Analysis of *Alu* Insertion Polymorphism in South Morocco (Souss): Use of Markers in Forensic Science

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Abstract: The aim of the present study is the assessment of the utility of *Alu* insertions in forensic DNA typing within the South Moroccan population. We report on a comprehensive study of seven loci of interest due to the insertion or the deletion of human-specific *Alu* fragments. The study was carried out on 215 unrelated healthy individuals, including five Souss Berber groups and one Saharawi. The accurate genotype frequency data and other genetic parameters of forensic interest were obtained. The frequencies of the *Alu* insertion in the total sample population were found to be 0.176 (ACE), 0.455 (TPA25), 0.196 (PV92), 0.697 (APO), 0.267 (FXIIIB), 0.373 (B65) and 0.804 (HS 3.23). The power of discrimination ranged from 0.4540 for ACE to 0.6223 for TPA25. The power of exclusion for these seven loci ranged from 0.1240 to 0.1865. The combined discrimination power and the joint power of exclusion were respectively 0.9959 and 0.6910. The results indicated that the application of *Alu* insertion polymorphism will assist in forensic identification and paternity testing that is performed by current STR technology.

Keywords: *Alu* insertion, forensic application, South Moroccan population.

1. INTRODUCTION

Forensic identification and paternity testing are routinely performed using Short Tandem Repeat (STR) or Variable Number Tandem Repeat (VNTR) techniques. Their high capacity discriminating power is related to the polymorphism of each locus and is their principal advantage [1]. Recently, the Single Nucleotide Polymorphisms (SNPs) technique has found its use in forensic testing. Despite the fact that they are biallelic and, therefore, of limited discriminatory value, SNPs are developed as an alternative to the classical markers, as they present several attractive features [2- 4].

The *Alu* fragments are abundant in the human genome, with more than 1 million copies comprising 11% of the total human genome sequence [5]. *Alu* elements are stable insertion and their presence represents identity by descent. The ancestral state of an *Alu* insertion is known with certainly to be the absence of the *Alu* element at a particular locus [6]. Polymorphisms were easy to genotype in a format consisting of the presence or absence of an *Alu* element at a particular locus. *Alu* insertion polymorphisms are robust markers for evolutionary and phylogenetic studies [7-22]. The use of polymorphic *Alu* insertions in forensic identification and paternity testing is limited [23-25].

Here, we report on a comprehensive study of seven *Alu* insertion polymorphisms as a new method or as a complement to existing systems within the South Moroccan population. Specific primer pairs were designed to amplify and detect the insertion or the deletion of the *Alu* fragments at the loci ACE, TPA25, PV92, APO, FXIIIB, B65 and HS3.23. A total of 215 individuals, including five Souss Berber groups and one Saharawi, were analysed. The accurate genotype frequency data and other genetic parameters of forensic interest were evaluated.

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2. MATERIALS AND METHODS

A total of 215 blood samples from unrelated healthy individuals from the South Moroccan population were collected. The population studied included Berber-speakers, who constitute the major group of the Souss valley and one from south Morocco. Genomic DNA was extracted from peripheral blood by the salting out method [26].

Human-specific *Alu* polymorphic elements were genotyped in each sample by using the primer sequences and annealing temperature described previously for the ACE, TPA25, PV92, APO, FXIIIB [9], B65 and HS3.23 [27,28] (Table **1**). The PCR amplifications were performed in a final volume of 25µl, containing 100ng genomic DNA, 1X buffer, 1.5mM $MgCl_2$, 0.2mM d'NTP, 0.24 pM of each primer and 1unit of Taq polymerase (Promega Corporation). PCR cycling temperature protocol was: 35cycles × (denaturing at 94°C for 1min, annealing at *x*°C (see Table **1** for the value of *x*) for 2 min and extension at 72°C for 2 min).

The PCR products were analysed by electrophoresis on 2% agarose gels and were stained with ethidium bromide and visualised under UV fluorescence. Fig. (**1**) was one of the examples for Alu tests in this work.

