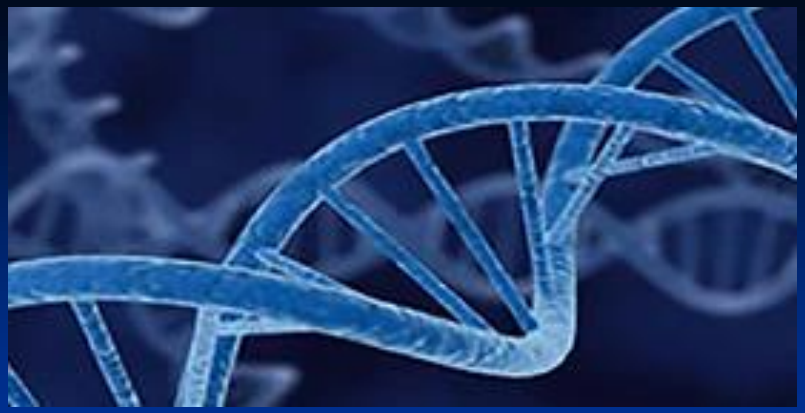

IDENTIFICATION OF THE LIVING AND THE DEAD

Forensic Medicine Lecture series

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Lecture outline

- Blood serology
  - Principles of BG inheritance
  - Applications of BG/BG serology in identification

- Identification by DNA profiling
  - Methods of DNA typing
  - DNA technologies used in forensic investigations
  - Disputed parentage
  - DNA forensics databases
  - Ethical, legal & social concerns; limitations

BLOOD GROUP SEROLOGY.

- Blood groups: Classification of RBC by antigens on RBC membrane
- 41 formally registered blood group systems (ISBT) (e.g. ABO, Lewis, MNSs etc.)
- 45 genes responsible for these systems have been identified and sequenced; associated polymprphysms known
- ~339 BG Ag identified
- A person has the same blood group for life
- Very rarely an individual's BG may change through addition/suppression of an antigen by infection, malignancy, autoimmune disease, BMT/organ transplant

Applications of BG and BG serology:

- To show whether blood stains (on weapon, clothing etc) are from a particular suspect
- Matching fragmented human remains
- Help resolve parentage/inheritance

PRINCIPLES OF BG INHERITANCE.

- Autosomal inheritance* BG genes passed from parents → offspring
- One gene/allelic pair derived from each parent.
- BG antigen in child must be present in at least one parent.
- If parent homozygous for allelic pair (AA, BB) one gene must be present in his/her offspring.

*Except for XG blood group system (Xg (a); MIC2 gene (CD 99) on pseudoautosomal region of X and Y chromosome

BLOOD GROUP INHERITANCE

Parent 1	Parent 2	Possible offspring	Impossible offspring *
O	O	O	A, B, AB
A	A	O, A	B, AB
A	AB	A, B, AB	O
B	AB	B, A, AB	O
O	AB	A, B	AB, O

BG applications

- ABO major blood group system
- 80% of individuals secrete water soluble BG in body fluids
- Other BG systems useful – Rh, Lewis etc (Obst./BT)
- Disease setting/associations – eg AIHA
- MN blood group legal paternity cases

BG application

- Red cell antigens that can be typed:

- MNS, Rh, ABO, Duffy, Kidd, Kell, Lutheran

- Serum proteins systems that can be typed

- Haptoglobulins, C3, Gm, Gc, ADA, AK and others

- Red cell enzymes that can be typed:

- Erythrocyte acid phosphatase, glutamate pyrurate transaminase, phosphoglucomutase and others.

- Using above combined systems one can exclude in 93%. (i.e. the more systems used the better)

- BG systems can exclude a man from parentage but cannot totally confirm paternity
- DNA profiling has now become widely available and is more often used
- Initial serologic testing may be employed where DNA is not available although it has major limitations

DNA FINGERPRINTING/PROFILING

- Jeffrey Glassberg (American biologist), first patented use of DNA variation for forensics in 1983

- In 1984 Sir Alec Jeffreys (British geneticist) with others used DNA to help solve the rape and murder of two teenagers in Leicestershire in 1983 and 1986.

