

RECENT TRENDS IN DENTAL FORENSICS

Elza Auerkari

Department of Oral Biology, Faculty of Dentistry
University of Indonesia, Jakarta

Abstract

Teeth are the most robust tissues of the human body, and usually most resistant to post-mortem decay. The dental patterns tend to be highly individualized and are therefore very useful for identification if appropriate records are available for comparison. For these reasons, dental evidence remains important in forensic cases after accidents, crime or prolonged exposure to the environment. Routine antemortem dental records are applied for storage and retrieval of such information but unfortunately not always available. The same is true for DNA typing, which is an increasingly useful approach with decreasing cost of analysis. This paper aims to review some of the new developments, particularly in biochemical forensic tools and methods that can be applied also for dental samples. No new tool will solve all cases, and it remains necessary to apply an array of techniques for post-mortem identification. It is also very important to use established protocols for efficiency and quality assurance in forensic investigation. Some challenges are highlighted for forensic application in Indonesia.

Key Words: dental forensics, dental records

Introduction

Teeth are the hardest and most robust tissues of the human body, and often resistant to decay even in difficult cases of accidents, crime, burial or other severe exposure to the elements. In addition, the dental patterns are characteristic for individual identification and therefore available for useful comparison if records exist for the purpose.¹ Therefore, dental characteristics often provide valuable information to establish postmortem individual identification of unknown human remains, especially when conventional soft-tissue criteria do not provide satisfactory results. Routine antemortem dental records can be used for information storage and retrieval to assist in establishing postmortem identity. Therefore, a specialist in dental forensics is often a part of the forensic team that is dispatched to identify and characterise the victims of crime, accidents and other cases where forensic services are needed.

The tools and techniques of dental forensics have naturally developed with the general advances of biological and medical sciences. In the early days, much of the comparative work had to be based on observations on e.g. missing teeth, other dental records if available, dental posture indicating likely ethnic origin or other more or less general characteristic features.¹ More recently, the dentists' records include x-ray images on dental postures and also records on restorative effort that tend to make every individual practically unique for identification – provided that a sufficient set of teeth and the records are found for the comparison.^{2,3} This is unfortunately not self-evident in all cases in the developing countries like Indonesia, where dental services and systematic records do not necessarily cover the whole population. However, much indirect evidence may still be amenable for forensic reasoning, when all clues and evidence are carefully combined. In recent disasters with thousands of victims, under circumstances where dental, fingerprint and even some DNA records were rather

ideally available, identification was mainly based on dental evidence in some 50-70% of the cases, on fingerprints in 20-35%, and on DNA evidence in 3-20% of the cases. Other physical evidence was applicable typically only in one to few percent of the cases.^{1,2,3} While all evidence is important and the relative weight varying by case, this clearly shows the potential and importance of dental evidence in forensic identification (Figs 1-3). However, such a disaster will require a major effort for successful victim identification: for example after the 2004 tsunami hundreds of dentists were involved for more than a year in all countries where the major impact was felt. This disaster was also an example of a case

that required wide collaboration not only between different professions and disciplines – which is in any case the rule in forensic work - but also across national boundaries. Interdisciplinary and cross-border collaboration as well as traditional comparisons are far easier with common databases that can be referred to. Build-up of such databases is one aspect of past, recent and on-going development to support efficient forensic identification.

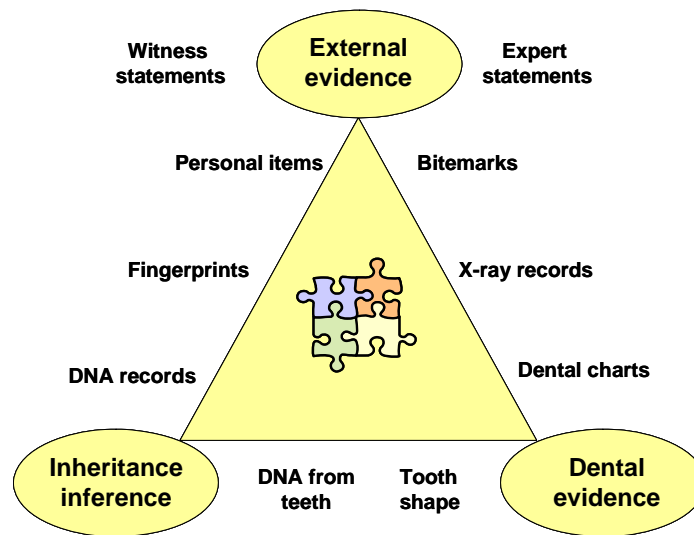


Fig 1. The essential elements of evidence for forensic identification of e.g. victims or culprits of crime or accidents

