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Y-SNP haplogroups in the Antalya population in Turkish Republic

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Abstract: SNPs are known to be the most abundant source of sequence variation in the human genome. The SNPs in the NRY (non-recombining Y-chromosome) region which passes from father to son as unchanged haplotype-blocks escaping recombination, provides important advantages in the investigations of sexual assault crimes, in the cases of parentage testing especially if the mother or alleged father is unavailable for testing and in the evolutionary studies. The aim of this study, was to determine the frequencies of Y SNP markers and the haplogroups, in order to define the Y-chromosome SNP markers which are polymorphic, have high discrimination power and can be used in forensic investigations in the Antalya population. For each of 75 unrelated males from Antalya, 35 different Y-SNP markers were amplified in a single reaction using multiplex minisequencing method. In the study, 18 markers of them were found to be polymorphic. The most frequent Y-SNP markers with mutations were M139 (100%), SRY_{10831}/SRY_{1532} (92%), M89 (85.3%), M213 (85.3%), M9 (44%), 92R7 (30.6%), 12F2 (30.6%), M45 (29.3%), M172 (26.6%) and M173 (22.6%). The Y-chromosome haplogroups of Antalya population were defined by these 18 Y-SNP polymorphic loci and the frequencies and the distribution of haplogroups were determined. J2*(xJ2F2) (26.6%), K*(xN3,O,P) (13.3%), E3b (9.3%), F*(xH,I,J,K) (8%), R1a1*(xR1a1b) (8%), R1b*(xR1b1, R1b6, R1b8) (8%), P*(xQ3a,R1) (8%) haplogroups were identified as the most abundant in Antalya population. These haplogroups are reported as widespread also in European and neighboring Near Eastern populations.

Key words: SNP, Turkish population, haplogroups

SNPs are base substitutions, insertions, or deletions which occur at single positions in the human genome at a density of 1-10 per 1000 nucleotides and therefore embody the most abundant source of genetic variation [1-20]. SNPs have very low mutation rates ($2x10^{-8}$ mutation/generation/year) and this characterisitic can increase the reliability in paternity tests and SNP markers can give more information about the history of human being in human evolution studies than STR markers [15-23].

SNPs can be analysed in short amplicons and this advantage makes it possible to analyse heavily degraded biological samples which can usualy be big problems in forensic investigations [12-17,19, 21, 24-30].

SNPs located in the non-recombining region of the Y-chromosome (NRY) which escapes recombination and is inherited from generation to generation as unchanged haplotype-blocks, can provide important advantages in investigations of rape cases with

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mixed stain evidences, in complex paternity testing of male children where the mother or alleged father is unavailable [9, 20, 31]. Y-SNPs are especially useful in human population studies because mutations which are recorded in the molecule are transferred from generation to generation as a genetic fingerprint of the evolutionary history of the locus [31].

Defined by characteristic Y-SNP alleles, Y chromosomes can be assigned to different haplogroups [32]. Some Y-SNP haplogroups show geographical spesificity and this regional spesificity provides important advantages in human evolution, population studies, forensic investigations and molecular anthropology [9, 30, 33-35].

The aim of this study, was to determine the frequencies of Y SNP markers and the haplogroups, in order to define the Y-chromosome SNP markers which are polymorphic, have high discrimination power and can be used in forensic investigations in the Antalya population.

1. Materials and Methods

1.1. Volunteers and DNA Isolation

The study was performed using peripheral blood samples of 75 healthy, unrelated volunteer males from various parts of Antalya. DNA was isolated from 300 µl of whole peripheral blood using the Gentra Puregene DNA Isolation Kit (Gentra Systems Inc., Minneapolis, MN, USA) according to the manufacturer's protocol. The extracted DNA samples were kept in -20°C until use.

1.2. Selection of PCR Amplification Primers

The sequence information of the primer pairs used to amplify the 25 Y-chromosome DNA segments including 35 different Y-SNP loci were obtained from the published article of J.J. Sanchez et al. (Table 1) [21]. Each primer pair was checked using the reference data of GenBank (www.ncbi.nlm.nih.gov).

1.3. PCR Conditions

Each primer pair was tested in singleplex PCR. Ampli Taq DNA polimerase (Promega) was used instead of Ampli Taq Gold DNA polimerase (Applied Biosystems) in the control amplification reactions to reduce the costs.

The PCR products of singleplex reactions were analysed by electrophoresis in % 8 polyacrylamide gels (PAGE) and the gels were stained with silver nitrate using silver staining (SS) method [36]. After successful amplifications of each primer pair, the primers of 25 Y chromosome DNA fragments were sorted in 3 different multiplex PCR groups (Table 2).

