

# Vertical and Horizontal Autosomal Sharing of Alleles in First Degree Relationship: A Case Study

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## Abstract

This case study details the identification of a deceased individual through paternity and sibship analysis using allele sharing among alleged relatives. Bone and blood samples from the deceased, as well as reference blood samples from an alleged son and brother, were provided. DNA was extracted, quantified, amplified, and analysed using an 8-capillary array DNA analyzer. Remarkably, each autosomal locus in the DNA profiles of the alleged son and brother shared at least one allele with the corresponding loci in the deceased's DNA profile. Y-STR analysis confirmed their immediate patrilineal relationship. To assess the paternity and sibling relationships, respective indices were calculated, confirming the relationships to the deceased. This is the first case observed where both the son and brother shared autosomal DNA fragments at all loci without the mother's reference. The study underscores the importance of DNA statistics in establishing relationship strength solely based on STR results, enabling reliable conclusions without additional testing.

**Keywords:** Allele sharing, Autosomal DNA, Kinship analysis, Paternity index, Sibship index.

## Introduction

Kinship analysis for first-degree relationships (FDR) using Short Tandem Repeat (STR) technology is a powerful method in genetics for determining familial connections. STRs are simple sequence repeats of DNA, where a short sequence of base pairs (1-6 bp) is repeated, constituting about 3% of the human genome<sup>1</sup>. The number of repeats varies significantly among individuals (~5-50), with 4 bp repeat STRs being particularly useful for genetic profiling, kinship analysis, and crime investigation<sup>2,3</sup>.

Kinship is established through degrees of relationship, which refer to the closeness of familial connections, determined by genetic analysis methods such as STR analysis, SNP analysis and sibship analysis either manually, or through software<sup>4-7</sup>. FDR include parent-child and full siblings, who share about 50% of their DNA segments in contrast to half-siblings who share only one common parent and therefore share only 25% of their DNA. During meiosis, numerous double-strand breaks are generated per chromosome, with some resulting

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in crossing-over events<sup>8</sup>. This leads to the random segregation of each parent's half genome into their offspring, causing full siblings to receive a more or less similar half of their genome from each parent<sup>9</sup>. Second-degree relationships, such as grandparent-grandchild and half siblings, share about 25% of their DNA. Third-degree relationships, like first cousins and great-grandparent to great-grandchild, share about 12.5% of their DNA, and so on.

In sibling cases, the STR alleles (the alternative forms of the DNA segments, usually two in case of diploid autosomal DNA) play a crucial role in determining the likelihood of a sibling relationship<sup>10</sup>. Among full siblings, the chance of sharing alleles at a given STR locus is distributed as follows: there is a 25% chance that they will share both alleles, a 50% chance that they will share one allele, and a 25% chance that they will share no alleles<sup>11</sup>. These probabilities stem from the fact that full siblings inherit their genetic material from the same set of parents, leading to a predictable pattern of allele sharing. By analysing multiple STR loci, geneticists can use these probabilities to accurately determine and confirm sibling relationships, which is crucial in forensic and genealogical investigations. In this particular case, while analysing 21 autosomal STR loci, we observed only the first and second scenarios of allele sharing, making this a unique instance of allele inheritance among two siblings.

In kinship analysis, DNA profiles are created by analyzing multiple STR loci and comparing them statistically to calculate the likelihood of various relationships. A primary statistic in kinship analysis is the paternity index (PI), which evaluates the probability of a man being the biological father of a child. The child's DNA profile is compared to the alleged father's profile at each STR locus. For each locus, a likelihood ratio is determined by comparing the probability of the child's allele given the alleged father's alleles against that of a random individual from the population. The product of these ratios across all loci results in the combined paternity index (CPI). A CPI above 1 supports paternity, while values exceeding 100 are generally considered conclusive evidence. The sibship index is another critical metric, assessing the likelihood of two individuals being biological siblings. It compares the genetic similarity

observed between them against expected values for siblings versus unrelated individuals. Likelihood ratios for each STR locus are calculated based on allele frequencies and combined into a combined sibship index (CSI). A higher CSI indicates a greater probability of a sibling relationship. This approach is particularly valuable in scenarios where paternity or maternity data is absent, such as inheritance cases or identifying unknown relatives.

