Molecules and Cells

Journal Club

Beethoven, Infected with Hepatitis B, Inspired the "Beethoven Virus."

Sequencing a high-coverage genome recovered from Ludwig van Beethoven's locks of hair reveals his high genetic risk of liver disease, which may be aggravated by hepatitis B infection and alcohol consumption.

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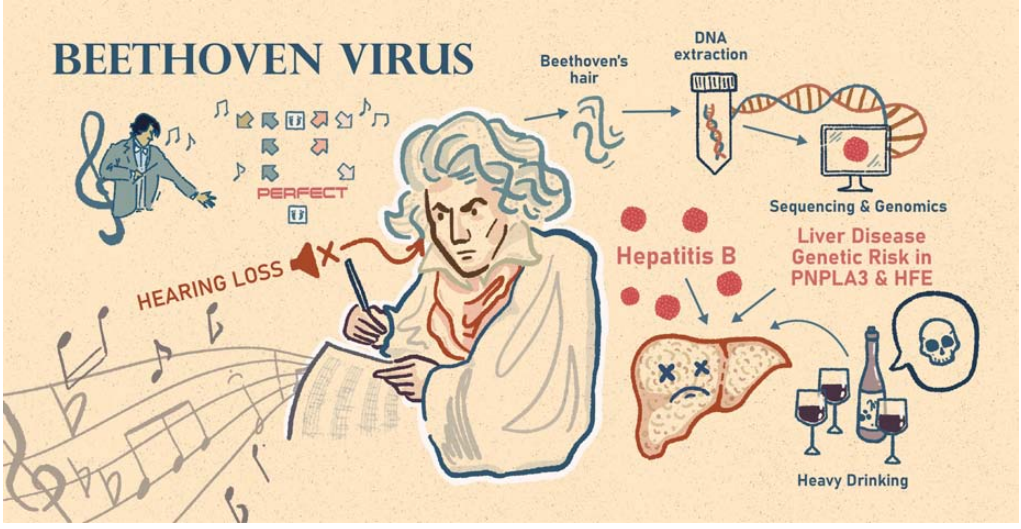

Modern genomics has revealed that Beethoven was at risk for liver disease. Contracted with the hepatitis B virus, having high-risk alleles in *PNPLA3* and *HFE*, and heavily drinking, Ludwig van Beethoven suffered from liver disease, which probably largely contributed to his demise. Beethoven's music greatly inspires human cultures, as exemplified by the K-drama and the arcade game music "Beethoven Virus." Together with other dramatic outcomes, the story built up by modern genomic analyses of DNA samples isolated from his locks of hair has made Beethoven's music even more viral than ever.

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Ludwig van Beethoven is one of the most admired composers. His works, including Fate Symphony No. 5, the piano sonata Pathetique, and Für Elise, are among the most performed repertoires of classical music. Additionally, legacy and music also provide insights and motifs for "Beethoven Virus", the modern K-drama series (Oh, 2008) and the world-famous arcade game music (BanYa, 2000), which are widely watched and played worldwide. He suffered from hearing loss since his 20s (Bauer, 2003; Cooper, 2009; Mai, 2007). Eventually, he developed functional deafness during his prime time as a composer. However, this did not prevent him from composing numerous masterpieces, including Choral Symphony No. 9. The legendary composer's health steadily declined over time, accompanied by gastrointestinal problems and liver disease symptoms. Upon his death at the age of 56, some friends and visitors came to his deathbed and collected a lock of his hair. The autopsy revealed that he had a severely damaged liver and swollen nerves in his auditory system (Mai, 2006). A modern forensic analysis of one of the hair locks indicated that lead poisoning may have contributed to his ailing health (Martin, 2001). It has also been hypothesized that his severe alcohol consumption contributed to his medical condition. In a letter, Beethoven requested an explanation of why and how he was "hopelessly afflicted" (Anderson, 1961). More than two centuries later, scientists responded by elucidating Beethoven's genetic and pathological status through means he never could have imagined. DNA extracted from his hair was analyzed genetically (Begg et al., 2023).

Begg et al. (2023) collected hair strands from eight locks of hair attributed to Beethoven and performed shallow shotgun sequencing to assess DNA preservation and authenticity. Five samples were found to have identical mitochondrial genomes with a private mutation, XY male karyotypes, and terminal C–T deamination resulting from DNA degradation over time, consistent with their provenances in the early 19th Century. Therefore, they concluded that these five independent hair strands belong to Beethoven and sequenced the nuclear genome of the best-preserved sample to an average of 24-fold coverage using laboratory and bioinformatics protocols optimized for ultrashort DNA fragments with an average fragment length of 29.62 bp, typical of historical hair samples. They analyzed an additional 1.64 Gb of the genome to which short reads could be confidently mapped. Principal component analyses (PCAs) performed on the high coverage Beethoven genome placed it among Europeans, clustering with modern Germans. The geographic clustering of ancestors of 665 FamilyTreeDNA customers who share a long autosomal IBD (identical by decent) segment with Beethoven is strongly correlated with regions near the Rhine River in Germany, which are also consistent with the birthplaces of Beethoven's German ancestors. One of the tested locks of hair had different mitochondrial haplotype and Y chromosome from the other five matched locks, indicating that it was not authentic. Another did not preserve sufficient DNA to be identified. Surprisingly, the highly publicized Hiller lock is not Beethoven's but originated from a woman who had a mitochondrial haplotype frequently detected in Ashkenazi Jews (Kreier, 2023). Previous toxicological analyzes of Hiller and a couple of other locks have been used to argue that Beethoven suffered from lead poisoning because all three locks shared patterns of longitudinally distributed lead isotope concentrations (Reiter and Prohaska, 2021). The inauthenticity of the Hiller lock does not support the notion that the other two are authentic. These results inarguably indicate that the five locks of hair are authentic Beethoven's, and all previously reported test results using Hiller locks should be revisited.

