Ancient DNA Analysis and Its Relevance in Forensic DNA Fingerprinting

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Abstract

The chapter presents the timeline, developments, nature, challenges, applications and prospects of ancient deoxyribonucleic acid (aDNA) technology. It also highlights the vision of future perspective that makes the field of aDNA technology so important and applicable to the people today. DNA is a blueprint of book of life that encodes genetic information. Human DNA consists of 3.2 billion nucleotides, comparable to letters in a book. DNA, the language of life, is present in all biological material—animals, plants. It degrades very slowly even after the so called death of living beings. This degrading DNA remains preserved in dead and fossilized living material is extractable and analyzable, called as ancient DNA (aDNA). Mostly aDNA is recovered from archaeological, anthropological, paleontological, museum specimen and clinical material. The age of aDNA ranges from 100 years, or even less, to millions of years and it has undergone natural environmental impact. Ancient DNA technology can be used in evolutionary biology, anthropology, medicine, agriculture, ancient diseases, living descendants by establishing a direct genetic link between a dead and a living individual, sex identification, animal or plant identification, kinship analysis, law enforcement and even determining the migration patterns of human populations. Due to recent ongoing development, the aDNA technology has become a factual, truthful, reliable and reproducible research field that recuperate DNA information

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from paleontological, anthropological and archaeological finds allowing us to study the genetic associations between past and the existent organisms.

Keywords

aDNA · Paleontology · Archaeology · Genetics · Inter disciplinary research

7.1 Introduction

DNA, abbreviation for deoxyribonucleic acid, is the basic life giving material in all species of animal and plants. It is generally present in the nucleus of cell in the form of a double helix and called as nuclear DNA. Besides nuclei, DNA is also present in mitochondria and hence referred as mitochondrial DNA. Genome is the name given to the total amount of DNA in a species. In human genome, mitochondrial DNA (mtDNA) consists of 16.5 kbp (kilo base pair) and nuclear DNA is 3 billion bp (Lander et al. 2001). DNA in humans is present in 46 chromosomes, which exist in the nucleus of all body cells except the red blood cells (RBCs). The human nuclear genome contains 5% coding region referred as exons and 95% noncoding region called as introns. The exons are highly conserved and the introns are highly polymorphic (Daniel and Walsh 2006). In each human body cell, DNA is present as a 6–9 ft long coiled thread. On unwinding of all DNA in human body cells it would reach to the sun and back one thousand times. It is interesting to note that sequencing reveals 99% similar DNA in all humans. Of the remaining 1% only, it's one-tenth, is responsible to make us unique (Feuk et al. 2006). Remember that human DNA is not unique as more or less of it is shared with many other past and present species of animals and plants. Thus 98% of human DNA is similar with that of chimpanzee and 50% is similar to that of banana. If we write the information in a single cell on paper it needs 46 volumes of Webster dictionary. DNA is a blueprint or book of life. Human DNA consists of 3.2 billion letters. Human genome sequence was completed in 2000, by a consortium of 18 laboratories in five countries. There are three chemical substances in DNA: (a) "deoxyribose sugar", (b) "phosphate group" and (c) "nitrogenous base". Of these three, deoxyribose, sugar and phosphate group, are similar in DNA of all persons, but the nitrogenous base differs as it characterizes individual constituent of the polymer. This variation gives differentiating characters in each person, which helps in individualistic identification. Each nitrogenous base consists of, as a general rule, one of the four letters of nucleotide; A, T, C and G. These four nucleotides—adenine, thymine, cytosine and guanine are complement to each other in a permanent order or sequence: A pairs with T and C pairs with G (Fig. 7.1). This complementation of four nucleotides makes a DNA molecule (Luftig and Richey 2001).