Ampli Taq Gold DNA polimerase was used in the multiplex reactions. The multiplex PCR reaction mix contents and PCR conditions are given in Table 3 and 4. The multiplex PCR products of 3 groups were analysed by PAGE (% 8) and SS. The primer concentrations in the multiplex reactions were optimized according to the results of electrophoregrams in order to obtain equal amount of each PCR product.

1.4. Purification

In order to remove the unused primers and unincorporated dNTPs from the PCR products, E. coli exonuclease I (Exo I) and shrimp alkaline phosphatase (SAP) was used. 1 µl PCR product of each 3 multiplex PCR group was taken into another PCR tube, 1.2 µl EXOSAP-IT kit (USB Corporation, Cleveland, USA) was added and incubated at 37°C for 1 hour. The enzymes were inactivated at 75 °C for 15 minutes.

1.5. Selection of PCR Minisequencing Primers

The sequences of the minisequencing primers were also obtained from the published article of J.J. Sanchez et al. (Table 5) [21]. The primers were designed with lengths between

19-106 nucleotides with intervals of 4 nucleotides so that the extension products could differ in length from each other [21].

The sequences of the chosen primers were checked for the possibility of primer-dimer formation. After the suitabilities of the primers were confirmed all the minisequencing primers were synthesized and HPLC purified.

Table 1. Y chromosome SNPs and and primer sequences for PCR amplification of 25 Y chromosome DNA fragments with SNPs. This table was taken from the study of JJ.Sanches et al. [21]

Locus	GenBank or dbSNPs accession	Mutation	PCR primers (5'→ 3') Forward primer	Reverse primer	μM	Amplicon size (bp)
M2/sY81	Rs3893	A/G	acggaaggagttctaaaattcagg	aaaatacagctccccctttatcct	0.15	128
M9	Rs3900	C/G	aggaccctgaaatacagaactg	aaatatttcaacatttcacaaaggaa	0.36	186
M17	Rs3908	4G/3G	cctggtcataacactggaaatc	agctgaccacaaactgatgtaga	0.09	170
M18	Rs3909	2 bp insertion	cctggtcataacactggaaatc	agctgaccacaaactgatgtaga	0.09	170
M19	Rs3010	T/A	cctggtcataacactggaaatc	agctgaccacaaactgatgtaga	0.09	170
M32	AC009977	T/C	tgaccgtcataggctgagaca	ttgaagcccccaagagagac	0.07	160
M33	AC009977	A/C	tgaccgtcataggctgagaca	ttgaagcccccaagagagac	0.07	160
M35	Rs1179188	G/C	agggcatggtccctttctat	tccatgcagactttcggagt	0.42	96
M40/SRY4064	AC006040	G/A	tggtctcaatctcttcaccctgt	catttcagtaaatgccacacaaga	0.18	119
M45	Rs2032631	G/A	gagagaggatatcaaaaattggcagt	tgacagtggcaccaaaggtc	0.03	138
M46/Tat	AC002531	T/C	tatatggactctgagtgtagacttgtga	ggtgccgtaaaagtgtgaaataatc	0.46	115
M52	AC009977	A/C	cctcaacttcccagagtgttg	gacgaagcaaacatttcaagagag	0.03	152
M78	AC010889	C/T	tgcattactccgtatgttcgac	tggaagcttaccatctttttatga	0.08	132
M81	Rs2032640	C/T	catctcttaacaaaagaggtaaattttgtcc	cattgtgttacatggcctataatattcagt	0.24	179
M89	Rs2032652	C/T	tggattcagctctcttcctaaggttat	ctgctcaggtacacacagagtatca	0.03	135
M96	AC010889	G/C	tgccctctcacagagcactt	ccacccactttgttgctttg	0.27	143
M123	AC010889	G/A	gttgcccaggaatttgcat	cacagagcaagtgactctcaaag	0.02	88
M139	AC010137	5G/4G	ccccgaaagttttattttattcca	ttctcagacaccaatggtcctatc	0.06	113
M151	AC010889	G/A	catctcttaacaaaagaggtaaattttgtcc	cattgtgttacatggcctataatattcagt	0.24	179
M153	AC010137	T/A	ccccgaaagttttattttattcca	ttctcagacaccaatggtcctatc	0.06	113
M154	AC010889	T/C	catctcttaacaaaagaggtaaattttgtcc	cattgtgttacatggcctataatattcagt	0.24	179
M157	AC010889	A/C	gagagaggatatcaaaaattggcagt	tgacagtggcaccaaaggtc	0.03	138
M163	AC009977	A/C	aggaccctgaaatacagaactg	aaatatttcaacatttcacaaaggaa	0.36	186
M167/SRY2627	AC006040	C/T	cggaaccactaccagcttca	agttaaggccccacgcagt	0.03	113
M170	Rs2032597	A/C	cagctcttattaagttatgttttcatattctgtg	gtcctcattttacagtgagacacaac	0.07	119
M172	Rs2032604	T/G	tgagccctctccatcagaag	gccaggtacagagaaagtttgg	0.16	179
M173	Rs2032624	A/C	ttttcttacaattcaagggcatttag	ctgaaaacaaaacactggcttatca	0.10	81
M175	Rs2032678	-5 bp	gatttaaactctctgaatcaggcacat	ttctactgatacctttgtttctgttcattc	0.02	79
M212	Rs2032664	C/A	ccatataaaaacgcagcattctgtt	tggagagaacttgagaaaaagtagagaa	0.12	176
M213	Rs2032665	T/C	ccatataaaaacgcagcattctgtt	tggagagaacttgagaaaaagtagagaa	0.12	176
M224	AC010889	T/C	tgcattactccgtatgttcgac	tggaagcttaccatctttttatga	0.08	132
SRY10831/SRY1532	Rs2534636	A/G	tcatccagtccttagcaaccatta	ccacataggtgaaccttgaaaatg	0.06	150
12f2	AC005820	Present/ Absent	cactgactgatcaaaatgcttacagat	ggatcccttccttacaccttataca	0.06	90
92R7	Rs2535813	GA/A	ttaaatccctcctatttgtgctaacc	aatgcatgaacacaaaagacgtaga	0.04	89
P25	Rs150173	C/CA	tggaccatcacctgggtaaagt	ggcagtataaggttgtcacatcacat	0.01	109