Sir Alec Jeffreys

DNA fingerprinting/profiling

How forensic ID works:

- Identification of DNA sequences that are unique to a species can identify any organism
- DNA is present in nucleated cells
- DNA is extractable from body fluids
- 99.9% of the genome is the same in humans; the remaining 0.1% shows variations between individuals
- These variable DNA sequences - polymorphic markers - can be used to both differentiate and correlate individuals

DNA forensics cont

- DNA regions that vary from person to person are scanned and used to create a DNA profile of that individual (DNA fingerprint). Usually 13 regions used
- Extremely small chance that another person has the same DNA profile for a particular set of regions
- Sufficient DNA is different to distinguish one individual from another, unless they are monozygotic twins

DNA in identifying persons

- Portions of the DNA sequence that vary the most among humans must be used
- Portions must be large enough to overcome the fact that human mating is not absolutely random
- A match at all would then be rare enough for confidence "beyond reasonable doubt"

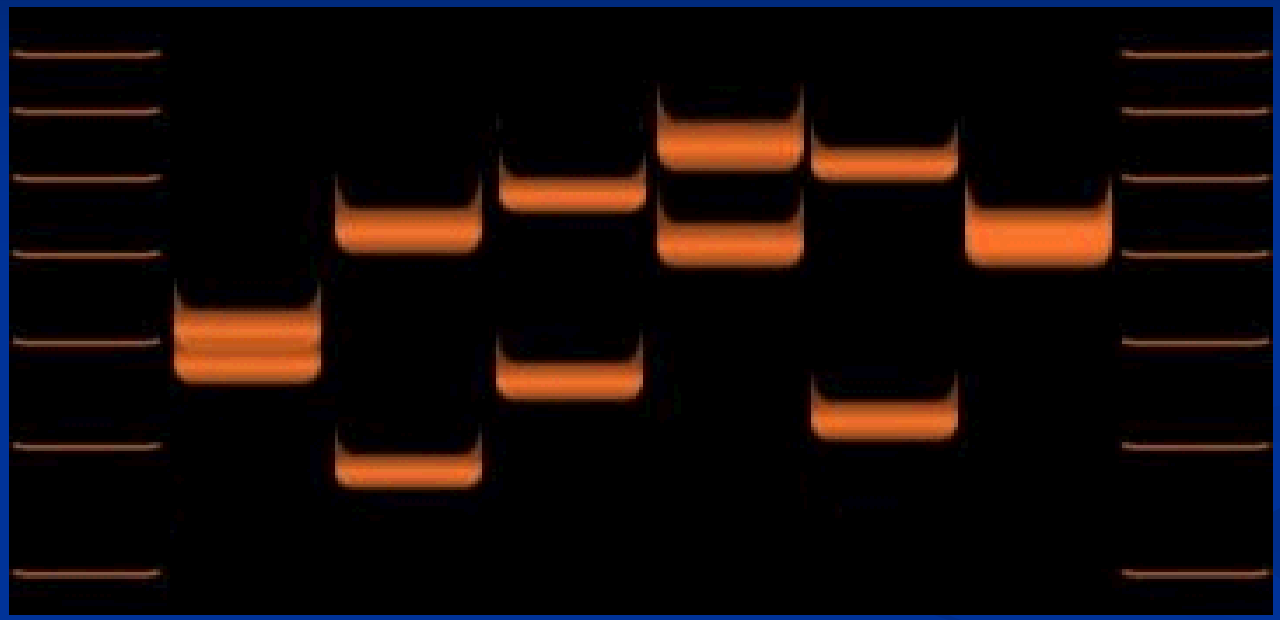
DNA profiling

- Only 0.1 % of DNA (about 3 million bases) differs from one person to the next
- 10% DNA molecule used for genetic coding
- These variable regions are used to generate DNA profile of individual
- Chances of 2 unrelated individuals having same sequence 1:1 million billion
- Siblings (not identical twins) 1:10, 000 million

DNA profiling cont

DNA profiling uses

- Variable number tandem repeats (VNTR)
  - Repetitive sequences that are highly variable,
  - VNTRs loci are very similar between closely related humans, but extremely variable in unrelated individuals
- Short tandem repeats (STR)
  - A type of VNTR also called microsatellite or minisattelites
  - consists of a unit of two to thirteen nucleotides **repeated** hundreds of times in a row on the DNA strand.



Variations of VNTR allele lengths in 6 individuals.

Technologies used in DNA profiling

RFLP analysis

(Restriction fragment length polymorphsm)

- The first methods for finding out genetics used for DNA profiling involved restriction enzyme digestion, followed by Southern blot analysis.
- Southern blot technique is laborious, and requires large amounts of un-degraded sample DNA
- Method not much used now

DNA profiling cont.

