuals with K=5. EUR = European; EAS = East Asian; ASW = African Southwest; AFR = African; AMR = American; SAS = South Asian. See also Figure S4.

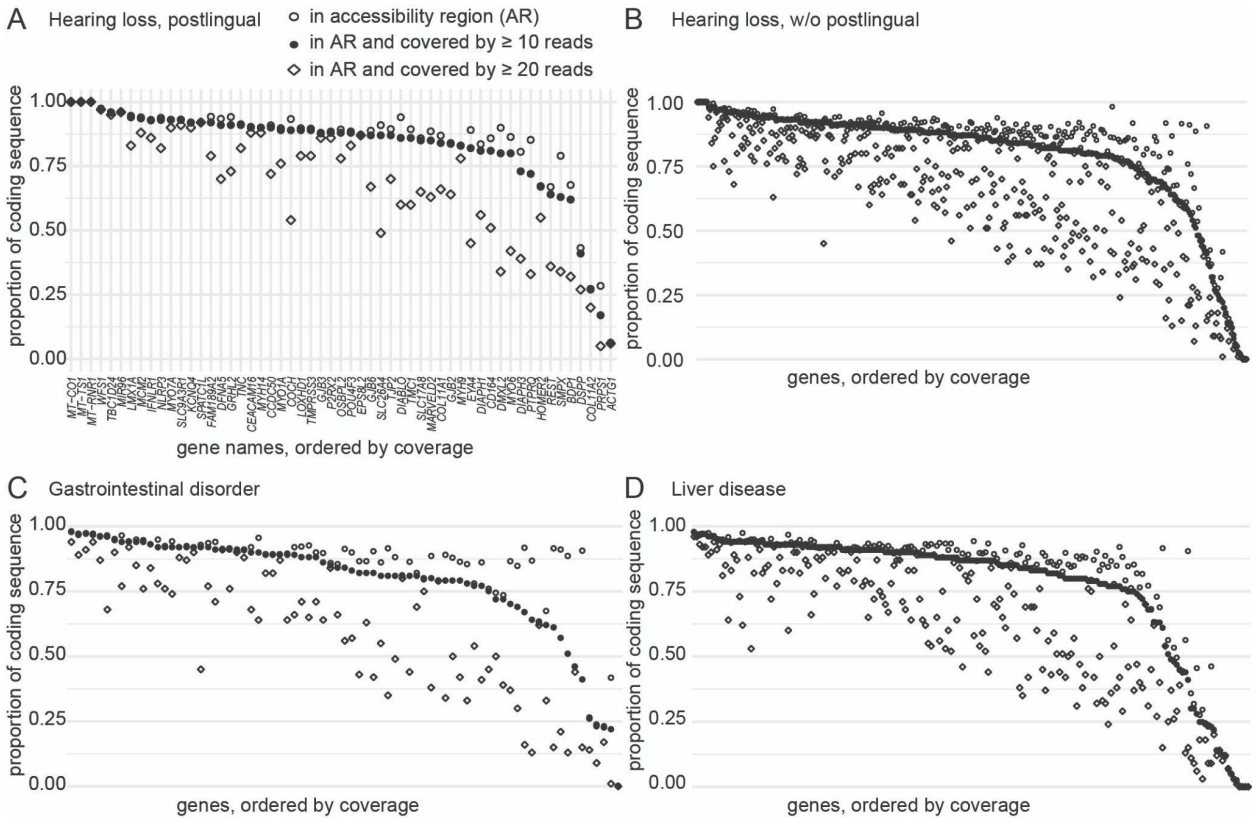

Figure S6. Coverage of genes that were analyzed for rare coding variants, related to Results: Genetic variants associated with somatic disease, Discussion: Origins of Beethoven's diseases and STAR Methods: Variant Effect Predictor and Analysis of Coverage: (A-D) Each panel displays the coverage in one gene set: (A) post-lingual hearing loss, (B) hearing loss but not specifically post-lingual hearing loss, (C) gastrointestinal disease or (D) liver disease. The y-axis corresponds to the proportion of the coding sequence of each gene that met criteria as encoded by the shapes of the markers. Each position on the x-axis corresponds to an individual gene. In panel A individual gene symbols are given, due to space limitations gene symbols were removed from panel B-D. Genes are ordered according to their proportion of coding sequence that is both in accessibility regions and covered by >= 10 reads with values decreasing from left to right. Note that for each gene the ensembl transcript with the longest coding sequence was used. Coding sequence refers to the protein coding sequence of protein coding genes or to the tRNA encoding sequence of mitochondrial genes. See also Data S1F, S1G and Table S7.

