MOLECULAR AND EXPERIMENTAL BIOLOGY IN MEDICINE

# GENETIC VARIATIONS OF D15S541 AND D15S11 LOCI IN THE CROATIAN POPULATION AND THEIR APPLICATION IN THE DETECTION OF UNIPARENTAL DISOMY 15

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**Abstract:**

**Aim**: Prader Willi (PWS) and Angelman (AS) syndromes are two clinically distinct genetic disorders, both mapping to chromosome region 15q11-q13. PWS and AS can be caused by a de novo derived deletion of this region, uniparental disomy (UPD) of chromosome 15, or silencing of alleles.

**Methods:** Polymorphism of two STR loci (D15S11 and D15S541) was studied in a sample of 178 healthy unrelated Croatian individuals. The group of 28 patients with the clinical presentation of PWS or AS was also tested for these two loci, as well as for additional two loci (D15S642 and D15S659) for the detection of UPD15. Alleles at the tested loci were determined by the PCR-STR method.

**Results**: Among healthy individuals, 13 and 17 different alleles were identified at the D15S11 and D15S541 locus, respectively. The most frequent alleles at D15S11 were the D15S11-2 allele (50.0%), followed by the D15S11-5 allele (12.6%) and the D15S11-6 allele (10.4%). Among D15S541 alleles, the most frequent was D15S541-5 (36.5%), followed by D15S541-9 (34.8%). The observed heterozygosity for tested STRs was 0.624 for D15S11 and 0.719 for D15S541, while PIC values were as follows: 0.712 (D15S11) and 0.727 (D15S541). No significant deviations from Hardy–Weinberg equilibrium could be observed for these systems.

In the patients' group, the parental origin of chromosome 15 was determined in 26 out of 28 patients, while for two patients only one STR locus was informative, and therefore not sufficient for the diagnosis of UPD.

**Conclusion:** The combination of four STR loci at chromosome 15 is in most cases sufficient for the detection of UPD15.

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## INTRODUCTION

Uniparental disomy (UPD) is defined as the inheritance/presence of both homologous chromosomes of a pair from one parent and no copy from the other parent in a diploid genome.[1] UPD is classified as maternal or paternal, depending on the origin of the disomic chromosome. Identification of uniparental disomy has been based on the observation of the patient's parental alleles inheritance. UPD is detected using DNA polymorphism analysis. This analysis typically determines single nucleotide polymorphism (SNP) or microsatellite loci, known also as Short Tandem Repeats (STRs), and it requires a DNA sample from both parents and the proband. STR loci are highly informative, easily detectable by the polymerase chain reaction (PCR), have a large number of alleles, and do not require a complicated laboratory procedure. Currently, more than 60 STR loci are mapped to the chromosome 15. One of the advantages of the application of STRs in the field of UPD diagnostics is the possibility of resolving the origin of chromosomes. The presence of either maternal Prader-Willi syndrome (PWS) or paternal Angelman syndrome (AS) alleles of chromosome 15 is indicative of UPD15.[2, 3] UPD 15 represents the most frequently observed UPD.[4] Two syndromes, PWS and AS, belong

to UPD 15. Prader-Willi syndrome affects an estimated 1 in 10,000 to 30,000 people worldwide, while Angelman syndrome affects an estimated 1 in 12,000 to 20,000 people. Prader-Willi syndrome is caused by the loss of active genes in a specific chromosome 15 region. This region is located on the long (q) arm of the chromosome and is designated as 15q11-q13. It is also the same part of chromosome 15 that is usually affected in people with Angelman syndrome, although different genes are associated with the two disorders. Approximately 70% of individuals with PWS have a 15q11.2-q13 deletion on the paternally inherited chromosome 15, 25% have maternal uniparental disomy (UPD), <5% have an imprinting center sequence variant, and 1% have a structural chromosome rearrangement involving 15q11.2-q13.[5, 6] At the same time, around 70% of individuals with AS have a 15q11.2-q13 deletion of the maternal-origin chromosome, 11% have a sequence variant in UBE3A, 7% of AS patients have paternal UPD, and 3% have an imprinting center sequence variant.[6, 7] For the detection of UPD 15, STR loci within and outside the PWS/AS critical region are used. STR loci inside the region are used for the identification of the origin of chromosome 15, while microsatellites outside the PWS/AS region are used to distinguish deletion from UPD. Deletion is suggested if there is a uniparental inheritance of STR loci (loci are derived from a single parent) within the PWS/AS region and biparental inheritance of STR loci (loci are derived from a both parents) outside the PWS/AS critical region. The presence of uniparental inheritance of both STR loci (within and outside the critical region) reveals a UPD. The origin of UPD depends primarily on nondisjunction (ND) events that can occur in either meiosis I (MI) or meiosis II (MII). STR loci localized close to the centromere also have to be analyzed for UPD patients because they allow us to disclose the meiotic origin of the nondisjunction.[8] The heterodisomic state (two different homologous chromosomes and their frequently different alleles are inherited from the same parent) of these polymorphic markers indicates a MI error, and their isodisomy (two copies of the same chromosome are inherited from one parent) indicates a nondisjunction in the MII stage or a postzygotic event.[9]