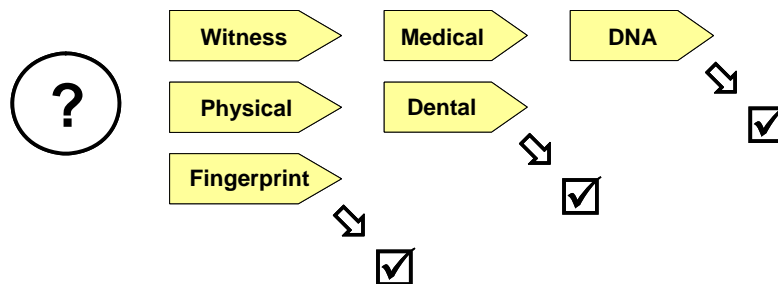


Fig 2. Typical order of applied tools for forensic identification according to availability and cost; any individual item can be decisive but combinations provide strength to evidence

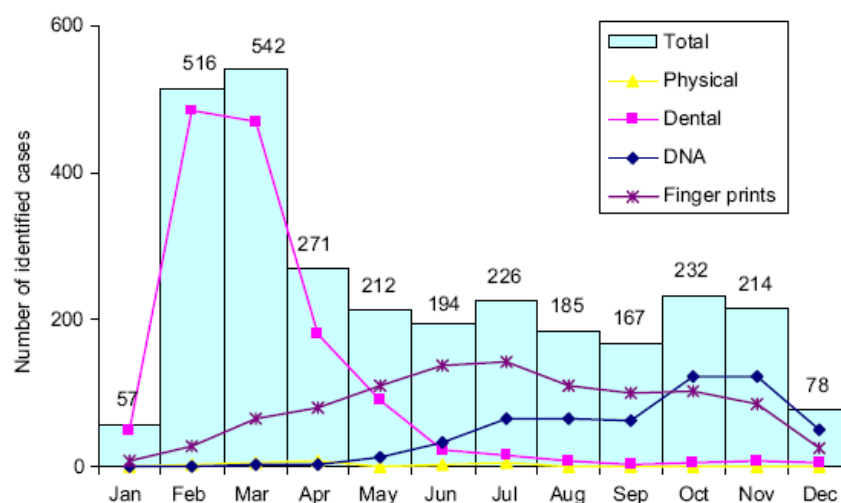


Fig 3. Principal identification tools after the December 2004 tsunami for the victims in Thailand, where 46% of the identifications were based on dental evidence³

The advances in molecular chemistry and genetics also provide increasingly powerful tools for the forensic services. Such tools are in principle not limited to particular tissue types, but post-mortem decay and other damage may well result in difficulties to obtain uncontaminated or otherwise useful tissue material for identification. In such cases it is osseous and dental tissues that again tend to retain the material of best quality for individual positive identification.

Below, a brief review is outlined of the recent developments in dental forensics regarding organised collaboration, databases for forensic comparison, and applied molecular chemistry and genetics.

Developments in cross-border collaboration and databases for comparison

It is a well established rule that a conclusion is progressively strengthened by increasing number of independent evidence pointing to this conclusion. This alone makes the case for interdisciplinary forensic effort that can provide evidence from different views. In the past the forensic work used to be an effort of a single person, as it still can be in simple cases and in local areas with limited resources. This has grown to systematic organised work of increasingly complex forensic teams involving people with varying professional backgrounds. Such organisation has brought more dental expertise to the forensic teams, and has also

required that the dental professionals involved are well aware of the organisation, requirements and practices of forensic work. Although the input from dental evidence can be very important, the forensic teams or departments are mostly run by people with legal or police backgrounds, and in case of biological type of evidence, mostly by pathologists. Any dentist in the team must know some principles that are important to the working principles of the other members of the team, and the proper treatment and order of treatment for potential evidence to obtain maximum benefit of the teamwork.