Nucleic acid molecule, as we know, is very long and called as macromolecules, which means that it is a molecule made up of smaller molecular units, which are called nucleotides. The nucleotide linked chemically to each other to form a chain. In DNA, the four nucleotides named as adenine, thymine, cytosine and guanine, as

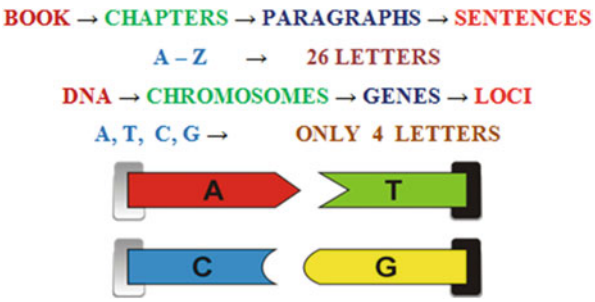

Fig. 7.1 Fundamentals of DNA and its constituents

mentioned earlier and A, T, C, G, respectively is their abbreviation. Another major nucleic acid, present in the cells, called as ribonucleic acid, abbreviated as RNA, which differs from DNA by replacing thymine with uracil (U). The sequence of the RNA nucleotides allows nucleic acid to encode genetic blueprint of an organism.

7.2 Ancient Deoxyribonucleic Acid (aDNA)

Ancient Deoxyribonucleic Acid, abbreviate as "aDNA", is a nuclear material, which is extracted from long dead biological organisms—plants, animals and humans, that were not preserved for the purpose of DNA isolation. Examples are all archaeological finds of biological origin, fossil remains, museum specimen and any other biological material not preserved for genetic or genomic studies but remained lying under natural environmental conditions over substantial period of time. DNA is very complex molecular structure, which starts degrading just after the death of organism as bacteria initiate the process of decomposition. Ancient DNA material sources are herbarium, museum specimens, bones, teeth, soft tissue and ancient plant seeds or compression fossils (Sutton et al. 1996; Poinar et al. 2001). Mostly ancient DNA samples possess less amount and highly degraded quality of DNA because of negative impact of various environmental factors such as microorganisms, humidity, light and heat, which may be both quantitatively and qualitatively inadequate (Bender et al. 2004). In archaeological, paleontological and anthropological finds, teeth and skeletal material are the best for sampling ancient DNA analysis as well as for forensics identification of missing persons and mass disasters. Biological material buried under debris for a long time has the ability of preservation for long time (Loreille et al. 2007; Seo et al. 2010).

7.3 Timeline of DNA Research

Let us go to the timeline of DNA research, which led us to successfully isolate and interpret the result of hereditary material. About one and a half century ago, in 1869, a young Swiss biologist, Johannes Friedrich Miescher, accidently found various phosphate-rich chemicals from the white blood cell nuclei. Friedrich Miescher got

his MD in 1868 from the University of Basal Switzerland. During his studies in 1865, Miescher worked in the organic chemistry laboratory of Adolf Stecker at Gottingen University, Germany, but due to his illness he had to interrupt his studies. Unfortunately his illness of typhoid fever impaired his hearing ability and he could not pursue medical profession like his father and uncle who were chair of Anatomy at University of Basal. Because of partial deafness, Miescher said good bye to medical profession and decided to pursue his studies towards physiological chemistry and joined University of Tübingen, Germany, at newly established faculty of natural science, under Felix Hoppe-Seyer, a chemist and physiologist, who later became the founder of biochemistry. Miescher was interested to study the chemistry of the nucleus of the white blood cell, but Hoppe-Seyer encouraged him to study neutrophil instead for the simple reason that it is difficult to get sufficient number of lymphocytes while it was known to get sufficient number of neutrophils in the pus, which could be obtained from the bandage of patients. The problem was how to wash cells off the bandage without damaging them. Miescher solved the problem, isolated the nuclei and on analyses of the contents he found a new compound from the nuclei of lymphocytes in 1868.

Young 24-year-old Miescher must have been amazed to find a compound that could not be characterized as a protein or lipid or a carbohydrate. He simply named it as "Nuclein" as it was isolated from the nucleus of blood cell. Interestingly Felix Hoppe-Seyer did not allow Miescher to publish it before his personal verification that took some time and finally Miescher published his findings in 1871. Nuclein remained in obscurity for 20 years till 1889, when Richard Altman, a German Pathologist, found the acidic properties of nuclein and renamed it as "nucleic acid". Today, this nucleic acid is known as deoxyribonucleic acid (DNA).

