# Case Analysis of 44 Autosomal STR Ternary Allele Genotypes

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**Abstract: This study investigates the distribution characteristics and genetic mechanisms of three-allele genotypes at STR loci. Using the PowerPlex 21 Amplification Kit and the Human DNA Typing Kit (Platinum) from Shenzhen Huada Forensic Technology Co., Ltd., 44 cases of three-allele genotypes identified in routine paternity testing cases handled by our institute from 2018 to 2025 were analyzed to summarize their distribution and genetic features. Results: The D6S1043, D5S818, D12S391, and vWA loci were detected in 1 case each; the D19S433, D8S1179, D78S820, TPOX, and CSFIPO loci were detected in 2 cases each. The D18S51 locus was detected in 3 cases, the Penta E locus in 6 cases, the D21S11 locus in 10 cases, and the Penta D locus in 11 cases. The three-allele genotypes at the Penta E, D21S11, and PentaD loci were relatively common. Patients with trisomy 21 were found to have type II three-allele genotypes at both the PentaD and D21S11 loci. In forensic DNA analysis, Type 1 tri-allelic pattern refers to the phenomenon of three alleles appearing at a single locus, typically caused by chimerism or somatic mutation. Chimerism and sampling procedures can indeed affect the ratio of the original gene to the mutated gene, leading to various forms of tri-allelic peak patterns, but the peak height of the dominant allele is the sum of the other two. The formation of Type 2 tri-allelic patterns may originate from non-allelic homologous recombination. This study provides statistical analysis of tri-allelic data from routine cases, offering data for research on the genetic characteristics of STR tri-allelic patterns.**

**Keywords: Short Tandem Repeat; Three-Allele Genotype; Genetic Mechanism**

## 1. Introduction

Due to its adherence to Mendelian co-dominant inheritance patterns, high detection sensitivity, and stable results, STR (short tandem repeat) has become one of the most commonly used molecular markers in forensic medicine, paternity testing, and scientific research. Paternity testing follows the Mendelian co-dominant inheritance pattern, where each STR locus in a child's genome carries two alleles, one from each parent, with each parent contributing one allele. According to normal genetic principles, each STR locus on human autosomes carries two alleles. In capillary electrophoresis (CE) patterns, a single peak indicates a homozygote, while two peaks with similar heights indicate a heterozygote. In rare cases, abnormal inheritance patterns may lead to conditions such as "triple alleles, microvariations, invalid alleles, or chromosomal abnormalities." Forensic scientists must be aware of these rare scenarios to ensure appropriate handling and minimize the impact of genetic variations on the accuracy of results.

One of the abnormal inheritance patterns—triple allele genotyping—can result from the limitations of the kit and individual specificity, such as allele deletion due to rare mutations at primer binding sites, slippage-induced mutations, and events like gene conversion and copy number variations (CNVs) [1]. Among these, the triple allele pattern caused by individual specificity was classified into two types, Type I and Type II, by Clayton et al. [2]. Type I manifests as an imbalanced triple allele pattern, which is the result of early somatic mutations. During embryonic development, somatic mutations lead to the formation of chimeras, where some cells contain the original allele and others contain the mutated allele. It is generally believed that the peak ratios of the three allele loci are 1:2, n:1, and n:2, respectively. Type II exhibits highly similar peaks, which is the result of chromosomal local duplications or chromosomal aneuploidy [2]. This article analyzes the typing characteristics and genetic features of triple allele genotypes through case studies, and the findings are reported as follows.

 

## 2. Study Subjects and Detection Methods

### 2.1 Study Subjects

From 2018 to 2025, the Guangdong Boxin Forensic Medical Evidence Judicial Appraisal Institute routinely accepted paternity testing cases involving three-allele genotypes, with the test materials being human peripheral blood spots.

### 2.2 Detection Methods

All blood spot samples were extracted using the Chelex method and subjected to multiplex PCR amplification with the PowerPlex 21 Human Fluorescent Labeling STR Multiplex Amplification Kit (Promega). Capillary electrophoresis was performed on an AB3500 genetic analyzer (AB), and the results were analyzed using GeneMapper® ID-X software to obtain mutation information for 20 STR loci (D19S433, D5S818, D21S11, D18S51, D6S1043, D3S1358, D13S317, D7S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX, Penta E, TH01, D12S391, D2S1338, FGA) and one sex locus (AMEL) for genotyping. Cases with detected three-allele genotypes were validated using the Human DNA Genotyping Kit (Platinum) from Shenzhen BGI Forensic Technology Co., Ltd.