Genotypic frequencies and statistical parameters for each locus and each group were calculated. The Power of Discrimination (P_D) is the probability that two individuals randomly taken in the same population would not have the same genotype at the particular locus. The Power of Exclusion (P_E) is the probability that a falsely-accused putative parent

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Table 1. The Analyzed Loci, Primer Sequences, Annealing Temperatures, Chromosome Location, Sub Family and Products Size Name for Each HS *Alu* Loci

Name	5' end Primer (5'→3')	3' end Primer (5'→3')	Annealing Temperature	Chr. Ban. Position	Products Size	Sub Family
ACE	CTGGAGAC-CACTCCCATCCTTTCT	GATGTGGCCATCA-CATTCGTCAGAT	58 °C	17q23	480-191	HS-1
TPA25	GTAAGAGTTCCGTAAC AGGACAGCT	CCCCACCCTAGGAG AACTTCTCTTT	58 °C	8p11.2	400-110	HS-2
PV92	AACTGGGAAAATTTG AAGAGAAAGT	TGAGTTCTCAACTC CTGTGTGTTAG	54 °C	16q24.2	437-122	HS-1
APO	AAGTGCTGTAGGCCAT TTAGATTAG	AGTCTTCGATGACA GCGTATACAGA	50 °C	11q23-q24	409-96	HS-1
FXIIIB	TCAACTCCATGAGATT TTCAGAAGT	CTGGAAAAAATGTA TTCAGGTGAGT	56 °C	1q31-q32.1	720-450	HS-1
HS3.23	GGTGAAGTTTCCAACG CTGT	CCCTCCTCTCCCTTT AGCAG	52 °C	7	410-110	Ya5
B65	ATATCCTAAAAGGGA CACCA	AAAATTTATGGCAT GCGTAT	60 °C	11q14.2	420-81	HS-1

would be excluded as the biological parent for a particular child. The joint power of discrimination and exclusion was also calculated.

3. RESULTS AND DISCUSSIONS

The loci are biallelic and the frequency of each genotype within each group studied is shown in Table **2**. The discrimination powers P_D and the power of exclusion P_E are also calculated by locus and population. Hardy-Weinberg equilibrium was assessed. Five out of 42 tests for Hardy-Weinberg equilibrium showed a significant departure from equilibrium. After correcting for multiple tests (Bonferroni correction), only one test is significant (B65 locus in Ouarzazate). Since these departures do not cluster by locus or by populations, they probably reflect random statistical fluctuations. The frequency of the presence of *Alu* insertion from all the 215 samples were 0.176 for ACE, 0.455 for TPA25, 0.196 for PV92, 0.697 for APO, 0.267 for FXIIIB, 0.373 for B65 and 0.804 for HS 3.23 (Table **3**). The discrimination powers P_D of the seven loci were 0.4540 for ACE, 0.6223 for TPA25, 0.4816 for PV92, 0.5766 for APO, 0.5529 for FXIIIB, 0.6064 for B65 and 0.4816 for HS3.23. It is shown that all loci were informative, with TPA25, B65, APO and FXIIIB being the most informative. The joint power of discrimination for the seven loci included in this study was 0.9959. The power of exclusion P_E ranged from 0.1240 to 0.1865, respectively for ACE and TPA25. The joint power of exclusion for the seven loci was 0.6910.

4. CONCLUSION

Polymorphic *Alu* insertions have proven to be useful for population genetic studies. In the Moroccan population the *Alu* inserts have previously been described. The distribution

