The Forensic DNA Identification of the two missing Romanov children (Alexei and Maria)

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University of North Texas Health Science Center
Fort Worth, Texas

Overview of the Center for Human Identification

Who We Are

- UNTCHI is an accredited forensic laboratory that provides nuclear and mitochondrial DNA testing services to law enforcement agencies, medical examiners and coroners at no charge.
- Designated as the State Missing Persons lab in 2002 (Senate Bill 1304)
- Designated as a criminal justice agency in 2011 (House Bill 2385)
Center for Human Identification
- Evidence Handling Unit
- Forensic Case Working Unit
- Missing Persons Unit
- Laboratory of Forensic Anthropology
- CODIS Unit
- Research and Development
- CHI is currently working to create a Forensic Genetic Genealogy Unit

Evidence Handling Unit
- The Evidence Handling Unit is responsible for:
  - Receipt, accessioning, transfer and return of evidence.
  - Communicating and interacting with law enforcement, medical examiners/coroners.

Forensic Unit
- Currently working to reduce the backlog of sexual assault kits in Texas
- Biological screening and DNA analyses for UCR, Part 1, Crime cases in Texas
  - Murder
  - Forcible Rape
  - Robbery
  - Aggravated assaults
  - Property crimes
Missing Persons Unit

• On behalf of the State of Texas, UNTCHI generates DNA data, makes associations and manages the TX Missing Persons DNA Database, which contributes to identifying human remains found in Texas.

Anthropology Unit

• In addition to DNA testing, UNTCHI also utilizes the expertise of four forensic anthropologists to aid investigations of missing persons and unidentified remains.

CODIS Databases

• Provides a means for crime laboratories throughout the country to electronically compare DNA profiles.
  – Convicted Offenders
  – Forensic Crime Scene Samples
  – Unidentified Human Remains
  – Missing Persons Direct Reference Samples
  – Family Reference Samples
Research and Development

- Current research projects include:
  - next generation sequencing
  - mixture interpretation
  - applications of microbial forensics
  - bioinformatics solutions such as familial searching software, and
  - molecular medicine

Forensic DNA Testing

An Introduction

The Human DNA Genome

Nuclear DNA
~3.2 billion bp
High Power of Discrimination

Mitochondrial DNA
16.5 Kbp
High Copy #
**Short Tandem Repeat (STR) Typing**

Fluorescent dye-labeled primer

STR Repeat Region

GATA  GATA  GATA  GATA  GATA

(Maternal) (Paternal)

Forward primer hybridization region

Reverse primer hybridization region

PCR Amplification

Capillary Electrophoresis

ABI 3100 – 16 capillary instrument

Capillary Electrophoresis

Electropherogram

Size of Fragments (bp)

Peak 1: 136 bp

Peak 2: 144 bp

Marker XYZ = 6, 8
The Forensic DNA Identification of the
Two Missing Romanov Children

Position of Forensic STR Markers on
Human Chromosomes

13 CODIS Core STR Loci
20 CODIS Core STR Loci

AMEL

Sex-typing

Random Match Probability = 1 in 129 quadrillion

Multiplexing of STR Markers

Promega Corporation PowerPlex 16 Multiplex STR kit

Autosomes – 22 pairs – 2 copies per cell

Sex Chromosomes (XX or XY)

mitochondria – in cell cytoplasm

100s of mtDNA copies per cell

Cell Nucleus – 3.2 billion bp
Lineage Markers

Autosomal DNA
1/8 from Great-grandparents

Lineage Markers

Y chromosome passed along paternal lineage

Autosomal DNA
1/8 from Great-grandparents

Forensic Y-STR Testing

Y-filer (Applied Biosystems) -17 Y-STRs
The Forensic DNA Identification of the Two Missing Romanov Children

Historical Investigation DNA Study

Figure 9.10, J.M. Butler (2005), Forensic DNA Typing, 2nd Edition © 2005 Elsevier Academic Press

Lineage Markers

Y chromosome passed along paternal lineage

mtDNA genome passed along maternal lineage

Autosomal DNA 1/8 from Great-grandparents

Historical Investigation DNA Study

Y chromosome passed along paternal lineage

mtDNA genome passed along maternal lineage

Autosomal DNA 1/8 from Great-grandparents

Forensic Scientists have focused on ~600bp of sequence information in the non-coding control region of the mtDNA.