PCR analysis

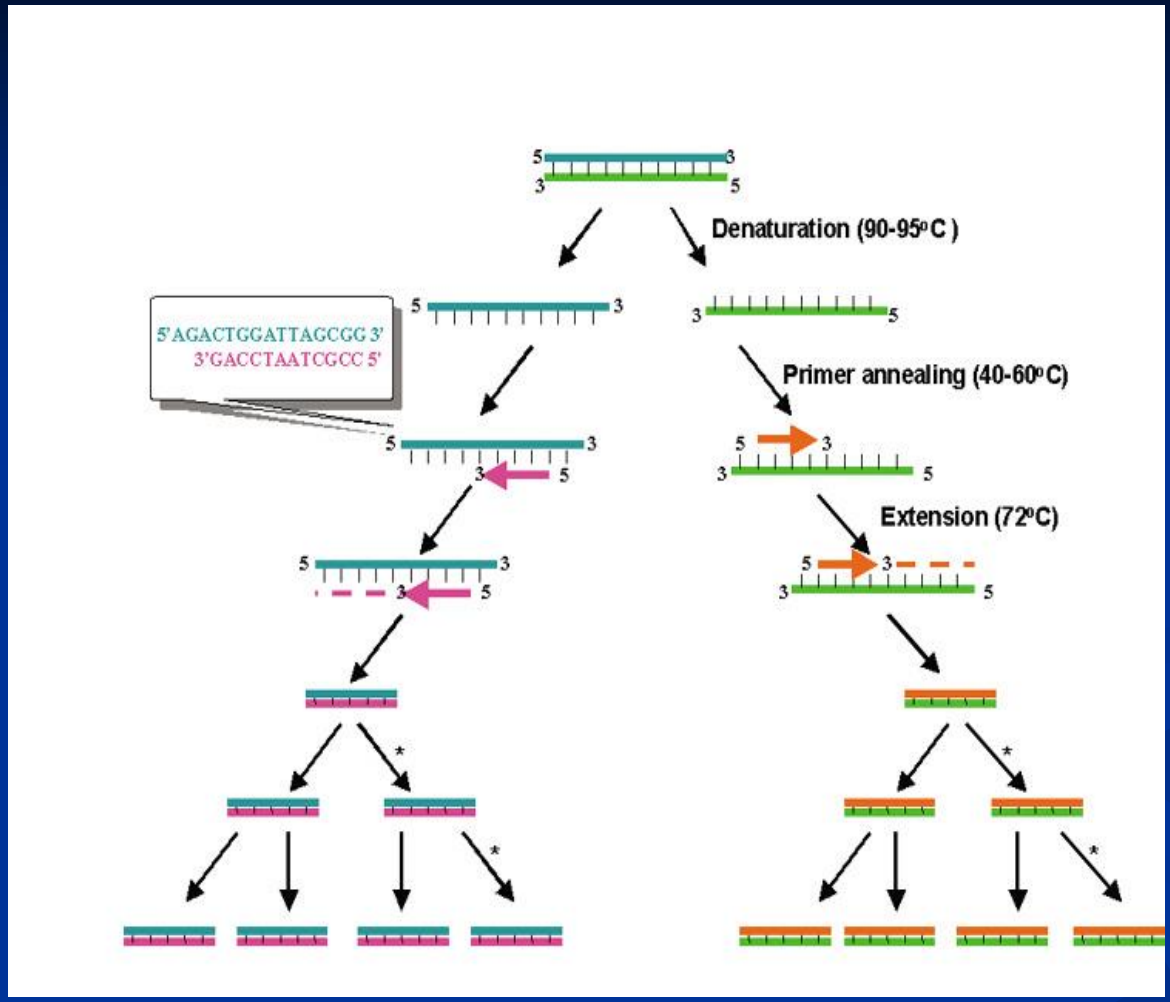
- DNA profiling using PCR analysis gives better discriminating power and the ability to use minute (or degraded) samples.
- PCR greatly amplifies the amounts of a specific region of DNA, using oligonucleotide primers and a thermostable DNA polymerase
- After amplification, DNA Gel electrophoresis is usually performed for analytical purposes
- DNA profiling used today is based on PCR and uses STR

1. The sample of DNA is placed in a solution containing free nucleotides and the appropriate enzyme

2. The complementary DNA strands are seperated

3. New complementary strands are formed using nucleotides from the solution

4. The cycle is repeated (usually 20-30 times)



Copying DNA using polymerases: requires 2 primers, each complimentary to the opposite strand of the denatured DNA