The aim of the present study was to analyze polymorphisms of D15S11 and D15S541 loci in the Croatian population and their possible application in diagnostics of UPD.

## MATERIAL AND METHODS

Blood samples from 178 unrelated healthy individuals from the Croatian population were obtained for the population study. Genomic DNA was isolated from peripheral blood using the NucleoSpin Blood isolation kit (Machery-Nagel, Duran, Germany).[10]

Twenty patients with a clinical presentation of PWS and eight patients with a clinical presentation of AS were also included in the present study. For the population study, two STR loci (D15S11 and D15S541) were analyzed while UPD patients were tested for additional two STR loci (D15S642 and D15S659). The origin of nondisjunction was established using the most centromeric markers (D15S511 and D15S541).

Amplification of all samples was performed in two separate PCR reactions as described previously.[2, 11] After amplification, the PCR products (1.5 μl) were mixed with 3 μl of loading buffer and 1μl of each of the two internal size markers and applied on a 6% standard denaturing polyacrylamide gel in an automated laser fluorescence DNA sequencer (ALFexpress, Pharmacia Biotech, Uppsala, Sweden). On each gel, external, commercial sizers (100bp and 200 bp) were included. Alleles were determined and analyzed using the AlleleLocator software (Pharmacia Biotech).

The Hardy-Weinberg equilibrium test was performed by the $\chi 2$-test. Allele and genotype frequencies for each STR locus were determined by direct counting. The power of exclusion (PE) was calculated as described by Crow,[12] while polymorphism information content (PIC) value was obtained as suggested by Hearne.[13] The average power of discrimination (PD) was estimated as proposed by Desmarais.[14]

## RESULTS AND DISCUSSION

D15S11 and D15S541 loci were characterized in the Croatian population, regarding the allele and genotype

**Table 1. The distribution of allele frequencies at D15S11 and D15S541 loci in the Croatian population (N=178)**

| D15S11 | | D15S541 | |
|---|---|---|---|
| **allele (bp)** | **frequency** | **allele (bp)** | **frequency** |
| **1** (240) | 0.0056 | **1** (134) | 0.0084 |
| **2** (242) | 0.5000 | **2** (136) | 0.0028 |
| **3** (244) | 0.0169 | **3** (138) | 0.0028 |
| **4 (**246) | 0.0449 | **4** (140) | 0.0871 |
| **5** (248) | 0.1264 | **5** (142) | 0.3652 |
| **6** (250) | 0.1039 | **6** (144) | 0.0281 |
| **7** (252) | 0.0590 | **7** (146) | 0.0253 |
| **8** (254) | 0.0253 | **8** (148) | 0.0365 |
| **9** (256) | 0.0421 | **9** (150) | 0.3483 |
| **10** (258) | 0.0449 | **10** (152) | 0.0618 |
| **11** (260) | 0.0169 | **11** (154) | 0.0056 |
| **12** (262) | 0.0112 | **12** (156) | 0.0169 |
| **13** (264) | 0.0028 | **13** (158) | 0.0028 |
| | | **14** (160) | - |
| | | **15** (162) | - |
| | | **16** (164) | 0.0056 |
| | | **17** (166) | 0.0028 |

