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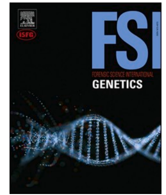

Research paper

Uniparental disomy of chromosome 21: A statistical approach and application in paternity tests

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ABSTRACT

Considering the overall frequency of paternity investigation cases including mutational events, there is a real possibility that at least a fraction of all inconsistencies reported in paternity cases are caused not by polymerase slippage mutations, but to chromosomic abnormalities, as Uniparental Disomy (UPD). We report here the investigation of a trio paternity case (mother, child and alleged father), with observed inconsistencies that can alternatively be explained by occurrence of maternal uniparental isodisomy of chromosome 21 (miUPD21). A total of 350 short tandem repeat (STR) and single nucleotide polymorphism (SNP) markers were tested, statistically suggesting true biological linkage within the trio. Additionally, we propose miUPD21 explains, with significantly greater probability, the occurrence of detected inconsistencies, when compared to alternative hypothesis of multiple and simultaneous slippage mutations. Similar cases could have their statistical conclusions improved or even altered by including unusual chromosomal segregation patterns in the hypothesis formulation, as well as in mathematical calculations. Such reports of allelic inconsistencies being explained by chromosomal alterations are common in clinical genetics, and such situations might have impact on forensic investigation.

1. Introduction

Forensic investigations of typical genetic relatedness (as in paternity trio tests including mother, child and alleged father) starts from the premise that the child always inherits an allele from his mother and another from his father. By convention, alleles found in the child that are not present in maternal genetic profile are considered to be obligate paternal alleles (OPAs). Thus, when the analysis and comparison of genotypes of those involved in a case of genetic kinship are carried out, it is sought to identify if the individual indicated as the alleged father presents all OPAs necessary to complement the child genetic profile. When genetic inconsistencies are not observed in studied loci between OPAs and alleles found in the man who is claimed to be the father, statistical calculations are performed to evaluate Combined Paternity Index (CPI) and probability of this man to be the biological father of the child in question. Such calculations give the alleged father a statistical probabilistic value, considering population allelic frequency of investigated markers. For this, likelihood ratio analysis is used, between the probability of observing genetic data considering that alleged father is the biological father of the child (null hypothesis) and the probability of genetic findings occurrence giving that the true biological father is any other individual in the population, unrelated and randomly selected (alternative hypothesis). [1–3]

Cases where one, two or even three allele inconsistencies are detected between alleged father and the OPAs usually require an increase in number of evaluated loci, in order to definitively exclude paternity or to consider inconsistencies as possible mutational events. [4,5] Technical recommendations mention slippage mutations as the

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main cause of inconsistencies in genetic paternity investigations, and also address occurrence of chromosomal alterations responsible for atypical results, including situations of triallelic patterns or trisomy. [1,6–9] However, no information is available on other variations that deviate from expected classical Mendelian inheritance. Occurrence of Uniparental Disomy (UPD) and other chromosomal abnormalities commonly found in clinical genetics are still poorly reported by forensic genetics studies and can lead to drawbacks in tests results interpretation or even misinterpretation, entailing inconclusive or even false reports. [1,9]

UPD constitutes inheritance of a chromosome pair from a single parent. Four proposed mechanisms describe autosomal UPD in a zygote: 1- Trisomy rescue: when a non-disjunction in meiosis-I (heterodisomy - hUPD) or in meiosis-II (isodisomy - iUPD), in the ovule (maternal UPD - mUPD) or spermatozoid (paternal UPD - pUPD), generates a dysfunctional gamete (24, X + A or 24, Y + A) that binds to a normal gamete (23, X or 23, Y), following trisomy rescue with expelling of third chromosome; 2 - Gamete complementation: when non-disjunction in spermatogenesis generates a nullisomic gamete (22, X-A or 22, Y-A) that binds to a normal ovule (23, X), and there is rescue of monosomy with two secondary oocyte chromatids complementing the 2n = 46 configuration; 3 - Post fertilization error: when, after the zygote formation, there is a segregation error in blastocyst mitotic divisions, which maintains two identical chromatids; 4 - Monosomic rescue: when non-disjunction in meiosis generates a nullisomic gamete (22, X-A or 22, Y-A) that binds to a normal gamete, followed by monosomy rescue where the chromatid copied during the mitotic division phase is kept. [10,11]