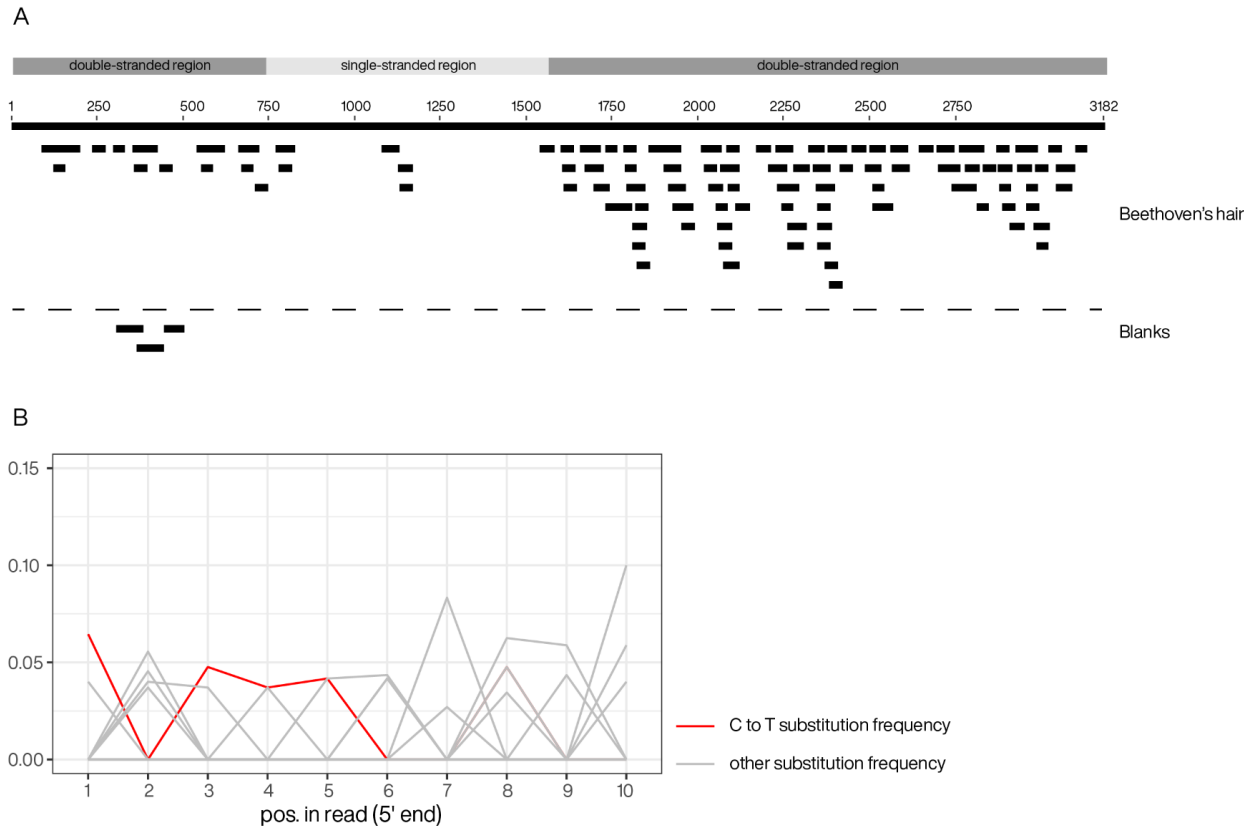

Figure S7. Hepatitis B virus DNA mapping and damage profile, related to Results: Hepatitis B virus DNA recovered from Beethoven's hair, Discussion: Hepatitis B virus DNA in Beethoven's hair and STAR Methods: Screening, capture, sequencing and analysis of hepatitis B virus DNA: (A) Position of reads mapping to the HBV reference sequence and (B) HBV DNA damage profile, after combining data obtained from all libraries. See also Figure 7 and Data S1I and S1J.