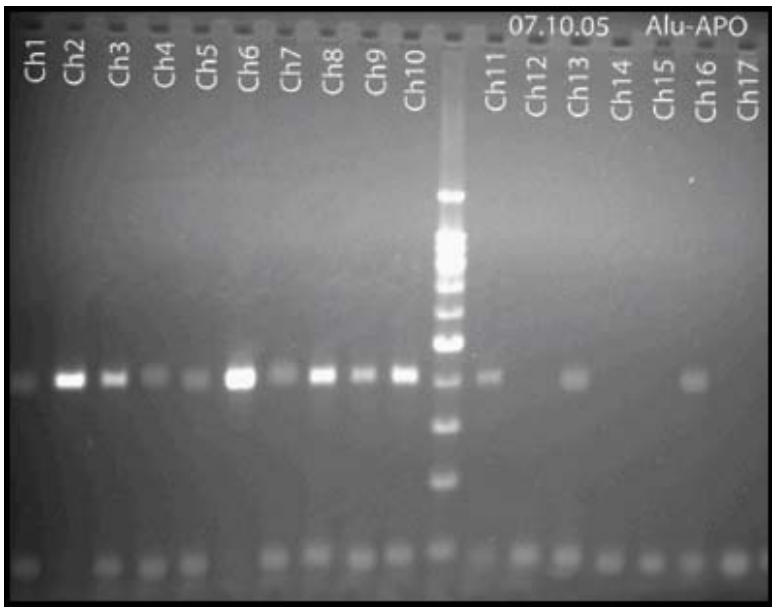

Fig. (1). Electrophoresis of PCR products on Alu-APO from 17 samples (homozygote with Alu insertion (Ch2, Ch6), Homozygote without Alu insertion (Ch12, Ch14, Ch15, Ch17) and heterozygote (Ch1, Ch3, Ch4, Ch5, Ch7, Ch8, Ch9, Ch10, Ch11, Ch13 and Ch16).

Table 2. The Frequency of Each Genotype, The Power of Discrimination P_D and The Power of Exclusion P_E Within Each Group Studied in South Moroccan Populations

	Polymorphism						
	ACE	TPA25	PV92	APO	FXIIIB	B65	HS3.23
Ouarzazate (2n = 76)							
Ppp	0.784	0.143	0.355	0.063	0.438	0.029	0.031
Ppq	0.135	0.543	0.452	0.125	0.406	0.971	0.438
Pqq	0.081	0.314	0.193	0.812	0.156	0.000	0.531
P_D	0.411	0.614	0.614	0.366	0.600	0.621	0.538
P_E	0.110	0.184	0.184	0.097	0.177	0.187	0.152
Joint PD	0.9961						
Joint PE	0.6970						
Tata (2n = 68)							
Ppp	0.515	0.485	0.576	0.161	0.690	0.647	0.107
Ppq	0.394	0.364	0.303	0.161	0.241	0.265	0.143
Pqq	0.091	0.151	0.121	0.678	0.069	0.088	0.750
P_D	0.566	0.590	0.556	0.531	0.473	0.510	0.458
P_E	0.163	0.173	0.159	0.150	0.130	0.142	0.125
Joint PD	0.9948						
Joint PE	0.6769						
Zagora (2n = 36)							
Ppp	0.824	0.167	0.611	0.111	0.647	0.571	0.000
Ppq	0.176	0.333	0.389	0.389	0.294	0.286	0.167
Pqq	0.000	0.500	0.000	0.500	0.059	0.143	0.833
P_D	0.285	0.588	0.480	0.575	0.494	0.563	0.273
P_E	0.157	0.173	0.132	0.167	0.137	0.162	0.070
Joint PD	0.9895						
Joint PE	0.6614						
Haha (2n = 62)							
Ppp	0.710	0.313	0.774	0.071	0.609	0.235	0.069
Ppq	0.226	0.531	0.226	0.393	0.261	0.706	0.276
Pqq	0.064	0.156	0.000	0.536	0.130	0.059	0.655
P_D	0.456	0.615	0.342	0.552	0.547	0.609	0.495
P_E	0.125	0.184	0.090	0.158	0.156	0.183	0.137
Joint PD	0.9945						
Joint PE	0.6745						
Chtouka (2n = 124)							
Ppp	0.652	0.240	0.790	0.200	0.581	NA	0.061
Ppq	0.326	0.500	0.184	0.400	0.355	NA	0.212
Pqq	0.022	0.260	0.026	0.400	0.064	NA	0.727
P_D	0.467	0.622	0.353	0.612	0.531	NA	0.441
P_E	0.128	0.187	0.093	0.182	0.150	NA	0.120
Joint PD	0.9867						
Joint PE	0.6068						
Guelmim (2n = 64)							
Ppp	0.750	0.594	0.867	0.161	0.517	0.000	0.056
Ppq	0.250	0.281	0.133	0.484	0.345	0.900	0.444
Pqq	0.000	0.125	0.000	0.355	0.138	0.100	0.500
P_D	0.367	0.551	0.227	0.612	0.579	0.609	0.558
P_E	0.097	0.157	0.058	0.183	0.168	0.186	0.160
Joint PD	0.9938						
Joint PE	0.6672						