Depending on which chromosome displays a disomic pattern, UPD patients may present syndromic phenotypes or punctual abnormalities, such as delays in physical and mental development; however, UPD cases may also have phenotypes compatible with normality. [10,12] Among chromosomal alterations in this category the mUPD15, which causes Prader-Willi syndrome, is reported to have the highest frequency (1.25×10^{-5}), followed by Angelman's syndrome (pUPD15) (1×10^{-6}). [13] Almost two decades ago, Robinson inferred expected total UPD frequency for total set of chromosomes would be 2.9×10^{-4}, which would be equivalent to a mean UPD frequency per chromosome of 1.32×10^{-5}, regardless of maternal or paternal origin. [13] In addition, UDP cases frequency may vary according to maternal age. This data indicates UPD occurrence is not extremely rare and may, therefore, be underestimated. [14–17]

Population prevalence of uniparental disomy cases, as well as its impact on some physical traits, were investigated in a recent study performed by Nakka and collaborators. [17] Analysis were performed on population databases comprised of over four million individuals, which includes nearly one million parent/sibling pairs. Estimations suggest UPD frequency as usual as 1 in 2000 births, with different frequencies for each chromosome. Maternal origin is significantly more common, and mother age seems to be a factor influencing event probability. UPD prevalence described in this study seems to be more reliable than previously proposed ones, since it is based not only in subjects displaying clinical manifestations due to presenting UPD, but in general population data instead.

Considering average frequency of UPD cases, there is a real possibility that a fraction of detected inconsistencies in paternity cases is caused not by polymerase slippage mutational events, but due to uniparental disomy instead. In this line of thought, this article reports the investigation of a paternity case with inconsistencies that could be explained by occurrence of maternal uniparental isodisomy of chromosome 21 (miUPD21). A total of 350 short tandem repeat (STR) and single nucleotide polymorphism (SNP) markers were tested, statistically suggesting true biological parental relations within the trio. In addition, over 100,000 paternity investigation cases were reviewed, in search of chromosome 21 inconsistencies which could also be explained by UPD.

2. Material and methods

2.1. Ethical statements

This study was submitted to CEP / CONEP (Comitê de Ética em Pesquisa / Conselho Nacional de Saúde) system and has been approved by the Research Ethics Review Boards of Pontifícia Universidade Católica do Rio Grande do Sul (CAAE: 87198618.3.0000.5336) and Secretaria da Saúde do Estado do Rio Grande do Sul (CAAE: 87198618.3.3001.5312), Brazil.

2.2. Case Study - DISOMY OF CHROMOSOME 21 (DC21)

2.2.1. Samples, DNA extraction and Quantification

Mother, child and alleged father were assisted by Secretaria da Saúde do Estado do Rio Grande do Sul – SES/RS –, Brazil for the purpose of paternity investigation. During anamnesis, all three individuals presented a phenotype compatible with normality. DNA from blood and oral mucosa was collected in FTA (Whatman, Maidstone, United Kingdom) and swabs, respectively, and was extracted using the QIAmp DNA Investigator kit (Qiagen, Hilden, Germany), according to the manufacturer's recommendations. [18] DNA quantification was performed with the Qubit dsDNA High Sensitivity assay kit (Thermo Fisher Scientific Inc., Massachusetts, United States) on the Qubit Fluorometric Quantitation (Thermo Fisher Scientific Inc.). [19]

2.2.2. Amplification of STRs markers with commercial kits

DNA samples were amplified with commercially available kits for human identification-oriented STRs markers Globalfiler (Thermo Fisher Scientific Inc.), PowerPlex Fusion (Promega Corporation, Madison, WI, United States) and PowerPlex Y23 (Promega Corporation), according to the manufacturer's instructions. [20–22] An additional analysis of chromosome 21 was performed by multiplex PCR, with a set of fluorescently labeled primers for the simultaneous amplification of four 21-STR loci: D21S1435 (21q21.1), D21S1437 (21q21.1), D21S2052 (21q21.1) and D21S2055 (21q22.2). Primer sequences are available in Supplementary Table 1. PCR was performed on Veriti 96-well Thermal Cycler equipment (Thermo Fischer Scientific Inc.) under following conditions: 95 °C 15′; 10 cycles 94 °C 30″, 60 °C 90″, 72 °C 60″; 15 cycles 94 °C 30″, 58 °C 90″, 72 °C 60″; 60 °C 60′. PCR products were separated by capillary electrophoresis in 3500 Genetic Analyzer (Thermo Fisher Scientific Inc.). Allele call and genetic profile analysis were performed on GeneMapper ID-X v.1.4 software (Thermo Fisher Scientific Inc.). [23]