The comparison of the Y chromosome brings even more surprising results. They compared Beethoven's high-coverage Y chromosome sequences with those from five living men belonging to the van Beethoven patrilineage. They found that these five men share nearly identical Y chromosomes. However, those Y chromosome sequences do not match those from any of the tested locks. The analysis indicates at least one extra-pair paternity event on Beethoven's direct paternal line, between the conception of his fifth great-grandfather, Hendrik van Beethoven, and that of the composer Beethoven. The simulation analyses, including genomic information from Beethoven's nephew's descendants, did not conclusively determine whether Beethoven and his brother were full siblings.

Further, Beethoven's polygenic risk of famous hearing loss was assessed. Summary statistics from the genome-wide association study (GWAS) for disease risk stratification through polygenic risk score (PRS) could not be obtained for otosclerosis, Paget's disease of bone, sarcoidosis, and some of the previously proposed conditions. Although his polygenic risk for Crohn's disease or ulcerative colitis was not elevated, only the PRS for systemic lupus erythematosus (SLE) notably was, placing Beethoven within the 93rd polygenic risk percentile (Jeong et al., 2023). They also evaluated monogenic variants that cause hearing loss but could not identify any disease-causing gene variants. Therefore, the investigation did not generate conclusive information regarding multifactorial or monogenic causes of Beethoven's hearing loss.

The effects of alcohol and viral infection are highly attributable to liver disease, which can also occur in other multifactorial diseases, such as SLE, and in several monogenic etiologies. The PRS analysis placed Beethoven within the 96th percentile of risk for liver disease. Additionally, Beethoven was homozygous for the variant implicated as the most strongly associated locus of liver cirrhosis in GWASs at rs738409 in *PNPLA3*, encoding patatin-like phospholipase domain-containing protein 3, an enzyme that hydrolyzes triacylglycerol. He also lacked rs2294918, an allele that reduces risk among carriers of rs738409. Monogenic analyses revealed that the ill-fated composer was a compound heterozygote for two variants of the human homeostatic iron regulator (*HFE*) gene that were causal to hereditary hemochromatosis (HH), resulting in excess dietary iron absorption. This condition can develop liver cirrhosis. They analyzed cohorts of UK BioBank men, including heavy drinkers, who matched Beethoven's genotypes at PNPLA3 and HFE. The prevalence of all-cause liver disease or liver cirrhosis among heavy-drinking males homozygous for Beethoven's *PNPLA3* diplotype or with compound heterozygosity in *HFE* was approximately 50% higher than that among all heavy drinkers. These results indicate that Beethoven was at high risk of liver disease.

Beethoven was probably lactose-tolerant, heterozygous for two lactose persistence alleles near the lactase gene (*LCT*).

However, Beethoven's polygenic scores for Crohn's disease and ulcerative colitis placed him in the 36th and 61st percentile, respectively. His PRS for irritable bowel syndrome placed him within the ninth percentile. He also lacked variants for celiac disease or cystic fibrosis. Therefore, we await an answer to the cause of Beethoven's gastrointestinal conditions.

All sequence data from validated locks of hair were screened using the metagenomic screening pipeline MALT (MEGAN ALignment Tool) (Herbig et al., 2016). They detected genomic sequences of the hepatitis B virus (HBV) in DNA libraries prepared from the Stumpff Lock, which was cut near Beethoven's death. For the initial analysis, only four reads were mapped to the HBV genome. After sequencing, deduplication, and filtering low-copy-number reads of libraries enriched by hybridization capture for HBV DNA, 92 matching reads were recovered and well distributed along the HBV genome. The sequence reads reconstruct 63% of the genome sequence, indicative of DNA fragments originating from a target HBV, unlikely from contamination. Phylogenetic analysis indicates that the Beethoven HBV genome was placed among subgenotype D2, one of Europe's most prevalent variants currently and has been present there since at least the Middle Ages. However, the results do not determine when or how Beethoven contracted HBV.

Modern genomics has been widely used to rewrite history by revealing secrets from archaic humans and the Inka ice maiden to endangered animals (Hyun et al., 2022; Liu et al., 2021; Wilson et al., 2007). Extensive genomic analyses have revealed that Beethoven was born with a substantial genetic risk for liver disease and was infected with HBV. Beethoven's severe liver disease may no longer be so puzzling when one considers his apparent heavy-drinking habit. However, the dataset still permits numerous additional inquiries, such as what caused Beethoven's deafness and his historical relatives. Hopefully, additional samples of Beethoven's hair will be analyzed for infectious agents, intrinsic biomarkers, and external environmental factors that contribute to diseases. More sophisticated methods and technologies are being further developed in bioinformatics, and medical genetic research is continuously progressing (Heo et al., 2021; Jung et al., 2020; Lee et al., 2021; Park and Jung, 2022). More people deposit their genetic databases to make them available to the public. Further testing of Beethoven's living and deceased relatives will yield clearer ideas. Science provides novel primary information clarifying who Ludwig van Beethoven is and the significance of his life, aiding in the propagation of Beethoven's forever viral music.

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AUTHOR CONTRIBUTIONS

S.K.L. and J.A. conceived commentary concepts, wrote the manuscript, and secured funding. S.H.K. provided expertise and feedback.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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