The application of NGS and CE systems in identification of human remains from rebels' graves found in Gediminas Hill: the Lithuanian historical mystery since 1863 year

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Introduction



For almost a century and a half, Lithuania has been trying to discover the uprising victims' graves, however, unsuccessfully until the beginning of 2017, when the emergency maintenance works started on the top of the Gediminas Hill, following a dangerous landslide and gave a new push to the investigation.

The Gediminas Hill in the heart of Vilnius, LT © DELFI.LT

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Place of archaeological research





The Gediminas Hill in the heart of Vilnius, LT \bigcirc <u>DELFI.LT</u>

Gediminas Hill Place until 2017 November 14 An area of 123.5 square meters was investigated, and eleven graves with 17 remains were detected and investigated.





The Gediminas Hill in the heart of Vilnius, LT © DELFI.LT

Background and some historical data (I):

- The findings have set a scientific buzz, with some of the historians pondering that the fragile bones excavated on the hill while executing maintenance works could belong to the participants of the 1863-1864 uprising against tsarist Russia.
- Furthermore, some scholars believe the remains could be of its leaders, including Zigmantas Sierakauskas (Zygmunt Sierakowski) and Konstantinas Kalinauskas (Konstanty Kalinowski).
- Participants on the 1863–1864 uprising sought to revive the former Polish-Lithuanian Commonwealth, which was annexed by Russia in 1795.



Background and some historical data (II):

- Some of the human bodies were covered in lime, buried without coffins, with their hands tied. In the graves, archaeologists found pieces of clothing and buttons from the shirt and trousers.
- In one grave a silver medallion was found, containing an image of the Blessed Virgin Mary, of the Chapel of the Gates of Dawn, a famous shrine in the old town of Vilnius, on one side and on the other side - a picture of the Snipiskes Chapel.
 - Also one male's right hand contained a golden ring with an inscription inside: "Zygmont Apolonija 11 Sierpnia/30 Lipca 1862". This evidence enabled to identify that the remains belong to Zigmantas Sierakauskas, the leader of the uprising.

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Main target Issue:

• To solve one of Lithuania's greatest historical mysteries by analyzing some bones from a grave on Gediminas Hill.





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Main target Issue of the project:

- Developing a next-generation sequencing (NGS) platform application in identification of human remains from rebels' graves buried in Gediminas Hill in 1863 to resolve Lithuanian history's big mysteries in collaboration with the historians and anthropologists closely examining the human remains recently found on the top of the Gediminas Hill in the heart of Vilnius.
- The human remains identification from aged genetic material using NGS, CE systems and Converge-integrated data analysis & management system for concordance between NGS & CE profiles.

Aim of research:

• The aim of the research was to determine and evaluate the concordance between NGS & CE profiles.

New approach:

- The conducted research study presents a new approach to the assessment of the aged genetic material using NGS, CE systems and Converge-integrated data analysis & management system for concordance between NGS & CE profiles.
- Analysis was performed using the the Capillary electrophoresis (CE) with GlobalFiler and Yfiler Plus kits with GeneMapper® ID-X Software and the NGS with Precision ID GlobalFilerTM NGS STR Panel v2 with the Ion S5TM System.



Material and methods (I):

- During the excavation, expecting bones of 21 individuals from the hill.
- The skeletal remains of 17 individuals were excavated.
- Analysis of samples taken from 17 femurs was conducted.
- 17 performed with CE and 11 with NGS (not final ...).

Material and methods (II):

Sample Preparation and Extraction

- Bones preparation
- Mechanical cleaning
- Chemical treatment: water, chloride solution and ethanol
- Milling and pulverization
- 0,7-1,0 g of the powder of each sample were subjected to DNA extraction
- Min. 4 repeats of each sample

Demineralization

- o 1% demineralization buffer (N-Lauroylsarcosinesodiumsalt, 0.5 M EDTA pH 8.0)
- o Proteinase K
- Incubate at 56° C with approx. 700 rpm. minimum of 12 hours (maximum 48 h)
- 2 hours centrifuge 3000 rpm.
- Concentration
- QIAquick PCR Purification kit from QIAGEN by protocol
- Final extract / eluate of 50 ul



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Material and methods (III):

DNA Quantification

- The 7500 Real-Time PCR System (Applied BiosystemsTM)
- Applied BiosystemsTMQuantifilerTMTrio
 DNA Quantification Kit