2.2.3. Massive parallel sequencing (MPS)

Three MPS panels designed for Ion Torrent Platform (Thermo Fischer Scientific Inc.) were used: Precision ID Identity (comprising 90 HID A-SNP and 34 Y-SNP), Precision ID Ancestry (165 AIM A-SNP) and Precision ID Globalfiler NGS STR (31 STRs), using 1 ng of total genomic DNA per reaction. Libraries were constructed with Ion AmpliSeq Library Kit 2.0 (Thermo Fischer Scientific Inc.) according to manufacturer's instructions using a different barcodes for each sample in a same run (Ion Xpress Barcode Adaptors 1–96 Kit or IonCode Barcode Adapters 1–384 Kit) (Thermo Fisher Scientific Inc.). [24] Library purification was performed with magnetic beads (Agencourt Ampure XP Magnetics Beads, Beckman Coulter, FL, United States) and final quantification of amplified samples was performed in Applied Biosystems 7500 Real-Time PCR System, using Ion Library TaqMan ™ Quantitation Kit (Thermo Fischer Scientific Inc.). An equimolar pool was prepared with final concentration of 20 pM of each library. Amplicons pool was amplified by emulsion PCR using Ion One Touch 2 Instrument (Thermo Fischer Scientific Inc.) with Ion PGM HiQ Template Kit, following manufacturer's protocol. PCR products underwent an ISP (Ion Sphere Particles) enrichment process using Ion Touch Enrichment System (ES) (Thermo Fischer Scientific Inc.). Finally, 318 Chip Kit v2 (Thermo Fisher

Scientific Inc.) was loaded with a volume of 30 µl. Sequencing reaction was performed on Ion Torrent PGM ™ Sequencer using PGM Hi-Q Sequencing Kit (Thermo Fischer Scientific Inc.). Sequencing results were generated by Torrent Suite Software v5.0 (Thermo Fischer Scientific Inc.) using Hg19 as reference genome. Secondary analysis, such as number of reads, was calculated through Coverage Analysis v5.0 plugin. For allele call and analysis of polymorphisms included in both SNPs panels, HID SNP Genotyper v4.3.1 plugin was used. BAM (Binary Alignment Map) files for all samples were visualized through the IGV (Integrative Genomic Viewer) software. [24–26] All SNPs were independently reviewed by two collaborators and manual corrections were applied, when necessary. For STR markers, HID STR Genotyper v.4.0 plugin (Thermo Fischer Scientific Inc.) was used, and all markers presenting coverage under 50x were reviewed. Amplicons presenting sequences with a single repeat motif shorter or longer than corresponding alleles and with read number under 20 % of main peaks were considered stutters.

