Evolutionary Medicine: Ancient biological samples to study the evolution of disease

Frank Rühli
MD, PhD
Outline

Evolutionary Medicine

Swiss Mummy Project

The field of aDNA

Skeletal series

Future

bone microdamage of archaeological cortical bone (Tomils, 11th–15th AD) fuchsin stained
Papageorgopoulou et al., J Archeol Sci, 2009
Aim

To present the research potential of clinically-oriented evolutionary medicine using unique, ancient samples (mummies, skeletons)

The Neolithic Iceman, ca 5300 BP
Evolutionary medicine

Evolutionary medicine investigates human disease vulnerability and disease aetiologies (genetics, behaviour, environment, pathogens, etc.) from evolutionary perspective.

It also addresses future developments in human health as a result of present-day medical and socio-economic practices.

Humans still evolve, in terms of anatomical structures + disease patterns/prevalence.
As a transdisciplinary bridge between the past, the present and the future, researchers at the ZEM study the general evolutionary aspects of, e.g. disease aetiology and disease patterns (prevalence, socio-economic stratifications, etc.). Primarily, musculoskeletal and joint diseases as well as the molecular evolution of disease pathogens are studied.
Impact of the Centre for Evolutionary Medicine

- Increasing interest in evolutionary medicine in leading scientific journals

- Value of ancient samples to study the evolution of disease is widely recognized

- Integral part of medical curricula in Anglo-Saxon universities (Johns Hopkins, Harvard, Durham, Auckland, Michigan etc.)

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**Nat Med, 2010**

**Nat Med, 2010**

**Science, 2010**
Evolutionary Medicine and clinically-oriented research on ancient samples

• Idea: to combine clinical methodology, clinical issues with physical anthropology / paleopathology

• “No biological problem is solved until both the proximate and the evolutionary causation has been elucidated.” (Mayr, 1982, p. 117)

• Not only about ancient health

• Hypothesis:
Samples consisting of mummified soft tissue and dry bone are crucial to the advances of clinically oriented evolutionary medicine research

Pyramid cell, Hippocampus area, ca. 1300 AD, (Papageorgopoulou et al., Neuroimage, 2010)
The value of ancient samples / mummies

- The value of historic remains for the study of the evolution of human morphology and disease patterns has become more and more recognized (Bosch, Lancet, 2000)
- Mummies unique: preservation of soft tissue
- Ancient mummies are an especially rich source of information about past human biological conditions as they originated world-wide and from different cultures and time periods (Cockburn and Cockburn, 1980; Aufderheide, 2003)
- Men is men’s best archive, tissues better than e.g. just visual or written records
- Both, mummies and skeletal remains are thus “ancient biobanks” of enormous scientific value
Specific contributions of mummy studies

• Mummies preserve soft tissues and are likely to yield more ancient DNA than skeletal remains.

• The examples of contributions made by mummy studies to our medical knowledge are increasing, particularly in the study of infectious diseases (Aufderheide, 2003, Lynnerup; 2007)

• Examples
  • Genetic analysis of “Spanish flu” from lung of frozen Inuit (Taubenberger et al., Nature, 2005; Reid et al., PNAS, 2000)
  • Prevention; “Influenza Immunity: New Insights from Old Studies” (Kilbourne, J Infect Dis, 2006, p.193)
  • Origin and evolutionary adaptation of Mycobacterium tuberculosis (Zink et al., J Clin Microbiol, 2003)
Mummy studies
Swiss Mummy Project

- Research Project at the University of Zürich since 1995
- Doctors, Egyptologists, Anthropologists, Chemists, molecular biologists etc.
- Egyptian / Roman-Greek / Peruvian / Medieval / Iranian Salt Mummies / Ice Mummies etc.
- Collections in Switzerland, Germany, Italy, France, USA, Australia etc.
Disease (e.g. cardiovascular / arthritis)
Questions for mummies

Analyses of different types of tissue
  • Differentiation
  • Pathologies, anatomical norm variations
  • Taphonomical changes

Individual level
  • Definition of sex and age
  • Cause of death
  • Facial reconstructions
  • Reconstruction of body height, progression of growth
  • Life and disease history
  • Archeological findings

Population level / Epidemiology
  • Social stratification
  • Evolution / prevalence of disease
  • Nutritional information

Hair of a roman-greek mummy (Confocal laser microscope 3D image, Papageorgopoulou et al. J Archeol Sci 2009.)

