APPLICATION OF FORENSIC DNA FINGERPRINTING METHODS FOR GENETIC DISCOVERY OF MEDIEVAL HUMAN SKELETAL REMAINS

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Introduction

In 2001/02 the municipal Archaeology of Hall (Tyrol, Austria) accomplished successful excavations of an Early Middle Age cemetery in Volders (Tyrol, Austria) under the auspices of the Institute of Archaeologies (University of Innsbruck). The majority of burials are believed to be from the late 6th to the early 7th century, where we still lack historical information from this region. This cemetery represents one of the largest and most ancient series of human remains being unearthed in Tyrol with 153 graves harbouring 144 well preserved complete human skeletons on an area of 140 m². Of great scientific interest is the burial situation in a tight area and the orientation of the skeletons with a prevalent restriction to East-West and only few North-South directions (Figure 1, [1]). There is a heterogeneous pattern of the sparse grave goods and the dress elements which perhaps originates from different social structures and the religious denomination of the buried individuals. Since prehistoric times Volders was a major settlement in the Inn Valley with more than 450 cremation burials and thrived during the medieval ages by mining activities [2]. The reconstruction of these early medieval rustic populations has been limited to archaeological and historical research with the aim of characterizing the familial organization and the social structures. However, many questions remain unanswered. This exceptional collection with respect to the number of individuals as well as the preservation state of the human remains perfectly lends itself to the application of molecular methods (typing of autosomal Short Tandem Repeats, haplotypic markers and ancestry informative Single Nucleotide Polymorphisms) to better characterize the remains (Figure 2) and to gain increased insight.

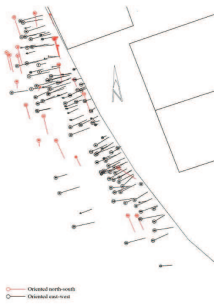

Figure 1: Simplified burial plan indicating the grave orientation. Burials in red are oriented in a north to south direction with the head to the north and those in black are oriented east to west with the head to the east [1].

Figure 2. Close-up view of a complete skeleton from grave number 132 and 123. The preservation state indicates successful application of molecular markers [2].

References

[1] McGlynn G. 2007. Using 13C-, 15N- and 18O stable isotope analysis of human bone tissue to identify transhumance, high altitude habitation and reconstruct palaeodiet for the early medieval Alpine population at Volders, Austria. Dissertation Ludwig-Maximilians-Universität München, Supervisor: Prof. Dr. Gisela Grupe, p. 7-9, 12-15, 17, 160

[2] Stadler H, Berger C, Zanesco A, McGlynn G, Haider PW, Appler H, Patzelt G. 2007. Forschungen zum frühmittelalterlichen Gräberfeld von Volders, Augasse (Nordtirol) Bd 1. Nearchos Sonderheft 15, p. 12, 40

[3] Niederstätter H, Köchl S, Grubwieser P, Pavlic M, Steinlechner M, Parson W. 2007. A modular real-time PCR concept for determining the quantity and quality of human nuclear and mitochondrial DNA. FSI:Genetics 1:29-34

[4] Eichmann C, Parson W. 2008. 'Mitominis': multiplex PCR analysis of reduced size amplicons for compound sequence analysis of the entire mtDNA control region in highly degraded samples. Int J Legal Med 122:385-8

[5] Sanchez JJ, Phillips C, Børsting C, Balogh K, Bogus M, Fondevila M, Harrison CD, Musgrave-Brown E, Salas A, Syndercombe-Court D, Schneider PM, Carracedo A, Morling N. 2006. A multiplex assay with 52 single nucleotide polymorphisms for human identification. Electrophoresis 27:1713–24

[6] Pereira R, Phillips C, Alves C, Amorim A, Carracedo Á, Gusmão L. 2009. A new multiplex for human identification using insertion/deletion polymorphisms. Electrophoresis 30:3682-90

[7] Walsh S, Liu F, Ballantyne KN, Van Oven M, Lao O, Kayser M. 2011. IrisPlex: A sensitive DNA tool for accurate prediction of blue and brown eye colour in the absence of ancestry information. FSI:Genetics 5:170–80

Material and Methods

Extraction: Prior to DNA extraction all samples were cleaned and treated with bleach and UV light to avoid surface contamination. Then bones (mainly femur) and teeth (preferably molars) were grinded using an oscillating blender mill (Essa, Vienna, Austria), followed by incubation of the powder in lysis buffer containing EDTA, N-Lauroylsarcosine and Proteinase K at 56°C overnight. DNA was concentrated with Centrifugal Filter Devices (Millipore, Billerica, MA, USA), for DNA purification the MinElute Purification Kit (Qiagen, Hilden, Germany) was used with additional washing steps. This was performed applying stringent scrutiny (isolation of work areas, work with laminar air flow) to avoid contamination by modern human DNA.

