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Could rapidly mutating Y-STRs be a potential forensic tool in discriminating Lebanese monozygotic twins?

Paula Romanos[1*], Jamilah Borjac[1]

[1] *Beirut Arab University, Faculty of Sciences, Debbieh, Lebanon*

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***Correspondence**
E-mail: paula_romanos84@hotmail.com

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Abstract

Despite the high power of discrimination that characterizes the well identified 16 to 24 autosomal short tandem repeat markers, monozygotic twins differentiation is generally limited. Arising from a single fertilized egg, monozygotic twins share the same genotype therefore the same DNA profile. This situation imposes a challenge in forensics especially considering that lineage markers are in general less informative than autosomal ones. Although in some cases Y haplotype is considered a powerful investigative tool, it cannot distinguish males belonging to the same paternal lineage. The use of rapidly mutating Y-STRs with mutation rate above $1x10^{-2}$ that were recently included in forensic casework is presumed helpful. Since science is always dynamic and each population has its own characteristics, we aim in this study to distinguish between Lebanese monozygotic male twins using rapidly mutating Y-STRs. For this purpose, fourteen unrelated pairs of male monozygotic twins were recruited. Participants filled in a well-designed questionnaire and signed an informed consent. DNA was extracted using PureLink Genomic DNA Mini kit, genotyped using the Identifiler Plus kit, and separated on 3500 Genetic Analyzer to confirm the monozygosity status. DNA samples underwent a second amplification using the Y Filer Plus kit. According to our results, all the Y Filer Plus DNA profiles showed complete match for each twin pair. By consequence, the use of rapidly mutating Y-STRs in this study did not improve discrimination.

Introduction

Accurate DNA typing results are nowadays the basis of increasing number of judicial decisions (Vidaki et al., 2017). Still, the main limitation in forensic genetics involves distinguishing individuals within a pair of monozygotic twins in criminal or paternity cases (Stewart et al., 2015). Monozygotic twins arise from a single fertilized egg and as a consequence they share the same genotype (Xu et al., 2015). While DNA profile is unique to each person, with the probability of a DNA match between 2 unrelated persons chosen randomly in a certain population being 1 in a trillion, identical twins share the same DNA profile (Butler, 2005). This scenario poses an urgent challenge to forensic genetics especially that

monozygotic twinning accounts for 1 in 250 live births on average (Fraga et al., 2005). Till the present day, and despite the high power of discrimination that characterizes the well identified 16 to 24 autosomal short tandem repeat (STR) markers, monozygotic twins discrimination is still limited in forensics (Xu et al., 2015; Vidaki et al., 2017). Furthermore, lineage markers are in general less informative than autosomal ones. The human Y chromosome is male specific, haploid and typically escapes meiotic recombination (Yadav et al., 2014). Although considered the third smallest human chromosome with only 57 MB in size, Y chromosome has a huge role in forensics including missing persons investigation, and mass disaster identification (Pratap Singh et al., 2011; Diegoli, 2015). Y-STRs are largely used to resolve the male component of a DNA mixture when a high female background is present (Alghafri et al., 2015). In addition, it guides the inspection to build any paternal relationship among male individuals (Butler, 2010). However, this reconstruction fails to differentiate among males belonging to the same paternal lineage since they share the same Y haplotype (Ballantyne et al., 2014).

Thirteen rapidly mutating Y-STRs (RM Y-STRs) with mutation rate above 1×10^{-2} were recently introduced in forensics with a significant improvement over the available classical Y-STRs with estimated mutation rate of 1×10^{-3} (Ballantyne et al., 2010; 2012). The purpose of using such markers is to increase the differentiation of Y-STR profiles among close relatives and enhancing the level of male lineage resolution (Ballantyne et al., 2013). These 13 RM Y-STRs showed significant distinction in 50% of father-son, 60% of brothers, and 75% of cousins-cases (Ballantyne et al., 2012).