In general, methods with roughly comparable identification capability are usually applied in the order of increasing cost (cf. Fig 2). This is currently one aspect of relative strength for dental forensics in comparison with e.g. DNA analysis, but one should not forget that the first line methods of physical evidence are cheaper still and applied first. The first line techniques are mostly used by the law enforcement professionals that initiate the process of identification, and also coordinate the collaboration between different disciplines, including decisions whether particular effort needs to be sought on e.g. dental evidence.

Well organised smooth collaboration is not only needed in those individual cases where conclusive evidence is hard to find, but also and especially in mass disasters and other extensive accidents with multiple victims that can be in poor state because of severe trauma, dismemberment, or effects of fire, impacts or extended decay. It is such

cases that will test the performance of the forensic teams, or teams because in very large disasters many teams will have to combine their forces. To facilitate good performance, the forensic teams must work to well defined procedures that document every step of the analysis and its outcome. As crime and disasters do not respect regional or national borders and because there can be benefits in pooling information from different sources, there is internationally wide interest in commonly understood forensic procedures. As the problem setting is similar in different areas and countries, there is indeed considerable scope for standardisation or unification of procedures. In the same time, unification should not hinder further technical development. Recommended procedures have been established for

common techniques and the overall identification process in most countries including Indonesia. In addition, international recommendations exist e.g. as Interpol documents.

Identification can be obtained directly from witness statements, accompanying documentation or other strictly personal items. However, in many cases positive identification is only possible by comparison of the personal features of the subjects with some pre-existing recorded data that can be used for differentiation. For example, fingerprints, lip prints, DNA analysis results or dental patterns are not very powerful tools unless they also exist in some accessible database from which data can be retrieved for forensic comparison (Fig 4).

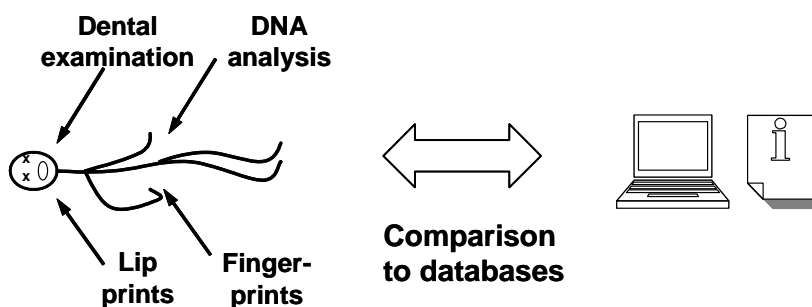


Fig 4. Databases for forensic comparison are usually necessary for positive identification

Another established rule suggests that the identification power of an item of evidence is strengthened by increasing uniqueness, i.e. decreasing likelihood of the indication to occur by chance (or error). This is why for example fingerprints and DNA can provide strong evidence, as they are very rarely similar for two different individuals. However, dental evidence can be even more powerful because the dental patterns are not equal even for identical twins. As noted above, this requires databases for meaningful comparison, and this is where there appears to be scope for further development in Indonesia. The dental records that are prepared should be stored in such a way that they are centrally retrievable later for possible forensic purposes. Even then, a relatively large fraction of the population is likely to remain outside the database for a considerable time to the future.

Developments in molecular biology for forensic identification

The post-mortem forensic identification aims to use any available information to confirm the identity of the subject. In this process, positive identification may depend on very small fragments of such information, however using first the information that is most easily obtained and only if necessary moving to methods of higher cost. For example, if the subject can be identified from identity documents or recognition by relatives or friends, there may be no need to proceed to more complex, slower and costly comparisons with biochemical, medical or other testing.