Quantification: Both human nuclear DNA and mitochondrial DNA were quantified with a modular real time PCR system [3].

Kinship analysis: STR typing (Amelogenin and 16 STR markers) was performed using the ESX/ESI17 Systems (Promega, Madison, WI, USA) using 35 amplification cycles and analyzed with an ABI 3100 Genetic Analyzer (Applied Biosystems (AB), Foster City, CA, USA).

mtDNA analysis: The entire human mitochondrial control region was amplified in 10 overlapping fragments (amplicon sizes from 144 to 237 bp) in two separate multiplex PCRs and sequenced with the individual amplification primers according to the protocol detailed in [4].

Ancestry informative markers were analyzed with a 52 SNP-plex assay [5] using SNaPshot (AB) and with 38 non-coding bi-allelic autosomal insertion/deletion (indel) polymorphisms on the basis of a commercial PCR Master Mix (Qiagen, Hilden, Germany) [6]. Phenotypically informative markers for skin, hair and eye pigmentation (SHEP) were analyzed according to the protocol provided in [7].

Number of samples	[mtGE/µL] mtGE143bp
13	0 - 100
7	100 - 600
10	600 -1000
13	1001 - 1500
6	1500-2000
5	2000 - 3000
2	3000 - 4000
1	4000 - 5000
2	5000 - 6000
3	6000 - 7000
2	7000 - 8000
3	9000 - 13947

Table 1: Summary of mitochondrial DNA yields.

Number of samples	[pg/µL] AluYb8
15	0 - 1,5
8	1,5 - 10
3	10 - 20
10	20-30
2	30 - 40
4	40 - 50
5	60 - 70
3	70 - 80
2	80 - 90
1	90 - 100
6	100 - 150
2	150 - 200
2	200 - 300
2	300 - 500
2	500 - 615

Table 2: Summary of nuclear DNA yields.

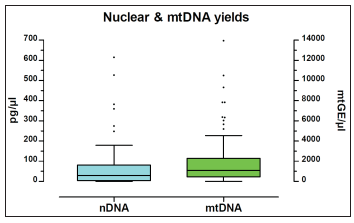

Figure 3: Comparison of mtDNA and nuclear DNA yields (nDNA…nuclear DNA, mtGE…mitochondrial genome equivalent).

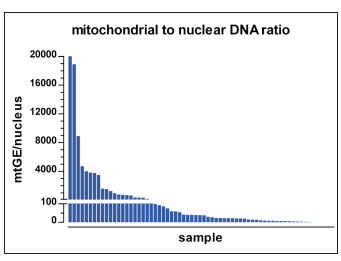

Figure 4: Mitochondrial to nuclear DNA ratio in all samples.

grave	tissue	mtGE/µL	haplotype	haplogroup
10	tooth	4548	16189C 16194N 16195N 16293G 16356C 16519C 263G 315.1C	H1b
15	tooth	1354	16126C 16294T 16296T 16304C 16519C 73G 195C 309.1C 315.1C	T2b
16	tooth	1082	16189C 16264G 16356C 16362C 16519C 263G 523AC	H1b
22	tooth	566	16189C 16192T 16270T 16398A 73G 150T 204C 263G (309T) (310C)	U5b2b2
25	tooth	7448	16519C 152C 263G	H*
27	tooth	6328	73G 263G 523ACdel	R*
32	tooth	551	16126C 16163G 16186T 16189C 16294T 16519C 73G 152C 195C 263G 573.1C	T1a
35	bone	33,5	16356C 16519C 73G 195C 263G	U4
40	tooth	7836	16224C 16311C 16519C 73G 309.1C 315.1C 497T 524.1ACAC	K1a
42	tooth	3086	16304C 16519C 207A 263G 456T	H5
48	tooth	528	16067T 200G 263G 498G 549N	HV1
50	tooth	6375	16189C 16311C 16519C	J
52	tooth	3665	16126C 16163G 16186T 16189C 16294T 16355T 16519C 73G 152C 263G	T1a
56	tooth	803	16069T 16126C 16222T 73G 185A 263G 295T 315.1C 462T 489C	J1b
57	bone	21	16298C 16519N 16519G 72C 263G	HV0

Table 4: Mitochondrial haplotypes and haplogroups of 15 bone and teeth samples.