## 3. Results

### 3.1 Characteristics of Allele Typing Distribution

This study analyzed 44 third-order genotypes identified in paternity testing cases routinely accepted by our institute from 2018 to 2025. The D6S1043, D5S818, D12S391, and vWA loci each detected 1 case, while the D19S433, D8S1179, D78S820, TPOX, and CSFIPO loci each detected 2 cases. The D18S51 locus detected 3 cases, the Penta E locus 6 cases, the D21S11 locus 10 cases, and the PentaD locus 11 cases. A total of 21 trisomy 21 patients were found to have type II third-order genotypes at the PentaD and D21S11 loci. (See Table 1)

**Table 1. Distribution Characteristics of Detected Three-allele Genotypes**

| STR locus | STR Genotyping | | | tertiary genotype peak-to-peak ratio / fractionation (subfraction) | tertiary genotype peak-to-peak ratio / fraction (parent) | tertiary genotype peak-to-valley ratio/fractionation (parent) |
|---|---|---|---|---|---|---|
| | son | father | mother | | | |
| Penta E | 12/13 | 5/12/13 | 12/17 | | 3:2:1 (Type I) | |
| | 12/12 | - | 12/13/18 | | | 2:1:3 (Type I) |
| | 17/20 | 16/17/20 | 11/17 | | 1:2:3 (Type I) | |
| | 11/12 | 12/18 | 10/11/20 | | | 1:2:3 (Type I) |
| | 15/19 | 14/15 | 12/19/20 | | | 3:2:1 (Type I) |
| | 17/19/20 | 17/20 | 14/19 | 2:1:1 (Type I) | | |
| D7S820 | 8/12 | 10/12 | 7/8/10 | | | 3:2:1 (Type I) |
| | 9/10/12 | 8/10 | - | 1:1:1 (Type II) | | |
| D18S51 | 13/14/15 | - | 13/15 | 3:2:1 (Type I) | | |
| | 13/14 | - | 14/15/20 | | | 1:1:2 (Type I) |
| | 14/16 | 16/23/24 | - | | 2:1:1 (Type I) | |
| D6S1043 | 13/13 | 13/13 | 13/14/18 | | | 1:1:2 (Type I) |
| vWA | 16/17/18 | 17/18 | 16/18 | 1:1:2 (Type I) | | |
| CSFIPO | 10/11/12 | 10/11 | 12/12 | 2:1:3 (Type I) | | |
| | 10/14 | 10/13/14 | 12/14 | | 2:2:1 (Type I) | |
| D19S433 | 14/15.2 | 12/14/15.2 | 14/15.2 | | 1:2:3 (Type I) | |
| | 15/15.2 | 13/15/16 | 13/15.2 | | 3:2:1 (Type I) | |
| D8S1179 | 11/12 | 11/16/17 | 12/14 | | 2:1.2:1 (Type I) | |
| | 10/16/17 | 14/16 | 10/14 | 3:2:1 (Type I) | | |
| D12S391 | 19/19 | 19/20/22 | 19/20 | | 1:1.1:2 (Type I) | |
| D5S818 | 9/10 | 9/10 | 9/13/14 | | | 3:2:1 (Type I) |
| TPOX | 8/11/12 | 8/11 | 8/12(8/8/12) | 1:1:1 (Type II) | | 2:1 (Type II) |
| Penta D | 8/13 | 8/13 | 9/10/13 | | | 1:1:2 (Type I) |
| | 9/14 | 9/10/13 | 9/14 | | 1:2:3 (Type I) | |
| D21S11 | 30/33.2 | - | 29/30/33.2 | | | 1:1:1 (Type II) |
| Penta D | 9/12(9/9/12) | 9/9 | 12/12 | 2:1 (Special Type II) | | |
| D21S11 | 30/32 | 31.2/32/33 | 30/31 | | 2:1.5:1 (Type I) | |
| *Penta D | 9/11(9/9/11) | 9/9 | 9/11 | 2:1 (Special Type II) | | |