2.2.4. Statistical Analysis

Familias v.3.2.4 software was used to evaluate statistical parameters and calculate the LR (Likelihood Ratio) for A-STRs and A-SNPs markers and, after that, Combined Parenthood Index (CPI). [27] Calculations were verified using in-house scripts developed by the authors, using the formulas proposed by Balding and Nichols (1994, 1995) and Ayres (2000). [28–30] For Y-STR and Y-SNP markers, the formula $PI = 1/f$ was used, being f the frequency found for the Y-STR haplotype in Brazilian population, according to available population databases. [1,31] Kappa method was used, which estimates haplotype frequency based on the proportion of singletons within a sample of a population, and resulting expected frequency for obtained haplotype is 4.5×10^{-5}, according to database available for Brazilian population *via* YHRD online platform. [31] All calculations were performed adopting a conservative approach, where corrections for population substructuration ($\theta = 0.01$) and minimum allele frequency (5/2 N) were observed. [1] Paternity probability was estimated using an *a priori* probability of 0.5 for both null and alternative hypotheses. For mUPD21 occurrence hypothesis, a frequency of 1.09×10^{-5} was used, which was estimated for this kind of occurrence from a large population dataset including almost a million parent/child duos. [17] This specific population prevalence was estimated for 21-chromosome UPD with maternal origin only, and excluded disomies where paternal chromosome is duplicated. Allele frequencies used in CPI calculations, for autosomal STRs markers, where based on available Brazilian frequencies. A comprehensive list of references adopted for each marker is presented in Supplementary Table 2. [32–38] For inconsistency analysis in STRs, equal probability mutation model was used for all loci (where number of motif repetitions are not considered for calculations), as well as the following mutation rates: CSF1PO $= 1.5 \times 10^{-3}$, D21S11 $= 1.6 \times 10^{-3}$, PENTA D $= 6.5 \times 10^{-4}$. [39,40] Markers D21S1435, D21S1437, D21S2052, D21S2055 and D5S2800 were not included in CPI calculations, since they have unknown frequencies in Brazilian population and no studies of linkage equilibrium were performed, for the same population. HID autosomal SNP markers frequencies were described for Brazilian population by Avila et al. (2019). [41] For AIM autosomal SNPs, Brazilian population frequencies were obtained and internally validated in Laboratory of Human and Molecular Genetics (data to be published). Since no information regarding ancestry of individuals examined in this case was available, ethnic bias was minimized by constructing a reference database where ethnic composition mimics overall biogeographical ancestry found in Brazilian population. Therefore, we applied general Brazilian frequencies for each locus, instead of specific ethnic group allele frequencies. For SNPs in which mutations were detected, general mutation rate of 1×10^{-8} was used, as mutation rates per locus are yet to be determined. [2]

2.3. ANALYSIS OF OTHER PATERNITY CASES

A total of 116,224 paternity cases were reviewed, referring to information contained in results reports and electropherograms (8,307 cases from 2015 to 2017 from Secretaria da Saúde do Estado do Rio Grande do Sul – SES/RS, Brazil; 107,917 cases from 2008 to 2017 of Instituto de Medicina Social e Criminalística de São Paulo, IMESC, SP, Brazil). In this evaluation, all paternity trio cases (mother, child and alleged father) with results proposing a true biological relationship, verified by CPI $\geq$ 10,000 and PP $\geq$ 99.99 %, and simultaneously presented mutations and/or chromosomal alterations in chromosome 21 loci, were computed.

3. Results and discussion

3.1. CASE STUDY DC21

During our routine paternity test protocol execution, genetic compatibility between mother and child was found in 100 % of the 23 autosomal STR loci evaluated; regarding OPA and alleged father alleles, concordance was found in 20 of 23 autosomal STR. The three inconsistencies detected between alleles present in child and in alleged father profiles were (in bold, the child inconsistent allele) CSF1PO: M = 11–14; C = **10**-11; AF = 11–12; D21S11: M = 31.2–31.2; C = **31.2–31.2**; AF = 29–30 and PENTA D: M = 12–15; C = **15–15**; AF = 9–10. When considered 23 Y-STR markers, child and alleged father share the same haplotype. This data was considered insufficient for issuance of a conclusive report, since LR values for paternity hypothesis provided weak evidence of paternity (LR close to 2540 for STR markers only). Alternatively, we tested for the possibility of a close relative of alleged father to be the child biological father. LR for the hypothesis of paternity attributed to a father/son or to a full sibling of alleged father was tested and, in both cases, results favored the conclusion of paternity assignment to a different, unrelated man in Brazilian population.