SNP markers on the same DNA fragment: (M9 and M163), (M17, M18 and M19), (M32 and M33), (M45 and M157), (M78 and M224), (M81, M151 and M154), (M139 and M153), (M212 and M213).

Table 2. The 3 multiplex reaction groups of primers for PCR amplification of 25 Y chromosome DNA fragments

Multiplex PCR Group I :

Locus	Mutations	PCR primers (5'→ 3') Forward primer	Reverse primer	Amplicon size (bp)
M2/sY81	A/G	acggaaggagttctaaaattcagg	aaaatacagctccccctttatcct	128
M9	C/G	aggaccctgaaatacagaactg	aaatatttcaacatttcacaaaggaa	186
M32	T/C	tgaccgtcataggctgagaca	ttgaagcccccaagagagac	160
M40/SRY4064	G/A	tggtctcaatctcttcaccctgt	catttcagtaaatgccacacaaga	119
M45	G/A	gagagaggatatcaaaaattggcagt	tgacagtggcaccaaaggtc	138
M52	A/C	cctcaacttcccagagtgttg	gacgaagcaaacatttcaagagag	152
M78	C/T	tgcattactccgtatgttcgac	tggaagcttaccatctttttatga	132
M81	C/T	catctcttaacaaaagaggtaaattttgtcc	cattgtgttacatggcctataatattcagt	179
M89	C/T	tggattcagctctcttcctaaggttat	ctgctcaggtacacacagagtatca	135

Multiplex PCR Group II :

Locus	Mutations	PCR primers (5'→ 3') Forward primer	Reverse primer	Amplicon size (bp)
M19	T/A	cctggtcataacactggaaatc	agctgaccacaaactgatgtaga	170
M96	G/C	tgccctctcacagagcactt	ccacccactttgttgctttg	143
M123	G/A	gttgcccaggaatttgcat	cacagagcaagtgactctcaaag	88
M167/SRY2627	C/T	cggaaccactaccagcttca	agttaaggccccacgcagt	113
M170	A/C	cagctcttattaagttatgttttcatattctgtg	gtcctcattttacagtgagacacaac	119
M172	T/G	tgagccctctccatcagaag	gccaggtacagagaaagtttgg	179
SRY10831/SRY1532	A/G	tcatccagtccttagcaaccatta	ccacataggtgaaccttgaaaatg	150
12f2	Present/ absent	cactgactgatcaaaatgcttacagat	ggatcccttccttacaccttataca	90

Multiplex PCR Group III :

Locus	Mutations	PCR primers (5'→ 3') Forward primer	Reverse primer	Amplicon size (bp)
M35	G/C	agggcatggtccctttctat	tccatgcagactttcggagt	96
M46/Tat	T/C	tatatggactctgagtgtagacttgtga	ggtgccgtaaaagtgtgaaataatc	115
M139	5G/4G	ccccgaaagttttatttttattcca	ttctcagacaccaatggtcctatc	113
M173	A/C	ttttcttacaattcaagggcatttag	ctgaaaacaaaacactggcttatca	81
M175	-5bp	gatttaaactctctgaatcaggcacat	ttctactgatacctttgtttctgttcattc	79
M212	C/A	ccatataaaaacgcagcattctgtt	tggagagaacttgagaaaaagtagagaa	176
92R7	GA/A	ttaaatccctcctatttgtgctaacc	aatgcatgaacacaaaagacgtaga	89
P25	C/CA	tggaccatcacctgggtaaagt	ggcagtataaggttgtcacatcacat	109