As is known now, nucleic acid research is the hub of current biomedical research. The reason of anonymity of nucleic acid in the nineteenth century was the publication of Charles Darwin's book in 1859, entitled "*On the Origin of Species*". The book generated hot debates on the theory of evolution, nevertheless by the 1870s, not only the scientific community but also people from the general public had started believing in evolution as a scientific fact. Another scientific investigation which was ignored at that time is the work of Gregor Mendel, "*Versuche über Pflanzenhybriden*" (Experiments on Plant Hybridization), who read it at two meetings (8 February and 8 March 1865) at the "Natural History Society of Brno", and published it in "Vehandlungen des naturforschenden Vereins Brünn" in 1966. However, Mendel work remained ignored till its rediscovery in 1900, by De Vries, Correns and Tsehermak.

7.4 Timeline of Ancient DNA Research

In the early 1980s, researchers started extracting DNA from different ancient sources such as fossil remains, archaeological finds, museum specimens, permafrost ice cores, fossilized faeces, and other uncommon antique biological sources. Such extractions were named "Ancient DNA". In 1984, the field of ancient DNA research

began with genetic analysis of Quaga (*Equas quagga*) DNA, an 140-year-old museum specimen (Higuchi et al. 1984). In 1985, the 2400 years old Egyptian mummies (*Homo sapiens*) were analyzed to understand ancient DNA technology (Pääbo 1985). In 1989, ancient DNA study divulged closest living relatives to extinct Tasmanian tiger (*Thylacinus cynocephalus*). In 1990, ancient DNA technology was introduced to readers by the best-selling novel Jurassic Park. In 1992, the analysis of aDNA from 3550 years old museum species revealed the relationships between moas (*Moa* sp.) and living flightless birds, e.g. ostriches (*Struthio camelus*) and kiwis (*Actinidia deliciosa*). In 1994, DNA of *Mycobacterium tuberculosis was detected in* 1000 years old archaeological specimens from Peruvian mummy (*Homo sapiens*). In 1994, DNA was extracted from 9000 to 50,000 years old Siberian woolly mammoth (*Mammuthus primigenius*) (Hänni et al. 1994). In 1997, mitochon drial DNA of Cheddar Man (*Homo sapiens*) from Britain's 9000-year-old complete skeleton was matched to a local Cheddar school teacher. In 1997, ancient malarial parasite (*Plasmodium falciparum) DNA was detected in a 60-year-old human* (*Homo sapiens*) *skeletal remains*. *In 1998, DNA extracted from* fossilized dung revealed the ecology (diet) of long extinct ground sloths (*Megatherium* sp.).

In 2003, archaeologists studied domestication of corn (*Zea mays tortillas*) from 6300-year-old Mexican wild grass teosinte. In 2003, DNA of ancient plants and animals was detected in 10,000–400,000-year-old Ice cores of Siberia. In 2005, researchers analyzed ancient DNA from 40,000-year-old cave bear (*Ursus spelaeus*) samples and their environment using a novel metagenomic approach. In 2005, DNA of ancient bottle gourds (*Lagenaria siceraria*) was compared with modern Asian and African varieties, and hence the scientists concluded that about 10,000 years ago, Paleoindians (*Homo sapiens*) brought gourds from Asia to Americas (Nicholls 2005). In 2007, researchers isolated oldest DNA (at least 450,000 years old) from southern Greenland cores that were compared with DNA of existing animals and plants. The comparison revealed DNA of conifers (Briggs et al. 2007). In 2008, for the first time, the woolly mammoth's (*Mammulus primigenius*) nuclear genome reported from an extinct animal. In 2010, both nuclear and mitochondrial DNA extracted from fossilized eggs of extinct elephant-birds (*Aepyonus* sp.), owl, ducks, emu (*Dromalius novaehollandiae*) and extinct moas. In 2010, scientists conducted genetic study on five 100-year-old royal Egyptian mummies to find out the relationship of these mummies to King Tutankhamen family and to indicate inherited disorders and infectious diseases in these mummies. The parents of king Tutankhamun were identified from these mummies and genes specific to *Plasmodium falciparum* (malaria pathogen) were found in four mummies. In 2010, scientists published the draft of genome sequence of 40,000-year-old material of Neanderthal man (*Homo neanderthalensis*), which play a role in the understanding of human evolution.

