Part III: Molecular Analysis of People in a Mass Grave

1. The first mass grave found in Yekaterinburg contained Nicholas II and Alix, four of their personal staff members (family physician, valet, maid, and cook), and three of their daughters. One daughter and son Alexei were missing from the first mass grave. The missing Romanov children were later discovered together approximately 70 meters away in a smaller grave. There is still debate about the identity of the missing daughter since there is no DNA reference from each sister. Russian experts claim the missing daughter was Maria while U.S. experts claim she was Anastasia.

2. The larger mass grave with 9 people including the Romanov family's four personal staff members was officially known to the public in 1991 after Dr. Alexander Avdonin located the mass grave in the late 1970s. The smaller mass grave with the missing daughter and Alexei was discovered in 2007 by a group of amateur archeologists.

3. The Ural Soviets leading the Bolshevik Revolution feared that the White Russian Army was going to rescue the Romanov family from exile in July of 1918. This led to the Ural Soviets executing the Romanov family on July 17, 1918, so that any hope by those loyal to Nicholas II and his ruling will be dissipated. The White Russian Army was the first major counter-revolutionary group against the Red Army of the Bolsheviks. They were described as a "loose confederation" of anti-communist militarized forces who fought against the Bolsheviks in the Russian Civil War (1917-1923) and stayed militarized within and outside Russian borders up to World War II. The White Army forces consisted of landowners, republicans, conservatives, the middle-class, reactionaries, pro-monarchists supporting Nicholas II, liberals, army generals, non-Bolshevik socialists, and democratic reformists.

4. Mitochondrial sequences, autosomal STR, and Y-STR DNA profiles linked the remains of Nicholas II and Alix to their children in both graves as well as living relatives like HRH Prince Philip. Sex relationships among the remains in the 1991 mass grave were confirmed by anatomical tooth or bone analysis as shown in Table 2. Nuclear DNA testing of five STR markers confirmed the sex relationships of the skeletons in the first mass grave, and this also confirmed familial relationships between Nicholas II, Alix, and three of the Tsar and Tsarina's daughters. Alix's mtDNA confirmed her maternal relationship to her three daughters in the mass grave. The mtDNA of Nicholas II's maternal relatives, the Duke of Fife and Princess Xenia Cheremeteff Sfiri, were used to confirm his identity as their mtDNA was identical to his with the exception of the Tsar having a single point heteroplasmy at position 16169.

5. Forensic DNA profiling of skeletal remains uses multiple simple sequence repeat (SSR) loci that are highly polymorphic. The likelihood of two random people sharing the same combination of two alleles of a particular SSR locus is 10%, and at 13 SSR loci, the chance of two people sharing the same allele combinations is about 1 in 10 trillion. A genotype for 13 unlinked SSR loci gives an individual a unique DNA profile or genetically related individuals confirmation of their familial relationship by an index of genetic relatedness. STR stands for short tandem repeat and uses polymorphic SSRs in forensic nuclear DNA profiling. STR markers or a microsatellite

of short repeats of 3bp-4bp repeats in the profiling of 4-6 loci up to a 15 STR loci examination are used in an unrelated individual's DNA profile or matching related individuals.

6. Mitochondrial DNA testing, nuclear STR testing, and Y-STR (Y-chromosome) testing were used to test the remains in the second grave, and these three genetic systems confirmed that one daughter who could be Maria or Anastasia and son Alexei were in the second grave. The missing children from the second grave had matching mtDNA with Alix and her three daughters from the mass grave. STR analysis was conducted on the two individuals in the smaller grave discovered in 2007 to confirm they were related to each other on the Sibship Index (SI). These two siblings were confirmed to be related to the Romanov remains in the first mass grave by STR testing on skeletal remains from those five members of the family. More microsatellites showed evidence for the parental relationship between Nicholas II and Alix and their children from both graves. One of their daughters and Alexei from the second grave have the same half allele sharing genotypes with Nicholas II and Alix. Y-STR testing was conducted on Nicholas II and his son Alexei to compare to a paternally living cousin of the Tsar, Prince Andrew Andreevich Romanov, over 17 markers to find an exact match between the three of them, confirming the relationship of Alexei to his father.

7. Y-STR testing was used to identify Alexei by his genetic relationship with his father and his father's distant living cousin, Prince Andrew Andreevich Romanov.