Table 3. The content of the first step multiplex PCR reaction mix

25 µl multiplex reaction volume contained:
• 10 ng DNA
• 1 x PCR buffer
• 8 mM $MgCl_2$
• 400 µM of each dNTP,
• 0.01-0.42 µM of each primer,
• 2.5 U Ampli Taq Gold DNA polimerase.

Table 4. The PCR program used for the multiplex reactions

94°C → 5 dk	
95°C → 30 sn	33 cycles
60°C → 30 sn	
65°C → 30 sn	
65°C → 7 dk	

Table 5. Minisequencing primer sequences for typing of 35 Y chromosome SNP markers (21)

Locus	Poly (dc)	Neutral Sequence (5' → 3')	Target spesific sequence (5' → 3')	Orientation	µM	Primer Size (nt)
M170	-	-	caacccacactgaaaaaaa	Reverse	0.02	19
M45	-	caa	ctcagaaggagctttttgc	Reverse	0.02	22
M139	-	aa	taatctgacttggaaagggg	Forward	0.01	22
M2/sY81	-	gacaa	ctttatcctccacagatctca	Reverse	0.28	26
M46/Tat	-	None	gctctgaaatattaaattaaaacaac	Reverse	0.25	26
M167/SRY2627	-	tgaaagtctgacaa	aagccccacagggtgc	Forward	0.35	30
M213	-	tgacaa	tcagaacttaaaacatctcgttac	Reverse	0.02	30
M52	-	tctgacaa	aatatcaagaaacctatcaaacatcc	Reverse	0.02	34
P25	-	tcgtgaaagtctgacaa	tgcctgaaacctgcctg	Forward	0.04	34
M78	-	gaaagtctgacaa	cttattttgaaatatttggaagggc	Reverse	0.02	38
92R7	-	gtgaaagtctgacaa	catgaacacaaaagacgtagaag	Reverse	0.01	38
M89	-	cacgtcgtgaaagtctgacaa	aactcaggcaaagtgagagat	Reverse	0.09	42
M123	-	acgtcgtgaaagtctgacaa	atttctaggtattcaggcgatg	Reverse	0.03	42
M35	-	ggtgccacgtcgtgaaagtctgacaa	tcggagtctctgcctgtgtc	Reverse	0.25	46
M153	-	ggtgccacgtcgtgaaagtctgacaa	gctcaaagggtatgtgaaca	Forward	0.02	46
M40/SRY4064	-	aaactaggtgccacgtcgtgaaagtctgacaa	tccaccctgtgatccgct	Reverse	0.08	50
M154	-	gccacgtcgtgaaagtctgacaa	gttacatggcctataatattcagtaca	Reverse	0.03	50
M32	-	taggtgccacgtcgtgaaagtctgacaa	agacaagatctgttcagtttatctca	Forward	0.50	54
M151	-	aggtgccacgtcgtgaaagtctgacaa	caatctactacatacctacgctatatg	Forward	0.02	54
M17	-	actaaactaggtgccacgtcgtgaaagtctgacaa	ccaaaattcacttaaaaaaaccc	Reverse	0.02	58
M96	-	aactgactaaactaggtgccacgtcgtgaaagtctgacaa	ggaaaacaggtctctcataata	Forward	0.15	62
M172	7	aactgactaaactaggtgccacgtcgtgaaagtctgacaa	caaacccattttgatgctt	Forward	0.10	66
M173	3	aactgactaaactaggtgccacgtcgtgaaagtctgacaa	tacaattcaagggcatttagaac	Forward	0.03	66
M19	4	aactgactaaactaggtgccacgtcgtgaaagtctgacaa	aaactatttttgtgaagactgttgta	Forward	0.10	70
M224	7	aactgactaaactaggtgccacgtcgtgaaagtctgacaa	aattgatacacttaacaaagatacttc	Forward	0.13	74
SRY10831/SRY1532	10	aactgactaaactaggtgccacgtcgtgaaagtctgacaa	ttgtatctgacttttcacacagt	Forward	0.03	74
M18	17	aactgactaaactaggtgccacgtcgtgaaagtctgacaa	gtttgtggttgctggttgtta	Forward	0.05	78
M157	18	aactgactaaactaggtgccacgtcgtgaaagtctgacaa	caccaaaggtcatttgtggt	Reverse	0.20	78
M81	14	aactgactaaactaggtgccacgtcgtgaaagtctgacaa	cttggtttgtgtgagtatactctatgac	Reverse	0.03	82
M163	25	aactgactaaactaggtgccacgtcgtgaaagtctgacaa	cacaaaggaatttttttgag	Reverse	0.51	86
M212	20	aactgactaaactaggtgccacgtcgtgaaagtctgacaa	gcattctgttaatataaaacacaaaa	Forward	0.20	86
M9	22	aactgactaaactaggtgccacgtcgtgaaagtctgacaa	catgtctaaattaaagaaaaataaagag	Reverse	0.40	90
12f2	29	aactgactaaactaggtgccacgtcgtgaaagtctgacaa	aacatgtaagtctttaatccatctc	Forward	0.02	94
M33	29	aactgactaaactaggtgccacgtcgtgaaagtctgacaa	cagttacaaaagtataatatgtctgagat	Reverse	0.18	98
M175	46	aactgactaaactaggtgccacgtcgtgaaagtctgacaa	cacatgccttctcacttctc	Forward	0.28	106