Therefore, only when the simpler techniques are insufficient, it may make sense to apply more tedious methods such as comparisons to dental radiographs or genetic fingerprints. This is not only a question of cost, because the background data for such comparison may be also difficult to acquire. As noted above, X-ray images may not exist or they can be inaccessible for the forensic comparison. The genetic fingerprints have here some advantage if relatives can be tested, because close relatives share

much of their genetic traits and therefore indicate kinship in DNA testing. This will also require access to consenting relatives, which may not be easy or trivial in all cases.

The methods that have seen most significant development during the recent two decades are relying on DNA-based identification. The principle is the same in all of its forms: the DNA is practically unique to all humans, except for identical twins. Therefore, by analysing in sufficient detail the structure of the DNA, its owner can be identified beyond any reasonable doubt provided that a comparison can be done to archives, relatives or other sources.

A traditional method of DNA analysis (typing) involves extraction of DNA, which is denatured and hybridized with a primate-specific aliphoid DNA repeat probe D17Z1 (slot blot hybridization). This procedure can be completed within one day to detect human DNA in quantities less than one nanogram.⁷ An even more sensitive semi-automatic method that applies direct real-time polymerase chain reaction (PCR) has become the approach of choice to identify mass disaster samples. After PCR amplification, DNA analysis can be done by typically typing 10 to 17 different loci of short tandem repeats (STR), and many commercial kits are available for this purpose.⁷ In this way, STRs are sufficiently variable to provide powerful differentiation between individuals.

When the environmental degradation is so severe that the usual STR typing does not result in reliable identification, other alternatives are also available. One now common alternative is typing the mitochondrial DNA (mtDNA) instead of the DNA in the chromosomes. There are thousands of copies of mtDNA in each cell, and the circular structure of mtDNA may also give some protection against damage. In particular, mtDNA typing is reportedly successful in more than 95% of skeletal samples.⁷ There are some disadvantages also: due to smaller genetic diversity it can also happen that mtDNA also

does not the required level of certainty for positive identification. There is another approach, however: chromosomal DNA lodged inside bones and teeth can be better protected and useful for very long time unlike the DNA from soft tissues. Successful DNA typing from skeletal bone has been done for Neanderthal individuals who lived some 30 000 years ago.²¹

It is also possible that neither usual STR typing not mtDNA methods are sufficient for positive identification when the sample is very small or very badly damaged. New methods are under development to further improve the situation, for example DNA profiling with arrays of single nucleotide polymorphisms (SNP's) from chromosomal or mitochondrial DNA. Using SNP's instead of STR's requires more loci (about 100) to be analysed but offers the option to avoid electrophoresis and to use in future a 'lab-on-chip' method for fast and simple profiling on-site.⁹

Mitochondrial DNA polymorphism has been widely used for identification and maternity testing, because mtDNA is strictly maternally inherited. Haplogroups that have been determined with SNPs of mtDNA are major monophyletic clades in the mtDNA tree. Haplogrouping is not done by sequence analysis from forensic samples because the mitochondrial SNPs related to haplogroups are scattered throughout mtDNA. Instead, assays like primer extension technique (SNaPshot), flow cytometry or amplified product-length polymorphism (APLP) are used for the purpose. Haplogroups are often surprisingly region-specific (Fig 5), and therefore can be applied in evaluating the ethnic background of the subjects when this is of interest.¹⁶

Much of the traditional forensic work has used physical traits such as general appearance, stature, facial, cranial and skeletal morphology to support identification. Some of these features have been found to be linked with certain SNPs that can in principle then be used to track the traits.¹⁷