In this case study, we used DNA statistics of amplified STR markers for kinship analysis to determine the likelihood of biological relationships (paternity and brotherhood) between individuals, utilizing allele frequency data from the population of Madhya Pradesh, India<sup>12</sup>. Blood and bone samples from the deceased person, along with reference blood samples from two putative relatives (the son and the brother), were provided for comparison, but no reference sample from the deceased's wife was available. Autosomal and Y-STR profiling were performed on all three individuals, revealing a unique pattern of allele DNA inheritance among the deceased person and his alleged brother, similar to paternal DNA inheritance observed in vertical transmission of alleles to his son.

## Materials and Methods

### *DNA Extraction*

In the laboratory, blood sample as well as a sternum bone piece of deceased individual were received, accompanied by reference blood sample from the alleged son and a brother, for DNA profiling and comparison. The sternum bone was thoroughly cleaned using chemical-grade water followed by 70% ethanol and allowed to dry at room temperature. Bone powder was prepared from the surface of the sternum using a hacksaw blade, and approximately 2 grams of the powder was placed in a 15 ml propylene tube. The sample was incubated with 0.5M EDTA (3-5 volumes) on a rotary mixer at 30 rpm at room temperature (RT), with EDTA renewed twice daily for three days, resulting in a paste-like consistency due to significant demineralization. Lysis of the bone powder and reference blood samples was performed in 1.5 ml microcentrifuge tubes at 56°C using a lysis buffer containing 10 mM Tris-Cl (pH 8.0), 200 mM NaCl, 1% SDS, 10 mM EDTA, 1 mM DTT, and

20 mg/ml proteinase K<sup>13</sup>. Following lysis, samples were centrifuged, and the supernatant was mixed with equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1), pH 8.0. After gentle mixing and centrifugation, the aqueous phase was collected, and DNA was precipitated with absolute ethanol at -20°C for at least 30 minutes and then centrifuged at 14000 rpm. The DNA pellet was washed with 70% ethanol, centrifuged at 14000 rpm at RT, pellet was dried, and resuspended in TE buffer for subsequent analysis. The extracted DNA was quantified using real-time polymerase chain reaction (RT-PCR) with the Quantifiler® Trio DNA Quantification kit (Applied Biosystems)<sup>14</sup>.

### STR amplifications and Fragment Analysis

For the amplification of autosomal STRs and Y-STRs, we used the Investigator® 24 Plex kit and the Investigator® Argus Y-28 QS kit (Qiagen Inc.) for multiplex analysis<sup>15,16</sup>. The autosomal STR kit allowed the simultaneous amplification of 21 autosomal STR loci, one Y-STR locus, a gender marker (Amelogenin), and two quality sensor (QS) markers. The 25 µl PCR mixture included 7.5 µl of Fast Reaction Mix, 2.5 µl of Primer Mix, and 15 µl of DNA/water, ensuring a final DNA template concentration of 1 ng per reaction. Positive and negative controls were included but are not detailed here. The amplification protocol involved an initial denaturation at 98°C for 30 seconds, annealing at 64°C for 55 seconds, and extension at 72°C for 3 seconds for 3 cycles. This was followed by

27 cycles at 96°C for 10 seconds, 61°C for 55 seconds, and 72°C for 5 seconds, with a final extension at 68°C for 5 minutes and 60°C for 5 minutes. PCR products (1.0 µl) or ladder were mixed with 10 µl of Hi-Di™ formamide and 0.4 µl BTO Size Standard dye in a 96-well plate, denatured at 95°C for 5 minutes, snap chilled, and loaded onto the Genetic Analyzer 3500 (Applied Biosystems). The Investigator® Argus Y-28 QS Kit was used for Y-STR profiling, co-amplifying 27 Y-STR markers and a QS marker as per the kit protocol.

### Calculation for Paternity and Sibling Index

Data analysis for calculating the duo paternity index and sibship index was performed on a comparative table (Supplementary Material -1). The paternity index (PI) was determined by the ratio of A to B, where A represents the likelihood ratio of the alleged father being the biological father (null hypothesis), and B represents the population allele frequency of a random man (alternative hypothesis). The value of B (autosomal STR frequency) was derived from the STR frequency data of Madhya Pradesh State<sup>7</sup>. The combined paternity index (CPI) was calculated by multiplying all the individual paternity indices (PI) for each locus (refer to Table 1). The CPI indicates the strength of genetic evidence supporting the hypothesis that the alleged man is the child's father, rather than any other man from the population.

