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Expanding Horizons

Establishing Kinship in Transplant Patients: Adherence to Guidelines

In India, human organ and tissue transplantation was started in 1962. At the outset, organ trafficking was rampant since organ transplant was unregulated. The primary legislation related to organ donation and transplantation in India, Transplantation of Human Organs Act (THOA), was passed in 1994 which was further amended in 2011 and the rules were notified in 2014 subsequently as Transplantation of Human Organs and Tissues Act (THOTA). It is aimed at regulation of removal, storage, and transplantation of human organs for therapeutic purposes and for prevention of organ trafficking.

THOA 1994 versus THOTA 2014

There are 21 forms described in the 2014 rules. As per THOTA, it is pertinent for the laboratory performing the genetic testing to establish relationship of the living donor with the recipient, to send the applications in proper format (termed Form 5) for avoiding delays. Without Form 5, the approval committee for live donor and organ transplant may not accept the application and consider it as incomplete.

Another major difference in the scope for donors includes the addition of grandparents and grandchildren as 'near-relatives.'

THOA 1994	THOTA 2014
'Near-relative' includes spouse, son, daughter, father, mother, brother or sister	'Near-relative' includes spouse, son, daughter, father, mother, brother, sister, grandparents, or grandchildren

Establishing Kinship with Living Donors

It is mandatory to provide –

1. documentary evidence of relationship
2. documentary evidence of identity and residence of the proposed donor
3. family photograph depicting the proposed donor and the proposed recipient along with another 'near-relative'

If the relationship is not conclusively established after evaluating the above evidence, human leukocyte antigen (HLA) matching or DNA fingerprinting from a laboratory accredited with National Accreditation Board for Testing and Calibration Laboratories (NABL) should be done.

Inadequacy of HLA Typing in Establishing Kinship

Two unrelated individuals may have matching HLA genes. This makes it difficult to use HLA typing as a sole means to prove a direct relationship. Moreover, as HLA is most polymorphic gene identified in human genome with an extremely clustered and patchwork assembly of sequence motifs, sequencing 'ambiguity' remains an issue despite the advancement in sequencing technologies. In some cases, the variations appear inconsistent with the genetic relationship that the pair report due to a variety of uncommon genetic explanations including chimerism, mosaicism, null alleles, uniparental disomy or misattributed parentage. Thus, the objective of HLA and ABO testing in the transplant setting is to determine donor eligibility and to make decisions about immunosuppresion, and not to confirm or refute parentage.

Kinship Analysis Using DNA Profiling

DNA test plays a vital role to prove kinship (biological relationship). It can be possible through –

1. STR
2. Y-STR (paternal side relative)
3. mt-DNA (maternal side relative)

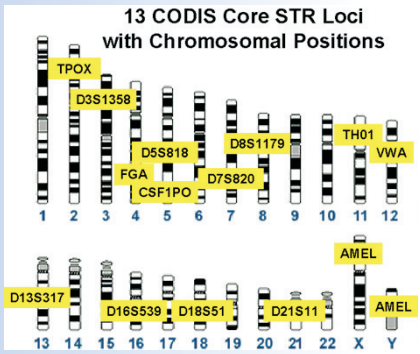
STR Analysis

DNA profiling by STR (short tandem repeats) analysis using a 15 genetic marker test is considered as a gold standard DNA testing having reliable result with a sensitivity of 99.99%. STRs are highly polymorphic, tremendous in number throughout the genome, and short enough (including mono-, di-, and tetra-nucleotide repeats) to be easily amplifiable by PCR. Because unrelated people almost certainly have different numbers of repeat units, STRs can be used to discriminate between unrelated individuals. The nomenclature of the STR loci and the allelic variants was established in 1993 by the DNA Commission of the International Society of Forensic Genetics (ISFG).

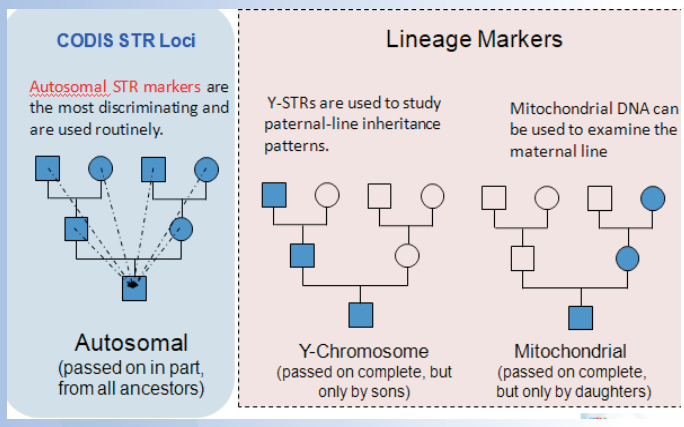
In the development of STR typing system, in 1997, the Federal Bureau of Investigations (FBI) introduced the database named CODIS (Combined DNA Index System) that included 13 autosomal loci and the amelogenin sex test. Most commercially available kits utilize these set of markers and additional markers. The Scientific Working Group on DNA Analysis Methods (SWGDAM) provides practice guidelines to the Quality Assurance Standards for Forensic DNA Testing Laboratories and the Quality Assurance Standards for DNA Databasing Laboratories.