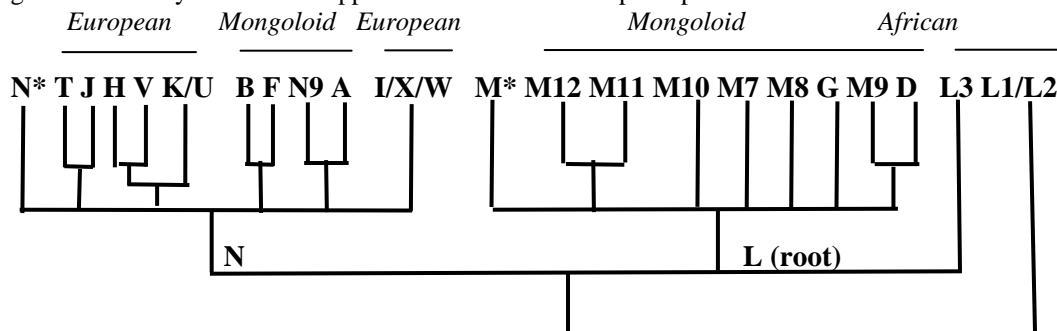


Fig 5. Principal mtDNA haplogroups in the world (simplified to show major groups only)¹⁶

Like any human activity, identification with mtDNA is not 100% error-free. Typical errors in mtDNA sequencing and documentation can be classified as shown in Table 1.¹⁵ Because of the potential for human or technical errors, quality control procedures are very important to establish and maintain in any forensic laboratory that applies DNA-based identification. Nevertheless, because of the large numbers sequenced since 1981^{13,14,15,19}, mtDNA is the most thoroughly tested and understood genetic marker.

The Y chromosome is mostly inherited unaltered from father to son, and is again similarly useful for the paternal lineage as mtDNA is for the maternal lineage. Y-specific STR's can be used not only in cases of sexual crime and paternity testing, but also in identifying the paternal haplotypes of populations and ethnic groups. Identification from blood or semen stains can be surprisingly effective, with positive results reported after more than 10 years of storage at room temperature.¹⁰

Table 1. Error types in forensic identification with mtDNA

Error type	Description
I: base shift	Position(s) misscored by alignment, reading or column shift
II: ref. bias	Overlooked nucleotide variants relative to the reference sequence
III: phantom mutations	Uncommon variants appearing in different lineages of the data set
IV: base misscoring	Miswriting nucleotide, or transition as transversion or vice versa
V: recombination error	Compound haplotype with fragments from more than one sample

As a result of established successful application, until now the molecular forensic science has been overwhelmingly dominated by DNA analysis. There are also other complementing methods that are not so widely applied.^{8,18} For example, corresponding RNA analysis has been traditionally avoided in stability of RNA. However, because of this instability, RNA analysis may actually be useful in determining e.g. the age of wounds and other injury, and possibly for assessing mechanisms of death and underlying disease.¹⁸ In spite of the developments in helpful technology, there are significant challenges remaining for the forensic expert. First, all new tools are not widely available, or the cost of using them remains beyond the available means in most cases, particularly in the developing world. Even the standard DNA fingerprinting is not particularly cheap, although the real cost has been much reduced over the last 20 years. Further significant cost reduction is expected (Fig 6), and what also helps is the general simultaneous growth in GDP, resulting in improving

affordability of DNA analysis. Nevertheless, it should be noted that no technique is likely to be 100% certain in all cases, and that combination with conventional forensic methods remains invaluable.

Discussion and concluding remarks

Two main lines of recent development continue to hold much promise in forensic identification, including dental forensics. The first is the development in the supporting technology, particularly in biotechnology, biomarkers and genetic typing. Although well established for some 20 years in some countries, such technology was initially too expensive for widespread application in the developing world, for example in Indonesia. While the cost issue is still significant, such technology is gradually becoming cheaper and thereby more widely accessible. In the same time, the other important issue is being addressed, i.e. personnel training to properly apply such technology.