Polymerase chain Reaction

- PCR – copies small DNA fragments a million fold
- Can be done on very small degraded old samples e.g. licked envelope, stamp, single hair root.
- DNA fingerprinting 1st developed as ID technique 1985
- Useful in – identification of human remains

  comparing crime scene evidence with suspect
- Great care needed to prevent contamination with other biological materials

Short tandem repeats (STR)

- Typically each STR allele will be shared by around 5 - 20% of individuals.
- Unrelated people have different numbers of repeat units so STRs can be used for discrimination
- STR loci are targeted with sequence-specific primers and amplified using PCR.
- Separation and identification of STRs is done using electrophoresis (capillary electrophoresis instrumentation is the gold standard for human identification)

Next-generation sequencing

- Crime laboratories can analyze targeted and relevant forensic markers to generate investigative leads and help determine the number of contributors in a mixture analysis

- The application of NGS is particularly helpful with those degraded samples that may not provide a full profile using traditional capillary electrophoresis–based methods

Short tandem repeat (STR) analysis

- STR used to evaluate specific regions (loci) within nuclear DNA
- High variability in STR regions can be used to distinguish one DNA profile from another
- Use in Criminal investigations
  - FBI uses standard set of 13 specific STR regions for CODIS (Combined DNA Index System)- software program operating in local, state, and national databases of DNA profiles from convicted offenders, unsolved crime scene evidence, and missing persons.

Amplified fragment length polymorphism

- AmpFLP, started early 1990s.
- Uses VNTR polymorphisms
- Relatively low cost, easily set-up and operation makes it popular in lower income countries.

IDENTIFICATION BY DNA PROFILING.

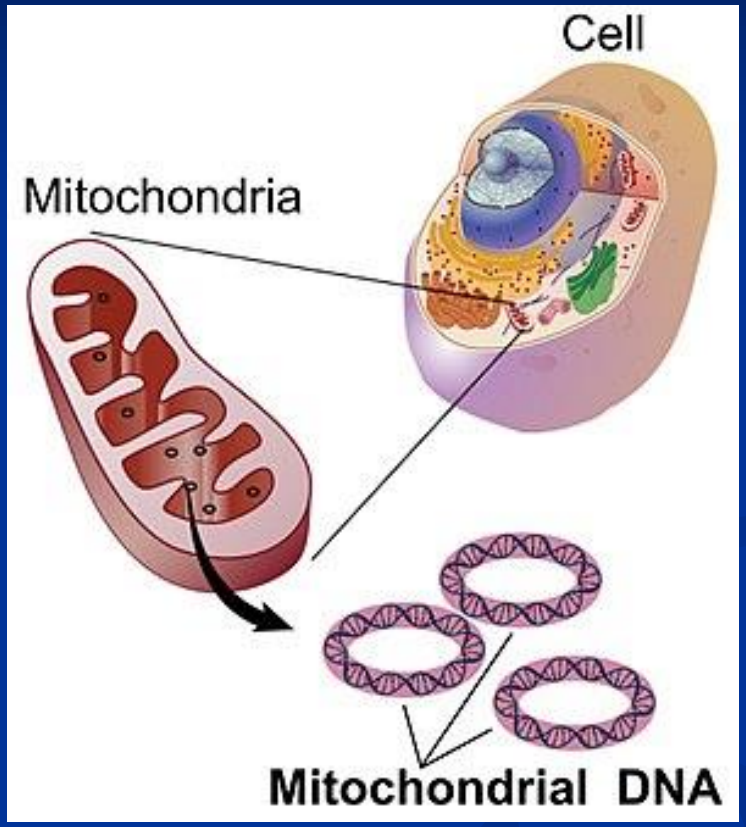
- DNA in hair root bulbs, nucleated hair can be profiled
- DNA from different sources can be matched (i.e. hair bulb/semen/ blood/ saliva etc)
- DNA data base can be created.