Lock Name	Owner and Item ID (2021)	Provenance Date (m/d/y)	Authenticity
Baroni-Cavalcabo	N/A	N/A	N/A
Bermann	Kevin Brown*	1815-1821	Authentic
Bernard	BHBa; Obj 225	3.1827	N/A
British Library	BL	N/A	N/A
Cramolini-Bodmer	BHBo; HCB V 10	3.27.1827	N/A
Cramolini-Brown	Kevin Brown*	3.27.1827	Inauthentic
Cramolini-Schechner	N/A	3.27.1827	N/A
Danhauser 1	N/A	3.27-28.1827	N/A
Danhauser 2	N/A	3.27-28.1827	N/A
Erdödy	Erdödy Estate?	N/A	N/A
Gräffer	BHBo; R 1	3.29.1827	N/A
Halm	N/A	4.25.1826	N/A
Halm-Epstein	N/A	4.25.1826	N/A
Halm-Thayer	Kevin Brown*	4.25.1826	Authentic
Hartmann 1	N/A	3.28.1827	N/A
Hartmann 2	N/A	3.28.1827	N/A
Hase	LC	3.29.1827	N/A
Heiligenstadt	BMV	N/A	N/A
Hiller	BC* & Alfredo Guevara*	3.27.1827	Inauthentic
Holz	BHBo; HCB V 11	N/A	N/A
Hummel 1	BC*	3.23.1827	N/A
Hummel 2	BHBo; R 1 e	3.23.1827	N/A
Hüttenbrenner-Seydler	N/A	3.26.1827	N/A
Hüttenbrenner-Teltscher	BHBo; R 39	3.26.1827	N/A
Kessler	ISU*	10.1863	Indeterminate
Moscheles	BC*	3.24.1827	Authentic
Schlesinger	BHBo; HCB V 6	1825	N/A
Schelsinger-Carrino	BB	1825	N/A
Schumann	BHBo; HCB Br 115	1819?	N/A
Simrock	BHBo; HCB BBi 11/28	1816?	N/A
Theile	NYPL; b21706307	1827?	N/A
Müller	BHBo; HCB V 12	11.4.1820	Authentic
Stumpff	Kevin Brown*	3.26-28.1827	Authentic
Becker	Simon Hayes	11.1803	N/A

Table S1. Catalog of known and/or identified locks of Beethoven's hair, related to STAR Methods: Beethoven hair samples: List of known locks of hair including present owners and original provenance date. GMV: Gesellschaft für Musikfreunde, Vienna; BHBo: Beethovenhaus Bonn (HCB = Hans-Conrad Bodmer Collection; WG = Wegeler Collection); BHBa: Beethovenhaus Baden; BC: Ira F. Brilliant Center for Beethoven Studies; ISU:Illinois State University; LC: Library of Congress; BL: British Library; BMV: Beethoven Museum, Vienna; NYPL: New York Public Library; BB: Biblioteca Beethoveniana, Collezione Carrino, Muggia. *Member or affiliate of American Beethoven Society. See also Methods S1A to S1H.

Sample ID	Haplogroup	Quality	Not Found Polymorphisms	Found Polymorphisms	Private Mutations
Müller	H1b1+16362	96.91%	NA	263G 750G 1438G 3010A 3796G 4769G 8860G 15326G 16189C 16356C 16362C	16176T
Bermann	H1b1+16362	96.91%	NA	263G 750G 1438G 3010A 3796G 4769G 8860G 15326G 16189C 16356C 16362C	16176T
Halm-Thayer	H1b1+16362	96.91%	NA	263G 750G 1438G 3010A 3796G 4769G 8860G 15326G 16189C 16356C 16362C	16176T
Moscheles	H1b1+16362	96.91%	NA	263G 750G 1438G 3010A 3796G 4769G 8860G 15326G 16189C 16356C 16362C	16176T
Stumpff	H1b1+16362	96.91%	NA	263G 750G 1438G 3010A 3796G 4769G 8860G 15326G 16189C 16356C 16362C	16176T
Cramolini-Brown	H79	90.60%	NA	263G 750G 1438G 4769G 8860G 12397G 15326G	11215C
Hiller	K1a1b1a	98.41%	16093C	73G 114T 263G 497T 750G 1189C 1438G 1811G 2706G 3480G 4769G 7028T 8860G 9055A 9698C 10398G 10550G 10978G 11299C 11467G 11470G 11719A 11914A 12308G 12372A 12954C 14167T 14766T 14798C 15326G 15924G 16224C 16234T 16311C	5585A

Table S2. Mitochondrial haplogroup determinations, related to Results: Authentication of hair samples and STAR Methods: Mitochondrial contamination estimation, haplogroup assignment, and regional haplogroup frequency analysis: Haplogroup determinations for seven locks of hair attributed to Ludwig van Beethoven using Haplogrep2.0[S4]. See also Table S3.