HV1
HV2

263 A – G
16519 T – C
315.1 C

http://www.mitomap.org/
The Romanovs – Russia’s Royal Family (1913)

Historical Background

• After spending several months in Tobolsk, the family is finally exiled to Siberia (Ekaterinburg).

The Romanovs in Tobolsk, Russia

The Romanov Family in captivity (left to right: Tatiana, Tsarvitch Alexei, Maria (standing), Tsar Nicholas II, Anastasia, Olga)
Historical Background

- After spending several months in Tobolsk, the family is finally exiled to Siberia (Ekaterinburg).
- "I would go anywhere at all, only not to the Urals." - Tsar Nicholas II

From the Sokolov collection at Harvard
The Romanov family was kept in Ekaterinburg under house arrest by the Bolsheviks from the end of April 1918 until their murder on July 17, 1918. From the Sokolov collection at Harvard.
“Here (we) ignited a fire, and while the grave was being prepared, we cremated two corpses: Alexei and by mistake, instead of Alexandra Fedorovna, (we) cremated, apparently, Demidova. At the cremation site (we) dug a pit, laid down the bones, leveled it, again lit a large fire and with the ashes concealed any traces.”
Excerpt from the Yurovsky Report

“Before laying down the other corpses, we doused sulfuric acid over them, filled the pit, sealed it with sleepers, the empty lorry drove over, (and) somewhat packed down the sleepers and (then we) finished. At 5-6 o’clock in the morning, (I) gathered every one and having declared to them the importance of the completed matter, having warned (them), that everyone must forget about what they saw and never talk about it with anybody.”

Investigator Nikolay Sokolov 1919

Photo from Dr. Alexander Avdonin

Basement Room of the Ipatiev House where the Russian Imperial family was murdered on July 17, 1918 by members of the Ural Soviet

From the Sokolov collection at Harvard
The Forensic DNA Identification of the Two Missing Romanov Children

1919 Site Excavation at Four Brother's Mine Shaft

1919 photo taken by Sokolov of the small bridge at Pig's Meadow
Sokolov’s photo of the Koptiaki Road standing on the wooden bridge

From the Sokolov collection at Harvard

1920 photo of Peter Ermakov standing on the bridge

1978 - Dr. Avdonin and his group discover the mass grave in Pig’s Meadow
1979 – Dr. Avdonin and his group planted bushes to hide their find.

1991 – Official discovery of the mass grave and excavation of the remains.
Identifications results comparison of Russian (Abramov) and U.S. (Maples) teams

<table>
<thead>
<tr>
<th>Skeleton #</th>
<th>Sex</th>
<th>Age</th>
<th>Size</th>
<th>Abramov conclusion</th>
<th>Maples conclusion</th>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>40-50</td>
<td>161-168</td>
<td>Demidova</td>
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<td>2</td>
<td>M</td>
<td>50-60</td>
<td>171-177</td>
<td>Botkin</td>
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<td>3</td>
<td>F</td>
<td>20-24</td>
<td>156-165</td>
<td>Olga</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>45-55</td>
<td>165-170</td>
<td>Nikolai</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>~20</td>
<td>166-171</td>
<td>Tetiana</td>
<td>Maria</td>
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<tr>
<td>6</td>
<td>F</td>
<td>~20</td>
<td>162-171</td>
<td>Anastasia</td>
<td>Tetiana</td>
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<tr>
<td>7</td>
<td>F</td>
<td>45-55</td>
<td>163-168</td>
<td>Alekandra</td>
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<tr>
<td>8</td>
<td>M?</td>
<td>40-50</td>
<td>172-181</td>
<td>Kharitonov</td>
<td></td>
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<tr>
<td>9</td>
<td>M</td>
<td>&gt;60</td>
<td>172-181</td>
<td>Trupp</td>
<td></td>
</tr>
</tbody>
</table>

http://www.romanov-memorial.com/Final_Chapter.htm

Previous DNA Testing of the 1991 Remains

Identification of the remains of the Romanov family by DNA analysis

Peter Gill\textsuperscript{1}, Pavel L. Ivanov\textsuperscript{1}, Colin Kimpton\textsuperscript{1}, Romelle Piercy\textsuperscript{1}, Nicola Benson\textsuperscript{1}, Gillian Tully\textsuperscript{1}, Ian Evert\textsuperscript{1}, Erika Hagelberg\textsuperscript{1} & Kevin Sullivan\textsuperscript{1}