Omentum majus, Mammut „Lyuba“ (Masson-Goldner staining; Institute of Anatomy, University of Zürich)
Basic research: The issue of diagnostic imaging
Clinical MRI of ancient dry mummies without rehydration (Rühli et al., JAMA, 2007)

- 3D ultra-short TE sequence (UTE)
- Utilizes non-selective rectangular RF pulses of 60 µs
- Minimum echo time of 70 µs
- Transverse relaxation times (T2) ca. 300 µs
- Sagittal relaxation time (T1) ca. 5 ms
- 1.5 T (Magnetom Avanto®)
- Acquisition time: 0.5 - 3 h
- Knee coil, body arrays
- Spatial resolution: 0.8-1.1 mm
- Siemens Medical Solutions, Erlangen, Germany
How about ancient biomolecules?

Ancient DNA – Least stable biomolecules, most informative, has been recovered from plants, animals and pathogens.

Proteins – More stable than DNA, but less informative, classifying it to identify species for example. Secondly, looking at the elements, such as carbon, nitrogen, and strontium, to assess the diet and environment when then proteins were formed.

Lipids – Most stable but least informative. Lipids include fats, oils and hormones. Structures, such as cell walls contain lipids, which can be differentiated in different species.
Ancient DNA

Genetic ancestry and kinship analysis
- mtDNA similarity
- Y-chromosome similarity

Functional genetic variants, origin
- Lactase persistence
- G6PD deficiency

Paleoproteomics
- Soft tissue proteomics

Medieval skull, 1100 AD
Recent “ancient” studies

Substitutions in woolly mammoth hemoglobin confer biochemical properties adaptive for cold tolerance

Kevin L Campbell, Jason E E Roberts, Laura N Watson, Jörg Steterfeld, Angela M Sloan, Anthony V Signore, Jesse W Howatt, Jeremy R H Tama, Nadin Rahland, Tong-Jian Shen, Jeremy J Austin, Michael Hofreiter, Chien Ho, Ray E Weber & Alan Cooper

Affiliations | Contributions | Corresponding authors
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Proc Natl Acad Sci USA
Vol. 11, pp. 12657-12660, October 1998
Microbiology

Detection of 400-year-old Yersinia pestis DNA in human dental pulp: An approach to the diagnosis of ancient septicaemia

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3Institut Pasteur de Lille, France
4Biopolis, Institut Pasteur de Lille, France

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Immunological evidence of Plasmodium falciparum infection in an Egyptian child mummy from the Early Dynastic Period

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5Institut Pasteur de Lille, France

The emergence of HIV/AIDS in the Americas and beyond

M. Thomas P. Gilbert1, Andrew Rambaut2, Gabriela Wasiuk2, Thomas J. Spiro3, Arthur E. Pitchenik4, and Michael Worobey1
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State of the field

• Phase 1: Identification
  – Methods development
  – Identification and verification
  – Presence/absence of targets

• Phase 2: Characterization
  – Genetic diversity, diachronic characterization
  – Population-level analysis

• Phase 3: Application
  – Apply evolutionary insights to modern treatments
aDNA Clean Lab (Dr Warinner et al.)