In 2011, DNA of the Black Death pathogen, *Yersinia pestis*, recovered from the 660-year-old skeletal remains of the people who died due to the "Black Death", and buried in East Smithfield Cemetery London. In 2011, fossilized fingertips and two teeth found in Siberia revealed the missing link of human species (*Homo*). In 2012, ancient syphilis pathogen (*Treponema pallidum*) DNA was isolated from

sixteenth- to seventeenth-century-old two newborns deceased affected by syphilis from birth. In 2012, genetic analysis of the 5300 year old frozen mummy of the Tyrolean Iceman, "Otzi", (*Homo sapiens*) carried out. In 2013, comparison of complete genomes of 1000-year-old *Mycobacterium leprae* (leprosy causing bacteria) with modern strains exposed little genomic changes, and indicated that leprosy in the Americas originated in Europe. In 2013, the genome of the oldest known European fungus, *Phytophthora infestans*, was compared with modern strains. The study revealed that *Phytophthora infestans* is an aggressive pathogen, which mutates rapidly in response to fungicides and use of new resistant potato crops. In 2013, the analysis of the 560,000–780,000 years old ancient horse (*Equas* sp.) genome exposed that the common lineage of present day zebra (*Equus zebra*), donkeys (*Equus asunus*) and horses (*Equus Caballus*) arose 4–4.5 million years ago. In 2013, analysis of DNA obtained from ancient dental plaque traced human diet from Neolithic European skeletons through modern times. In 2013, a unique family reunion was celebrated by the Australian and British descendants of Cheddar man in the cave where his 9000-year-old skeleton remains were discovered. In 2014, the analysis of DNA obtained from old bones of Clovis boy linked to American Indians and other native peoples in the Americas. In 2014, the genetic study revealed that Kiwis (*Actinidia deliciosa*) are the closest living relatives of the massive elephant-birds (*Aepyonus* sp.) (Gansauge and Meyer 2014).

In 2016, researchers were able to find out that all human beings are descendant of one common female ancestor called "The Mitochondrial Eve", who lived circa 150,000 years ago in Kenya (Jones 2016). Ancient DNA research has exposed the evolutionary history of earlier man, *Homo denisova*, *Homo neanderthalensis,* etc. and in future aDNA studies will further increase our knowledge about the history of human (Slatkin and Racimo 2016). The field of ancient DNA is an emerging field and currently passing through a phase of exponential growth (Woods et al. 2017). We, human beings, share our DNA genome with other living beings, e.g. chimpanzee (*Pan troglodytes*) [98%], slug (*Arion distictus*) [75%] and banana (*Musa* sp.) [50%] (Haviland et al. 2013).

7.4.1 Development of Ancient DNA Research

To extract and analyze DNA and/or genomes of ancient biological specimens, last decade saw a substantial development of approaches, that opened a new window to clearly understand the genotypes of past organisms, to know the place and time of their extinction and biological variation of species that took place as well as to gain clear insights that how they were evolved from the previous position/stage towards the existing (Orlando et al. 2013).

A massive, methodological transformation has undergone in the study area of ancient DNA technology since 1984 Higuchi and his colleagues initiated its research. The analysis of DNA from ancient biological samples was a laborious process at that time. However, the discovery of polymerase chain reaction (PCR) initiated a rapid progress in the field of ancient DNA research (Mullis and Faloona

1987). The organeller DNA, such as chloroplast DNA in plants, and mitochondrial DNA in animals, is protected by extra membranes and its multiple copies are found in the cells, therefore, the early analyses of the past organisms were focused on organeller DNA. However, the analysis of the nuclear DNA/genomes of ancient material made possible with the discovery of NGS, i.e. next-generation sequencing techniques. Insights into the geographical area and its extents, the types of organisms and taphonomy of ancient organisms were clearly understood with advancement in bioinformatics tools. The advancement in all these technologies provides new information about the identification of fossils and the precise association between past and present organisms, communities and populations, and gives insights into past fossilized life as well as present living organisms from the evolutionary point within a tree of life with the passage of time and environmental changes.