Worldwide, the male identical twins account for 6 per 1000 males (Weber-Lehmann et al., 2014). In Lebanon, the live twinning births rate is relatively high as estimated by the Lebanese Ministry of Health in 2016. Lebanon is an eastern Mediterranean country with a small population of 4 million (El Andari et al., 2013). It is well known for its distinct human genetic, cultural, ethnic and religious diversity (Haber et al., 2011). Because of the Islamic expansion in the 7th century, the Y haplogroup J2 is more frequent in Lebanese Muslims than in Lebanese non-Muslims with Arab peninsula as its origin.

The same study also elucidated that the crusaders activity in the 11th-13th century is the cause for higher frequency of the Y haplogroup R1b in Lebanese Christians compared to non-Christians with Eastern Europe as its origin (Zalloua et al., 2008). Moreover, some of 23 Y-STR studied markers were proven to be highly discriminative in the Lebanese population (Al-Azem et al., 2017).

Nevertheless, none of the Lebanese studies dealt with monozygotic twins enigma up till now. For this purpose, we used the Y Filer Plus kit, a new multiplex PCR kit shown to be well applicable in other Arab populations (Alghafri et al., 2013; Almohamd et al., 2015), to explore whether Lebanese monozygotic male twins could be discriminated using rapidly mutating Y-STRs.

Materials and methods

Participants

Fourteen unrelated pairs of Caucasian Lebanese male monozygotic twins were chosen randomly to participate in this study. After the Beirut Arab University IRB committee approval, the participants or the guardians of under-aged participants signed a written informed consent to participate in the study. The volunteers were geographically distributed over the five Lebanese governorates: the capital Beirut, Mount Lebanon, East Bekaa, North, and South Lebanon. They were subdivided according to their religion. Christians included the Maronite and Catholic fractions. Muslims included the Shiite and Sunnite fractions. Three pairs of the participants were under or equal 10 years of age, five pairs were under or equal 20 years of age, and six pairs were under or equal 30 years. Relevant data including age, marital status, health conditions, life styles, and education levels were obtained through the questionnaire.

Kits

Genomic DNA purification kit was procured from Invitrogen (PureLink Genomic DNA Mini kit Cat. No. K182001). The genotyping kits Identifiler Plus (Cat. No. A26182) and Y Filer Plus (Cat. No. 4484678) were procured from Applied Biosystems. The autosomal STRs of Identifiler Plus kit are D8S1179, D21S11, D7S820, CSF1PO, D3S1358,

THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA, and the sex determination Amelogenin. The 27 plex Y Filer Plus kit uses 6 dyes chemistry and includes the Y Filer kit loci (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439,DYS448, DYS456, DYS458, DYS635, and GATA H4), three new highly polymorphic loci (DYS460, DYS481, and DYS533) and seven new rapidly mutating Y-STRs (DYS387a/b, DYS449, DYS518, DYS570, DYS576, and DYS627). All chemicals and instrumentation used in the separation and detection of the amplified PCR products were procured from Applied Biosystems.

Sample collection, DNA extraction & quantification

Buccal swabs, from left and right cheeks of the twenty-eight volunteers, were collected using sterile cotton swabs. Participants washed thoroughly their mouth clean ten minutes before taking the swab. Buccal swabs were allowed to dry at room temperature and stored at 4ºC before DNA isolation. Genomic DNA was extracted according to the manufacturer's instructions. DNA samples were quantified using NanoDrop 2000 (Thermo Scientific).

PCR amplification

All the genomic DNA samples were first genotyped with Identifiler Plus kit to confirm their monozygotic status. The total volume of the reaction was 25 µl. It consisted of 10 µl of master mix, 5 µl of primer set, and 1 ng of extracted DNA (in a maximum input volume of 10 µl). The standard thermal cycling conditions applied were: enzyme activation at 95ºC for 11 min; 28 cycles of denaturation at 94ºC for 20 sec and annealing/extension at 59ºC for 3 min, followed by a final extension step at 60ºC for 10 min using Thermo Px2 96-well Thermal Cycler (Thermo Scientific).