8. A 17 Y-STR loci profile was generated for Alexei and Nicholas II from his tooth. A 17 Y-STR loci profile was then generated for Nicholas II's second cousin's son, Prince Andrew Andreevich Romanov. All three males had an exact match over all 17 markers, which confirmed Alexei's identity as Nicholas II's son.

9. As the mtDNA of the missing daughter confirmed her relationship to her mother Alix in the first mass grave, the daughter could be either Maria or Anastasia in the second grave. Because of the absence of a DNA reference from each sister to confirm each of their identities, it is still up to debate whether Maria or Anastasia was in the grave with Alexei. According to Russian experts, the missing daughter was Maria, while U.S. experts claim the missing daughter was Anastasia.

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Part IV: Who Wants to Be Anastasia?

10. Anna Anderson was the most well-known self-proclaimed Grand Duchess Anastasia. Anna Anderson was a Polish-German factory worker who convinced many that she escaped the July 17,1918 execution and left Russia. This imposter story was further perpetuated when one princess was missing as her three sisters were in the mass grave. The disbelievers of Anderson's claims believed she was Franzisca Schanzkowska, who was born in 1896 and lived in Pomerania, Germany close to the Polish border. This information was found out by the Tsarina Alexandra's brother, Grand Duke of Hesse's private investigator. During WWI, she worked in a munitions factory in Berlin where she was injured in an explosion and disappeared in 1920 after being admitted to two mental hospitals and hiding her birth name. Soon after, in 1922, Anna Anderson became public with claims she was Anastasia, and she was released from asylum with supporters. Five STR marker analyses of Anna Anderson's intestine sample and mtDNA sequences of her hair samples confirmed she was the same person as Franzisca Schanzkowska. Schanzkowska's grandnephew, Carl Maucher, who had maternal relatedness, donated a blood sample with mtDNA that identified Anna Anderson as Franzisca Schanzkowska.

11. Anna Anderson eventually settled in Charlottesville, Virginia where she had undergone surgery and gave intestine samples to the Forensic Science Service (FSS) and the Armed Forces Institute of Pathology (AFIP).

12. Anna Anderson married an American history professor, John E. Manahan, in 1968 and moved to the U.S.

13. The sources of Anna Anderson's nuclear DNA were from intestine tissue samples preserved in formaldehyde and preserved in paraffin wax blocks.

14. The sources of Nicholas II's and Alix's nuclear DNA were from the STR DNA profiles of their bones in the mass grave.

15. STR analysis over five markers was done on nuclear DNA from Anna Anderson, Nicholas II, and Alix. Four out of five of Anna Anderson's different STRs were inconsistent with Nicholas II's and Alix's SSR loci for genetic relatedness of parentage.

16. Anna Anderson's mtDNA from her six hair samples and intestine sample was sequenced and compared to the blood sample donated by HRH Prince Philip as the grandnephew of Alix and the blood sample donated by Franzisca Schanzkowska's grandnephew, Carl Maucher.

17. Hypervariable regions of mtDNA are sites that evolve at a significantly higher rate than average in noncoding human mtDNA. A hypervariable region has more sequence variation or polymorphisms than normally observed analyses of mtDNA variation.

18. There were six differences found in hypervariable region 1 between Anna Anderson and HRH Prince Philip, who is close to the mtDNA reference sequence for Alix as her grandnephew. These results do not give Anna Anderson a maternal relationship with Alix. However, the mtDNA from Carl Maucher's blood sample shows maternal relatedness to Anna Anderson with an exact match to a hypervariable region. These results conclude that Anna Anderson is Franzisca Schanzkowska at birth, and Carl Maucher is her grandnephew.

19. Nicholas II's and Alix's remaining children missing from the mass grave were accounted for in 2007 in a smaller grave about 70 meters away. The identities of the missing daughter and Alexei were confirmed by mtDNA and STR testing for both children and Y-STR testing for Nicholas II's paternal relationship with Alexei.

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Part V: Current Hemophilia Treatment

20. A current treatment that is effective and widely used for hemophilia is to inject clotting factor concentrates into a patient's vein. This treatment replaces the missing clotting factor IX or X for proper blood coagulation. Clotting factor treatment products such as plasma-derived factor and recombinant factor concentrates are available for home use and at hemophilia treatment centers. Treatment options prescribed by physicians and nurses include episodic care to stop a patient's severe bleeding episodes and prophylactic care to prevent severe bleeding episodes from reoccurring. All these treatment options for hemophilia are effective for short-term and long-term use that results in less severe bleeding and other symptoms of hemophilia.