 

| | | | | | | |
|---|---|---|---|---|---|---|
| *D21S11 | 30/31/33.2 | 29/31 | 30/33.2 | 1:1:1 (Type II) | | |
| *Penta D | 9/10(9/9/10) | 9/13 | 9/10 | 2:1 (Special Type II) | | |
| *D21S11 | 29/31/31.2 | 29/29 | 31/31.2 | 1:1:1 (Type II) | | |
| *Penta D | 11/13(11/11/13) | - | 11/13 | 2:1 (Special Type II) | | |
| *D21S11 | 29/31/33.2 | - | 29/33.2 | 1:1:1 (Type II) | | |
| *Penta D | 9/10/14 | 9/14 | 9/10 | 1:1:1 (Type II) | | |
| *D21S11 | 31/33.2 (31/33.2/33.2) | 29/31 | 31/33.2 | 1:2 (Special Type II) | | |
| *Penta D | 9/10/12 | 9/12 | 10/12 | 1:1:1 (Type II) | | |
| *D21S11 | 29/30/32.2 | 29/30 | 29/32.2 | 1:1:1 (Type II) | | |
| *Penta D | 9/10/13 | 10/10 | 9/13 | 1:1:1 (Type II) | | |
| *D21S11 | 28/30/32.2 | 32.2/32.2 | 28/30 | 1:1:1 (Type II) | | |
| *Penta D | 9/10/12 | - | 9/10 | 1:1:1 (Type II) | | |
| *D21S11 | 28/32/33.2 | - | 29/32 | 1:1:1 (Type II) | | |
| *Penta D | 9/12/13 | 9/9 | 12/13 | 1:1:1 (Type II) | | |
| *D21S11 | 29/31/32.2 | 31/32.2 | 29/32.2 | 1:1:1 (Type II) | | |

Note: "*" indicates a case of trisomy 21

### 3.2 Genetic Analysis of Trisallelic Genotypes

Table 1 observed that parents carrying the I-type trisomic genotype randomly transmitted one allele to their offspring; parents carrying the II-type trisomic genotype simultaneously transmitted both alleles to their offspring. In cases where both parents were involved in the identification, the offspring had a trisomic genotype of type I, with three alleles in the trisomic genotype carrying mutations from either the mother or the father. The offspring had a trisomic genotype of type II, with all three alleles in the trisomic genotype derived from both the mother and the father.

The most frequently detected triploid alleles at the Penta D locus were Type II in 9 cases and Type I in 2 cases; at the D21S11 locus, Type II was observed in 9 cases and Type I in 1 case; the triploid alleles detected at the Penta E, D18S51, D6S1043, vWA, CSFIPO, D19S433, D8S1179, D12S391, and D5S818 loci were all Type I. At the TPOX locus, 2 cases were Type II, 1 case was Type I, and 1 case was Type II. (See Table 2)

Two cases of type II three-allele genotype at the TPOX locus were detected in a mother-daughter pair with established paternity. Similar phenomena have been reported in Picanco et al.'s study [3] on the occurrence of three-allele genotypes at the TPOX locus. Additionally, type II three-allele genotypes were simultaneously detected at the PentaD and D21S11 loci in a patient with trisomy 21 (see Figure 1-8).

**Table 2. Allele Genotype Pattern Counts**

| locus | Counting the patterns of third-allele genotyping | | Total number of alleles |
|---|---|---|---|
| | I mould | II mould | |
| Penta E | 6 | 0 | 6 |
| D7S820 | 1 | 1 | 2 |
| D18S51 | 3 | 0 | 4 |
| D6S1043 | 1 | 0 | 1 |
| vWA | 1 | 0 | 1 |
| CSFIPO | 2 | 0 | 2 |
| D19S433 | 2 | 0 | 2 |
| D8S1179 | 2 | 0 | 2 |
| D12S391 | 1 | 0 | 1 |
| D5S818 | 1 | 0 | 1 |
| TPOX | 0 | 2 | 2 |
| Penta D | 2 | 9 | 11 |
| D21S11 | 1 | 9 | 10 |

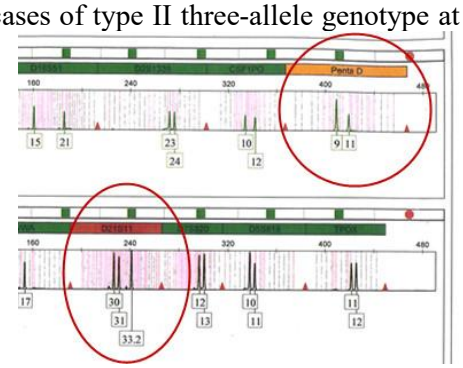

**Figure 1. STR Genotyping Map of Penta D and D21S11 Loci in Case 1**

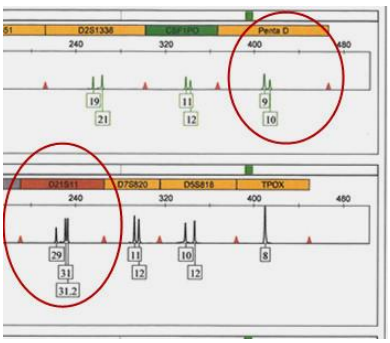

**Figure 2. STR Genotyping Map of Penta D and D21S11 Loci in Case 2**

 