- State-of-the-art laboratory for the extraction and analysis of ancient DNA from historical samples such as bone, mummified tissue or coprolites.
- To minimise contamination – apart from the constantly updated protocols of extraction and PCR – the access to this clean lab has been exclusively restricted.
• Grinding bone to powder (freezer mill with liquid nitrogen)
• Treat bone powder with sodium hypochlorite (decontamination)
• Decalcification with EDTA (1-3 days)
• Concentration and purification of DNA extract
• Pipetting a PCR reaction

• PCR amplification genes of interest
• Purification of PCR products
• Cloning
• Plasmid purification
• Sequencing
• Analysis
What can we learn from ancient biomolecules?

Human ancestry and migration

With the human genome project we understand much more of our genetic similarities and differences. This has allowed us to gain an oversight as to how modern humans spread across the globe. However, modern human populations have gone through a number of processes, such as bottlenecks and long distance exchange, which have obscured our past.

Evolution of human diet

Although the Neolithic revolution is known to have changed our diet, humans have had many more dietary shifts. For example, the introduction of fish in the Mesolithic, and the utilization of milk some time after mammal domestication.

Origins and spread of plant and animal domestication
Evolution of health and disease

Disease is caused by a number of factors. With infectious disease there is the pathogen and the body’s defense against the pathogen. Most serious pathogens have been investigated and an evolutionary scenario put forth, however, by adding to modern data with ancient DNA we have a much clearer idea of the evolution of disease.

For example, it was thought that three different strains of Yesinia pestis were responsible for the three major pandemics, however this was proven wrong, showing that the only way to identify the evolution of pathogens is to retrieve it from ancient samples.
Molecular evidence of HLA-B27 allele in a case of spondylitis ankylosans

m, 65yrs, ca. 1600 AD

Haak et al., Arthr Rheum, 2005

Table 1. Genotyping results in the historical individual “La Neuveville” and all researchers involved in the study*

<table>
<thead>
<tr>
<th>Researchers</th>
<th>Amelogenin</th>
<th>D3S1358</th>
<th>D8S179</th>
<th>D5S818</th>
<th>vWA</th>
<th>D21S11</th>
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<td>11/13</td>
<td>12/13</td>
<td>17</td>
<td>30/32.2</td>
<td>8/9</td>
<td>19/21</td>
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<td>l'd</td>
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<tr>
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<td>13/14</td>
<td>11/13</td>
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<td>15</td>
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<td>10/13</td>
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<td>28/30</td>
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<td>19/21.2</td>
<td>9/10</td>
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<tr>
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<td>14/15</td>
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<td>8/9</td>
<td>21/25</td>
<td>11</td>
<td>12/13</td>
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* Results shown are the typed alleles from 9 autosomal short tandem repeat loci and the sex-determining locus amelogenin. Individual “La Neuveville” was typed 6 times (3 times per extraction), and the researchers were typed at least twice. l'd = locus dropout (no amplification of long polymerase chain reaction products due to DNA degradation).
Molecular research at the Centre for Evolutionary Medicine?

Origins of European Dairying and Lactase Persistence

We are looking for the lactase persistance mutation in human remains from Europe in order to better understand when this mutation originally appeared.

Diet and Health Reconstruction from Ancient Calculus

We are looking different at dental calculus from a Swiss Medieval population. Proteins and DNA will inform us of the oral biota and of the diet of these peoples.

European Evolution of HIV-Resistance (CCR5 gene)

This mutation is present in Western Eurasia in high frequencies and predates HIV. It has been linked to resistance to a number of diseases, including bubonic plague and smallpox. We are investigating the frequency of this mutation in historic European populations.

Origins of East Asian Alcohol Intolerance

We are looking at three of these mutations that are common in Eastern Eurasia to attempt to understand their origin.
Δ32 heterozygosity does not protect against Plague.
Plague epidemics not cause of increased Δ32.
Smallpox responsible for the selective advantage?

G6PD-deficiency SNP analysis

Partially mummified toe, 2500 BP

G6PD gene, X chromosome, Xq28
What do we need as target pathogen?