The samples underwent another PCR amplification using the Y Filer Plus kit. The total volume of the reaction was 25 µl. It consisted of 10 µl of master mix, 5 µl of primer set, and 1 ng of extracted DNA (in a maximum input volume of 10 µl). The standard thermal cycling conditions applied were: enzyme activation at 95ºC for 1 min; 30 cycles of denaturation at 94ºC for 4 sec and annealing/extension at 61.5ºC for 1 min; followed by a final extension step at 60ºC for 22 min using the Veriti 96-well Thermal Cycler with the 9600 emulation mode (Applied Biosystems).

Positive controls (Identifiler Plus kit DNA control 9947A and Y Filer Plus kit DNA control 007) as well as negative controls were applied.

Capillary electrophoresis

All amplified samples were run in duplicate on ABI 3500 Genetic Analyzer using the standard injection parameters. Samples were prepared for fragment analysis by adding 3 µl of amplicon to 9.6 µl HiDi Formamide with either 0.4 µl the size standard Liz 500 for the Identifiler Plus Kit or Liz 600 for the Y Filer Plus Kit. Samples were then denatured at 95º C for 3 min and chilled on ice prior to electrophoresis. Identifiler Plus results were analyzed using Pop 7 polymer, a 50 cm capillary array and Gene mapper v4.1 software. Y Filer Plus results were analyzed using Pop 4 polymer, a 36 cm capillary array, and Gene mapper ID-X 1.4 software.

Allele designations were made using the allelic ladders provided with the PCR kits following the recommendations of the DNA commission of International Society of Forensic Genetics (ISFG) on autosomal and Y STRs analysis. The threshold of 50 RFU was used for peak calling.

Statistical Analysis

Discrimination capacity (DC), calculated as the number of different observed haplotypes over the total number of sampled haplotypes, is determined (Ballantyne et al., 2012). Haplotype frequency (HF) is also calculated through the Y Haplotype Reference Database (YHRD). Haplotype frequency is estimated by the counting method using the equation $f = X/N$ where X is the number of times a Y haplotype is observed in a database containing N profiles (Butler, 2010).

Results and Discussion

The fourteen pairs in this study, all lived in Lebanon since birth with Muslims majority, were monozygotic as confirmed by Identifiler Plus kit genotyping. Figure 1 shows a representative DNA profile match for a 24 years old monozygotic pair twin.

Similarly, all the 14 pairs showed complete match between individuals within the pair as obtained by their Y Filer Plus DNA profiles. Figure 2 shows a representative Y Filer Plus DNA profile match for a 14 years old monozygotic pair twin.