1.6. Testing Minisequencing Primers with Gel Electrophoresis

To check the minisequencing primers, minisequencing reaction was performed with unlabeled terminator ddNTP (Fermentas), instead of SNaPshot kit (Applied Biosystems). The PCR products were analysed by electrophoresis in PAGE (% 8) and SS. PGEM DNA was used as size standart and minisequencing primers were used as control ladder for each of their primer extension products. The detected bands of reaction products were just 1 bp behind their ladder because of the successful primer extension. In this way, we checked all the minisequencing primers without wasting the SNaPshot kit (Applied Biosystems).

1.7. SnaPshot-Minisequencing Reaction

After the test reactions, all the primers were used in one multiplex minisequencing reaction performed with SNaPshotTM kit (Applied Biosystems). The minisequencing reaction mix contents and PCR conditions are given in Table 6 and 7. After the minisequencing reaction a further purification step with 1 Unit SAP was performed to remove the 5' phosphoryl groups of the unincorporated [F]ddNTPs for 1 hour at 37°C. SAP was inactivated by incubation at 75°C for 15 minutes.

Table 6. The contents of the minisequencing reaction mix

The 5.2 µl total rection volume contained:
• 10 ng purified first step PCR product mixture
• 2.6 µl of SNaPshot™ reaction mix,
• 0.01-0.5 µM of minisequencing primers (Table 5).

Table 7. The PCR program for the minisequencing reaction

96°C → 1 dk	
96°C → 10 sn	26 cycles
50°C → 5 sn	
60°C → 30 sn	

Table 8. Frequencies of 35 Y-SNP markers in Antalya population

Locus	Polymorphism	Frequency (number)	Frequency (%)
M2/sY81	A/G	75/0	100.0/0.0
M9	**C/G**	**42/33**	**56.0/44.0**
M17	**4G/3G**	**69/6**	**92.0/8.0**
M18	No ins./ 2 bp İnsertion	75/0	100.0/0.0
M19	T/A	75/0	100.0/0.0
M32	T/C	75/0	100.0/0.0
M33	A/C	75/0	100.0/0.0
M35	**G/C**	**68/7**	**90.7/9.3**
M40/SRY4064	**G/A**	**67/8**	**89.4/10.6**
M45	**G/A**	**53/22**	**70.7/29.3**
M46/Tat	T/C	75/0	100.0/0.0
M52	A/C	75/0	100.0/0.0
M78	**C/T**	**71/4**	**94.7/5.3**
M81	C/T	75/0	100.0/0.0
M89	**C/T**	**11/64**	**14.7/85.3**
M96	**G/C**	**67/8**	**89.4/10.6**
M123	**G/A**	**73/2**	**97.3/2.7**
M139	**5G/4G**	**0/75**	**0.0/100.0**
M151	G/A	75/0	100.0/0.0
M153	T/A	75/0	100.0/0.0
M154	T/C	75/0	100.0/0.0
M157	A/C	75/0	100.0/0.0
M163	A/C	75/0	100.0/0.0
M167/SRY2627	C/T	75/0	100.0/0.0
M170	**A/C**	**73/2**	**97.3/2.7**
M172	**T/G**	**55/20**	**73.4/26.6**
M173	**A/C**	**58/17**	**77.4/22.6**
M175	-5 bp	75/0	100.0/0.0
M212	C/A	75/0	100.0/0.0
M213	**T/C**	**11/64**	**14.7/85.3**
M224	T/C	75/0	100.0/0.0
SRY10831/SRY1532	**A/G**	**6/69**	**8.0/92.0**
12f2	**Present/ Absent**	**52/23**	**69.4/30.6**
92R7	**GA/A**	**52/23**	**69.4/30.6**
P25	**C/CA**	**69/6**	**92.0/8.0**