Maternal Country of Origin	Match Count (GD 1)	mtFull Database Count	Frequency
Reunion	1	5	20.0000%
British Virgin Islands	1	6	16.6667%
Czech Republic	3	741	0.4049%
Australia	1	270	0.3704%
Norway	9	2,919	0.3083%
Austria	2	710	0.2817%
Northern Ireland	1	360	0.2778%
United States	28	11,489	0.2437%
United Kingdom	8	3,304	0.2421%
England	21	10,168	0.2065%
Netherlands	2	1,033	0.1936%
France	5	3,386	0.1477%
Wales	1	730	0.1370%
Ukraine	2	1,465	0.1365%
Canada	2	1,566	0.1277%
Hungary	1	836	0.1196%
Unknown Origin	115	104,331	0.1102%
Scotland	4	3,729	0.1073%
Ireland	8	7,838	0.1021%
Switzerland	1	1,007	0.0993%
Germany	8	8,809	0.0908%
Finland	5	5,692	0.0878%
United States (Native American)	1	2,285	0.0438%
Italy	1	2,427	0.0412%
Sweden	1	7,272	0.0138%
Other	0	21,136	0.0000%
Total	232	203,514	0.1140%

Table S3. Mitochondrial haplogroup regional frequencies, related to Results: Authentication of hair samples and STAR Methods: Mitochondrial contamination estimation, haplogroup assignment, and regional haplogroup frequency analysis: Regional frequencies of the mitochondrial haplotype in haplogroup H1b1+16,362C (n=232) that is ancestral to Beethoven's whole mitochondrial genome sequence. Note frequencies are reported from FTDNA's mtFull database (n=203,514). Beethoven's mitochondrial sequence differs from the reported haplotype by a genetic distance (GD) of 1, owing to a single private mutation at position 16,176T. See also Table S2.

	Length		Coverage	
Chromosome	**Pre-Filter**	**Post-Filter**	**Pre-Filter**	**Post-Filter**
1	249,250,621	136,536,174	19.77	25.14
2	243,199,373	150,520,806	20.04	22.94
3	198,022,430	124,293,132	19.29	21.78
4	191,154,276	117,112,689	17.93	20.13
5	180,915,260	111,178,454	19.14	21.85
6	171,115,067	103,576,174	18.72	21.49
7	159,138,663	90,688,410	19.92	23.56
8	146,364,022	90,494,129	19.95	22.73
9	141,213,431	68,640,086	17.60	25.05
10	135,534,747	80,093,152	20.96	24.95
11	135,006,516	81,044,903	21.47	25.55
12	133,851,895	79,617,869	20.15	23.28
13	115,169,878	61,588,661	15.67	20.63
14	107,349,540	54,345,089	17.26	23.80
15	102,531,392	47,670,581	17.18	25.23
16	90,354,753	44,568,607	22.01	30.22
17	81,195,210	43,324,434	25.12	31.98
18	78,077,248	48,816,717	19.33	22.19
19	59,128,983	25,972,743	28.66	41.08
20	63,025,520	38,318,153	24.10	28.89
21	48,129,895	21,003,328	15.85	24.76
22	51,304,566	19,597,804	20.06	36.95
X	155,270,560	79,084,913	9.64	10.91
Y	59,373,566	8,819,704*	2.97	8.74*
Autosomal	**2,787,053,225**	**1,639,002,095**	**19.68**	**24.06**
Total	**3,095,677,412**	**1,718,087,008**	**18.60**	**23.38**

Table S4. Coverage per nuclear chromosome, related to STAR Methods: High-coverage Beethoven autosomal genome sequencing and genotype calling: Coverage per nuclear chromosome determined using Qualimap 2[S5], total coverage for all autosomes, and all nuclear chromosomes (including sex chromosomes) before and after the application of quality filters. *Indicates a separate filter for the Y-chromosome[S6]. See also Data S1B.