Regarding autosomal STRs, the inconsistency found in CSF1PO marker was considered as an independent, one-step slippage mutational event. Sequence analysis in MPS data exhibits both maternal and paternal alleles are identical, for CSF1PO-11 allele, and therefore mutation origin could not be determined. Inconsistencies in two 21-chromosome STR markers (D21S11 and PENTA D) show that the child presents only one maternal allele in homozygozis, which is suggestive of miUPD21 occurrence. Thus, to evaluate the possibility of miUPD21, an assay was performed with amplification of extra markers, including four new 21-chromosome STRs and MPS genotyping of 289 SNPs (255 A-SNPs and 34 Y-SNPs), plus 11 STRs, one Y-STR, one Y-INDEL and amelogenin locus. Complete results show that: i- in all four 21-chromosome STRs, child's profile presented a homozygous pattern for a single maternal allele, and all of them were inconsistent with alleged father alleles; ii- genetic compatibility in all 255 autosomal SNPs analyzed between mother and child, and 254 of 255 in autosomal SNPs between child's OAP and alleged father alleles; this single allelic inconsistency occurred at locus 21q22.2 (rs914165: M=AG; C=AA; AF=GG). This result once again repeats the pattern observed in child profile, were only a single maternal allele is present in homozygosis. iii- Y-chromosome SNP markers confirmed that child and alleged father have the same haplotype, classified as haplogroup "E" (African origin) by genotyping plugins. iv- autosomal STR markers, evaluated by the MPS system included in analysis, confirmed the results found in previous genotyping performed by capillary electrophoresis; in an extra locus, however, a single inconsistency between mother and child was identified (D1S1677: M = 14–14, C = 12–12, AF = 12–13), which was considered as maternal drop-out allele, due to the low reading coverage observed at the locus (<30x) or, with less possibility, as a null allele or maternal two-step slippage mutation. Table 1 presents all genotypes supporting miUPD21 occurrence hypothesis. It is important to notice that, while some SNP markers genotypes found for all three individuals do not

Table 1
Chromosome 21 genotypes for evaluated trio. All observed results are compatible with miUPD21 occurrence. All genotypes observed for child are homozygous, as determined by peak height ratio analysis. Data regarding inconsistencies found in other chromosomes (in number of 2) are not presented.

MARKER	LOCATION	MOTHER	CHILD	OPA	FATHER
D21S11	21q21.1	31.2–31.2	31.2–31.2	31.2	29–30
PENTA D	21q22.3	12–15	15–15	15	9–10
D21S2052*	21q21.1	210–214	210–210	210	224–224
D21S2055*	21q22.2	356–360	360–360	360	352–352
D21S1437*	21q21.1	534–534	534–534	534	528–542
D21S1435*	21q21.1	172–180	180–180	180	164–172
rs722098	21q21.1	AG	AA	A	AG
rs2830795	21q21.1	AA	AA	A	AG
rs2831700	21q21.1	AG	AA	A	AG
rs914165	21q22.2	AG	AA	A	GG
rs221956	21q22.3	CC	CC	C	CC
rs2835370	21q22.2	TT	TT	T	TT

*analyzed by allele size (pb); OPA: Obligate paternal allele.

unmistakably support an miUPD21 event (since normal genetic inheritance can also explain these genotypes), all of them are compatible with miUPD21 hypothesis, with all the child alleles found in homozygosis. Only a single 21-chromosome SNP marker was consistent with UPD hypothesis and would not fit Mendelian segregation model. This result exhibits that biallelic SNP markers have a lower discrimination power for evidencing this kind of occurrence than the more polymorphic STR counterparts. For these markers set, Brazilian frequencies (when available) for alleles present in the child profile shows that probability of a random person in Brazilian population to be homozygous for all these markers with the same alleles is approximately 1 in 80 million.

With all genetic results found, statistical calculations were carried out to verify obtained CPI for alleged father in this trio paternity test. LR (likelihood ratio) was computed using traditional methods, and divergences were assigned as mutational events. Results are presented as follows:

(i)- LR calculated under traditional mutation model (LR_{μ}):

LR_{μ} .= Null Hypothesis/Alternative Hypothesis (Table 2) where Null hypothesis considered the probability of the child being a biological son of both mother and alleged father; Alternative Hypothesis considers the child is a son of the mother and of another random man in Brazilian population, unrelated to alleged father. Equal probabilities mutation model was used to explain inconsistencies in CSF1PO, D21S11, Penta D and rs914165. Results for all different markers and technologies applied are summarized in Table 2.

Paternity Probability (with a *prior* of 0.5) is > 99,9999999999 %. This result was considered robust enough to support a paternity inclusion conclusion in report issued by the laboratory. Despite that, all observed results are compatible with occurrence of miUPD21. In order to evaluate impact of such uncommon event in paternity investigation reports, we demonstrate a statistic model considering reported frequency of mUPD21. In this model, all 21-chromosome markers are not accounted as events with independent probabilities; instead, they are considered together as a single probabilistic event, with a frequency equivalent to the incidence of a single chromosome isodisomy with maternal origin. Thus, we designed the following model, and LR values are presented in Table 3.