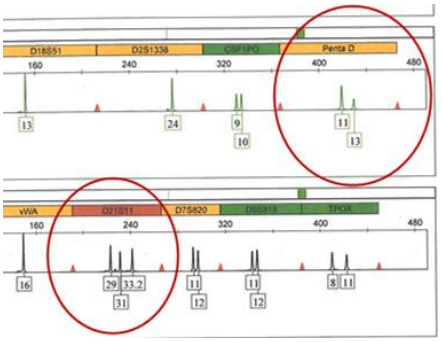

**Figure 3. STR Genotyping Map Of Penta D and D21S11 Loci in Case 3**

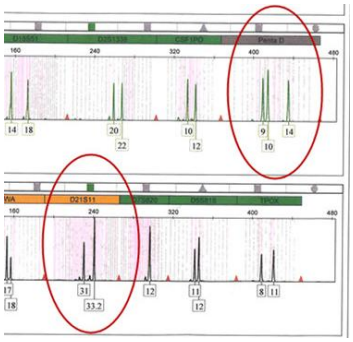

**Figure 4. STR Genotyping Map of Penta D and D21S11 Loci in Case 4**

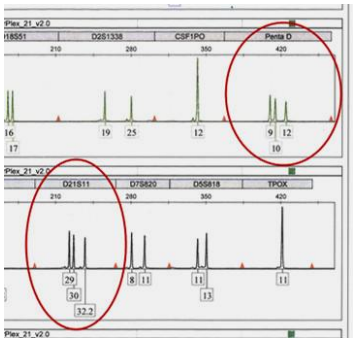

**Figure 5. STR Genotyping Map of Penta D and D21S11 loci in Case 5**

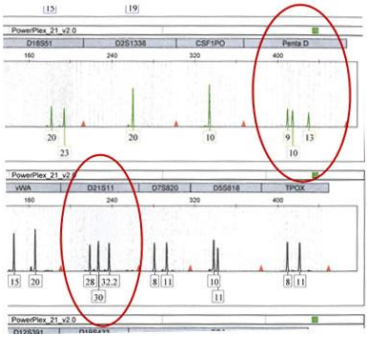

**Figure 6. STR Genotyping Map of Penta D and D21S11 Loci in Case 6**

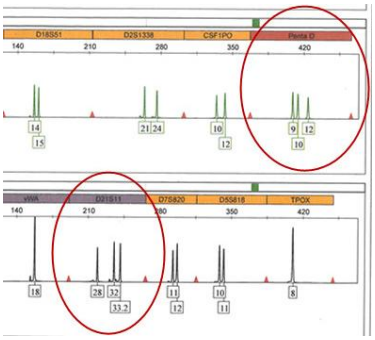

**Figure 7. STR Genotyping Map of Penta D and D21S11 Loci in Case 7**

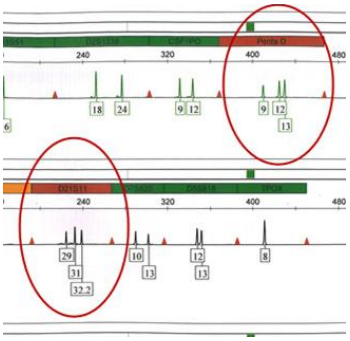

**Figure 8. STR Genotyping Map of Penta D and D21S11 Loci in Case 8**

## 4. Discussion

### 4.1 Discovery of Ternary Alleles

The majority of individuals (90%) exhibit STR genotyping with two bands, indicating heterozygosity. A minority (10%) show a single band, representing homozygosity, while a very small proportion display three bands, corresponding to a three-allele genotype [4]. The three-allele genotype primarily manifests in two peak patterns: Type 1, where the combined heights of the first two peaks approximate the third peak height, is more prevalent and may be associated with somatic mutations at the heterozygous locus; Type 2, characterized by three peaks of equal height, may result from chromosomal rearrangements at the heterozygous locus [5]. Currently, STRBase contains approximately 220 autosomal STR loci, among which 35 loci (CSF1PO, D1S1656, D2S1338, D2S441, D3S1358, D5S818, D6S1043, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D20S482, D21S11, D22S1045, FGA, Penta D, Penta E, SE33, TH01, TPOX, vWA, D1S1627, D3S3053, D4S2366, D5S2500, D6S474, D9S1122, D11S4463, D14S1434, D17S974, DYS612) have reported third allelic genotypes. The exact number of third allelic genotype combinations is not publicly disclosed due to dynamic updates, but literature suggests that each locus may report 1–5 different combinations (e.g., D21S11 has 4 common combinations), totaling approximately 50–100 types. The third allelic genotypes for loci vWA,

D5S818, D19S433, D8S1179, TPOX, Penta D, and the special type of third allelic genotype II for D21S11 [6] have not been reported domestically.