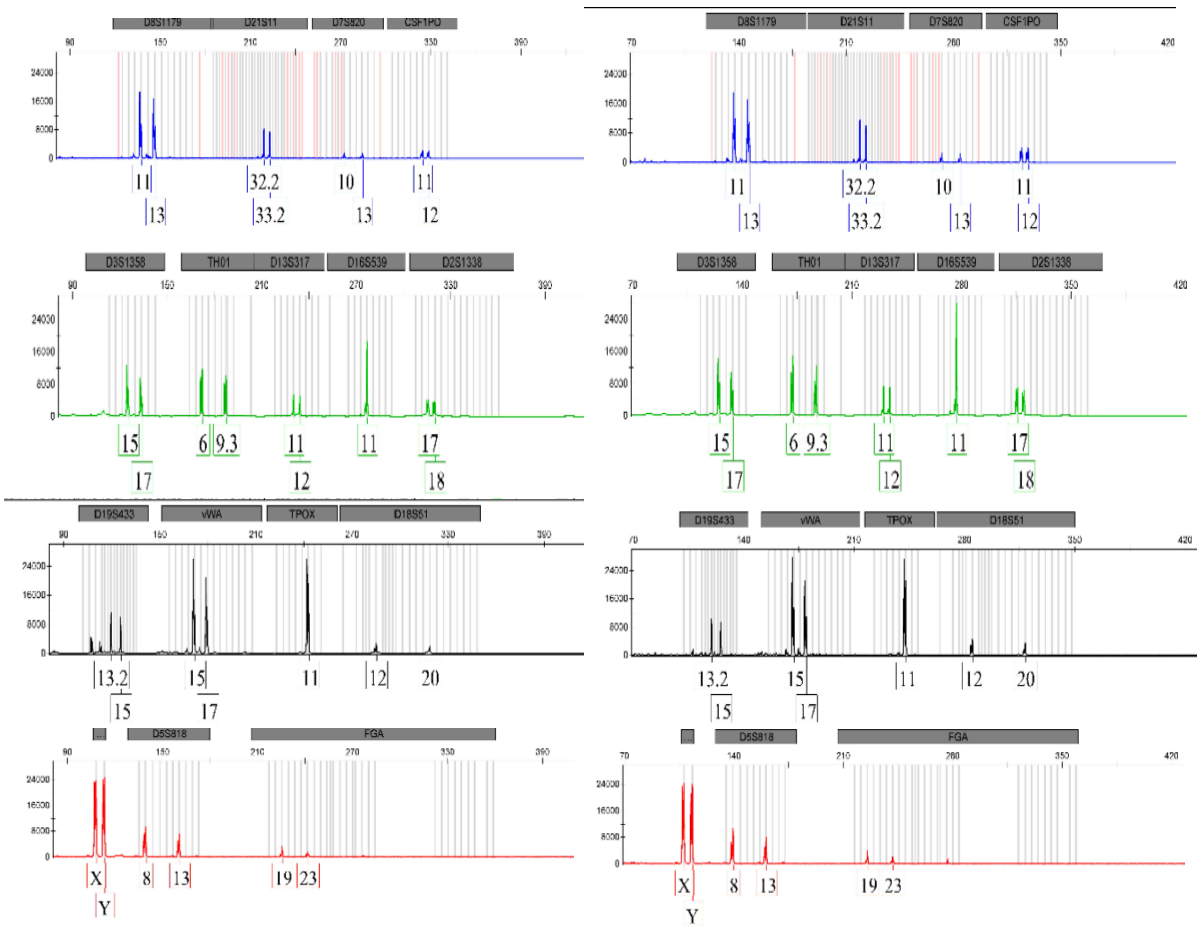
Our findings indicate 0 discrimination capacity for individuals within pair where the total of sampled haplotypes is 2. This result is valid for all the monozygotic twin pairs. According to our results, the used rapidly mutating Y-STR in this study showed no discrimination between the individuals within each pair.

One of the reasons could be that the frequency of mutation/locus/generation is high and easier to detect in father-son cases. This is due to the fact that the number of mutations increases as the number of meioses increases. In father-son cases, an improved discrimination capacity of 50% was observed while genotyping samples of 305 male relatives originated from Germany, Belgium, Poland and Canada (Ballantyne et al., 2012). Another study, including 74 father-son pairs in Serbia, detects 23 mutations of which 22 were one step mutations and 1 was 2 step mutation with DYF387S1 as one of the most mutable markers (Zgonjanin et al., 2017).

The efficiency of the rapidly mutating Y-STRs was examined worldwide not only in discriminating paternally related individuals like father-son cases but also in unrelated male cases. After genotyping of 250 and 600 samples in Qatar and UAE populations respectively, an increase in discrimination capacity was also obtained in both populations (Alghafri et al., 2013; Almohamd et al., 2015). In our research, a DC of 1 was obtained for unrelated twin pairs since the total of different observed haplotypes is 14 and the total of sampled haplotypes is 14.

It is worth noting that a complete Y Filer Plus DNA profile match between unrelated monozygotic pairs 4 and 5 was observed except at two loci, DYS389II and DYF387S1 as shown in Figure 3. It is most probably that twin pair 4 and 5 shared once a common ancestor Y haplotype.

Figure 1. DNA profiles of a 24 years old monozygotic pair twin matched with the Identifiler Plus



Both pairs 4 and 5 showed similar characteristics including age, living area, and religion. Genealogical records to trace their family history are needed to help in identifying if they are paternal cousins. Twin pair 4 presents allele 30 at DYS389II while twin pair 5 presents allele 31 at the same locus. In addition, twin pair 4 is homozygous (37, 37) at DYF387S1 while twin pair 5 is heterozygous (37, 38) at the same locus. At both loci, one step mutation which is a gain or loss of one repeat unit has been detected. DYFS3871S1 is a multicopy rapidly mutating Y-STR marker, having a large allele ranges and high diversities with mutation rate of 1.55 x 10-2 (Ballantyne et al., 2010).