(ii)-LR considering the occurrence of miUPD21 ($LR_{\mu+miUPD21}$)

$LR_{\mu+miUPD21}$= Null Hypothesis/Alternative Hypothesis (Table 3) are the same as in previous applied model. Equal probabilities mutation model was used to explain inconsistency in CSF1PO marker only. The paternity index for each evaluated 21-chromosome allele markers were not included in LR calculations. Instead, a single event of isodisomy, with a frequency equivalent to the probability of occurrence for this kind of event in the general population, was included in CPI calculations. Therefore, for the miUPD event (italic in Table 3), the LR value was calculated using the prevalence of miUPD21 in the general population (1.09×10^{-5}) as the numerator, and the product of OPA frequencies for inconsistent chromosome 21 markers used as the denominator. Only markers with known population frequencies were included in this assessment, comprising 8 out of 12 inconsistent markers in 21-chromosome, as shown in Table 1.

Since paternity inclusion was proposed, an extra comparison was executed, based in results presented in Tables 2 and 3. A new LR was determined, based on the following hypothesis: LR = H_0 / H_1, where H_0 considers the chance of alleged father is the biological father including an occurrence of miUPD21 in addition to mutation model (CSF1PO), and H_1 defines the chance of alleged father is the biological father under assumption that all inconsistencies are derived from mutations only. Obtained LR was equivalent to 1.29×10^{13}, showing that the chance of explaining the true biological relation between alleged father and child in the DC21 case with the occurrence of miUPD21 is 12.91 trillion times more likely than the chance of explaining the true biological relation between alleged father and child in case of DC21 based on mutations only. This ratio may still be underestimated, since proposed mutation model used to explain inconsistencies in loci CSF1PO, D21S11, Penta D accounts for equal probabilitiy mutations only. If a stepwise model were considered, mutation rates for both chromosome 21 STR markers would be significantly smaller, since adjusts in mutation frequency would consider 1 and a half steps and 5 steps mutations, respectively.

As far as we know, aneuploidy/euploidies are events arising from non-disjunction errors in random meiosis and, by theory, would occur with the same frequency for any human chromosome. [42,43] Drastic changes, such as monosomies and most autosomal chromosome trisomies, interfere in the viability of gametes, zygotes, embryos and/or fetuses, and therefore are not commonly observed. Human chromosomes 21, 18 and 13 have the lowest gene numbers (232, 269 and 321 genes, respectively), and present the highest rates of trisomy occurrences. Individuals presenting 21-chromosome trisomy are the only ones, among trisomic subjects, able to complete development to

Table 2
Likelihood Ratio of Null hypothesis including STR mutation rates to CSF1PO, D21S11 and Penta D loci, and SNP mutation rate to rs914165 locus *versus* Alternative hypothesis.

MARKERS (TECHNOLOGY)	LR_{μ}	ACCUMULATED LR	INCONSISTENCIES
STRs (CE)	2.54×10^{3}	2.54×10^{3}	3 mutations
Y-STR + Y-SNP (CE + MPS)	2.33×10^{4}	5.89×10^{7}	None
STRs (MPS)	8.33×10^{4}	4.91×10^{12}	1* (not defined)
SNPs HID (MPS)	1.38	6.78×10^{12}	1 mutation
SNPs AIM (MPS)	1.47×10^{17}	9.97×10^{29}	None
	$\mathbf{LR_{\mu}}$	$\mathbf{9.97 \times 10^{29}}$	

CE = Capillary Electrophoresis; MPS = Massive Parallel Sequencing; *Inconsistency in D1S1677 marker was not included in calculations (see above).

Table 3
Likelihood Ratio of Null hypothesis including STR mutation rate to CSF1PO locus, and miUPD21 *versus* Alternative hypothesis.