Material, methods and results (III):

DNA Quantification results (table)

| Sample | Quantity (ng/µL) | Degradation Index |
|-----------|------------------|-------------------|
| Sample 1 | 0,997 | 6,241 |
| Sample 2 | 0,005 | 4,848 |
| Sample 3 | 0,179 | 4,703 |
| Sample 4 | 0,313 | 1,003 |
| Sample 5 | 0,114 | 1,927 |
| Sample 6 | 0,076 | 0,832 |
| Sample 7 | 0,578 | 66,805 |
| Sample 8 | 0,337 | 1,196 |
| Sample 9 | 0,444 | 1,779 |
| Sample 10 | 0,548 | 2,923 |
| Sample 11 | 0,673 | 14,043 |
| Sample 12 | 0,145 | 2,133 |
| Sample 13 | 0,120 | 2,836 |
| Sample 14 | 0,107 | 1,638 |
| Sample 15 | 0,531 | 1,872 |
| Sample16 | 0,068 | 1,175 |
| Sample 17 | 0,159 | 1,665 |





Material and methods (IV):

Polymerase chain reaction (PCR)

- Applied BiosystemsTMGlobalFilerTM PCR Amplification Kit
- Applied BiosystemsTMYfilerTM Plus PCR Amplification Kit
- Applied Biosystems ProFlexTM PCR System

Capillary Electrophoresis (CE) and data analysis

- The Applied BiosystemsTM 3500 Series Genetic Analyzer
- Applied BiosystemsTM GeneMapper® ID-X Software Version 1.4.

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Material and methods (V):

Next-generation sequencing (NGS)

- Applied BiosystemsTM Ion Chef System TM
- Applied BiosystemsTM Ion S5TM System
- Precision ID GlobalFilerTM NGS STR Panel v.2



Material and methods (V):







The Serology and DNA Lab of SFMS, Vilnius, LT www.vtmt.lt

Material & methods (VI):

Comprehensive analysis platform

- The data from CE and NGS profiles compered by using the ConvergeTM Software v2.0 (Applied BiosystemsTM)as a comprehensive analysis platform that integrates answers.
- The data on human remains identification from aged genetic material using NGS, CE systems and Converge-integrated data analysis & management system for concordance between NGS & CE profiles, done in Serology and DNA Lab of SFMS, Vilnius, LT.





CE Data(I):



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CE Data(I):



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Project: Gedimino

CE Data(I):







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NGS Data(I):

Sample Name: lonCode_0108 : 5_24_6b_12_05 Batch Id: Batch-1001 Case Id: Case-1007



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Sample Name: IonCode_0110 : S_24_8 Batch Id: Batch-1001 Case Id: Case-1007



CE Data(I): (with partial profile)





NGS Data(I): (with partial profile)

Sample Name: IonCode_0119 : S_24_7a Batch Id: Batch-1001 Case Id: Case-1007



Converge Data (I) CE and NGS profiles compered by using the Converge

| Locus | Profile-1245 (Precision_ID_GlobalFiler_NGS_STR_Panel_v1.0) | Profile-1278 (GlobalFiler_Panel_v1) | Profile-1279 (GlobalFiler_Panel_v1) |
|------------|---|--|--|
| AMEL | Y, X | Y, X | Y, X |
| CSF1P0 | 10 | 10 | 10 |
| D10S1248 | 14 | 14 | 14 |
| D12ATA63 | 13, 18 | • | * |
| D12S391 | 17, 19 | 17, 19 | 17, 19 |
| D135317 | 9, 11 | 9, 11 | 9, 11 |
| D14S1434 | 10,13 | • | |
| D16S539 | 12 | 12 | 12 |
| D18S51 | 12, 15 | 12, 15 | 12, 15 |
| D19S433 | 13 | 13 | 13 |
| D1S1656 | 15,16 | 15, 16 | 15, 16 |
| D1S1677 | 13, 15 | • | 141 |
| D21S11 | 30, 30 | 30 | 30 |
| D22S1045 | 11,15 | 11, 15 | 11, 15 |
| D2S1338 | 17 | 17 | 17 |
| D2S1776 | 9, 10, 11 | • | ata |
| D2S441 | 11,14 | 11, 14 | 11, 14 |
| D3S1358 | 13, 15 | 13, 15 | 13, 15 |
| D3S4529 | 14, 15 | • | |
| D4S2408 | 8,10 | • | |
| D5S2800 | 14, 17 | • | |
| D5S818 | 11,12 | 11, 12 | 11, 12 |
| D6S1043 | 14, 21 | • | * |
| D6S474 | 15, 18 | • | |
| D7S820 | 9,10 | 9, 10 | 9, 10 |
| D8S1179 | 10, 16 | 10, 16 | 10, 16 |
| DY\$391 | 10 | 10 | 10 |
| FGA | 19, 20 | 19, 20 | 19, 20 |
| Penta_D | 12 | • | • |
| Penta_E | 5 | • | · |
| SRY | 1 | 1 | |
| TH01 | 8, 9 | 8, 9 | 8,9 |
| трох | 8, 9 | 8, 9 | 8,9 |
| Yindel | 2 | 2 | 2 |
| vWA | 15, 18 | 15, 18 | 15, 18 |
| SE33 | | 15, 24.2 | 15, 24.2 |
| Allele Mai | tch % | 100% | 100% |