Ins: insertion, bp: base pair

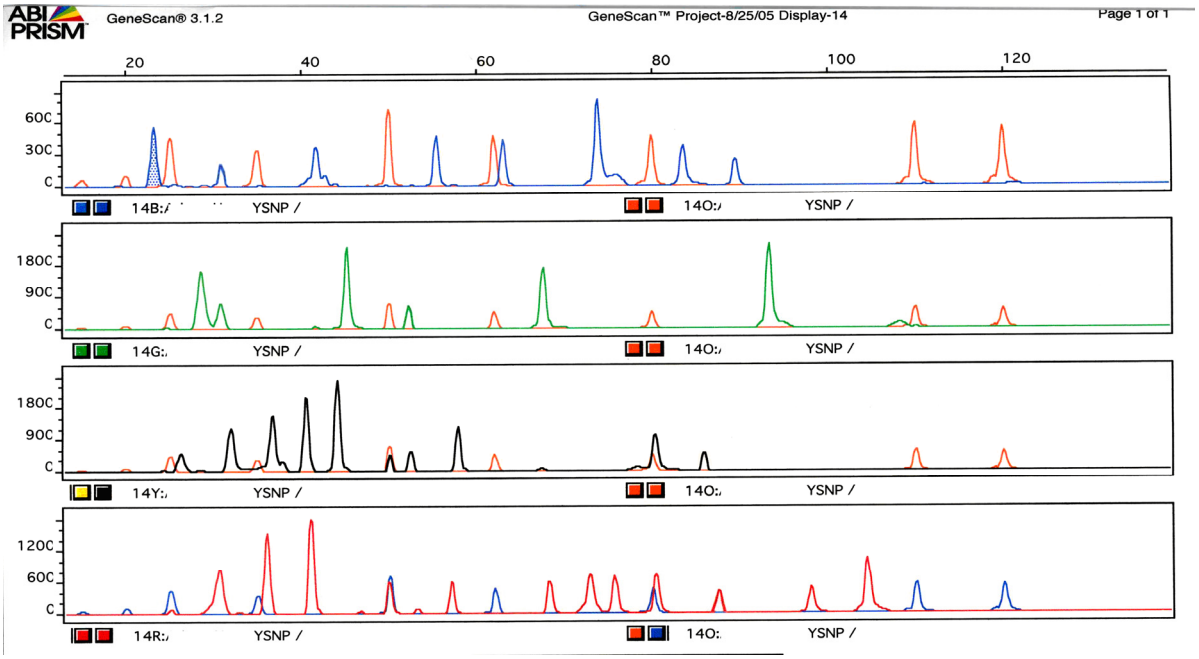
1.8. Capillary Electrophoresis

ABI Prism 310 Genetic Analyzer (Applied Biosystems), a 36 cm capillary aray, POP 4 polymer in conjunction with GS POP-4 (1mL) E5 module and GeneScan-120 LIZ as internal size standart was used for the capillary electrophoresis.

1.9. Analysis of Data

The datas obtained from the Genetic Analyser were analysed using GeneScan Analysis software 3.1.2 (Applied Biosystems). The SNP markers were represented as peaks with different colors in electropherogrammes (Fig.1). The peaks were sorted into bins according to the sizes compared with the internal size standart and SNPs were determined.

Fig. 1 Electrophoregramme representing 35 Y-SNP profiles of a male donor analysed by GeneScan Analysis software 3.1.2 (Applied Biosystems).



2. RESULTS

In the investigation of 35 Y-SNPs in 75 male individuals from Antalya, a total of 18 SNPs showed variation while 17 SNPs were monomorphic. The frequency distribution of 35 SNPs in 75 individuals from Antalya is given in Table 8. Mutation was determined in M139 in all of the participants.

The other informative markers with high mutation frequencies were SRY_{10831}/ SRY_{1532} (92%), M89 (85.3%), M213 (85.3%), M9 (44%), 92R7 (30.6%), 12F2 (30.6%), M45 (29.3%), M172 (26.6%), M173 (22.6%), in descending order.

According to the last Y-chromosome haplogroup classification of Y Chromosome Consortium (YCC), 13 different haplogroups were determined with frequencies ranging from 1.3% to 26.6 % in this study. Table 9 shows the phylogenetic relationships and frequency distribution of the haplogroups.

J2*(xJ2F2) (26.6%), K*(xN3,O,P) (13.3%), E3b (9.3%), F*(xH,I,J,K) (8%), R1a1*(xR1a1b) (8%), R1b*(xR1b1, R1b6, R1b8) (8%), P*(xQ3a,R1) (8%) haplogroups were determined as haplogroups with highest frequencies in Antalya population (Table 9.).