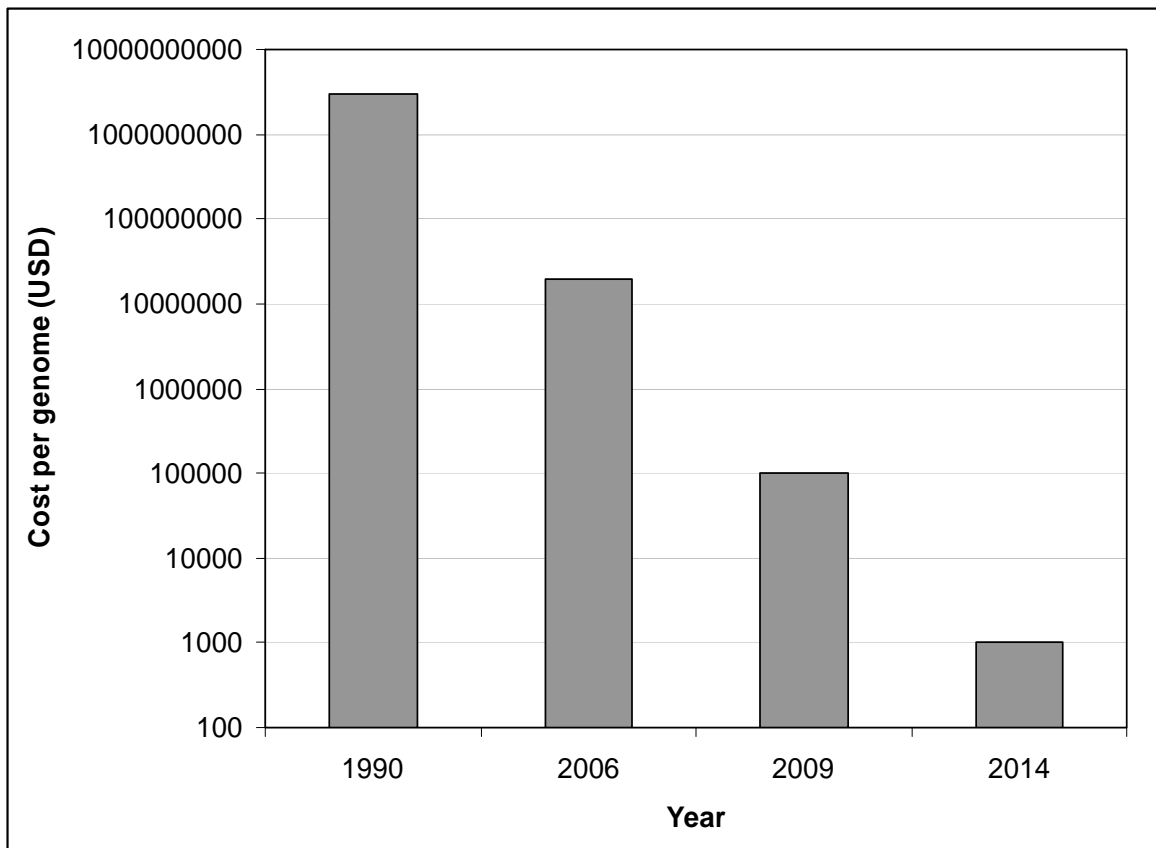


Fig 6. Cost reduction in sequencing the whole human genome of one individual²⁰ (values for 2009 and 2014 are goal estimates only); common partial DNA analysis for forensic identification is much cheaper but still relatively costly in the Indonesian context.

However, the new technology is not the panacea or final solution for all forensic cases. Apart from cost and availability of trained personnel, there are two reasons for this. First, there are always cases where complementing information will be needed even if the new biotechnology tools are used. For example, the samples may be too badly damaged or contaminated, or lack background comparison for such methods to work, and then other tools are needed anyway. Also, the principle of building a solid identification case with evidence from multiple sources will also require that several independent approaches or tools are used for the purpose. Here the conventional methods like comparison to dental records remain powerful and necessary even if newer biotechnology methods are also applied. Whichever approach is used, also some of the problems are common such as the difficulties in finding the required background information for useful comparison. In case of DNA typing, the method is most useful when the DNA profile of the person to

be identified is recorded and retrievable. Similarly, dental records can only be useful if they are recorded and later accessible for the forensic comparison.

The second line of important development is the systematic and “standardised” approach to forensic work. General guidelines are available for developing action plans for identification of human remains after disasters. The protocols typically include the phases of (1) on-site documentation, sample collection, preservation, shipping and storage; (2) description of tracking and chain of custody issues; (3) description of applied laboratory facilities, software etc.; (4) establishment of quality assurance and quality control practices; (5) sample analysis and identification, with evidence documentation; (6) interpretation of results; (7) training, communication and privacy issues; and (8) reporting.