Converge Data (I) CE and NGS profiles compered by using the Converge

| Locus | Profile-1282 (GlobalFiler_Panel_v1) | Profile-1251 (Precision_ID_GlobalFiler_NGS_STR_Panel_v1.0) | Profile-1250 (Precision_JD_GlobalFiler_NGS_STR_Panel_v1.0) |
|------------|--|---|---|
| AMEL | Y, X | Y, X | Y, X |
| CSF1P0 | 11, 12 | 11, 12 | 11, 12 |
| D10S1248 | 15,16 | 15, 16 | 15, 16 |
| D12S391 | 17,18 | 17, 18 | 17, 18 |
| D13S317 | 11, 14 | 11, 14 | 11, 14 |
| D16S539 | 11, 13 | 11, 13 | 11, 13 |
| D18S51 | 14, 16 | 14, 16 | 14, 16 |
| D19S433 | 14, 15 | 14, 15 | 14, 15 |
| D1S1656 | 11, 15.3 | 11, 15.3 | 11, 15.3 |
| D21S11 | 29, 30.2 | 29, 30.2 | 29, 30.2 |
| D22S1045 | 13, 17 | 13, 17 | 13, 17 |
| D2S1338 | 24, 26 | 24, 26 | 24, 26 |
| D2S441 | 10,14 | 10, 14 | 10, 14 |
| D3S1358 | 14, 16 | 14, 16 | 14, 16 |
| D5S818 | 11,13 | 11, 13 | 11, 13 |
| D7S820 | 10, 11 | 10, 11 | 10, 11 |
| D8S1179 | 9, 15 | 9, 15 | 9, 15 |
| DYS391 | 11 | 11 | 11 |
| FGA | 21, 23 | 21, 23 | 21, 23 |
| SE33 | 18, 22 | | |
| TH01 | 9, 9.3 | 9, 9.3 | 9, 9.3 |
| трох | 8 | 8 | 8 |
| Yindel | 2 | 2 | 2 |
| vWA | 17, 19 | 17, 19 | 17, 19 |
| D12ATA63 | | | 13, 15 |
| D14S1434 | | | 13, 14 |
| D1S1677 | | | 13, 15 |
| D2S1776 | • | | 11, 12 |
| D3S4529 | * | | 13, 16 |
| D4S2408 | - | | -10, 11 |
| D5S2800 | - | | -17, 18 |
| D6S1043 | | | 12, 14 |
| D6S474 | • | | 16, 17 |
| Penta_D | | | |
| Penta_E | | | 9 |
| SRY | | 1000 | |
| Allele Mat | tcn % | T00% | 100% |

Results and Conclusion on the application of NGS and CE systems in identification

- The CE and NGS systems useful for HID on aged genetic material.
- CE 17 profiles, NGS System 11 profiles.
- Autosomal and Y STRs on CE and used Precision ID NGS System for autosomal STRs.
- The Converge Software system allows to combine and compare data as a comprehensive analysis platform that integrates answers.
- The concordance of results 100%.

Perspectives and Challenges for future projects

- Question: What criteria would be used for future projects to decide whether to use CE or NGS analysis?- it's related on target issue.
- Related degradation level (Degradation Index) SNP, mtDNA
- The development Ancestry panel and kinships
- The Phenotyping
- The NGS validation process

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Thank you for your attention

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the Lithuanian historical mystery since 1863 year

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