The new era of ancient DNA technology is very important as it provides new avenues in understanding the genomes of fossils and past organisms and even explore about their associations with their existing living organisms. To get new knowledge and breakouts in the field of aDNA technology, the collaborations and combination of multiple fields such as genomics, taphonomy, archaeology, anthropology, biochemistry, bioinformatics and chronology are very critical for making accurate predictions about the future status of existing organisms under future climatic change scenarios.

The increasing collaborations in multiple research fields, improvement of sophisticated sequencing, coupled with use of tools of bioinformatics, development of "New Generation Sequencing" based aDNA data sets, improvement of phylogenetic, denser ancient DNA material sampling around times of extinction and changes in the environment make it possible to use a potpourri of phenotypic and genotypic characters in both fossils and existing organisms. This approach allows us to know as to how populations and ancient species diverge, how diversification patterns develop and how they interact, which pave the way to evolve independently through periods of environmental change (Sjögren et al. 2017).

7.5 Techniques Used for DNA Extraction from Past Organisms

Extraction of DNA from past organisms has obvious impact in archaeology, anthropology and forensic DNA studies, however, the existing protocols used for DNA extraction are far from satisfaction (Kalmár et al. 2000). The extraction process of ancient DNA from the old specimens is very difficult because antique archaeological material is strongly influenced not only by environmental factors but also by microbial contamination, sample age, substrate properties, minimum amount of material to start, as well as presence of PCR inhibitors. From the plant fossils extraction of DNA is more difficult compared to animal fossils, because generally animal old skeletal fossils material possesses well-preserved DNA (Brown and Barnes 2015). In plant fossils, pollen grains are not only well preserved but found in abundance for ancient DNA extraction. The old skeletal remains contain inorganic minerals, which create hurdles during the extraction of DNA, therefore, the buffer

containing EDTA is used for incubation to demineralize these specimens and inactivates DNAses by chelating bivalent Ca^{2+} or Mg^{2+} cations (Hochmeister et al. 1991; Loreille et al. 2007; Barbaro et al. 2011). The extraction of DNA from ancient specimens and its amplification are issues that are important in current DNA technology. Occasionally, the history and condition of the old specimens is not known, however, to get good, reliable and reproducible results a standard and perfect protocol for ancient DNA extraction is needed. A wide range of DNA extraction protocols is available, e.g. alcohol precipitation, spin columns or silica columns. Davoren and his colleagues in 2007, made a comparison of the silica column-based and the phenol/chloroform-based DNA extraction technology for old skeletal materials. Significant results were obtained with silica column-based DNA extraction technique. Loreille et al. (2007) compared the phenol/chloroform and total demineralization DNA extraction methods. Maximum quantity of DNA was obtained with total demineralization extraction protocol using old bone specimens. In 2012, phenol/chloroform, total demineralization and crystal aggregates DNA extraction methods were compared for both fresh and old bone specimens. The phenol/chloroform extraction method was found excellent for fresh bones while the total demineralization extraction method was significantly better for old specimens (Jakubowska et al. 2012). Based on the previous history, silica column and total demineralization extraction methods were merged to develop a new modified silica column-based total demineralization extraction method for DNA extraction from ancient skeletal remains, which were highly degraded (Zar et al. 2013, 2015).