Results and Discussion

We here report intermediate results based on the successful analysis of 67 specimens. 20 of them were analyzed via STR typing, 15 mitochondrial haplotypes were established and 13 were characterized using ancestry informative markers.

Quantification: The 67 analyzed samples gave a wide range of nuclear and mitochondrial DNA yields, most of which gave useful quantities for successful forensic analysis (Tables 1 and 2). In most samples mitochondrial DNA showed higher genome equivalent numbers than nuclear DNA (Figure 3) and few showed some greater discrepancies as seen in mitochondrial and nuclear DNA ratio (Figure 4).

STR typing: Four out of 20 samples (70%) analysed with autosomal STRs gave full 17 loci genotypes. In the remaining samples we observed drop-outs of SE33 most likely due to its high molecular weight (Table 3). Regarding size length of amplicons both ESX and ESI showed drop outs but ESX achieved better results than ESI (Table 3, Figure 5).

Ancestry informative markers: The 52 SNP-plex and the indelDIPlex kit are powerful methods for population and forensic genetic purposes when STR typing fails due to the degradation state of the samples. Figure 6 compares STR and DIPlex derived data and demonstrates the increased success rate with the shorter indel-markers.

mtDNA analysis: Mitochondrial control region haplotypes of 15 bone and teeth samples were established by direct sequencing (Figure 7) and 14 could be assigned to mitochondrial haplogroups typical for the West Eurasian phylogeny (Table 4).

STR-System	size range ESX	size range ESI	drop out total	drop out ESX	drop out ESI
SE33	267-417	309-459	16	8	8
D2S1338	197-269	223-295	10	5	6
D12S391	130-182	291-343	7	3	4
D2S441	88-124	347-383	8	-	8
D21S11	203-259	203-259	8	4	4
D10S1248	83-127	286-330	7	-	7
D1S1656	137-184	226-273	7	4	3
D22S1045	79-118	306-345	6	-	6
D19S433	193-245	163-215	6	5	1
FGA	143-289	264-410	6	4	2
D18S51	286-366	134-214	5	3	2
D16S539	273-321	84-132	4	4	-
vWA	124-180	124-180	3	1	2
D8S1179	203-251	76-124	3	3	-
TH01	152-195	72-115	2	2	-
D3S1358	103-147	103-147	1	-	1
		total	99	46	54

Table 3: Summary of STR-Markers, size ranges and drop-outs.

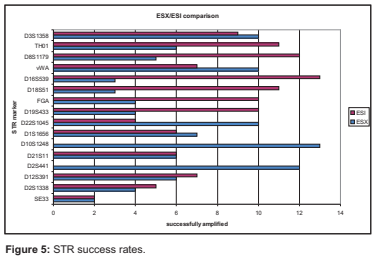

Figure 5: STR success rates.

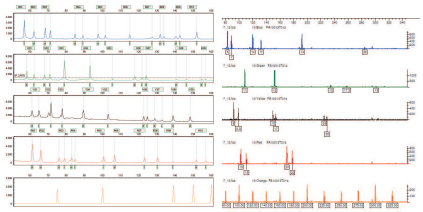

Figure 6: Comparative depiction of STR and INDEL results.

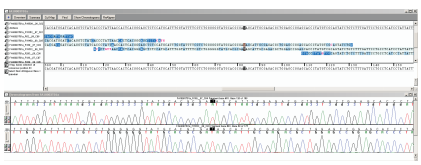

Figure 7: Example of a sequencing electropherogram of an early medieval tooth sample.

Acknowledgements:
This study is supported by the FWF Austrian Science Fund – Stand-alone project (P 228800-B12).