of polymorphic *Alu* insertions in the Arab, Berber and Saharawis populations from Morocco and their relationships with other Mediterranean populations were evaluated [11, 29]. The use of Polymorphic *Alu* insertions in forensic identification and paternity testing is limited. The aim of the present study is the assessment of the utility of *Alu* insertions for forensic DNA typing within the Moroccan population. We report on a comprehensive study of seven loci of interest due to the insertion or the deletion of human-specific *Alu* fragments. The combined discrimination power of these seven loci (ACE, TPA25, PV92, APO, FXIIIB, B65 and HS3.23) was 0.9959. The combination of these seven *Alu* loci could assist in forensic identification and paternity testing in the Moroccan population. It is therefore expected that

Table 3. The Frequency of the Presence of *Alu* Insertion from the 215 Samples; Observed and Expected Heterozygosity, Power of Discrimination P_D; Power of Exclusion P_E and Joint Power of Discrimination and Exclusion for the Seven Loci Including in this Study

	Polymorphism						
	ACE	TPA25	PV92	APO	FXIIIB	B65	HS3.23
Allele freq.	0.1760	0.4550	0.1961	0.6974	0.2670	0.3727	0.8038
Obs. Het.	0.2615	0.4372	0.2710	0.3300	0.3312	0.6182	0.2739
Exp. Het.	0.2971	0.4972	0.3162	0.4295	0.4034	0.4697	0.3141
P_D	0.4540	0.6223	0.4816	0.5766	0.5529	0.6064	0.4816
P_E	0.1240	0.1865	0.1327	0.1666	0.1574	0.1792	0.1327
Joint PD	0.9959						
Joint PE	0.6910						

the application of *Alu* insertion polymorphism will assist in routine STR forensic testing. The addition of more *Alu* insertion loci can be used to increase the power of discrimination. Indeed, in a recent work, Ray *et al*. [30] developed *Alu* insertion for the inference of human geographical origins. This study, based on 100 *Alu* insertion polymorphisms, made it possible to correctly infer the geographic affiliation of 18 unknown human individuals with high levels of confidence. The technique to infer the geographic affiliation of unknown human DNA samples will be a useful tool in identifications in investigative forensics. *Alu*-insert based technologies will undoubtedly remain essential to identification in investigative forensics in the future.

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REFERENCES

[1] Butler, J.M. *Forensic DNA typing: Biology and Technology behind STR markers*. Academic Press: London, **2001**.

[2] Gill, P. An assessment of the utility of single nucleotide polymorphisms (SNPs) for forensic purposes. *Int. J. Legal Med*., **2001**, *114*, 204-210.

[3] Petkovski, E.; Keyser, C.; Ludes B.; Hienne, R. Validation of SNPs as markers for individual identification. *Int. Congr. Ser*., **2003**, *1239*, 33-36.

[4] Frudakis, T.; Venkateswarlu, K.; Thomas, M.J.; Gaskin, S.; Ginjupalli, S.; Gunturi, V.; Ponnuswamy, S.; Natarajan, P.K.; Nachimuthu A. A classifier for the SNP-based inference of ancestry. *J. Forensic Sci.*, **2003**, *48*, 771-782.

[5] International Human Genome Consortium. Initial sequencing and analysis of the Human Genome. *Nature*, **2001**, *409*, 860-921.

[6] Batzer M.A.; Deininger P.L. Alu repeats and human genomic diversity. *Nat. Rev. Genet.*, **2002**, *3*, 370-9.

[7] Perna, N.T.; Batzer, M.A.; Deininger P.L.; Stoneking M. Alu insertion polymorphism: a new type of marker for human population studies. *Hum. Biol*., **1992**, *64*, 641-648.