In SRL, we use the CODIS 13 STR-based DNA-profiling system with the probability of a random match being 1 in trillions. This in link with the STR human identity software, Genemarker HID is a multiplex approach which gives likelihood ratio (LR) also called the relationship index (RI) or kinship index (KI) i.e. how strongly the genotypes support one relationship over the other.

Each independent locus tested produces its own relationship index, which can be multiplied by those of other independent loci to calculate a combined relationship index (CRI). By the definition of a LR: CRI > 1 supports the numerator (claimed relationship); CRI < 1 supports the denominator (alternative relationship). Larger CRI values provide more support for the claimed relationship. It also provides flexibility to analyze data and calculate relationship levels across three generations.



The limitation of autosomal DNA analysis is that it may only be used to prove direct relationship as autosomal DNA is only half matched between first degree relatives and complicated to evaluate and to prove a distant relationship. In the cases of distant relationship, Y-STR and mtDNA analysis are being employed for paternal and maternal lineage, respectively.



Y-STR Analysis

Y-STRs are derived solely from the male sex-determining Y chromosome. Profiles based on autosomal STRs provide far stronger statistical power than profiles based on Y-STRs, because autosomal DNA is randomly exchanged between matched pairs of chromosomes in the process of making egg and sperm cells. That's how, with billions of humans on the planet, no two people who are not identical twins are exactly alike. Profiles based on Y-STRs are statistically weaker because only males have a Y chromosome and all males get theirs from their fathers, so all males in any paternal line have nearly identical Y chromosomes. Y-STR analysis is used when two or more male individuals are related through their paternal lineage. All the individuals in the paternal lineage such as grandfather, father and brother will share the same Y-STR profile.

In SRL, Yfiler® Plus is used which utilizes 27 loci, including 7 rapidly mutating (RM) Y-STRs making it more sensitive, with more discriminatory and exclusionary power than the previous version (Yfiler).

mtDNA Analysis

mtDNA (mitochondrial DNA) analysis is usually used to prove maternal lineage relationship, as mtDNA is passed from the cytoplasm of the ovum; therefore, it is maternally inherited from mothers to all their offsprings. The mitochondrial genome is 16,569 base pairs (bp) long and contains the most variable segments of the human genome, the hyper-variable (HV) region, which is 1200 bp long. The variability in this region is used to distinguish the maternal relations. Mitochondrial DNA can be very useful in India, where about 90% of organs are obtained from living donors, whereas 10% are obtained from deceased donors.

However, mode of inheritance and subsequent lack of heterologous recombination make the mtDNA analysis inadequate to retrace evolutionary relationships unambiguously down the maternal lineage without the confounding effects of recombination. Also, considering the following inherent limitations of mtDNA, it should only be used as a last resort when all other tests have failed.

Characteristics	Disadvantage
Sometimes heretoplasmic	Destroys advantage of clonality
Insertions in nuclear genome	Nuclear insertions of mitochondrial sequences (nuMts) cause horizontal evolution problems
Different regions of genome often show	This is caused because mtDNA is clonal
Phylogenetic incongruence with each other	Interpretation problems

Conclusion

Organ transplantation from living donors raises a series of ethical questions. First it breeches the principal precepts of medicine – primum non nocere (above all, do no harm) – as it involves the removal of an organ from a healthy person for implantation into another person. Yet other physicians feel that it's their duty to help patients find living donors. On the contrary, commercial dealings of organs pose another threat to the medical fraternity. In lieu of these aspects, it is essential for clinicians and physicians to know and understand the guidelines and legal requirements for establishing kinship for transplantation purpose.

Tests Offered at SRL

Test Name	Methodology	Test Code
Kinship Analysis	Multiplex PCR	RD1502

References

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SRL Ltd., Prime Square, Plot No. 1, S. V. Road, Goregaon (West), Mumbai 400 062, India • Tel.: +91-22-30811111 • Web: www.srlworld.com

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