Pairwise Comparison	Normalized2 Allele Difference	Standard Error	Non-Normalized P0	Non-Normalized Standard Error	Pairwise SNP Comparisons	Relatedness
Bermann : Stumpff	0.5974331095	0.003448925846	0.2092014706	0.001207700657	1,104,505	Monozygotic Twin/Same Individual
Halm-Thayer : Stumpff	0.5893274501	0.003533103657	0.2063631347	0.001237176964	923,662	Monozygotic Twin/Same Individual
Moscheles : Stumpff	0.5935977527	0.01216839699	0.2078584545	0.00426097333	16,266	Monozygotic Twin/Same Individual
Müller : Stumpff	0.5660191628	0.005700411404	0.1982013373	0.001996097018	66,855	Monozygotic Twin/Same Individual
Bermann : Halm	0.5805703312	0.00371317633	0.203296679	0.001300232505	407,971	Monozygotic Twin/Same Individual
Bermann : Moscheles	0.5558925249	0.01616349753	0.194655321	0.005659926441	8,392	Monozygotic Twin/Same Individual
Bermann: Müller	0.5670086767	0.008554057522	0.1985478326	0.002995350249	30,775	Monozygotic Twin/Same Individual
Halm-Thayer : Moscheles	0.5378421603	0.01674197628	0.1883346756	0.005862490715	7,401	Monozygotic Twin/Same Individual
Halm-Thayer : Müller	0.5640162987	0.009761510283	0.1975	0.003418160584	26,829	Monozygotic Twin/Same Individual
Moscheles : Müller	0.5345848128	0.0470196457	0.1871940594	0.01646473701	648	Monozygotic Twin/Same Individual
Stumpff : Cramolini-Brown	1.008821146	0.00388941494	0.3532560617	0.001361945484	2,443,423	Unrelated
Stumpff : Hiller	1.010085786	0.004068774897	0.3536988971	0.001424751455	973,538	Unrelated
Stumpff : Kessler	0.9922709028	0.02089831803	0.3474607095	0.007317905211	6,082	Unrelated
Cramolini-Brown : Hiller	0.9911736819	0.004082349805	0.3470764987	0.001429504942	838,494	Unrelated
Cramolini-Brown : Kessler	0.9692967047	0.02262025817	0.3394158992	0.007920872143	4,992	Unrelated
Hiller : Kessler	0.980606256	0.03236352576	0.3433761329	0.01133264474	2,175	Unrelated

Table S5. Summary statistics for relatedness testing among eight locks of hair attributed to Beethoven, related to Results: Authentication of hair samples and STAR Methods: Relatedness testing of autosomal and X-chromosomal DNA among locks of hair: Summary statistics from autosomal relatedness testing using READ[S7] for pseudo-haploid genotype calls for all eight locks of hair attributed to Ludwig van Beethoven. See also Figure 2.

Study Sample	Origin	Haplogroup	Private SNVs	Age SNVs	Intersect FTBED Coverage (bp)	LvB intersect Haplogroup	TMRCA with LvB
LvB	b. 1770	I-FT396000	~5	N/A	N/A	I-FT396000	N/A
FT1	ca. 1600 DE	I-FT396000	9	5	9,488,344	I-FT396000	1003 CE (602-1307 CE)
FT2	ca. 1820 PA, US	I-FT396000	10	7	9,885,013	I-FT396000	1003 CE (602-1307 CE)
FT3	ca. 1870 DE	I-FT396000	18	13	9,799,709	I-FT396000	1003 CE (602-1307 CE)
FT4	ca. 1940 DE	I-FT396000	18	13	9,808,477	I-FT396000	1003 CE (602-1307 CE)
FT5	ca. 1680 SK	I-FT415000	10	8	9,385,239	I-FT396000	1003 CE (602-1307 CE)
FT6	ca. 1730 PL	I-FT244582	15	15	9,875,384	I-FT244582	833 CE (405-1163 CE)
FT7	*ca. 1755 VA, US*	I-Y7043	40	30	9,614,561	I-Z2541	1905 BCE (2547-1352 BCE)
FT8	*US*	I-FT126507	40	28	9,637,559	I-BY52426	1711 BCE (2390-1134 BCE)
FT9	*ZA*	I-PH2666	9	4	7,368,378	I-BY52426	1711 BCE (2390-1134 BCE)

Table S6. Summary information for FamilyTreeDNA customers sharing patrilineal ancestry with Beethoven, related to STAR Methods: Time to most recent common ancestor estimates: Information on nine FamilyTreeDNA customers (FT1-9) sharing patrilineal ancestry with Beethoven within the last 2,000 years. Age SNVs are those from regions considered for TMRCA estimates. See also Methods S1Q and Data S1E.

Category	Average proportion in accessibility filters	Average proportion in accessibility filters and covered > 10X	Average proportion in accessibility filters and covered > 20X
Post-lingual hearing loss genes	0.85	0.82	0.67
Other genes relevant to hearing loss	0.82	0.80	0.64
Genes relevant to the gastrointestinal disease	0.84	0.79	0.59
Genes relevant to the liver disease	0.78	0.76	0.59

Table S7. Coverage information for groups of genes that have been prioritized, related to Genetic variants associated with somatic disease, Discussion: Origins of Beethoven's diseases and STAR Methods: Variant Effect Predictor and Analysis of coverage: Coverage information for groups of genes that have been prioritized for querying of potentially deleterious variants. See also Figure S5, Data S1F and S1G.

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