[8] Batzer, M.A.; Stoneking, M.; Alegria-Hartman, M.; Bazan, H.; Kass, D.H.; Shaikh, T.H.; Novick, G.E.; Ioannou, P.A.; Scheer, W.D.; Herrera, R.J.; Deininger P.L. African origin of human-specific polymorphic Alu insertions. *Proc. Natl. Acad. Sci. USA*, **1994**, *911*, 2288-12292.

[9] Batzer, M.A.; Arcot, S.S.; Phinney, J.W.; Alegria-Hartman, M.; Kass, D.H.; Milligan, S.M.; Kimpton, C.; Gill, P.; Hochmeister, M.; Ioannou, P.A.; Herrera, R.J.; Boudreau, D.A.; Scheer, W.D.; Keats Bronya, J.B.; Deininger, P.L.; Stoneking M. Genetic Variation of Recent Alu Insertions in Human Populations. *J Mol Evol*., **1996**, *42*, 22-29.

[10] Stoneking, M.; Fontuis, J.J.; Clifford, S.L.; Soodyall, H.; Arcot, S.S.; Saha, T.; Jenkins, M.A.; Tahir, P.L.; Deininger, M.A. Alu insertion polymorphisms and human evolution: evidence foor a larger population size in Africa. *Genome Res*., **1997**, *7*, 1061-1071.

[11] Comas, D.; Calafell, F.; Benchemsi, N.; Helal, A.; Lefranch, G.; Stoneking, M.; Batzer, M.A.; Bertranpetit, J.; Sajantila, A. Alu insertion polymorphisms in NW Africa and the Iberian Peninsula: Evidence for a strong genetic boundary through the Gibraltar straits. *Hum. Genet.*, **2000**, *107*, 312-319

[12] Comas, D.; Schmid, H.; Braeuer, S.; Flaiz, C.; Busquets, A.; Calafell, F.; Bertranpetit, J.; Scheil, H.G.; Huckenbeck, W.; Efremovska, L.; Schmidt; H. Alu insertion polymorphisms in the Balkans and the origins of the Aromuns. *Ann. Hum. Genet.*, **2004**, *68*, 120-127.

[13] Bamshad, M.; Kivisild, T.; Watkins, W.S.; Dixon, M.E.; Ricker, C.E.; Rao, B.B.; Naidu, J.M.; Prasad, B.V.; Reddy, P.G.; Rasanayagam, A.; Papiha, S.S.; Villems, R.; Redd, A.J.; Hammer, M.F.; Nguyen, S.V.; Carroll, M.L.; Batzer, M.A.; Jorde, L.B. Genetic evidence on the origins of Indian caste populations. *Genome Res.*, **2001**, *11*, 994-1004.

[14] Bamshad, M.J.; Wooding, S.; Watkins, W.S.; Ostler, C.T.; Batzer, M.A.; Jorde, L.B. Human population genetic structure and inference of group membership. *Am. J. Hum. Genet.*, **2003**, *72*, 578-589.

[15] Watkins, W.S.; Ricker, C.E.; Bamshad, M.J.; Carroll, M.L.; Nguyen, S.V.; Batzer, M.A.; Harpending, H.C.; Rogers, A.R.; Jorde L.B. Patterns of ancestral human diversity: an analysis of *Alu* insertion and restriction site polymorphisms. *Am J. Hum Genet.*, **2001**, *68*, 738-752.

[16] Watkins, W.S.; Rogers, R.A.; Ostler, T.C.; Wooding, S.; Bamshad, M.J.; Brassington, A.M.; Carroll, M.L.; Nguyen, S.V.; Walker, J.A.; Prasad, B.V.; Reddy, P.G.; Das, P.K.; Batzer, M.A.; Jorde, L.B. Genetic variation among world populations: inferences from 100 *Alu* insertion polymorphisms. *Genome Res.*, **2003**, *13*, 1607-1618.

[17] Antùnez-de-Mayolo, G.; Antùnez-de-Mayolo, A.; Antùnez-de-Mayolo, P.; Papiha, S.S.; Hammer, M.; Yunis, J.J.; Yunis, E.J.; Damodaran, C.; Martinez de Pancorbo, M.; Caeiro, J.L.; Puzyrev, V.P.; Herrera, R.J. Phylogenetics of worldwide human populations as determined by polymorphic Alu insertions. *Electrophresis*, **2002**, *23*, 3346-3356.