Legend: bp-base pairs

**Table 2. Observed genotype frequencies for D15S11 and D15S541 loci in the Croatian population (N=178)**

| D15S11 | | | | D15S541 | | | |
|---|---|---|---|---|---|---|---|
| **g** | **f** | **g** | **f** | **g** | **f** | **g** | **f** |
| **1-4** | 0.006 | **5-5** | 0.028 | **1-9** | 0.017 | **6-9** | 0.011 |
| **1-9** | 0.006 | **5-6** | 0.017 | **2-5** | 0.006 | **6-10** | 0.022 |
| **2-2** | 0.281 | **5-7** | 0.017 | **3-4** | 0.006 | **6-11** | 0.006 |
| **2-4** | 0.017 | **5-10** | 0.011 | **4-4** | 0.011 | **7-9** | 0.022 |
| **2-5** | 0.140 | **5-11** | 0.006 | **4-5** | 0.051 | **7-10** | 0.006 |
| **2-6** | 0.084 | **5-12** | 0.006 | **4-8** | 0.006 | **8-8** | 0.017 |
| **2-7** | 0.062 | **6-6** | 0.022 | **4-9** | 0.073 | **8-9** | 0.006 |
| **2-8** | 0.017 | **6-8** | 0.006 | **4-10** | 0.017 | **8-10** | 0.006 |
| **2-9** | 0.034 | **6-10** | 0.011 | **5-5** | 0.118 | **9-9** | 0.118 |
| **2-10** | 0.062 | **6-11** | 0.011 | **5-6** | 0.017 | **9-10** | 0.017 |
| **2-11** | 0.011 | **6-12** | 0.011 | **5-7** | 0.022 | **9-12** | 0.006 |
| **2-12** | 0.006 | **7-7** | 0.006 | **5-8** | 0.017 | **10-10** | 0.011 |
| **2-13** | 0.006 | **7-8** | 0.006 | **5-9** | 0.309 | | |
| **3-6** | 0.011 | **8-8** | 0.006 | **5-10** | 0.034 | | |
| **3-7** | 0.011 | **8-10** | 0.006 | **5-12** | 0.028 | | |
| **3-8** | 0.006 | **9-9** | 0.017 | **5-13** | 0.006 | | |
| **3-11** | 0.006 | | | **5-17** | 0.006 | | |
| **4-4** | 0.017 | | | | | | |
| **4-6** | 0.011 | | | | | | |
| **4-7** | 0.011 | | | | | | |
| **4-9** | 0.011 | | | | | | |

Legend: g - genotype; f - frequency

frequencies. The allele frequencies observed for each of the two STR loci are listed in Table 1.

At D15S11 locus 13, different alleles with frequencies ranging from 50.0% (D15S11-2 allele) to 0.3% (D15S11-13 allele) were observed. PCR fragment length ranged from 240-264 bp. The most frequent allele was the D15S11-2 allele (50.0%) followed by the D15S11-5 allele (12.6%) and the D15S11-6 allele (10.4%). A total of 37 genotypes were observed, with D15S11 2-2 being the most frequent genotype (28.1%). The D15S11 genotype frequencies are presented in Table 2.

Seventeen alleles with frequencies ranging from 36.5% (D15S541-5 allele) to 0.3% (D15S541-2, D15S541-3, D15S541-13, D15S541-17 alleles) were identified at the D15S541 locus. PCR fragment length ranged from 134-166bp. D15S541-14 and D15S541-15 alleles have not been detected in our population. The most frequent allele was the D15S541-5 allele (36.5%) followed by the D15S541-9 allele (34.8%). The genotype frequencies are presented in Table 2. At D15S541 locus, out of the 30 determined genotypes, genotype D15S541 5-5 (30.9%) was the most frequent.

The results of statistical calculations and additional forensic data for the loci D15S11 and D15S541 are shown in Table 3.

The studied population sample is in Hardy-Weinberg equilibrium. The expected and observed numbers of heterozygosities do not differ significantly. The observed heterozygosities of D15S11 and D15S541 were 62.4% and 71.9% respectively. The values for both observed and expected heterozygosity of these two markers were similar to those described for other Caucasian populations.[2, 11, 15]

The strategy adopted for the analysis of AS/PWS patients in different laboratories varies. In our laboratory, a routine karyotype analysis which detects translocations and inversions predisposing to deletions or other chromosomal abnormalities is performed first. Following that, a fluorescent in situ hybridization (FISH) analysis is carried out. FISH analysis ensures the detection of deletions but not of uniparental disomies. For that reason, the PCR-STR method is the next step performed for all samples negative for deletion.

The parental origin of chromosome 15 was analyzed in the group of 20 patients with a clinical presentation of PWS and in the group of eight patients with a clinical presentation of AS using D15S11 and D15S541 loci, as well as two previously tested loci (D15S642 and D15S659).[16] All patients demonstrated normal FISH results (no deletion was found). As the American College of Medical Genetics strongly recommends, we considered at least two fully informative loci showing either matUPD15 or biparental inheritance for diagnostic reporting.[17] Based on this criterion, we were able to identify the parental origin of chromosome 15 in 26 out of 28 patients; while for the remaining two patients, only one STR locus was fully informative. In a total of 20 patients with a clinical presentation of PWS, we discovered seven patients with maternal UPD (Table 4). At the same time, among eight patients with a clinical presentation of AS, only one patient demonstrated paternal UPD (Table 5). Heterozygosity at the PWS critical region for all PWS patients excludes the possibility of deletion. The origin of non-disjunction was established using the most centromeric loci, D15S11 and D15S541. It is important to mention that the number of tested STR loci is questionable, but a similar study was performed in 2000, in which authors used only two STR loci.[18] The used loci, D15S11 and D15S541, are the closest to the centromere of chromosome 15.