Ubiquitous

DNA obtainable from multiple tissues

Endemic / co-evolutionary relationship with human populations

Global health consequences

Well-studied genome
Diagnostic challenges

• “Dirty” samples
  – High contamination, high bacterial load, metal contamination, soil acids and other chemicals

• Damaged samples
  – Molecular degradation, strand breaks, cross-linking

• Low quantity of target
  – Typical yield of ancient DNA from bone is only 4pg/mg bone, or about 1 copy of the human nuclear genome

• High frequency of non-specific reactions
  – Non-specific amplification of PCR products
    • New bacterial species discovered every day in our lab!
  – Cross-reactivity of immunological targets

Archaeobotanical extract, 6000 BP
Recent breakthroughs

- “Dirty” samples
  - Champlot et al. 2010: effective removal of contaminants in reagents
  - Kermekchiev et al. 2009 and Matheson et al. 2010: new DNA polymerases that can function in the presence of metals and soil acids
- Damaged samples
  - Heyn et al. 2010: new method for DNA repair
  - Gilbert et al. 2006, Hansen et al. 2006: better characterization of damage
- Low quantity of target
  - Roland et al. 2009: new high-efficiency extraction methods for bone
  - Gilbert et al. 2007: new extraction methods for hair
- High frequency of non-specific reactions
  - Next-generation sequencing: overcomes problem through massive parallel sequencing
What is still needed

Small target optimization protocols, 40-200bp
Low copy optimization of protocols (pg)
Robust recombinant enzymes
Faster, easier cloning
Cheaper Next-Gen sequencing
Better paleoimmunological tests
Future: Paleoproteomics (together with Functional Genomics Centre UZH ETHZ)

Soft tissue proteomics

- Protein preservation and identification
  - Little is known about protein preservation at the molecular level in mummified soft tissues
  - Ancient proteins have the potential to inform us about important medical states in the past that do not leave genetic traces
  - Will develop new methods for extracting, identifying, and quantifying paleoproteins
  - Will investigate protein preservation in the salt mummy soft tissues as well as in modern control samples
Experimental mummification of fresh human tissue
(Swiss National Science Foundation)

PCR amplification of fragments of different sizes within the HPRT1 gene in muscle at different time points. Amplicons from nt 14700-18206 (fragment A) and nt 31519-32802 (fragment B).
STR degradation ratio

* STR ratio: peak height of the smallest STR locus/peak height of largest STR locus

\[ y = 0.161 \ln(x) + 0.861 \]
\[ R^2 = 0.311 \]

\[ y = 0.215 \ln(x) + 1.096 \]
\[ R^2 = 0.329 \]

P. Cézanne, Young Man with a Skull, 1896-98
Skeletal studies

osteocytes, macerated bone
Papageorgopoulou et al.,
J Archeol Sci, 2009
In the skeletal system, opening of the sacral canal - called spina bifida occulta - became more common in cohorts born in the second half of the 20th century (Lee, Solomon, Ruhli et al., Eur Spine J, 2010).

Also more often in modern times:

- **Tarsal coalitions** (Solomon, Rühli et al., J Orthop Res, 2003).

- Skeletal pathologies such as **ossification of the posterior longitudinal ligament** (Hukuda et al., J Rheumatol, 2000) …

- … or **diffuse idiopathic skeletal hyperostosis** (Arriaza, AJPA, 1993).
Secular trend of incisurae vertebrales, ca. 1800 - 1950 AD

Rühli and Henneberg, Eur Spine J, 2004
Rühli et al., Am J Phys Anthropol, 2006
Dry bone series

- for population studies (morphology, microscopy, aDNA)

- as reference series

  - Reference series from Institute of Pathology, University of Zurich (UZH)(> 2000 specimen of bone pathologies, all major pathology categories represented, confirmed diagnosis (by autopsy reports), ca. 1830-1970
Spondylosis deformans: risk factors