Mitochondrial DNA Analysis (mtDNA)

- Mitochondrial DNA - small circular chromosome found inside mitochondria
- Passed from mother to offspring through egg
- mtDNA – useful in examination of DNA from samples that cannot be analyzed by RFLP or STR
- mtDNA analysis uses DNA extracted from mitochondria
- Older biological samples that lack nucleated cellular material – e.g. hair, bones, and teeth, can be analyzed with mtDNA

Mitochondrial DNA

- 'Powerhouse' of the cell
- Human mtDNA includes 16,569 base pairs and encodes 13 proteins
- mtDNA permits an examination of the relatedness of populations
- important in anthropology, biogeography



mtDNA cont…

- Mothers have the same mitochondrial DNA as their daughters (Embryo's mitochondria come from egg)
- Useful in missing-person investigations.
- mtDNA is extremely useful in the investigation of old unsolved cases

Y-Chromosome Analysis

- Y chromosome is passed directly from father to son
- Analysis of genetic markers on the Y chromosome useful for:
  - Tracing relationships among males
  - Analyzing biological evidence involving multiple male contributors

Examples of DNA Uses for Forensic ID

- DNA probes designed that will each seek out and bind to the complementary DNA sequence
- A series of probes create a distinctive pattern
- In criminal cases involves obtaining samples from crime-scene evidence and a suspect, extracting the DNA, and analyzing it for the presence of a set of specific DNA regions (markers)

DNA ANALYSIS cont…

- Identify potential suspects whose DNA may match evidence left at crime scenes
- Exonerate persons wrongly accused of crimes
- Identify crime and catastrophe victims
- Establish paternity and other family relationships
- Identify endangered and protected species as an aid to wildlife officials (could be used for prosecuting poachers)

DNA ANALYSIS cont…

- Detect bacteria and other organisms that may pollute air, water, soil, and food
- Match organ donors with recipients in transplant programs
- Determine pedigree for seed or livestock breeds
- Authenticate consumables such as caviar, champagne and wine

DNA ANALYSIS cont…

- Monitoring bone marrow transplants
- Detecting fetal cells in a mother's blood
- Tracing human history
- Development of newer instrumentation and techniques making DNA profiling easier, faster, cheaper and more widely available

Some Interesting Uses of DNA Forensic ID

- Identifying the victims of the September 11, 2001
- Various air crashes: Ethiopian Airlines in 2019 etc
- Disappeared Argentina children 1976-1983
- Migration Patterns (genetic anthropology, migrations, human lineage)
- Wine heritage (18 of world's great wines are closely related)
- Poached animals
- Burns victims in various tragedies
- Tracing ones roots – African Americans

Disputed parentage

- In practice this means disputed paternity as maternity rarely in doubt.
- Exceptions exist: Where claims of swapped or stolen babies (Bishop Deya saga!)
  - A married man claiming wife has committed adultery and disputes being father of child/children
  - A woman with illegitimate offspring may allege that a certain man is the father to obtain an affiliation order

Paternity testing cont…

- Court may request paternity determination
- Submission to blood testing agreed upon (court orders needed otherwise)
- Child's consent necessary (over 16/18 years)
- DNA profiling is used as more positively discriminating than BG serology
- Blood taken from child and putative father and mother
- No blood transfusion should have been administered within 3 months of sampling

Disputed parentage analysis

LOCUS	CHILD	FATHER	MATCH
DSS1358	14/16	16/16	YES
VW3	18/18	17/18	YES
FGA	22/23	22/23	YES
AMELOGENIN	X/X	XY	YES
D881179	14/15	14/15	YES

- Use of several loci (depending on race) e.g. 16
- If all match 99.999% probability of paternity
- A calculated probability of > 99.9 is taken as proof of paternity

CONROVERSIES OF DNA FINGERPRINTING.

- Accuracy
  - DNA segments not complete strands (whole genome sequencing can be done)
  - Standards of the procedure
  - Human error
- Costs
- Misuse
  - Use of DNA information by unauthorized persons for unauthorized purposes

Ethical, legal & social concerns of DNA data banking

- The primary concern is privacy:
  - Susceptibility to particular diseases, legitimacy of birth, and predispositions to certain behaviors and sexual orientation
  - Potential for genetic discrimination
  - Who is chosen for sampling also is a concern:
    - Police officers provide the state with intimate evidence that could lead to "investigative arrests."
  - Retention of possible innocent persons DNA

Conclusion

- BG may be used to identify the living and the dead
- Currently identification is by DNA profiling
- Only ~0.1 % of DNA differs from one person to the next
- Portions of DNA sequences that vary the most among humans must be used for identification
- DNA profiling uses VNTR and STR
- mtDNA - examination of DNA from samples that cannot be analyzed by RFLP or STR
- DNA technologies are used in various forensic investigations, disputed parentage
- There are ethical, legal & social concerns and limitations in DNA profiling

Questions?