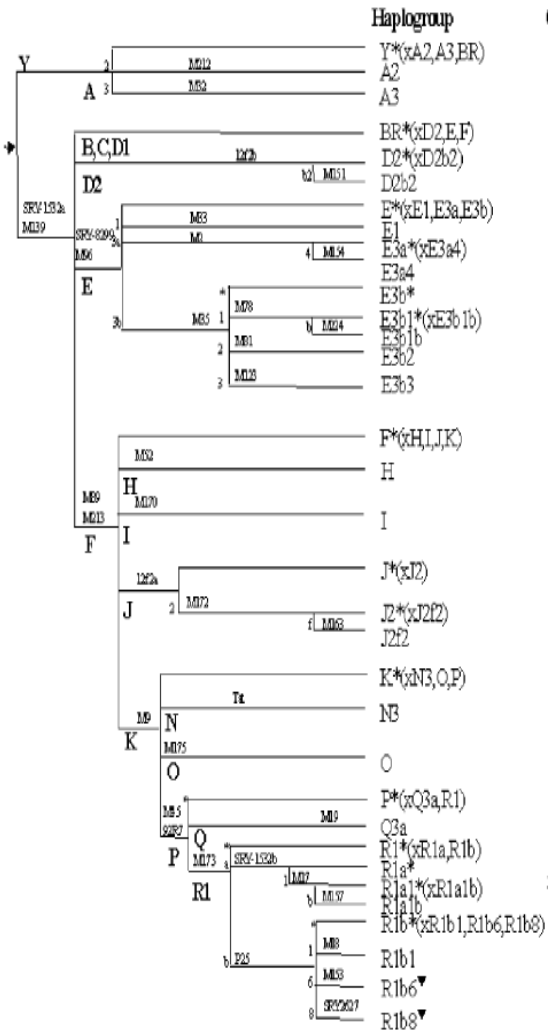
3. DISCUSSION

We used the SNaPshot minisequencing method in this study. As expected, we determined more extra bands in the PCR products amplified by Ampli Taq DNA polimerase (Promega) (used in the control reactions, to reduce the costs) than in the PCR products amplified by Ampli Taq Gold DNA polimerase (Applied Biosystems) which is known to minimize primer dimer formations.

The first step amplification primers could not be HPLC purified because of the limited financial fund provided for researches in our university. In order to minimize non-spesific products, the first amplification step was performed in 3 different multiplex PCRs. The multiplex PCR groups included maximal 9 primer pairs. This solution resulted in clear PCR products without aspesific products.

Table 9. The phylogenetic relationships* and frequency distribution of the haplogroups in Antalya population.

Haplogroup	N=75	Frequency (%)
Y*(xA2,A3,BR)		
A2		
A3		
BR*(xD2,E,F)	3	4
D2*(xD2b2)		
D2b2		
E*(xE1,E3a,E3b)	1	1.3
E1		
E3a*(xE3a4)		
E3a4		
E3b*	1	1.3
E3b1*(xE3b1b)	4	5.3
E3b1b		
E3b2		
E3b3	2	2.7
F*(xH,I,J,K)	6	8
H		
I	2	2.6
J*(xJ2)	3	4
J2*(xJ2f2)	20	26.6
J2f2		
K*(xN3,O,P)	10	13.3
N3		
O		
P*(xQ3a,R1)	6	8
Q3a		
R1*(xR1a,R1b)	5	6.6
R1a*		
R1a1*(xR1a1b)	6	8
R1a1b		
R1b*(xR1b1,R1b6,R1b8)	6	8
R1b1		
R1b6		
R1b8		



* Diagram of phylogenetic relationships was performed according to Sanchez et al.

Onofri et al. reported a great number of non-spesific amplification products mainly due to the use of none-HPLC purified primers [30]. Thus, the minisequencing reaction primers were all HPLC purified and 35 Y-SNP markers were successfully amplified in one minisequencing reaction.

A lack of correlation in expected/measured sizes for shorter extension products on avarage 4-5 nucleotides more was observed in the electropherogrammes obtained by GeneScan Analysis software. Onofri et al. also observed a lack of correlation in expected/measured sizes for the shortest extension products in their study. The reason of this determination was reported as the influence of the various masses of fluorochromes on the mobility of shorter DNA molecules and the secondary structure assumed by extension fragments in capillary electrophoresis. They solved the problem by increasing the extension primers up to 40-50 nucleotides [30]. The same problem was observed in the study of Sanches et al. and the high influence of various fluorochromes, purines and pyrimidines on the mobility of short DNA molecules was given as the potential reason of this problem [21].