**Table 1: Paternity index and sibship index calculation of deceased person with alleged son and the alleged brother respectively.**

S. No	STR LOCUS	Sample Source & Alleles Called			Paternity Index	Full Sibship Index
		Deceased Person	Alleged Son	Alleged Brother		
1	Amelogenin	X, Y	X, Y	X, Y	-	-
2	TH01	8, 9	6, 8	9, 9.3	1.969	0.590
3	D3S1358	16, 16	14, 16	16, 16	2.024	6.373
4	vWA	14, 16	16, 19	16, 17	1.196	0.848
5	D21S11	28, 30	29, 30	28, 30	1.232	6.732
6	TPOX	8, 11	11, 11	8, 11	1.222	1.736
7	DYS391	11	11	11	--	-
8	D1S1656	13, 16.3	13, 16	13, 16.3	1.786	55.429
9	D12S391	23, 23	20, 23	21, 23	6.757	3.628

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10	SE33	20, 20	20, 29.2	20, 30.2	5.556	3.028
11	D10S1248	13, 14	14, 14	12, 14	1.779	0.695
12	D22S1045	11, 15	11, 15	11, 15	1.430	1.984
13	D19S433	13, 14	14, 15	13, 14.2	1.111	0.606
14	D8S1179	10, 16	10, 10	10, 13	2.778	0.944
15	D2S1338	18, 20	20, 26	18, 20	2.119	8.565
16	D2S441	10, 11	10, 11	9, 10	1.328	0.582
17	D18S51	16, 16	12, 16	16, 16	3.906	19.273
18	FGA	24, 24	24, 25	23, 24	2.994	1.747
19	D16S539	12, 13	11, 12	12, 13	1.389	7.257
20	CSF1PO	10, 11	11, 11	10, 11	1.773	3.704
21	D13S317	11, 12	11, 12	11, 12	2.173	3.681
22	D5S818	12, 12	11, 12	11, 12	1.572	1.031
23	D7S820	11, 12	11, 12	11, 12	2.709	4.930
					(CPI)= $2.83 \times 10^6$	(CSI)= $1.52 \times 10^9$

NB: The Combined Paternity Index (CPI) and the Combined Sibship Index (CSI) are here the likelihoods that the deceased person is the biological father or biological full sibling of the alleged individuals. This indicates that the alleged father has  $2.83 \times 10^6$  times more possibility to be the father of the alleged son compared to an arbitrary person or  $1.52 \times 10^9$  times more to be the full sibling of the alleged brother compared to an arbitrary person.

2. Formula for calculating Sibship Index				
S. No.	Sharing of Alleles	Full sibling (F)	Half sibling (H)	Unrelated (U)
1	Share two alleles (both are pq)		$p+q+4pq$	$8pq$
2	Share two alleles doubly (both are pp)	$(1+p)^2$	$2p(1+p)$	$2p^2$
3	Share one allele double (both are pp or pq)	$1+p$	$1+2p$	$4p$
4	Share one allele (both are heterozygous, pq and pr)	$1+2p$	$1+4p$	$8p$
5	Share no alleles (pq or pp vs rs or rr)	1	2	4

**Supplementary Material - 1. Formula used in the case study for calculation of duo paternity index and sibship index (Courtesy: <https://dna-view.com/>).**

1. Formula for calculating Duo Paternity Index			
S. No.	Father	Alleged Child	Duo Paternity Index
1	q	q	$1/q$
2	q	pq	$1/2q$
3	qr	q	$1/2q$
4	pq	pq	$p+q/4pq$
5	qr	pq	$1/4q$
6	r	q	0

Foreestablishment of sibling relationship, sibship index was calculated using the formula available at DNA view website (<https://dna-view.com/>) which were derived from the symbolic iKinship program<sup>17</sup>(Supplementary Material -1). The proportion  $F: U$  was used for estimating the likelihoods of the full

sibling versus unrelated person using amplified STR types assuming full-sibship and unrelated respectively. From these proportions likelihood ratios of full to half-sibship (F/H) was calculated for each set of alleles for each amplified locus. The combined sibship index (CSI) was calculated by multiplying

the individual values obtained for each locus studied (refer to Table 1). Paternity and sibship indices for each locus were computed based on the population data of Madhya Pradesh<sup>18</sup>.