7.6 Applications of Ancient DNA (aDNA) Technology

Ancient DNA technology is an emerging field, which plays a key role in anthropology, archaeology, palaeontology, medicine, agriculture and law enforcement (Papiha et al. 1999; Burger et al. 2000; Pääbo et al. 2004). Some major applications of aDNA are mentioned as follow:

- **Historical Mysteries**: aDNA is used to identify an unknown person from his/her old skeletal material by matching his/her DNA to a known relative.
- **Human Evolution**: aDNA is analyzed to study human evolution.
- **Ancient Diseases**: The old skeletal remains and tissues of dead peoples may still contain pathogens. Therefore, aDNA is used to identify ancient diseases such as Bubonic Plague, Tuberculosis, etc.
- **Living Descendants**: aDNA is analyzed to establish a direct genetic link between a dead and a living individual.
- **Sex Identification**: aDNA is used to distinguish between male and female from the analysis of old skeletal remains or aDNA samples.
- **Animal or Plant Identification**: aDNA can be used to gain insight into prehistoric subsistence practices and the local economy through identification of archaeological/palaeontological remains of plant and animal species.

- **Migration Patterns**: aDNA can be used to ascertain the migration patterns of human populations by comparing the DNA of ancestral remains to that of people living there today.

7.7 Requirements and Challenges of Ancient DNA Research

Genetic markers are required for ancient DNA analysis. These markers must be polymorphic, i.e. marker level differences must be identifiable in the populations that are compared or among the individuals being studied. If differences are not perceived, then it will not be possible to differentiate individuals and about their movements, relationships and evolution. Several calculations are made to know genetic distance, which depends on genetic discrepancies and their number between individuals and/or populations. These genetic discrepancies are used to measure resemblance or dissimilarity between individuals or populations. Often, DNA data interpretation is carried out through comparison of the new data with previous data obtained from previous studies on the same genomic region. For this purpose, access to the previous data stored in the comparative database is crucial. Mitochondrial genome has the most widespread comparative database, because mitochondrial DNA (mtDNA) has been studied more than any other genomic DNA.

Mitochondrial DNA molecule is the preferred organelle for the analysis of ancient DNA because mtDNA persist in multiple copies per cell, having large number of DNA than nuclear DNA, which has single copy and diploid in any given specimen. Nuclear DNA is inherited, through haploid number of chromosomes, from both parents while mitochondrial DNA is inherited only through mitochondria of the maternal genome. Single-copy-chromosome markers (Y-Chromosomal DNA) are inherited through the paternal-lineage. If the analysis of mtDNA is successful, subsequent analysis of nuclear DNA (paternal-lineage or autosomal nuclear loci) may be attempted for ancient DNA studies.

Based on the results, ancient DNA studies are grouped as (a) DNA yield authentic and endogenous, (b) DNA yield contaminated or (c) DNA yield nil. This classification is actually carried out for authenticity of aDNA analysis. As clarified earlier that precise interpretation of ancient DNA data needs a well-defined comparative database for identification and classification of the new data. If no resemblance persists between the new ancient DNA (aDNA) sequences and other earlier reported aDNA sequences, then the interpretation of significance of newly sequenced aDNA becomes extremely difficult. In that case, removal of contamination also becomes very difficult. The uniqueness/variation of a sequence is considered as authentic ancient DNA data, provided all sources of contamination are recognized and eradicated. Some time, the novelty of sequences is so thrilling that contamination looks extremely doubtful, for example, the DNA sequences of *Homo neanderthalensis* were clustered outside the range of the current *Homo sapiens* DNA sequences (Krings et al. 1997, 1999, 2000; Ovchinnikov et al. 2000; Schmitz et al. 2002; Serre et al. 2004).

The analysis of ancient DNA, isolated from past specimens, is an emerging technology which is growing very rapidly. Regardless of pervasive interest in analysis of ancient DNA, the problems occur in ancient DNA analysis. The main problems in ancient DNA analysis are firstly, the contamination of samples with exogenous DNA and secondly, the inability to successfully produce good results from many past specimens. In most cases, majority of samples failed to produce results due to low copy number and highly degraded DNA, and fruitful analysis of past specimens is not achieved. Contamination of DNA samples is another big problem of DNA analysis, therefore, research strategies must be carefully designed to remove and identify all foreign DNA contaminants. Generally, ancient DNA study is extremely specialized and technical. It is fast emerging and quite expensive and needs widespread training. In ancient DNA technology, each step should be carefully planned, DNA samples are needed to be well preserved, and should focus on well-trained physical anthropologists, palaeontologists, archaeologists, geneticists, linguists, related researchers (Kelman and Kelman 1999; Kolman and Tuross 2000; Pääbo et al. 2004; Mulligan 2006).