MARKERS (TECHNOLOGY)	$LR_{\mu+miUPD21}$	ACCUMULATED LR	INCONSISTENCIES
STRs (CE)	6.18×10^{9}	6.18×10^{9}	1 mutation
Y-STR + Y-SNP (CE + MPS)	2.33×10^{4}	1.44×10^{14}	None
STRs (MPS)	8.33×10^{4}	1.20×10^{19}	1* (not defined)
SNPs HID (MPS)	9.84×10^{7}	1.18×10^{27}	miUPD21
SNPs AIM (MPS)	1.12×10^{17}	1.32×10^{44}	None
miUPD event	9.75×10^{-2}	1.29×10^{43}	8 in 12 markers
	TOTAL $\mathbf{LR_{\mu+miUPD21}}$**:**	$\mathbf{1.29 \times 10^{43}}$	

CE = Capillary Electrophoresis; MPS = Massive Parallel Sequencing; *Inconsistency in D1S1677 marker was not included in calculations (see above).

adulthood. [44] Based on this understanding, as well as in anamnesis results of the DC21 case individuals (which have phenotype compatible with normality), and considering the genetic data (child likely presenting a duplicated maternal chromosome), the following possibility is suggested to explain observed results: formation of a 47XY + 21 trisomy karyotype zygote (or blastomeres) due to non-disjunction of maternal sister chromatids of 21-chromosomes during meiosis II, which allowed the continuity of development, followed by trisomy rescue with the loss of paternal chromosome 21.

3.2. ANALYSIS OF OTHER PATERNITY CASES

In view of scarcity of UPD forensic reports, a data review was performed in routines kinship analysis results to investigate possible unreported or unidentified UPD21 cases. [5,45–50] In a total of 116.224 trial exams, 121 cases involving inconsistencies in loci of chromosome 21 were found: 16 cases with trialellic pattern (15 of maternal origin and one of non-determined origin) and 105 inconsistencies denominated as mutational events (54 of paternal origin, 34 of maternal origin and 17 of undetermined origin). Only two of those cases were determined to be compatible with a possible UPD event, where the child's profiles for 21-chromosome markers exhibits a single parental allele in homozygosis and not shared by the other parent. This phenomenon was observed for at least two different 21-STR markers in each trio. Genotypes for those cases are: case A (piUPD21) D21S11: M = 28–29; C = 28−28; AF = 28–30; Penta D: M = 12–12; C = 10–10; AF = 10–10, which was interpreted as a two-steps maternal mutation event in Penta D locus; and case B (miUPD21) D21S11: M = 29–29; C = 29–29; AF = 30–31; Penta D: M = 9–15; C = 15–15; AF = 10–15, which was reported as a paternal one-step mutation at the D21S11 locus. Both cases could be actually UPD cases, but due to allele sharing between parents and child in one of tested markers, slippage mutation hypothesis was accepted. Visualization of UPD occurrence, and its possible identification, is only possible when at least two or more markers for a single chromosome are genotyped.

In regular paternity trio tests, a significant number of UPD cases probably remain undetected and are neither properly identified, nor misinterpreted for a mutation event. This situation may happen when father and mother share a common allele, also present in the offspring disomic chromosomes. In such cases, UPD is usually regarded as a classic inheritance pattern, and is not detected unless additional markers in the same chromosome are evaluated. For this reason, the number of cases identified in our analysis as possible UPD occurrences (two) is significantly lower than what was expected for the total number of cases evaluated, based on estimated UPD prevalence in the general population (where six UPD events were anticipated).

4. Conclusion

In DC21 case study, 350 markers were analyzed and statistically supported a biological kinship within the trio (mother, child, alleged father), and demonstrated that miUPD21 explains, with a significantly greater probability, the occurrence of observed inconsistencies, when compared to alternative hypothesis of multiple and simultaneous slippage mutations. Similar cases could have statistics results improved by considering UPD or other abnormalities occurrence probability in hypotheses formulation and mathematical calculations, with allelic inconsistencies being explained by well-known, and not so rare in clinical genetics, chromosomal alterations. Recent reports and population studies that measure the frequency of UPD for each chromosome with greater precision show that UPD events are more frequent than originally thought, with prevalence in some chromosomes equivalent to usual STR mutation rates. We reinforce the importance that research groups and institutions include the occurrence of classic chromosomal abnormalities in their technical recommendations and other publications.

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CRediT authorship contribution statement

C.P Cavalheiro: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing - original draft. **E. Avila:** Formal analysis, Investigation, Validation, Writing - review & editing. **A.Z. Gastaldo:** Formal analysis, Investigation, Validation, Writing - review & editing. **P. Graebin:** Data curation, Investigation. **C.H.A. Motta:** Data curation, Formal analysis, Resources. **R. Rodenbusch:** Data curation, Formal analysis, Resources. **C.S. Alho:** Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fsigen.2020.102368.

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