Table 10. The comparison of the Y-SNP markers with high mutation frequencies in different populations

Researchers	Population	Y-SNP's with high mutation frequencies
Timur S. et al.	Antalya – 75 men	M139, SRY_{10831}/SRY_{1532}, M89, M213, M9, 92R7, **12F2**, M45, **M172**, M173
Sanches J.J. et al. [21]	Denmark – 194 men	M139, SRY_{10831}/SRY_{1532}, M89, M213, M9, 92R7, M45, M173
Raitio M. et al. [37]	Finland – 5 population – 300 men (Saami, Karelya, Ob-Ugric, Mansi, Khanti)	M9, SRY_{10831}/SRY_{1532}, 92R7, **M46-Tat, M17** **M12 only in Saami popultion**

Table 11. The general geographical distribution of some haplogroups determined by Y-SNP's in the world (3,38)

Population	Most frequently determined haplogroups
European and neighboring Near Eastern populations	E3b, G, J, I, L, N, K2, R1
Central Asian	C, Q, O
Indian	H, R2
African	A, E3*, E3a

Table 12. Comparison of Y-Chromosome haplogroups determined in different populations and researches

Researchers	Region-Population	Number of participants	Number of Y-SNP'S	Haplogrops with high frequency
Timur S. et al.	Turkey-Antalya	75	35	J, K*(xN3,O,P), E3b, F*(xH,I,J,K), R1a1*(xR1a1b), R1b*(xR1b1,R1b6, R1b8), P*(xQ3a, R1)
Cinnioğlu C.et al. [38]	Turkey	523	89	J, R, E3b, G, I
Onofri V. et al. [30]	European Asian African South American	68 17 9 3	37 37 37 37	R1, E3b, J2, I K*(xN3,O,P), P*(xQ3a, R1), R1, D A, B**,** E3b P*(xQ3a, R1)
Sanches J.J. et al. [39, 40]	Danes Greenlanders Somalis Canary Islands	194 215 201 348	35 35 35 35	I, R1b*(xR1b1,R1b6,R1b8),R1a1*(xR1a1b), P*(xQ3a, R1), R1b*(xR1b1, R1b6, R1b8), I E3b1*(xE3b1b), K*(xN3, O, P) R1b*(xR1b1,R1b6, R1b8), I, E, J2*(xJ2f)
Zegura S.L. et al. [41]	51 population in America, Asia, Europe American Asian	2344	63	Q (% 76.4), C (% 5.8) Q (% 17), C (% 22)
Butler J.M. et al. [42]	U.S. Caucasian African American	20 20	42 42	R (%38) E3a (%40)

We observed that 92R7, $M167/SRY_{2627}$ and M52 Y-SNP primers did not yield the corresponding peaks. 92R7 Y-SNP extension product was detected as a peak at 40 nucleotide size level with extra peaks at 40, 42 size levels, with wide peak bases. A similar problem was also seen in the peaks of $M167/SRY_{2627}$ and M52 Y-SNP extension products. In the study of Onofri et al. 2 couples of primers did not yield ideal peaks either. The reason was thought to be synthesis problem during manufacture by the researchers. The problem was solved by purchasing the oligonucleotides from another commercial source in their study [30].

The problem with $M167/SRY_{2627}$, M52 and 92R7 Y-SNP loci was tried to be solved by increasing the EXOSAP-IT kit and SAP concentrations during the purification steps, but no improvement could be achieved. So the minisequencing primers of $M167/SRY_{2627}$, M52 and 92R7 had to be resynthesized and HPLC purified by another commercial source. In the control minisequencing reaction and capillary electrophoresis with the new primers, ideal peaks were obtained.

We determined two signals for each 92R7 and P25 in some individuals. Similar results for 92R7 and P25 SNPs were published in many other studies. It is reported that this result depends on paralogous sequence variants [21, 29].

A general comparison of our results with other results of similar studies is given in table 10 and table 12 and table 11 shows the general geographical distribution of some haplogroups determined by Y-SNP's in the world.

The results we determined in our study are nearly similar to those of the research of C. Cinnioğlu et al. The haplogroups with high frequencies in Antalya population have also high frequencies in European and neighboring Near Eastern populations.

The discrimination power of STRs is about 4 times higher than SNPs, so in forensic cases we need more or less 60 SNP markers to reach the discrimination power of a routine used multiplex STR analysis kit with 15 STR markers [12, 13, 16, 24, 25, 27, 43,44]. In this study we determined only 18 polymorphic Y-SNP markers for Antalya population. Similar researches has to be performed and more polymorphic Y-SNP markers has to be determined for Antalya population in order to reach a sufficient discrimination power for routine forensic Y-SNP analysis in Antalya.

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