Nature Genetics – Feb. 1994
9 unique DNA profiles – Skeletons 4 (Tsar) and 7 (Tsarina) are consistent with being parents of skeletons 3, 5, and 6.

NOTE: STRs were used as a sorting tool here
Concerns About the 1st DNA Testing

- Heteroplasmy – not well understood at the time. We now know that it is quite common.
- Relatively low statistical power – mtDNA database size of 200-300 individuals (LR = 70).
- STRs – in their infancy – only 5 markers were examined.

Despite the concerns – the evidence was overwhelming

http://www.foxnews.com/story/0,2933,294360,00.html

Remains of Czar Nicholas II's Son May Have Been Found
FRIDAY, AUGUST 24, 2007

MOSCOW — The remains of the last czar's hemophiliac son and heir to the Russian throne, missing since the royal family was gunned down nine decades ago by Bolsheviks in a basement room, may have been found, an archaeologist said Thursday.
The Forensic DNA Identification of the Two Missing Romanov Children

2007
2 sets of remains
Alexei and Maria

~ 70 meters away

9 bodies:
Tsar Nicolas II
Tsaarina Alexandra
Olga
Tatiana
Anastasia
Anna Demidova
Alexei Trupp
Dr. Botkin
Ivan Kharitinov
The Forensic DNA Identification of the Two Missing Romanov Children

mtDNA Analysis

16111 C-T
16357 T-C
263 A-G
315.1 C

Gill et al. (1994)
Table 1. Sequences of the samples recovered from "Grave Z2" in August 2007 and tested in this study.

<table>
<thead>
<tr>
<th>Source</th>
<th>Mutation</th>
<th>Region Sequenced</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right homunculus</td>
<td>141</td>
<td>16024-16139 and 35-369</td>
<td>16111 C-T, 16357 T-C</td>
</tr>
<tr>
<td>Isolated fragment</td>
<td>120</td>
<td>no results</td>
<td></td>
</tr>
<tr>
<td>Right homunculus</td>
<td>144 T</td>
<td>16024-376</td>
<td></td>
</tr>
<tr>
<td>Left female</td>
<td>146 T</td>
<td>16024-376</td>
<td></td>
</tr>
<tr>
<td>Right homunculus</td>
<td>147 T</td>
<td>16024-376</td>
<td></td>
</tr>
</tbody>
</table>

Cable et al. (2009)

The “Tsarina” mtDNA Sequence

- Has not been observed in a database of **21,546** individuals (4,839 individuals in the US FBI mtDNA database and 16,707 individuals from an internal AFDIL Research Section database).

- mtDNA results agree with previous sequence data from Gill *et al.* 1994
nuclear DNA (STR) Testing

Sample 146.1

Sample 147

Scenario: Samples 146.1 and 147 as Sibs

\[
LR = \frac{\Pr(E | H_1)}{\Pr(E | H_2)}
\]

(The samples are siblings)

(The samples are NOT siblings)

Cumulative LR (CSI) = 5.6 Million

The evidence is 5.6 million times more likely to be observed if the samples are siblings rather than if the samples were from two unrelated individuals.
Can These Remains be Children of Tsar Nicholas II and Tsarina Alexandra?

Table 2. Samples recovered from “Grave 61” in the early 1990s and tested in this study.

