

Researchers publish DNA identification of czar's children

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Cutting edge science has finally put to rest a 90-year-old mystery that involved nobility, revolution, murder and the long-romanticized story of a child's escape from the firing squad. Genomic analysis performed at the University of Massachusetts Medical School in cooperation with Institutions of Russian Academy of Science (VIGG) and Academy of Medical Sciences (MHRC) have confirmed that human remains found in the Ural Mountains in July 2007 are indeed those of the two "missing" children of Nicholas II, the last czar of Russia, whose family was murdered in 1918 during the Bolshevik Revolution.

The final evidence was presented in a paper published this week in the online Early Edition of the *Proceedings of the National Academy of Sciences*, "Genomic identification in historical case of Nicholas II Royal family" by Evgeny I. Rogaev, PhD, professor of psychiatry at UMass Medical School's Brudnick Neuropsychiatric Research Institute and professor of genetics at the Russian Academic Institutions, and his colleagues.

Rogaev was asked by the Russian officials to examine DNA from the newly discovered bone fragments; comparing remains from the first grave found in the 1990s to those found more recently, he and his colleagues completed basic studies of mitochondrial DNA inherited through the maternal lines and linked the remains genetically to Empress Alexandra, wife of the Czar, indicating that the remains were very likely those of the czar's children. Rogaev and his UMMS colleagues, including Anastasia Grigorenko and Yuri Moliaka, were able to determine the

complete mitochondrial genome sequences from the remains. After doing so, they determined the gender of the bone fragments and recovered profiles of nuclear DNA, including that inherited exclusively through the paternal lines. The data conclusively demonstrated that the remains found belonged to children of the Romanovs, 13-year-old Crown Prince Alexei and one of his older sisters. This study, along with prior work, concluded that all five children—four daughters and one son along with their parents, last Russian Czar Nicholas II and his wife Empress Alexandra have been located and identified and thus none of the family members survived the 1918 murders.

"Children were murdered along with their parents, the Czar and Empress, and their attendants, in a basement of Ipatyev House in Yekaterinaburg in 1918. Their bodies were transported to a forest and were badly damaged in an attempt to eliminate evidence of this crime," said Rogaev, who used as little as possible of the bone fragments in order to preserve the remains for proper burial. The murderers apparently attempted to destroy two of bodies and thus, they were not found in the first grave. "The newly found bone fragments were fragile and difficult to work with, but we were able to extract enough uncontaminated genetic material to conduct the tests. Thus we were able to test the genetic connections through both maternal and paternal lineages," said Rogaev.

Rogaev and colleagues reconstructed the complete mitochondrial genome sequences from the remains and, analyzing specific Y-chromosome markers, were able to compare those with descendents of the paternal line. A critical link to the Romanovs was established via the DNA profiles from bloodstain specimens—known to be from Czar Nicholas—held at the State Hermitage Museum in St. Petersburg, Russia. [In 1891, while he was heir to the throne, Nicholas II was attacked in an apparent assassination attempt during a visit to Osaka, Japan. His bloodstained shirt was recently discovered in the museum.]

"The bloodstain DNA profile for all DNA systems matched with that of the bone fragment DNA profiles, and so we could conclude the identity of the putative remains of Nicholas II with a great degree of confidence. The analysis of Y- chromosome markers demonstrated further the link between the bone fragment of putative Alexei to his father Tsar Nicholas II and the distant paternal relatives which must have inherited the same Y-chromosome markers from their common Imperial ancestor."

While DNA is present inside the nucleus of every cell of the body, mitochondrial DNA is distinct from nuclear DNA in that it possesses its own genome that exists outside of the cell nucleus. And whereas autosomal cchromosomal DNA undergoes the process of recombination, where sections of DNA from the mother and the father are mixed resulting in a more distorted genetic history, mitochondrial DNA, which is inherited only from the mother, or non-recombinant part of Y- chromosome DNA, inherited only from the father, allows for the tracing of a more direct genetic lines. All of the Romanov children, including Crown Prince Alexei and his sisters, have the same type of mitochondrial DNA as their mother, Empress Alexandra, who was a granddaughter of Queen Victoria. Prince Philip of England is also a distant relative on his mother's side. Rogaev's team compared the mitochondrial DNA types extracted from the remains and also reviewed published information about the prince's DNA and, in addition, compared with mitochondrial DNA sequences from other distant maternal relatives of Queen Victoria in their work.

Czar Nicholas II, his wife Alexandra and their five children were assassinated as civil war broke out; numerous stories circulated over the years that Anastasia, then 17, somehow escaped. In 1991, five bodies were found and later identified as the Romanovs. Independent studies done in the US and England and later by Rogaev compared extracted DNA samples to those from descendents of the royal family. Two bodies, however, were missing, leaving some question as to the fate of

two of the czar's children. Bone fragments were found in the summer of 2007, not far from the original discovery site, about 900 miles east of Moscow, but had been badly damaged by not only time and natural decomposition, but also by acid and fire, as the murderers apparently sought to fully destroy the bodies and evidence of the murder.

Source: University of Massachusetts Medical School

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