**Table 3. Statistical parameters for D15S11 and D15S541 loci**

| Parameter | D15S11 | D15S541 |
|---|---|---|
| ***Observed heterozygosity*** | 0.623596 | 0.719101 |
| ***Expected heterozygosity*** | 0.664644 | 0.685835 |
| ***Expected probability of exclusion*** | 0.897643 | 0.887548 |
| ***Matching probability*** | 0.102357 | 0.112452 |
| ***Polymorphism information content*** | 0.711893 | 0.72723 |

Mutirangura et al. suggested that heterozygosity for maternal alleles at a locus close to the centromere suggests (with a 90% likelihood) that the nondisjunction is a MI error, since the probability of a recombination between this locus and the centromere is low.[2] The heterodisomic state of these loci indicates an

**Table 4. Microsatellite analysis of uniparental disomy**

a) Prader-Willi syndrome patients

| sample | D15S541 | D15S11 | D15S642 | D15S659 |
|---|---|---|---|---|
| mother | 4 8 | 2 5 | 7 10 | 8 10 |
| patient 1 | 4 8 | 2 5 | 7 10 | 8 10 |
| father | 5 9 | 2 6 | 2 9 | 3 3 |
| mother | 15 15 | 1 2 | 2 9 | 6 7 |
| patient 2 | 15 15 | 1 2 | 2 9 | 6 7 |
| father | 6 8 | 12 12 | 4 10 | 5 6 |
| mother | 3 3 | 1 4 | 9 10 | 5 9 |
| patient 3 | 3 3 | 1 4 | 9 10 | 5 9 |
| father | 4 5 | 2 2 | 4 7 | 3 11 |
| mother | 5 9 | 4 12 | 9 9 | 5 6 |
| patient 4 | 5 9 | 4 12 | 9 9 | 5 6 |
| father | 8 12 | 8 8 | 7 10 | 6 8 |
| mother | 4 5 | 3 6 | 4 14 | 6 8 |
| patient 5 | 4 5 | 3 6 | 4 4 | 6 8 |
| father | 10 12 | 3 8 | 4 13 | 7 7 |
| mother | 6 7 | 1 5 | 8 8 | 4 4 |
| patient 6 | 6 7 | 1 5 | 8 8 | 4 4 |
| father | 4 5 | 2 2 | 4 8 | 3 8 |
| mother | 5 8 | 1 7 | 3 8 | 2 3 |
| patient 7 | 5 8 | 1 7 | 3 8 | 2 3 |
| father | 4 8 | 1 8 | 3 3 | 4 5 |

b) Angelman syndrome patient

| sample | D15S541 | D15S11 | D15S642 | D15S659 |
|---|---|---|---|---|
| mother | 3 7 | 5 7 | 3 5 | 2 8 |
| patient | 3 3 | 1 1 | 4 4 | 3 3 |
| father | 3 3 | 1 4 | 4 7 | 3 6 |

Legend: alleles of fully informative loci are underlined

MI error and its isodisomy indicate nondisjunction in the MII stage or a postzygotic event.[9] Using the analysis of STR loci, we identified the meiotic origin of nondisjunction in six out of eight cases. We were not able to identify the meiotic origin of nondisjunction for patients no. 2 and no. 3, because in their cases the locus D15S541 was not informative for hetero-/isodisomy.

However, the D15S11 locus (which is mapped only 3cM from the centromere) showed heterodisomy for both patients, so we can consider these cases as heterodisomy. Thus, among patients that presented a meiotic error, our results suggest that all of the cases are the result of non-disjunction at MI. A mitotic error was considered if all STR loci throughout the chromosome showed a reduction to homozygosity. We found one child (UPD AS patient) with a reduction to homozygosity for all STR loci, which is attributable to a post-zygotic event.

In conclusion, the combination of four STR loci at detection of UPD15. Our results also confirm that most maternal ND events resulting in UPD15 PWS are associated with MI errors, with rare cases being MII or due to postzygotic errors, whereas most paternal UPD15 seem to be postzygotic events.

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