The two other important challenges in ancient DNA analysis are Informed Consent and Intellectual Property. It is important to decide as to who has the right to give informed consent for ancient DNA research and who owns the information obtained from ancient DNA research are very difficult questions. Since the past specimens cannot provide consent, therefore, we must go to their descendants, but how can we come to know about their descendants, who controls the information about their descendants and past specimens? Will the information created or source of information are authentic, and the way of information sharing is appropriate or according to the descendant communities?

7.8 DNA Evidence Overview

The role of DNA in discrimination or elimination of a suspect in a criminal offense is becoming an important forensic tool with new advancement in its technology. In 1987, DNA was allowed as legal evidence in the United States of America judicial system. DNA as evidence on crime scene can be isolated from saliva, tissue, blood, hair or semen. DNA is also an investigative tool to solve problem of identification of individual human being. Some of the sources which contain DNA as evidence are mentioned below: carpets (analyzed for biological DNA evidence), automobiles and automobile seats (kidnapping, carjacking, rapes, etc.), Guns (for blood and associations made in assaults, battery, homicide case, etc.), Shoes (almost all types of cases), cigarette butts, hairs (battery, armed robbery, sexual assault and homicides), bones (identification of missing people, missing children, war casualties, reconstruction of bodies and mass disaster such as airplane crashes), teeth (identification of missing people, missing children, war casualties, reconstruction of bodies and mass disaster such as airplane crashes), fingernail (sexual assaults, rapes or murders), blood stains (sexual assaults, rapes or murders), semen stains (sexual assaults, rapes or murders), vaginal secretions (sexual assaults, rapes or

murders), chewing gum (saliva), razor blades (missing kids, missing females, etc.), ski masks (saliva and hair, bank robberies, carjacking, rapes or whenever the suspect wants to hide his identity), caps (hair), tooth brush (saliva), stamps and envelops (saliva), ear rings, nasal secretions, ear wax, watches, coffee cups, soft drink cans, beer bottles, etc., shirts (neck area for sweat), tooth picks, eye glasses (nose piece and the back of arm), hearing aids, condoms, saliva stains (threatening letters, or claiming the responsibility of certain crime in a letter), finger rings, bracelets, telephones, cell phones, computer keyboards, steering wheels and dashboards, forks and spoons, date pits and mango stones, gun triggers and magazines. *Locard's principle* states that "Wherever there is contact between two objects, there is a transfer of material between them".

7.9 DNA Database

A DNA database/databank contains profiles of DNA to be used in the analysis of DNA, fingerprinting, genetic genealogy and genetic diseases. DNA databases may be private or public, however, many developed countries have developed their large national database. In 1997, forensic DNA scientists prepared a standardized set of STR markers for identification of *Homo sapiens*. This is called "Combined DNA Index System" abbreviated as "CODIS". For CODIS project as many as 17 STR loci were identified and studied as candidates but only 13 were finally selected as part of the CODIS system. The 13 CODIS STR loci include: "TH01, TPOX, FGA, D21S11, CSF1PO, D7S820, vWA, D18S51, D8S1179, D13S317, D5S818, D16S539, and D3S1358". Among them, D21S11, D18S51 and FGA are the most polymorphic STR markers while TPOX displays the least variation between persons (Chakraborty et al. 1999; Butler 2001).