[18] Romualdi, C.; Balding, D.; Nasidze, I.; Risch, G.; Robichaux, M.; Sherry, S.T.; Stoneking, M.; Batzer, M.A.; Barbujani, G. Patterns of human diversity, within and among continents, inferred from biallelic DNA polymorphisms. *Genome Res.*, **2002**, *12*, 602- 612.

[19] Gonzalez-Perez, E.; Via, M.; Esteban, E.; Lopez-Alomar, A.; Mazieres, S.; Harich, N.; Kandil, M.; Dugoujon, J.M.; Moral, P. Alu insertions in the Iberian Peninsula and North West Africa-Genetic Boundaries or Melting Pot ? *Coll. Antropol*., **2003**, 27, *2*, 491-500.

[20] Santovito, A.; Marin, A.; Zampicinini, G.P.; Girotti, M.; Biggatti, M.P.; Lamberti, L.; Del Pero, M.; Selvaggi, A.; Cervella; P. Polymorphismes par insertion Alu dans trois populations du Nord de l'Italie. *Antropo*, **2004**, *7*, 45-53.

[21] Terreros, M.C.; Martinez, L.; Herrera, R.J. Polymorphic Alu insetions and genetic diversity among African populations. *Hum. Biol.*, **2005**, *77*, 675-704.

[22] Garcia-Obregon, S.; Alfonso-Sanchez, M.A.; Pérez-Miranda, A.M.; Vidales, C.; Arroyo, D.; Pena J.A. Genetic Position of Valencia (spain) in the Mediterranean Basin According to Alu insertions. *Am. J. Hum. Biol.*, **2006**, *18*, 187-195.

[23] Novick, G.E.; Menendez, C.M.; Novick, C.C.; Duncan, G.; Yunis, J.; Yunis, E.; Deininger, P. L.; Batzer, M. A.; Herrera, R. J. The use of polymorphism Alu insertions as a new methodological alternative in human paternity testing and child identification. *Int. Pediatr.*, **1994**, *2*, 60-8.

[24] Novick, G.E.; Novick, C.C.; Yunis, J.; Yunis, E.; Martinez, K.; Duncan, G.; Troup, G. M.; Deininger, P.L.; Stoneking, M.; Batzer M.A.; Herrera, R.J. Polymorphic human specific Alu insertion as marker for human identification. *Electrophoresis*, **1995**, *16*, 1596-1601.

[25] Hsieh, H.M.; Su, C.W.; Tsai, L.C.; Huang, N.E.; Tai-Ping Shih, R.; Linacre, A.; Chun-I Lee, J. Analysis of the Alu insertion polymorphism in Taiwanese Han population. *Forensic Sci. J.*, **2002**, *1*, 27-30.

[26] Miller, S.A.; Dykes, D.D., Polesky, H.F. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.*, **1988**, *16*, 1215.

[27] Arcot, S.S.; Wang, Z.; Weber, J.L.; Deininger P.L.; Batzer M.A. Alu repeats: a source for the genesis of primate microsatellites. *Genomics*, **1995**, *29*, 136-144.

[28] Arcot, S.S.; Adamson, A.W.; Lamerdin, J.E.; Kanagy, B.; Deininger, P.L.; Carrano A.V.; Batzer, M.A. Alu fossil relics-Distribution and insertion polymorphism. *Genome Res.*, **1996**, *6*, 1084-92.

[29] Chbel, F.; De Pancorbo, M.M.; Marinez-Bouzas, C.; Azeddoug, H.; Alvarez-Alvarez, M.; Rodriguez-Tojo, M.J.; Nadifi; S. Polymorphism of six Alu-insertions in residents of Morocco: comparative study in Arab and Berber populations and residents of Casablanca. *Genetika*, **2003**, *39*, 1398-405.

[30] Ray, D.A.; Walker, J.A.; Hall, A.; Llewellyn, B.; Ballantyne, J.; Christian, A.T.; Turtetaub, K.; Batzer, M.A. Inference of human geographic origins using Alu insertion polymorphisms. *Forensic Sci. Int.*, **2005**, *153*, 117-124.

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