Dahlheim
Ca. 1100 n. Chr.
N = 151

Figure 8: Sex-related probability of spondylosis deformans by individual stature and age group (adult and mature not significantly different).
The value of such a bone reference collection

- as a medical education tool
- allows an unprecedented insight in the natural history of a large number of diseases and is invaluable to paleopathologists
- many of its specimens demonstrate bone disease rare today
- bone pathology from before the introduction of treatment that has cured or changed its course in developed countries
- some aspects of the history of medicine as it evolved in central Europe during the formation of this collection
- number of autopsies decrease
- Less / no new non-forensic specimens
The Galler series

- DISH
- Tuberculosis
- M. Bamberger
- Gonarthrosis
- Osteomalacia
- Osteomyelitis
- Osteogenesis imperfecta
- Osteolytic tumor with compression of medulla
Database

Galler Collection

Object ID: 4217
Sex: female
Height: 130 cm
Patient #: 1064
Age: 64
Weight: 42 kg
Autopsy Nr./Yr.: 748/62
Profession: None

Object type: Whole bone
Anat. localisation: Right femur
Number of pieces: 1
Condition: good
Box: 88
Box location: Institute of Anatomy, University of Zurich


Further information (Patient):
Patient retarded from birth, sister was of normal mental health.

Further information (This Object):

Diagnosis (general):

Diagnosis (osteological):
Skeletal Lesion Healing Through Time (Holloway et al., unpublished)

Percentage of total lesions

- Not healed
- Healed by fusion of vertebrae (typical for TB in the past)
- Healed by bone deposition (e.g. Lipping on vertebrae)
- Healed by fusion of posterior elements

<table>
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<td>1960-1975</td>
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</table>
Biological Standards of Living – Swiss Armed Forces Data
Population of conscripts is almost identical (80-100%) with Swiss resident male population at age 19.

Staub et al., Eur J Clin Nutr, 2010
Staub et al., Swiss Med Wkly, 2010
Possible future issues

- Evolution of pathogens, corresponding human genome > personalized medicine (pharmacotherapy)
- Drug resistance (origins of common variants), ancient medicinal (identification from archaeobotanical remains)
- Secular trends in disease prevalence
- Impact of climate, society on human health
- Microevolution of human morphology (skeletal robustness, soft tissue structures)
- Incorporate into teaching at Medical Faculties / Health Sciences
No informed consent / posthumous harm?

- Appropriateness vs. invasiveness
- Code of ethics

Kaufmann and Rühli, J Med Ethics, 2010
Summary

State-of-the-art ancient DNA laboratories allow nowadays to analyse nuclear and mitochondrial DNA of several thousand year old human corpses.

Particularly infectious disease can be genetically analysed and also, forensic research questions can be addressed.

Diagnostic challenges such as highly fragmented target DNA is typical for any such sample and modern contamination a major issue.

New diagnostic tools such as next-generation sequencing shall help to overcome at least some pitfalls in the future.

Also, historic bone pathology reference series or other skeletal samples offer a unique glimpse into eg. pre-antibiotic era and allow addressing alternative treatment concept.

By studying human diseases and disorders directly through ancient molecular and morphological research, we can gain unique insights into the nature of human illness and better adjust medical treatments and public health policies.
Newsletter, webpage

- Semiannual ZEM Newsletter
- Webpage:  www.anatom.uzh.ch/zem
  www.evolutionäremedizin.ch
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• Institut für Technische und Makromolekulare Chemie, RWTH Aachen (Blümich et al.)
• Institut für Mumien und den Eismann, Bozen (Zink et al.)
• Swiss light source, Paul-Scherer-Institut, Villigen (Stampanoni et al.)
• Institut für Biomedizinische Technik, UZH /ETHZ (Müller et al.)
• Anatomical Sciences, University of Adelaide (Henneberg et al.)
• Departemente Radiologie / Pathologie, Krankenhaus Bozen (Egarter et al.)
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