The application of the new rapidly mutating marker DYFS3871S1 revealed a second mutation leading to a better discrimination. DYF3871S1 tends to be very informative in discriminating unrelated individuals.

As for the haplotype frequency, our data found 0 match in the multinational 40070 Y Filer Plus haplotypes (95% CI) for all twin pairs except for twin pair (Willuweit et al., 2015). By deduction, 13 out of 14 haplotypes were found to be very discriminative in the Lebanese population. Twin pair 10 haplotype found 7 matches in the multinational 40070 Y Filer Plus haplotypes. Concerning the national database "Lebanon", it found 7 matches in 50 Y Filer Plus haplotypes. Since all the matches appear to be in Lebanon, the multinational haplotype frequency would be 1.74 x 10^{-4} versus 0.14 for the national haplotype frequency. By consequence, this specific haplotype is more frequent in the Lebanese population than other ones thus less discriminative. The ancestry information, based on the minimal haplotype database, declares 33 matches in 255811

Figure 2. DNA profiles of a 14 years old monozygotic pair twin matched using the Y Filer Plus

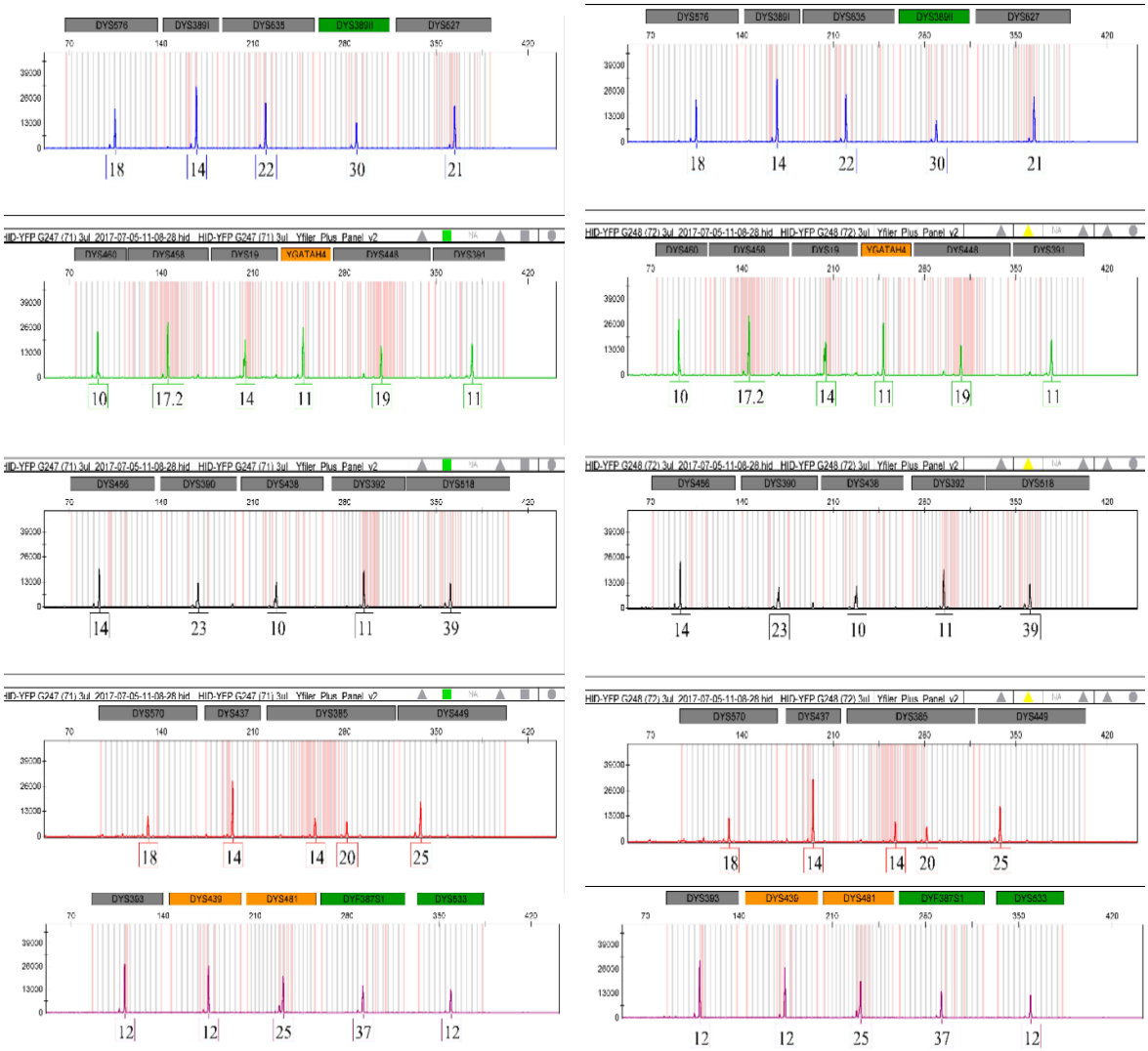
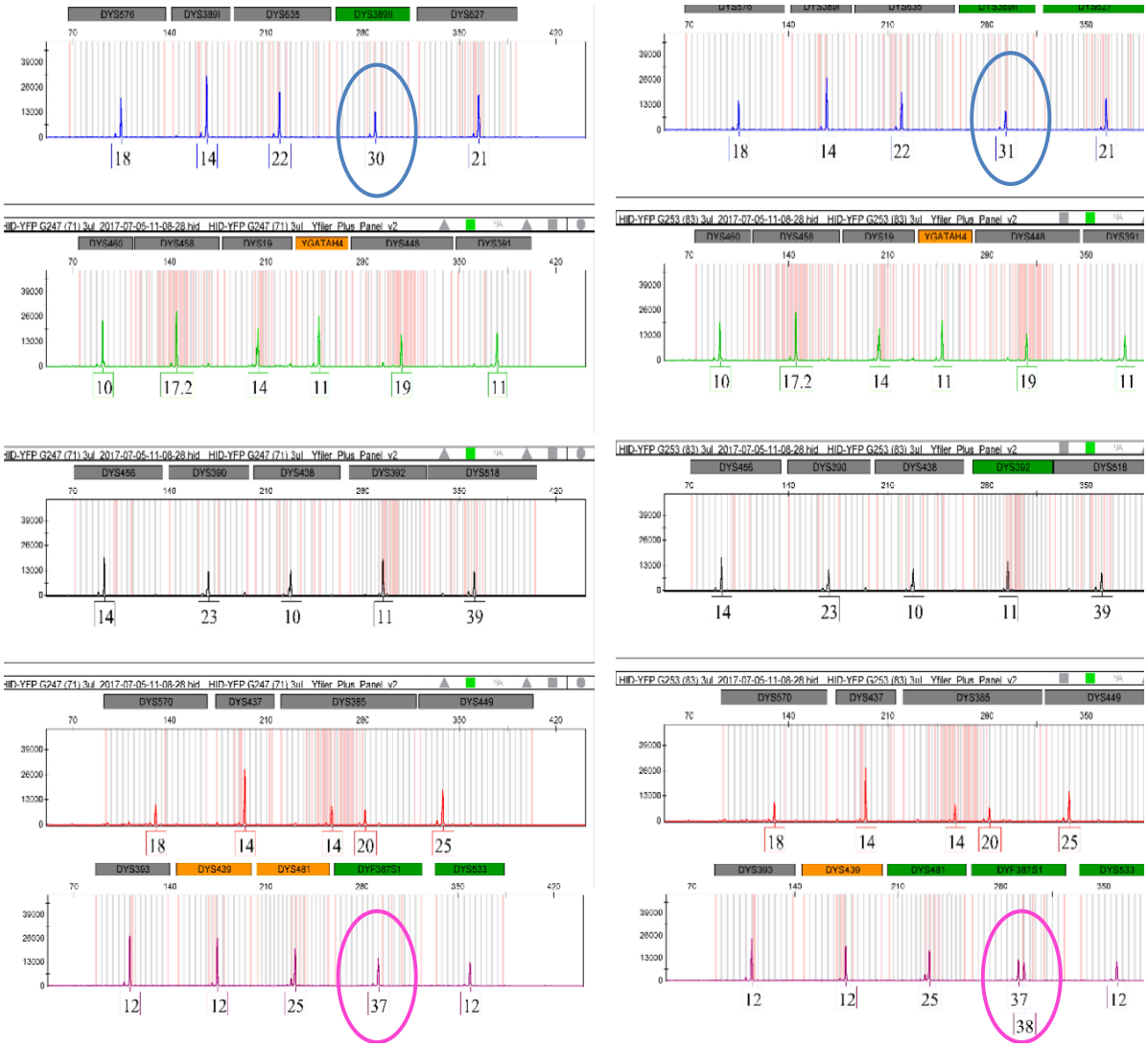