<table>
<thead>
<tr>
<th>Skeleton</th>
<th>Attribution</th>
<th>Sample</th>
<th>Bone/Teeth</th>
</tr>
</thead>
<tbody>
<tr>
<td>d3</td>
<td>Oleg</td>
<td>5.40”</td>
<td>Fragment of a left femur</td>
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<tr>
<td>d4</td>
<td>Nicholas</td>
<td>6.29”</td>
<td>Fragment of a rib</td>
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<tr>
<td>d5</td>
<td>Petal teeth</td>
<td>6.51”</td>
<td>Fragment of a clavicle</td>
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<tr>
<td>d4</td>
<td>444</td>
<td></td>
<td>Fragment of a pelvis</td>
</tr>
<tr>
<td>d5</td>
<td>Tulaia</td>
<td>5.20”</td>
<td>Fragment of a left femur</td>
</tr>
<tr>
<td>e4</td>
<td>Anastasia</td>
<td>6.14”</td>
<td>Fragment of the diaphysis of a left femur</td>
</tr>
<tr>
<td>e7</td>
<td>Alexander</td>
<td>7.48”</td>
<td>Fragment of a pelvis</td>
</tr>
<tr>
<td>e7</td>
<td>7.40”</td>
<td></td>
<td>Fragment of the diaphysis of a left femur</td>
</tr>
<tr>
<td>e7</td>
<td>Tsarina</td>
<td>7.6”</td>
<td>Fragment of a right femur</td>
</tr>
<tr>
<td>e7</td>
<td>7.40”</td>
<td></td>
<td>Fragment of the diaphysis of a left femur</td>
</tr>
</tbody>
</table>

Coble et al. (2009)
Scenario: Samples 146 and 147 are members of the Romanov Family

Cumulative LR = **4.36 Trillion to 80 Trillion**

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<table>
<thead>
<tr>
<th>Sample 4.3</th>
<th>Sample 7.4</th>
<th>Sample 3.46</th>
<th>Sample 5.21</th>
<th>Sample 6.14</th>
<th>Sample 147</th>
<th>Sample 146.1</th>
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</thead>
<tbody>
<tr>
<td><strong>Markers</strong></td>
<td>Tsar Nicholas II</td>
<td>Tsarina Alexandra</td>
<td>Olga</td>
<td>Tatiana</td>
<td>Anastasia</td>
<td>Maria</td>
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<td><strong>DYS19585</strong></td>
<td>14, 17</td>
<td>16, 18</td>
<td>17, 18</td>
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<td><strong>TH01</strong></td>
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<td>6.0</td>
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<td><strong>vWA</strong></td>
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<td><strong>TPOX</strong></td>
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</tbody>
</table>

Coble et al. (2009)
The Forensic DNA Identification of the Two Missing Romanov Children

21 January 1923 – 28 November 2021
The “Romanov” Y-STR Type

- Has not been observed in a database of $30,000+$ individuals.
- (http://www.yhrd.org )

<table>
<thead>
<tr>
<th>Y-STR</th>
<th>DYS391</th>
<th>DYS390</th>
<th>DYS389</th>
<th>DYS388</th>
<th>DYS387</th>
<th>DYS386</th>
<th>DYS385</th>
<th>DYS384</th>
<th>YATM349</th>
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<tbody>
<tr>
<td>Value</td>
<td>15</td>
<td>3</td>
<td>30</td>
<td>19</td>
<td>10</td>
<td>12</td>
<td>12</td>
<td>11, 14</td>
<td>24, 12</td>
</tr>
</tbody>
</table>

Coble et al. (2009)

Sverdlovsk Regional Forensic Bureau
Forensic DNA Team

Tsarevich Nicholas at Nagasaki

May 11, 1891

Sanzo Tsuda
The Forensic DNA Identification of the Two Missing Romanov Children

STR Profile of the blood stain from the 1891 shirt

Complete Concordance with the Post-mortem Evidence!

Y-STR Profile of the blood stain from the 1891 shirt
The Forensic DNA Identification of the Two Missing Romanov Children

Y-STRs

Mitochondrial DNA

Forensic STRs

The Imposters

Anna Anderson Manahan
Total number of "Russian imperial children" claimants since 1918:

- Olga: 28 claimants
- Tatiana: 33 claimants
- Maria: 53 claimants
- Anastasia: 33 claimants
- Alexei: 81 claimants

All of the Romanovs were executed in the early morning of July 17, 1918

For more information…

http://www.plosone.org/article/info:doi/10.1371/journal.pone.0004838
Thank you!

Osher Lifelong Learning Institute at UNT
Dr. Stephanie Reinke

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