The six Penta E trisomic genotypes identified in this study—17/19/20 (Type I), 12/19/20 (Type I), 10/11/20 (Type I), 12/13/18 (Type I), 16/17/20 (Type I), and 5/12/13 (Type I)—along with one D7S820 trisomic genotype (7/8/10, Type 1), two D18S51 trisomic genotypes (14/15/20 (Type I) and 16/23/24 (Type I)), one D12S391 locus trisomic genotype (19/20/22 (Type I)), one D8S1179 locus trisomic genotype (11/16/17 (Type I)), and one D6S1043 locus trisomic genotype (13/14/18 (Type I)) were all previously unreported and not included in the STRbase database. These represent newly discovered trisomic genotype alleles. Domestic scholars Liu Fang et al. [7] detected that the three-allele genotypes of the D18S51 locus were 13/18/19 and 12/22/23, both belonging to Type1, while the three-allele genotype of the D7S820 locus was 8/9/11, belonging to Type2; Lan Feifei et al. [8] reported that the three-allele genotype of the D7S820 locus was 8/11/12, belonging to Type2; Xu Zehui et al. [9] found one case of the three-allele genotype of the D18S51 locus (17/18/19) belonging to Type1 through testing 3,600 paternity identification cases. Chen Ling et al. [10] detected three types of three-allele genotypes of the D18S51 locus in 29,111 individuals from the China population, which were 14/15/17 (Type2),13/19/20 (Type1), and 13/14/15 (Type1). Zeng Yanhong et al. [11] detected two three-allele genotypes of D18S51 in 5,500 individuals: 14/20/21 and 16/21/22, both belonging to Type 1; Zhang Xiaoyan et al. [12] identified three-allele genotypes of the D6S1043 locus as 12,18,19 (Type 1),14,18.19 (Type 2), and the Penta E locus three-allele genotype as 14,23,24 (Type 1).

### 4.2 Clinical Application Value of Ternary Alleles

In this study, 8 patients with trisomy 21 (Down syndrome) were simultaneously detected to carry type II trisomic alleles at both the PentaD and D21S11 loci. All patients exhibited intellectual disability and characteristic clinical features of the condition. Among the 8 patients, the trisomic allele patterns at both D21S11 and PentaD loci were either conventional type II (peak height ratio 1:1:1) or special type II (peak height ratio 2:1/1:2). It has been reported that the extra chromosome 21 in trisomy 21 is predominantly maternal in origin, with maternal inheritance [13]. This is attributed to the prolonged oocyte development process and the extended interphase between meiosis II (approximately 12–40 years [14]), which increases the likelihood of meiotic homologous chromosome non-disjunction in oocytes compared to spermatocytes, leading to chromosomal numerical abnormalities. According to this classification of trisomic alleles, the trisomic alleles detected at both PentaD and D21S11 loci in this study all belong to type II. Additionally, the three-allele genotype 31.2/32/33 (Type I) of locus D21S11, the three-allele genotypes 29/31/31.2, 29/31/33.2, 31/33.2/33.2, 28/32/33.2, 29/31/32.2 of Type II, and the three-allele genotype 9/9/12,9/9/10,11/11/13,9/10/14 of locus Penta D II have not been reported or included in the STRbase database, representing newly discovered three-allele genotypes. The loci Penta D and D21S11 exhibit high population genetic polymorphism in China, both located on chromosome 21, with no linkage, and hold significant application value in forensic identification of trisomy 21 syndrome [15].

## 5. Summary

Through the analysis of 44 cases with three alleles, this study reports for the first time multiple novel allele combinations. Consistent results were obtained when re-verified using two different reagent kits, excluding false-positive results caused by non-specific PCR amplification, lane leakage, and experimental contamination. These variants may originate from chromosomal instability or population-specific mutations, challenging forensic STR typing standards. Future studies should integrate NGS technology and large-sample validation to further elucidate their genetic mechanisms and application boundaries.

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