Figure 3. Y Filer Plus matched DNA profiles of monozygotic pairs 4 and 5 aged 14 and 15 respectively. DNA profiles differed at loci DYS389II and DYF387S1



worlwide haplotypes with 24 out of 33 found in Lebanon. Therefore, Lebanon presents the highest relative frequency of 4.3 x 10^{-2} with 24 matches out of the 555 national minimal haplotypes. The second high relative frequency, 2.5 x 10-3, was found in Bosnia and Herzegovina for 1 match out of 400 haplotypes. Regarding the ancestry information, based on the Y Filer Plus neighbors database, 11 neighbors were revealed out of 40070 haplotypes. One locus mismatch at DYS439 with allele 11 for twin pair 10 and allele 12 for its neighbors was discovered. Table 1 shows the twin pair 10 with one locus mismatch at DYS439 compared to its neighbors haplotype. Despite the outstanding results mentioned above, some studies like the one accomplished in Brasil and Italy showed that the expected haplotype mutation rate is considered insignificant and may still not be enough for the distinction (Palha et al., 2012; Boattini et al., 2016).

A research concerning eleven mono and dizygotic Serbian male twin pairs, using 13 rapidly mutating Y STRs instead of the 7 rapidly mutating Y STRs used in our study, was performed. Their results were concordant with ours. The Y Filer Plus kit loci did not reveal any differences among the monozygotic twins (Zgonjanin et al., 2017). Larger sample number may be needed to assess the possibility of using this kit in discriminating monozygotic twins.

Table 1. Twin pair 10 haplotype with one locus mismatch at DYS439 compared to its neighbors haplotype

Markers	Twin pair 10 haplotype	The neighbors haplotype
DYS576	18	18
DYS389I	13	13
DYS635	23	23
DYS389II	29	29
DYS627	21	21
DYS460	10	10
DYS458	16	16
DYS19	14	14
YGATAH4	11	11
DYS448	20	20
DYS391	10	10
DYS456	17	17
DYS390	25	25
DYS438	11	11
DYS392	13	13
DYS518	40	40
DYS570	15	15
DYS437	15	15
DYS385	12,14	12,14
DYS449	29	29
DYS393	12	12
DYS439	**11**	**12**
DYS481	22	22
DYF387S1	35,36	35,36
DYS533	12	12

Conclusions

In the present study, authors investigated the power of discrimination of 7 new rapidly mutating Y-STRs among 14 unrelated pairs of Lebanese monozygotic twins. While discrimination capacity of individuals within pair was 0, it shows to be 1 for unrelated twin pairs. Our results elucidate the importance of the new rapidly mutating Y-STR DYF387S1 application in discriminating unrelated individuals. Also our results showed that 13 out of 14 haplotypes are very discriminative in the Lebanese population. These facts highlight the importance of rapidly mutating Y-STRs use in forensics. Therefore much attention should be addressed to the ability of rapidly mutating Y-STRs to differentiate between unrelated individuals in general and monozygotic twins in specific.

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