These protocols and adherence to them are precisely what is needed to overcome some of the most persistent difficulties, such as varying

reliability of identification, and limited access to personal DNA profiles and dental records. Databanks of such personal data are available for forensic comparisons in a growing number of countries, although the progress in coverage for the whole population can be slow. However, the systematic approach in general can go a long way to overcome the obstacles. There are many recent examples on this, from the tsunami disaster to aircraft accidents and other major incidents with many victims. Equally well problematic cases can only involve one victim at the time. Experts of dental forensics will retain their useful role in such investigations.

References

1. Singh IJ, Pentel L, Goren AD, Lowell, JL. The use of dental characteristics in the identification of human remains. *Oral Surgery, Oral Medicine, Oral Pathology* 35 (2) 1973: 275-281
2. Naiman M, Larsen AK, Valentin PR. The role of the dentist at crime scenes. *Dental Clinics of North America* 51 (4) 2007: 837-856
3. Petju M, Suteerayongprasert A, Thongpud R, Hassiri K. Importance of dental records for victim identification following the Indian Ocean tsunami disaster in Thailand. *Public Health* 121; 2007: 251-257
4. Sweet D. Solving certain dental records problems with technology – the Canadian solution in the Thailand tsunami response. *Forensic Science International* 159S; 2006: S20-S23
5. Perrier M, Bollmann M, Girod A, Mangin P. Swiss DVI at the tsunami disaster: Expect the unexpected. *Forensic Science International* 159S; 2006: S30-S32
6. Piccinini A, Betti F, Capra M, Cattaneo C. The identification of the victims of the Linate air crash by DNA analysis. *International Congress Series* 1261; 2004: 39-41
7. Budowle B, Bieber FR, Eisenberg AJ. Forensic aspects of mass disasters: Strategic considerations for DNA-based human identification. *Legal Medicine* 7; 2005:230-243
8. Yasuda T, Kawai Y, Ueki M, Kishi K. Clinical applications of DNase I, a genetic marker already used for forensic identification. *Legal Medicine* 7; 2005: 274-277
9. Tamaki K, Jeffreys AJ. Human tandem repeat sequences in forensic DNA typing. *Legal Medicine* 7; 2005: 244-250
10. Iida R, Kishi K. Identification, characterisation and forensic application of novel Y-STRs. *Legal Medicine* 7; 2005: 255-258
11. von Wurmb-Schwark N, Bosinski H, Ritz-Timme S. What do the X and Y chromosomes tell us about sex and gender in forensic case analysis? *Journal of Forensic and Legal Medicine* 14; 2007: 27-30
12. Szibor R. X-chromosomal markers: Past, present and future. *Forensic Science International: Genetics* 1; 2007: 93-99
13. Salas A, Bandelt H-J, Macaulay V, Richards MB. Phylogeographic investigations: The role of trees in forensic genetics. *Forensic Science International* 168; 2007: 1-13
14. Yuasa I, Umetsu K. Molecular aspects of biochemical markers. *Legal Medicine* 7; 2005: 251-254
15. Salas A, Carracedo A, Macaulay V, Richards M, Bandelt H-J. A practical guide to mitochondrial DNA error prevention in clinical, forensic, and population genetics. *Biochemical and Biophysical Research Communications* 335; 2005: 891-899
16. Umetsu K, Yuasa I. Recent progress in mitochondrial DNA analysis. *Legal Medicine* 7; 2005: 259-262
17. Pulker H, Lareu MV, Phillips C, Carracedo A. Finding genes that underlie physical traits of forensic interest using genetic tools. *Forensic Science International: Genetics* 1; 2007: 100-104
18. Bauer M. RNA in forensic science. *Forensic Science International: Genetics* 1; 2007: 69-74
19. Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, Young IG. Sequence and organisation of the human mitochondrial genome. *Nature* 290; 1981: 457-465
20. Church GM. Genomes for all. *Scientific American* 294; 2006: 46-54
21. Wall JD, Hammer MF. Archaic admixture in the human genome. *Current Opinion in Genetics & Development* 16; 2006: 606-610