### Discussion

The DNA profiles of the deceased person, his alleged son, and his alleged brother were generated using validated Qiagen Kits (Investigator® 24 Plex Kit and the Investigator® Argus Y-28 QS Kit) to establish their biological relationships. Y-STR profiling confirmed their shared patrilineal ancestry. The alleged son shared at least one allele at all the amplified loci with the deceased person. The Duo paternity index was calculated, indicating that the deceased person is  $2.83 \times 10^6$  times more probability to be the father compared to an arbitrary man from the same population.

The alleged brother's autosomal profile was also compared, revealing that he shared at least one allele at all the autosomal amplified loci with the deceased

person. This is a rare occurrence, as in a randomly mating population, the segregation of alleles is independent, and the chances of sharing of all 21 forensic STR loci among siblings are extremely low. The probability distribution for allele sharing among full siblings at a given STR locus is: 25% chance of sharing both alleles, 50% chance of sharing one allele, and 25% chance of sharing no alleles. In this particular case, while analysing 21 autosomal STR loci, we observed only the first scenario (9 events) and the second scenario (12 events) of allele sharing, making this a unique instance of allele inheritance among two siblings. The Combined Sibship Index (CSI) indicates that the alleged brother is  $1.52 \times 10^9$  times more probability to be a full sibling compared to an arbitrary man from the same population. Additionally, Y-STR profiling of the deceased person, the alleged son, and the alleged brother revealed an identical profile across 27 loci (see Table 2), confirming their common immediate patrilineal ancestor.

**Table 2: Y-STR profiling of deceased, alleged son and alleged brother confirming their same patrilineage.**

S. No.	STR LOCUS	Sample Source & Alleles Called		
		Deceased Person	Alleged Son	Alleged Brother
1	DYS389I	13	13	13
2	DYS391	11	11	11
3	DYS389II	29	29	29
4	DYS533	12	12	12
5	DYS390	22	22	22
6	DYS627	18	18	18
7	DYS458	19	19	19
8	DYS393	12	12	12
9	DYS19	16	16	16
10	DYS437	14	14	14
11	DYS449	34	34	34
12	DYS460	10	10	10
13	DYS576	17	17	17
14	YGATAH4	13	13	13
15	DYS481	24	24	24
16	DYS448	19	19	19
17	DYS518	36	36	36
18	DYS439	11	11	11
19	DYS549	12	12	12

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20	DYS438	9	9	9
21	DYS456	16	16	16
22	DYS643	9	9	9
23	DYS570	17	17	17
24	DYS635	20	20	20
25	DYS385	15, 16	15, 16	15, 16
26	DYS392	11	11	11

### Conclusion

In this forensic case study, the autosomal STR profile of an alleged brother showed an unusual resemblance to a paternal relationship. At each of the 21 loci amplified from the deceased's bone sample and the alleged brother's sample, they shared at least one allele, a rare occurrence attributed to identity by descent from a common ancestor. In the absence of the child's mother's reference blood sample, the deceased shared at least one allele at all loci with the alleged son, while the son did not share alleles at two loci with his uncle, ruling out a first-degree relationship with the uncle.

Further analysis revealed a higher paternity index for the alleged brother when considered as the son, and a significantly lower sibship index when the alleged son was considered as the brother, reflecting their paternally bonded relationship. The half-sibship index between the deceased and the alleged brother was less than one, excluding such a relationship. This case, lacking the mother's reference sample and mitochondrial DNA analysis, highlights a unique challenge for forensic analysts. The results suggest that based on the shared alleles, the alleged son and brother cannot be excluded as the true son and brother of the deceased. This study highlights the forensic importance of STR statistics in kinship analysis and highlights the potential for integrating genealogical studies in the future to resolve complex patterns of STR inheritance and establish relationships more accurately.

### Ethics Approval and consent to participate

This article does not include any studies involving human participants or animals conducted by any of the authors. The authorization for examining the samples (bone sample and reference blood samples) was granted by the Superintendent of Police, Hyderabad, Telangana.

**Competing interests:** All the authors have declared that there is no competing interest in publication of this case report.

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### Authors' contributions

DB extracted DNA and carried out capillary electrophoresis. PCK carried out electrophoresis and statistical calculations. AKR conceptualized the article, analysed the results, and wrote the paper.

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