The benefits of DNA database are as follows:

- In case without any lead, DNA can efficiently and effectively identify suspects by eliminating possible suspects
- Identification of serial offenders by determining the links with different crime scene
- DNA analysis would serve as an investigative tool in tandem with finger prints
- Hard evidence in addition to soft evidence to be presented in the court of law
- DNA database profiles may act as deterrence and create awareness to some habitual offenders from committing crime in future
- Solution of past unsolved cases where DNA evidence was found and available but could not be used because of non-availability of DNA technology but linked with a present suspect offender and matched with the present crime scene
- DNA analysis could not be used in pre-DNA-database era and hence offenders from such cases could not be identified

Jerry Miller Case (DNA That Cleared One Man Implicate Another) The DNA profile obtained from Item(s) M16 and M17 was searched in the Indiana DNA

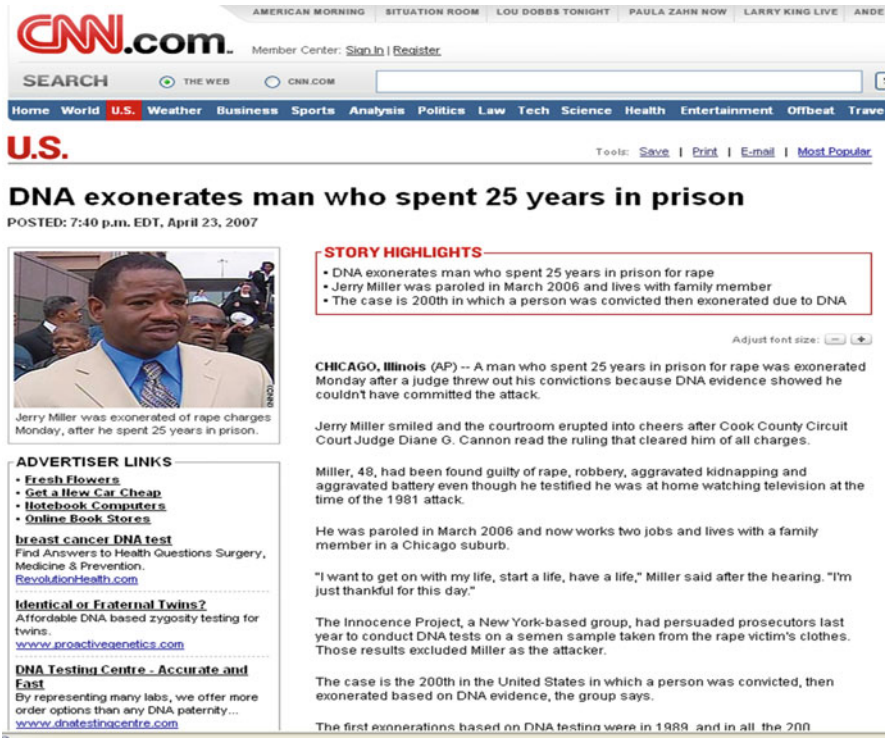

Fig. 7.2 Newspaper cutting highlighting Jerry Miller case

Database and was found to be consistent with the convicted offender sample of Jeffery Jones (DOC inmate #974912) (Fig. 7.2).

7.9.1 Importance of Forensic DNA Database Legislation

There are over 50 countries throughout the world that have already passed DNA Database legislation such as Europe (every country in Europe required by EU legislation for data sharing), the United States (every state has passed separate legislation), Australia (every state has passed separate legislation), Canada, Russia, Kenya, Malaysia, etc. No country has ever reduced the scope of DNA database testing done (Doleac 2017). Countries have only expanded database utilization based on:

- The effectiveness of the databases
- The security of the database systems
- NO example of database misuse anywhere in the world

Each country and state has had to address similar issues that arise in the context of Forensic DNA data basing in the context of its own legal, historical and cultural frameworks, for example—destruction of samples in Germany. Each DNA database legislation is different; there are many issues which, after extensive scientific and legal research, have been addressed in very similar ways (Levitt 2007; Wallace et al. 2014). Some of these issues include:

- The protection of Human/Constitutional and other legal rights.
- Sample and profile retention
- The type of sample to be collected and who may collect it

7.9.2 Common Features of DNA Database Authorizing Legislation

- Entry criteria
- Sample collection
- Removal criteria
- Sample retention
- Database access

Legislation is required for:

- Effective utilization of DNA analysis facility of all forensic labs
- Eradicate the confusion of admissibility of DNA evidence
- Ensuring security of DNA profile
- Linking victim and suspect
- Linking suspect/victim with crime scene

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